# GENETIC POTENTIAL AND LIMITATIONS OF ETHIOPIAN CHICKPEA (*CICER ARIETINUM* L.) GERMPLASM FOR IMPROVING ATTRIBUTES OF SYMBIOTIC NITROGEN FIXATION, PHOSPHORUS UPATKE AND USE EFFICIENCY, AND ADZUKI BEAN BEETLE (*CALLOSOBRUCHUS CHINENSIS* L.) RESISTANCE

**A THESIS SUBMITTED**

**TO**

**THE SCHOOL OF GRADUATE STUDIES**

**FACULTY OF LIFE SCIENCES**

**ADDIS ABABA UNIVERSITY**

**BY**

**GEMECHU KENENI WAKEYO**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF PHILOSOPHY (PhD) IN BIOLOGY (APPLIED GENETICS)**

**ADDIS ABABA** **JUNE 2012**

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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF PHILOSOPHY (PhD) IN BIOLOGY (APPLIED GENETICS)**

**MAJOR SUPERVISOR: PROF. ENDASHAW BEKELE**
**CO-SUPERVISORS: DR MUHAMMAD IMTIAZ, DR FASSIL ASSEFA, DR EMANA GETU AND DR KIFLE DAGNE**

# DEDICATION

This thesis is dedicated to the immortal love and memories of my beloved father, Keneni Wakeyo, who, to me, never died but simply departed!

"We have seen that man by selection can certainly produce great results, and can adapt organic beings to his own uses, through the accumulation of slight but useful variations, given to him by the hand of Nature".

"This preservation of favourable individual differences and variations, and the destruction of those which are injurious, I have called Natural Selection, or the Survival of the Fittest."

Charles Darwin (From *On the Origin of Species, 1859*)

## DECLARATION

I declare that the thesis hereby submitted by me for the Degree Doctor of Philosophy (PhD) in Biology (Applied Genetics) to the School of Graduate Studies of Addis Ababa University is my own independent work and has not previously been submitted by me or anybody else at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

Signed on 21st of June 2012, The School of Graduate Studies, Faculty of Life Sciences, Addis Ababa University, Arat Kilo.

PhD Candidate _____

Gemechu Keneni

Supervisor _____

Dr Kifle Dagne

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They both unveiled to me that the "secret" of life is situated nowhere else in an organism but inside the miniature unit, i.e. the "center" of the cell, associated with the DNA or the RNA. They armed me with the courses of applied genetics, the sum total of which makes the whole background concepts and principles of plant breeding. As nothing else I can do in my capacity to reciprocate the special favors I received from these doyens of genetics, as a plant breeder, I wish to gratify them by meaningfully applying the knowledge I acquired from them in the difficult task underway to improve the overall wellbeing of humankind, particularly by best serving the betterment of the livelihoods of the poor farmers in Ethiopia.

The impetus of my academic achievement, a move from a little shepherd to a PhD holder, was first conceived at a time in 1974 when my beloved father, Keneni Wakeyo, placed me in Meta Jara Elementary School in Bacho, West Shewa Zone, Ethiopia. Unfortunately, my father was not fortunate enough to live a little more to see his ambitions being realized with the eventual success of his son. Beyond nurturing me with affection and love, I consider my father a giant of my professional life as he taught me all out of his kindness without himself being taught by his father. I wish to dedicate this thesis to his immortal love and memories which always reside deep into my heart.

I also wish to place on record my deep sense of gratitude and due respect to a number of elite scholars who taught me all the way from elementary schools to advanced university levels. A number of good professional fathers at different levels not only dragged me out of illiteracy but also educated me to the level of a matured professional. Some of my teachers and instructors educated me not only on academic and technical skills but they also socially reshaped me as life models to a mentally and psychologically matured citizen. I cannot keep on mentioning their names as they are too numerous to list but I would like to express my sincere respect and gratitude to all of them wherever they may be by now.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| AAU | = Addis Ababa University |
| AFLP | = Amplified fragment length polymorphism |
| AFRNA | = Alley Farming Research Network for Africa |
| AMOVA | = Analysis of molecular variance |
| ANOVA | = Analysis of vaiance |
| AOAC | = Association of Official Agricultural Chemists |
| APA | = Arcelins, phytohemagglutinins and α-amylases |
| CIAT | = International Center for Tropical Agriculture |
| CSA | = Central Statistics Agency |
| CTAB | = Cetyltriethylammonium bromide |
| CV | = Coefficient of variation |
| DAP | = Diammonium phosphate |
| DARC | = Debre Zeit Agricultural Research Center |
| DFID | = Department for International Development |
| $dH_2O$ | = Deionized water |
| DMRT | = Dunkan's Multiple Range Test |
| dNTPs | = Deoxynucleoside triphosphate |
| EC | = Exchangeable cation |
| EIAR | = Ethiopian Institute of Agricultural Research |
| EMA | = Ethiopian Mapping Authority |
| FAO | = Food and Agriculture Organization (of the United Nations) |
| GA | = Genetic advance from selection |
| IAEA | = International Atomic Energy Agency |
| IBC | = Institute of Biodiversity Conservation |

## ACRONYMS AND ABBREVIATIONS (CONTINUED)

| | |
|---|---|
| IBPGR | = International Board for Plant Genetic Resources |
| ICARDA | = The International Center for Agricultural Research in the Dry Areas |
| ICRISAT | = The International Crop Research Institute for the Semi-Arid Tropics |
| IPM | = Integrated pest management |
| μ | = Population mean |
| Meq | = Milli equivalent |
| MoA | = Ministry of Agriculture |
| MSE | = Mean square of error |
| MT | = Metric ton |
| NPL | = Number of polymorphic loci |
| PCs | = Principal components |
| PIC | = Polymorphic information content |
| ppm | = Part per million |
| QTL | = Quantitative trait loci |
| RAPD | = Random amplified polymorphic DNA |
| RFLP | = Restriction fragment length polymorphism |
| RILs | = Recombinant inbred lines |
| rpm | = Round per minute |
| SAS | = Statistical Analysis System |
| So4 S | = Sulphate sulfur |
| SSR | = Simple sequence repeat |
| TNL | = Total number of loci |
| TSP | = Triple supper phosphate |
| UV | = Ultra violet illumination X-ray |
| VAM | = Vesicular arbuscular micorrhizae |
| WHO | = World Health Organization |

# ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important food legumes grown all over the world. In Ethiopia, chickpea is among the most important food legumes both in terms of area coverage and volume of production. The production of chickpea is important not only in terms of human and animal nutrition but also in terms of ecological sustainability through symbiotic nitrogen fixation. Despite the importance, productivity of chickpea is constrained at least in part by production problems related to the inherent low-yielding potential of the local cultivars, production without application of adequate inputs including fertilizers and post-harvest insect pest damage particularly adzuki bean beetle (*Callosobruchus chinensis* L.).

Ethiopia is known as one of the centers of secondary diversity for chickpea. Genetic resources with proven performance for better symbiotic nitrogen fixation, phosphorus uptake and use efficiency and adzuki bean beetle resistance are limited for focused utilization in chickpea breeding programs. Characterization and evaluation of genetic make-up of the Ethiopian chickpea germplasm accessions is, therefore, essential to designing effective breeding programs.

Genetic diversity and population structure of 155 chickpea genotypes were studied using 33 microsatellite (SSR) markers. A series of field and laboratory experiments were also undertaken in 2009-2010 in Ethiopia to characterize and evaluate Ethiopian chickpea germplasm for attributes of symbiotic nitrogen fixation, phosphorus use efficiency and adzuki bean beetle resistance. The field studies for symbiotic nitrogen fixation and phosphorus use efficiency were undertaken at Ambo and Ginchi while the adzuki bean beetle resistance study was conducted in entomology laboratories at Debre Zeit, Holetta and Ambo in Ethiopia. The tests for symbiotic nitrogen fixation and phosphorus uptake and use efficiency were conducted with 155 genotypes and 130 genotypes were tested for adzuki bean beetles resistance. Randomized complete block designs with 4 replications for symbiotic nitrogen fixation, 2 for phosphorus uptake and use efficiency and 3 for resistance to adzuki bean beetle were used. The difference technique, with a non-nodulating reference genotype was employed to estimate nitrogen fixation and the balance method was used to estimate phosphorus uptake and use efficiency.

Molecular analysis of variance showed variation of 73% within and 27% between populations. Introduced genotypes had higher polymorphism (70.27%) than the local accessions (36-57%). Collections from Shewa, Harargie, W. Gojam and S. Gonder regions showed the second higher polymorphism (50-57%) than the rest of the local accessions (36-45%). Accessions from adjoining eco-geographical origins mostly showed tendencies for more genetic similarity than those from far isolated origins.

Cluster analyses at the molecular level grouped the genotypes into five clusters. The first cluster ($C_1$) constituted accessions from Arsi, the second ($C_2$) from Gojam and Gonder, the third ($C_3$) from Harargie and E. and N. Shewa, the fourth ($C_4$) from W. Shewa, Tigray and Wello regions and the fifth ($C_5$) all improved genotypes. Improved Kabuli and Desi types fell into a distinct cluster ($C_5$) regardless of the difference in seed types.

Analysis of variance of symbio-agronomic characters, phosphorus uptake and use efficiency and adzuki bean beetle resistance showed significant differences among the genotypes, locations and genotype by location interaction effects for a number of traits. Genotype by phosphorus level interaction effects were non-significant except in a few cases.

A number of landraces superior to introduced genotypes were identified for attributes of symbio-agronomic characters except for seed size where the best genotypes were all from exotic sources. The amount of fixed nitrogen ranged from 13-49% in foliage, 30-44% in grain and 28-40% in total above ground biomass. Grain yield performance varied from 31-70 g 5 plants$^{-1}$ and seed size from 82-288 g/1000 seeds. The top 5% best accessions for total (shoot + grain) nitrogen fixation include Acc. Nos. 41222, 41029, 41021, 41074, 41075, 41129, 41320 and 41026. There were also some other genotypes which had better fixations either in their shoots (e.g. 41103) or grains (e.g. 207734). Two introductions from ICRISAT, namely ICC 5003 and ICC 4973, were also among the top 5% best fixers of nitrogen in their shoot. The best assimilators of fixed nitrogen were Acc. Nos. 41115, 207659, 219799, 207150, 41277, 41113 and 207894.

The application of phosphorus fertilizer increased a number of characters including symbiotic nitrogen fixation. Yield increments of 15% at Ambo and 17% at Ginchi were recorded due to application of phosphorus. The top 5% best efficient, responder genotypes for grain yield include Acc. No. 41274, 41111, 207742, 207563, 207763, 231328, ICC 19180 and 41114. Three of these accessions, namely 41274, 207563 and 41111, also repeated best performances as efficient, responder genotypes for biomass weight. Other efficient, responder genotypes for biomass weight include: Acc. Nos. 207743, 41015, 41066, 41185 and Ejere.

Complete resistance to adzuki bean beetle was not observed among the genotypes but Acc. Nos. 41320, 41289, 41291, 41134, 41315, 207658, 41103, 41168, 41142, 41174, 41029, 41207, 209087, 231327, 41161 and 41008 showed partial resistance. Significant progresses were achieved in grain yield and seed size from past breeding efforts but the same efforts had inadvertently increased seed susceptibility to adzuki bean beetle.

Cluster analysis grouped the genotypes into six cluster for symbio-agronomic characters, five clusters in the absence and six clusters in the presence of phosphorus, respectively, for attributes of phosphorus uptake and use efficiency and three clusters for attributes of adzuki bean beetle resistance. The higher number of clusters when the crop was grown with phosphorus may be a manifestation of more genetic diversity due to the application of phosphorus. The limited genetic diversity for response characters to infestation by adzuki bean beetle may imply the need for characterization of additional landraces and exotic genotypes.

The Mahalanobis's $D^2$ statistics mostly showed significant genetic distances between clusters constituted local landraces on the one hand and introduced genotypes on the other. This indicated that there were distinct multivariate differences between landraces and introduced genotypes. No clear interrelationship was observed between the origins of the landraces within

Ethiopia and the pattern of genetic diversity. Different characters had different contribution to the total differentiation of the populations in all the cases.

The study on symbio-agronomic traits showed significant positive correlations between a number of characters. Grain yield was positively associated with fixed nitrogen assimilation efficiency, shoot, grain, and above ground biomass nitrogen yields and nitrogen harvest index. Agronomic characters like grain filling period, pod and seed numbers, shoot, and total above ground biomass accumulation, harvest index, grain production efficiency and biomass production and economic growth rates also positively correlated with grain yield. Increased nitrogen yield, nitrogen harvest index, fixed nitrogen assimilation efficiency and above ground biomass may be more important than the *per se* concentration of nitrogen in plant tissue. Characters like shoot, grain and total biomass nitrogen contents and fixation, fixed nitrogen assimilation efficiency, seed size, grain filling period showed higher genetic variation, broad-sense heritability and expected genetic gains from selection.

The study on phosphorus uptake and use efficiency revealed significant positive correlations within plant tissue (shoot, seed and biomass) phosphorus contents (r = 0.22-0.85), between plant tissue phosphorus contents and phosphorus yields (r = 0.22-0.99), within plant tissue phosphorus yields (r = 0.23-0.89) and within parameters of phosphorus uptake and use efficiency in a number cases. Grain yield and economic growth and biomass production rates, grain production efficiency, and shoot and biomass dry weight showed significant positive correlations (r = 0.70-0.99) with phosphorus yield efficiency. Broad sense heritability values ranged from 60-93% and genetic advance values ranged from 4-62% in the absence of phosphorus. The corresponding broad sense heritability and genetic advance values in the presence of phosphorus ranged from 59-93% and 4-79% in that order.

In the study of bruchid resistance seed-related traits (seed size, percent seed coat weight and seed weight loss) exhibited larger heritable variation than insect-related traits (number of eggs and adults, days to adult emergence, number of uninfected seed and adult recovery). Broad-sense heritability for seed and insect-related traits varied from 43-76% and 0.20-11%, respectively. The corresponding expected genetic gains from selection as percent of mean ranged from 28-42% and 0.01-6% in the same order. Significant positive correlations were found among seed weight loss and three component characters, i.e. number of eggs and adults emerged and seed size.

The results from these series of studies suggests possibilities for identification of chickpea genotypes superior for symbio-agronomic characters, phosphorus uptake and use efficiency and adzuki bean beetle resistance to the varieties released so far, indicating the need for the initiation of a planned breeding program. Further implications of the findings as regard germplasm collection, conservation and eco-geographical pattern evolution are also discussed.

# 1. INTRODUCTION

## 1.1. Background and justification

Chickpea (*Cicer arietinum* L.; 2n = 2x = 16) has been grown in the Mediterranean, Southeastern Asia and East African sub-continents since antiquity (Muehlbauer and Tullu, 1997). Currently, the crop is grown in over 40 countries of the world on 11 million ha of land from which over 8 million tons of seed is annually harvested (Kassie *et al.*, 2009). It is the third most important pulse crop after haricot bean (*Phaseolus vulgaris*) and field pea (*Pisum sativum*) at global level (Joshi *et al.*, 2001; Graham and Vance, 2003). The major producers are India, Pakistan, Turkey, Australia, Iran, Myanmar, Canada, Ethiopia, Mexico and Iraq with over 93% of the global production (Upadhyaya *et al.*, 2008). Most of the chickpea is produced with rainfall under marginal situation in developing countries (Ali *et al.* 2002; Serraj *et al.*, 2004; Toker *et al.*, 2007).

In Africa, chickpea is widely grown in Ethiopia, Sudan, Eritrea, Kenya, Tanzania and Malawi (Bejiga and van der Maesen, 2006). Ethiopia, with a total area of 208,381 ha and the productivity of 1.55 t $ha^{-1}$ (CSA, 2011), contributes around 46% of the total production in Africa (Kassie *et al.*, 2009). Chickpea ranks second in area coverage from among the pulses grown in Ethiopia preceded only by faba bean (*Vicia faba*) and third in volume of production next to faba bean and haricot bean (CSA, 2011). It is grown in several regions of the country with residual moisture on black vertisol soils (Bejiga and van der Maesen, 2006; Kassie *et al.*, 2009).

Chickpea is produced for different purposes including food and feed, cash and foreign currency earnings. In addition, it replenishes soil fertility as it fixes a substantial amount of atmospheric nitrogen in symbiotic association with two species of root nodule bacteria, namely *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* (Rivas *et al.*, 2006; Willems, 2006). Chickpea may fix well over 100 kg of nitrogen $ha^{-1}$ (Crouch *et al.*, 2004; Shiferaw *et al.*, 2004) with a contribution of over 500,000 MT of nitrogen every year in developing countries (Hardarson, 2004) thereby result in savings for smallholder farmers from less fertilizer use (IFPRI, 2010).

Despite the significant economic and ecological importance, however, the productivity of chickpea in Ethiopia is far below its potential (Bejiga and van der Maesen, 2006). It is assumed that genetic potential yields of chickpea under ideal condition on experimental plots may reach 5 t $ha^{-1}$ (Muehlbauer and Tullu, 1997). Improved varieties released in Ethiopia are reported to yield 2.8 t $ha^{-1}$ on research stations (Kassie *et al.*, 2009) and 1.8 t $ha^{-1}$ on farmers' fields (Jarso *et al.*, 2011). Nevertheless, the national average yield is low (1.55 t $ha^{-1}$) (CSA, 2011; Kassie *et al.*, 2009; Jarso *et al.*, 2011). Even though 100 kg $ha^{-1}$ of DAP (diammonium phosphate) is recommended in Ethiopia to increase production (Eshete, 1994), the crop is mainly produced under marginal conditions with no application of external inputs including fertilizer (Bejiga and Daba, 2006; Agegnew *et al.*, 2006).

Generally, biotic and abiotic stresses cause significant economic losses to crop plants (Slater *et al.*, 2003). Low nutrient stress preceded only by drought may be among the most important abiotic stresses with worldwide distribution (Singh, 2002; Cattivelli *et al.*, 2008). Nitrogen and phosphorus deficiency stresses are important problems worldwide, particularly in the tropics and the subtropics (Ali *et al.*, 2002; Beebe *et al.*, 2006; Gunes *et al.*, 2006; Ojo *et al.*, 2006). Nutrient deficiency problem is widespread in East Africa (Sanchez, 2002) and Ethiopia is not an exception (Mamo *et al.*, 1988; Tsegaye, 1992).

Most legume crops perform poorly under poor soil fertility (Ali *et al.*, 2002; Velayutham and Kalpana, 2004; Beebe *et al.*, 2006; Ojo *et al.*, 2006; McKnight Foundation, 2008). For instance, the production of each ton of soybean (*Glycine max*) seed may require the assimilation of 100 kg of nitrogen (Keyser and Li, 1992).

Nitrogen and phosphorus are components of amino acids, nucleic acids and phospholipids that condition plant growth, development and yield formation (Sinclair and Vadez, 2002; Hubbell and Kidder, 2003; Lindemann and Glover, 2003; Bucher, 2006). Phosphorus is also important in adenosine tri-phosphate (ATP) synthesis for nitrogen fixation (Sinclair and Vadez, 2002; Muhammad *et al.*, 2004; Serraj, *et al.*, 2004; Bucher, 2006; Qiao *et al.*, 2007).

In addition to nitrogen and phosphorus deficiencies, storage insect pests, particularly the adzuki bean beetle (*Callosobruchus chinensis* L.), are important constraints of chickpea production in Ethiopia (Ali and Habtewold, 1994; Damte and Dawd, 2006). Some reports indicate that the adzuki bean beetle in chickpea may cause a loss of up to 50% in Ethiopia (Ali and Habtewold, 1994; Damte and Dawd, 2006) and 28% in Eritrea (Haile, 2006). In addition to the actual loss, the chickpea grain, once damaged by bruchid, fits neither for planting (due to poor germination) nor for food or feed (due to spoilage and bad smell) (Aslam, 2004; Haile, 2006).

Plant nutrition and storage insect pest management will continue to be problems of chickpea production particularly by resource-poor farmers in the future (Ali *et al.*, 2002; Sithanantham and Rao, 2006). The breeding of chickpea genotypes with better symbiotic nitrogen fixation, phosphorus use efficiency and resistance to the adzuki bean beetle could, therefore, prove one of the possible approaches both for cost effectiveness, agricultural sustainability and food safety (Pearson *et al.*, 1995; Aryal *et al.*, 2003; Burger *et al.*, 2008; Löschenberger *et al.*, 2008; Osman *et al.*, 2008; Somta *et al.*, 2008; Wolfe *et al.*, 2008).

Genotypic differences for attributes of symbiotic nitrogen fixation, phosphorus use efficiency and resistance to the adzuki bean beetle have been observed in many legumes including chickpea (Ali *et al.*, 2002; Krasilnikoff *et al.*, 2003; Walley *et al.*, 2005; Shaheen *et al.*, 2006; Chen *et al.*, 2007; Somta *et al.*, 2008). Examples of success stories in terms of varietal identification and/or release have also been reported. For instance, phosphorus efficient genotypes of chickpea (Singh, 1990) and haricot bean (Aráujo *et al.*, 1998) were identified and improved varieties of soybean with potentials of doubling yield without additional nutrients have been released to farmers in China and Africa (McKnight Foundation, 2008).

A few reports also indicated the existence of cultivars with multiple nutrient use efficiency (Bassam, 1998) and those with combined nutrient deficiency stress tolerance and use efficiency (Górny, 2001). Existence of complete resistance to adzuki bean beetle among the cultivated species of haricot bean, field pea, cowpea (*Vigna unguiculata*), black gram (*Vigna*

*mungo*) and chickpea have also been reported as noble opportunities to be exploited in plant breeding programs (Redden and McGuire, 1983; Pacheco *et al.*, 1994; Dongre *et al.*, 1996; Goossens *et al.*, 2000; Morton *et al.*, 2000; Shaheen *et al.*, 2006).

Large number of root nodule bacteria from different legume species have been collected and characterized in Ethiopia, with results confirming existence of adequate genetic diversity (Wolde-meskel *et al.*, 2004; 2005; Wolde-meskel, 2007; Belay and Assefa, 2011). Efforts made to enhance nitrogen fixation through selection of effective and competitive strains of *Rhizobia* have also resulted in the identification of many effective and competitive strains (Hailemariam and Tsige, 2006). Some strains developed by the National Soils Laboratory have already been under commercial use including in chickpea (Bejiga, 2004).

However, development of better strains alone may not provide the required productivity gains without compatible host genotypes (Rengel, 2002; Chen *et al.*, 2003; Crouch *et al.*, 2004; Giongo *et al.*, 2007). Some reports indicated that host plants are also equally important as strains in enhancing the symbiotic process (Vance *et al.*, 1988; Shantharam and Mattoo, 1997; Hardarson, 2004). Despite possibilities for improving host-strain symbiotic efficiency through breeding in a number of legumes (Pearson *et al.*, 1995; Hardarson. 2004), the role of host plants attracted little attention in Ethiopia (Bejiga, 2004) and elsewhere (Hardarson, 2004; Winter *et al.*, 2004).

Ethiopia, as the secondary center of genetic diversity for many crops, owns an immense wealth of genetic diversity for many legumes (Hagedorn, 1984; Mekibeb *et al.*, 1991). For effective utilization in breeding programs of these germplasm, however, genetic characterization and evaluation should make an integral part of germplasm collection and conservation programs (Carvalho, 2004). Genetic characterization and evaluation of the available germplasm not only unveils the magnitude and pattern of genetic diversity available in the germplasm for conservation but also enables the determination of useful genes in germplasm and the possible progresses that can be made through future breeding activities (Arumuganathan and

Earle, 1991; Hayward and Breese, 1993; de Vicente *et al.*, 2005). Screening and selection would generate promising genotypes only if the source germplasm is genetically diverse. Crossing is also likely to produce higher heterosis, desirable genetic recombination and segregation in progenies when it is made between genetically diverse parents (Singh, 2002). Despite the large number of chickpea collections available in Ethiopia, however, most of these collections have not yet been well characterized and evaluated (Tanto and Tefera, 2006).

### 1.2. General objectives

- To characterize and evaluate the genetic diversity of Ethiopian chickpea germplasm accessions at molecular and morpho-agronomic levels
- To assess the potential and possibilities for genetic improvement of Ethiopian chickpea germplasm accessions for attributes of symbiotic nitrogen fixation, phosphorus uptake and use efficiency and adzuki bean beetle resistance

### 1.3. Specific objectives

- To assess the magnitude and pattern of genetic diversity among the chickpea germplasm using microsatellite (SSR) markers
- To assess the magnitude and pattern of genetic diversity among the chickpea germplasm for attributes of symbiotic nitrogen fixation, phosphorus uptake and use efficiency, adzuki bean beetle resistance and morpho-agronomic traits
- To identify source of desirable genes among the chickpea germplasm accessions for symbiotic nitrogen fixation, phosphorus uptake and use efficiency and adzuki bean beetle resistance
- To classify the test genotypes into homogeneous groups and thereby examine if there is any definite relationship between geographical origin and genetic diversity at both molecular and morpho-agronomic levels; and
- To determine the magnitude of genetic variability, heritability and expected genetic gains from selection for symbiotic nitrogen fixation, phosphorus uptake and use efficiency and adzuki bean beetle resistance.

# 2. LITERATURE REVIEW

## 2.1. The Role of Legumes in Sustaining Crop Production

### 2.1.1. Agricultural sustainability

It is generally agreed that agricultural productivity should be increased in environmentally friendly and sustainable ways in order to ensure food availability to the ever growing human population. Agricultural sustainability is not only difficult to define but also ambiguous to explain (Pearson *et al.*, 1995). Some consider it as the exploitation of natural resources to satisfy human needs while at the same time maintaining the environment (TAC, 1988). Others state it as maintaining production at levels necessary to meet the increasing human aspirations without degrading natural resources (FAO, 1989). For others agricultural sustainability is nothing but meeting the needs of the currently existing human population without compromising the ability of future generations to meet their own needs (Tilman *et al.*, 2002; DFID, 2004). A sustainable cropping system is, therefore, said to maintain natural resources such as soil and water while providing adequate production for human consumption (Hoare, 1992). From the biological perspectives, sustainable agriculture is that which is managed toward greater resource efficiency and conservation while maintaining an environment favorable for the evolution of all species (Golley *et al.*, 1992).

Modern agriculture is based on maximum output from optimal production conditions (Bohlool *et al.*, 1992). There is a general feeling that more priority has been given to immediate productivity at the expense of the natural resource base and agricultural sustainability (Pearson *et al.*, 1995; DFID, 2004). The successes of the "Green Revolution" were, for instance, partially based on the use of high inputs with responsive varieties to the inputs (Shantharam and Mattoo, 1997; Singh, 2000). Nitrogen and phosphorus fertilizers are the most important external inputs for maximum

outputs in modern crop production. Nevertheless, experiences from efforts like the Green Revolution could not be sustainably repeated in poor countries of the tropics and the subtropics (Borlaug, 2000; 2002) mainly because the input levels required along with the new varieties were too high for poor farmers (Singh, 2000).

The use of nitrogen and phosphorus fertilizers is drastically increasing with time (World Phosphate Institute, 2006). This may be because many countries aspire to increase crop production as a result of the increasing demand for food with population growth (Keneni and Wakjira, 2004). However, in addition to the immediate costs, the continued use of high fertilizer input accelerates depletions of the non-renewable raw materials and energy resources required for fertilizer production (Bøckman, 1997; Syers *et al.*, 2008). The harmful effect of fertilizers as pollutants of ground or surface water (eutrophication) is also an issue of great concern, particularly in the developed countries (Isfan *et al.*, 1995; Roy, 1995; Beem and Smith, 1997; Quinones *et al.*, 1997; Sharpley *et al.*, 2003). This can be witnessed from different legislations enacted by countries like the USA to reduce the amount of nutrients going to drinking water (U.S. Environmental Protection Agency, 1996; Helland, 2004).

A number of workers advise that agricultural sustainability should rely on the use and effective management of internal resources including crop rotation and intercropping of cereals with legumes with more efficient use of fertilizers (Pearson *et al.*, 1995; Beebe *et al.*, 2006; Srinivasarao *et al.*, 2006), which is considered as economically feasible and ecologically sound (Bohlool *et al.*, 1992; El-Sharkawi *et al.*, 2007). Biological nitrogen fixation, defined as the enzymatic reduction of $N_2$ gas to ammonia (Beringer *et al.*, 1988), is considered as safest and sustainable source of nitrogen for organic agriculture and sustainable farming system for the production of healthy food for human consumption (Wolfe *et al.*, 2008).

Crop productivity can basically be improved by genetic modification of crops or by altering the growing environments (Coffman and Smith, 1991; Wallace and Yan, 1998). Genetic modification of crops to increase yields is often preferred to the continual manipulation of the growing environment

not only because of cost but also because of concerns for agricultural sustainability (Keneni, 2007). Symbiotic nitrogen fixation can be improved, among others, by host plant breeding for enhanced nitrogen fixation, selection of effective strains able to fix more nitrogen and use of different agronomic methods that improve soil conditions for the crop and microbial symbionts (Brockwell *et al.*, 1988; Montañez, 2000). Phosphorus deficiency problems can also be overcome in part through development and use of integrated plant nutrition systems involving use of nutrient efficient genotypes as a major component (Sanginga *et al.*, 2000; Ahmad *et al.*, 2001; Gill *et al.*, 2005).

### 2.1.2. The role of legumes in sustaining crop production

The soil fertility restoring ability of legumes was realized and exploited by farmers since antiquity (Hirsch, 2009), even before symbiotic association between bacteria and the host plants was scientifically known (Lim and Burton, 1982; Hirsch, 2009). Two types of biological nitrogen fixation are recognized based on the level of association between the host plant and the bacteria.

The first is symbiotic nitrogen fixation, resulting from symbiotic association of certain nodule bacteria with the roots of legume plants. Symbiotic association between legumes and *Rhizobium* was discovered in the 1880s by two German chemists, Herman Hellriegel and Herman Wilfarth (Lim and Burton, 1982; Fomeg-as, 2004). Symbiotic host-bacteria association is a relationship between legume host plants and the bacteria whereby both associates mutually benefit from the relationship with each other. The bacteria gets energy from the host plant and reduces atmospheric nitrogen to ammonium, which eventually is exported to plant tissues for use by the plant for protein synthesis (Keyser and Li, 1992). The genus *Rhizobium* was first assumed as the only fixer of atmospheric nitrogen (Lim and Burton, 1982) until further investigations revealed additional genera of soil bacteria, namely *Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium* as fixers of atmospheric nitrogen symbiotically with legumes (Rincón *et al.*, 2008).

The second type is non-symbiotic association of micro-organisms like *Azotobacter* and *cyanobacteria* (blue-green algae) with non-nodulating host plants like rice. Almost about the same time when symbiotic nitrogen fixation was discovered in Germany, a Russian biologist Sergei Nikolaievich Winogradsky also discovered non-symbiotic nitrogen fixation (Delwiche and Wijler, 1956). Non-symbiotic nitrogen fixers have been categorized as rhizosphere organisms, facultative endophytes and obligate endophytes (Baldani *et al.*, 1997). It is a type of biological nitrogen fixation where the microorganisms are freely and independently living in the soil.

Biological nitrogen fixation makes an integral part of sustainable agro-ecosystems by contributing well over hundred million metric tons of renewable nitrogen per year to the nitrogen economy of the globe (Hardarson and Atkins, 2003; Hubbell and Kidder, 2003; Jensen and Hauggaard-Nielsen, 2003). Symbiotic nitrogen fixation is considered as the most important type of fixation for contribution to agricultural sustainability (Hubbell and Kidder, 2003). The amount of nitrogen annually fixed with symbiotic association is estimated to be far more than the amount industrially produced each year (Ishizuka, 1992; Bøckman, 1997; Rengel, 2002; Jensen and Hauggaard-Nielsen, 2003). The contribution of free-living bacteria should also not be undermined but it is far less than the amount from symbiotic associations. The lower contribution by the latter is attributed to the low bacterial populations in the soil and the limited availability of organic substrates as source of energy for the bacteria (Hubbell and Kidder, 2003).

A number of studies showed that some legumes may also enhance nutrient availability for associated or subsequently grown cereals (Li *et al.*, 1997; Nuruzzaman *et al.*, 2005; Jemo *et al.*, 2006; Vesterager *et al.*, 2007; Kirkegaard *et al.*, 2008) by producing exudates like citrate and malate that solublize soil minerals including fixed forms of soil phosphorus (Ascher *et al.*, 2001; Ali *et al.*, 2002; Ojo *et al.*, 2006; Vesterager *et al.*, 2006; Fageria *et al.*, 2008). For instance, white lupine (*Lupinus albus*) improved phosphorus uptake for wheat (*Triticum aestivum*) (Gardner and Boundy, 1983); pigeon pea (*Cajanus cajan*) for sorghum (*Sorghum bicolor*) (Ae *et al.*, 1990); faba

bean, cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*) for maize (*Zea mays*) (Li *et al.*, 2003b; Vesterager *et al.*, 2007; Waddington *et al.*, 2007); and soybean, chickpea and faba bean for wheat (Herridge *et al.*, 2000; Li *et al.*, 2003a; Rao *et al.*, 2004; Fan *et al.*, 2006).

Nitrogen fixation by faba bean was found to have significance spillover effect to subsequently grown wheat in Ethiopia (Gorfu, 1998). Some reports also indicated that growing legumes after cereals with only residual nutrients resulted in harvesting full yields of both crops (Bahl and Pasricha, 1998; Ahlawat *et al.*, 2007). Beyond replenishing soil fertility through symbiotic nitrogen fixation and mineral solublization, legumes also play important roles as "break" crops in rotation with cereals as they can break the life cycles of crop-specific pathogens of cereals as non-host crops (Malhotra *et al.*, 2004; Kirkegaard *et al.*, 2008).

In order to keep the farming system healthy and sustainable, therefore, legumes should occupy a significant portion of area currently under cereals both as components of multiple cropping and in crop rotation (Parr *et al.*, 1983; Pearson *et al.*, 1995). Despite the importance being already realized, nevertheless, legumes are grown so far as sources of food and feed but not to the level required for soil fertility amendment as their main purpose (Bøckman, 1997). Production statistics show that only a small portion of land is allotted to legumes particularly in Sub-Saharan Africa (Serraj *et al.*, 2004; Twomlow, 2004). In Ethiopia, for instance, of the total 11,822,786 ha of land currently cultivated to grains, pulses occupy only 1,357,523 (11.48%) while cereals occupy 81.97% (CSA, 2011). Even though Ethiopia has a huge potential for growing and exporting various pulses, the achievements to date are very low because of least share of land and input allotment to these crops resulted in low production (Adenew, 2009) and consumption of only 12 kg of pulses per capita per year (FAO, 1996). The trend is more or less the same in many other developing countries like India (Commodityonline, 2009).

At farm level, nitrogen fixation from annual legumes may contribute 20 to 120 kg of nitrogen $ha^{-1}$ in a growing season in the temperate region (Pearson *et al.*, 1995) or 15–120 kg of nitrogen $ha^{-1}$ in Africa (Dakora and

Keya, 2000) even though higher values were reported for soybean (Keyser and Li, 1992) even in Africa (Shoko *et al.*, 2007). The contribution from symbiotic nitrogen fixation may rise by 3-4 folds for perennial forage and tree legumes where leaves can annually be pruned and incorporated into the soil (Pearson *et al.*, 1995; Carlsson and Huss-Danel1, 2003; Sanginga, 2003). Non-symbiotic bacteria may also contribute 1-3 kg of nitrogen $ha^{-1}$ in a year (Crouch *et al.*, 2004).

A number of reports prove that much of the nitrogen may be removed when legumes are harvested with still significant amount remaining in the soil for future crops (Lo´pez-Bellido *et al.*, 2006; Liu *et al.*, 2010). Studies from different countries in different parts of the world like Syria (Keatinge *et al.*, 1998), Australia (Armstrong *et al.*, 1997; Holford and Crocker, 1997; Herridge *et al.*, 2000; Evans *et al.*, 2001; Smith *et al.*, 2009), Ghana (Adjei-Nsiah *et al.*, 2008), Guinea (Sanginga *et al.*, 1997), India (Ghosh *et al.*, 2007; Sharma and Behera, 2009), Nigeria (Adeboye *et al.*, 2007; Yusuf *et al.*, 2007; 2009), Ethiopia (Gorfu, 1998), Tunisia (Romdhane *et al.*, 2008) and Denmark (Jørgensen *et al.*, 1999) have clearly revealed benefits of different cereals grown after different legumes. Even in dry areas, identification of legume cultivars tolerant to drought for attributes of symbiotic nitrogen fixation was proven to be a possibility of practical significance (Purcell *et al.*, 1997; Serraj and Sinclair, 1998; Serraj *et al.*, 2004; Devi *et al.*, 2010).

## 2.2. Conceptual Frameworks for Breeding Success

### 2.2.1. Choice of germplasm

Breeding progress depends on the magnitude of genetic variability among the genetic material under consideration, heritability of a given trait in a given environment and the level of selection intensity applied (Falconer, 1989; Hayward and Breese, 1993; Singh, 2002).

The source of genetic variability for host symbiotic nitrogen fixation ability could be created by introduction from exotic and collection of

germplasm from local sources. According to Provorov and Tikhonovich (2004), better nitrogen fixing genotypes may often be found not in commercial varieties but in old cultivars, landraces or wild relatives which may not be good for *per se* agronomic performance. Adgo and Schulze (2002), who found that field pea landraces introduced from Ethiopia were more nitrogen fixers than a commercial cultivar under greenhouse condition in Germany, also confirmed the relative importance of landraces for symbiotic nitrogen fixation over the commercial cultivars they studied. A similar trend was observed for haricot bean from Austria (de Oliveira *et al.*, 1998).

Superiority of improved cultivars to primitive forms has also been shown in other reports. For instance, Keneni *et al.* (2010) compared improved cultivars of field pea (*P. sativum var. Sativum*) with primitive forms (*P. sativum var. Abyssinicum*) on red and black soils and found out that the former were apparently superior to the latter. In chickpea, both nodulation efficiency and grain yield were improved as the result of selection from released cultivars (Ali *et al.*, 2002).

Existence of good nitrogen fixation ability in commercial cultivars would make life easier for breeders as commercial varieties have already desirable agronomic attribute for direct use in production without much genetic manipulation and modification (Acevedo and Fereres, 1993; Singh, 2002). Crossing genotypes from different sources was also found to be promising for breeding programs to increase nodule number and nodule dry weight in haricot beans (Franco *et al.*, 2001). Nevertheless, it is generally believed that landraces as sources of initial breeding materials have considerable breeding values under sub-optimal production as they contain valuable adaptive genes to different circumstances (Ceccarelli, 1994; Bunder *et al.*, 1996; Chahal and Gosal, 2002; Provorov and Tikhonovich, 2004).

Existence of high heritability (broad sense, narrow sense, realized) and genetic gains from selection for symbiotic nitrogen fixation have also been reported from previous studies (Provorov and Tikhonovich, 2004). Many reviews have showed that effective symbiotic nitrogen fixation could be achieved from genetic improvement of the host and the strain for genetic

variation exists in both the host species and bacterial strains (Beringer *et al.*, 1988; Clark *et al.*, 1988; Schmidt, 1988; Witty *et al.*, 1988; Pearson *et al.*, 1995).

Similarly, bruchid resistance can be screened from cultivated varieties, germplasm collections and related wild species (Ku-Hwan *et al.*, 2002; Somta *et al.*, 2006; 2007; Chen *et al.*, 2007). Genes for complete resistance to insect pests in general and storage pests in particular may either be under represented or under expressed in cultivated species of legumes, but exist in a number of wild relatives as they have coexisted with and constantly exposed to insect pests on an evolutionary time scale (Acosta-Gallegos *et al.*, 2008). Complete resistance is not common in cultivated species but complete resistances against bruchid have been reported in related wild species of a number of legumes (Clement *et al.*, 2002; Ku-Hwan *et al.*, 2002; Somta *et al.*, 2006; 2007; 2008; Chen *et al.*, 2007).

Nevertheless, a few cases of complete resistance were also reported in cultivated or germplasm collections of haricot bean (Ishimoto *et al.*, 1996; Goossens *et al.*, 2000), field pea (Morton *et al.*, 2000), cowpea (Redden, 1983; Redden and McGuire, 1983; Redden *et al*, 1983), black gram (Dongre *et al.*, 1996) and chickpea (Pacheco *et al.*, 1994; Shaheen *et al.*, 2006). Some reports also showed improved varieties are more susceptible than landraces (Desroches *et al.*, 1995; Lale and Kolo, 1998) because improvements associated with grain quality might have inadvertently resulted in more susceptibility of the improved cultivars as compared to local cultivars (Shaheen *et al.*, 2006).

### 2.2.2. Characterization and evaluation of germplasm

Genetic characterization of germplasm by gene banks may be considered as recording of distinctly identifiable and heritable characteristics (Upadhyaya *et al.*, 2008). It could also be stated as the process of detecting genetic diversity (in genotypes or genes) existing within or among germplasm accessions using descriptor lists of morphological characters or at the level of gene expression (proteins) or DNA sequences (de Vicente *et al.*, 2005). Genetic evaluation, on the other hand, may involve evaluation of agronomic

performance of accessions for different morpho-agronomic and physiologic characters under various environmental conditions that assist effective future utilization of the accessions in plant breeding programs (de Vicente *et al.*, 2005; Upadhyaya *et al.*, 2008).

Characterization and evaluation by genebanks mostly focus on morphological differentiation of accessions using qualitative traits that show little genotype by environment interaction (Carvalho, 2004). However, for genetic evaluation to be useful, evaluation must be related to the breeders' needs in terms of encompassing relevant characters like high yield, resistance to pests and diseases, adaptation to different environments and improved quality. The range of characters is very diverse and would require a multidisciplinary team and an interdisciplinary approach. However, although time-consuming and expensive, systematic evaluation is necessary if use and benefits derived from the conserved germplasm is to be maximized (Carvalho, 2004).

Genetic characterization is a measure of genetic diversity within species (Rubenstein *et al.*, 2005). The application of information on genetic diversity may vary from discipline to discipline. Evolutionary biologists may be more interested to the process of speciation or the formation of new species, ecologists to the number and distribution of species within a given habitat and plant breeders may put more focus on diversity within a crop species of interest including secondary and tertiary gene pools (Rubenstein *et al.*, 2005). At intra-specific level, genetic diversity arises either as a result of geographic separation or genetic barriers to crossability (Singh, 2002).

Genetic characterization and evaluation as intra-specific diversity provides essential information for germplasm utilization, establishment of core collections and the detection of duplications in collections (Carvalho, 2004). The effectiveness of breeding programs largely depends, on the genetic structure of a given population, may be defined as a community of individuals which share a common gene pool (Hayward and Breese, 1993).

Knowledge of the determination of the eco-geographic distribution of genetic diversity is also rewarding in terms of effective germplasm collection, conservation utilization of genetic resources (Ford-Lloyd and Jackson, 1986;

Chahal and Gosal, 2002; Singh, 2002) and in studies of crop evolution (Singh, 2002). Joshi and Dhawan (1966) suggested that differences in geographical origins of crop plants may serve as an index of genetic distance in parental selection. It is believed that crosses between genetically diverse parents are likely to produce higher heterosis, desirable genetic recombination and segregation in progenies (Chahal and Gosal, 2002; Singh, 2002).

Genetic characterization and evaluation of accessions may be carried out by using different methods ranging from the traditional practices of descriptor lists of morpho-agronomical characters, biochemical and modern molecular methods (Carvalho, 2004; de Vicente *et al.*, 2005). Prior to the discovery of PCR-based markers, cytogentic hybridization techniques, seed protein electrophoresis and isozyme analysis were used to characterize and evaluate crop germplasm including that of chickpea (Upadhyaya *et al.*, 2008). Recently, markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), have been used to study the genetic diversity and species relationships in many legumes (Upadhyaya *et al.*, 2008). However, it is advisable to combine both morpho-agronomic, biochemical and molecular methods of characterization as all approaches have their own comparative advantages and disadvantages.

Even though characterization and evaluation based on quantitative characters may be more valuable for germplasm utilization (Carvalho, 2004), it may not necessarily reveal the performance at the gene level (Zhu, 1996). It is generally believed that the use of biochemical and molecular markers is more reliable and repeatable than genetic characterization and evaluation based on morpho-agronomic markers, but the existence of molecular markers may not necessarily imply knowledge of their expression in the phenotype (Carvalho, 2004). Therefore, morpho-agronomic characterization and evaluation of genetic materials, complemented by biochemical and molecular methods, may better satisfy different scientific needs.

Genetic characterization using morpho-agronomic characters was undertaken on chickpea (Talebi *et al.*, 2008; Dwevedi and Lal, 2009) and

most of the results indicated the existence of adequate genetic diversity at phenotypic level (Upadhyaya *et al.*, 2008). In Ethiopia, agronomic and molecular characterization (Workeye, 2002; Dadi, 2005) and evaluation for bruchid resistance (Alemayehu, 2005; Lemma, 1990) have been done on chickpea germplasm accessions. However, their studies did not involve attributes of symbiotic nitrogen fixation and phosphorus use efficiency. The results also may not be inferred for Ethiopian germplasm at large because of less representation in terms of number of accessions and sources of eco-geographical origins. Furthermore, results of characterization and evaluation of germplasm based on quantitative morpho-agronomical characters are not stable because of polygenic nature of the quantitative characters and the genotype by environment interaction. Generally, qualitative characters like flower color are more preferable for differentiation of germplasm than quantitative characters like the yielding potential. This is because qualitative characters controlled by major genes are less affected by changing environments and, as a result, they are more stable than the quantitative characters which are controlled by the combined effects of polygenes whose differential responses result in a continuous distributions of phenotypic values (Falconer, 1989).

A number of studies also reported high morphological variability and limited genomic variability in chickpea (Millan *et al.*, 2006; Upadhyaya *et al.*, 2008). The cause of limited genetic variability was partially attributed to the "founder effect" of its monophyletic descendence from its wild progenitor, *C. reticulatum* (Abbo *et al.*, 2003). Nevertheless, high level of genetic diversity has been revealed with the advent of simple sequence repeat (SSR) or microsatellite markers which are co-dominant, polymorphic, abundant and randomly distributed in the genome (Millan *et al.*, 2006; Upadhyaya *et al.*, 2008; Celucia *et al.*, 2009). The simple sequence repeats are tandem repeat sequences with less than six base pairs in length (Celucia *et al.*, 2009). Simple sequence repeat markers are widely used in genetic characterization of different plants including horticultural crops (Creste *et al.*, 2003; Fernandez-Gonzalez *et al.*, 2007), cereals (Huang *et al.*, 2007; Bagge and Lübberstedt, 2008), oil crops (Celucia *et al.*, 2009) and pulses (Mian *et al.*,

2008; Sarıkamış *et al.*, 2009) including chickpea (Winter *et al.*, 1999; Millan *et al.*, 2006; Upadhyaya *et al.*, 2006; Upadhyaya *et al.*, 2008).

### 2.2.3. Choice of selection environment

The concept of direct and indirect selection environments was first suggested by Falconer (1960) and later on it has been used in several investigations related to the determination of optimum selection environments (Ceccarelli, 1989; Ceccarelli and Grando, 1996; Banziger and Edmeades, 1997; Banziger *et al.*, 1997; Banziger and Lafitte, 1997). Two kinds of selection methods are recognized as related to selection environments: direct and indirect selection. Direct selection refers to a kind of selection made directly under the target production environment or under simulated condition as the target environment. Indirect selection, conversely, refers to selection made under distinctly different environment from the actual target production environment but still to improve productivity under the latter; for example, selection under optimum soil fertility level to improve productivity under low fertility level.

Methods have been designed to determine the efficiency of selection under favorable selection environments for improving performance in stressed target environments (Falconer, 1989). To determine the efficiency of selection under favorable environments for improving performance under unfavorable target environments, the procedure assumes a character measured in two different environments not as one but as two characters with genetic correlation between them since the physiological mechanisms and the genes required for high performance may be different. If the genetic correlation between them is high, then performances in two different environments represent nearly the same character, determined nearly by the same set of genes. If it is low, however, the characters are likely to differ to a great extent, and high performance requires a different set of genes.

It is still debatable whether selection under more favorable condition is likely to result in better yields than if selection were done under stress condition (Rosielle and Hamblin, 1981; Hawtin, 1982). Nevertheless, there is

a tendency of preference among breeders to select genotypes under favorable condition including for the unfavorable target environments because heritability and genetic variance usually decrease under stressed condition and the expected genetic gains are, therefore, less than at under favorable conditions (Rosielle and Hamblin, 1981; Simmonds, 1991; Banziger and Edmeades, 1997; Singh, 2002).

Possibilities to forecast the performance of genotypes under one condition on the basis of performance obtained under another can assist breeders in allocation of the scarce resources and to decide whether to develop varieties for wide or specific adaptation and in recommending their final release. However, many studies disproved the concept that stipulates cultivars selected under favorable environments also suit to the unfavorable ones (Ceccarelli, 1989; Ceccarelli and Grando, 1996; Banziger and Edmeades, 1997; Banziger *et al.*, 1997; Banziger and Lafitte, 1997). Many of such varieties developed under potential conditions have also failed to succeed under marginal condition (Ceccarelli, 1989; Reijntjes *et al.*, 1992; Ceccarelli and Grando, 1996) because it is practically impossible to collect together genes responsible for superior performance in all environments into a single genotype (Annicchiarico, 2002).

There are also some reports with compromising ideas as far as the concept of direct and indirect selection environments are considered. The use of "intermediate" environments as primary selection sites was suggested as a good alternative over either selection at high or low yielding environments (Allen *et al.*, 1978) but there is no clear criterion to determine the intermediate type of environment. Testing of varieties under both stressed and non-stressed conditions could be one of the stable options to create alternative varieties that suit both conditions but the cost of germplasm evaluation would obviously be doubled.

### 2.2.4. Choice of selection criteria

In order to achieve the goal of plant breeding, there is no single approach serving as a "magic bullet" for a "quick-fix" of all sorts of production

problems and breeders follow different breeding philosophies. Breeding to improve grain yield, as stated by Sedgley (1991), may be based on three approaches: defect removal, direct or indirect selection for yield (empirical *vis-à-vis* analytical breeding) and breeding for pre-defined ideotypes. The concept of "component optimization" based on the "balanced" combination of all desirable characters was also recently suggested (Wallace and Yan, 1998). Defect removal, like breeding for disease or lodging resistance, involves elimination of specific limiting traits and the other approaches are briefly discussed below:

#### 2.2.4.1. Empirical vis-à-vis analytical breeding approaches

Selection applied on one character to improve another character is termed as indirect selection (analytical or reductionist approach), whereas selection for the ultimate product like grain yield is called direct selection (the so called empirical or traditional approach). Studies on the establishment of comparative yield advantages from empirical and analytical methods of selection do not show similar trends.

The analytical approach has emanated from the concept that heritability and genetic variance of grain yield are typically lower under stress versus non-stress conditions (Rosielle and Hamblin, 1981). Direct selection for yield *per se* is often not sufficiently effective as yield is a complicated character more polygenic than its components such as pod and seed numbers (Lawes *et al.*, 1983). The use of secondary traits that are positively associated with grain yield, genetically variable and highly heritable is advisable under such conditions (Edmeades *et al.*, 1997; Edmeades *et al.*, 1998) when yield is the ultimate expression of these traits.

Despite lower heritability and genetic variance of grain yield under stress, it is assumed that heritability and genetic variation of some secondary traits may remain high and at the same time the traits may maintain good level of positive correlation with grain yield (Banziger and Lafitte, 1997). Nevertheless, most of the recent reports confirm that it is not sufficient for a breeder to identify secondary traits as determinants of grain yield. They suggest that the relative efficiency of indirect selection for these

determinants over selection for grain yield *per se* must be systematically quantified (Banziger and Edmeades, 1997; Banziger and Lafitte, 1997; Banziger *et al.*, 1997) as shown by Wricke and Weber (1986).

The genetic expression of different traits and the extent and pattern of their relationship with grain yield may also vary with changes in the environment (Rosielle and Hamblin, 1981; Hawtin, 1982; Lawes *et al.*, 1983; Singh, 2002; Simmonds, 1991; Banziger and Edmeades, 1997; Banziger and Lafitte, 1997). Some traits may become more influential to grain yield than others with changes in environment (Richards, 1987; Edmeades *et al.*, 1998).

The empirical approach, on the other hand, is based on the concept that direct selection for grain yield has been a very successful approach to be considered as the only dependable route to improve yields (Richards, 1987). This concept emanated from the assumption that direct selection for yield will always be effective because yield is the ultimate output from the integration of all components of the holistic plant system (Wallace and Yan, 1998). However, direct selection for grain yield may not be possible in the early stages of segregating materials in breeding nurseries where the breeder is forced to select individual plants on the basis of their phenotypic performance for yield attributes (Singh, 2002). In addition, direct selection for yield *per se* may not be effective because of its more polygenic nature than the component traits (Lawes *et al.*, 1983).

#### 2.2.4.2. The ideotype breeding approach

The concept of ideotype breeding was first suggested by Donald (1968). An ideotype may be defined as a hypothetical plant frame or architecture described in terms of characters that can exploit available resources efficiently to produce maximum economic yield (Chahal and Gosal, 2002). Selection in this case is totally based on yield components (Smith, 1987; Singh, 2002). However, the desirability of a trait may be environment specific and a common ideotype may not suit all environments as mentioned earlier.

The traits to be considered in the ideotype breeding approach are generally morphological, physiological, molecular, biochemical, anatomical, phenological or their combinations (Singh, 2002). While the theory of ideotype breeding itself is attractive, the required characterization of "model" components for the target environment has been difficult to achieve, as different genotypes may give the same yield through different yield component pathways (Smith, 1987).

#### 2.2.4.3. The concept of "component optimization"

This approach supplements the analytical approach which assumes that indirect improvement of a yield-contributing trait would result in a higher yield. Each component trait, for a given level of expression, must compete and compromise with all the other traits sharing photosyntates which has only a "constant capacity". In relation to the analytical approach which deals with a single or a few components of a complex system, the component optimization approach rather deals with the holistic plant system. According to this concept, superior cultivars are required to have favorably balanced level of combination of component traits that maximize the system functioning for optimal use of the growth factors (Wallace and Yan, 1998).

This concept recognizes that optimal combination of component traits exists for different environments. Even though the concept is theoretically appealing, particularly for breeding in predictable environments with minimal temporal and spatial variability, the optimum combination of the component traits will shift with changes in the level of growth resources and with alteration in genetically and physiologically established optimal level of even one of the traits (Wallace and Yan, 1998). For instance, cultivars constituted for terminal drought may not be tolerant when the drought stress comes early in the growing season or when it comes in the middle of the season (Ceccarelli *et al.*, 2004).

## 2.3. The Chickpea Crop

The Genus *Cicer* has eight annual and 34 perennial species (Van der Maesen, 1987) of which *Cicer arietinum* is the only cultivated species (Millan

*et al.*, 2006). An earlier study on the cytogenetic relationships of different species indicated that the wild *C. reticulatum* was the progenitor of the cultivated chickpea (Ladizinsky and Adler, 1976). Nevertheless, the ancestry of C. *reticulatum* could not be reconfirmed with another study (Ohri and Pal, 1991). The cultivated chickpea belongs to family Fabaceae (formerly Leguminosae) and subfamily Faboideae. Different chromosome numbers were reported from early cytogenetic studies (van der Maesen, 1987) but it was later confirmed that chickpea is a diploid species having chromosome number of 2n = 2x = 16 (van der Maesen, 1987; Upadhyaya *et al.*, 2008) with comparatively a small genome size of 740 Mbp (Arumuganathan and Earle, 1991).

Based on the presence of wild relatives, namely *C. reticulatum* and *C. echinospermum*, South-Eastern Turkey and the adjoining areas of Syria was considered as the most probable center of origin for chickpea (van der Maesen, 1987). Archeological evidences show that chickpea was first domesticated in the Middle East before the late Neolithic period (as early as 3500 BC) in Turkey (Tanno and Willcox, 2006). The crop was probably diffused from where it was originated and first domesticated, i.e. the Middle East, to different continents of the world by the Phoenicians (The Worldwide Gourmet, 2010). As early as 1520 BC, chickpea was known to be grown in Ethiopia (Joshi *et al.*, 2001), which is now considered as the secondary center of diversity for the crop (van der Maesen, 1987).

Chickpea is a strictly self-pollinated crop (Muehlbauer and Tullu, 1997; Romeis *et al.*, 2004; Toker *et al.*, 2006) with two types of cultivars, *Desi* and *Kabuli*. The *Desi* type has small darker seeds with a rough seed coat while the *Kabuli* has larger seeds with lighter colour and a smoother seed coat. Existence of a pea-shaped third type characterized by medium to small seed size and creamy colour was also recognized (Upadhyaya *et al.*, 2008), which may be a result of crossing of *Desi* with *Kabuli* types that has resulted in a sort of intermediate types (Muehlbauer and Tullu, 1997). Currently, about 75% of the area all over the world is covered by the *Desi* and the remaining 25% by the *Kabuli* types (Kassie *et al.*, 2009). The *Desi* type is grown mostly in Indian subcontinents, Ethiopia, Mexico and Iran while the *Kabuli* type is

grown in Southern Europe, Northern Africa, Afghanistan, Pakistan and Chile (Upadhyaya *et al.*, 2008; Kassie *et al.*, 2009).

The crop may grow to a height of 30-70 cm depending on the suitability of the growing environment. It has small feathery leaves on both sides of the stem and sometimes with 2-3 bisexual flowers in a node. The flowers may be white, pink, purple or blue in color. The crop may set 1-3 seeds in a pod and the seed is a good source of protein (23%), carbohydrates (64%), fat (5%) and crude fiber (6%). It also contains 340 mg of phosphorus, 190 mg of calcium, 140 mg of magnesium, 7 mg of iron and 3 mg of zinc in 100 g of seed (Bejiga and van der Maesen, 2006). Chickpea needs a subtropical or tropical climate with well drained fertile soils having pH of 5.5-8.6 (Muehlbauer and Tullu, 1997; Kassie *et al.*, 2009).

## 2.4. Breeding Legumes for Sustainable Cropping System

The full yield potential of legume crops is rarely attained particularly in tropical and sub-tropical Africa where limitations imposed by abiotic and biotic stresses are severe (Kramer, 1980; Buddenhagen and Richards, 1988) and, consequently, global agriculture in general and African agriculture in particular is at a cross roads (Sinha, 1987). Food security is totally unsustainable without yield increases under marginal conditions (Lorieux, 2005).

The breeding of legume genotypes with better symbiotic nitrogen fixation, phosphorus use efficiency and resistance to pests could prove one of the dependable approaches to address the problem of the majority of the resource-poor farmers. Such genotypes have been suggested for cost effectiveness and agricultural sustainability (Beringer *et al.*, 1988; Clark *et al.*, 1988; Schmidt, 1988; Witty *et al.*, 1988; Pearson *et al.*, 1995) with particular relevance under low input and organic agriculture (Gahoonia and Nielsen, 1996; Aryal *et al.*, 2003; Burger *et al.*, 2008; Löschenberger *et al.*, 2008; Osman *et al.*, 2008; Wolfe *et al.*, 2008).

### 2.4.1. Breeding for symbiotic nitrogen fixation

#### 2.4.1.1. Factors limiting symbiotic nitrogen fixation

It is established that a number of elements of growing environment interplay to determine the extent to which yield potential of a given crop genotype is expressed (Harris *et al.*, 1987; Collaku *et al.*, 2002). The elements of growing environment, in this sense, may include climatic, paedological, biological and crop management factors that affect crop growth and development (Annicchiarico, 2002). In nature, these elements of the growing environment do not exist in a proportion that is required for potential productivity of the crop plants.

The interaction between the microsymbiont and the legume host plant are known to be complicated by unfavorable edaphic, climatic and management factors (Abaidoo *et al.*, 1990; Broughton *et al.*, 2003). It is believed that any environmental or physical stress that reduces host plant growth and development may also negatively affect the associated bacterial strain and, thereby, the amount of nitrogen fixation (Lindemann and Glover, 2003). It is a 'rule of thumb' that symbiotically fixed nitrogen alone may not increase production if some other nutrients are limiting unless the latter are ameliorated (Bohlool *et al.*, 1992). Generally, improved management (exclusive of nitrogen fertilizer) as compared to traditional practices may increase the level of symbiotic nitrogen fixation and the uptake of the fixed nitrogen (Keatinge *et al.*, 1998). For instance, a greenhouse study on chickpea showed that symbiotic nitrogen fixation was enhanced with the balanced application of important nutrients, particularly phosphorus (Shukla and Yadav, 2000).

Climatic factors like extreme temperatures, excessive soil moisture and moisture stress and edaphic factors like soil acidity, existence of mineral nitrogen in the soil and deficiencies of phosphorus, calcium, molybdenum, copper, cobalt, zinc and boron may cause poor nitrogen fixation (Abaidoo *et al.*, 1990; Pearson *et al.*, 1995; Shukla and Yadav, 2000; Serraj *et al.*, 2004; Pimratch *et al.*, 2008). Legumes normally require more phosphorus than nitrogen non-fixing crops (Serraj *et al.*, 2004) as phosphorus is important in

ATP synthesis for nitrogen fixation (Sinclair and Vadez, 2002; Muhammad *et al.*, 2004; Bucher, 2006; Qiao *et al.*, 2007). Molybdenum is also considered an important constituent of the Nitrogenase enzyme (Pearson *et al.*, 1995)

Application from external sources of these nutrients may increase nodulation, nitrogen fixation and thereby seed yield in legumes (de Oliveira *et al.*, 1998; Bambara and Ndakidemi, 2010). Toxicity by heavy metals was also found to reduce symbiotic nitrogen fixation which is compounded by acidity (Wani *et al.*, 2007). The negative side effects of seed treatment with certain fungicides on the legume–rhizobia symbiosis was also reported by a number of workers as reviewed by Broughton *et al.* (2003) and Fatou *et al.* (2003).

The existence of mineral nitrogen may inhibit the *Rhizobium* infection process, nodule development and thereby nitrogen fixation probably by impairment of the host-strain recognition mechanism and diversion of photosynthates towards nitrate assimilation (Pearson *et al.*, 1995; Gulden and Vessey, 1998; Broughton *et al.*, 2003). On the contrary, some studies showed that low concentrations of nitrogen in the soil also inhibit nitrogen fixation and plant growth (Gulden and Vessey, 1998), showing the need for the "starter" amount of nitrogen (Malhotra *et al.*, 2004). It is assumed that the physiologically optimum range of most of the physical factors limiting symbiotic nitrogen fixation may not follow a definite pattern as it depends on the species of *Rhizobia,* the host legume, and other components of the environment and their interaction (Sanginga *et al.*, 1995).

A number of biological factors also determine the level of symbiotic nitrogen fixation in food legumes. The success of symbiotic nitrogen fixation depends on the genetic potential of the host and strain and on how they interact with each other and with other components of the growing environment (Abaidoo *et al.*, 1990; Bohlool *et al.*, 1992; Pearson *et al.*, 1995; Lindemann and Glover, 2003). For instance, some reports indicated existence of host genotypes whose nitrogen-fixing effectiveness may not be impaired by the application of nitrogen to the soil (Herridge and Rose, 2000; Singh and Usha, 2003). Such genotypes are considered

desirable as they may impose less competition when they are intercropped with cereals (Singh and Usha, 2003).

Another observation in haricot bean showed low nodulation and yields regardless of the amount of nitrogen applied from external sources (Lindemann and Glover, 2003). This may be considered as an indication that nodules may somehow help the plant to efficiently use nitrogen but the mechanism is not yet clear. Intercropping of legumes with non-legume crops increased the amount of symbiotic nitrogen fixation by the legumes and the total nitrogen uptake and use by both component crops (Pineda *et al.*, 1994; Hardarson, 2004). However, it is not yet systematically confirmed whether there is a sort of demand based "triggering" effect from the cereals to initiate the legumes to fix more nitrogen.

Nodule longevity, i.e. the time that elapses between the first appearance of fixation and the final disintegration of the symbiotic tissue (nodule senescence), may vary with the ontogeny of the host plant (Sutton, 1983). The role of strain competitiveness, its ability to survive in the soil and its compatibility with the host plant are important to fully realize the potential of symbiotic nitrogen fixation (Abaidoo *et al.*, 1990). Identification of host and strain genotypes adapted to low phosphorus soils and inoculation by mycorrhizal fungi was also suggested as a good strategy to enhance nodulation and symbiotic nitrogen fixation (Pearson *et al.*, 1995; Lindemann and Glover, 2003) but little is known about the mechanism of improved nitrogen uptake by the mycorrhizal fungi (Javaid, 2009). The potential host legume for symbiotic nitrogen fixation may also be negatively affected by biotic stresses particularly insect pests and diseases (Serraj *et al.*, 2004).

#### 2.4.1.2. Mechanism of symbiotic nitrogen fixation

The mechanism of symbiotic association and specificity between bacteria and the legume host plant are extensively studied. Nitrogen fixation ($N + 3H_2 + Energy \rightarrow 2NH_3$) is dependent on the presence of Nitrogenase enzyme, which catalyzes the conversion of nitrogen into a reduced form notably to ammonia ($NH_3$) (Hubbell and Kidder, 2003).

The Nitrogenase system consists of two different enzymes, i.e. azoferredoxin (an iron containing) and molybdoferredoxin (iron and molybdenum) containing enzymes (Pearson *et al.*, 1995; de Oliveira *et al.*, 1998; Hubbell and Kidder, 2003). The two components combine and function together as a single system (Hubbell and Kidder, 2003).

The host-rhizobium association is known to be effected by a "nod factor" secreted by the rhizobia and transmembrane receptors on the cells of the roots of the legume (Broughton *et al.*, 2003). Recognition between the two associates is controlled by structural and regulatory nod genes (Crouch *et al.*, 2004), initiated following exchange of signals between the rhizobia–legume symbiotic system (Rengel, 2002; Broughton *et al.*, 2003).

First, legume plants secrete root exudates (Broughton *et al.*, 2003) in which the flavonoids components are active in inducing rhizobial *nod* genes (Rengel, 2002; Broughton *et al.*, 2003) and then nodulation (Crouch *et al.*, 2004). As nod-factors also stimulate the synthesis and release of flavonoids from legume roots, the response to inoculation is amplified (Broughton *et al.*, 2003). Second, the product of the regulatory rhizobial nod gene induces transcription of a number of rhizobial structural genes, which code for extracellular nod factors (Broughton *et al.*, 2003). Thirdly, endogenous plant hormones, as well as factors produced by rhizobia, are important mediators of nodule initiation. So far, only flavonoids and Nod factors have been chemically synthesised and of these only the former are available in large quantities (Broughton *et al.*, 2003).

Field trials in North America show that seed application of flavonoids stimulates nodulation and nitrogen fixation in soybeans (Broughton *et al.*, 2003). The ammonia resulting from fixation is rapidly incorporated into certain amino acids, such as glutamine or alanine (Broughton *et al.*, 2003). The nitrogen may then be transferred to other amino acids and nitrogen-containing compounds by a variety of commonly occurring amino transfer reactions (Hubbell and Kidder, 2003).

Specificity results when different strains of rhizobia produce different nod factors and different legumes produce different receptors (Crouch *et al.*,

2004). Symbiotic association occurs only when the combination between the nod factors of bacteria is compatible with the receptors of the host plant.

The bacteria enter through epithelial cells of the root and migrate into the cortex. Once the bacteria enter the plant, other sets of signals are exchanged between the symbionts (Broughton *et al.*, 2003). Infection thread is constructed by the root cells in response to the infection. When the infection thread reaches a cell deep in the cortex, it bursts and the bacteria are engulfed by endocytosis into endosomes. The cortex cells then begin to divide rapidly forming a nodule. This response is driven by the translocation of cytokinins from epidermal cells to the cells of the cortex.

The legume is certainly helpful in that it supplies nutrients to the bacteroids with which they synthesize the large amounts of ATP needed to convert nitrogen ($N_2$) into ammonia ($NH_3$). Because of the specificity of the interaction between the nod factor and the receptor on the legume, specific strains of rhizobia will infect specific legumes.

Some attribute the host-rhizobium specificity in symbiotic association to the differential expression of plasmid controlling fixation in genetically dissimilar backgrounds (Sadowsky and Bohlool, 1985). *Bradyrhizobium* and *Rhizobium* species infect plant roots forming galls or nodules, and fix nitrogen from the soil atmosphere directly to the plants (Pearson *et al.*, 1995). Not all symbioses fix $N_2$ with equal effectiveness. The mechanisms that confer competitive advantage to a strain are not fully understood. Some of the factors that have been suggested are: faster growth in the rhizosphere of the host; preferential selection by the host; tolerance of climate, pH, and other soil factors; antagonism between competing strains; and the relative ratio of inoculants to resident strains as reviewed by Mayt and Bohool (1983).

#### 2.4.1.3. The energy cost for symbiotic nitrogen fixation

Nitrogen exists in the atmosphere in the form of dinitrogen molecule ($N_2$) where the two atoms of nitrogen are linked by a very strong triple bond (Hubbell and Kidder, 2003; Lindemann and Glover, 2003). The triple bond needs to be "broken" for hydrogen atoms to be added to the nitrogen. The

molecule is almost inert due to this triple bond and large amount of energy is required to break such bonds (Hubbell and Kidder, 2003). The plant must contribute a significant amount of energy in the form of photosynthate (photosynthesis derived sugars) and other nutritional factors for the bacteria (Hubbell and Kidder, 2003; Lindemann and Glover, 2003). Then, dinitrogen must first be combined with hydrogen, the process of reduction of nitrogen referred as "nitrogen fixation" (Lindemann and Glover, 2003).

Adenosine triphosphate (ATP), from oxidative degradation of sugars and related molecules, manufactured by the host-plant during photosynthesis and transferred to the nodules are the main source of energy in symbiotic nitrogen fixation (AFRNA, 1992). Plants usually use the ammonia (NH3) form of nitrogen to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing components necessary for life (Hubbell and Kidder, 2003; Lindemann and Glover, 2003).

Some studies show that for each gram of nitrogen fixed by *Rhizobium,* the plant contributes 1-20 grams of fixed carbon from photosynthesis (Hubbell and Kidder, 2003). For instance, a soybean plant may divert 20-30 percent of its photosynthate to the nodule instead of to other plant functions when the nodule is actively fixing nitrogen (Lindemann and Glover, 2003). A review by Keyser and Li (1992) also indicated that for each kg of nitrogen fixed, 10 kg of carbohydrate is required. Therefore, the high requirement for phosphorus in legumes is partially related to the high rates of energy required in the form of ATP for symbiotic nitrogen fixation (Sinclair and Vadez, 2002). The extra energy cost of nitrogen fixation can be safely carried by most field-grown legumes with little or no loss of production (AFRNA, 1992).

#### 2.4.1.4. Genetics of symbiotic nitrogen fixation

Plant breeding by its nature is a costly and lengthy process (Moreno-González and Cubero, 1993) and, despite all that, it is also a difficult job with minimum recovery of desirable genotypes. The best combination of agronomically desirable characteristics is usually of rare occurrence in nature and their artificial constitution needs high technical dedication and

endurance. Even though the inheritance of the process of nodulation was considered to be relatively simple as reviewed by Rengel (2002), the genetic control of the whole process of symbiotic nitrogen fixation is complicated due to the polygenic nature of inheritance (Austin, 1993; Tate, 2000).

The exact number of genes involved in symbiotic nitrogen fixation and their mode of regulation of the process is not yet clearly known (Shantharam and Mattoo, 1997). Some sources indicated that nitrogen fixation depends on at least 17 genes in the bacterium and 50 genes in the plant and even if all the genes required for these traits could actually be identified and transferred, the problems of genetic instability could increase as a result (Johnston, 1989). For instance, a total of 22 genes interacting with symbiotic bacteria in nodule formation have been identified in field pea (Tate, 2000).

A recent review showed 43 symbiotic genes controlling nodulation in the same crop, of which eight are sequenced and already cloned (Borisov *et al.*, 2007). Although Singh and Rupela (1998) identified a monogenic recessive gene, controlling non-nodulation in *Kabuli* chickpea, other non-nodulating genes were also identified (Davis *et al.*, 1985; 1986; Rupela, 1992). Tsai *et al.* (1998) studied a mapping population from two contrasting haricot bean genotypes (BAT-93 and Jalo EEP558) for nitrogen fixation and identified at least six chromosomal regions controlling nodule number. Efforts to clone the genes and use in transformation of cereals are also under way even though such efforts are extremely complex due to the different groups of genes involved (signalling, nodulation and fixation) and the polygenic nature of the process of symbiotic nitrogen fixation (Dixon *et al.*, 1997).

#### 2.4.1.5. Challenges of breeding for symbiotic nitrogen fixation

Possibilities for breeding success in developing the best matching host plant by bacteria symbiotic association is amenable to certain technical limitations. In many of the other breeding programs, the usual breeding task is to genetically manipulate attributes of only a single organism for a single primary character like yielding potential. As if this itself may not be

simple, particularly when the inheritance is polygenic, breeding for symbiotic nitrogen fixation suffers multiples of technical complexities. Many basic issues about symbiotic interactions are yet unresolved and the necessary baseline information for effective breeding are limited (Parker, 2008).

The major challenge to the plant breeder, or microbiologists for that matter, is that breeding for improved symbiotic nitrogen fixation demands dealing simultaneously with many independent attributes not in one but in two different organisms differently interacting between them and with the environment (Crouch *et al.*, 2004).

Host specificity is another barrier for improving the nodulation capacity of rhizobial strains (Shantharam and Mattoo, 1997). While the breeding for one character, particularly polygenic character, itself is sufficient to complicate the task of plant breeding, the situation in this case is rather worsened by the need to simultaneously improve not only the *per se* performances but also the symbiotic association of the two organisms. At the top of that, the best host with one strain may not be the best with another strain and vice versa (Thies *et al.*, 1991).

This means that a given legume cultivar nodulated by different strains of the same species of root nodule bacteria would fix different amounts of nitrogen and, similarly, a given strain of *Rhizobium* will nodulate and fix different amount of nitrogen in symbiosis with a range of cultivars of the same plant species (AFRNA, 1992).

Therefore, in order to improve symbiotic nitrogen fixation in legumes, there is a need to develop the best matching host-bacterial association. Even then, there is another challenge that the best matching association in one environment (both spatial and temporal variation) may not repeat the best performance in another environment. The pattern in which the phenotypic value of the symbiotic association changes with changes in the environment is not well understood (Mytton and SkØt, 1993).

There are some technical possibilities to overcome problems associated with a high level of genotype by environment interaction. The environment for which breeding is undertaken should be clearly defined and

systematically classified into fairly homogeneous categories to reduce the magnitude of genotype by environment interaction to a "tolerable" level for stability analysis, and thereby increase gains from breeding crops including chickpea (Collaku et al., 2002; Malhotra and Singh, 2004). Classification of the test environments should be based on crop biological responses, edaphic and climatic factors. Alternative approaches have also been suggested and widely applied in different crops to stratify environments into homogeneous groups by cluster analysis based on the similarity of crop biological responses and edaphic and climatic factors (Campbell and Lafever, 1980; Fox and Rosielle, 1982; Yau et al., 1991; van Oosterom et al., 1993). We can follow the same procedure in order to reduce bacterial strain by environment interaction effects (Lindemann and Glover, 2003).

### 2.4.2. Breeding legumes for phosphorus uptake and use Efficiency

#### 2.4.2.1. Mechanism of phosphorus uptake and use efficiency in legumes

The mechanisms of efficient uptake and use of nutrients by legumes may basically be associated with root size, microbial symbioses and surface chemical characteristics like root exudates to solublize the limiting nutrient (Ascher *et al.*, 2001; Ali *et al.*, 2002; Ojo *et al.*, 2006; Vesterager *et al.*, 2006; Fageria *et al.*, 2008), and the so called "uptake kinetics" of the crop (Gill *et al.*, 2005). The main exudates include malonate, citrate and malate (Veneklaas *et al.*, 2003). Chickpea reported to produce extensive roots and substantial quantities of organic acids to solubilize phosphorus from the soil (Alloush *et al.*, 2000; Veneklaas *et al.*, 2003; Gahoonia *et al.*, 2007).

Many studies showed that nutrient use efficiency is associated with root growth and development in many crops including haricot bean (Beebe *et al.*, 2006), soybean (Ogoke *et al.*, 2006), chickpea (Srinivasarao *et al.*, 2006) and white clover (*Trifolium repens*) (Blair and Godwin, 1991). On the other hand, Vesterager *et al.* (2006) reported mechanisms of phosphorus

uptake and use efficiencies in pigeon pea and cowpea involving the release of some organic acids.

It is generally believed that root growth and development plays more vital role to improving phosphorus acquisition efficiency in most crop plants than root exudation (White *et al.*, 2005). Therefore, genes regulating root growth and development might be manipulated to improve nutrient uptake and use efficiency by these crops. The association with various fungi, particularly vesicular arbuscular micorrhizal fungi (VAM), facilitates uptake and use of nutrients including phosphorus by legume plants (Goicoechea *et al.*, 1997; Nogueira *et al.*, 2007; Chen *et al.*, 2010).

Generally, even though a number of specific mechanisms of nutrient use efficiencies have been identified in different legumes, it yet appears that much remains the subject of future investigation in understanding the dynamics and detailed mechanisms underlying the efficient use of soil nutrients in crops (Rosswall and Paustain, 1984; Graham, 1988; Ali *et al.*, 2002). For instance, factors which affect the magnitude and pattern of root exudation rates are not yet clearly known (Veneklaas *et al.*, 2003).

#### 2.4.2.2. Genetics of phosphorus uptake and use efficiency in legumes

Improving adaptation to a marginal environment like nutrient deficiency stress may be considered as the genetic modifications in structures or functions of crop plants to improve the ability to survive and reproduce under such condition (Kramer, 1980). It is generally believed that sufficient heritable variation exists for root morphological traits to improve phosphorus acquisition and yield on soils with low phosphorus availability by plant breeding (White *et al.*, 2005).

Genetic control, heritability and genetic gains from selection have also been determined in many cases (Ascher *et al.*, 2001; Ara´ujo *et al.*, 2005; Beebe *et al.*, 2006; Ojo *et al.*, 2006; Kimani *et al.*, 2007; Kimani and Tongoona, 2008). The study made on the inheritance of root traits and

phosphorus uptake in haricot bean under limited soil phosphorus supply showed considerable additive genotypic effects with broad-sense heritability values ranging from 0.43 - 0.61 for root area, for root length, for root mass and total phosphorus content with high correlation coefficients between the traits (Ara´ujo *et al.*, 2005).

Another study made on genetics of phosphorus utilization in cowpea, based on a cross involving a phosphorus responsive (IT90K-277-2) and non-responsive (IT89KD-288) genotypes using generation mean analysis of the parents, their F1, F2 and the two backcross generations showed existence of highly significant differences among the six generations with respect to seed phosphorus concentration and grain yield with positive heterosis for both traits (Ojo *et al.*, 2006). The same study also showed seed phosphorus concentration was under polygenic control with no maternal and cytoplasmic effects and narrow-sense heritability for seed phosphorus concentration of 50.51%. Wang *et al.* (2004) studied recombinant inbred lines (RILs) from two contrasting genotypes of soybean (CN4 and XM6) for some important root hair traits in China and found low heritability for root hair density (27.32-33.97%) and high for average root hair length (53.85 - 60.98%). Aráujo *et al.* (1998) reported existence of genetic diversity for traits related to phosphorus use efficiency among wild and cultivated genotypes of common bean.

Several studies identified the traits and genes/QTL responsible for efficiency in utilization of a number of nutrients in a range of crops, including legumes (Ascher *et al.*, 2001; Li *et al.*, 2005; White *et al.*, 2005; Beebe *et al.*, 2006; Dita, 2006; Miklas *et al.*, 2006; Zhang *et al.*, 2010). However, these traits and genes are not yet widely exploited in breeding programs. For instance, Li *et al.* (2005) in soybean and Beebe *et al.* (2006) in haricot bean identified QTLs associated with attributes of phosphorus use efficiency. About 26 Quantitative Trait Loci (QTL) for root architecture traits correlated with phosphorus acquisition have also been identified using RFLPs and microsatellites and mapped in common bean (Beebe *et al.*, 2006). Ojo *et al.* (2006) suggested the backcross breeding method would be an

effective means of transferring the genes for seed phosphorus concentration among tropical cowpea genotypes.

#### 2.4.2.3. Strategies to breeding for phosphorus-plant relation

Many strategies may be sought in order to improve plant-phosphorus relations, each strategy with its own comparative advantages and disadvantages (Keneni and Imtiaz, 2010). The first strategy targets cultivars that are responsive to optimum soil fertility levels. The ultimate goal of this approach is yield potential based on the full exploitation of productivity improvements from the best-bet combination of genotype, fertilizer and the synergistic interaction between the two. This strategy is advantageous in terms of boosting crop productivity but could be useful only for a few resource-rich and progressive farmers in potential areas who can afford costs of fertilizer inputs (de Boef and Ogliari, 2008).

The second strategy is breeding crop cultivars that are tolerant to nutrient deficiency stress, either under farmers own condition or simulated circumstances. The ultimate goal here is not to exploit the genetic potential of the crop but it is rather to enhance resistance to and performance under nutrient deficiency stress (de Boef *et al.*, 1996). Nevertheless, one can not anticipate dramatic results from breeding efforts in marginal environments but one can expect only small gradual changes (Buddenhagen and Richards, 1988).

It is generally believed that the development of suitable genotypes to marginal management situations may not provide the required productivity levels for lower genetic gains expected from selection under such circumstances may limit yield improvements (Rosielle and Hamblin, 1981; Buddenhagen and Richards, 1988). Some workers argued that breeding for nutrient deficiency stress tolerance may result in continuous depletion and mining of the soil nutrients in the long-run and thereby put at risk the desires of the poor nations to double or triple productivity in order to feed the increasing population (Sinebo and Yirga, 2002). This would make it difficult to simultaneously achieve productivity and sustainability under such conditions. However, the issue of nutrient mining varies according to

particular nutrients, macro-and micronutrients, and depends on soil type (Graham, 1984).

The third approach is breeding varieties that consistently better perform under various soil fertility levels. The goal is not only for higher average performance but also for consistency in higher average performances of crop varieties under a range of soil fertility levels, assuming that these traits could concurrently be improved through selection. It is, therefore, important to focus on specific varieties that fit to each fertility level and also on a stable variety that is suitable for general cultivation over a wide range of fertility levels.

The concept of performance consistency theoretically seems attractive but it is unlikely that one can easily recover genotypes that consistently better perform across distinctly different soil fertility regimes due to the differential response of genotypes to such varying soil fertility levels. In the tropics and the sub-tropics in general and Ethiopia in particular (EMA, 1988), where environmental differences are great, it may be expected that the genotype by environment interaction, or the differential response of genotypes in different environments, is also very high (Falconer, 1989).

Legumes are normally believed to be more liable to the negative impacts of high genotype by environment interaction (Hawtin *et al.*, 1988). Therefore, the best genotype under one soil fertility level may not also be the best under another. A number of studies in Ethiopia reported the existence of significant genotype by soil fertility level interaction in a number of legume crops like chickpea (Yadeta, 2004), faba bean (Agegnehu and Ghizaw, 2004) and field pea (Ghizaw *et al.*, 2005). Although these studies in legume crops claimed to have proved that cultivars selected at one fertility level may not consistently repeat the same performance under another, it is practically impossible to collect together genes responsible for superior performance in all fertility levels into a single genotype (Annicchiarico, 2002).

The fourth strategy involves breeding genotypes for nutrient use efficiency which was defined slightly in different ways by different authors (Ahmad *et al.*, 2001; White *et al.*, 2005; Fageria *et al.*, 2008; Liao *et al.*, 2008). Some consider as genotypes that are able to mobilize the limiting

nutrients in greater amounts (acquisition efficiency) and better use of the absorbed nutrient for yield formation (use efficiency) (Bowen and Zapata, 1991; Gahoonia and Nielsen, 1996; Ascher *et al.*, 2001; Beebe *et al.*, 2006; Liao *et al.*, 2008) or simply as the ability of genotypes to yield better under nutrient deficient conditions (Graham *et al.*, 1992; Gunes *et al.*, 2006).

Others describe it as genotypes which have high yield at both high and low soil fertility levels as opposed to nutrient use inefficient genotypes which have high yield only at high soil fertility levels but greatly reduced yield at low fertility levels (Ransom *et al.*, 1993). Basically, most of the definitions are based on yield per unit of nutrient input or yield per plant tissue content of nutrient (White *et al.*, 2005). Additional definitions encompassing biochemical aspects have also been suggested (Römer and Schenk, 1998; Ahmad *et al.*, 2001; Fageria *et al.*, 2008).

The breeding of nutrient efficient cultivars based on minimizing the intensive use of fertilizers along with genotypes that are able to mobilize the limiting nutrient in greater amounts is getting wide acceptance particularly, in marginal areas where farmers could not afford to apply adequate amount of fertilizers (FAO, 1984; Bowen and Zapata, 1991; Zapata and Hera, 1995; Beem and Smith, 1997). Breeding for nutrient use efficiency as a strategy may be considered as a compromise between breeding for optimum soil fertility level and for nutrient deficiency stress tolerance. It is assumed that a certain level of productivity could be achieved from appropriate genotypes that are capable of efficiently exploiting low fertilizer that is affordable by the small scale farmers. The genetic potential for the improvement of nutrient use efficiency in crops including legumes is reported by a number of authors (Graham, 1988; Bowen and Zapata, 1991; Zapata and Hera, 1995; Ali *et al.*, 2002; Srinivasarao *et al.*, 2006).

### 2.4.3. Breeding legumes for adzuki bean beetle resistance

Examples of possibilities for effective storage insect pest management through genetic improvement of the host for resistance has thoroughly been reviewed for different legume crops (Lin *et al.*, 2005; Shaheen *et al.*, 2006; Sithanantham and Rao, 2006; Chen *et al.*, 2007; Somta *et al.*, 2006; 2007;

2008). The tremendous negative impacts of using pesticides in both developed and developing countries show that food security is totally unsustainable without the use of environmentally friendly crop protection approaches.

#### 2.4.3.1. The reproductive biology of the adzuki bean beetle

The adzuki bean beetle is considered the most important cosmopolitan storage insects in many food legumes (Kashiwaba *et al.* 2003). Losses due to storage insect pests in general, and adzuki bean beetle in particular, are much greater in tropical and subtropical regions than in the temperate areas (Gwinner *et al.*, 1990; Singh, 2002).

According to Gwinner *et al.* (1990), a single female may lay as large number of eggs as 100 depending on the environment. The larvae, after hatching, bore into cotyledons where it develops into adult within a month. It is the larvae (not adult) that feeds and damages the seed. For normal reproduction, growth and development, the insect requires temperature ranges of 18-35 $^{o}$C (optimum = 30 $^{o}$C), relative humidity of 25-90% (optimum = 80%) and only 23 days to complete its life cycle (Gwinner *et al.*, 1990). In Ethiopia, Lemma (1990) reported that oviposition started the same day the adult was emerged from the seeds and lasted for five days. He also indicated that a female laid 43-69 eggs on chickpea of which 83-96% were hatched. The insect causes significant quantitative and qualitative damage and loss to chickpea in Ethiopia (Ali and Habtewold, 1994; Damte and Dawd, 2006). The damage to seeds by *C. chinesis* is manifested by the round exit holes with the 'flap' of seed coat made by emerging adults.

#### 2.4.3.2. Mechanism of seed resistance to storage insects

Insect pest resistance in crops in general comprises of four important mechanisms, i.e. antixenosis, antibiosis, tolerance and escape but insect pest tolerance and escape may be relevant resistance mechanisms for field infestation and not for storage insects of grain crops. Resistance processes involve morphological, physiological and/or biochemical mechanisms which range from simply minimizing the effect of insect attack to adversely

affecting the insects' cellular processes, growth and development (Singh, 2002).

According to Edwards and Singh (2006), legumes as a group employ an extraordinary array of direct and indirect defenses including structural defenses, secondary metabolites and anti-nutritional compounds. Morphologically, varieties with smooth, soft, and thin seed coats are preferred for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Ahmed *et al.*, 1989; Shaheen *et al.*, 2006). In faba bean, for instance, Desroches *et al.* (1995) found that the seed coat acted like a physical barrier for two bruchid species (*C. chinensis* and C. *maculatus*) and only 45-58% of the neonate larvae perforated the seed coat and reached the cotyledons. A similar type of resistance to *C. maculatus* on cowpea was reported by Edde and Amatobi (2003). However, there are some controversies and doubts to consider these features as universal indicators of resistance (Lale and Kolo, 1998; Somta *et al.*, 2007; Srinivasan and Durairaj, 2007). Lale and Kolo (1998) observed that resistance to *C. maculatus* in three cultivars of cowpea was conferred mainly by a combination of reduced oviposition and reduced egg-hatching which may be a reflection of the chemical rather than the physical characteristics of the seed coat. Edde and Amatobi (2003) also found that seed coat has no value in protecting cowpea seed against attack by *C. maculatus*.

Host plants may pose nutritional, physiological and ecological hurdles on the insects while insects have also evolved mechanisms to overcome the multiple hurdles posed by the hosts (Panda and Khush, 1995). Antixenosis refers to non-preference of the insect pest due to one or more unattractiveness or unsuitability of the host for colonization, ovi-position or both due to some morphological or biochemical features of the host. For instance, Shaheen *et al.* (2006) found that morphological characters like rough, wrinkled, hard and thick seed coat may contribute to bruchid resistance in chickpea cultivars as mentioned above.

Resistance may also be expressed with the adverse effect of feeding on a resistant host plant (Panda and Khush, 1995) as antibiosis, which may also involve morphological, physiological and biochemical features of the host

plant or their combination. Green plants utilize principal metabolic pathways and biochemical cofactors for converting carbon dioxide and water to sugars, nitrogen to amino acids, and to synthesize nucleotides, lipids and simple organic acids. These primary plant metabolites serve as the starting materials for the biosynthesis of secondary metabolites (polymers like lignin and tannin, alkaloid, quinine, etc.) that play important role in the defense against insects as repellents, feeding inhibitors and toxins (Panda and Khush, 1995).

In severe cases, antibiosis may lead the insect pest to death. In haricot bean, for instance, arcelins, phytohemagglutinins and α-amylases (major digestive enzymes in bruchids that infest seeds of starchy grain legumes) inhibitor (APA) genes or anti-nutritional proteins collectively called lectins, were identified, cloned and sequenced in cultivated and wild species (Morton *et al.*, 2000; Tomooka *et al.*, 2000; Yamada *et al.*, 2003; Edwards and Singh, 2006; Acosta-Gallegos *et al.*, 2008).

Lectins are often resistant to proteolytic activity and function by binding to chitin or to carbohydrate targets in the insect mid gut, thereby blocking nutrient assimilation (Edwards and Singh, 2006). Nevertheless, even though arcelins were assumed seed storage proteins conditioning resistance to storage insects, Goossens *et al.* (2000) fed artificial seeds of haricot bean into which purified arcelin-5 was incorporated to *Zabrotes subfasciatus* and found that there was no correlation between the presence of arcelin-5 and insecticidal effects.

The α-amylases are major digestive enzymes in some bruchids like the adzuki bean beetle that infest seeds of legumes and, to cope with such insect pests, some species of *Phaseolus* and *Pisum* have developed seed protection systems involving secondary metabolites like α-amylases inhibitors (Morton *et al.*, 2000; Yamada *et al.*, 2003; Edwards and Singh, 2006). However, for each defense mechanism by host plants, there are anti-counter mechanisms like detoxification of toxic food by enzymes or avoidance of poisoning food by the insects (Panda and Khush, 1995; Lin *et al.*, 2005).

#### 2.4.3.3. Genetics of legumes for storage insects' resistance

The genetic control of resistance to storage insect pests ranges from monogenic and oligogenic for insects like *C. chinensis* and *C. maculatus* in mungbean (Redden *et al.*, 1983; Dongre *et al.*, 1996; Chen *et al.*, 2007; Somta *et al.*, 2007) to polygenic in others like resistance of *Pisum fulvum* accessions to pea weevil (*B. pisorum*) (Somta *et al.*, 2007; 2008). Dongre *et al.* (1996) studied the inheritance of cowpea weevil (*Callosobruchus maculatus*) resistance in black gram (*Vigna mungo*) and found a ratio of 15:1 in the $F_2$ generation, indicating the presence of two dominant genes controlling resistance.

Monogenic/oligogenic resistance is characterized by differential reaction, simple inheritance and liable to breakdown whereas polygenic resistance is characterized by cumulative effects of many genes and better durability but more difficult to breed. Mostly additive and dominant genes govern storage insect pest resistance in many legume crops (Somta *et al.*, 2006; 2007; 2008) but, in a few cases, cytoplasmic gene effects have also been reported (Redden *et al.*, 1983; Somta *et al.*, 2006; 2007).

Recently, the use of QTL based marker-assisted selection has also shown promise in a number of legume crops (Chen *et al.*, 2007; Somta *et al.*, 2006; 2008). Problems associated with the need for long cycles of backcrossing with the conventional breeding method have already been resolved (Higgins *et al.*, 1999). For example, the application of marker-assisted selection in haricot bean to transfer resistance genes to *Zabrotes subfasciatus* from a wild background into elite cultivars through backcrossing at CIAT was proved to be simpler and more effective than the conventional methods (Blair *et al.*, 2007). Efforts to transform chickpea for *a*-amylase inhibitor gene isolated from haricot bean seeds was reported to significantly reduce the survival rate of both *C. Chinensis* (Sarmah *et al.*, 2004) and *C. maculatus* (Sarmah *et al.*, 2004; Ignacimuthu and Prakash, 2006).

#### 2.4.3.4. Limitations of breeding for storage insects' resistance

Breeding efforts to improve storage insect pest resistance have been underway for long period of time (Panda and Khush, 1995; Lale and Kolo, 1998). However, it is hard to say that these efforts have resulted in varietal breakthrough as desired because of certain limitations in making major advances (Bushara, 1988) as little attention is given to insect pest resistance and limited genetic resources available for some species (Clement *et al.*, 2002; Edwards and Singh, 2006).

Collections of wild accessions of crop species or their non-domesticated relatives may provide the answer to the problems posed by the limited genetic resources in legume crops (Clement *et al.*, 2002). There are also other technical challenges like biotypic variation, undesirable genetic linkages, lack of inter-specific cross-compatibility, selection pressure and failure of resistance and limited knowledge of genetic bases of resistance (Singh, 2002).

Breeding for insect pest resistance is complicated by the genetic variability of the pest population itself. Biotypes of each species may differ for their ability to infest or attack varieties of the same host species. Cultivars resistant to one biotype may fail to resist another biotype and it would be challenging to plant breeders to develop cultivars that simultaneously resist all possible combination of biotypes. For instance, laboratory studies on geographically distinct population of *C. maculatus* have repeatedly highlighted significant intra-specific variation in performance on resistant cowpeas (Appleby and Credland, 2004), indicating that the effectiveness of a resistant variety in one geographical location cannot necessarily be taken as an indication of its effectiveness in other locations.

The incorporation of different resistance genes into new cultivars (gene pyramiding) may control different biotypes as possibilities to develop the same breeding line with multiple resistances to different species of bruchids (Chen *et al.*, 2007; Somta *et al.*, 2007). The use of multiline varieties and varietal mixtures may also be suggested as each component of the multiline and/or the varietal mixtures may carry separate genes for resistance

(Welish, 1981). The introduction of two insecticidal proteins that act at different sites in the insect as a strategy to slow the rate of insect pest resistance development also served to overcome problems of biotic variation (Morton *et al.*, 2000; Chen *et al.*, 2007; Somta *et al.*, 2007).

Achieving pest resistance without reducing agronomic quality has been among technical problems of significant concerns (Edwards and Singh, 2006). Even though it is evident that a vast amount of genetic diversity within crop plants and in their wild types is available, its proper utilization is limited by existence of undesirable genetic linkage (Edwards and Singh, 2006; Acosta-Gallegos *et al.*, 2008), a situation whereby genes for desirable and undesirable characters show a tendency to be inherited together. Therefore, breeding for resistance against storage pest using wild relatives as gene sources not only improves resistance but it often reduces the quality of the produce or may even make it unfit for consumption as the biochemical features associated with resistance may also make the product unattractive for consumption by human beings (Singh, 2002).

Varieties carrying genes for insect pest resistance from related wild species are generally lower yielder with inferior quality. According to Tomooka *et al.* (1992), Tomooka *et al.* (2000), Chen *et al.* (2007) and Somta *et al.* (2007), wild species have several defects not only in terms of agronomic performance, but also in terms of food safety. It is particularly difficult to breed for pest resistance when the mechanism of resistance in itself reduces crop quality. This can be true for many physical resistance mechanisms including pod thickness (e.g. pea weevil resistance), and for chemical resistance when the compounds involved are also toxic to mammals (e.g. alkaloids in lupines) (Edwards and Singh, 2006). The problem of "genetic drag" is particularly serious when resistance traits are under polygenic control or have low dominance, resulting in many undesirable qualities being introduced along with the desired trait during the breeding process (Edwards and Singh, 2006).

Genetic linkage could be broken and two linked genes could be separated when there is crossing-over between two homologous chromosomes during meiosis. The breeder would have, therefore, to grow

large number of $F_2$ population in order to increase the level of recovery of new recombinants as a result of possible crossing-over. A modified recurrent selection procedure was also used by CIAT to successfully overcome this problem and improve resistance and tolerance to leafhoppers in common beans (Edwards and Singh, 2006). Backcross breeding could be another approach to minimize the deleterious effects of linkage drag.

Inter-specific hybridization is normally made to transfer specific characters like pest-resistance traits from wild species to the cultivated ones. Nevertheless, the transfer of genes governing resistance to pests in general from wild relative to the cultivated varieties presented inter-specific cross-incompatibility problems which may not be easy to overcome. In general, species with close taxonomical relations are expected to cross more easily than those more distantly related ones. For instance, very low cross compatibility was reported between *Vigna umbellata* and adzuki bean (*V. angularis*) and between *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* (Somta *et al.*, 2006).

In some cases where inter-specific cross-incompatibility is not a problem, failure of zygotic formation (failure of fertilization) and development or failure of $F_1$ germination may be encountered. This is due to the existence of lethal genes, genotypic disharmony (genetic imbalance in the resultant hybrid) between the two parental genomes, chromosome elimination (mitotic irregularities), incompatible cytoplasm or endosperm abortion (Singh, 2002).

The recovery of inter-specific hybrids may normally be improved by making sufficiently large number of crosses from given parents. Obtaining $F_1$ may still not be possible at all in cases when distant species particularly those with different ploidy levels are crossed (Singh, 2002). For instance, even though many accessions of the cultivated rice bean (*V. umbellata*) showed complete resistance to bruchids, the transfer of bruchid resistance from this cultivated species to susceptible adzuki bean was found to be difficult due to cross-incompatibility between the two species (Somta *et al.*, 2006). In such cases, mismatches in chromosome number may be adjusted by increasing or reducing chromosome number in one of the parents or their $F_1$ crosses or both or by using another bridging species.

When two species cannot be crossed directly, the third species which is compatible with both parental species may be used as a bridge in such a way that the bridge species is first crossed to one of the parental species and then the resulting $F_1$ will be crossed to the other parental species. For example, species of the *V. minima*, *V. riukiuensis* and *V. nakashimae* have been suggested as the most suitable bridging species between *V. umbellata* and *V. angularis* to transfer bruchid resistance from *V. umbellata* to *V. angularis* (Tomooka *et al.*, 2000; Somta *et al.*, 2006). Therefore, *V. nakashimae*, that crosses with both *V. umbellata* and *V. angularis* without the need for embryo rescue could be used as a bridging species (Somta *et al.*, 2006).

Failure of seed set in inter-specific crosses may also be overcome by the use of embryo culture techniques (Singh, 2002). Ease of hybridization among *V. angularis*, *V. nakashimae* and *V. Umbellate*, for instance, may differ in that crosses between *V. angularis* and *V. umbellata* were found to require embryo rescue as there is a high level of abnormality in segregating populations (Kaga *et al.*, 2000). The same report indicated that the level of abnormal segregation is high in the cross between *V. umbellata* and *V. nakashimae* but hybrids do not require the application of embryo rescue techniques.

## 2.5. The Rationale and Relevance of the Present Study

Challenges due to low soil fertility and storage insect pest problems can be overcome through the mutual manipulation of the environment by the use of inorganic and organic fertilizers, insecticides and by genetic manipulation of the crop itself (Sanginga *et al.*, 2000; Ahmad *et al.*, 2001; Gill *et al.*, 2005; Lin *et al.*, 2005; Sithanantham and Rao, 2006; Damte and Dawd, 2006; Syers *et al.*, 2008).

Breeding of cultivars with better symbiotic nitrogen fixation, phosphorus use efficiency and resistance to storage insect has also more comparative advantages in that the existing crop management and cropping

systems may not need to be changed with the adoption of such cultivars. Experience shows that seed based technologies are easier to transfer to farmers than more complex knowledge based agronomic and crop protection practices (Edmeades *et al.*, 1998). Host resistance can also be used on its own or as one of the most important components of integrated pest management (IPM) because of its compatibility with cultural, biological and chemical control measures (Panda and Khush, 1995).

Application of fertilizers and insecticides are not affordable to the resource-poor farmers due to shortage of supplies and high costs (Macduff and White, 1984; Isfan *et al.*, 1995; Roy, 1995; Beem and Smith, 1997). Unfortunately, the cost of agrochemicals may continue to rise to the level farmers in developing countries in general and, in Ethiopia in particular, may not afford to purchase (FAO, 1984). It is likely that prices will rather rise in the future due to the dwindling oil reserves (Beem and Smith, 1997; Bøckman, 1997; Syers *et al.*, 2008). Organic sources like farmyard manure are also limited (Quinones *et al*, 1997). The rock phosphate deposit considered affordable to poor farmers (Sanchez, 2002) could also be depleted within some decades to come (Beebe *et al.*, 2006). The local construction and operation of fertilizer factories is not affordable to most developing countries (Williams and Schultz, 1990). In the developed countries, environmental pollution from excessive use of agrochemicals is of considerable concern. Not only has the harmful residual effects, but also, food safety has been issues of great concern in modern agriculture.

The use of resistant varieties against storage insect pest has also, among others, a number of comparative advantages over other methods. First, use of other methods like insecticide chemicals application may need to be periodically repeated and are hence more expensive as compared to genetic manipulation. The storage structures in use under small-scale production in the tropics and sub-tropics also do not suit for the use of chemical pesticides particularly for formulations like fumigants (Chen *et al.*, 2007). In most cases, the storage structure is built within a dwelling house which makes use of insecticides more hazardous to human health. Since

seeds are used for consumption, use of chemicals to protect the seeds is usually not recommended (Ignacimuthu and Prakash, 2006; Chen *et al.*, 2007). Additionally, the availability of sub-standard and poor quality insecticides further aggravates the situation.

The long-term use of insecticides may result in insecticide resistance of target pests and resurgence of minor pest. Resistant individuals to insecticides in a population continue to multiply while susceptible ones are eliminated by the insecticide and, eventually, resistant insects outnumber the susceptible ones and the pesticide may no longer be effective. The continuous use of insecticides may also reduce the population of natural enemies (predators and parasitoids) of insect pests (Panda and Khush, 1995). As there is no economic threshold level for storage insect pests, insecticides are applied as prophylactic measure which further aggravates the problem. Moreover, legumes are export commodities of several countries and the use of insecticides is strictly governed by the importer and by the maximum residual levels set by WHO/FAO Codex Committee on pesticide residue and the Codex of Alimentarium Committee (Evans, 1987).

It is believed that Debre Zeit Research Center (then under Alemaya College of Agriculture) started chickpea breeding in the 1950's. The research efforts were strengthened and organized on a multidisciplinary basis in early 1980's when collaborated efforts were started among different research centers at different locations and the program was further strengthened by supports from ICARDA and ICRISAT (Telaye *et al.*, 1994). Despite the wide genetic diversity existing in Ethiopia, the potential of the Ethiopian chickpea landraces has not been properly exploited by the hitherto breeding efforts (Telaye *et al.*, 1994). This could be witnessed from the fact that most of the released varieties in Ethiopia trace their genetic base to the introductions either from ICARDA or ICRISAT (MoA, 2010).

It is generally believed that landraces as sources of initial breeding materials have considerable breeding values under sub-optimal production systems like that of Ethiopia as they contain valuable adaptive genes to different circumstances (Ceccarelli, 1994; Bunder *et al.*, 1996; Chahal and

Gosal, 2002; Singh, 2002; Provorov and Tikhonovich, 2004). In the present study, Ethiopian chickpea germplasm accessions that can serve as genetic resources for better symbiotic nitrogen fixation, phosphorus uptake and use efficiency and adzuki bean beetle resistance would be identified. In addition, baseline information that can assist in future genetic improvement would be generated and the extent and eco-geographic pattern of distribution of genetic diversity in the Ethiopian chcikpea landraces would be established.

# 3. MATERIALS AND METHODS

## 3.1. Genetic characterization for microsatellite markers

### 3.1.1. Plant materials

One hundred fifty five chickpea entries were considered for the study. While 139 of these were Ethiopian germplasm accessions collected by the Institute of Biodiversity Conservation (IBC) from the major chickpea production areas ranging in altitude from 1220 to 3120 meters above sea level, eight were nationally released varieties and the other eight were breeding lines introduced, from the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT).

The local accessions thus represent over 12% of the 1150 chickpea germplasm holding of the Ethiopian Institute of Biodiversity Conservation (Tanto and Tefera, 2006). Even though each germplasm collection is considered a single population, it represents a bulk of different individuals or populations which are (genetic pools) and is treated, hereafter, as genotypes for experimental or practical purposes. Descriptions of the test entries are given in Table 1 along with the map of the origins of the geographical regions of the Ethiopian materials in Figure 1. The genotypes from specific origins (Arsi, East Gojam, West Gojam, North Gonder, South Gonder, West Harerge, East Shewa, North Shewa, West Shewa, Tigray, Wello and introductions) were considered as belonging to 12 initial groups for reclassification into genetic populations.

### 3.1.2. DNA extraction

Twenty seeds of each of the entries were planted in pots on sterile sand in the growth chamber under regulated conditions at ICARDA. Two weeks after planting, approximately equal amount of bulk leave samples were collected from 5-10 plants of each entry as suggested by Gilbert *et al.* (1999).

Table 1. Passport description of the test genotypes

| S. No. | Accession/genotype | Region | Zone | District | Altitude (masl) |
|---|---|---|---|---|---|
| 1 | Acc. No. 231327 | Oromiya | Arsi | Merti | 1540 |
| 2 | Acc. No. 231328 | Oromiya | Arsi | Jeju | 1600 |
| 3 | Acc. No. 209093 | Oromiya | Arsi | Dodota Sire | 1710 |
| 4 | Acc. No. 208829 | Oromiya | Arsi | Dodota Sire | 1740 |
| 5 | Acc. No. 209094 | Oromiya | Arsi | Dodota Sire | 1750 |
| 6 | Acc. No. 209092 | Oromiya | Arsi | Dodota Sire | 1770 |
| 7 | Acc. No. 209096 | Oromiya | Arsi | Dodota Sire | 1850 |
| 8 | Acc. No. 209097 | Oromiya | Arsi | Dodota Sire | 1860 |
| 9 | Acc. No. 209098 | Oromiya | Arsi | Dodota Sire | 1860 |
| 10 | Acc. No. 41002 | Oromiya | Arsi | Tena | 2080 |
| 11 | Acc. No. 207761 | Oromiya | Arsi | Tena | 2080 |
| 12 | Acc. No. 207763 | Oromiya | Arsi | Tena | 2080 |
| 13 | Acc. No. 207764 | Oromiya | Arsi | Tena | 2080 |
| 14 | Acc. No. 41268 | Amahara | E. Gojam | H. Ej Enese | 1770 |
| 15 | Acc. No. 41026 | Amahara | E. Gojam | Hulet Ej Enese | 2280 |
| 16 | Acc. No. 41074 | Amahara | E. Gojam | Hulet Ej Enese | 2450 |
| 17 | Acc. No. 41075 | Amahara | E. Gojam | Hulet Ej Enese | 2410 |
| 18 | Acc. No. 41073 | Amahara | E. Gojam | Hulet Ejenese | 2400 |
| 19 | Acc. No. 41076 | Amahara | E. Gojam | Hulet Ej Enese | 2470 |
| 20 | Acc. No. 41021 | Amahara | E. Gojam | Enarj Enawga | 2510 |
| 21 | Acc. No. 41027 | Amahara | E. Gojam | Shebel Berenta | 2450 |
| 22 | Acc. No. 41222 | Amahara | E. Gojam | Dejen | 2460 |
| 23 | Acc. No. 207734 | Amahara | E. Gojam | Goncha Siso Enese | 2560 |
| 24 | Acc. No. 41103 | Amahara | E. Gojam | Enemay | 2570 |
| 25 | Acc. No. 41320 | Amahara | E. Gojam | Debay Telatgen | 2400 |
| 26 | Acc. No. 41029 | Amahara | E. Gojam | Enarj Enawga | 2880 |
| 27 | Acc. No. 41015 | Amahara | W. Gojam | Jabi Tehnan | 2020 |
| 28 | Acc. No. 41271 | Amahara | W. Gojam | Adet | 1880 |
| 29 | Acc. No. 41272 | Amahara | W. Gojam | Adet | 1960 |
| 30 | Acc. No. 41276 | Amahara | W. Gojam | Adet | 2230 |
| 31 | Acc. No. 207745 | Amahara | W. Gojam | Adet | 2230 |
| 32 | Acc. No. 41275 | Amahara | W. Gojam | Adet | 2240 |
| 33 | Acc. No. 41277 | Amahara | W. Gojam | Adet | 2240 |
| 34 | Acc. No. 207743 | Amahara | W. Gojam | Adet | 2240 |
| 35 | Acc. No. 207744 | Amahara | W. Gojam | Adet | 2240 |
| 36 | Acc. No. 41273 | Amahara | W. Gojam | Adet | 2300 |
| 37 | Acc. No. 41274 | Amahara | W. Gojam | Adet | 2360 |
| 38 | Acc. No. 207741 | Amahara | W. Gojam | Adet | 2360 |
| 39 | Acc. No. 207742 | Amahara | W. Gojam | Adet | 2360 |
| 40 | Acc. No. 41316 | Amahara | N. Gonder | Gonder Zuria | 1900 |
| 41 | Acc. No. 41298 | Amahara | N. Gonder | Gonder Zuria | 1920 |
| 42 | Acc. No. 41311 | Amahara | N. Gonder | Dembia | 1925 |
| 43 | Acc. No. 41313 | Amahara | N. Gonder | Gonder Zuria | 1925 |
| 44 | Acc. No. 41280 | Amahara | N. Gonder | Gonder Zuria | 1940 |
| 45 | Acc. No. 41312 | Amahara | N. Gonder | Gonder Zuria | 1950 |
| 46 | Acc. No. 41315 | Amahara | N. Gonder | Gonder Zuria | 1900 |
| 47 | Acc. No. 41308 | Amahara | N. Gonder | Dembia | 2010 |
| 48 | Acc. No. 41299 | Amahara | N. Gonder | Gonder Zuria | 1920 |
| 49 | Acc. No. 41046 | Amahara | N. Gonder | Chilga | 2160 |
| 50 | Acc. No. 41047 | Amahara | N. Gonder | Chilga | 2160 |
| 51 | Acc. No. 41304 | Amahara | N. Gonder | Dabat | 2610 |
| 52 | Acc. No. 41303 | Amahara | N. Gonder | Wegera | 2710 |
| 53 | Acc. No. 41295 | Amahara | S. Gonder | Fogera | 1820 |
| 54 | Acc. No. 41296 | Amahara | S. Gonder | Kemekem | 1850 |
| 55 | Acc. No. 41289 | Amahara | S. Gonder | Kemekem | 1855 |
| 56 | Acc. No. 41290 | Amahara | S. Gonder | Kemekem | 1880 |
| 57 | Acc. No. 41284 | Amahara | S. Gonder | Dera | 1900 |
| 58 | Acc. No. 41291 | Amahara | S. Gonder | Kemekem | 1900 |
| 59 | Acc. No. 41297 | Amahara | S. Gonder | Kemekem | 1950 |
| 60 | Acc. No. 41293 | Amahara | S. Gonder | Kemekem | 2040 |
| 61 | Acc. No. 41019 | Amahara | S. Gonder | Este | 2500 |
| 62 | Acc. No. 41048 | Amahara | S. Gonder | Farta | 2640 |
| 63 | Acc. No. 41049 | Amahara | S. Gonder | Farta | 2640 |
| 64 | Acc. No. 41053 | Amahara | S. Gonder | Lay Gayint | 3120 |
| 65 | Acc. No. 41054 | Oromiya | W. Harerge | Chiro | 1500 |
| 66 | Acc. No. 41052 | Oromiya | W. Harerge | Mieso | 1510 |
| 67 | Acc. No. 209082 | Oromiya | W. Harerge | Kuni | 1680 |
| 68 | Acc. No. 209083 | Oromiya | W. Harerge | Kuni | 1700 |
| 69 | Acc. No. 209084 | Oromiya | W. Harerge | Kuni | 1700 |
| 70 | Acc. No. 209091 | Oromiya | W. Harerge | Habro | 1730 |
| 71 | Acc. No. 209087 | Oromiya | W. Harerge | Kuni | 1740 |
| 72 | Acc. No. 209088 | Oromiya | W. Harerge | Habro | 1740 |
| 73 | Acc. No. 209089 | Oromiya | W. Harerge | Habro | 1740 |
| 74 | Acc. No. 209090 | Oromiya | W. Harerge | Habro | 1740 |
| 75 | Acc. No. 209081 | Oromiya | W. Harerge | Girawa | 2130 |
| 76 | Acc. No. 41159 | Oromiya | E. Shewa | Ada'a Chukala | 1910 |
| 77 | Acc. No. 41160 | Oromiya | E. Shewa | Ada'a Chukala | 1910 |

Table 1. Continued

| S. No. | Accession/genotype | Region | Zone | District | Altitude (masl) |
|---|---|---|---|---|---|
| 78 | Acc. No. 41161 | Oromiya | E. Shewa | Ada'a Chukala | 1940 |
| 79 | Acc. No. 207661 | Oromiya | E. Shewa | Ada'a Chukala | 1850 |
| 80 | Acc. No. 207667 | Oromiya | E. Shewa | Akaki | 2180 |
| 81 | Acc. No. 207666 | Oromiya | E. Shewa | Akaki | 2060 |
| 82 | Acc. No. 41141 | Oromiya | E. Shewa | Lome | 2040 |
| 83 | Acc. No. 207665 | Oromiya | E. Shewa | Akaki | 2060 |
| 84 | Acc. No. 41134 | Oromiya | E. Shewa | Akaki | 2080 |
| 85 | Acc. No. 41128 | Oromiya | E. Shewa | Akaki | 2130 |
| 86 | Acc. No. 41168 | Oromiya | E. Shewa | Ada'a Chukala | 2150 |
| 87 | Acc. No. 41129 | Oromiya | E. Shewa | Akaki | 2170 |
| 88 | Acc. No. 41130 | Oromiya | E. Shewa | Akaki | 2190 |
| 89 | Acc. No. 41110 | Amara | N. Shewa | Kewet | 1220 |
| 90 | Acc. No. 207657 | Amara | N. Shewa | Efratana Gidim | 1400 |
| 91 | Acc. No. 41111 | Amahara | N. Shewa | Efratana Gidim | 1400 |
| 92 | Acc. No. 41106 | Amahara | N. Shewa | Mafudmezezo Mojana | 1820 |
| 93 | Acc. No. 207658 | Amahara | N. Shewa | Efratana Gidim | 1400 |
| 94 | Acc. No. 41142 | Amahara | N. Shewa | Minjarna Shenkora | 2290 |
| 95 | Acc. No. 41207 | Amahara | N. Shewa | Siyadebrina Wayuense | 2580 |
| 96 | Acc. No. 41215 | Amahara | N. Shewa | Moretena Jiru | 2640 |
| 97 | Acc. No. 41216 | Amahara | N. Shewa | Moretena Jiru | 2640 |
| 98 | Acc. No. 41066 | Oromiya | N. Shewa | Wara Jarso | 2550 |
| 99 | Acc. No. 41011 | Oromiya | N. Shewa | Gerar Jarso | 2558 |
| 100 | Acc. No. 41007 | Oromiya | N. Shewa | Yaya Gulale | 2670 |
| 101 | Acc. No. 41008 | Oromiya | N. Shewa | Yaya Gulale | 2700 |
| 102 | Acc. No. 41186 | Oromiya | W. Shewa | Waliso Goro | 1960 |
| 103 | Acc. No. 209035 | Oromiya | W. Shewa | Alem Gena | 2010 |
| 104 | Acc. No. 41176 | Oromiya | W. Shewa | Ambo | 2020 |
| 105 | Acc. No. 41175 | Oromiya | W. Shewa | Ambo | 1970 |
| 106 | Acc. No. 41174 | Oromiya | W. Shewa | Ambo | 2120 |
| 107 | Acc. No. 209027 | Oromiya | W. Shewa | Kersa Kondaltiti | 2060 |
| 108 | Acc. No. 41170 | Oromiya | W. Shewa | Dendi | 2160 |
| 109 | Acc. No. 41171 | Oromiya | W. Shewa | Dendi | 2230 |
| 110 | Acc. No. 41185 | Oromiya | W. Shewa | Woliso Goro | 2000 |
| 111 | Acc. No. 209036 | Oromiya | W. Shewa | Alem Gena | 2220 |
| 112 | Acc. No. 41190 | Oromiya | W. Shewa | Woliso Goro | 2080 |
| 113 | Acc. No. 41195 | Oromiya | W. Shewa | Becho | 2160 |
| 114 | Acc. No. 41197 | Oromiya | W. Shewa | Becho | 2120 |
| 115 | Acc. No. 207150 | Tigray | S. Tigray | Enderta | --- |
| 116 | Acc. No. 207151 | Tigray | S. Tigray | Enderta | --- |
| 117 | Acc. No. 207563 | Tigray | S. Tigray | Hintalo Wajirat | 1960 |
| 118 | Acc. No. 207564 | Tigray | C. Tigray | Laelay Maychew | 2150 |
| 119 | Acc. No. 207894 | Tigray | S. Tigray | Endamehoni | 2600 |
| 120 | Acc. No. 207895 | Tigray | S. Tigray | Alaje | --- |
| 121 | Acc. No. 213224 | Tigray | E. Tigray | Wukro | 2100 |
| 122 | Acc. No. 219797 | Tigray | C. Tigray | Laelay Maychew | 2150 |
| 123 | Acc. No. 219799 | Tigray | C. Tigray | Laelay Maychew | 1970 |
| 124 | Acc. No. 219800 | Tigray | C. Tigray | Adwa | 2400 |
| 125 | Acc. No. 219803 | Tigray | W. Tigray | Tahtay Koraro | 1880 |
| 126 | Acc. No. 221696 | Tigray | S. Tigray | Enderta | --- |
| 127 | Acc. No. 41114 | Amahara | S. Wello | Werebabu | 1560 |
| 128 | Acc. No. 212589 | Amahara | S. Wello | Kalu | 1600 |
| 129 | Acc. No. 41113 | Amahara | S. Wello | Kalu | 1650 |
| 130 | Acc. No. 207659 | Amahara | S. Wello | Dessie Zuria | 1950 |
| 131 | Acc. No. 207660 | Amahara | S. Wello | Dessie Zuria | 1950 |
| 132 | Acc. No. 41115 | Amahara | S. Wello | Kutaber | 2290 |
| 133 | Acc. No. 225878 | Amahara | S. Wello | Debresina | 2420 |
| 134 | Acc. No. 225873 | Amahara | S. Wello | Debresina | 2445 |
| 135 | Acc. No. 225874 | Amahara | S. Wello | Debresina | 2450 |
| 136 | Acc. No. 225877 | Amahara | S. Wello | Kelala | 2450 |
| 137 | Acc. No. 207645 | Amahara | S. Wello | Debresina | 2510 |
| 138 | Acc. No. 207646 | Amahara | S. Wello | Debresina | 2510 |
| 139 | Acc. No. 225876 | Amahara | S. Wello | Kelala | 2540 |
| 140 | ICC 5003* | India | --- | --- | --- |
| 141 | ICC 4918 | India | --- | --- | --- |
| 142 | ICC 4948 | India | --- | --- | --- |
| 143 | ICC 4973 | India | --- | --- | --- |
| 144 | ICC 15996 | ICRISAT | --- | --- | --- |
| 145 | Shasho (ICCV 93512) | ICRISAT | --- | --- | --- |
| 146 | Arerti (FLIP 89-84C) | ICARDA | --- | --- | --- |
| 147 | Worku (DZ-10-16-2) | ICRISAT | --- | --- | --- |
| 148 | Akaki (DZ-10-9-2) | ICRISAT | --- | --- | --- |
| 149 | Ejere (FLIP-97-263C) | ICARDA | --- | --- | --- |
| 150 | Teji (FLI 97-266C) | ICARDA | --- | --- | --- |
| 151 | Habru (FLIP 88-42c) | ICARDA | --- | --- | --- |
| 152 | Natoli (ICCX-910112-6) | ICRISAT | --- | --- | --- |
| 153 | ICC 19180 | ICRISAT | --- | --- | --- |
| 154 | ICC 19181 | ICRISAT | --- | --- | --- |
| 155 | PM 233 (155) | ICARDA | --- | --- | --- |

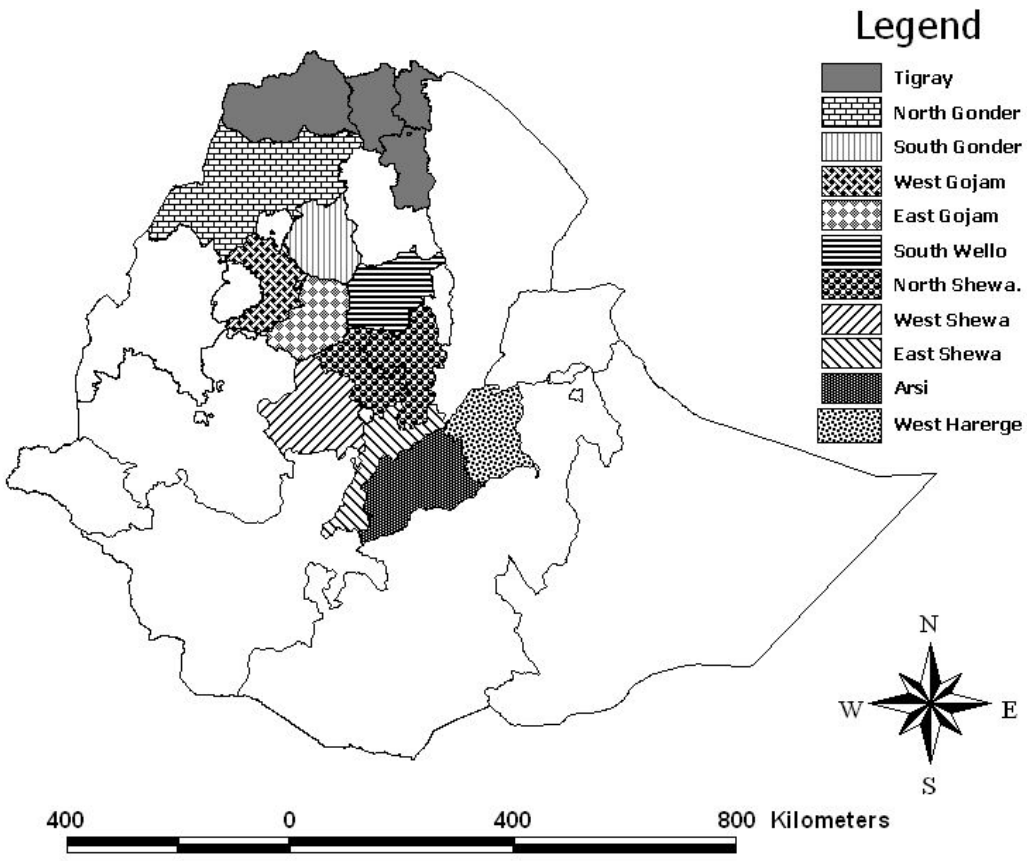



Figure 1. Map of Ethiopia showing the approximate areas of origins (shaded region) of the 139 germplasm accessions (NB: all boundaries are approximate and nothing to do with political borders)

About 100 mg of fresh leaves were placed in 2 ml autoclaved and labelled Eppendorf tubes, covered by paraffin paper with a small slot at one side for air circulation and freeze dried for three days at -80 $^{o}$C. The samples were ground using a mixer mill (Retsch MM® 200) and genomic DNA was extracted using the cetyltriethylammonium bromide (CTAB) method (Doyle and Doyle, 1990) with some minor modifications. About 1 ml of 1X CTAB was added to each sample to break open plant cells and solubilize the contents. The sample was shortly mixed using vortex and kept in gently shaking water bath (GFL 1086) for 1h at 65 $^{o}$C. After the samples were taken out of the water bath and kept on ice for 5 minutes, chlorophyll and some denatured proteins were removed from the samples by dissolving in 1 ml mixture of phenol, chloroform and isoamylalcohol at the ratio of 25:24:1.

The organic phase was separated by centrifugation (with Eppendorf Centrifuge 5417R) for 20 minutes at 14000 rpm. RNA was removed by transferring the supernatant to new 2 ml Eppendorf tubes and incubating it at 37 $^{o}$C with 5 µl the enzyme RNAse. About 1 ml of isopropanol was added and the DNA was precipitated by centrifugation. The isopropanol was removed and the DNA was cleaned by repeated washing with 75% ethanol. Then the pellet was air dried and dissolved in 100 µl of 1X TE.

### 3.1.3. Procedures of polymerase chain reaction (PCR)

The quality of DNA was tested and the amount in each sample was quantified on agarose gel (1%) and optimized for PCR reactions using a lambda DNA standard, pUC 19 (50 µg). About 50 simple sequence repeat (SSR) markers distributed throughout the chickpea genome and previously reported to be polymorphic with other chickpea genetic materials including world collections were selected from published sequences and genetic maps (Hüttel *et al.*, 1999; Winter *et al.*, 1999; Winter *et al.*, 2000; Millan *et al.*, 2006; Sethy *et al.*, 2006; Radhika *et al.*, 2007; Upadhyaya *et al.*, 2008; Nayak *et al.*, 2010) as described in Table 2.

PCR reaction was performed with a thermal cycler (GeneAmp®PCR System 9700) in a total volume of 10 µl containing 50 ng DNA, 1µl dNTPs, 1µl buffer 10x, 1µl forward and reverse primer (unlabelled) and 0.1µl Taq DNA polymerase in 4.9 µl $dH_2O$. The PCR was programmed at an initial denaturation step of 2 minutes at 94°C followed by 35 cycles of 20 seconds denaturation at 94°C, annealing at 55 or 60°C (depending on the primer) for 50 seconds, initial extension at 72°C for 50 seconds and final extension at 72°C for 7 minutes. PCR amplification for primers NCPGR 21, NCPGR 33, NCPGR45, NCPGR 53, NCPGR 94, NCPGR 98, NCPGR 99 and NCPGR 100 was carried out using 'touchdown' methodology. The touchdown methodology involved initial denaturation for 3 minutes at 94°C followed by 18 cycles at 94°C for 30 seconds, 64°C for 50 seconds with 0.5 °C decrease in each subsequent cycle at 72 °C for 50 seconds.

Table 2. Description (denomination, sequence, repeats, fragment size, linkage group and number of alleles) of the polymorphic microsatellite markers used in the analysis of the chickpea genotypes

| Microsatellite | Primer sequences | | Repeat | Fragment size (bp) | Linkage Group* | No of allele |
|---|---|---|---|---|---|---|
| | Forward | Reverse | | | | |
| GA 11 | GTTGAGCAACAAAGCCACAA | TTCTTGTCTGGTTGTGTGAGC | (CT)21 | 159 | 1/2/6 | 3 |
| GA 24 | TTGCCAAAACCAATAACTCTG | TCCCTTTTACACAAGGCCAG | (GA)19 | 203 | 1/2/4 | 2 |
| NCPGR 45 | TGTTTTCAAATCAAACAGGC | GATACACACCAAGGCACAGT | (CT)2GTCAT(CT)5CC(CT)2CC(CT)17 | 223 | 2 | 1 |
| NCPGR 53 | CCCTCCTTCTTGCTTACAAA | TAATGGTGAACGAATCATGG | (CT)5CA(CT)CA(CT)10CA(CT)4 CA(CT)TA(CT)4 GTCA(CT)12 | 194 | 1 | 2 |
| TA 144 | TATTTTAATCCGGTGAATATTACCTTT | GTGGAGTCACTATCAACAATCATACAT | (TAA)27 | 241 | 6 | 5 |
| NCPGR 94 | GGTTTGATGTGTTCTTGGCT | CCCTCAATTCCCTCGATTTA | (CT)25 | 176 | 5 | 1 |
| NCPGR 100 | CCATTTTCTACAATCTCATGTCT | GTAGAAAGAGCCAAGAGGCA | (CT)15N42(CT)2CC(CT)5TT(CT)6AT(CT) 7 | 263 | 1 | 2 |
| TA 18 | AAAATAATCTCCACTTCACAAATTTTC | ATAAGTGCGTTATTAGTTTGGTCTTGT | (TAA)24 | 147 | 5/7 | 6 |
| CaSTMS 11 | GTATCTACTTGTAATATTCTCTTCTCT | ATATCATAAACCCCCCAC | (GA)20 | 232 | 4 | 2 |
| TA 59 | ATCTAAAGAGAAATCAAAATTGTCGAA | GCAAATGTGAAGCATGTATAGATAAAG | (TAA)29 | 258 | 2/7 | 4 |
| TA 1 | TGAAATATGGAATGATTACTGAGTGAC | TATTGAAATAGGTCAGGCTTATAAAAA | (TAA)32 | 243 | 1 | 5 |
| TA 27 | GATAAAATCATTATTGGGTGTCCTTT | TTCAAATAATCTTTCATCAGTCAAATG | (TAA)21 | 241 | 2/7 | 4 |
| TA 37 | ACTTACATGAATTATCTTTCTTGGTCC | CGTATTCAAATAATCTTTCATCAGTCA | (TTA)20 | 282 | 2/3/7 | 6 |
| TA 76 | TCCTCTTCTTCGATATCATCA | CCATTCTATCTTTGGTGCTT | (AAT)7 (AAT)4[ACT(AAT)11]2ACT(AAT)3 TAT(AAT)2(ATT)5 | 206 | 3 | 4 |

Table 2. Continued

| Microsatellite | Primer sequences | | Repeat | Fragment size (bp) | Linkage group | No of allele |
|---|---|---|---|---|---|---|
| | Forward | Reverse | | | | |
| TA 110 | ACACTATAGGTATAGGCATTTAGGCAA | TTCTTTATAAATATCAGACCGGAAAGA | (TTA)22 | 220 | 2 | 6 |
| TR 1 | CGTATGATTTTGCCGTCTAT | ACCTCAAGTTCTCCGAAAGT | (TAA)31 | 224 | 6 | 5 |
| TR 2 | GGCTTAGAGTTCAAAGAGAGAA | AACCAAGATTGGAAGTTGTG | (TTA)36 | 210 | 3 | 3 |
| TR 3 | GAAGTATCAGTATCACGTGTAATTCGT | CTTACGGAGAACATGAACATCAA | (TTA)27 | 244 | 6/10/13 | 4 |
| TR 7 | GCATTATTCACCATTTGGAT | TGTGATAATTTTCTAAGTGTTTT | (TTA)25 | 204 | 6 | 6 |
| TR 19 | TCAGTATCACGTGTAATTCGT | CATGAACATCAAGTTCTCCA | (TAA)27 | 227 | 2 | 5 |
| TR 29 | GCCCACTGAAAAATAAAAAG | ATTTGAACCTCAAGTTCTCG | (TAA)8TAGTAATAG(TAA)32 | 220 | 1/5 | 7 |
| TR 43 | AGGACGAAACTATTCAAGGTAAGTAGA | AATTGAGATGGTATTAAATGGATAACG | (TAA)24 | 297 | 1/2 | 3 |
| TS 19 | TTTCTTTTGTTAGAGTTAAAAAAAATT | TCTCATGTTTTGCTTTTTATTATTATTA | (TAA)27 | 117 | 1/3/5 | n.a** |
| NCPGR 21 | TCTACCTCGTTTTTCGTGCC | TTGCTCCTTCAACAAAACCC | (CT)15 | 137 | 2 | 2 |
| TA 11 | CATGCCATAAACTCAATACAATACAAC | TTCATTGAGGACAATGTGTAATTTAAG | (TTA)17 | 230 | n.a | 6 |
| NCPGR 33 | ACATCTTGAAGTGCCCC | TGCAAGCAGACGGTTACAAG | (GA)20 | 248 | 4 | 1 |
| NCPGR 98 | CATCTTATTTTTCATTTTAGAGGAGG | AGGAAGTGTTATGGAGATGCC | (GA)20GG(GA)14 | 141 | 2 | 1 |
| NCPGR 99 | ATCATGAAGCAAATCCTCAC | TGAACCCAACATAGCATACA | (GA)18 | 227 | n.a | 1 |
| TA 117 | GAAAATCCCAAATTTTTCTTCTTCT | AACCTTATTTAAGAATATGAGAAACACA | (ATT)52 | 248 | 5 | 5 |
| TA 5 | ATCATTTCAATTTCCTCAACTATGAAT | TCGTTAACACGTAATTTCAAGTAAAGAT | (TTA)29 | 205 | 1/3/5 | 5 |
| TA 64 | ATATATCGTAACTCATTAATCATCCGC | AAATTGTTGTCATCAAATGGAAAATA | (TAA)39 | 239 | 1/3 | 8 |
| TA 72 | GAAAGATTTAAAAGATTTTCCACGTTA | TTAGAAGCATATTGTTGGGATAAGAGT | (ATT)36 | 256 | 2/4 | 7 |
| TS 35 | GGTCAACATGCATAAGTAATAGCAATA | ACTTTCGCGATTCAGCTAAAATA | (TAA)9T(A)3(TAA)13 | 247 | 1/5 | 2 |

*Different markers located on different linkage groups as reported by different investigators are separated by *slash*; **n.a = not available

This was followed by 20 cycles of 94 °C for 30 seconds, 55 °C annealing temperature for 50 seconds, 72 °C for 50 seconds and a final extension for 10 minutes at 72°C.

Samples were loaded on 8% polyacrylamide gel with a 6x DNA loading dye. Electrophoresis was carried out on a vertical electrophoresis set up (EPS-300 IIV) using a standard DNA ladder (GeneRuler ™ 50bp) with known reference bands. The gel was stained with Ethidium bromide (on SK-71 shaker) for 20 minutes and the amplification was visualised under UV illumination X-ray (Alphalmager® HP). Primers with unclear and missing bands were sorted and repeated. Non-polymorphic, missing, faint and distorted gels (in this specific case) were disregarded at scoring and only records of 33 primers with clear polymorphic bands were considered for statistical analysis (Table 2).

### 3.1.4. Data scoring and analysis

The amplified products were visually scored using binary numbers (1 for presence of band and 0 for absence) from the gel photographed under UV illumination (Warburton and Crossa, 2002; Saeed *et al.*, 2011). The data from all entries was combined for statistical analysis. The software GeneAlex version 6 (Peakall and Smouse, 2006) was used for analysis of molecular variance (AMOVA) to determine the following genetic parameters.

Average PhiPT = $\frac{VAP}{VWP + VAP} = \frac{VAP}{VT}$, where PhiPT coefficient denotes the proportion of the variance among populations relative to the total variance, $V_{AP}$ is the variance among populations, $V_{WP}$ the variance within populations and $V_T$ is the total variance or the sum of $V_{AP} + V_{WP}$.

Pairwise between populations PhiPT = $\frac{VAP+VAR}{VWP+VAP+VAR} = \frac{VAP+VAR}{VT}$, where PhiPT, $V_{AP}$ and $V_{WP}$ are as mentioned above; $V_{AR}$ stands for variance between geographical regions and $V_T$ is the total variance or the sum of $V_{AP} + V_{WP} + V_{AR}$.

Expected and unbiased expected heterozygosity values were estimated based on Nei (1978) assuming each band to correspond with a single bi-allelic locus but genetic interpretation of individuals as

heterozygous/homozygous for a particular locus was not attempted throughout because each sample from an accession constituted of a bulk of leaves from different individual plants.

He = $1-\Sigma p^2$, where He is the expected heterozygosity, assuming Hardy-Weinberg Equilibrium where an amplified allele (band present) has frequency p = 1-q, a null allele (band absent) has frequency q = 1 - p, frequency of genotype with null allele is $q^2$ = frequency of absence = 1-frequency of presence and q = √(frequency of absence).

UHe = $\frac{2N}{2N-1}$*He, where UHe is unbiased expected heterozygosity and N is number of population.

I = - 1*[p*ln(p)+q*ln(q)]

Where I Shannon's information index and p and q as given above.

% P = $\frac{NPL}{TNL}$ x 100, where % P is percent polymorphism, NPL is number of polymorphic bands and TNL is total number of bands.

The polymorphic information content (PIC) values were estimated employing the method suggested by Roldan-Ruiz *et al.* (2000) as:

$PIC_i = 2f_i(1-f_i)$, where $f_i$ is the frequency of amplified allele (band present) and $(1-f_i)$ is the frequency of the null allele (band absent) of marker i.

GD = -ln(GI), GI = $\frac{Jxy}{\sqrt{(Jx*Jy)}}$, $J_{xy} = \sum_{i=1}^{k} p_{ix} * p_{iy}$, $J_x = \sum_{i=1}^{k} p^2_{ix}$ and $J_y = \sum_{i=1}^{k} p^2_{iy}$,

where GD is Nei's genetic distance, GI is Nei's genetic identity, $P_{ix}$ and $P_{iy}$ are the frequencies of $i^{th}$ allele (band present) in populations x and y.

The population structure of the genotypes was defined by the Bayesian model-based clustering method of Pritchard *et al.* (2000) using the Structure 2.2 software assuming population admixture through inferred ancestry. For each of the K=2 to K=12 settings, 20 independent simulations were performed using the admixture model and 5000 replicates for burn-in and post burning sampling by Markov Chain Monte Carlo of 50,000 runs to estimate the number of subpopulations for each of the K values. The appropriate number of clusters (K) was determined according to Evanno *et al.* (2005).

## 3.2. Genetic characterization and evaluation for symbio-agronomic characters

### 3.2.1. Plant materials

In this study, the same genotypes as characterized with SSR markers (section 3.1) were characterized and evaluated for the symbiotic and agronomic performance. The passport description of test genotypes is given in Table 1 above with the geographical sources of origin of the local landraces in Figure 1. All genotypes were rejuvenated during 2008/9 under the same condition at Ginchi to minimize initial variation due to difference in seed age and indigenous seed nitrogen (Liao *et al.*, 2008).

### 3.2.2. Test environments

The experiment was conducted under field conditions at two locations (Ginchi and Ambo) in central part of Ethiopia for one year during the main cropping season of 2009/10 (September to January). The two locations are assumed to represent the major chickpea production areas of Ethiopia. Climatic data of the two locations during the growing period were taken from Ambo and Holetta Research Centers. Soil samples from both locations were collected from the rhizosphere (top 20 cm) for physico-chemical characterization (Table 3).

It was witnessed that more or less the weather variables recorded did not deviate much from the long-term trends at both locations (data not shown), indicating the possible reproducibility over seasons of the results in this study. Although the presence of high content of nitrogen in the soil may inhibit *Rhizobium* infection, nodulation and nitrogen fixation (Pearson *et al.*, 1995; Gulden and Vessey, 1998; Broughton *et al.*, 2003), tests of the physico-chemical properties of the soil showed equal level of low nitrogen contents of the soils at both locations. In Mexico, for instance, symbiotic nitrogen fixation in cowpea, soybean and faba bean was found to be inhibited with the application of more than 22-33 kg of nitrogen per acre (Lindman and Glover, 2003).

Based on the critical levels suggested by Jones (2003), P was high at Ambo but low at Ginchi while K, Ca, Mg, Na and Fe also existed in variable amounts of high levels at both locations. There were also high levels of exchangeable cations with pH values more or less closer to neutral. The descriptions of the two locations (during the growing season) are presented in Table 3. Similar results were reported from previous analysis of soils of the same locations (Dibabe *et al.*, 2001). The two sites received more or less similar amount of rainfall with different pattern of distribution but Ambo was more humid than Ginchi (Figures 2 and 3).

### 3.2.3. *Rhizobium* inoculant and inoculation

An effective isolate of *Rhizobium* for chickpea, CP EAL 004, originally isolated by the National Soil Laboratory from a collection of Ada'a District of East Shewa Zone, Ethiopia, was used for the study. The isolate was found to be efficient in nodulation and symbiotic nitrogen fixation in previous studies on chickpea (Hailemariam and Tsige, 2006). The inoculum was received at the concentration of approximately $10^9$ cells $g^{-1}$ of peat carrier. The concentration and purity of the inoculum was confirmed in the Soil Microbiology Laboratory at Holetta immediately before planting. Seeds of all genotypes were coated with the inoculant at the rate of approximately 2 g of inoculum for 80 seeds using 40% gum Arabic as an adhesive (Somasegaran and Hoben, 1985).

### 3.2.4. Experimental design and layout

The experiment was conducted under field conditions for one year during the main cropping season of 2009/2010 (September to January). A randomized complete block design with 4 replications was used. A blanket basal application of phosphorus was made to all plots in the form of triple supper phosphate (TSP) at the recommended rate (20 g for a single row of 4 meters) (Eshete, 1994). All other crop management practices were applied uniformly to all genotypes as required. Seed rate was 5 cm between plants and 40 cm between adjacent rows. Plot size was 1 row 4 m long. The

genotypes were assigned to plots at random within each block. Nitrogenous fertilizer was totally omitted and all other crop management and protection practices were applied uniformly to all genotypes as required.

Table 3. Description of the test locations for geographical position and physico-chemical properties of the soils

| Parameter | Source of soil | |
|---|---|---|
| | Ambo | Ginchi |
| Latitude | 09° 00′ N | 09° 00′ N |
| Longitude | 37° 22′ E | 38° 10′ E |
| Altitude (m.a.s.l.) | 2225 | 2200 |
| % Clay | 70.00 | 65.83 |
| % Silt | 15.00 | 20.42 |
| % Sand | 15.00 | 13.75 |
| Organic C (%) | 1.53 (low) | 1.30 (low) |
| N (%) | 0.103 (low) | 0.103 (low) |
| C/N ratio | 14.85 (high) | 12.62 (high) |
| P (ppm*) | 18.07 (high) | 4.49 (low) |
| K (Meq/100 g soil) | 2.438 (high) | 2.485 (high) |
| Ca (Meq/100 mg soil) | 59.03 (high) | 39.62 (high) |
| Mg (Meq/100 mg soil) | 11.20 (high) | 9.00 (high) |
| Na (Meq/100 mg soil) | 0.70 (high) | 0.61 (high) |
| $SO_4$ S (ppm) | 5.23 (optimum) | 5.62 (optimum) |
| Fe (ppm) | 27.73 (high) | 51.50 (high) |
| pH (1:1 $H_2O$) | 7.23 (optimum) | 6.18 (optimum) |
| EC (µS)** | 650.00 (high) | 547.33 (high) |

*ppm = parts per million; **µS = micro Siemens

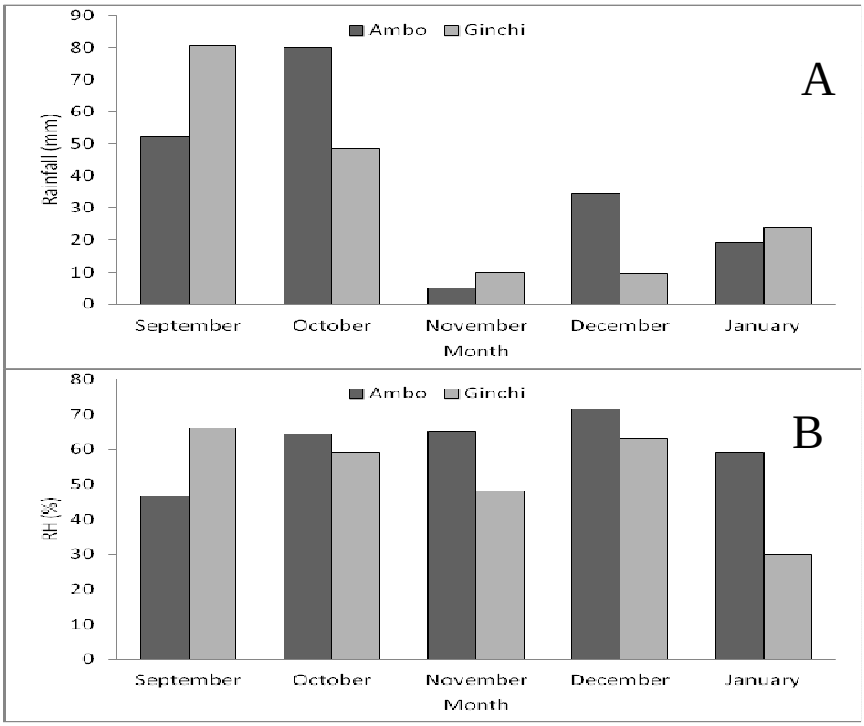



Figure 2. Rainfall (mm) and relative humidities (%) of (A) Ambo and (B) Ginchi during the growing season

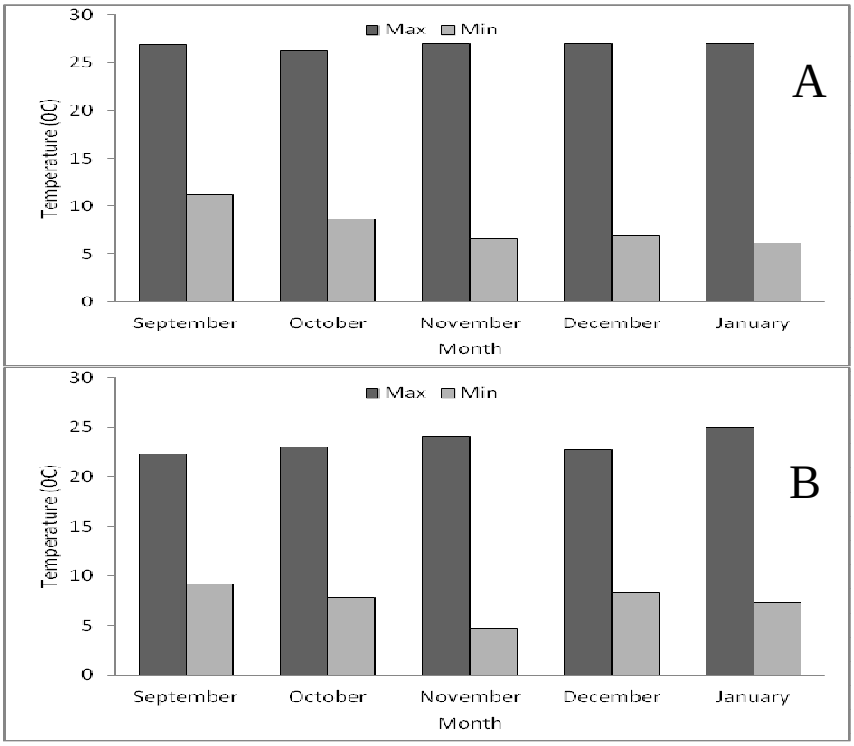



Figure 3. Maximum and minimum temperatures (°C) at (A) Ambo and (B) Ginchi during the growing season

### 3.2.5. Shoot and grain nitrogen analysis

Forty-five days after emergence (shortly before flowering), five plants from each plot were carefully dug up and their root washed free from soils with water running over a sieve for collection of nodules. Another five plants from each plot were sampled at 90% physiological maturity and shoot samples at maturity and grain samples from each plot were oven-dried to constant moisture at 70°C for 18 hours. The dry samples were ground to pass through 1 mm size mesh sieve to determine nitrogen using the Kjeldahl technique (AOAC, 1970) at Holetta and Debre Zeit Soil Science Research Laboratories.

Even though the initial plan was to use the average nitrogen content of the three genetically non-nodulating reference checks received from ICRISAT and ICARDA (ICC 19180, ICC 19181 and PM 233), it was concurrently observed that one of the reference genotypes, ICC 19180, had nodules indicating probable existence of symbiotic nitrogen fixation. The second reference genotype, ICC 19181, also showed excessively long vegetative period and it was assumed that it might explore and assimilate disproportionally more nitrogen from the soil than the nodulating test entries. Therefore, two of the reference checks were excluded for technical reasons and the amount of nitrogen fixed was estimated using PM 233 as a reference as it had no nodules and showed better morpho-agronomic similarity with the test entries.

The amount of total nitrogen accumulated in shoots and grains of the nodulating test genotypes was estimated by the difference method using a non-nodulating reference check (difference in shoot and grain nitrogen contents between the nodulating test genotypes and a non-nodulating reference) (Smith *et al.,* 1984; Zapata, 1990). The assumption is that the fixing plants explore and assimilate the same amount of soil mineral nitrogen as the neighbouring non-fixing reference (Herridge *et al.*, 2008). This method has been used widely for many years as a preferred procedure to obtain information for general purposes (Syers *et al.*, 2008).

Protein contents of shoot and grain were determined by multiplying nitrogen percentages by the standard conversion factor of 6.25 (AOAC, 1970). Based on the nitrogen contents, the following parameters were calculated:

$$N\ fixed\ in\ shoot = \frac{(Nsfg - Nsnfg)\ X\ 100}{Nsfg}$$

Where Nsfg = amount of nitrogen in shoot of fixing genotype and Nsnfg = amount of nitrogen in shoot of non-fixing genotype.

$$N\ fixed\ in\ grain = \frac{(Ngfg - Ngnfg)\ X\ 100}{Ngfg}$$

Where Ngfg = amount of nitrogen in grain of fixing genotype and Ngnfg = amount of nitrogen in grain of non-fixing genotype.

$$N\ fixed\ in\ biomass = \frac{(N\ fixed\ in\ shoot + N\ fixed\ in\ grain)\ X\ 100}{Nsfg + Ngfg}$$

$$N\ assimilation\ efficiency = \frac{(N\ fixed\ in\ grain)\ X\ 100}{Nsfg + Ngfg}$$

Grain N yield = Grain N content × grain yield

Shoot N yield = Shoot N content × shoot yield

Biomass N yield = Grain N yield + shoot N yield

Nitrogen harvest index (NHI) was estimated as:

$$NHI = \frac{\text{Grain N yield}}{\text{Biomass N yield}}$$

### 3.2.6. Data collection on symbiotic and agronomic characters

Data were collected either on plot basis or from randomly selected five plants (IBPGR, ICRISAT and ICARDA, 1993). Records were taken on: (1) early vigor as shoot dry weight before flowering, (2) shoot dry weight at physiological maturity, (3) shoot dry weight ratio (of nodulating to non-nodulating genotypes) before flowering and maturity, (4) nodulation index

(nodule weight to shoot weight ratio), (5) number of nodules, (6) nodule dry weight, (7) shoot nitrogen and protein contents, (8) shoot nitrogen fixation, (9) grain nitrogen and protein contents, (10) grain nitrogen fixation, (11) biomass nitrogen content, (12) biomass nitrogen fixation, (13) fixed nitrogen assimilation efficiency, (14) shoot, grain and biomass nitrogen yields, (15) nitrogen harvest index (the ratio of the amount of the element in the grain relative to the amount of the element in the total above-ground biomass), (16) days to 50% flowering and 90% maturity, (17) grain filling period (the number of days from 50% flowering to 90% physiological maturity), (18) numbers of pods and seeds, (19) total biomass weight, (20) harvest index (proportion of shoot dry matter that is grain), (21) grain production efficiency (seed filling duration divided by duration of vegetative period and then multiplied by grain yield), (22) biomass production rate (above ground biomass weight divided by days to 90% physiological maturity and then multiplied by 100), (23) economic growth rate (grain weight divided by seed fill duration and then multiplied by 100), (24) thousand seed weight and (25) grain yield.

## 3.3. Genetic characterization and evaluation for phosphorus uptake and use efficiency

### 3.3.1. Plant materials

In this study, the same 155 chickpea genotypes evaluated for symbio-agronomic characters (section 3.2) were used (Table 1 and Figure 1).

### 3.3.2. The test environment

The experiment was conducted under field conditions at two locations (Ginchi and Ambo) in central part of Ethiopia for one year during the main cropping season of 2009/10 (September to January). The two locations are characterized by Vertisol soils (Dibabe *et al.*, 2001) and assumed to represent the major chickpea production areas in Ethiopia. Chickpea is mostly grown on Vertisol soils with residual moisture in Ethiopia as

mentioned above. Climatic data of the two locations during the growing period are presented in Figures 2 and 3 and the physico-chemical properties of the soils is given in Table 3.

### 3.3.3. Phosphorus application and experimental layout

The experiment was laid down in a randomized complete block design with 2 replications. Each block was divided into two adjacent sub-blocks to accommodate both the phosphorus fertilized and unfertilized plots. The sub-blocks were separated 1.5 m apart. Whole set of genotypes were planted separately in alternating adjacent sub-blocks with and without phosphorus in side-by-side pairs. Undamaged clean seeds of each genotype selected to a reasonably uniform size by hand sorting were planted on the seedbeds.

Plot size was 1 row 4m long. One sub-block in each block received basal application of phosphorus in the form of triple supper phosphate (TSP) containing 46% $P_2O_5$ in water soluble form at the recommended rate (calculated as 20 g for a single row of 4 meters) and not to the other sub-block. The genotypes were assigned to plots at random within each block. As a source of nitrogen, all genotypes were inoculated with an effective isolate of *Rhizobium* for chickpea, CP EAL 004, as mentioned above (section 3.2.). All other crop management practices were applied uniformly to all genotypes.

### 3.3.4. Shoot and grain phosphorus analysis

Shoot and grain samples collected from five random plants at 90% physiological maturity were oven-dried to constant moisture at 70°C for 18 hours and ground to pass through 1 mm size mesh sieve.

The determination of phosphorus content was made using the wet digestion technique (AOAC, 1970) at Holetta and Debre Zeit Soil Science Research Laboratories. Phosphorus uptake and use efficiency was estimated by the balance method following Cassman *et al.* (1998) and Syers *et al.* (2008) as:

The apparent use of P from fertilizer and soil sources (APUfs %) =

$$\frac{Biomass\ uptake\ of\ P\ in\ treated\ plants\ (g/5\ plants)\ X\ 100}{P\ applied\ to\ treated\ plants\ (g/5\ plants)}$$

The apparent use of P from fertilizer (APUf %) =

$$\frac{[Biomass\ uptake\ of\ P\ in\ treated\ plants\ (g/5\ plants) - Biomass\ uptake\ of\ P\ in\ untreated\ plants\ (g/5\ plants)]\ X\ 100}{P\ applied\ to\ treated\ plants\ (g/5\ plants)}$$

The apparent use of P from soil (APUs %) = APUfs – APUf

Phosphorus yield efficiency (PYE) =

$$\frac{Grain\ yield\ of\ treated\ plants\ (g/5\ plants)}{P\ applied\ to\ treated\ plants\ (g/5\ plants)}$$

Phosphorus physiological efficiency (PPE) =

$$\frac{Grain\ yield\ in\ treated\ plants\ (g/5\ plants)}{P\ in\ treated\ plants\ (g/5\ plants)}$$

Plant phosphorus yields were obtained by multiplying their tissue phosphorus concentration by dry matter yield as follows:

Grain P yield = Grain P content × grain yield

Shoot P yield = Shoot P content × shoot yield

Biomass P yield = Grain P yield × shoot P yield

The phosphorus harvest index (PHI), i.e. the ratio of the amount of the element in the grain relative to the amount of the element in the total above-ground biomass of the plant, was estimated as:

$$PHI = \frac{\text{Grain P yield}}{\text{Biomass P yield}}$$

Relative reductions of phosphorus related and agronomic characters in phosphorus untreated plants relative to the respective phosphorus treated plants were calculated to evaluate the sensitivities of the characters to phosphorus unavailability at both locations (Pimratch *et al.*, 2008) as:

$$\text{Relative reduction} = 1 - \left(\frac{\text{performance without P}}{\text{Performance with P}}\right)$$

### 3.3.5. Data collection

Data were collected either on plot basis or from randomly selected five plants mostly based on the descriptor developed by IBPGR, ICRISAT and ICARDA (1993) on phosphorus related traits which include: (1) shoot, grain and total biomass phosphorus contents (%), (2) phosphorus acquired from soil and fertilizer, (3) phosphorus uptake and use efficiency.

Similarly, records taken on agronomic traits include: (4) days to 50% flowering and 90% physiological maturity, (5) grain filling period (the number of days from 50% flowering to 90% physiological maturity), (6) number of pods and seeds in 5 plants, (7) shoot and total biomass dry weight in 5 plants, (8) harvest index, (9) grain production efficiency (grain filling duration divided by duration of vegetative period and then multiplied by grain yield), (10) biomass production rate (above ground biomass weight divided by days to 90% physiological maturity and then multiplied by 100), (11) economic growth rate (grain weight divided by grain fill duration and then multiplied by 100), (12) seed size and (13) grain yield (g/5 plants).

In order to examine the role of phosphorus in symbiotic nitrogen fixation, data on attributes of symbiotic nitrogen fixation were also collected as stated above (section 3.2.).

## 3.4. Genetic characterization and evaluation for adzuki bean beetle resistance

### 3.4.1. Test environments

The experiment was conducted in Entomology Laboratories under ambient temperature and relative humidity at Holetta, Ambo and Debre Zeit

Agricultural Research Centers, Ethiopia. The temperatures and relative humidity of the test period are summarized in Figure 4.

### 3.4.2. Plant materials

Seeds of 117 Ethiopian chickpea germplasm accessions collected from the major chickpea production areas all over the country were considered for the study. The accessions represent over 10% of the chickpea germplasm held by the Ethiopian Institute of Biodiversity Conservation. Thirteen improved varieties or breeding lines from the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were included for comparison. The list of the genotypes tested in this study is given in Table 4 and detailed passport data of the local landraces is given in Table 1 (section 3.1.).

All genotypes were rejuvenated during 2008/9 under the same condition at Ginchi to minimize initial variation due to difference in seed age. Freshly harvested seeds with moisture contents adjusted to 9.0-9.5% were disinfected in a deep freeze at about -20 $^{o}$C for a month prior to the study. Seeds with no insect damage were hand-sorted and exposed to infestation from April to June, 2009. The test period coincides with the period of the year when large loss is expected because of high temperature and relative humidity.

### 3.4.3. Mass rearing of the insects

Adzuki bean beetles were obtained from Holetta Agricultural Research Center (Entomology Research Section) and mass reared in the laboratory at the same center on a bulk of chickpea seeds of one of the susceptible *Kabuli* cultivars, Shasho, under ambient room temperature and relative humidity.

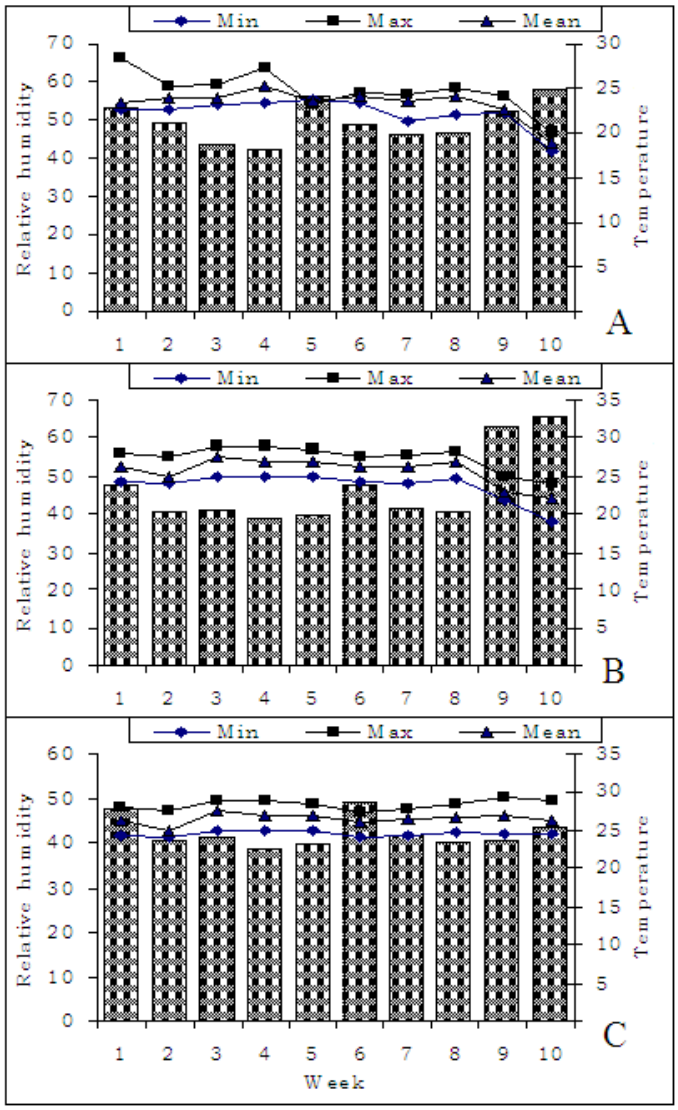



Figure 4. Relative humidity (%, bar graphs) and temperature (°C, line graphs) in laboratories at (A) Holetta, (B) Ambo and (C) Debre Zeit during the study period (for first and second progenies)

Table 4. Description of the chickpea genotypes evaluated for response to infestation by the adzuki bean beetle (note: further descriptions of the passport data and areas of geographic origins of the local landraces are given in Table 1 and Figure 1)

| Origin | No of genotypes | Name of genotypes |
|---|---|---|
| Arsi | 11 | Acc. Nos. 231327, 231328, 209093, 208829, 209094, 209096, 209097, 209098, 41002, 207761, 207763 |
| East Gojam | 10 | Acc. Nos. 41026, 41074, 41075, 41076, 41021, 41027, 207734, 41103, 41320, 41029 |
| West Gojam | 12 | Acc. Nos. 41015, 41271, 41276, 207745, 41275, 41277, 207743, 207744, 41273, 41274, 207741, 207742 |
| North Gonder | 13 | Acc. Nos. 41316, 41298, 41311, 41313, 41280, 41312, 41315, 41308, 41299, 41046, 41047, 41304, 41303 |
| South Gonder | 9 | Acc. Nos. 41295, 41296, 41289, 41290, 41291, 41297, 41293, 41048, 41053 |
| West Harerge | 8 | Acc. Nos. 41054, 41052, 209084, 209091, 209087, 209088, 209089, 209090 |
| East Shewa | 13 | Acc. Nos. 41159, 41160, 41161, 207661, 207667, 207666, 41141, 207665, 41134, 41128, 41168, 41129, 41130 |
| North Shewa | 10 | Acc. Nos. 41110, 41111, 207658, 41142, 41207, 41215, 41066, 41011, 41007, 41008 |
| West Shewa | 10 | Acc. Nos. 209035, 41176, 41175, 41174, 41170, 41171, 41185, 209036, 41190, 41195 |
| Tigray | 9 | Acc. Nos. 207151, 207563, 207564, 207895, 219797, 219799, 219800, 219803, 221696 |
| South Wello | 12 | Acc. Nos. 41114, 212589, 41113, 207659, 207660, 225878, 225873, 225874, 225877, 207645, 207646, 225876 |
| Improved genotypes (ICRISAT, ICARDA) | 13 | ICC 5003, ICC 4918, ICC 4948, ICC 4973, ICC 15996, Shasho (ICCV 93512), Arerti (FLIP 89-84C), Worku (DZ-10-16-2), Akaki (DZ-10-9-2), Ejere (FLIP-97-263c), Teji (FLIP-97-266c), Habru (FLIP 88-42c), Natoli (ICCX-910112-6) |

### 3.4.4. Infestation and experimental layout

About two hundred seeds of each genotype were put in a 250 ml (6 cm x 7 cm) plastic jar with a perforated lid for free air circulation. Fourteen 1-2 day old unsexed adults were randomly selected and placed in each jar. The male to female ratio in this insect being nearly 1:1 (Lemma, 1990), it was assumed that each jar received 7 males and 7 females. The ovipositing adults were kept in the jars for 10 days after introduction and then were removed from the jars. Records on the first progeny were taken until complete adult emergence. The first progeny was removed from the jars in the same way as the initial parents for further evaluation of the level of attack and loss incurred by the second progeny. The experiment was conducted in a randomized complete block design with 3 replications. The chickpea genotypes were assigned to jars at random within each block.

### 3.4.5. Data collection

Data were collected on: (1) number of eggs female$^{-1}$ (first progeny), (2) proportion of un-infested seeds (%) (first progeny), (3) days to adult emergence (first, second and total progenies), (4) number of adults emerged (first, second and total progenies), (5) adult recovery (%) (first progeny) and (6) seed weight loss (%) (first, second and total progenies). Days to adult emergence were recorded only at Holetta and Ambo. (7) Thousand seed weight (g) and (8) seed coat weight as percent of total seed weight of the same genotypes grown under the same conditions was also taken from replicated field trials at Ginchi and Ambo (9) Weight loss adjusted to 10% moisture was calculated for each genotype at the end of the experiment by separating healthy seeds (without holes) from each jar as suggested by Shaheen *et al*. (2006):

Weight loss = Initial weight – (Weights of healthy + damaged seeds).

### 3.4.6. Data analyses (for experiments in sections 3.2-3.4)

#### 3.4.6.1. Data preparation

The SAS computer package (SAS Institute, 1996) was used to test for presence of outliers and normality of residuals in all the cases. Data based on insect count and percentage values were log and ARCSINE transformed, respectively, for statistical analysis when necessary (Little and Hills, 1978; Gomez and Gomez, 1984) and untransformed means were presented otherwise. Data based on nodule (number, weight and nodulation index) were also log transformed to offset heterogeneity (Little and Hills, 1978; Gomez and Gomez, 1984) for statistical analysis (Doughton *et al.*, 1995). For combined analysis of variance, the homogeneity of error variance was tested using the F-max method of Hartley (1950), which is based on the ratio of the larger mean square of error (MSE) from the separate analysis of variance to the smaller mean square of error as:

$$\text{F} - \text{ratio} = \frac{\text{Larger MSE}}{\text{Smaller MSE}}$$

If the larger error mean square is not three-fold larger than the smaller error mean square, the error variance was considered homogeneous (Gomez and Gomez, 1984).

For cluster and distance analyses, records on all traits were pre-standardized to means of zero and variances of unity before clustering to avoid bias due to differences in measurement scales (Manly, 1986).

#### 3.4.6.2. Analysis of variance

For all experiments, pooled analysis of variance over location was conducted to quantify the total variation among the genotypes using the following model:

$P_{ijk} = \mu + (b/l)_{ik} + g_j + l_k + (gl)_{jk} + e_{ijk}$

Where $P_{ijk}$ = phenotypic observation on genotypes j in block i (at location) k (i = 1…B, j = 1…G, and k = 1…L) and G, L and B = number of genotypes, location and block, respectively, $\mu$ = grand mean, $(b/l)_{ik}$ = the effect of block i

(within location k), $g_j$ = the effect of genotype j, $l_k$ = the effect of location k, $(gl)_{jk}$ = the interaction effect between genotype j and location k and $e_{ijk}$ = the residual or effects of random error.

For the experiment on evaluation for resistance to adzuki bean beetle (Section 3.4), some means were adjusted for initial seed weight as a covariate whenever a significant covariance value was observed between initial seed weight and a given trait.

For the experiment on evaluation for phosphorus uptake and use efficiency, pooled analysis of variance over phosphorus level and location was conducted using the following model:

$$P_{ijkm} = \mu + (b/l)_{ik} + g_j + l_k + p_m + (gl)_{jk} + (gp)_{jm} + (pl)_{km} + (gpl)_{jkm} + e_{ijk}$$

Where $P_{ijkm}$ = phenotypic observation on genotype j in block i (at location k and phosphorus level m) (i = 1…B, j = 1…G, k = 1…L and p = 1…P) and G, L, B and P = number of genotypes, location, block and phosphorus level, respectively, $\mu$ = grand mean, $(b/l)_{ik}$ = the effect of block i (within location k), $g_j$ = the effect of genotype j, $l_k$ = the effect of location k, $p_m$ = the effect of phosphorus level m, $(gl)_{jk}$ = the interaction effect between genotype j and location k, $(gp)_{jm}$ = the interaction effect between genotype j and phosphorus level m, $(pl)_{km}$ = the interaction effect between phosphorus level m and location k, $(gpl)_{jkm}$ = the interaction effect between genotype j, phosphorus level m and location k, and $e_{ijkm}$ = the residual or effects of random error. Existence of significant difference among the genotypes, locations, phosphorus level and their interaction were determined using the F-test in all the cases.

#### 3.4.6.3. Mean separation

For the studies of symbiotic nitrogen fixation and phosphorus uptake and use efficiency (Sections 3.2 and 3.3), mean separation at 1% or 5% probability levels was done using Dunkan's Multiple Range Test (DMRT) following Gomez and Gomez (1984). However, for the evaluation of resistance to adzuki bean beetle (Section 3.4), mean separation was done using Tukey's honestly significant difference test as suggested by Sokal and

Rohlf (1997).

#### 3.4.6.4. Classification of the genotypes into phosphorus use efficiency groups

Two criteria were used to categorize the genotypes into phosphorus efficiency groups: biomass and grain yields under phosphorus fertilized and unfertilized conditions. The genotypes were categorized into four phosphorus efficiency groups using the method initially suggested by Gerloff (1977) and later applied by many others (e.g. Ortiz-Monasterio *et al.*, 2001; Gunes *et al.*, 2006). The categories include: (i) inefficient, non-responder; (ii) efficient, non- responder; (iii) inefficient, responder; and (iv) efficient, responder. This method assumes phosphorus responsiveness as the capacity to produce more yield as the result of more phosphorus uptake when the supply of the latter is increased (Ahmad *et al.*, 2001). An efficient cultivar has higher mean performance than the other cultivars under low nutrient supply, while a responder cultivar has higher mean performance under high nutrient supply. The genotypes were scattered using performances in the absence and presence of phosphorus fertilizer. Then, in order to categorize the genotypes into efficient, non-efficient and responder, non-responder groups, the mean performances in the absence and presence of phosphorus fertilizer were used as the cutting points (Gunes *et al.*, 2006).

#### 3.4.6.5. Impact of past breeding on bruchid resistance

The impact of past breeding efforts on pattern of response to bruchid infestation by the released varieties was examined by their disaggregated evaluation. Genetic progresses from breeding for grain yield, seed size, proportion of seed coat weight and the associated increase in susceptibility to infestation by adzuki bean beetle were estimated by regression analysis. Linear regression models were fitted and the mean performance of accessions/genotypes at all locations was regressed on years of release starting from 1994 to 2007 (Cox *et al.,* 1988) as:

Y = bx + a; where Y = the mean value of the dependent variable, x = the mean value of the independent variable, a = the constant value and b = the regression coefficient.

The coefficients of regression of varietal means on the years of release were used as estimates of the annual genetic progress of the traits; calculated as:

Annual rate of breeding gain (*b*) = Cov xy/var x; where x = the year of variety release, y = the mean value of each character for each variety, *Cov*= Covariance and *Var* = Variance. The variety *Worku* was considered as an oldest reference base for the estimation of the temporal changes as a result of genetic progresses.

#### 3.4.6.6. Cluster analysis and distance analyses

Clustering of genotypes was performed by average linkage method and principal component analysis using the SAS software (SAS Institute, 1996). Points where local peaks of the pseudo F statistic join with small values of the pseudo $t^2$ statistic followed by a larger pseudo $t^2$ for the next cluster fusion were examined to decide the number of clusters.

Genetic distances between clusters as standardized Mahalanobis's $D^2$ statistics were calculated as:

$D^2_{ij} = (xi - xj)' \, cov^{-1}(xi - xj)$

Where, $D^2ij$ = the distance between cases i and j; xi and xj = vectors of the values of the variables for cases i and j; and $cov^{-1}$ = the pooled within groups variance-covariance matrix.

The $D^2$ values obtained for pairs of clusters were considered as the calculated values of Chi-square ($X^2$) and were tested for significance both at 1% and 5% probability levels against the tabulated value of $X^2$ for 'P' degree of freedom, where P is the number of characters considered (Singh and Chaudhary 1985).

The dendrogram was built based on the average linkage method for phosphorus uptake and use efficiency (section 3.3) and the Ward's agglomerative hierarchical classification with Euclidian distance (Ward 1963) as a measure of dissimilarity for symbio-agronomic and resistance to adzuki bean beetle and, subsequently, the results were complemented with principal component analysis using the MINITAB 14 statistical package.

#### 3.4.6.7. Interrelationships between characters

Correlation coefficients between characters in all experiments (sections 3.2-3.4) were estimated based on the standard procedure as:

$r = Cov_{xy} / sqrt [\sigma_x^2 + \sigma_y^2]$

Where $Cov_{(xy)}$= co-variance of traits x and y, $\sigma_x^2$ = variance of x and $\sigma_y^2$ = variance of y.

The level of significance of correlation coefficients was determined from r table (correlation table) at appropriate degrees of freedom and probability level (Gomez and Gomez, 1984).

#### 3.4.6.8. Broad-sense heritability and gains from selection

Partitioning of the total variance into components due to genotype ($\sigma_g^2$), environment ($\sigma_e^2$) and genotype by location interaction ($\sigma_{gl}^2$) variances was performed from the analyses of variance by assuming various observed mean squares equal to their expected mean squares (Table 5) as suggested by Singh and Chaudhary (1985).

$\sigma_g^2 = [(\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2) - (\sigma_e^2 + R\sigma_{gl}^2)]/RL = (MS3-MS4)/RL$

$\sigma_e^2 = MS5$

$\sigma_{gl}^2 = [(\sigma_e^2 + R\sigma_{gl}^2) - (\sigma_e^2)]/R = (MS4-MS5)/R$

Where $\sigma_g^2$ = genotype variance, $\sigma_e^2$ = environmental variance and $\sigma_{gl}^2$ = genotype by location interaction variances.

Broad-sense heritability ($h_b^2$) was calculated as:

$$h_b^2 = \sigma_g^2 / [\sigma_g^2+\sigma_{gl}^2/L +\sigma_e^2/RL] \times 100$$

Where the components of the equation are as described above.

The predicted response to selection or the expected genetic advance (GA) was calculated, assuming the selection intensity of 5%, as:

$$GA = K. \sqrt{\sigma_P^2}. (\sigma_g^2/\sigma_p^2) = K. \sigma_P. h_b^2$$

$$\text{GA as \% of mean} = \frac{GA}{\bar{X}} \times 100$$

Where, GA = expected genetic advance from selection and K = the selection differential (K = 2.06 at 5% selection intensity), $\sigma_P$ = phenotypic standard deviation and $h_b^2$ = broad-sense heritability (Singh and Chaudhary, 1985).

Table 5. Combined analysis of variance (ANOVA) and calculation of components of variation for quantifying differences among genotypes and genetic variation and selection gains for symbio-agronomic characters and response to infestation by adzuki bean beetle in chickpea

| Source of variation | Degree of Freedom | Mean Square (MS) | Expected Mean Square (EMS) * |
|---|---|---|---|
| Locations | L –1 | MS1 | $\sigma_e^2 + G\sigma_r^2 + GRL\sigma_l^2$ |
| Replications/location | L(R -1) | MS2 | $\sigma_e^2 + G\sigma_r^2$ |
| Genotypes | G –1 | MS3 | $\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2$ |
| Genotype x Location | (G –1)( L -1) | MS4 | $\sigma_e^2 + R\sigma_{gl}^2$ |
| Error | L (G -1)(R –1) | MS5 | $\sigma_e^2$ |

*The explanation of abbreviations used is given above (sub-section 3.4.6.8)

### 3.4.6.9. Comparison of selected genotypes with the original population

To compare selected subsets of the 5% best genotypes within the whole population, they were sorted and means were independently computed for each character. The absolute value of Student's Z test was calculated to compare genotypic values of the 5% best selected genotypes with the base population as:

$$Z = \frac{\overline{X} - \mu}{\sigma/\sqrt{n}}$$

Where $\overline{X}$ is mean of selected genotypes, $\mu$ is mean of the base populations, $\sigma$ is the standard deviation calculated for the base populations and n is the number of genotypes selected from the base population for better performance.

The significance of the difference between the population parameter ($\mu$) and sample mean ($\overline{X}$) was tested using Z table, i.e. when the calculated value of Z-test was more than the tabulated Z value, the difference was considered significant (Singh, 2001).

# 4. RESULTS AND DISCUSSION

## 4.1. Genetic characterization of the genotypes for microsatellite markers

### 4.1.1. Magnitude of genetic diversity

Molecular analysis of variance (AMOVA) showed a 73% and 27% variation within and among populations, respectively (Table 6). Saeed *et al.* (2011) evaluated diversified populations of chickpea involving cultigens, landraces, internationally developed improved lines and wild relatives and found relatively lower within population variance of 59% and higher among population variance of 41% as compared to the present results.

The relatively higher among population variance they obtained could be attributed to the presence of wild relatives included in their study. Some reports indicate that the level of polymorphism depends on the type of germplasm (He *et al.* 2011), marker used (Baraket *et al.* 2011; Sharma *et al.* 2011), primers selected (Kong *et al.* 2011; Sharma *et al.* 2011) and the sampling strategy (Kong *et al.* 2011).

Table 6. Analysis of Molecular Variance (AMOVA)* showing the distribution of genetic diversity within and among populations of chickpea entries from different sources of origins

| Source of variation | df | SS | MS | Variance | | Statistic | Value | P |
|---|---|---|---|---|---|---|---|---|
| | | | | Estimated | % | | | |
| Among populations (AP) | 11 | 602.36 | 54.76 | 3.51 | 27 | | | |
| Within population (WP) | 143 | 1361.57 | 9.521 | 9.52 | 73 | PhiPT | 0.27 | 0.01 |
| Total (TOT) | 154 | 1963.92 | --- | 13.03 | 100 | | | |

* df stands for degrees of freedom; SS for sum of square and MS for mean of squares

The degree of polymorphism among the populations varied from 36.04% for the collections from East Gojam to 70.27% for the improved genotypes, with the average of 49.77%. Materials from all parts of Shewa, West Harerge, West Gojam and South Gonder showed more polymorphism (50-57%) than those from the rest of the country (36-45%) (Table 7).

Visual observations on the gels of the specific markers also revealed the existence of more polymorphism in the introduced genotypes than in the landraces (Figure 5). Chickpea, being a diploid (van der Maesen, 1987) and strictly self-pollinated plant (Muehlbauer and Tullu, 1997), the existence of accessions with more than two bands imply that such accessions constituted of different individuals (Figure 5). It appears that the level of polymorphism among the local collections increased as one goes from the southwest (Arsi) and north (Tigray and Wello) towards the center (Shewa), and northwestern (Gojam and Gonder) parts of the country (Figure 1).

Table 7. Summary of parameters for genetic diversity in chickpea populations from different sources

| Geographical region of origin | No of entries | Expected heterozygosity (He) | Unbiased expected heterozygosity (UHe) | Shannon's information index (I) | % P |
|---|---|---|---|---|---|
| Arsi | 13 | 0.118±0.017 | 0.122±0.018 | 0.179±0.025 | 38.74 |
| E. Gojam | 13 | 0.086±0.014 | 0.089±0.015 | 0.137±0.021 | 36.04 |
| W. Gojam | 13 | 0.147±0.018 | 0.153±0.018 | 0.227±0.025 | 52.25 |
| N. Gonder | 13 | 0.129±0.017 | 0.134±0.018 | 0.199±0.024 | 45.05 |
| S. Gonder | 12 | 0.163±0.018 | 0.170±0.019 | 0.247±0.026 | 50.45 |
| W. Harerge | 11 | 0.171±0.018 | 0.179±0.019 | 0.263±0.025 | 56.76 |
| E. Shewa | 13 | 0.157±0.017 | 0.163±0.018 | 0.244±0.025 | 56.76 |
| N. Shewa | 13 | 0.166±0.018 | 0.172±0.019 | 0.253±0.026 | 53.15 |
| W. Shewa | 13 | 0.150±0.017 | 0.156±0.018 | 0.231±0.025 | 52.25 |
| Tigray | 12 | 0.117±0.016 | 0.122±0.017 | 0.183±0.023 | 43.24 |
| S. Wello | 13 | 0.113±0.016 | 0.118±0.017 | 0.177±0.023 | 42.34 |
| Improved | 16 | 0.226±0.018 | 0.233±0.018 | 0.345±0.025 | 70.27 |
| Mean ± SE | 13 | 0.145±0.005 | 0.151±0.005 | 0.224±0.007 | 49.77±2.71 |

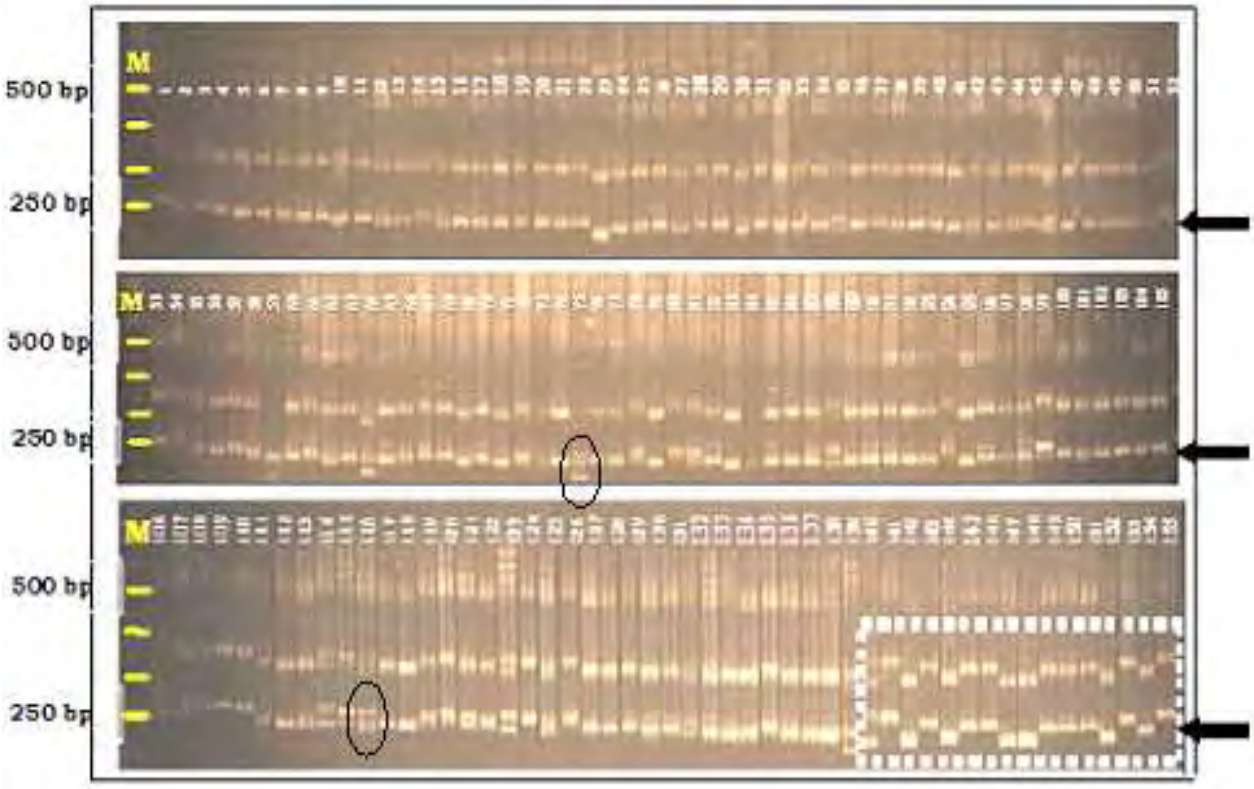



Figure 5. Electrophoretic bands of chickpea DNA of 155 genotypes as revealed by a polymorphic SSR marker, TA 144. The lane numbers identify serial No of genotypes as designated in Table 1 above, the broken rectangle at the right angle bottom shows improved genotypes, M stands for DNA ladder GeneRuler ™ 50bp and arrows indicate polymorphic bands. Some accessions (eg. circled) revealed more than 2 bands.

Based on the magnitude of the genetic distance (GD), more differentiations were revealed between the different populations from different geographical regions of Ethiopia and improved genotypes from ICARDA and ICRISAT (GD range = 0.077-0.138, $\overline{X}$=0.107). The second largest inter-regional distance range was observed between accessions from Arsi and those from the rest of the sources (GD= 0.081-0.134, $\overline{X}$=0.106). The highest values of GD (0.138 and 0.134) were recorded between accessions from East Gojam and the improved genotypes and those from Arsi and South Wello in that order. The smallest genetic distance (GD=0.016) was observed between accessions from West Gojam and North Gonder (Table 8).

Likewise, germplasm accessions from Arsi exhibited higher pairwise population PhiPT values with all groups of collections from other regions (Table 9), indicating the existence of more distinct differences between the two.

Positive and highly significant interrelationship (r = 0.845, P≤0.01) was observed between the pairwise GD and PhiPT coefficients (Figure 6). This indicated that higher distances between populations of different geographical origins entail higher proportional magnitudes of variation among the populations relative to total variation.

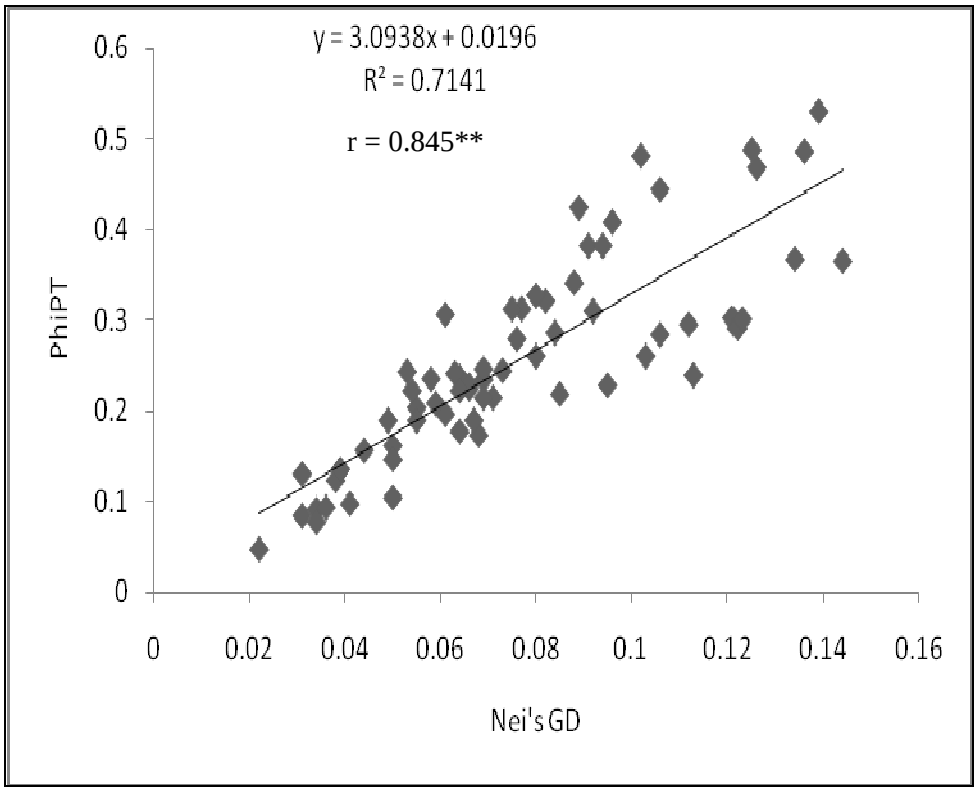



Figure 6. Interelationship of Nei's genetic distance and PhiPT showing a strong association between the proportional magnitudes of variation among population relative to total variation with genetic distance in chickpea populations from different sources

Table 8. Pairwise population Nei's genetic distance showing the magnitude of genetic differentiation between chickpea populations from different sources

| Geographical region of origin | Arsi | E. Gojam | W. Gojam | N. Gonder | S. Gonder | W. Harerge | E. Shewa | N. Shewa | W. Shewa | Tigray | S. Wello | Improved |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Arsi | 0.000 | | | | | | | | | | | |
| E. Gojam | 0.102 | 0.000 | | | | | | | | | | |
| W. Gojam | 0.106 | 0.039 | 0.000 | | | | | | | | | |
| N. Gonder | 0.089 | 0.031 | 0.022 | 0.000 | | | | | | | | |
| S. Gonder | 0.091 | 0.053 | 0.050 | 0.034 | 0.000 | | | | | | | |
| W. Harerge | 0.088 | 0.049 | 0.041 | 0.031 | 0.036 | 0.000 | | | | | | |
| E. Shewa | 0.136 | 0.082 | 0.055 | 0.069 | 0.071 | 0.050 | 0.000 | | | | | |
| N. Shewa | 0.094 | 0.064 | 0.038 | 0.039 | 0.050 | 0.034 | 0.061 | 0.000 | | | | |
| W. Shewa | 0.126 | 0.077 | 0.055 | 0.054 | 0.073 | 0.068 | 0.080 | 0.064 | 0.000 | | | |
| Tigray | 0.125 | 0.061 | 0.063 | 0.058 | 0.066 | 0.067 | 0.084 | 0.069 | 0.044 | 0.000 | | |
| S. Wello | 0.139 | 0.096 | 0.075 | 0.080 | 0.092 | 0.066 | 0.064 | 0.059 | 0.076 | 0.069 | 0.000 | |
| Improved | 0.134 | 0.144 | 0.106 | 0.112 | 0.103 | 0.113 | 0.121 | 0.085 | 0.095 | 0.122 | 0.123 | 0.000 |

Table 9. Pairwise population PhiPT values (below diagonal) and probability level based on 99 permutation (above diagonal) showing the proportional magnitudes of variation among population relative to total variation in chickpea populations from different sources

| Geographical region of origin | Arsi | E. Gojam | W. Gojam | N. Gonder | S. Gonder | W. Harerge | E. Shewa | N. Shewa | W. Shewa | Tigray | S. Wello | Improved |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Arsi | | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| E. Gojam | 0.481 | | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| W. Gojam | 0.444 | 0.135 | | * | ** | ** | ** | ** | ** | ** | ** | ** |
| N. Gonder | 0.424 | 0.130 | 0.047 | | ** | ** | ** | ** | ** | ** | ** | ** |
| S. Gonder | 0.382 | 0.243 | 0.161 | 0.091 | | ** | ** | ** | ** | ** | ** | ** |
| W. Harerge | 0.341 | 0.189 | 0.097 | 0.084 | 0.093 | | ** | * | ** | ** | ** | ** |
| E. Shewa | 0.486 | 0.321 | 0.203 | 0.234 | 0.214 | 0.104 | | ** | ** | ** | ** | ** |
| N. Shewa | 0.382 | 0.237 | 0.123 | 0.135 | 0.146 | 0.077 | 0.196 | | ** | ** | ** | ** |
| W. Shewa | 0.468 | 0.312 | 0.189 | 0.222 | 0.244 | 0.173 | 0.260 | 0.177 | | ** | ** | ** |
| Tigray | 0.488 | 0.306 | 0.241 | 0.235 | 0.224 | 0.189 | 0.286 | 0.214 | 0.156 | | ** | ** |
| S. Wello | 0.530 | 0.408 | 0.312 | 0.327 | 0.310 | 0.228 | 0.222 | 0.208 | 0.279 | 0.246 | | ** |
| Improved | 0.367 | 0.365 | 0.284 | 0.295 | 0.260 | 0.239 | 0.302 | 0.218 | 0.228 | 0.291 | 0.301 | |

*P $\leq$ 0.050 and **P $\leq$ 0.010

Other genetic parameters including expected heterozygosity (He), unbiased expected heterozygosity (UHe) and Shannon's information index (I) also showed the existence of high genetic variation within the improved genotypes (Table 7).

Differences among the original introductions, the nature and degree of both human and natural selection after introduction and/or specificities of ecological and agricultural conditions as major forces of evolution are normally expected to give rise to a distinct form of genetic diversity (Ford-Lloyd and Jackson, 1986; Spagnoletti and Qualset, 1987). The differences observed in landrace populations studied could also result at least partly from combined effects of drift, mutation, migration and selection (Felsenstein, 2007).

The highest genetic variability observed in the introduced genotypes as compared to landraces could also be explained by the broader geographic spectrum from where they were initially acquired and subsequent genetic recombination undertaken by ICARDA and ICRISAT before their introduction to Ethiopia. It was demonstrated, even with these limited numbers of samples (16 genotypes), that introduction of genetic materials from ICARDA and ICRISAT has practically broadened the genetic base of the national breeding programs.

The 33 microsatellite markers used to study genetic diversity revealed a total of 111 bands with a range of 2-5 or on average 3.364 bands marker$^{-1}$ among the studied 155 entries of chickpea. High PIC values of the markers ranging from 0.278-0.500 ($\overline{X}$ = 0.412) were obtained (Figure 7), indicating the usefulness of most of these markers in chickpea germplasm characterization and evaluation in the future (Saxena and Chandra, 2010; Sharma *et al.*, 2011).

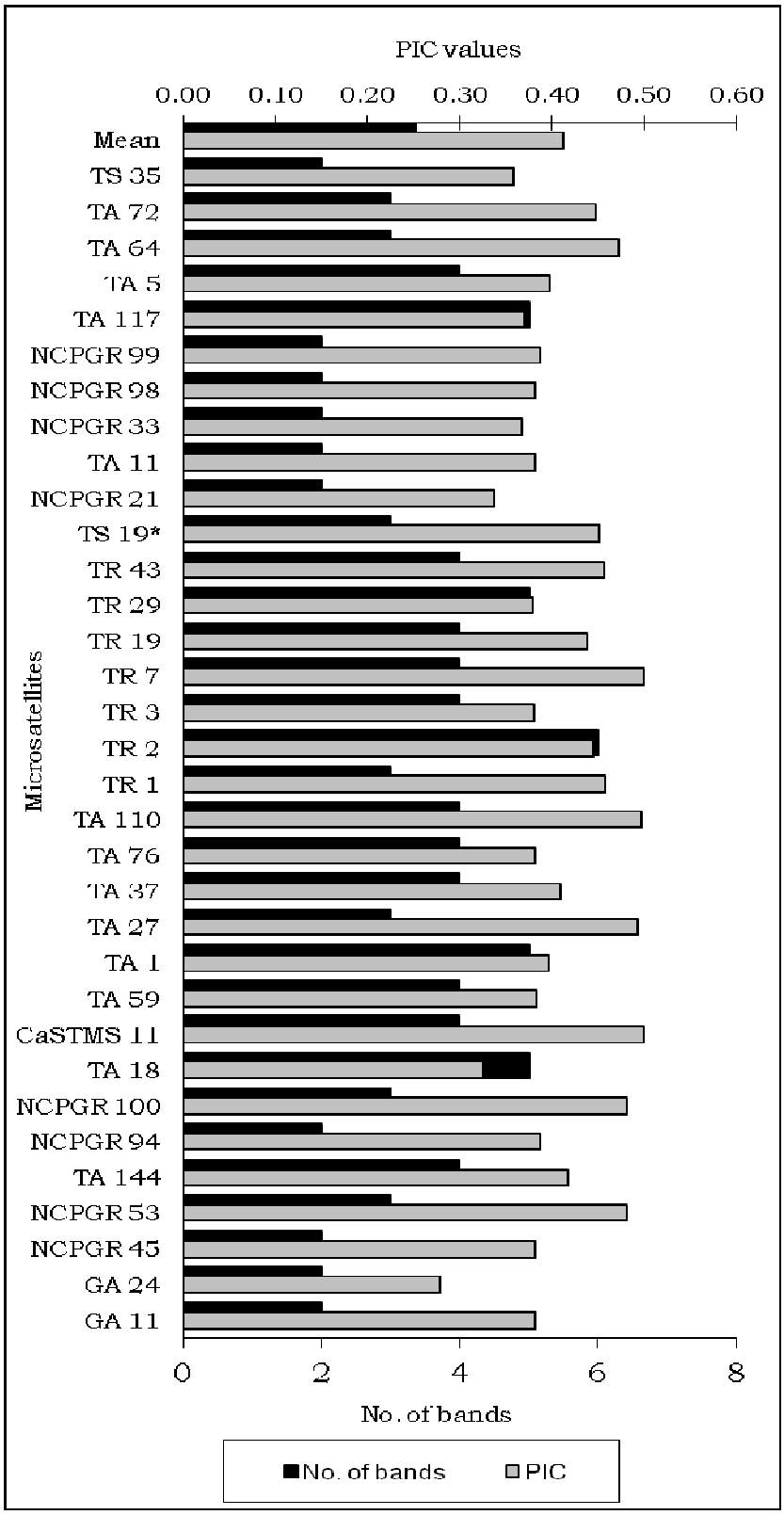



Figure 7. Comparison of No. of bands amplified and PIC values among 33 polymorphic microsatellite markers evaluated across 155 chickpea genotypes

With cluster analysis, it was possible to classify the 155 entries from 12 different sources of origins (considered as 12 initial groups) into five distinct clusters whereby the different members within a cluster are assumed to be more closely related with each other than with those members in different clusters. Populations from Arsi were generally assigned to cluster $C_1$.

The distinctness of Arsi may be related to the existence of the Great Rift Valley as a buffer zone of approximately over 100 km in all directions between the other regions. The ecological suitability of Arsi might also resulted in higher genetic diversity as environments that are more suitable may enhance species evolution (Singh, 2002). Arsi could also be considered as a relatively self-contained region where the local population is sufficient to satisfy its limited domestic needs for seed and grain in terms of chickpea. A study on biochemical diversity of barley also showed a distinct pattern of genetic diversity in landrace collections from Arsi as compared to those from Wello and Gojam (Demissie and Bjφrnstad, 1997).

### 4.1.2. Pattern of genetic diversity

The populations from the different sources were grouped into 5 clusters of distinct genetic populations (Figure 8) showing that they had evolved from different lines of ancestry or derived from independent events of evolutionary forces (genetic drift, mutation, migration, selection and in flux/out flux of genes in the form of germplasm exchange) that separated them into related but different gene pools. The clustering pattern showed the existence of definite pattern of relationships between geographical origins and genetic diversity for microsatellite markers. High levels of intra-regional similarities were observed within each origin or, in other cases, between adjoining geographical origins. Populations from the same geographical origin were observed to characteristically fall exclusively in a single or two clusters.

Some clusters constituted populations mostly from the same geographical origin while others had populations from more than one sources and, hence, the number of entries varied from cluster to cluster. The cross-border similarities between a few adjoining regions may be attributed, at

least in part, to seed movements among neighboring regions. He *et al.* (2011) also observed definite relationship between sources of geographic origin and genetic diversity in *Eriobotrya japonica*.

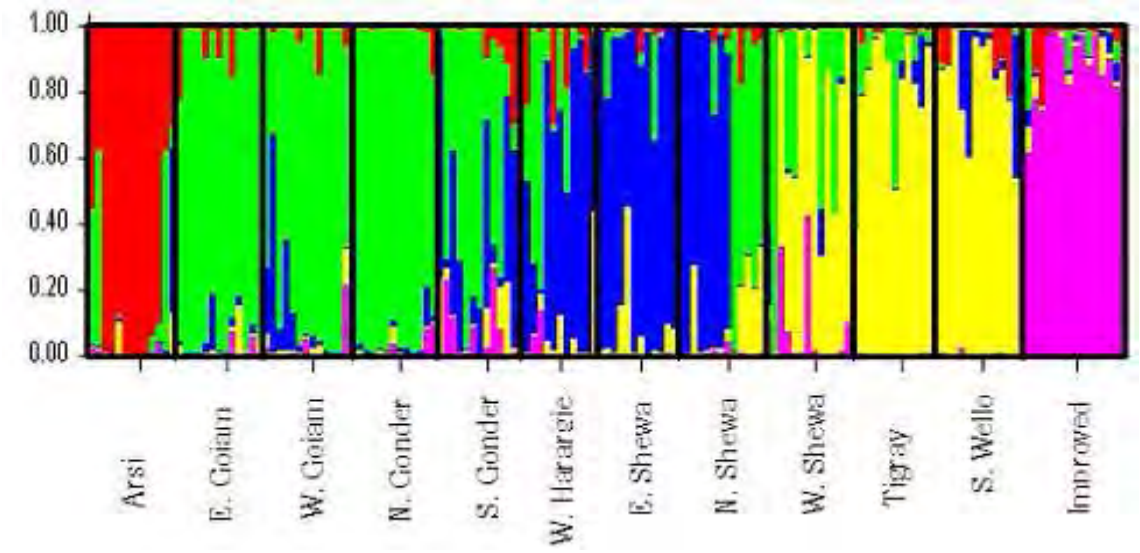



Figure 8. Structure bar-plot of the tested chickpea entries from different origins showing the pattern of assignment of the entries from the 12 sources of origins into 5 clusters

The first cluster, $C_1$, constituted accessions mainly from Arsi and, the last cluster, $C_5$, constituted almost entirely improved genotypes regardless of being *Kabuli* and *Desi* types. Likewise, the only *Kabuli* type landrace (Acc. No. 41197) collected from West Shewa Zone was grouped in the same cluster with the *Desi* types collected from the same origin rather than with the *Kabuli* types in cluster $C_5$. This indicated that genotypes from different seed types might have similar genetic background for microsatellite markers provided that they are exposed to similar events of domestication, both natural and artificial selection. The rest of the clusters (i.e. $C_2$-$C_4$) comprised accessions from two or more geographical origins grouped together showing closer genetic relationships.

The second cluster, $C_2$, constituted accessions mostly from Gojam and Gonder; the third cluster, $C_3$, comprised those from Harerge and East and North Shewa while the fourth cluster, $C_4$, consisted of accessions from West Shewa, Tigray and South Wello (Table 10). It can be discerned from Figure 8 that accessions from North Shewa share a significant portion of their ancestral gene pool with accessions from the adjoining regions of East Shewa on one side and those from Gojam (and Gonder via Gojam) on the other.

Table 10. Estimated probabilistic memberships of chickpea populations from different geographic origins to the 5 clusters

| Geographical region of origin | No of entries | Cluster | | | | |
|---|---|---|---|---|---|---|
| | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ |
| Arsi | 13 | 0.790 | 0.139 | 0.043 | 0.02 | 0.009 |
| E. Gojam | 13 | 0.048 | 0.891 | 0.025 | 0.022 | 0.014 |
| W. Gojam | 13 | 0.023 | 0.817 | 0.115 | 0.019 | 0.027 |
| N. Gonder | 13 | 0.017 | 0.901 | 0.049 | 0.014 | 0.019 |
| S. Gonder | 12 | 0.054 | 0.510 | 0.299 | 0.067 | 0.070 |
| W. Harerge | 11 | 0.101 | 0.228 | 0.611 | 0.038 | 0.022 |
| E. Shewa | 13 | 0.014 | 0.053 | 0.826 | 0.103 | 0.004 |
| N. Shewa | 13 | 0.030 | 0.304 | 0.549 | 0.107 | 0.011 |
| W. Shewa | 13 | 0.005 | 0.304 | 0.036 | 0.569 | 0.086 |
| Tigray | 12 | 0.013 | 0.145 | 0.033 | 0.804 | 0.005 |
| S. Wello | 13 | 0.053 | 0.009 | 0.095 | 0.837 | 0.006 |
| Improved | 16 | 0.043 | 0.039 | 0.020 | 0.031 | 0.866 |

Conversely, accessions from Tigray and Wello, which are frequently experiencing severe drought, showed a significant level of genetic similarity with accessions from the geographically non-adjoining West Shewa. The latter is in fact among the most important producers of chickpea. East Harerge, another drought-prone region, also shared similar ancestral gene pool with the adjoining East Shewa. The probable reason for these

impressive genetic similarities between accessions from Tigray and Wello with those from West Shewa, and those from West Harerge with those from East Shewa and implicitly North Shewa (via East Shewa) could be related to massive seed movements associated with response to recurrent drought.

According to McGuire and Sperling (2008), periodic provision of a huge amount of seeds of many crops including chickpea (as a suitable crop even after the failure of the long-season crop) has a long history in drought-prone areas in Ethiopia. The native accessions in these areas may become genetically eroded and significantly replaced with seeds purchased from other regions.

Traces of new "bloods" of improved genotypes within the local accessions almost from all over the country (Figure 8) including in the drought-prone areas like Tigray, Harerge and Wello, may not be a surprise as improved seeds have been distributed to farmers through both the formal and informal channels including through the "relief seed system" (McGuire and Sperling 2008). In Ethiopia, once improved varieties are introduced, there is no planned "generation control", i.e. replacement of the old seeds with new ones. As a result, the local accessions can harbor genes from the improved varieties through the limited natural hybridization and recombination over a long period of time of togetherness. Similarly, each region showed a few overlapping or admixtures with those from other regions.

Based on a cluster assignment probability of 0.80 (80%) as a cutting-point to assign a given population or individual to a given cluster (Garris *et al.* 2005), populations or individual members of a population were assigned fully or partially to clusters. The patterns of genetic diversity among individual accessions were also consistent with the pattern of genetic diversity in the populations at large. However, a few individual accessions slightly deviated from the general trend and were identified as admixtures. For instance, out of the thirteen accessions from Arsi, nine were characteristically grouped into a single cluster $C_1$. Three remaining accessions (Acc. Nos. 231327, 231328 and 207763) partly shared ancestral gene pools with

accessions from Gojam and Gonder in cluster $C_2$ and another accession (Acc. No. 207764) with accessions from Harerge and East and North Shewa in cluster $C_3$.

Rather than seed movement *per se*, the gene transport in this case could be related to the historical influx of people from the other parts to Arsi along with their chickpea seeds. Two accessions from Gojam (Acc. Nos. 41268 and 41222) and two from Gonder (Acc. Nos. 41303 and 41293) shared limited ancestry with accessions from Arsi. Similarly, a number of accessions from Gojam (Acc. Nos. 41015, 41271 and 41276) and Gonder (Acc. Nos. 41316, 41304, 41295, 41289, 41290, 41293, 41049 and 41053) showed limited genetic relations with accessions in clusters $C_2$ and $C_3$. Other examples from the rest of the sources of origins may show possibilities for close genetic interrelationships among the accessions regardless of their sources of origins (Appendix 1). The interchange of genes somehow through hybridization and interbreeding may have resulted in such sporadic cross-border genetic similarities.

## 4.2. Genetic characterization and evaluation for symbio-agronomic performance

### 4.2.1. Symbiotic performance

Depending on host genotype, the amount of fixed nitrogen varied from 13-49% ($\overline{X}$=30%) in foliage, 30-44% ($\overline{X}$= 36%) in grain and 28-40% ($\overline{X}$= 34%) in total above ground biomass. Differences among the genotypes were significant for symbiotic nitrogen fixation and a number of associated characters (Appendix 2 and Table 11).

The larger coefficients of variation (CV) values (> 20%) in many traits may be related to production on residual moisture where the crop was highly stressed or estimation of mean performances based on a few plant samples grown on small plots or both.

Table 11. Combined analysis of variance (over locations) for attributes of symbiotic and agronomic performances in 155 chickpea genotypes tested at two locations

| Character | Mean square | | | CV (%) |
|---|---|---|---|---|
| | L | G | G x L | |
| Early vigor (SDWF, g 5 plants$^{-1}$) | ** | ** | ** | 25.61 |
| Shoot dry weight ratio before flowering (SDWRF) | ** | ** | ** | 25.61 |
| Nodulation index (NI, mg g$^{-1}$) ( | ** | ** | ** | 21.65 |
| No of nodules (NN, 5 plants$^{-1}$) | ** | ** | ** | 24.38 |
| Nodule dry weight (NDW, mg 5 plants$^{-1}$) | ** | ** | ** | 14.69 |
| Shoot nitrogen content (SNC, %) | NS | ** | NS | 19.00 |
| Shoot protein content (SPC, %) | NS | ** | NS | 19.00 |
| Shoot nitrogen fixation (SNF, %) | ** | ** | NS | 19.25 |
| Grain nitrogen content (GNC, %) | NS | ** | NS | 9.53 |
| Grain protein content (GPC, %) | NS | ** | NS | 9.53 |
| Grain nitrogen fixation (GNF, %) | NS | ** | NS | 10.73 |
| Total biomass nitrogen content (BNC, %) | NS | ** | NS | 9.66 |
| Total nitrogen fixation (BNF, %) | ** | ** | NS | 9.35 |
| Fixed nitrogen assimilation efficiency (FNAE, %) | ** | ** | NS | 10.38 |
| Grain nitrogen yield (GNY, g 5 plants$^{-1}$) | NS | ** | ** | 26.70 |
| Shoot nitrogen yield (SNY, g 5 plants$^{-1}$) | ** | ** | ** | 19.55 |
| Biomass nitrogen yield (BNY, g 5 plants$^{-1}$) | ** | ** | ** | 27.13 |
| Nitrogen harvest index (NHI) | ** | ** | ** | 10.91 |
| Days to 50% flowering (DTF) | ** | ** | NS | 3.33 |
| Days to 90% maturity (DTM) | ** | ** | NS | 2.42 |
| Grain filling period (GFP) | ** | ** | NS | 5.71 |
| No of pods (NP, 5 plants$^{-1}$) | ** | ** | ** | 26.54 |
| No of seeds (NS, 5 plants$^{-1}$) | NS | ** | ** | 28.30 |
| Shoot dry weight at maturity (SDWM, g 5 plants$^{-1}$) | ** | ** | * | 29.64 |
| Shoot dry weight ratio at maturity (SDWRM) | ** | ** | * | 29.55 |
| Total biomass weight (BMW, g 5 plants$^{-1}$) | * | ** | * | 29.73 |
| Harvest index (HI) | ** | ** | ** | 15.01 |
| Grain production efficiency (GPE, g 5 plants$^{-1}$) | ** | ** | ** | 26.53 |
| Biomass production rate (BPR, %) | ** | ** | ** | 19.69 |
| Economic growth rate (EGR, %) | * | ** | ** | 25.00 |
| Thousand seed weight (TSW, g) | NS | ** | ** | 18.39 |
| Grain yield (YLD, g 5 plants$^{-1}$) | NS | ** | ** | 24.57 |

**=highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and NS = non-significant ($P > 0.05$)

The potential of fixation by the genotypes may be generally limited because of shortage in soil moisture level as the crop was grown with residual moisture. In Syria (Beck and Rupela, 1996), for instance, fixation in winter-sown chickpea reached over 80-81% while spring-sown chickpea, where moisture was a limiting factor, fixed only 8-27%.

Protein contents of the shoot, grain and biomass, being derivative characters of plant tissue nitrogen, obviously followed the same pattern of variation as nitrogen contents. Other associated characters also showed wider ranges of variation.

Comparison of the test genotypes with a recently released variety Natoli and with the overall mean performances of eight released varieties (Shasho, Arerti, Worku, Akaki, Ejere, Teji, Habru and Natoli) showed that a number of accessions had superior symbiotic performance including for the amount of nitrogen fixation in shoot, grain and total above ground biomass (Figures 9 and 10). The best 5% of the accessions for total (shoot + grain) nitrogen fixation include Acc. Nos. 41222, 41029, 41021, 41074, 41075, 41129, 41320 and 41026.

These landraces could register additional fixation ranging from 18.52-23.88% over the standard check, Natoli, and 17.44-22.75% over the mean performance of the released varieties. These accessions had also the highest above ground biomass nitrogen contents varied from 5.24-5.61%, the content by Natoli being 4.56%. As protein content follows the same trend (but different magnitude) as nitrogen contents, these accessions also contained the highest amount of protein in their biomasses (Appendix 2).

There were some other genotypes which had better fixations either in their shoots (e.g. Acc No. 41103) or grains (e.g. Acc No. 207734). Two introductions from ICRISAT, namely ICC 5003 and ICC 4973, were also among the top 5% best genotypes for amount of nitrogen fixation in the shoot. However, comparison of germplasm accessions with genetically "fixed" genotypes needs a careful interpretation. This is because genetic enhancement or increasing the frequency of desirable genes among

germplasm accessions through purification and selection is likely to produce more lines with more desirable than the parental accessions themselves, as a few of the accessions may constitute individual plants with better performance masked upon averaging.

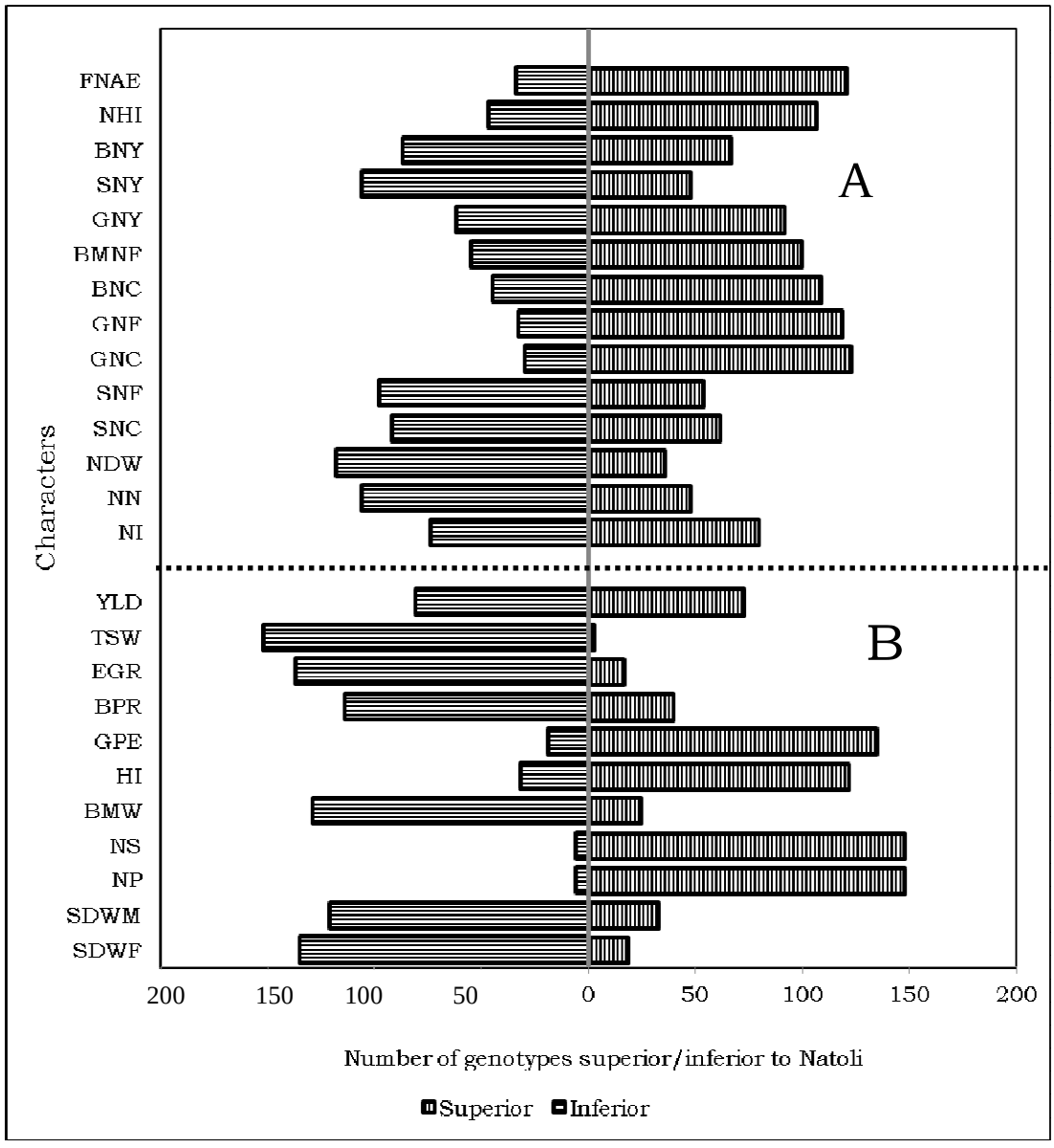



Figure 9. Proportion by number of the 155 chickpea genotypes superior and inferior to the recently released check, Natoli, for (A) symbiotic and (B) agronomic characters showing superiority of a number of landraces to the standard check (see Table 11 above for abbreviations of the characters)

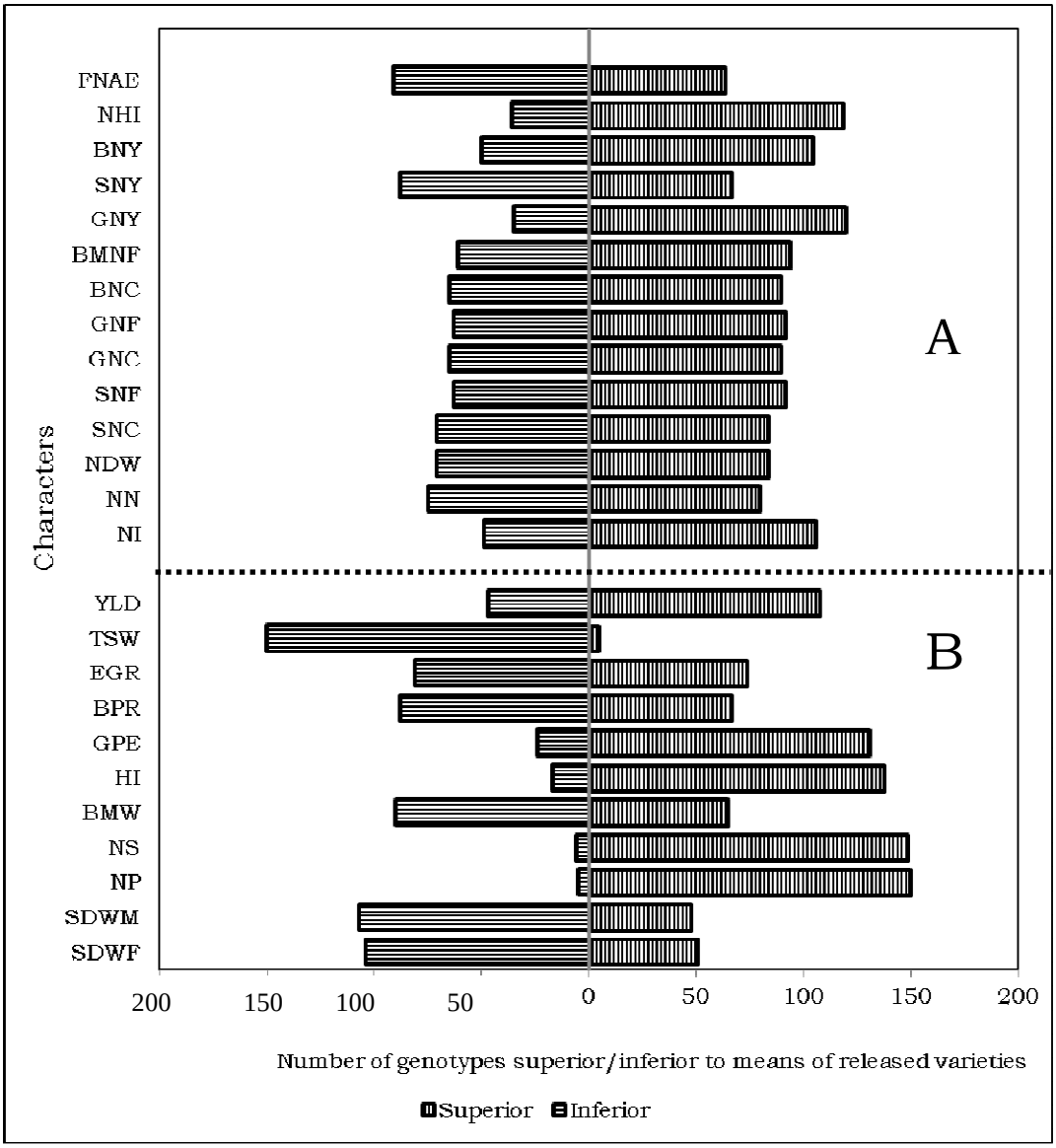



Figure 10. Proportion by number of the 155 chickpea genotypes superior and inferior to mean performances of all the released varieties for (A) symbiotic and (B) agronomic characters showing superiority of a number of landraces to the standard check (see Table 11 above for abbreviations of the characters)

The best landrace genotypes for nitrogen harvest index (NHI) were found to be superior by 13.79-18.97% to Natoli and 15.79-21.05% to the mean NHI performance of all the released varieties. This is contrary to the findings that modern cultivars usually show better nitrogen harvest index than landraces (Sinclair and Vadez, 2002). The best assimilators of fixed nitrogen were Acc. Nos. 41115, 207659, 219799, 207150, 41277, 41113 and 207894.

A genotype introduced from ICRISAT as a non-nodulating check, ICC 19180, was found to nodulate in our study and it was among the best for grain nitrogen yield and assimilation of nitrogen (Table 12). There are accessions that combined desirable attributes of both symbiotic and agronomic significance. For example, Acc. No. 41274 possessed both better attributes of symbiotic and agronomic significances (Tables 12 and 13).

Comparison of nitrogen fixation patterns between modern cultivars and landraces does not appear to follow a simple trend as there are conflicting reports. Provorov and Tikhonovich (2004) found better nitrogen fixing genotypes from among the old cultivars, landraces or wild relatives. In another study, field pea landraces introduced from Ethiopian to Germany were found to fix more nitrogen than a commercial cultivar (Adgo and Schulze, 2002). A similar result was also reported in *Phaseolus vulgaris* in Austria (de Oliveira *et al.*, 1998).

Our experience with primitive forms of *Pisum sativum* showed superiority of improved cultivars over the landraces (Keneni *et al.*, 2010). In chickpea, both nodulation efficiency and grain yield were improved as the result of selection from commercial cultivars (Ali *et al.*, 2002). Therefore, specific tests for specific genetic materials may be needed for better decision.

Table 12. Comparison of mean performances of 5% of the accessions selected for best symbiotic performance with Natoli, a recently released variety, and with mean performances of released varieties

| Acc. No. | Mean of selected accession* | Comparative advantage (% over) Natoli | MRV** | Acc. No. | Mean of selected accession | Comparative advantage (% over) Natoli | MRV |
|---|---|---|---|---|---|---|---|
| Shoot N fixation (%) | | | | Grain N fixation (%) | | | |
| 41222 | 48.67a | 53.73 | 70.59 | 41021 | 43.54a | 27.09 | 22.72 |
| 41026 | 47.29ab | 49.37 | 65.76 | 41029 | 43.41ab | 26.71 | 22.35 |
| 41074 | 46.05a-c | 45.45 | 61.41 | 41222 | 42.79a-c | 24.9 | 20.6 |
| 41103 | 45.63a-d | 44.13 | 59.94 | 41074 | 42.48a-d | 23.99 | 19.73 |
| 41075 | 45.04a-e | 42.26 | 57.87 | 41320 | 42.07a-e | 22.8 | 18.57 |
| ICC 4973 | 42.85a-f | 35.34 | 50.19 | 207734 | 42.05a-e | 22.74 | 18.52 |
| ICC 5003 | 42.80a-f | 35.19 | 50.02 | 41129 | 42.04a-e | 22.71 | 18.49 |
| 41320 | 42.11a-g | 33.01 | 47.6 | 41075 | 41.75a-f | 21.86 | 17.67 |
| Natoli | 31.66b-v | --- | 10.97 | Natoli | 34.26e-u | --- | -3.44 |
| MRV | 28.53f-x | -9.89 | --- | MRV | 35.48b-u | 3.56 | --- |
| Biomass N fixation (%) | | | | Nitrogen harvest index | | | |
| 41222 | 40.20a | 23.88 | 22.75 | 41115 | 0.69a | 18.97 | 21.05 |
| 41029 | 40.11ab | 23.61 | 22.47 | 207150 | 0.67ab | 15.52 | 17.54 |
| 41021 | 39.86a-c | 22.84 | 21.71 | 231328 | 0.66a-e | 13.79 | 15.79 |
| 41074 | 39.31a-d | 21.14 | 20.03 | 207741 | 0.66a-e | 13.79 | 15.79 |
| 41075 | 38.85a-e | 19.72 | 18.63 | 209036 | 0.66a-e | 13.79 | 15.79 |
| 41129 | 38.70a-e | 19.26 | 18.17 | 207895 | 0.66a-e | 13.79 | 15.79 |
| 41320 | 38.54a-g | 18.77 | 17.68 | 219799 | 0.66a-e | 13.79 | 15.79 |
| 41026 | 38.46a-g | 18.52 | 17.44 | 41113 | 0.66a-e | 13.79 | 15.79 |
| Natoli | 32.45e-y | --- | -0.92 | Natoli | 0.58d-z | --- | 1.75 |
| MRV | 32.75d-y | 0.92 | --- | MRV | 0.57g-z | -1.72 | --- |
| Shoot N yield (g 5 plants$^{-1}$) | | | | Grain N yield (g 5 plants$^{-1}$) | | | |
| 41275 | 1.83a | 37.59 | 45.24 | 41274 | 2.46a | 35.91 | 44.71 |
| 41103 | 1.82ab | 36.84 | 44.44 | 41111 | 2.34ab | 29.28 | 37.65 |
| 41026 | 1.76a-c | 32.33 | 39.68 | 207763 | 2.33a-c | 28.73 | 37.06 |
| 207734 | 1.71a-d | 28.57 | 35.71 | 207734 | 2.33a-c | 28.73 | 37.06 |
| 41289 | 1.70a-e | 27.82 | 34.92 | 207742 | 2.29a-d | 26.52 | 34.71 |
| 41185 | 1.69a-e | 27.07 | 34.13 | ICC 19180 | 2.28a-e | 25.97 | 34.12 |
| 41284 | 1.67a-f | 25.56 | 32.54 | 41268 | 2.23a-f | 22.65 | 30.59 |
| 41320 | 1.66a-g | 24.81 | 31.75 | 41316 | 2.22a-g | 22.65 | 30.59 |
| Natoli | 1.33a-o | --- | 5.56 | Natoli | 1.81a-p | --- | 6.47 |
| MRV | 1.26a-o | -5.26 | --- | MRV | 1.70b-q | -6.08 | --- |
| Biomass N yield (g 5 plants$^{-1}$) | | | | Fixed N assimilation efficiency (%) | | | |
| 207734 | 4.05a | 28.57 | 36.82 | ICC 19180 | 90.61a | 15.38 | 10.16 |
| 41274 | 3.87ab | 22.86 | 30.74 | 41115 | 90.15ab | 14.8 | 9.6 |
| 41275 | 3.80a-c | 20.63 | 28.38 | 207659 | 89.74a-c | 14.27 | 9.11 |
| 41185 | 3.80a-c | 20.63 | 28.38 | 219799 | 89.03a-d | 13.37 | 8.24 |
| 41111 | 3.75a-d | 19.05 | 26.69 | 207150 | 88.74a-e | 13 | 7.89 |
| 41284 | 3.70a-d | 17.46 | 25 | 41277 | 88.10a-f | 12.19 | 7.11 |
| 41103 | 3.69a-d | 17.14 | 24.66 | 41113 | 87.79a-g | 11.79 | 6.74 |
| 41289 | 3.68a-e | 16.83 | 24.32 | 207894 | 87.10a-h | 10.91 | 5.9 |
| Natoli | 3.15a-m | --- | 6.42 | Natoli | 78.53d-r | --- | -4.52 |
| MRV | 2.96a-m | -6.03 | --- | MRV | 82.25a-p | 4.74 | --- |

*Figures sharing the same letter(s) or ranges of letters with in the same column are non-significantly different; **MRV = mean of released varieties, ***significance levels of the mean performances for released varieties was approximated from equivalent values of the test genotypes for the same character

### 4.2.2. Agronomic performance

Differences among the genotypes were significant for a number of characters (Table 11). The comparison of the different genotypes with the recently released Natoli variety of chickpea showed the superior performances of a number of landraces for a number of agronomic attributes (Appendix 3). Accordingly, the top 5% best accessions for best yield include Acc. Nos. 41274, 207763, 41111, 207742, 231328, 207563, 41053 and 212589. Nevertheless, comparisons at both levels (with Natoli and average performances of all released varieties) showed that there was no landrace comparable to the improved genotypes for seed size (Figures 9), the top 5% best performing genotypes for this trait were ICC 4918, ICC 5003, ICC 19180, Natoli, Teji, Ejere, Arerti and Habru which are introductions either from ICRISAT or ICARDA.

Although there was no single genotype exhibiting consistent superiority for grain yield and other desirable characters, the landraces were among the top 5% best performers compared to the improved introduced genotypes for many attributes (Table 12). This was exemplified by Acc No. 41274 that displayed the best character in grain yield, pod and seed setting, biomass weight and production rate, grain production efficiency and economic growth rate. Another accession, Acc. No. 207563, was also among the top performers for grain yield, economic growth rate and pod setting. A number of other superior accessions for multiple traits of agronomic significance including grain yield were also identified (Table 12).

Detailed physiological reasons for the superiority of the landraces over the improved genotypes may need future study. From this observation, it was witnessed that landraces had relatively shorter period of vegetative growth and longer grain filling periods. The comparison of the top 5% best yielders for phenological characters with the 5% least yielders may also show at least in part that the genotypes tested here differed in grain yield for a similar reason (Figure 11).

Table 13. Comparison of mean performances of 5% of the accessions selected for best agronomic performance with Natoli, a recently released variety, and with mean performances of released varieties

| | Mean of selected accession* | Comparative advantage (% over) | | | Mean of selected accession | Comparative advantage (% over) | |
|---|---|---|---|---|---|---|---|
| Acc. No. | | Natoli | MRV** | Acc. No. | | Natoli | MRV |
| No. of pods (5 plants$^{-1}$) | | | | No. of seeds (5 plants$^{-1}$) | | | |
| 41289 | 515a | 94.34 | 105.18 | 41111 | 595a | 144.86 | 146.89 |
| 41274 | 497ab | 87.55 | 98.01 | 207658 | 575a | 136.63 | 138.59 |
| 41215 | 486a-c | 83.4 | 93.63 | 41185 | 566a-c | 132.92 | 134.85 |
| 41284 | 485a-c | 83.02 | 93.23 | 41274 | 556a-d | 128.81 | 130.71 |
| 209091 | 480a-d | 81.13 | 91.24 | 41215 | 556a-d | 128.81 | 130.71 |
| 41015 | 471a-e | 77.74 | 87.65 | ICC 4948 | 541a-e | 122.63 | 124.48 |
| 207563 | 466a-f | 75.85 | 85.66 | 207764 | 535a-f | 120.16 | 121.99 |
| 41114 | 464a-g | 75.09 | 84.86 | 209084 | 535a-f | 120.16 | 121.99 |
| Natoli | 265u-z | --- | 5.58 | Natoli | 243z | --- | 0.83 |
| MRV | 251w-z*** | -5.28 | --- | MRV | 241z | -0.82 | --- |
| Biomass weight (g 5 plants$^{-1}$) | | | | Harvest index | | | |
| 41284 | 224.32a | 32.55 | 45.1 | 231328 | 42.88a | 29.59 | 34.21 |
| 41274 | 204.41ab | 20.79 | 32.22 | 209093 | 42.58ab | 28.68 | 33.27 |
| 207734 | 198.76a-c | 17.45 | 28.56 | 209094 | 42.07a-c | 27.14 | 31.67 |
| 41275 | 194.41a-d | 14.88 | 25.75 | 41002 | 40.41a-d | 22.12 | 26.48 |
| Habru | 191.06a-e | 12.9 | 23.58 | 231327 | 40.27a-e | 21.7 | 26.04 |
| ICC 19180 | 188.92a-e | 11.64 | 22.2 | 207764 | 40.12a-f | 21.25 | 25.57 |
| 41185 | 183.47a-f | 8.41 | 18.67 | 207741 | 40.08a-f | 21.12 | 25.45 |
| 207563 | 182.83a-g | 8.04 | 18.26 | 41115 | 39.47a-g | 19.28 | 23.54 |
| Natoli | 169.23a-m | --- | 9.46 | Natoli | 33.09g-w | --- | 3.57 |
| MRV | 154.6b-n | -8.65 | --- | MRV | 31.95j-x | -3.45 | --- |
| Grain production efficiency (g 5 plants$^{-1}$) | | | | Biomass production rate (%) | | | |
| 207763 | 70.65a | 63.69 | 57.17 | 41284 | 198.82a | 37.65 | 47.06 |
| 41111 | 70.35ab | 63 | 56.51 | 41274 | 181.47ab | 25.64 | 34.22 |
| 41274 | 69.71a-c | 61.52 | 55.08 | ICC 19180 | 175.17a-c | 21.28 | 29.56 |
| 207742 | 69.14a-d | 60.19 | 53.82 | 207734 | 170.96a-d | 18.36 | 26.45 |
| 41053 | 68.21a-e | 58.04 | 51.75 | 41275 | 169.91a-e | 17.63 | 25.67 |
| 209093 | 67.70a-f | 56.86 | 50.61 | Habru | 169.65a-f | 17.45 | 25.48 |
| 207658 | 67.57a-f | 56.56 | 50.32 | 207743 | 162.38a-g | 12.42 | 20.1 |
| 219800 | 66.70a-g | 54.54 | 48.39 | 207563 | 161.98a-g | 12.14 | 19.81 |
| Natoli | 43.16q-z | --- | -3.98 | Natoli | 144.44b-n | --- | 6.83 |
| MRV | 44.95m-z | 4.15 | --- | MRV | 135.2b-o | -6.4 | --- |
| Economic growth rate (%) | | | | Grain yield (g 5 plants$^{-1}$) | | | |
| 41274 | 125.02a | 18.6 | 34.65 | 41274 | 70.10a | 30.76 | 42.22 |
| ICC 19180 | 118.90ab | 12.8 | 28.06 | 207763 | 66.05ab | 23.2 | 34 |
| 207763 | 113.75a-c | 7.91 | 22.51 | 41111 | 65.16a-c | 21.54 | 32.2 |
| 41268 | 112.48a-d | 6.71 | 21.14 | 207742 | 64.22a-d | 19.79 | 30.29 |
| 231328 | 111.83a-e | 6.09 | 20.44 | 231328 | 63.62a-e | 18.67 | 29.07 |
| 41293 | 111.58a-e | 5.85 | 20.17 | 207563 | 63.10a-f | 17.7 | 28.02 |
| 41111 | 110.44a-f | 4.77 | 18.94 | 41053 | 62.59a-g | 16.75 | 26.98 |
| 207563 | 109.29a-g | 3.68 | 17.71 | 212589 | 62.52a-g | 16.62 | 26.84 |
| Natoli | 105.41a-l | --- | 13.53 | Natoli | 53.61a-t | --- | 8.76 |
| MRV | 92.85b-v | -11.92 | --- | MRV | 49.29b-v | -8.06 | --- |

*Figures sharing the same letter(s) or ranges of letters with in the same column are non-significantly different; **MRV = mean of released varieties, ***significance levels of the mean performances for released varieties was approximated from equivalent values of the test genotypes for the same character

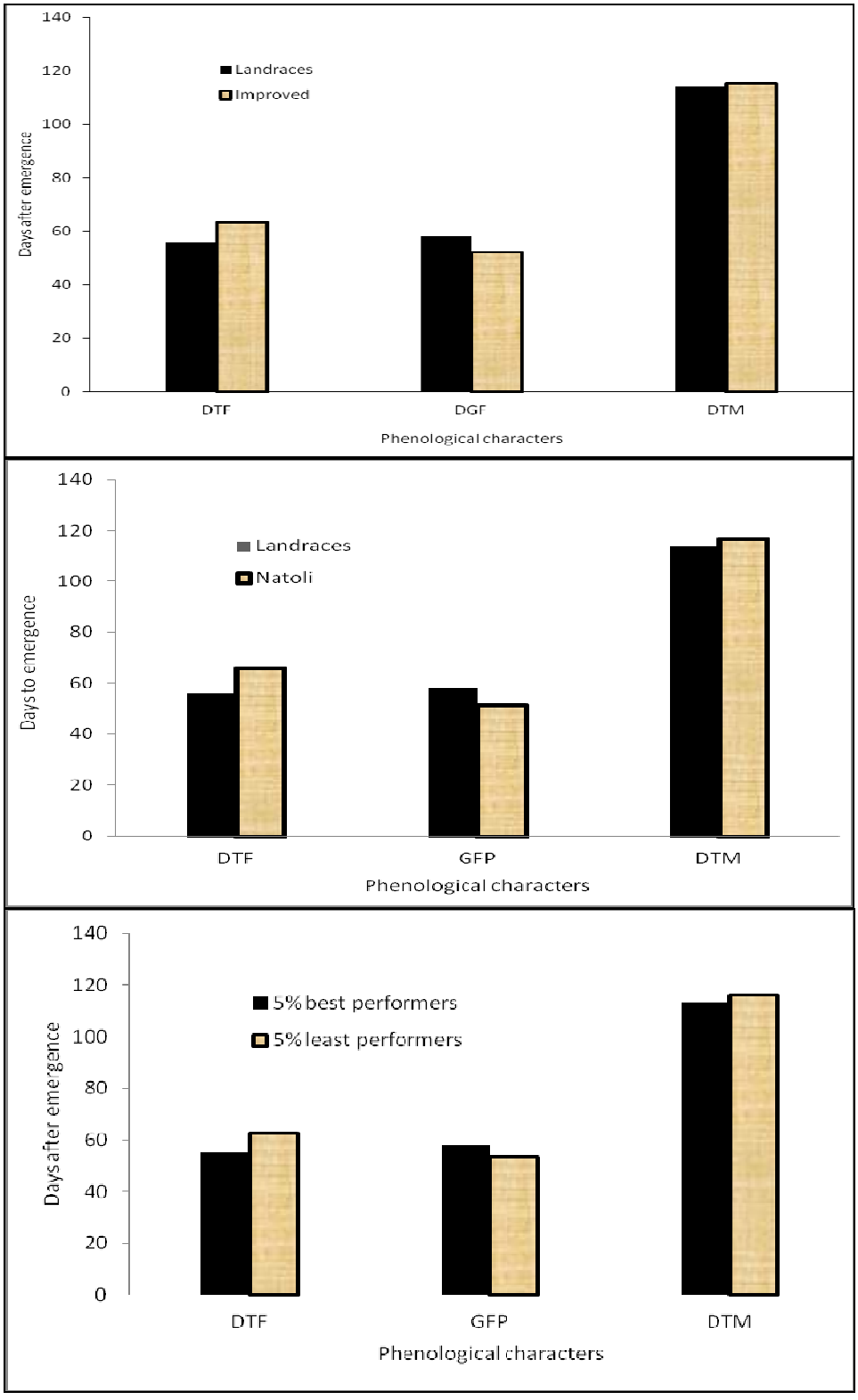



Figure 11. Comparison of phenological characters in 155 genotypes for days to flowering (DTF), grain filling period (GFP) and days to maturity (DTM) showing shorter relative periods of vegetative and longer grain-filling periods in landraces than in improved genotype.

Ideotypes with the faster developmental switch to reproductive growth earlier in the growing season when the soil moisture level is still in better situation might have better comparative advantage to mobilize and more efficiently use assimilates for reproductive growth. According to Richards (1991), crop phenology is the most important single factor influencing yield and adaptation particularly where the growth factors are limited.

The same mechanism might have provided the landraces with adequately longer period of time for grain filling. A similar observation was made in Ethiopian fenugreek (*Trigonella foenum-graecum*) landraces as compared to a released variety (Fikresilassie, 2008). On the contrary, improved genotypes showed initial lag to days to flowering; the consequence may be higher investment at vegetative growth and exposure of relatively longer part of their reproductive growth to an end-of-season moisture stress. It may imply, therefore, that landraces may have better structural and functional fitness to survive and reproduce under marginal condition than the improved genotypes.

### 4.2.3. Effect of location

The two locations displayed significant differences for a number of symbiotic and agronomic characters. However, number and size of seeds, shoot and grain nitrogen and protein contents, grain and total nitrogen fixation, grain nitrogen yield and grain yield did not show marked differences between locations (Table 14).

Number of nodule was higher at Ginchi and lower at Ambo but nodule weight was heavier at Ambo. As nitrogen fixation was also better at Ambo than at Ginchi, this may imply that weight of nodules may be more important (within a limit) for symbiotic nitrogen fixation than their mere number. Better fixation at Ambo may also be attributed particularly to the higher level of phosphorus in the soil (Table 3) since phosphorus is of paramount importance in fixation (Shukla and Yadav, 2000).

Table 14. Mean performances of 155 chickpea genotypes for attributes of symbiotic nitrogen fixation and agronomic performance at two locations in Ethiopia

| Character | Ambo | Ginchi | Mean |
|---|---|---|---|
| Early vigor (SDWF, g 5 plants$^{-1}$) | 30.78b | 44.03a | 37.4 |
| Shoot dry weight ratio before flowering (SDWRF) | 1.02b | 1.74a | 1.38 |
| Nodulation index (NI, mg g$^{-1}$) ( | 2.48a | 0.38b | 1.43 |
| No of nodules (NN, 5 plants$^{-1}$) | 12.33b | 14.06a | 13.19 |
| Nodule dry weight (NDW, mg 5 plants$^{-1}$) | 683.39a | 156.99b | 420.19 |
| Shoot nitrogen content (SNC, %) | 1.19a | 1.17a | 1.18 |
| Shoot protein content (SPC, %) | 7.41a | 7.30a | 7.36 |
| Shoot nitrogen fixation (SNF, %) | 35.77a | 24.18b | 29.97 |
| Grain nitrogen content (GNC, %) | 3.52a | 3.51a | 3.51 |
| Grain protein content (GPC, %) | 21.99a | 21.92a | 21.95 |
| Grain nitrogen fixation (GNF, %) | 35.74a | 36.15a | 35.95 |
| Total biomass nitrogen content (BNC, %) | 4.70a | 4.67a | 4.69 |
| Total nitrogen fixation (BNF, %) | 34.26a | 32.22b | 33.24 |
| Fixed nitrogen assimilation efficiency (FNAE, %) | 77.21b | 83.35a | 80.28 |
| Grain nitrogen yield (GNY, g 5 plants$^{-1}$) | 1.87a | 1.83a | 1.85 |
| Shoot nitrogen yield (SNY, g 5 plants$^{-1}$) | 1.30a | 1.20b | 1.25 |
| Biomass nitrogen yield (BNY, g 5 plants$^{-1}$) | 3.17a | 3.03b | 3.10 |
| Nitrogen harvest index (NHI) | 0.59b | 0.61a | 0.60 |
| Days to 50% flowering (DTF) | 55.00b | 58.22a | 56.61 |
| Days to 90% maturity (DTM) | 113.72a | 114.41a | 114.06 |
| Grain filling period (GFP) | 58.72a | 56.19b | 57.45 |
| No of pods (NP, 5 plants$^{-1}$) | 388.26a | 363.20b | 375.73 |
| No of seeds (NS, 5 plants$^{-1}$) | 423.72a | 419.10a | 421.41 |
| Shoot dry weight at maturity (SDWM, g 5 plants$^{-1}$) | 109.17a | 102.57b | 105.87 |
| Shoot dry weight ratio at maturity (SDWRM) | 1.55a | 1.53b | 1.54 |
| Total biomass weight (BMW, g 5 plants$^{-1}$) | 155.16a | 148.59b | 151.88 |
| Harvest index (HI) | 34.63b | 35.68a | 35.15 |
| Grain production efficiency (GPE, g 5 plants$^{-1}$) | 57.1a | 51.13b | 54.12 |
| Biomass production rate (BPR, %) | 136.56a | 130.07b | 133.32 |
| Economic growth rate (EGR, %) | 90.47b | 93.20a | 91.84 |
| Thousand seed weight (TSW, g) | 113.21a | 114.38a | 113.8 |
| Grain yield (YLD, g 5 plants$^{-1}$) | 52.85a | 52.20a | 52.53 |

*Figures within a row sharing the same letter are statistically non-significant

### 4.2.4. Genotype by location interaction effects

Genotypes by location interaction effects were significant for a number of symbiotic and agronomic characters (Table 11). When significant, genotype by location interaction effects were mostly a "cross-over" type; i.e. interactions were associated with rank order changes among the genotypes as demonstrated by grain nitrogen yield just as a single example (Appendix 4). This indicated that the two locations were distinctly different for some of the characters and that better genotypes at one location may not also be better performing at another.

While most of the traits related to yield and agronomic yield components, nodulation and nitrogen yields showed significant interaction, genotype by location interaction effects were non-significant for components related to plant tissue nitrogen contents including the amount of fixation. This may indicate lesser sensitivity of some of the main traits for symbiotic nitrogen fixation to changes in environment. In a previous study with primitive forms of *P. sativum* native to Ethiopia, similar results were recorded (Keneni *et al.*, 2010). Therefore, genotypes selected at one location for nitrogen (and hence protein) content and symbiotic nitrogen fixation levels may repeat similar performance under other locations as well.

### 4.2.5. Phenotypic diversity

#### 4.2.5.1. Cluster and distance analyses

Cluster analysis of the 155 genotypes distinguished six different groups of genotypes (Table 15). Hierarchical classification presented in a dendrogram also identified two main branches of six clusters (Figure 12). Members within a single cluster are considered similar while those in clusters with non-significant distance are assumed to have more close relationships with each other than they are with those in significantly distant clusters. The first cluster ($C_1$, n=51) had the largest number of accessions from all over the regions in Ethiopia. The fourth cluster ($C_4$, n=35) was the second largest in number of accessions constituted with accessions from all over the regions in

Ethiopia with the exception of Arsi. The second cluster ($C_2$, n= 29) was the third in terms of number of accessions with most of the accessions collected from Wello and Tigray and an introduction from India through ICRISAT.

The third cluster ($C_3$, n= 25) was the fourth in terms of number of accessions from all over the regions with the exception of East Gojam and Tigray. The fifth cluster ($C_5$, n= 13) was comprised of introduced genotypes that are nodulating. The sixth cluster ($C_6$, n=2) constituted only the two non-nodulating genotypes (Table 15 and Figure 12). The sharp distinctness of the non-nodulating genotypes from all the nodulating genotypes (both landraces and introductions) could be due to the small sample size of the former or due to the fact that the non-nodulating behaviour is associated with agronomic inferiority as reflected in this study.

There was high genetic diversity for both symbiotic and agronomic characters in the populations studied. The genetic distances as measured by the pairwise generalized $D^2$ statistics between each cluster is shown in Table 16. The standardized Mahalanobis $D^2$ statistics showed existence of high genetic distances among clusters. The first exceptionally divergent $D^2$ values were obtained between clusters ranging from $C_1$ to $C_5$ on the one hand and cluster C6 on the other hand with $D^2$ value ranging from 25465 to 25744. The uniquely high distance values in this case may stem from the presence of highly contrasting non-nodulating references together with nodulating test genotypes, which resulted in $D^2$ values disproportionately high among the clusters.

The maximum genetic distance was found between clusters $C_4$ and $C_6$ with $D^2$ = 25744. The second most divergent clusters were $C_1$ and $C_6$ with $D^2$ = 25718 and the third were $C_3$ and $C_6$ with $D^2$ = 25649. The fourth and fifth most divergent clusters were $C_2$ and $C_6$ with $D^2$ = 25612 and $C_5$ and $C_6$ with $D^2$ = 25465, respectively. The most divergent classes separated all genotypes ($C_1$-$C_5$) on one side from the non-nodulating references ($C_6$) on the other side (Table 16). However, it was witnessed that introductions from foreign sources relatively more closely related to the non-nodulating references ($D^2$ = 25465) than the local landraces ($D^2$ = 25612-25744) disregarding the

contribution of a single Indian introduction which was exceptionally grouped with the landraces in cluster $C_2$ (Table 15).

It is interesting to note from the hierarchical classification of the genotypes using a dendrogram that, no matter how many clusters above two are formed, the three genotypes initially planned as reference checks always go together to the individual level (Figure 12). The local accessions were also more closely related among themselves than with the introductions from foreign sources for both symbiotic and agronomic characters.

Table 15. Clustering of 155 chickpea genotypes into six clusters using mean of 32 symbiotic and agronomic characters

| Cluster | No. of genotypes | Genotypes |
|---|---|---|
| $C_1$ | 51 | Acc. Nos. 231327, 209093, 209096, 207761, 207763, 207764, 41268, 41076, 41027, 207734, 41015, 41272, 41276, 207745, 41275, 207743, 41274, 207742, 41316, 41298, 41313, 41312, 41047, 41295, 41284, 41293, 41019, 41049, 41053, 209084, 209091, 209087, 41160, 41161, 207661, 41141, 207665, 41128, 41110, 41111, 41106, 207658, 41215, 41066, 41176, 41185, 207563, 219803, 225877, 207645, 207646 |
| $C_2$ | 29 | 231328, 208829, 209094, 209092, 207741, 41052, 209082, 207666, 207657, 41186, 209036, 41190, 207150, 207151, 207564, 207894, 207895, 219797, 219799, 219800, 221696, 41114, 212589, 41113, 207659, 41115, 225873, 225876, ICC 4918 |
| $C_3$ | 25 | 209097, 209098, 41002, 41159, 41271, 41277, 207744, 41315, 41299, 41303, 41296, 41297, 209088, 209089, 209081, 41130, 41142, 41007, 41175, 41174, 209027, 41195, 41197, 207660, 225874 |
| $C_4$ | 35 | 41026, 41074, 41075, 41073, 41021, 41222, 41103, 41320, 41029, 41273, 41311, 41280, 41308, 41046, 41304, 41289, 41290, 41291, 41048, 41054, 209083, 209090, 207667, 41134, 41168, 41129, 41207, 41216, 41011, 41008, 209035, 41170, 41171, 213224, 225878 |
| $C_5$ | 13 | ICC 5003, ICC 4948, ICC 4973, ICC 15996, Shasho (ICCV 93512), Arerti (FLIP 89-84C), Worku (DZ-10-16-2), Akaki (DZ-10-9-2), Ejere (FLIP-97-263C), Teji (FLI 97-266C), Habru (FLIP 88-42c), Natoli (ICCX-910112-6), ICC 19180 |
| $C_6$ | 2 | ICC 19181, PM 233 |

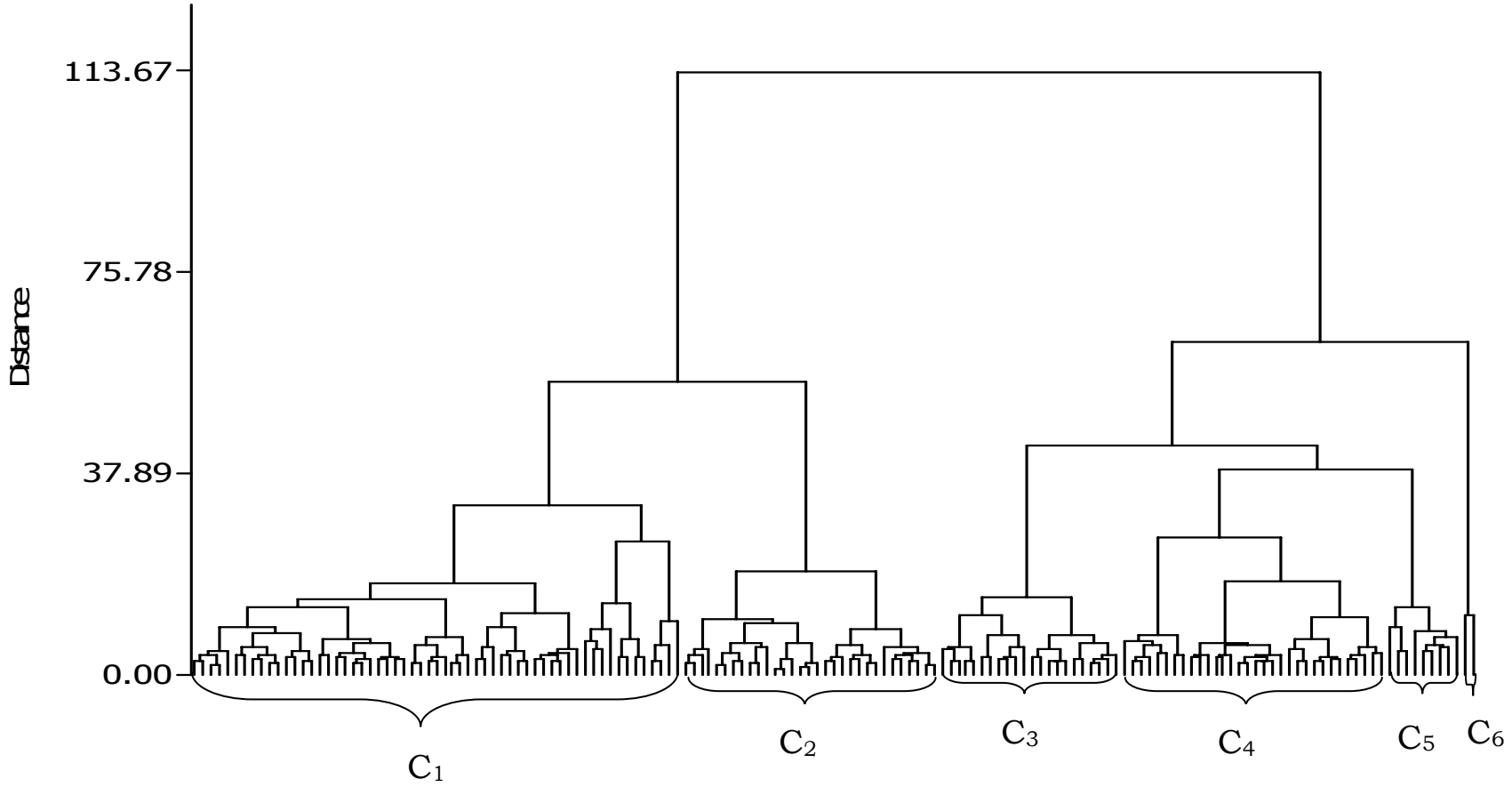



Figure 12. Dendrogram of hundred fifty five chickpea genotypes developed by Ward's agglomerative hierarchical classification method based on Euclidian distance using mean of 32 agronomic and symbiotic characters

In the second category, clusters $C_1$-$C_4$ which were comprised of only local accessions with the exception of a single genotype (i.e. ICC 4918 in cluster $C_2$) showed more divergence with cluster $C_5$ which constituted all improved genotypes introduced from ICRISAT and ICARDA ($D^2$ = 72-84). Clusters formed by the local accessions, i.e. $C_1$-$C_4$, more closely related with each other. The distances between the clusters consisting only of landraces may be in part underestimated because of the existence of extremely unique non-nodulating genotypes which were inferior for almost all symbiotic and agronomic traits.

Table 16. Pair-wise generalized squared distances between six clusters constituting 155 chickpea genotypes

| Cluster | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
|---|---|---|---|---|---|---|
| $C_1$ | 0 | 12 | 24 | 11 | 84** | 25718** |
| $C_2$ | | 0 | 18 | 20 | 78** | 25612** |
| $C_3$ | | | 0 | 15 | 72** | 25649** |
| $C_4$ | | | | 0 | 81** | 25744** |
| $C_5$ | | | | | 0 | 25465** |
| $C_6$ | | | | | | 0 |

**$P \leq 0.01$

### 4.2.5.2. Performances of genotypes in different clusters

Cluster mean performances showed existence of considerable variation among the different clusters for individual traits (Table 17). The first cluster ($C_1$) was characterized by the highest biomass accumulation at early (early vigour) and later (maturity) stages, pod and seed setting, economic growth rate and grain production efficiency, nitrogen (shoot, grain and biomass) yields and grain yield. This cluster was also superior to grand mean of all other traits averaged over all clusters, indicating that this cluster contained desirable genotypes according to multiple symbiotic and agronomic characters.

The second cluster ($C_2$) had the lowest nodulation index, nitrogen fixation and protein contents but the highest nitrogen and grain harvest indices and fixed nitrogen assimilation efficiency. The third cluster ($C_3$) showed the highest nodulation index, nodule number and dry weight and nitrogen harvest index but the least total above ground biomass (grain and shoot) weight, biomass and economic growth rates and nitrogen and grain yields. This result deviated from the previous report that the values of nitrogen harvest index were consistently higher in modern crops than in landraces (Sinclair and Vadez, 2002).

The fourth cluster ($C_4$) was characterized by the best nodulation and nodule dry weight, highest foliar and grain nitrogen and protein contents, highest percentage of foliar and grain nitrogen fixation, better nitrogen yield and the least for shoot dry matter accumulation (particularly early in the growing season) and seed size. Cluster $C_5$ showed better early vigour, long vegetative and short grain filling period, lower nitrogen harvest index, largest seed size, lower harvest index, and pod and seed setting. The analysis also reconfirmed that introduction of exotic genotypes provided the best genetic sources for increasing seed size.

The last cluster ($C_6$), constituted from the non-nodulating reference lines, was obviously the least for all characters including attributes of symbiotic and agronomic significance, characterized particularly by the least above ground biomass and shoot dry weight, number of seeds plant$^{-1}$, harvest index, foliar and seed protein contents, growth rate and production efficiency and low yield (Table 17).

#### 4.2.5.3. Geographical pattern of phenotypic diversity

The introduced genotypes were distinctly grouped into nodulating and non-nodulating types as clusters $C_5$ and $C_6$, respectively. However, the landraces collected from the regions within Ethiopia were dispersed over clusters $C_1$-$C_4$ indicating that there was no definite association between sources of origin and clustering pattern at least within the local accessions. The latter may be either related to extensive seed exchange between farmers or similarity of the initial germplasm introduced to different regions.

However, not all sources of origins of chickpea genotypes within Ethiopia had equal levels of diversity. Accessions from six zones of origins, namely West Gojam, West Harerge, Shewa and Wello were distributed all over the clusters $C_1$-$C_4$. Accessions from Arsi, Gonder and Tigray were distributed over three of the four clusters constituted by the landraces. Accessions from East Gojam were distributed over two of the four clusters (Table 18).

The grouping for symbiotic and agronomic characters of the genotypes in this case showed distinct clusters only between landraces and introduced genotypes. No clear interrelationship was observed between the origins of landraces in Ethiopia and the pattern of genetic diversity as there were a number of accessions from the same source of origin fell into different clusters and accessions from different origins overlapped in the same clusters.

Geographic diversity should, therefore, not serve as an index of genetic diversity in selecting suitable parents for hybridization. Jomová *et al.* (2005) also characterized chickpea accessions from four countries and reported that genetic divergence was not apparently related to geographic diversity for morpho-agronomic traits. Contrary to the present finding, in another study, cluster analysis based on morpho-agronomic traits separately grouped the *Kabuli* types from the *Desi* types (Upadhyaya *et al.*, 2007). However, the *Kabuli* types were obviously underrepresented in this study.

On the other hand, diversity and cluster analysis of the same genotypes at molecular level using microsatellite markers distinguished five clusters but there was more or less clear association between pattern of genetic diversity and geographic sources of origin (Figure 8). This may be related to better power and precision of differentiation of the simple sequence repeat markers over the morphological characters (Carvalho, 2004).

It may also be hypothesized that, at least within a limit, the same phenotypic differentiation and clustering pattern may be attained through different combination among morpho-agronomic characters as can be witnessed from the cluster mean performances (Table 17).

Table 17. Differences among the six clusters of 155 chickpea genotypes for mean performance of 32 symbiotic and agronomic characters

| Character | Clusters | | | | | | Grand mean |
|---|---|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ | |
| No of nodules 5 plants$^{-1}$ | 13.18 | 9.79 | 15.40 | 14.94 | 14.15 | 0.00 | 11.24 |
| Nodule dry weight (mg 5 plants$^{-1}$) | 427.56 | 270.06 | 510.47 | 511.13 | 372.34 | 0.00 | 348.59 |
| Nodulation index | 1.43 | 0.84 | 1.88 | 1.82 | 1.06 | 0.00 | 1.17 |
| Foliar nitrogen content (%) | 1.17 | 1.07 | 1.16 | 1.30 | 1.19 | 0.83 | 1.12 |
| Foliar protein content (%) | 7.29 | 6.72 | 7.24 | 8.16 | 7.44 | 5.19 | 7.01 |
| Foliar nitrogen fixation (%) | 29.82 | 24.34 | 29.13 | 36.87 | 30.78 | 0.00 | 25.16 |
| Seed nitrogen content (%) | 3.53 | 3.39 | 3.50 | 3.66 | 3.49 | 2.44 | 3.34 |
| Seed protein content (%) | 22.07 | 21.18 | 21.91 | 22.89 | 21.80 | 15.22 | 20.85 |
| Seed nitrogen fixation (%) | 36.65 | 33.76 | 36.18 | 38.74 | 35.62 | 0.00 | 30.16 |
| Above ground biomass nitrogen content (%) | 4.70 | 4.46 | 4.66 | 4.97 | 4.68 | 3.27 | 4.46 |
| Total nitrogen fixation (%) | 33.87 | 31.04 | 33.39 | 35.99 | 33.09 | 0.00 | 27.90 |
| Fixed nitrogen assimilation efficiency (%) | 81.63 | 82.90 | 81.83 | 79.48 | 80.67 | 0.00 | 67.75 |
| Grain nitrogen yield (g 5 plants$^{-1}$) | 2.05 | 1.89 | 1.63 | 1.79 | 1.69 | 0.78 | 1.64 |
| Shoot nitrogen yield (g 5 plants$^{-1}$) | 1.37 | 1.09 | 1.04 | 1.38 | 1.29 | 0.69 | 1.14 |
| Biomass nitrogen yield (g 5 plants$^{-1}$) | 3.42 | 2.98 | 2.67 | 3.17 | 2.99 | 1.46 | 2.78 |
| Nitrogen harvest index | 0.61 | 0.64 | 0.61 | 0.57 | 0.56 | 0.54 | 0.59 |
| Early vigor (g 5 plants$^{-1}$) | 38.85 | 37.59 | 38.15 | 34.66 | 38.84 | 27.05 | 35.86 |
| Shoot dry weight ratio before flowering | 1.45 | 1.39 | 1.43 | 1.26 | 1.41 | 0.95 | 1.32 |
| Days to 50% flowering | 55.82 | 55.28 | 55.84 | 56.71 | 62.69 | 71.50 | 59.64 |
| Days to 90% maturity | 114.04 | 113.83 | 113.44 | 114.51 | 114.38 | 121.00 | 115.20 |
| Grain filling period | 58.28 | 58.46 | 57.56 | 57.85 | 51.88 | 49.57 | 55.60 |
| No of pods 5 plants$^{-1}$ | 417.80 | 385.55 | 339.96 | 379.34 | 265.62 | 264.00 | 342.05 |
| No of seeds 5 plants$^{-1}$ | 475.04 | 436.21 | 378.96 | 428.23 | 269.31 | 245.00 | 372.13 |
| Shoot dry weight at maturity (g 5 plants$^{-1}$) | 117.40 | 101.21 | 89.57 | 104.14 | 110.62 | 82.54 | 100.91 |
| Shoot dry weight ratio at maturity | 1.71 | 1.47 | 1.31 | 1.52 | 1.60 | 1.21 | 1.47 |
| Above ground biomass weight (g 5 plants$^{-1}$) | 169.31 | 148.46 | 129.94 | 146.30 | 153.89 | 115.75 | 143.94 |
| Harvest index | 35.30 | 37.76 | 35.67 | 34.03 | 31.63 | 29.91 | 34.05 |
| Grain production efficiency (g 5 plants$^{-1}$) | 60.83 | 59.12 | 48.03 | 50.55 | 42.75 | 22.75 | 47.34 |
| Biomass production rate (%) | 148.77 | 130.66 | 114.73 | 127.92 | 134.76 | 95.38 | 125.37 |
| Economic growth rate (%) | 99.98 | 95.52 | 81.08 | 85.17 | 94.21 | 66.40 | 87.06 |
| Thousand seed weight (g) | 108.32 | 111.85 | 108.51 | 100.54 | 184.02 | 123.14 | 122.73 |
| Grain yield (g 5 plants$^{-1}$) | 57.90 | 55.63 | 46.45 | 49.00 | 48.86 | 32.26 | 48.35 |

Table 18. Clustering pattern of 155 chickpea genotypes from different origins over six clusters based on mean performance of 32 agronomic and symbiotic characters

| Origin | No of genotypes | No of genotypes in each cluster | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Arsi | 13 | 6 | 4 | 3 | - | - | - |
| East Gojam | 13 | 4 | - | - | 9 | - | - |
| West Gojam | 13 | 8 | 1 | 3 | 1 | - | - |
| North Gonder | 13 | 5 | - | 3 | 5 | - | - |
| South Gonder | 12 | 6 | - | 2 | 4 | - | - |
| West Haragie | 11 | 3 | 2 | 3 | 3 | - | - |
| East Shewa | 13 | 6 | 1 | 2 | 4 | - | - |
| North Shewa | 13 | 6 | 1 | 2 | 4 | - | - |
| West Shewa | 13 | 2 | 3 | 5 | 3 | - | - |
| Tigray | 12 | 2 | 9 | - | 1 | - | - |
| South Wello | 13 | 3 | 7 | 2 | 1 | - | - |
| Improved genotypes | 14 | - | 1 | - | - | 13 | - |
| Non-nodulating checks | 2 | - | - | - | - | - | 2 |
| Total | 155 | 51 | 29 | 25 | 35 | 13 | 2 |

#### 4.2.5.4. Principal component analysis

In this study, principal component analysis showed that the first five principal components accounted for 82.60% of the total variation among 155 genotypes for the thirty-two characters considered in this study. Of these, the first and the second principal components constituted 30.15% and 23.70% of the variation, respectively (Table 19).

Different characters accounted for different levels of differentiation among the populations into different clusters. Some very useful traits like pod and seed setting, dry matter accumulation, nitrogen and grain yields, growth rate and grain production efficiency and nitrogen fixation showed

greater absolute values of eigenvectors in the first and/or second principal components. This indicated that these traits had higher contributions to the total differentiation of the populations into clusters.

Selection efforts based on these traits may be more effective. It was observed that other characters like early vigor, crop phenological characters, number of nodules and grain nitrogen yield with more contribution to the third principal component which had a gross contribution of only 13.04% of the total variation had lesser contribution to the total differentiation of the populations into clusters.

#### 4.2.5.5. Implications for germplasm utilization

The present study may imply that there is no special "hot spot" region for multiple traits of symbiotic and agronomic characters in Ethiopia. Nevertheless, it may not be by coincidence that a few of the top best 5% accessions selected for specific symbiotic and agronomic characters existed in specific clusters and geographical regions. For attributes of symbiotic nitrogen fixation, cluster $C_4$ constituted much greater number of best accessions, followed by clusters $C_1$ and $C_2$. Cluster $C_1$ also constituted most of the best accessions for attributes of agronomic significance (Figure 13).

Similarly, the best accessions (41222, 41029, 41021, 41074, 41075, 41320 and 41026) selected for symbiotic nitrogen fixation had their origins in East Gojam while those for shoot, grain and total above ground biomass nitrogen yields were associated with East and West Gojam zones. Likewise, from among the agronomic traits, six (out of eight) best accessions (231328, 209093, 209094, 41002, 231327 and 207764) selected for harvest index were collected from Arsi. Even though the best performers for agronomic traits were more distributed over the regions than those for symbiotic traits, West Gojam was found to be better source of accessions for agronomic traits (Figure 14). Therefore, specific origins may exhibit best accessions for specific characters, which may facilitate selection of groups of accessions possessing specific usage in breeding programs.

Table 19. Eigenvalue, percentage and cumulative variances and eigenvectors on the first five principal components for 32 agronomic and symbiotic characters in hundred fifty five chickpea genotypes

| | Principal components (PCs) | | | | |
|---|---|---|---|---|---|
| Parameter | $PC_1$ | $PC_2$ | $PC_3$ | $PC_4$ | $PC_5$ |
| Eigenvalue | 9.95 | 7.82 | 4.30 | 2.75 | 2.44 |
| Variance (%) | 30.15 | 23.70 | 13.04 | 8.33 | 7.40 |
| Cumulative Variance (%) | 30.15 | 53.84 | 66.88 | 75.21 | 82.60 |
| Character | ---------- Eigenvectors ---------- | | | | |
| No of nodules 5 plants$^{-1}$ | 0.075 | 0.158 | -0.021 | 0.295 | -0.161 |
| Nodule dry weight (mg 5 plants$^{-1}$) | 0.108 | 0.119 | -0.077 | 0.396 | -0.323 |
| Nodulation index | 0.087 | 0.154 | -0.095 | 0.268 | -0.398 |
| Shoot nitrogen content (%) | 0.062 | 0.309 | 0.112 | -0.091 | -0.003 |
| Shoot protein content (%) | 0.063 | 0.309 | 0.112 | -0.090 | -0.002 |
| Shoot nitrogen fixation (%) | 0.092 | 0.304 | 0.067 | -0.079 | 0.031 |
| Grain nitrogen content (%) | 0.156 | 0.255 | -0.124 | -0.068 | 0.156 |
| Grain protein content (%) | 0.156 | 0.256 | -0.123 | -0.068 | 0.156 |
| Grain nitrogen fixation (%) | 0.192 | 0.223 | -0.164 | -0.039 | 0.173 |
| Total biomass nitrogen content (%) | 0.135 | 0.309 | -0.038 | -0.087 | 0.106 |
| Total biomass nitrogen fixation (%) | 0.190 | 0.240 | -0.142 | -0.039 | 0.163 |
| Fixed nitrogen assimilation efficiency (%) | 0.195 | 0.045 | -0.238 | 0.024 | 0.192 |
| Grain nitrogen yield (g 5 plants$^{-1}$) | 0.288 | -0.102 | -0.040 | -0.051 | 0.052 |
| Shoot nitrogen yield (g 5 plants$^{-1}$) | 0.212 | 0.134 | 0.287 | -0.032 | -0.022 |
| Biomass nitrogen yield (g 5 plants$^{-1}$) | 0.299 | 0.004 | 0.125 | -0.050 | 0.023 |
| Nitrogen harvest index | 0.062 | -0.210 | -0.346 | -0.004 | 0.035 |
| Early vigor (g 5 plants$^{-1}$) | 0.096 | -0.072 | -0.053 | 0.317 | 0.270 |
| Shoot dry weight ratio before flowering | 0.098 | -0.073 | -0.059 | 0.311 | 0.254 |
| Days to 50% flowering | -0.150 | 0.080 | 0.313 | 0.037 | -0.297 |
| Days to 90% maturity | -0.093 | 0.034 | 0.209 | -0.139 | 0.049 |
| Grain filling period | 0.123 | -0.067 | -0.230 | -0.130 | -0.126 |
| No of pods 5 plants$^{-1}$ | 0.208 | -0.066 | 0.009 | -0.207 | -0.134 |
| No of seeds 5 plants$^{-1}$ | 0.204 | -0.063 | -0.029 | -0.256 | -0.268 |
| Shoot dry weight at maturity (g 5 plants$^{-1}$) | 0.235 | -0.086 | 0.281 | 0.062 | -0.001 |
| Shoot dry weight ratio at maturity | 0.235 | -0.087 | 0.279 | 0.063 | -0.005 |
| Above ground biomass weight (g 5 plants$^{-1}$) | 0.247 | -0.135 | 0.216 | 0.076 | -0.021 |
| Harvest index | 0.069 | -0.160 | -0.316 | -0.104 | 0.007 |
| Grain production efficiency (g 5 plants$^{-1}$) | 0.234 | -0.195 | -0.111 | -0.062 | -0.046 |
| Biomass production rate (%) | 0.252 | -0.139 | 0.188 | 0.091 | -0.005 |
| Economic growth rate (%) | 0.223 | -0.187 | 0.088 | 0.023 | 0.071 |
| Thousand seed weight (g) | -0.044 | -0.072 | 0.130 | 0.310 | 0.364 |
| Grain yield (g 5 plants$^{-1}$) | 0.249 | -0.201 | -0.002 | -0.028 | 0.100 |

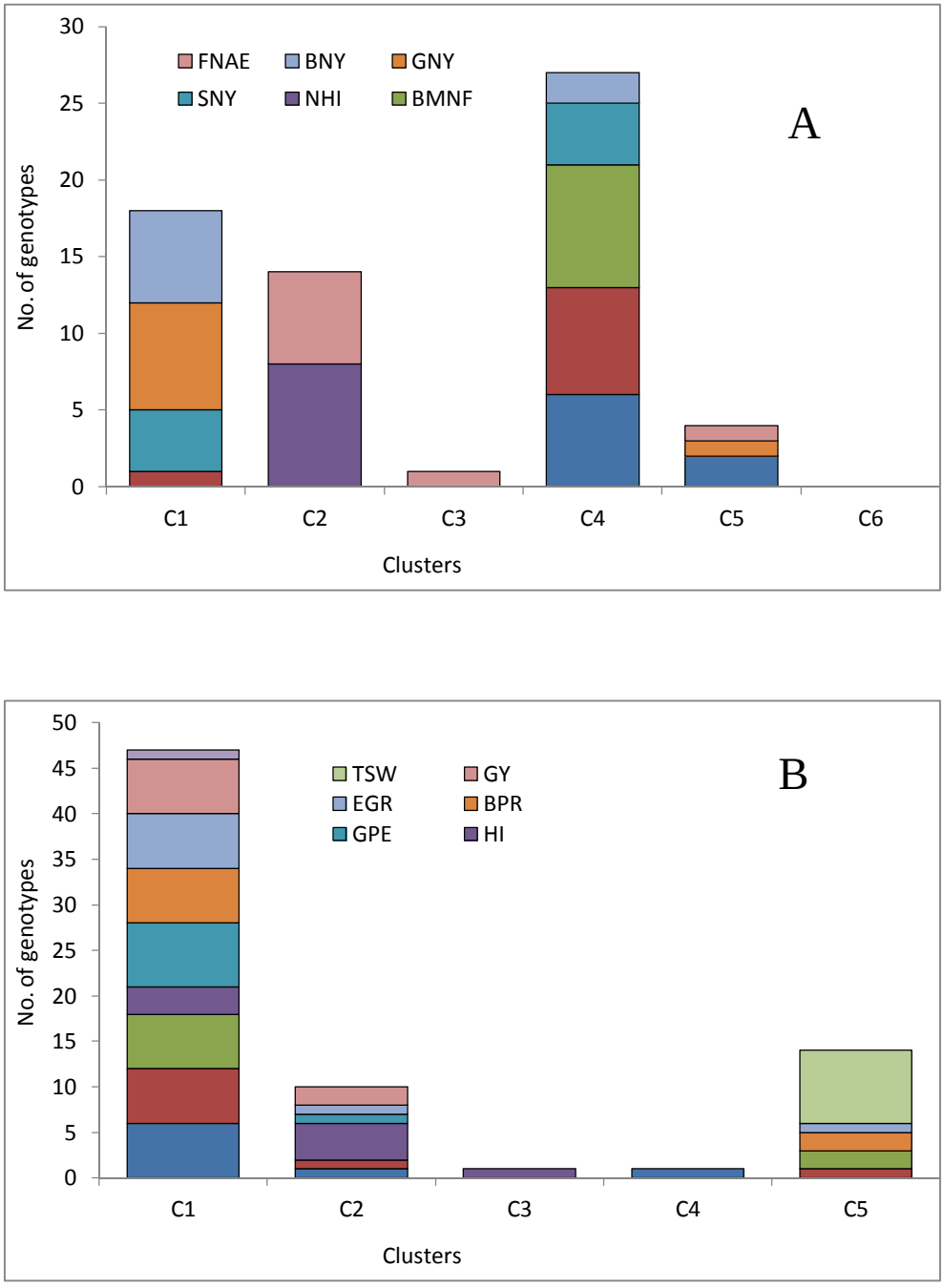
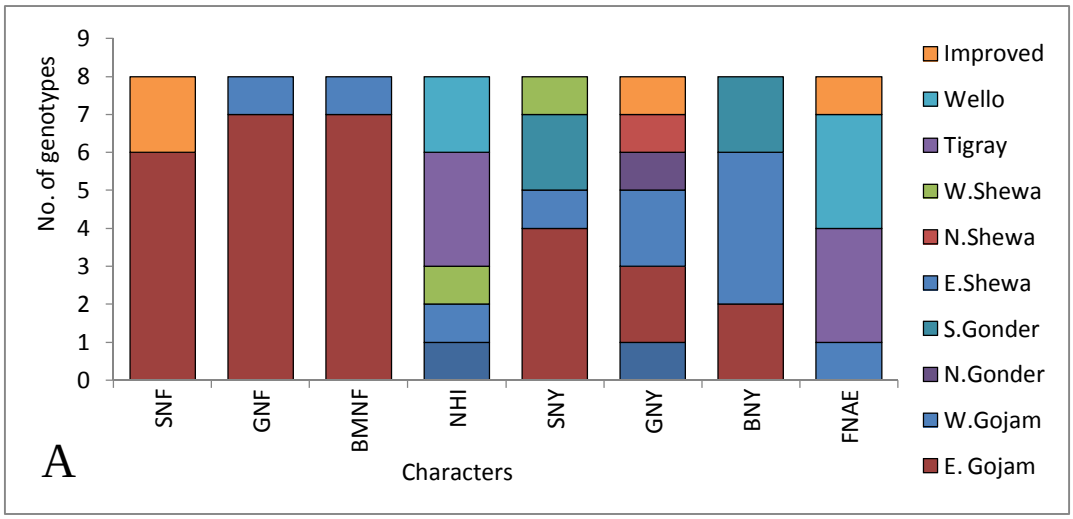



Figure 13. Number of best 5% of chickpea genotypes selected for (A) symbiotic and (B) agronomic characters in each cluster based on evaluation at two locations in Ethiopia (FNAE = fixed nitrogen assimilation efficiency, BNY = biomass nitrogen yield, GNY = grain nitrogen yield, SNY = shoot nitrogen yield, NHI = nitrogen harvest index, and BMNF = biomass nitrogen fixation; TSW = thousand seed weight and GY = grain yield; EGR = economic growth rate, BPR = biomass production rate, GPE = grain production efficiency and HI = harvest index.



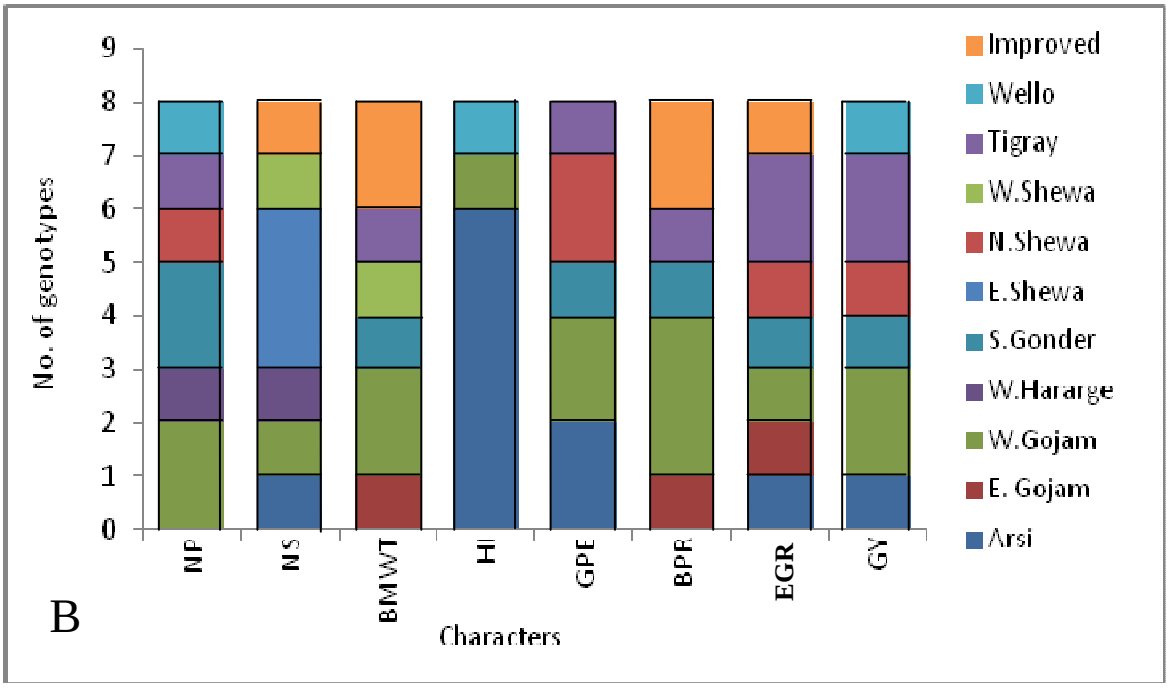




Figure 14. Distribution of the best 5% of chickpea genotypes selected for (A) symbiotic and (B) agronomic characters over different sources of origin based on evaluation at two locations in Ethiopia (SNF = shoot nitrogen fixation, GNF = grain nitrogen fixation, BMNF = biomass nitrogen fixation, NHI = nitrogen harvest index, SNY = shoot nitrogen yield, GNY = grain nitrogen yield, BNY = biomass nitrogen yield, FNAE = fixed nitrogen assimilation efficiency, NP = no of pods, NS = no of seeds, BMWT = biomass weight, HI = harvest index, GPE = grain production efficiency, BPR = biomass production rate, EGR = economic growth rate and GY = grain yield)

It may be difficult, to find out a single genotype combining all desirable characters to the required levels for direct selection and use as a cultivar. In order to produce demanded segregants, a step-wise combination and recombination of valuable genes should be made using intercrossing of selected parents from the accessions with desirable characters. Parents with one or more of the desired characters can be chosen from the sources grouped in this study based not only on group performance but also on performances of individual accessions for specific traits (Singh, 2002).

Once parental accessions are identified and purified, multiple crossing schemes of several parental lines and selection of improved varieties may help to recombine desirable symbiotic and agronomic characters into a single genotype. For example, best lines for nitrogen fixation to be developed from accession 41222 in cluster $C_4$ can be crossed with the best lines for yield to be developed from accession 41274 in cluster $C_1$.

Even though introduced genotypes gave lower yields when directly utilized in field trials as compared to the selected best landraces, they may also serve as donors with parents chosen from the adapted landraces because wider genetic recombination is expected in progenies of distant parents (Sneller *et al.*, 2005). For example, best lines combining nitrogen harvest index and fixed nitrogen assimilation efficiency to be developed from accession 41113 in cluster $C_2$ can be crossed with ICC 19180 in cluster $C_5$. The latter is the best genotype not for nitrogen fixation but for the assimilation of fixed nitrogen.

Further crossing of the single cross progenies to make multiple crosses followed by selection in segregating generation under inoculation with effective *Rhizobium* strains is expected to result in the recombination of the desirable symbiotic and agronomic traits from all parents into a single genotype. Alternatively, the most widely adapted parent may be selected as seed parent to make the genetic background for crossing all other parents as carriers of specific traits with it.

### 4.2.6. Interrelationships between characters

#### 4.2.6.1. Symbiotic characters

There were significant positive association between a number of component characters and symbiotic nitrogen fixation in shoot, grain and total biomass (Table 20). Nitrogen contents in the shoot, grain and total biomass consistently showed significant positive association with fixation in the respective component parts and with each other in possible pairs. Almost perfect positive correlation coefficients were observed particularly between shoot nitrogen content and shoot nitrogen fixation (r = 0.97), grain nitrogen content and grain nitrogen fixation (r = 0.95), grain nitrogen content and above ground biomass nitrogen fixation (r = 0.93) and biomass nitrogen content and biomass nitrogen fixation (r = 0.90).

Nitrogen fixation in grain also revealed perfect positive association with biomass nitrogen fixation (r = 0.99). This indicated that the higher the tissue nitrogen content the higher will be the amount of nitrogen from fixation and *vice versa*. The amount of nitrogen fixation in shoot, grain and above ground biomass also showed significant positive associations with fixed nitrogen assimilation efficiency, indicating that better fixers of nitrogen were also better assimilators of the fixed nitrogen. Better nodulation as expressed by number and weight of nodules and by the nodulation index showed consistently significant but relatively lower (as compared to tissue nitrogen content) positive associations with fixation in all component parts, i.e. shoot, grain and total above ground biomass (r = 0.24-0.35). However, despite the ease of observation without laboratory analysis and modest positive associations of nodule based traits (nodule number, weight and nodulation index), with the amount of symbiotic nitrogen fixation, the low level of genetic variation and heritability of those traits, as presented below, may not allow them to be good selection criteria.

Nitrogen harvest index was also positively associated with fixed nitrogen assimilation efficiency (r = 0.41) and grain nitrogen yield (r = 0.47). However, there were negatively significant associations between nitrogen harvest index and number of nodules (r = -0.16), nitrogen harvest index and shoot nitrogen

content (r = -0.68), nitrogen harvest index and shoot nitrogen fixation (r = -0.59), nitrogen harvest index and above ground biomass nitrogen content (r = -0.34), and between nitrogen harvest index and shoot nitrogen yield (r = -0.55).

Characters with both perfect positive association and higher heritable variation include; grain nitrogen yield, nitrogen harvest index and fixed nitrogen assimilation efficiency. It is interesting to note that these characters also showed positive relationships with a number of other symbiotic and agronomic components (Tables 20 and 21). In terms of total nitrogen output from a given genotype, it appeared that the gross amount of nitrogen as related to total biomass accumulation may be more important than the *per se* concentration of nitrogen in plant tissue. However, these characters which possessed higher positive association and higher heritable variation are not easily observable as they require destructive sampling and laboratory analysis.

#### 4.2.6.2. Agronomic characters

Grain yield was negatively associated with days to flowering (r = -0.45) and maturity (r = -0.24) but positively associated with grain filling period (r = 0.38). This may be attributed to the fact that long flowering and maturity durations might have directly reduced grain yield and indirectly have lowered other component characters like harvest index, grain production efficiency, and biomass production and economic growth rates. Longer flowering also negatively affected pod (r = -0.36) and seed (r = -0.39) setting. Similar results were reported from moisture stressed areas in Australia where higher yields were manifested by genotypes characterized by early flowering and rapidly setting pods (Berger *et al.*, 2003). According to Wallace and Yan (1998), days to maturity usually correlated negatively with harvest index.

In other environments where moisture is not as such a limiting factor, different scenario may be expected in that late flowering and maturing genotypes may effectively exploit the available moisture and perform better than early genotypes that cannot fully exploit the moisture (Wallace and Yan, 1998).

Table 20. Correlation coefficients (r) between symbiotic characters in 155 chickpea genotypes grown at two locations in Ethiopia

| | Characters[1] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characters | NI | NN | NDW | SNC | SNF | GNC | GNF | BMNC | BMNF | FNAE | GNY | SNY | BNY |
| NI | 1.00 | | | | | | | | | | | | |
| NN | 0.56** | 1.00 | | | | | | | | | | | |
| NDW | 0.88** | 0.60** | 1.00 | | | | | | | | | | |
| SNC | 0.31** | 0.32** | 0.21** | 1.00 | | | | | | | | | |
| SNF | 0.33** | 0.33** | 0.24** | 0.97** | 1.00 | | | | | | | | |
| GNC | 0.30** | 0.33** | 0.27** | 0.57** | 0.63** | 1.00 | | | | | | | |
| GNF | 0.32** | 0.34** | 0.30** | 0.51** | 0.61** | 0.95** | 1.00 | | | | | | |
| BMNC | 0.34** | 0.37** | 0.28** | 0.82** | 0.85** | 0.93** | 0.87** | 1.00 | | | | | |
| BMNF | 0.34** | 0.35** | 0.31** | 0.59** | 0.70** | 0.93** | 0.99** | 0.90** | 1.00 | | | | |
| FNAE | 0.16* | 0.21** | 0.20* | 0.03 | 0.16* | 0.57** | 0.78** | 0.41* | 0.74** | 1.00 | | | |
| GNY | 0.09 | 0.05 | 0.15 | -0.08 | 0.02 | 0.32** | 0.42** | 0.19* | 0.40** | 0.53** | 1.00 | | |
| SNY | 0.21** | 0.27** | 0.23** | 0.65** | 0.64** | 0.39** | 0.39** | 0.55** | 0.45** | 0.13 | 0.44** | 1.00 | |
| BNY | 0.17* | 0.18* | 0.22** | 0.30** | 0.35** | 0.42** | 0.49** | 0.42** | 0.50** | 0.41** | 0.88** | 0.81** | 1.00 |
| NHI | -0.07 | -0.16* | -0.02 | -0.68** | -0.59** | -0.07 | 0.04 | -0.34** | -0.04 | 0.41** | 0.47** | -0.55** | 0.011 |

[1]NI = nodulation index, NN = no of nodules, NDW = nodule dry weight, SNC = shoot nitrogen content, SNF = shoot nitrogen fixation, GNC = grain nitrogen content, GNF = grain nitrogen fixation, BMNC = biomass nitrogen content, BMNF = biomass nitrogen fixation, FNAE = fixed nitrogen assimilation efficiency, GNY = grain nitrogen yield, SNY = shoot nitrogen yield, BNY = biomass nitrogen yield and NHI = nitrogen harvest index; **=highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$)

For instance, in winter grown chickpea, where moisture use efficiency was better (Singh, 1990), late maturing varieties gave better yields because of the phenological advantages (Özdemir and Kardavut, 2003). The present study also showed that genotypes with longer relative grain filling period (days to maturity more or less kept constant) were characterized by better pod (r = 0.34) and seed (r = 0.42) setting, harvest index (r = 0.41), grain production efficiency (r = 0.69) and, hence, grain yield (r = 0.38). However, grain filling period was negatively associated with seed size and that may be due to the fact that the large-seeded genotypes are introduced genotypes which have relatively longer vegetative period at the expense of reproductive period.

Other component characters that positively influenced grain yield include pod (r = 0.57) and seed (r = 0.59) setting, shoot biomass (r = 0.67) and total biomass (r = 0.79), harvest index (r = 0.53), grain production efficiency (r = 0.92), and biomass production (r = 0.81), and economic growth (r = 0.93) rates.

It is generally believed that higher biomass combined with higher harvest index is the major physiological and genetic components of grain yield (Wallace and Yan, 1998). In this study, grain yield was not significantly influenced by seed size (r = 0.04), indicating that there is an independent genetic control between the two traits and that improvement in any one of the two would have little effect on the other. Yucel *et al.* (2006) also found positive association between grain yield and number of pods and seeds but did not show any relationship between grain yield and seed size.

Number of pods and seeds were also positively associated with a number of other characters but negatively related with seed size may be because of the expected reciprocal compensation (Wallace and Yan, 1998). The physiological parameters including shoot and biomass dry weight, harvest index, grain production efficiency, and biomass production and economic growth rates showed positive relationship among themselves in most of the cases (Table 21).

#### 4.2.6.3. Symbiotic vs. agronomic characters

Grain yield showed negative relationship with increased shoot nitrogen content (r = -0.28) and shoot nitrogen fixation (r = -0.20). This implies that improving protein content of the shoot as a source of feed may have negatively influenced grain yield. However, grain and above ground biomass nitrogen contents and nitrogen fixation did not show significant relationship with grain yield, indicating independent genetic control between the two traits and grain yield.

On the other hand, correlation coefficients showed that grain yield could be significantly increased by increasing fixed nitrogen assimilation efficiency (r = 0.39), grain nitrogen yield (r = 0.93), shoot nitrogen yield (r = 0.31), biomass nitrogen yield (r = 0.77), and nitrogen harvest index (r = 0.52). On the contrary, increments in shoot and above ground biomass did not influence the amount of symbiotic nitrogen fixation in all component parts of the shoot, grain and total above ground biomass.

Seed size had either negative or little relation with most of the symbiotic characters measured in this study with the exception of nodulation index which showed positive correlation (r = 0.23). This indicated that efforts to increase symbiotic nitrogen fixation may sometimes result in the reduction of seed size and *vice versa*.

Biomass nitrogen fixation, fixed nitrogen assimilation efficiency, shoot, grain and biomass yields, and nitrogen harvest index showed significant positive associations with a number of agronomic yield components, and either non-significant or negative association with other components (Table 22). Generally, it can be concluded that symbiotic characters can make better selection criteria for grain yield than agronomic characters can do for symbiotic nitrogen fixation in all components.

Table 21. Correlation coefficients (r) between agronomic characters in 155 chickpea genotypes grown at two locations in Ethiopia

| | Characters[1] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characters | SDWF | DTF | DTM | SFD | NP | NS | SDWM | BMWT | HI | PE | BPR | EGR | TSW |
| SDWF | 1.00 | | | | | | | | | | | | |
| DTF | -0.23** | 1.00 | | | | | | | | | | | |
| DTM | -0.22** | 0.54** | 1.00 | | | | | | | | | | |
| GFD | 0.15 | -0.82** | 0.03 | 1.00 | | | | | | | | | |
| NP | 0.01 | -0.36** | -0.1 | 0.34** | 1.00 | | | | | | | | |
| NS | -0.02 | -0.39** | -0.06 | 0.42** | 0.85** | 1.00 | | | | | | | |
| SDWM | 0.22** | -0.05 | -0.01 | 0.06 | 0.50** | 0.43** | 1.00 | | | | | | |
| BMWT | 0.27** | -0.17* | -0.10 | 0.15 | 0.55** | 0.49** | 0.97** | 1.00 | | | | | |
| HI | 0.11 | -0.56** | -0.37** | 0.41** | 0.20* | 0.27** | -0.20* | -0.01 | 1.00 | | | | |
| PE | 0.28** | -0.71** | -0.24** | 0.69** | 0.55** | 0.60** | 0.51** | 0.65** | 0.60** | 1.00 | | | |
| BPR | 0.29** | -0.23** | -0.22** | 0.13 | 0.55** | 0.48** | 0.95** | 0.99** | 0.03 | 0.66** | 1.00 | | |
| EGR | 0.26** | -0.17* | -0.28** | 0.02 | 0.47** | 0.47** | 0.71** | 0.80** | 0.40** | 0.72** | 0.82** | 1.00 | |
| TSW | 0.27** | 0.21** | -0.05 | -0.27** | -0.58** | -0.67** | 0.16* | 0.15 | -0.14 | -0.07 | 0.15 | 0.16* | 1.00 |
| YLD | 0.29** | -0.45** | -0.24** | 0.38** | 0.57** | 0.59** | 0.67** | 0.79** | 0.53** | 0.92** | 0.81** | 0.93** | 0.04 |

[1]SDWF = early vigor, DTF = days to 50% flowering, DTM = days to 90% maturity, GFD = grain filling duration, NP = no of pods, NS = no of seeds, SDWM = shoot dry weight at maturity, BMWT = biomass weight, HI = harvest index, PE = production efficiency, BPR = biomass production rate, EGR = economic growth rate, TSW = thousand seed weight and YLD = grain yield; **=highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$)

### 4.2.7. Genetic variation for symbio-agronomic characters

#### 4.2.7.1. Frequency distribution

The populations of chickpea genotypes studied here had normal distributions for most of the symbiotic and agronomic traits with only some levels of skewness for days to flowering and grain filling period, nitrogen fixed in grain and biomass, and fixed nitrogen assimilation efficiency (Figures 15, 16 and 17). This may be an indication that the Ethiopian chickpea germplasm accessions have normally distributed variation, and the individuals evaluated in this study represented the random samples of the Ethiopian chickpea germplasm collections (Welsh, 1981). It is expected that, among genetically diverse natural populations, the distribution of traits is usually normal (Simpson and Sedjo, 1998).

#### 4.2.7.2. Phenotypic and genotypic variation

Among the attributes of symbiotic nitrogen fixation, location and genotype by location interaction effects accounted for the largest part of the total variation for number of nodule, nodule dry weight, nodulation index and shoot and biomass nitrogen yields. Conversely, genotypic effects accounted for the largest part of the total variation for tissue nitrogen and protein contents and symbiotic nitrogen fixation in shoot, grain and biomass. The proportion of variation contributed by the genotypes was far less than that contributed by location and genotype by location interaction effects in shoot and above ground biomass dry weights and for biomass production and economic growth rates (Figure 18).

The greater proportion of location and genotype by location interaction variances relative to genotypic variance may suggest that selection in these traits would be less effective as compared to traits in which the largest proportion of the variation was contributed by the genotypes.

Table 22. Correlation coefficients (r) between symbiotic and agronomic characters in 155 chickpea genotypes grown at two locations in Ethiopia

| Agronomic characters | Symbiotic characters[1] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NI | NN | NDW | SNC | SNF | GNC | GNF | BMNC | BMNF | FNAE | SNY | GNY | BNY | NHI |
| SDMWF | -0.12 | 0.11 | 0.15 | -0.14 | -0.08 | 0.03 | 0.11 | 0.04 | 0.10 | 0.24** | 0.06 | 0.28** | 0.21** | 0.20* |
| DTF | -0.16* | 0.03 | -0.15 | 0.17* | 0.06 | -0.14 | 0.30** | -0.03 | 0.28** | -0.53** | 0.07 | 0.46** | 0.27** | 0.55** |
| DTM | -0.15 | 0.00 | -0.17* | 0.12 | 0.02 | -0.14 | -0.28** | -0.05 | -0.27** | -0.45** | 0.09 | -0.26** | -0.13 | -0.37** |
| SFD | 0.10 | -0.02 | 0.07 | -0.10 | -0.04 | 0.09 | 0.19* | 0.02 | 0.18* | 0.35** | 0.01 | 0.39** | 0.26** | 0.40** |
| NP | 0.15 | 0.01 | 0.12 | 0.01 | 0.04 | 0.13 | 0.19* | 0.10 | 0.18* | 0.24** | 0.39** | 0.59** | 0.59** | 0.18* |
| NS | 0.16* | -0.04 | 0.10 | 0.02 | 0.05 | 0.15 | 0.21** | 0.11 | 0.20* | 0.27** | 0.35** | 0.61** | 0.58** | 0.24** |
| SDMW | 0.03 | 0.08 | 0.15 | 0.01 | 0.03 | 0.06 | 0.12 | 0.04 | 0.13 | 0.19* | 0.74** | 0.66** | 0.82** | -0.15 |
| BMWT | 0.04 | 0.06 | 0.17* | -0.13 | -0.08 | 0.01 | 0.09 | -0.05 | 0.08 | 0.24** | 0.63** | 0.75** | 0.82** | 0.05 |
| HI | -0.07 | -0.16* | -0.06 | -0.34** | -0.29** | -0.07 | 0.03 | -0.20* | 0.01 | 0.29** | -0.37** | 0.46** | 0.11 | 0.82** |
| PE | 0.02 | -0.08 | 0.07 | -0.29** | -0.20* | -0.02 | 0.14 | -0.14 | 0.12 | 0.43** | 0.19* | 0.86** | 0.66** | 0.59** |
| BPR | 0.06 | 0.05 | 0.18* | -0.15 | -0.09 | 0.01 | 0.11 | -0.05 | 0.10 | 0.28** | 0.60** | 0.77** | 0.82** | 0.10 |
| EGR | -0.06 | -0.06 | 0.03 | -0.25** | -0.19* | -0.06 | 0.06 | -0.15 | 0.05 | 0.30** | 0.34** | 0.86** | 0.74** | 0.40** |
| TSW | 0.23** | -0.03 | -0.08 | -0.20* | -0.19* | -0.22** | -0.18* | -0.24** | -0.18* | -0.02 | -0.04 | -0.05 | -0.05 | -0.06 |
| YLD | -0.03 | -0.06 | 0.05 | -0.28** | -0.20* | -0.03 | 0.11 | -0.14 | 0.09 | 0.39** | 0.31** | 0.93** | 0.77** | 0.52** |

[1]Abbreviations of characters as given in Tables 11 and 14 above; **=highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$)

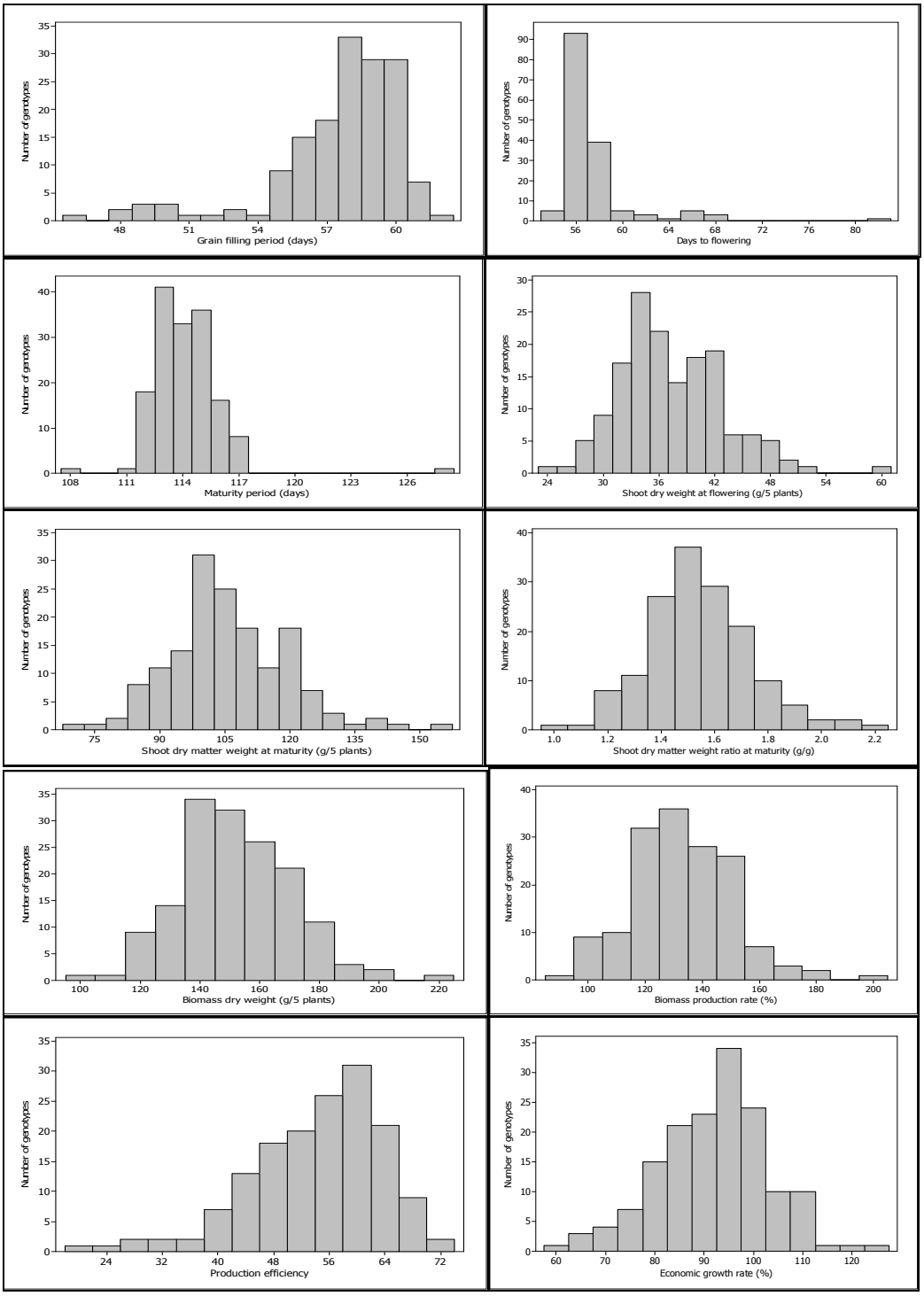



Figure 15. Frequency distributions for ten phenological and some physio-agronomic characters in 155 chickpea genotypes evaluated at two locations in Ethiopia

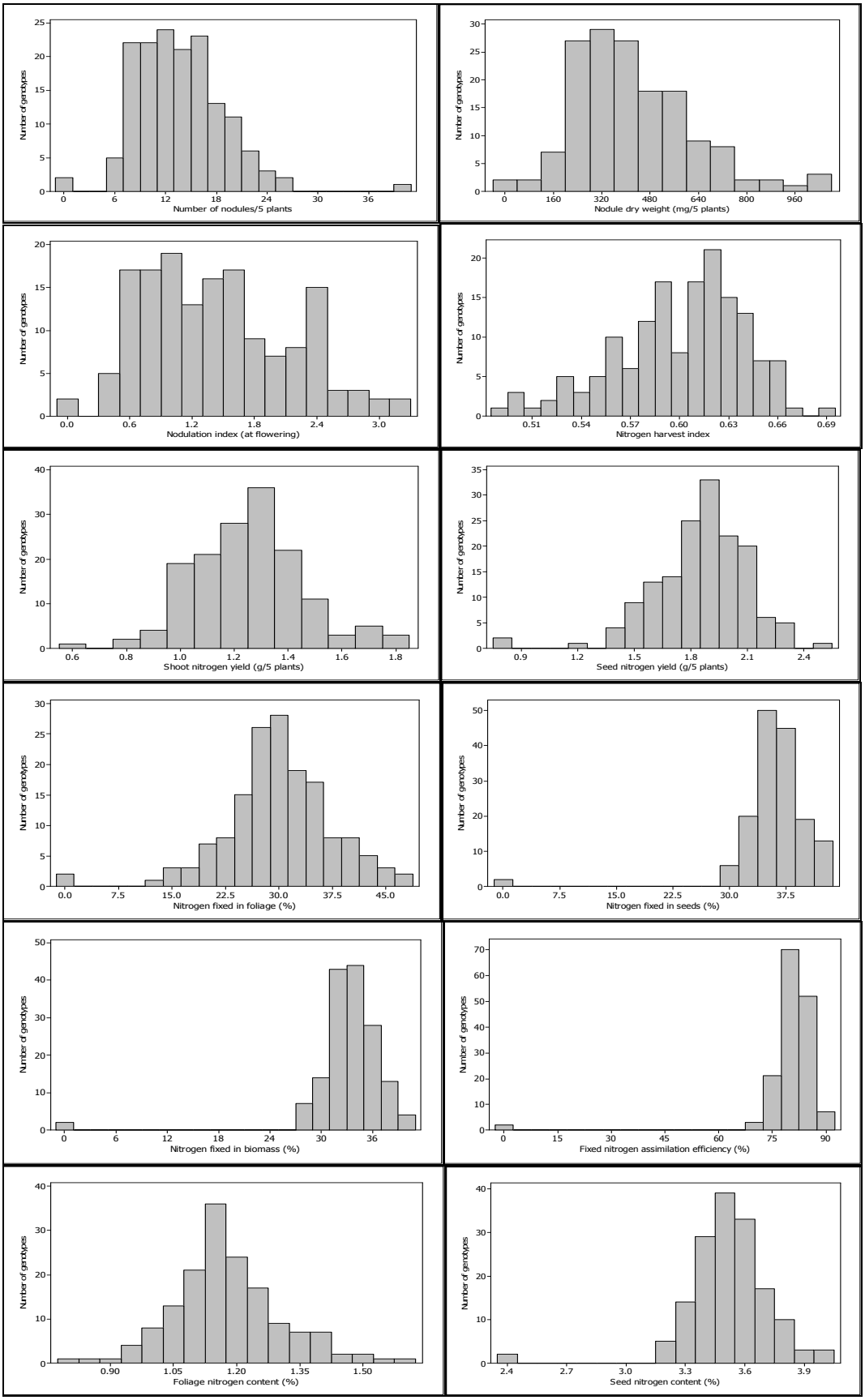



Figure 16. Frequency distributions for twelve symbiotic characters in 155 chickpea genotypes evaluated at two locations in Ethiopia

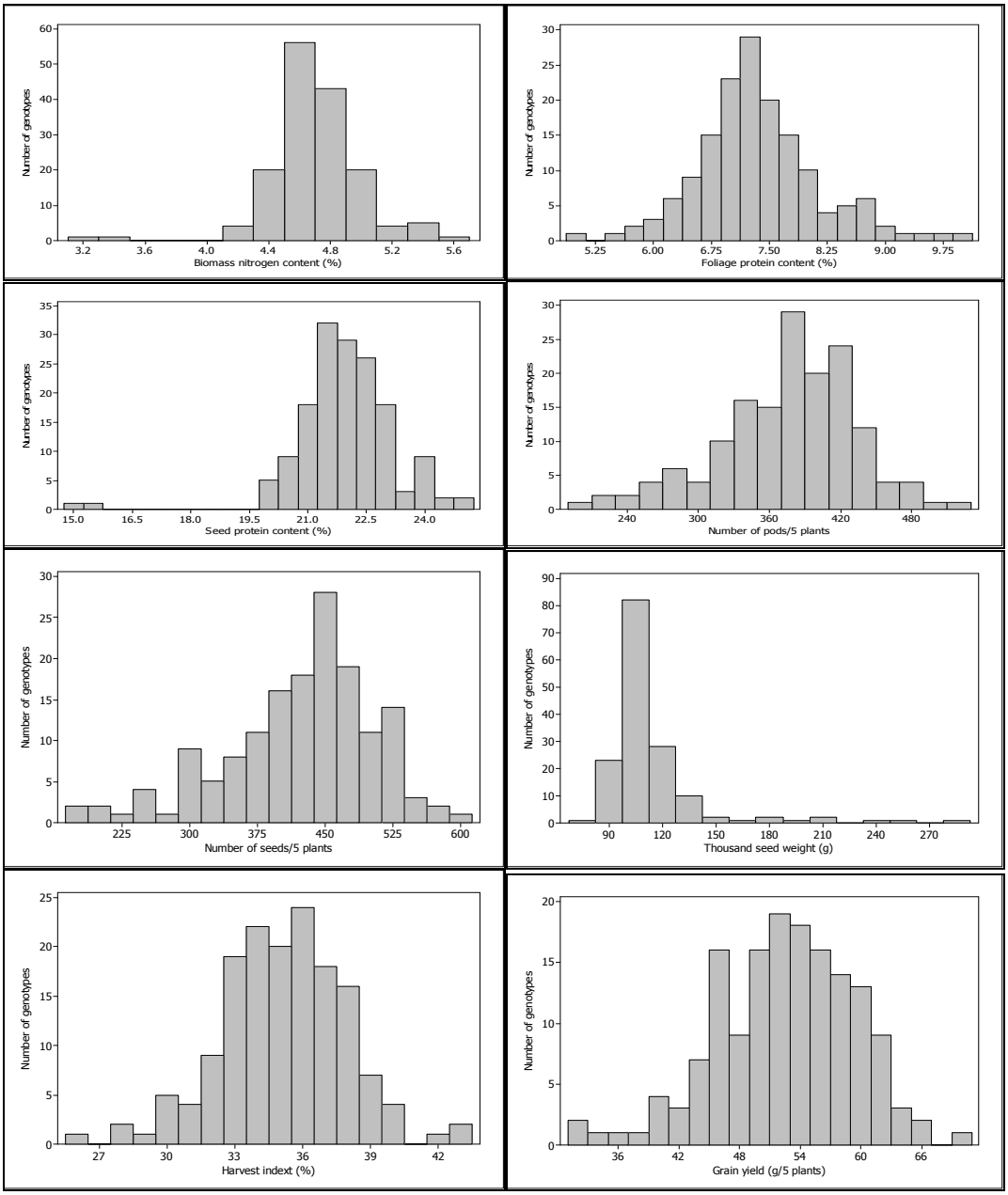



Figure 17. Frequency distributions for eight agronomic characters in 155 chickpea genotypes evaluated at two locations in Ethiopia

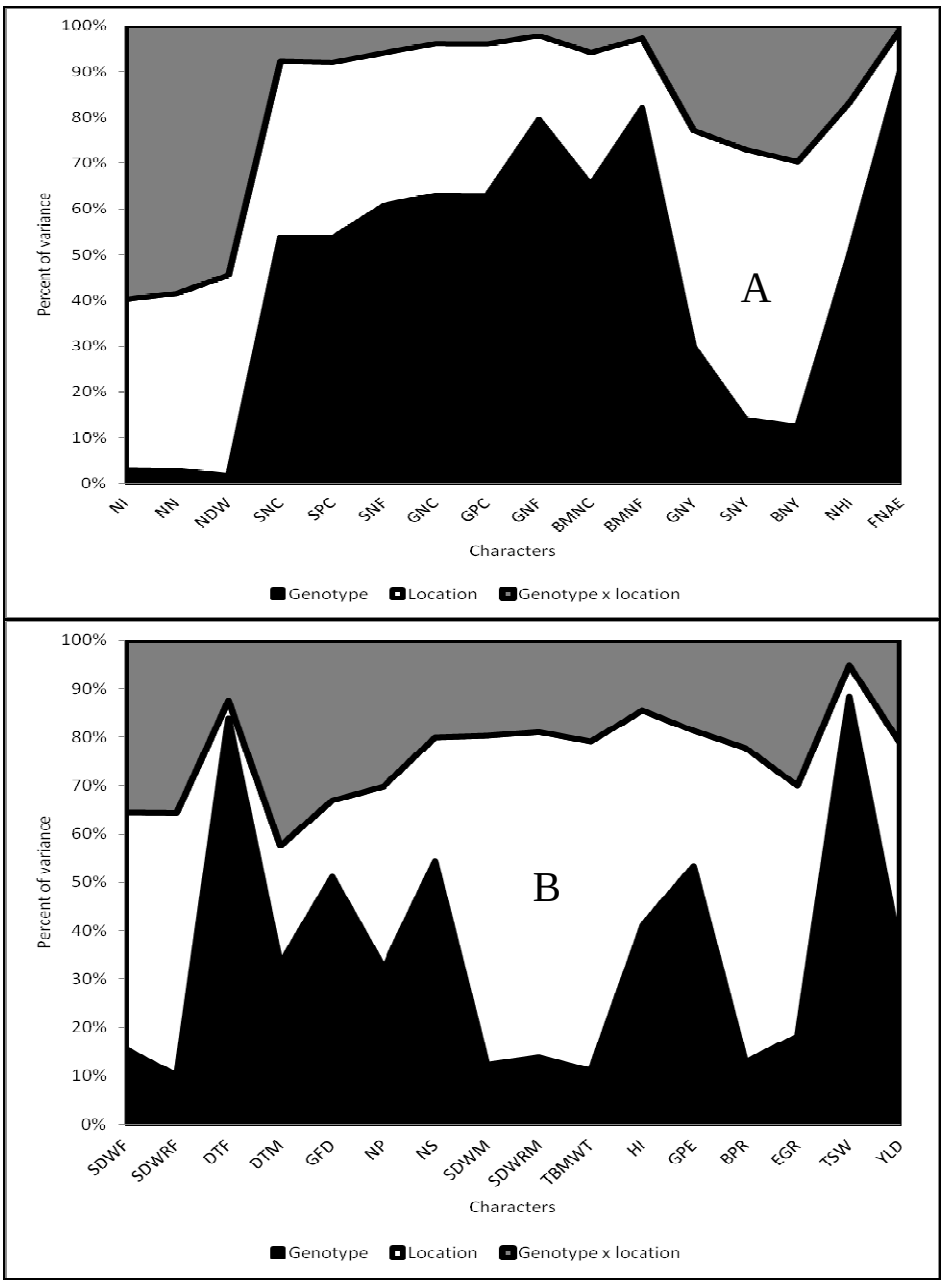



Figure 18. Proportional contribution of components of variation (genotypic, location and genotype by location interaction effects) to the total variability in 155 chickpea genotypes for (A) symbiotic and (B) agronomic characteristics (the abbreviations of characters as given in Tables 11 and 14 above).

#### 4.2.7.3. Broad-sense heritability and expected gains from selection

Broad-sense heritability and expected genetic gains from selection for traits of symbiotic and agronomic significance are presented in Figure 19. From among the symbiotic characters, the highest broad-sense heritability values ranging from 80-91% were obtained for fixed nitrogen assimilation efficiency, and above ground biomass and seed nitrogen fixation. Shoot, seed and biomass nitrogen contents showed slightly better broad-sense heritability values ranging from 54-65%. As derivative traits, values of broad-sense heritability and expected genetic gains from selection for shoot, seed and biomass protein contents followed the same pattern (but 6.25 times higher in magnitude) as nitrogen contents of the same components.

All characters related to nodulation (nodule number, nodule dry weight and nodulation index) generally exhibited relatively lower genotypic variation and broad sense heritability. Similarly, shoot and above ground biomass nitrogen yield had slightly better genotypic components of variation than nodulation but still very low as compared to the variation of location and genotype by location interaction components. The same traits obviously showed the least levels of broad-sense heritability values of 14% and 13%, respectively. This may necessitate the need for evaluation of additional germplasm materials for these traits.

At 5% selection intensity, nitrogen fixation in shoot, seed and biomass and nitrogen assimilation efficiency showed the highest expected genetic gains from selection as percent of mean, with values ranging from 34-58%. Even though shoot and biomass nitrogen contents showed high heritable variations, values for expected genetic gains from selection were low (Figure 19). Seed size and days to flowering showed the highest broad-sense heritability of 88% and 84%, respectively. Seed fill duration, number of seed and grain production efficiency showed modest broad-sense heritability values of 51% to 54%. Grain yield, harvest index, number of pods per plant and days to maturity had relatively lower heritability values ranging from 33-41%.

Other characters including shoot and biomass dry weights and biomass production and economic growth rates had the least heritability values of 11-18%. Seed size revealed the highest expected genetic gain from selection of 57% at 5% selection intensity. In this study, the highest broad-sense heritability and expected genetic gain from selection associated with seed size may be attributed to the existence of uniquely large-seeded introduced materials from ICRISAT and ICARDA. Expected gains from selection in genotypes tested here revealed that number of pods and seeds, grain production efficiency and grain yield could be improved by about 20-38% (Figure 19).

Genetic enhancement of these accessions to increase the frequency of best performing genes through further intra-accession selections would be expected to result in more promising lines. Nevertheless, it may be assumed that the effects of location and genotype by location interaction were found to influence traits with low broad-sense heritability and expected genetic gains from selection more strongly than the genetic effects. According to Singh (2002), selection for a trait with a high heritability (80% or more) can be fairly easy. There would be a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to the phenotype.

Selection could be difficult or virtually impractical for a trait with low heritability, say less than 40% due to the environment concealing genotypic effects. Thus, it would be very difficult, to improve symbiotic traits like number of nodules, nodule dry weight and nodulation index with the genotypes. Likewise, it would be difficult to improve agronomic characters like shoot and biomass dry weight, biomass production and economic growth rates in these genotypes because of higher genotype by environment and stronger environmental influences.

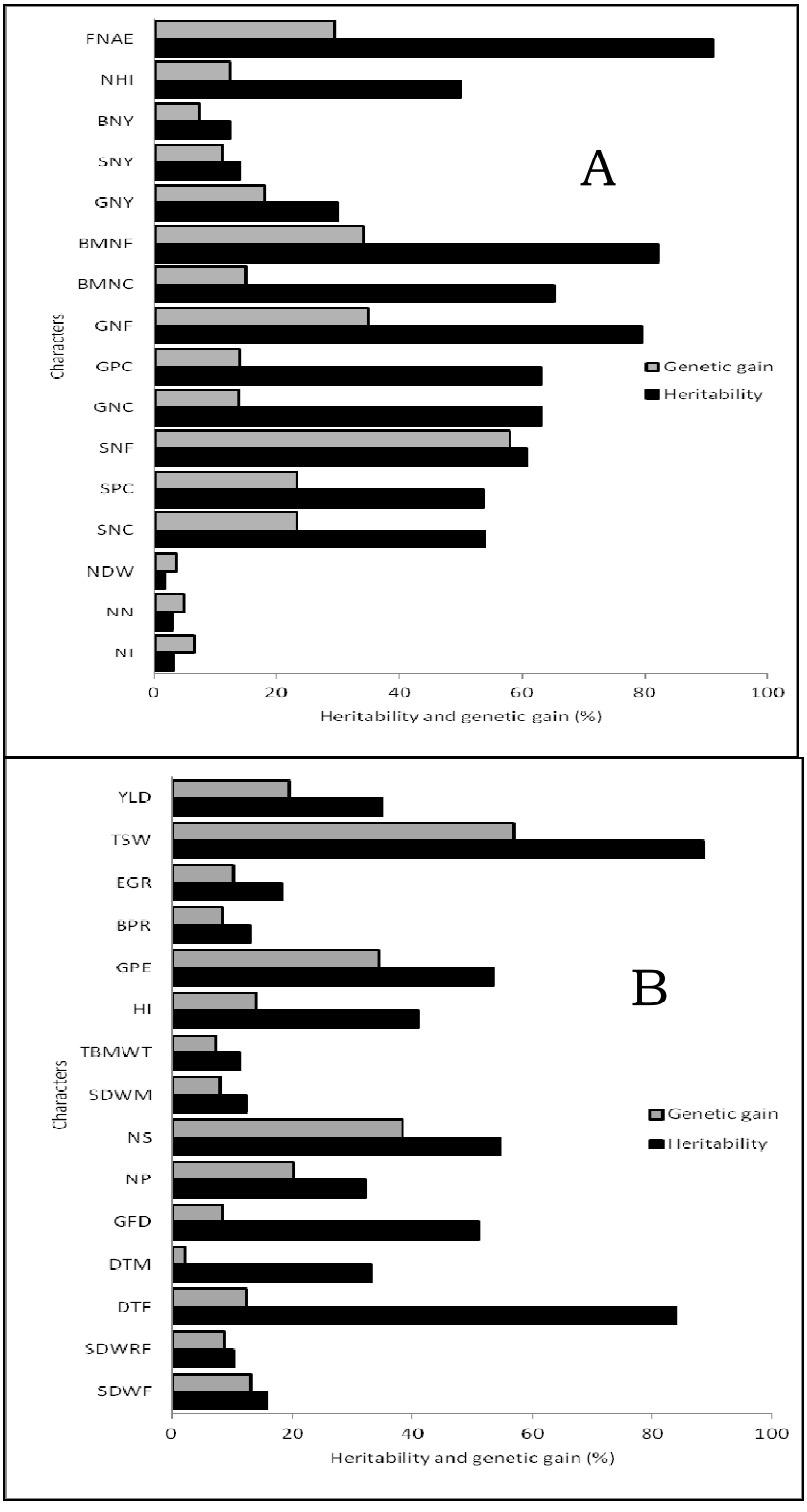



Figure 19. Estimates of broad-sense heritability ($h_b^2$) and expected genetic advance (GA) from selection in 155 chickpea genotypes for (A) symbiotic and (B) agronomic characteristics. SPC = shoot protein content, GPC = grain protein content, SDWRF = shoot dry weight ratio at flowering, and other abbreviations of characters as given in Tables 11 and 14 above.

#### 4.2.7.4. Comparison of selected genotypes with the original population

The Z-test showed significant differences between means of the selected subsets of the top 5% best genotypes ($\overline{X}$) and the population parameters (μ) for all traits at phenotypic level. Comparison of the average performance for respective characters of the selected subsets of the top 5% best genotypes with the average performances of the whole population for symbiotic characters revealed possibilities for different level of improvements through selection, ranging from 11% for fixed nitrogen assimilation efficiency to 119% for nodule dry weight. Above ground biomass nitrogen fixation can also be improved by 18%.

Likewise, possible improvements through selection for agronomic characters, disregarding phenological characters, also ranged from 17% for harvest index to 93% for seed size but the highest possible gain in the latter may be only due to existence of introduced varieties in the test genotypes. Grain yield can be improved by 23% (Table 23). This indicated that the selected accessions were not true representatives of the population and that almost all characters effectively responded to phenotypic selection (Singh, 2001).

It should be noted, however, that selection of superior genotypes based on the phenotypic performances of genotypes under one set of environments may not repeat under another set of environments at least for the characters that showed genotype by environment interaction effects. This is because the phenotype is the result of not only genetic but also environmental and genotype by environment interaction effects (Singh, 2002). When selecting for phenotypic performance in the presence of significant genotype by environment interaction, particularly for primary traits of economic importance like grain yield, the plant breeder should weigh the importance of performance consistency of genotypes across environments to its average performance over environments, i.e. performance stability.

Table 23. Comparison of the mean performances of selected subsets ($\bar{X}$) of the 5% best accessions for symbiotic and agronomic characters with the average performances of the whole population (μ) of 155 chickpea genotypes

| Characters[1] | Mean of selected genotypes ($\bar{X}$) | Population parameter (μ) | Change through selection ($\bar{X}$-μ) | Change as % of population parameter (μ) | Z |
|---|---|---|---|---|---|
| Symbiotic characters | | | | | |
| NI | 2.93 | 1.43 | 1.50 | 104.90 | 6.05** |
| NN | 25.50 | 13.21 | 12.29 | 93.04 | 6.66** |
| NDW | 918.91 | 420.19 | 498.72 | 118.69 | 7.18** |
| SNC | 1.48 | 1.18 | 0.30 | 25.42 | 6.59** |
| SNF | 45.06 | 29.97 | 15.09 | 50.35 | 5.66** |
| GNC | 3.90 | 3.51 | 0.39 | 11.11 | 5.37** |
| GNF | 42.52 | 35.95 | 6.57 | 18.28 | 3.67** |
| BMNC | 5.35 | 4.69 | 0.66 | 14.07 | 6.28** |
| BMNF | 39.25 | 33.24 | 6.01 | 18.08 | 3.67** |
| FNAE | 88.91 | 80.28 | 8.63 | 10.75 | 2.46* |
| GNY | 2.31 | 1.85 | 0.46 | 24.86 | 5.12** |
| SNY | 1.73 | 1.25 | 0.48 | 38.40 | 6.59** |
| BNY | 3.79 | 3.10 | 0.69 | 22.26 | 5.16** |
| NHI | 0.67 | 0.60 | 0.07 | 11.67 | 5.03** |
| Agronomic characters | | | | | |
| SDWF | 50.66 | 37.4 | 13.26 | 35.45 | 6.60** |
| NP | 483 | 376 | 107.00 | 28.46 | 5.27** |
| NS | 557 | 421 | 136.00 | 32.30 | 4.62** |
| SDWM | 137.34 | 105.87 | 31.47 | 29.73 | 6.61** |
| BMWT | 196 | 152 | 44.00 | 28.95 | 6.43** |
| HI | 40.99 | 35.15 | 5.84 | 16.61 | 5.91** |
| GPE | 68.75 | 54.12 | 14.63 | 27.03 | 4.31** |
| BPR | 174 | 133 | 41.00 | 30.83 | 6.67** |
| EGR | 114 | 92 | 22.00 | 23.91 | 5.52** |
| TSW | 220.03 | 113.8 | 106.23 | 93.35 | 10.35** |
| YLD | 64.67 | 52.53 | 12.14 | 23.11 | 4.97** |

[1] Abbreviation of characters as given in Tables 11 and 14 above;
** = highly significant ($P \leq 0.01$) and * = significant ($P \leq 0.05$)

## 4.3. Genetic characterization and evaluation for phosphorus uptake and use efficiency

### 4.3.1. Performance of the genotypes

Significant differences ($P \leq 0.01$) were observed among the genotypes for all characters (Table 24). The comparison of test genotypes with the standard variety Natoli, showed the existence of a number of superior landraces for plant tissue phosphorus contents, phosphorus yields, phosphorus uptake and use efficiency and for other agronomic characters including grain yield (Figures 20 and 21). However, no landrace was superior or even comparable to the released varieties in general and Natoli in particular for seed size. This is related to the special attention recently given to the introduction, evaluation and release of large-seeded chickpea varieties in response to the market demand (EARO, 2000; MoA, 2010).

The comparison of all genotypes for parameters of plant tissue phosphorus contents and phosphorus yields, and phosphorus uptake and use efficiency showed existence of better genotypes to the released varieties including Natoli (Appendices 5 and 7). This indicated the possibilities for developing superior varieties for phosphorus use efficiency to the released ones in using chickpea landraces collected from Ethiopia as source materials. A disaggregated comparison of the best responder, efficient genotypes for grain yield and their response to phosphorus application was made with the released varieties. The best genotypes gave yields of 63-80g/5 plants with phosphorus and 53-73g/5 plants without phosphorus. Similarly, the varieties released so far gave yield ranges of 32-72g/5 plants and 32-55 g/5 plants with and without phosphorus fertilizer, respectively.

The selected genotypes revealed yield responses ranging from 0-33%, the best being Acc. Nos. 207763 (33%), 207742 (26%) and 207563 (19%) (Figure 22). The released varieties showed ranges from no yield response in Shasho to 32% yield increment in Habru, with an average of 16% without phosphorus fertilizer. The three top released varieties with best yield response to application of phosphorus include Habru (32%), Arerti (28%) and Natoli (18%) in that order.

Table 24. Combined analysis of variance (over locations and phosphorus level) for attributes of phosphorus use efficiency and agronomic performance in 155 chickpea genotypes tested at two locations in Ethiopia

| Character | Mean square[1] | | | | | | | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | L | G | P | G × L | G × P | P × L | G × L × P | |
| Phosphorus contents and yields | | | | | | | | |
| Shoot P content (SPC, g/5 plants) | ** | ** | ** | ** | * | NS | NS | 22.04 |
| Grain P content (GPC, g/5 plants) | ** | ** | ** | NS | NS | ** | NS | 23.29 |
| Biomass P content (BMPC, g/5 plants) | ** | ** | ** | ** | NS | NS | NS | 25.04 |
| Shoot P yield (SPY, mg/5 plants) | ** | ** | ** | ** | * | NS | NS | 27.47 |
| Grain P yield (GPY, mg/5 plants) | ** | ** | ** | ** | NS | ** | NS | 24.31 |
| Biomass P yield (BMPY, mg/5 plants) | ** | ** | ** | ** | NS | NS | NS | 21.24 |
| Phosphorus harvest index | ** | ** | ** | * | NS | NS | NS | 11.53 |
| Phosphorus uptake and use efficiency | | | | | | | | |
| Apparent use of P from fertilizer and soil (APUfs, %) | ** | ** | --- | * | --- | --- | --- | 19.86 |
| Apparent use of P from fertilizer (APUf, %) | NS | ** | --- | NS | --- | --- | --- | 24.95 |
| Apparent use of P from soil (APUs, %) | ** | ** | --- | NS | --- | --- | --- | 21.91 |
| Phosphorus yield efficiency (PYE, GY/P applied) | NS | ** | --- | NS | --- | --- | --- | 24.95 |
| Phosphorus physiological efficiency (PPE, GY/P in plant) | ** | ** | --- | NS | --- | --- | --- | 15.98 |
| Agronomic characters | | | | | | | | |
| Days to 50% flowering (DTF) | ** | ** | NS | ** | NS | NS | NS | 3.92 |
| Days to 90% maturity (DTM) | ** | ** | NS | ** | NS | NS | NS | 2.95 |
| Grain filling period (GFP) | ** | ** | NS | ** | NS | NS | NS | 6.89 |
| No of pods (NP, 5 plants$^{-1}$) | ** | ** | ** | ** | NS | NS | NS | 21.58 |
| No of seeds (NS, 5 plants$^{-1}$) | NS | ** | ** | ** | NS | NS | NS | 23.35 |
| Shoot dry matter weight (SDMW, g 5 plants$^{-1}$) | ** | ** | ** | * | NS | NS | NS | 24.61 |
| Total biomass weight (BMWT, g 5 plants$^{-1}$) | ** | ** | ** | * | NS | NS | NS | 21.04 |
| Harvest index (HI) | ** | ** | NS | NS | NS | NS | NS | 16.03 |
| Grain production efficiency (GPE, g 5 plants$^{-1}$) | ** | ** | ** | ** | NS | NS | NS | 22.37 |
| Biomass production rate (BPR, %) | ** | ** | ** | ** | NS | NS | NS | 20.68 |
| Economic growth rate (EGR, %) | NS | ** | ** | * | NS | NS | NS | 21.12 |
| Thousand seed weight (TSW, g) | NS | ** | NS | * | NS | NS | NS | 18.43 |
| Grain yield (YLD, g 5 plants$^{-1}$) | NS | ** | ** | * | NS | NS | NS | 24.95 |

[1]L = location, G = genotype, P = phosphorus level; **=highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and NS = non-significant ($P > 0.05$)

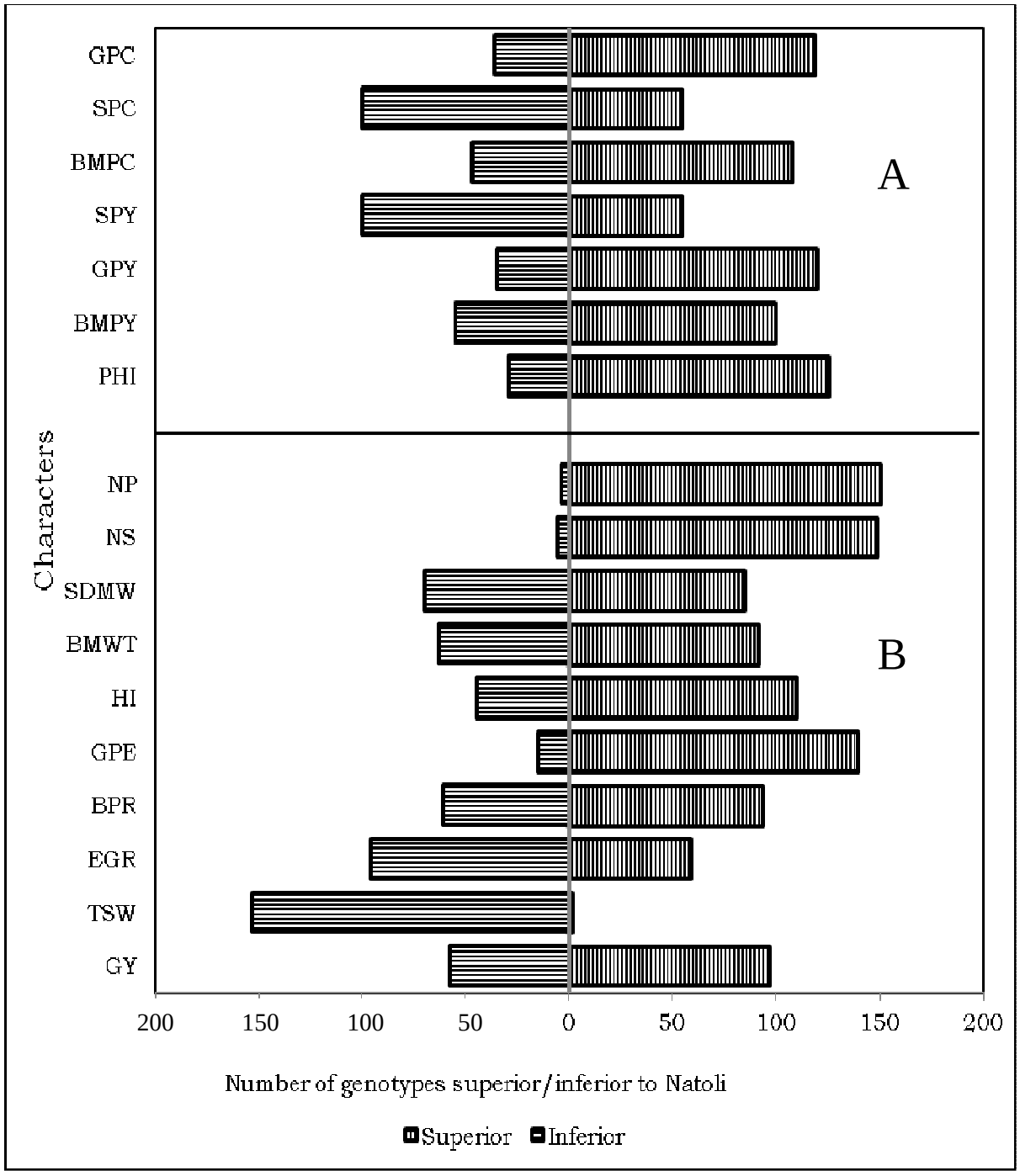



Figure 20. Proportion by number of the 155 chickpea genotypes superior and inferior to the recently released check, Natoli, for (A) plant tissue phosphorus contents and phosphorus yield, and (B) agronomic characters showing superiority of a number of landraces without phosphorus application at two locations in Ethiopia (see Table 24 above for abbreviations of the characters)

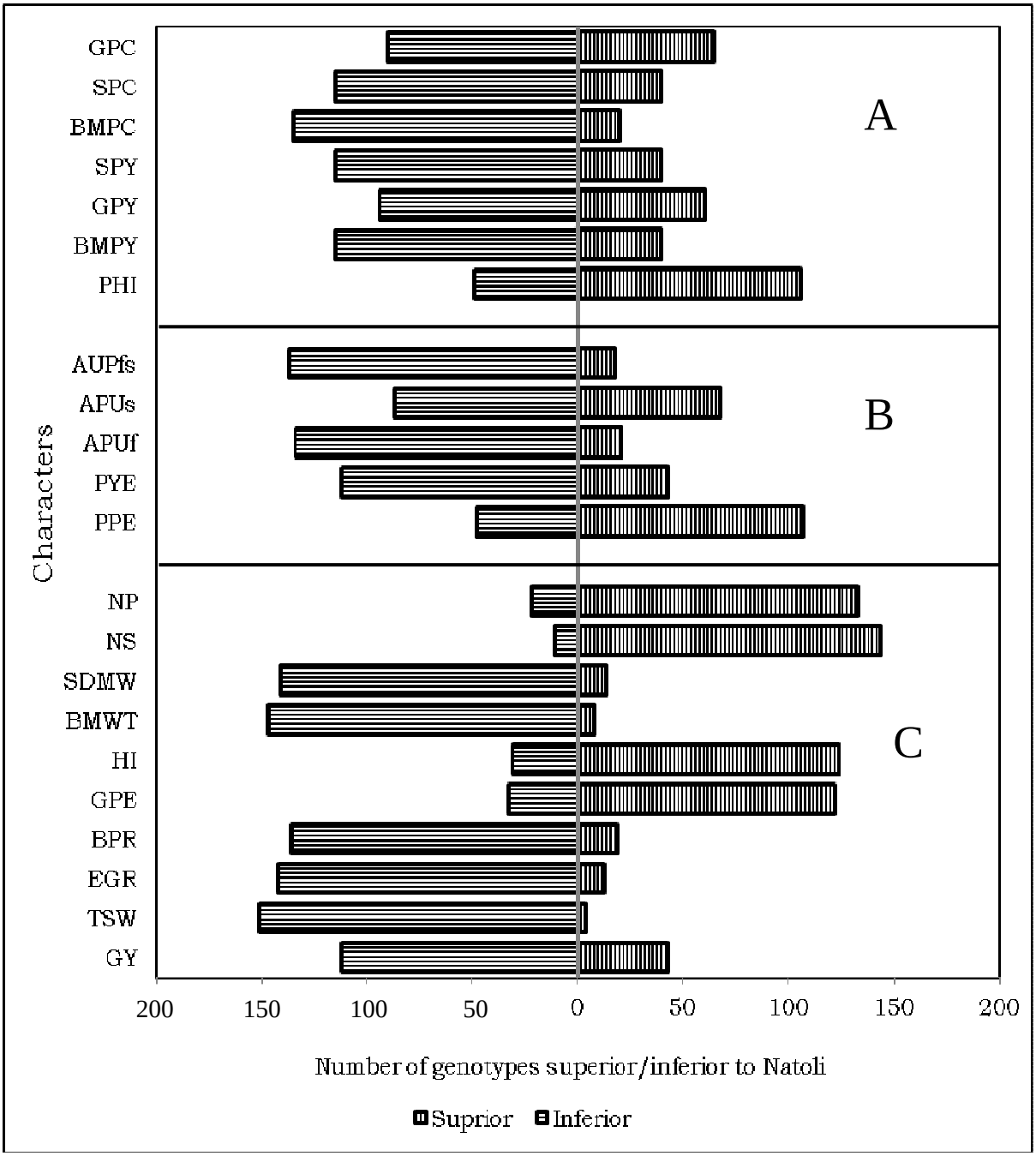



Figure 21. Proportion by number of the 155 chickpea genotypes superior and inferior to the recently released check, Natoli, for (A) plant tissue phosphorus contents and phosphorus yield, (B) phosphorus uptake and use efficiency and (C) agronomic characters showing superiority of a number of landraces with phosphorus application at two locations in Ethiopia (see Table 24 above for abbreviations of the characters)

Whether the yield increase associated with phosphorus application is related to the direct effect of phosphorus on yield and yield components or if it is also related to an indirect effect of phosphorus through enhanced symbiotic nitrogen fixation may need an in depth analysis. However, it was observed that two of the non-nodulating genotypes were not only grouped as inefficient, non-responder but also found to be among the most inferior for grain yield with and without phosphorus (Figure 22).

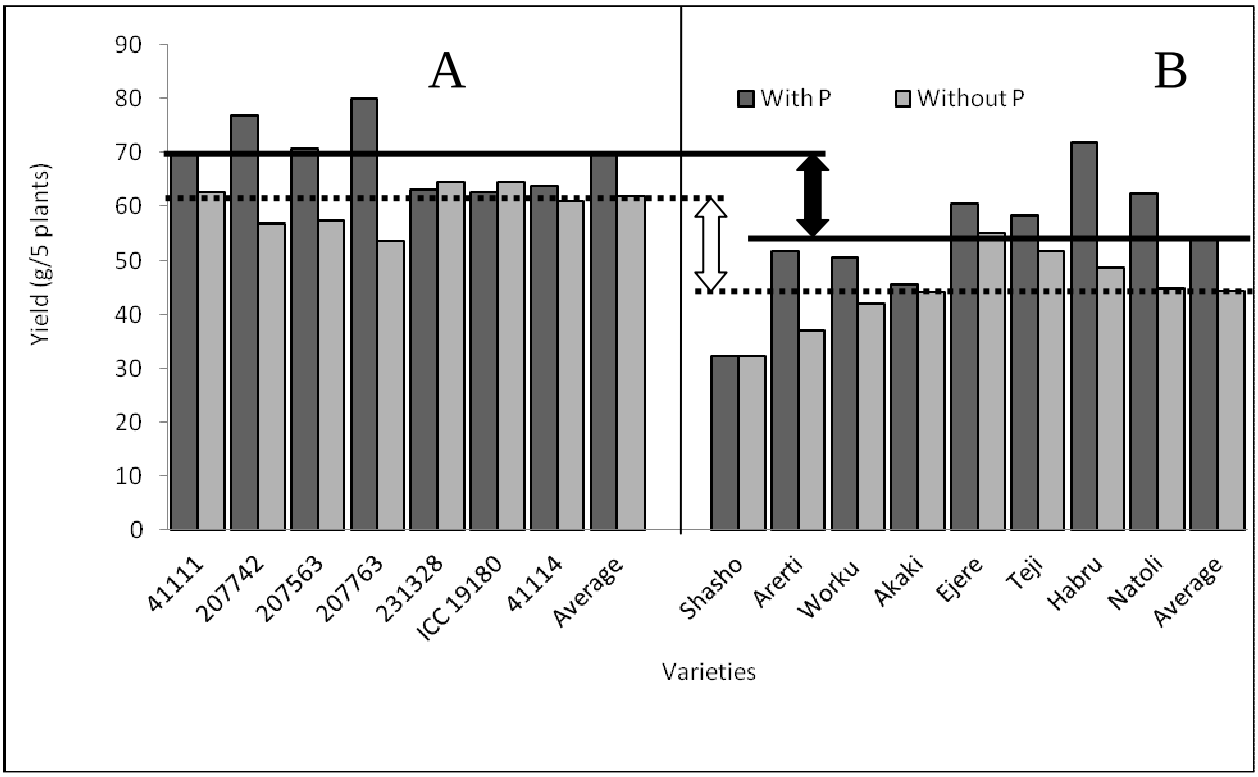



Figure 22. Grain yield performances and response to phosphorus application to the (A) 5% best efficient, responder genotypes as compared to (B) the released chickpea varieties grown with and without phosphorus at Ambo and Ginchi in Ethiopia. The double arrows show the yield advantages expected from the selected genotypes as compared to the released varieties under no phosphorus application (white) and with phosphorus (dark)

Whatever the case may be, this study clearly showed that the application of phosphorus alone can lead to certain levels of yield increment and that it is possible to improve yield performances of the Ethiopian

chickpea landraces through selection under both phosphorus fertilized and unfertilized conditions.

### 4.3.2. Effect of location

The location effects were significant in a number of cases but non-significant for apparent use of phosphorus from fertilizer, phosphorus yield efficiency, number of seeds, biomass and economic growth rates, seed size and grain yield. Likewise, many characters significantly varied ($P \leq 0.01$) between the two phosphorus levels with the exception of phenological characters (i.e. days to flowering and maturity and grain filling period), and harvest index and seed size (Table 24).

### 4.3.3. Genotype by location interaction effects

Genotype by location interaction effects also revealed significant differences ($0.01 \leq$ P or P $\leq 0.05$) for all characters except grain phosphorus content, apparent use of phosphorus from fertilizer, apparent use of phosphorus from soil, phosphorus yield and phosphorus physiological efficiency and harvest index. The genotype by phosphorus and phosphorus by location interaction effects were significant only in two cases each, i.e. for shoot phosphorus content and phosphorus yield, and grain phosphorus content and phosphorus yield, respectively. However, even this limited number of significant genotype by phosphorus level interaction effects observed in this study could be still manageable in breeding programs as the interactions were mostly a "non cross-over" type. That is, despite the existence of significant interaction effects, most of the genotypes more or less consistently maintained their relative rank orders with changes in phosphorus level.

When genotypes perform consistently across locations, on the other hand, breeders are able to effectively evaluate germplasm with a minimum cost in a few locations for ultimate use of the resulting varieties across wider geographic areas. However, with high genotype by location interaction effects, genotypes selected for superior performance under one set of environmental conditions may perform poorly under different environmental

conditions (Singh, 1990; Romagosa *et al.*, 1996; Ceccarelli, 1997). Therefore, it could be implicated that selection of better performing genotypes at one location may not enable the identification of genotypes that can repeat nearly the same performances at another location. However, separate evaluations with and without phosphorus fertilizer may not be necessarily needed at this level as evaluation under any one of the two may serve to identify appropriate genotypes for both conditions.

### 4.3.4. The role of phosphorus in genotypic performance

A similar pattern of genotypic response was observed with the application of phosphorus at Ambo and Ginchi but relatively higher values of plant tissue phosphorus contents, phosphorus uptake and use efficiency and agronomic responses were recorded at Ginchi (Table 25). This, despite better relative contents of phosphorus and other minerals in the soil of Ambo, may be related to the confounded effects of many other factors like better moisture holding capacity of the soil at Ginchi because of the gentler slope.

The comparison of the average genotypic performances with and without phosphorus fertilizer showed different levels of reductions among a number of characters when phosphorus was not applied. The average relative reductions due to phosphorus unavailability ranged from 25-38% in characters related to plant tissue phosphorus contents and phosphorus yields. The relative changes in magnitudes of yield and yield components under no phosphorus application as compared to their magnitude in the presence of phosphorus fertilizer showed that shoot phosphorus content, shoot and biomass phosphorus yields, biomass and grain phosphorus contents and grain phosphorus yield were more sensitive to phosphorus unavailability in that order.

Phosphorus harvest index rather showed a tendency to decrease with the application of phosphorus. This was attributed to the fact that the application of phosphorus fertilizer resulted in higher relative increase in the gross amount of phosphorus (but not concentration) in the shoot relative to the seed. Similarly, the average relative reductions in a number of

quantitative agronomic characters ranged from 0-20% (Table 25). The comparison of the whole set of genotypes grown without phosphorus showed a relative yield reductions of 15-17% or, on average, 16% as compared to the same genotypes grown with phosphorus fertilizer. Ali *et al.* (2002) also reviewed that, depending on the agroclimatic conditions and the genotype, phosphorus deficiency may cause yield losses of 0–45% in chickpea.

Other agronomic characters which showed more sensitive response to phosphorus unavailability include shoot dry matter weight, biomass production rate, number of pods, grain production efficiency, economic growth rate, biomass weight and number of seeds, their relative reductions being in the range of 15-20%. This indicated that a significant portion of yield reduction was attributed to the direct sensitivity of grain yield itself to phosphorus unavailability and to the indirect effects through a number of other component traits associated with grain yield. Differences in plant tissue phosphorus level and yield increments due to the application of phosphorus fertilizer were also previously reported in genetic resources of legume crops (Beebe *et al.*, 2006; Krasilnikoff *et al.*, 2003; Daoui *et al.*, 2012) including chickpea (Walley *et al.*, 2005; Srinivasarao *et al.*, 2006).

Phenological characters (i.e. days to flowering and maturity and grain filling period), harvest index and seed size were least influenced with phosphorus application. Like phosphorus harvest index, grain harvest index rather showed a tendency to reduce with the application of phosphorus fertilizer because phosphorus resulted in higher relative increase in shoot dry matter weight than it resulted in grain yield.

In many food legume crops, root size, microbial symbioses and surface chemical characteristics like root exudates are normally known mechanisms to solublize and mobilize the limiting nutrients including phosphorus (Ascher *et al.*, 2001; Ali *et al.*, 2002; Ojo *et al.*, 2006; Vesterager *et al.*, 2006; Fageria *et al.*, 2008). Chickpea was also reported to produce extensive roots and substantial quantities of organic acids to solubilize phosphorus from the soil (Alloush *et al.*, 2000; Veneklaas *et al.*, 2003; Gahoonia *et al.*, 2007) and mobilize it for the formation of assimilates (Srinivasarao *et al.*, 2006).

Table 25. Mean performances of 155 chickpea genotypes for attributes of phosphorus use and agronomic performance at two P levels and relative reductions due to lack of phosphorus at two locations in Ethiopia

| Character* | Ambo | | | Ginchi | | | Combined | | |
|---|---|---|---|---|---|---|---|---|---|
| | With P | Without P | Relative reduction | With P | Without P | Relative reduction | With P | Without P | Relative reduction |
| Phosphorus related characters | | | | | | | | | |
| GPC (g/5 plants) | 0.196a | 0.150b | 0.235 | 0.251a | 0.186b | 0.259 | 0.224a | 0.168b | 0.250 |
| SPC (g/5 plants) | 0.131a | 0.081b | 0.382 | 0.116a | 0.071b | 0.388 | 0.123a | 0.076b | 0.382 |
| BMPC (g/5 plants) | 0.774a | 0.571b | 0.262 | 0.882a | 0.653b | 0.260 | 0.828a | 0.612b | 0.261 |
| GPY (mg/5 plants) | 196.01a | 150.13b | 0.234 | 250.77a | 186.05b | 0.258 | 223.33a | 168.09b | 0.247 |
| SPY (mg/5 plants) | 131.23a | 81.98b | 0.375 | 115.51a | 71.42b | 0.382 | 123.37a | 76.30b | 0.382 |
| BMPY (mg/5 plants) | 327.24a | 231.32b | 0.293 | 366.28a | 257.47b | 0.297 | 346.76a | 244.39b | 0.295 |
| PHI | 0.600b | 0.650a | -0.083 | 0.688b | 0.723a | -0.051 | 0.644b | 0.686a | -0.065 |
| Agronomic characters | | | | | | | | | |
| DTF | 54.99a | 55.01a | 0.000 | 58.17a | 58.28b | -0.002 | 56.58a | 56.64a | -0.001 |
| DTM | 113.70a | 113.73a | 0.000 | 114.42a | 114.40a | 0.000 | 114.06a | 114.07a | 0.000 |
| GFP | 58.71a | 58.72a | 0.000 | 56.25a | 56.12a | 0.002 | 57.48a | 57.42a | 0.001 |
| NP | 424.19a | 352.32b | 0.169 | 392.56a | 333.84b | 0.150 | 408.37a | 343.08b | 0.160 |
| NS | 456.63a | 390.80b | 0.144 | 454.03a | 384.18b | 0.154 | 455.33a | 387.49b | 0.149 |
| SDMW(g /5 plants$^{-1}$) | 121.43a | 96.91b | 0.202 | 113.53a | 91.60b | 0.193 | 117.48a | 94.25b | 0.198 |
| BMWT (g/5 plants$^{-1}$) | 172.21a | 145.67b | 0.154 | 163.49a | 139.38b | 0.147 | 167.85a | 142.53b | 0.151 |
| HI | 33.75b | 35.51a | -0.052 | 35.52a | 35.84a | -0.009 | 34.63a | 35.67a | -0.030 |
| GPE (g/5 plants$^{-1}$) | 61.82a | 52.59b | 0.149 | 56.26a | 46.59b | 0.172 | 59.04a | 49.59b | 0.160 |
| BPR (%) | 151.21a | 121.92b | 0.194 | 143.16a | 116.98b | 0.183 | 147.19a | 119.45b | 0.188 |
| EGR (%) | 97.81a | 83.62b | 0.145 | 102.22a | 85.12b | 0.167 | 100.02a | 84.37b | 0.156 |
| TSW (g) | 110.43a | 115.99a | -0.050 | 113.46a | 115.31a | -0.016 | 111.94a | 115.65a | -0.033 |
| YLD (g/5 plants$^{-1}$) | 57.19a | 48.76b | 0.147 | 57.30a | 47.66b | 0.168 | 57.25a | 48.21b | 0.158 |

*see Table 24 above for abbreviations of the characters; **Figures within a row and location sharing the same letter indicate statistically non-significant response of the respective character to P application

### 4.3.5. Grouping of the genotypes into phosphorus use efficiency classes

Based on the criteria of nutrient efficiency classification suggested by Gerloff (1977), an efficient cultivar has higher mean performance than the other cultivars under low nutrient supply, while a responder cultivar has higher mean performance under high nutrient supply. Accordingly, 34% of the genotypes were grouped as inefficient, non-responder; 24% as inefficient, responder; 23% as efficient, responder; and 19% as efficient, non-responder for biomass yield (Figure 23). The corresponding classification for grain yield grouped 34% of the genotypes as inefficient, non-responder; 19% as inefficient, responder; 32% as efficient, responder; and 15% as efficient, non-responder (Figures 23).

From this result, it can be implicated that different possible breeding strategies may be sought in order to address different needs under different production domains. First, where farmers can apply adequate amount of phosphorus, varieties that are responsive to soil fertility level may be developed from the responsive sources in order to exploit the yield potential. Secondly, breeding phosphorus efficient chickpea cultivars under phosphorus deficient conditions where farmers cannot afford the application of phosphorus fertilizer could be considered as an alternative strategy. And, thirdly, developing genotypes which can compromise and consistently better perform at both high and low soil phosphorus levels could also be a possibility as such categories of genotypes also existed among the genotypes tested in this study (Figure 23).

Despite the existence of different categories of genotypes as genetic background for selection in different target production, however, plant breeders cannot change the genetic make of crops unless they have full control over the number and type of genotypes to be advanced from one stage of variety trial to another. From the breeding point of view, therefore, it is felt that this classification may not enable plant breeders to impose the proper amount of selection intensity.

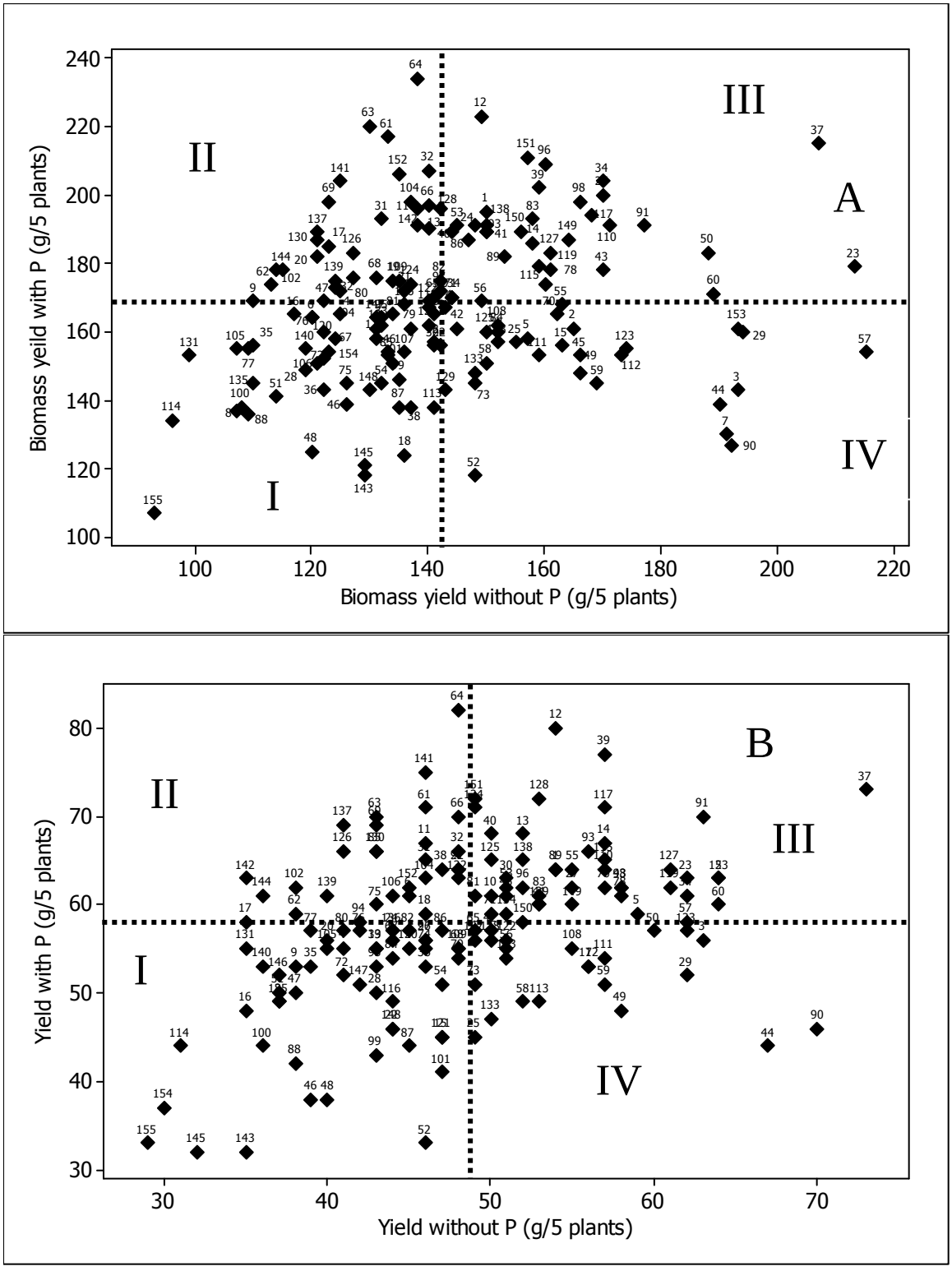



Figure 23. The relationship between (A) biomass and (B) grain yield in 155 chickpea genotypes grown with and without phosphorus at two locations in Ethiopia showing different phosphorus response and use efficiency groups: (I) inefficient, non-responder; (II) inefficient, responder; (III) efficient, responder; and (IV) efficient, non-responder. The serial Nos. Marking the data labels stand for genotypes as given in Table 1.

In the present case, for instance, the top 5% best of the efficient, responder genotypes for grain yield were identified as: Acc. Nos. 41274, 41111, 207742, 207563, 207763, 231328, ICC 19180 and 41114. Three of the accessions, namely Acc. Nos. 41274, 207563 and 41111, also repeated best performances as efficient, responder genotypes for biomass weight. Other efficient, responder genotypes for biomass weight include: Acc. Nos. 207743, 41015, 41066, 41185 and Ejere (Figure 22). This indicated that chickpea genotypes that were found to be efficient, responder based on grain yield may not necessarily repeat the same performance for biomass weight as reported by Srinivasarao *et al*. (2006).

### 4.3.6. Phenotypic diversity

#### 4.3.6.1. Cluster and distance analysis

Cluster analysis grouped the genotypes into five clusters in the absence and six clusters in the presence of phosphorus fertilizer, such that the genotypes within a cluster were more closely related than those belonging to different clusters (Table 26 and Figure 24). The higher number of clusters when the crop was grown with phosphorus may be a manifestation that the application of phosphorus resulted in better expression of genetic diversity than when phosphorus is not applied. The first and the second clusters were larger than the other clusters under phosphorus unfertilized and fertilized conditions.

Genetic distances ($D^2$) of the 155 chickpea genotypes grown without and with phosphorus are presented in Table 27. Inter-cluster $D^2$ values ranged from 11 (between clusters $C_1$ and $C_2$) to 132 (between clusters $C_4$ and $C_5$) when the crop is grown in the absence of phosphorus and from 10 (between clusters $C_1$ and $C_2$) to 162 (between clusters $C_4$ and $C_6$) were obtained with phosphorus. The maximum pairwise generalized squared distances ($D^2$) were found between clusters $C_4$ and $C_5$ ($D^2$ = 132) without phosphorus and between $C_4$ and $C_6$ ($D^2$ = 132) with phosphorus. It is interesting to note that $C_5$ and $C_6$ constituted the non-nodulating (i.e. ICC 19181 and PM 233) references without and with phosphorus, respectively.

Table 26. Clustering of one hundred fifty five chickpea genotypes grown without and with phosphorus into clusters using mean of seventeen response characters to phosphorus and agronomic performance

| Cluster | No of genotypes | Genotypes included in the cluster |
|---|---|---|
| Without phosphorus | | |
| $C_1$ | 106 | 231327, 208829, 209092, 209097, 209098, 41002, 207761, 207763, 207764, 41074, 41075, 41073, 41076, 41021, 41027, 41271, 41276, 207745, 41275, 41277, 207744, 41273, 207741, 41316, 41298, 41311, 41315, 41308, 41299, 41304, 41303, 41295, 41296, 41290, 41291, 41019, 41048, 41049, 41053, 41054, 41052, 209082, 209083, 209084, 209087, 209088, 209089, 209090, 209081, 41159, 41160, 207661, 207667, 207666, 41141, 41128, 41168, 41129, 41130, 41106, 41142, 41207, 41216, 41011, 41007, 41008, 41186, 209035, 41176, 41175, 41174, 209027, 41171, 41195, 41197, 207151, 207564, 207895, 213224, 219797, 219800, 219803, 221696, 212589, 41113, 207659, 207660, 41115, 225878, 225873, 225874, 225877, 207645, 207646, 225876, ICC 5003, ICC 4918, ICC 4948, ICC 4973, ICC 15996, Shasho, Arerti, Worku, Akaki, Habru, Natoli |
| $C_2$ | 42 | 231328, 209094, 41268, 41026, 41222, 207734, 41103, 41320, 41029, 41015, 41272, 207743, 41274, 207742, 41313, 41280, 41312, 41046, 41047, 41289, 41284, 41297, 41293, 209091, 41161, 207665, 41134, 41110, 207657, 41111, 207658, 41215, 41066, 41170, 41185, 209036, 41190, 207150, 207563, 207894, 219799, 41114 |
| $C_3$ | 2 | 209093, 209096 |
| $C_4$ | 3 | Ejere, Teji, ICC 19180 |
| $C_5$ | 2 | ICC 19181, PM 233 |
| With phosphorus | | |
| $C_1$ | 54 | 231327, 209098, 207761, 207763, 207764, 41073, 41027, 207734, 41103, 41015, 41276, 207745, 207743, 41273, 41274, 207742, 41298, 41311, 41047, 41295, 41289, 41284, 41019, 41049, 41053, 41052, 209083, 209084, 209091, 41160, 207667, 41141, 207665, 41128, 41168, 41111, 41106, 207658, 41215, 41216, 41066, 41176, 41171, 41185, 207563, 219800, 219803, 221696, 212589, 207659, 225877, 207645, 207646, ICC 4948 |
| $C_2$ | 88 | 231328, 209093, 208829, 209094, 209092, 209096, 209097, 41002, 41268, 41026, 41074, 41075, 41076, 41021, 41222, 41320, 41029, 41271, 41272, 41277, 207744, 207741, 41316, 41313, 41280, 41312, 41315, 41308, 41046, 41304, 41296, 41290, 41291, 41297, 41293, 41048, 41054, 209082, 209087, 209088, 209089, 209090, 209081, 41159, 41161, 207661, 207666, 41134, 41129, 41130, 41110, 207657, 41142, 41207, 41011, 41007, 41008, 41186, 209035, 41175, 41174, 209027, 41170, 209036, 41190, 41195, 41197, 207150, 207151, 207564, 207894, 207895, 213224, 219797, 219799, 41114, 41113, 207660, 41115, 225878, 225873, 225874, 225876, ICC 5003, ICC 15996, Arerti, Worku, Akaki |
| $C_3$ | 6 | ICC 4918, Ejere, Teji, Habru, Natoli, ICC 19180 |
| $C_4$ | 1 | 41275 |
| $C_5$ | 4 | 41299, 41303, ICC 4973, Shasho |
| $C_6$ | 2 | ICC 19181, PM 233 |

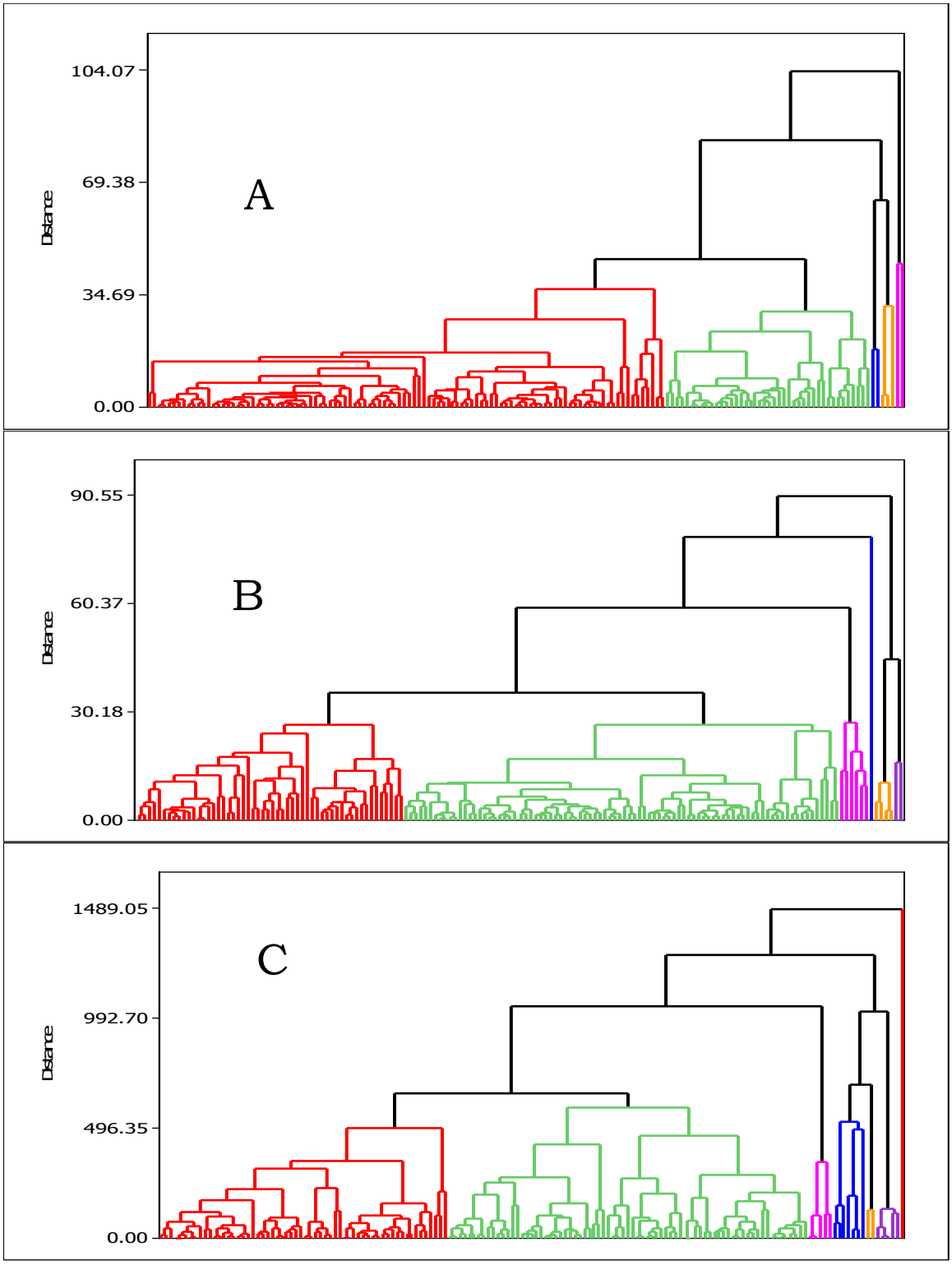



Figure 24. Dendrograms of hundred fifty five chickpea genotypes grown (A) without phosphorus, (B) with phosphorus and dendrogram (C) was built based on attributes of phosphorus uptake and use efficiency parameters (i.e. apparent use of phosphorus fertilizer and/or soil sources, phosphorus physiological and yield efficiencies). The dendrograms were developed by average linkage and Squared Euclidian distance using mean of 17 agronomic characters and attributes of phosphorus use efficiency ($C_1$ = red, $C_2$ = green, $C_3$ = light pink, $C_4$ = blue, $C_5$ = orange and $C_6$ = purple)

The second most divergent groups in the absence of phosphorus were clusters $C_3$ and $C_4$ ($D^2$ = 97) constituting local landraces and introductions, respectively. In the presence of phosphorus, the second most divergent groups were in clusters $C_4$ and $C_5$ ($D^2$ = 152), i.e. between a single local accession versus two local accessions and two introductions, respectively. The genetic divergences between a number of other clusters were also highly significant (Table 27).

Since maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances, crosses between the landraces and introduced genotypes constituted in divergent clusters are expected to provide relatively better genetic recombination and segregation in their progenies.

Selection of parents should, however, consider the special advantages of each cluster and each genotype within a cluster depending on the specific objectives of hybridization. Therefore, this study revealed that the desirable relationship between landrace collections and exotic introductions tends to be mutually complementary. The minimum inter-cluster distances between $C_1$ and $C_2$ indicated that members of these clusters closely related whether they were grown in the presence or absence of phosphorus.

Comparison of $D^2$ values in the absence and presence of phosphorus showed that, not only the number of clusters increased from five to six with the application of phosphorus, but also the $D^2$ values between some clusters also tended to increase under the latter.

More number of clusters and higher cluster distances were obtained when the crop was grown with phosphorus compared to when it was grown without phosphorus. It is generally believed that more conducive environments may be expected to result in better expression of the genetic potential of the genotypes for the traits under consideration (Rosielle and Hamblin, 1981; Simmonds, 1991; Singh, 2002) despite the controversy that there may be no interrelationship between the type of the environment and the magnitude of genetic variation (Ceccarelli and Grando, 1996).

Table 27. Pair-wise generalized squared distances ($D^2$) values between clusters constituting 155 chickpea genotypes grown in the absence and presence of phosphorus fertilizers

| Clusters | Clusters** | | | | | |
|---|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Without phosphorus | | | | | | |
| $C_1$ | 0.00 | 11.02NS | 65.46** | 74.06** | 60.69** | --- |
| $C_2$ | | 0.00 | 42.24** | 68.70** | 65.26** | --- |
| $C_3$ | | | 0.00 | 97.05** | 57.92** | --- |
| $C_4$ | | | | 0.00 | 132.18** | --- |
| $C_5$ | | | | | 0.00 | --- |
| With phosphorus | | | | | | |
| $C_1$ | 0.00 | 9.94 | 80.42** | 42.05** | 50.83** | 78.97** |
| $C_2$ | | 0.00 | 97.01** | 84.09** | 22.90 NS | 64.37** |
| $C_3$ | | | 0.00 | 116.65** | 115.58** | 140.52** |
| $C_4$ | | | | 0.00 | 152.84** | 161.88** |
| $C_5$ | | | | | 0.00 | 47.97** |
| $C_6$ | | | | | | 0.00 |

** = highly significant, $P \leq 0.01$, NS = non-significant, $P > 0.05$

#### 4.3.6.2. Pattern of phenotypic diversity

The pattern of distribution of the genotypes from different origins over different clusters was apparently random, showing that there was no clear association between geographic sources of origin and genetic diversity. Some genotypes from the same places of origin fell into different clusters and vice versa (Tables 28). This is as opposed to genetic diversity and cluster analysis of the same genotypes using microsatellite markers which showed definite association between pattern of genetic diversity and geographic sources of origin as already discussed (sub-section 4.1.2.).

The distribution of local accessions over the clusters was almost entirely limited to clusters $C_1$ and $C_2$, with the exception of two accessions from Arsi which fell in cluster $C_3$ when grown without phosphorus and one accessions

from West Gojam and other two from North Gonder which fell into clusters $C_4$ and $C_5$ that grew with phosphorus, respectively. The rest of the genotypes which were grouped into clusters $C_3$-$C_5$ without phosphorus and $C_3$-$C_6$ with phosphorus trace their genetic background back into introductions from ICARDA or ICRISAT. Despite this distinct pattern of variation in a number of cases, however, overlappings were found among local landraces and introductions mostly in clusters $C_1$ and $C_2$. The partial overlapping of genotypes across geographical boundaries is an indication that geographical isolation is not the only factor causing genetic diversity (Sharma and Mehta, 1990).

It should be noted here that the non-nodulating references were separately grouped from all the other genotypes into cluster $C_5$ without phosphorus and cluster $C_6$ with phosphorus. This may be related to their inferior multitrait performance for almost all traits, except maybe seed size. Even if relatively more distinct pattern of variation was revealed between the introduced genotypes and the local accessions, it was generally observed that genotypes may show morpho-agronomical similarities regardless of the differences in places of origin.

#### 4.3.6.3. Principal component analysis

Principal component analysis indicated that the first vectors were more important than the second and all the other vectors both in the absence and presence of phosphorus fertilizer. The first principal components accounted for 58% of the total multitrait standardized variations without phosphorus and 52% with phosphorus. The corresponding values for the second principal components were 21% and 23% in that order. The first two principal components of the parameters of phosphorus use efficiency accounted for 42% and 31%, respectively. Totally, the first five principal components accounted for 97% of the total variation without phosphorus, 94% with phosphorus and 100% for parameters of phosphorus use efficiency (Tables 29).

Table 28. Clustering pattern of 155 chickpea genotypes from different origins over six clusters based on mean performance of 17 characters

| Origin | No. of genotypes | No. of genotypes in each cluster | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Without phosphorus | | | | | | | |
| Arsi | 13 | 9 | 2 | 2 | - | - | - |
| East Gojam | 13 | 6 | 7 | - | - | - | - |
| West Gojam | 13 | 8 | 5 | - | - | - | - |
| North Gonder | 13 | 8 | 5 | - | - | - | - |
| South Gonder | 12 | 8 | 4 | - | - | - | - |
| West Haragie | 11 | 10 | 1 | - | - | - | - |
| East Shewa | 13 | 10 | 3 | - | - | - | - |
| North Shewa | 13 | 7 | 6 | - | - | - | - |
| West Shewa | 13 | 9 | 4 | - | - | - | - |
| Tigray | 12 | 8 | 4 | - | - | - | - |
| South Wello | 13 | 12 | 1 | - | - | - | - |
| Introduction | 16 | 11 | - | - | 3 | 2 | - |
| Total | 155 | 106 | 42 | 2 | 3 | 2 | - |
| With phosphorus | | | | | | | |
| Arsi | 13 | 5 | 8 | - | - | - | - |
| East Gojam | 13 | 4 | 9 | - | - | - | - |
| West Gojam | 13 | 7 | 5 | - | 1 | - | - |
| North Gonder | 13 | 3 | 8 | - | - | 2 | - |
| South Gonder | 12 | 6 | 6 | - | - | - | - |
| West Haragie | 11 | 4 | 7 | - | - | - | - |
| East Shewa | 13 | 6 | 7 | - | - | - | - |
| North Shewa | 13 | 6 | 7 | - | - | - | - |
| West Shewa | 13 | 3 | 10 | - | - | - | - |
| Tigray | 12 | 4 | 8 | - | - | - | - |
| South Wello | 13 | 5 | 8 | - | - | - | - |
| Introduction | 16 | 1 | 5 | 6 | - | 2 | 2 |
| Total | 155 | 54 | 88 | 6 | 1 | 4 | 2 |

Under no-phosphorus condition, the five top important characters responsible for genetic divergence in the major axis include biomass dry weight (+ 0.305), biomass production rate (+ 0.298), grain phosphorus content (+ 0.296), grain phosphorus yield (+0.296) and grain yield (+ 0.295). Economic growth rate, biomass phosphorus content and yield, shoot dry matter weight and grain production efficiency, with vector weights ranging from + 0.290-0.268, had almost equal contributions. The least contributors were seed size and phosphorus and grain harvest indices.

Similarly, under phosphorus fertilized condition, the five top important characters responsible for genetic divergence in the major axis include grain yield (- 0.314), grain phosphorus yield (- 0.303), grain phosphorus content (- 0.302), economic growth rate (-0.299) and biomass phosphorus yield (- 0.296). Biomass production rate, biomass dry weight, grain production efficiency, shoot dry matter weight and biomass phosphorus content were also important. The least contributors were again seed size and phosphorus and grain harvest indices.

Among the parameters of phosphorus use efficiency, phosphorus yield efficiency contributed the largest magnitude (+ 0.611) to the differentiation of the population into clusters followed by apparent use of phosphorus from fertilizer and soil (+ 0. 594) and apparent use of phosphorus from fertilizer (+ 0.475). Apparent use of phosphorus from soil (+ 0.132) and phosphorus physiological efficiency (+ 0.180) contributed the least amount. All parameters of phosphorus use efficiency significantly contributed to the differentiation of the population through the second principal component except phosphorus yield efficiency.

It is normally assumed that characters with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). Accordingly, many characters contributed to the total variation and, therefore, the differentiation of the genotypes into different clusters was rather dictated by the cumulative effects of a number of characters.

To examine the contribution of the traits to the total genetic divergence among the genotypes, an ordination was conducted between the first two principal components both in the absence and presence of phosphorus. The length of lines (i.e. vectors from the origin) indicates the importance of a given character to the total variation by the two principal components.

Based on their relative contribution to the first two principal components ($PC_1$ and $PC_2$) as reflected by the absolute values of vector weights, the characters may be stratified into four distinct groups. The first group included biomass phosphorus content and phosphorus yield, shoot and biomass dry matter weight, biomass production and economic growth rates, grain phosphorus content and phosphorus yield, grain production efficiency and yield. The second contributors included number of pod and seed. The third group included shoot protein content and phosphorus yield. The last group included seed size and phosphorus and grain harvest indices.

The important characters mentioned above had the same main characters had the same pattern of contribution both in the presence and absence of phosphorus except the change in direction. It is interesting to note that phosphorus yield efficiency had also maintained its best position in terms of contribution to the total genetic divergence by the attributes of phosphorus use efficiency showing the potential to improve this character through selection among the Ethiopian chickpea gene pool (Figure 25).

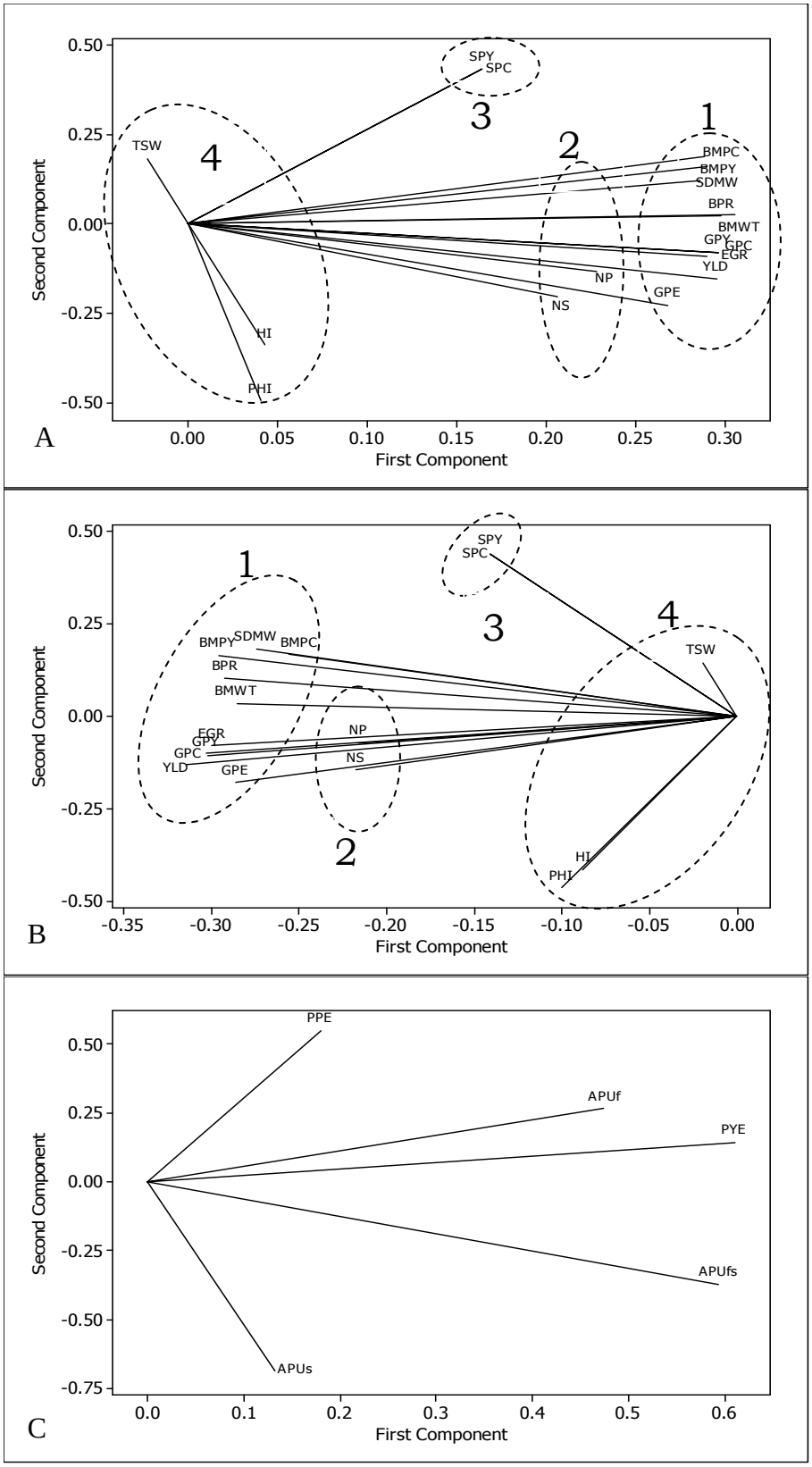



Figure 25. Loading plots of the first two principal components showing the contribution of different phosphorus-related and agronomic characters to the total variation by the two components (A) in the absence of phosphorus, (B) in the presence of phosphorus and (C) for attributes of phosphorus use efficiency in 155 chickpea genotypes. *Refer to Table 24 for the abbreviation of characters

### 4.3.7. Interrelationships between characters

#### 4.3.7.1. Relationships between phosphorus characters

There were significant ($P \leq 0.01$) positive associations of different degree within plant tissue (shoot, seed and biomass) phosphorus contents (r = 0.22-0.85), between plant tissue phosphorus contents and phosphorus yields (r = 0.22-0.99), and within plant tissue phosphorus yields (r = 0.23-0.89). Phosphorus harvest index also associated positively with grain phosphorus content and with grain phosphorus yields but showed negative association with shoot phosphorus content and shoot phosphorus yield. The association between phosphorus harvest index and biomass phosphorus content was also negative (r = -0.18) when phosphorus was not applied and positive (r = 0.64) when phosphorus was applied (Table 30).

This indicated that the proportional allocation of phosphorus to grain was increased with the external application of phosphorus in the form of fertilizer. Phosphorus harvest index was non-significantly associated with biomass phosphorus yield both in the absence and presence of phosphorus. Therefore, selection for more plant tissue phosphorus contents will increase total phosphorus yields while selection for better grain phosphorus content and grain phosphorus yield would result in better phosphorus harvest index. Selection for shoot phosphorus content and shoot phosphorus yield, on the other hand, could result in a negative response of phosphorus harvest index.

There were good levels of positive correlation coefficients (r = 0.29-0.96) between apparent uptakes of phosphorus from both soil and fertilizer and plant tissue phosphorus contents. Phosphorus yield efficiency was also increased with increased plant tissue phosphorus contents and phosphorus yields. Phosphorus physiological efficiency was positively associated with grain phosphorus content (r = 0.32) and grain phosphorus yield (r = 0.31), negatively with shoot (r = -0.39) and biomass (r = -0.23) phosphorus contents and with shoot biomass yield (r = -0.39) but not significantly associated with biomass phosphorus yield (r = 0.02).

Table 29. Eigenvalue, percentage and cumulative variances and eigenvectors on the first five principal components for phosphorus-related and agronomic characters* in hundred fifty five chickpea genotypes grown in the absence and presence of phosphorus

| | Principal components (PCs) | | | | |
|---|---|---|---|---|---|
| | $PC_1$ | $PC_2$ | $PC_3$ | $PC_4$ | $PC_5$ |
| Parameter | Without phosphorus | | | | |
| Eigenvalue | 9.88 | 3.51 | 1.69 | 1.00 | 0.39 |
| Proportion (%) | 58.10 | 20.60 | 9.90 | 5.80 | 2.30 |
| Cumulative (%) | 58.10 | 78.70 | 88.60 | 94.40 | 96.70 |
| Characters | ---------- Eigenvectors ---------- | | | | |
| GPC | 0.296 | -0.081 | -0.118 | 0.163 | 0.388 |
| SPC | 0.164 | 0.433 | 0.036 | 0.249 | -0.131 |
| BMPC | 0.288 | 0.188 | -0.001 | 0.093 | 0.320 |
| SPY | 0.164 | 0.433 | 0.036 | 0.249 | -0.130 |
| GPY | 0.296 | -0.080 | -0.113 | 0.170 | 0.387 |
| BMPY | 0.289 | 0.160 | -0.062 | 0.244 | 0.207 |
| PHI | 0.041 | -0.495 | -0.146 | -0.105 | 0.311 |
| NP | 0.228 | -0.134 | 0.406 | -0.015 | -0.169 |
| NS | 0.206 | -0.203 | 0.457 | -0.024 | -0.024 |
| SDMW | 0.286 | 0.121 | 0.050 | -0.328 | -0.120 |
| BMWT | 0.305 | 0.026 | -0.015 | -0.240 | -0.161 |
| HI | 0.043 | -0.339 | -0.264 | 0.610 | -0.452 |
| GPE | 0.268 | -0.227 | -0.118 | 0.050 | -0.123 |
| BPR | 0.298 | 0.021 | -0.016 | -0.322 | -0.100 |
| EGR | 0.290 | -0.089 | -0.153 | -0.123 | -0.276 |
| TSW | -0.022 | 0.180 | -0.666 | -0.282 | -0.053 |
| YLD | 0.295 | -0.155 | -0.131 | -0.041 | -0.213 |
| Parameter | With phosphorus | | | | |
| Eigenvalue | 8.90 | 3.82 | 1.81 | 0.94 | 0.52 |
| Proportion (%) | 52.30 | 22.50 | 10.60 | 5.50 | 3.10 |
| Cumulative (%) | 52.30 | 74.80 | 85.40 | 91.00 | 94.10 |
| Characters | ---------- Eigenvectors ---------- | | | | |
| GPC | -0.302 | -0.106 | -0.156 | 0.235 | -0.092 |
| SPC | -0.141 | 0.440 | 0.032 | 0.213 | -0.203 |
| BMPC | -0.256 | 0.168 | -0.057 | 0.329 | 0.601 |
| SPY | -0.141 | 0.440 | 0.032 | 0.214 | -0.202 |
| GPY | -0.303 | -0.101 | -0.156 | 0.234 | -0.087 |
| BMPY | -0.296 | 0.166 | -0.096 | 0.285 | -0.173 |
| PHI | -0.100 | -0.464 | -0.120 | -0.045 | -0.024 |
| NP | -0.216 | -0.071 | 0.465 | -0.106 | 0.070 |
| NS | -0.217 | -0.145 | 0.480 | 0.026 | -0.066 |
| SDMW | -0.274 | 0.182 | 0.065 | -0.407 | -0.111 |
| BMWT | -0.286 | 0.034 | 0.012 | -0.167 | 0.637 |
| HI | -0.088 | -0.414 | -0.161 | 0.328 | -0.132 |
| GPE | -0.286 | -0.180 | -0.019 | -0.048 | -0.093 |
| BPR | -0.292 | 0.105 | 0.034 | -0.397 | -0.215 |
| EGR | -0.299 | -0.080 | -0.130 | -0.158 | -0.057 |
| TSW | -0.020 | 0.145 | -0.649 | -0.311 | 0.048 |
| YLD | -0.314 | -0.131 | -0.078 | -0.112 | -0.077 |
| Parameter | Phosphorus use efficiency | | | | |
| Eigenvalue | 2.08 | 1.56 | 1.29 | 0.09 | 0.01 |
| Proportion (%) | 41.50 | 31.30 | 25.70 | 14.00 | 0.10 |
| Cumulative (%) | 41.50 | 72.80 | 98.50 | 99.90 | 100 |
| Characters | ---------- Eigenvectors ---------- | | | | |
| APUfs | 0.594 | -0.373 | 0.178 | 0.340 | 0.601 |
| APUs | 0.132 | -0.685 | -0.419 | 0.201 | -0.545 |
| APUf | 0.475 | 0.265 | 0.570 | 0.196 | -0.584 |
| PYE | 0.611 | 0.143 | -0.354 | -0.694 | -0.017 |
| PPE | 0.180 | 0.548 | -0.586 | 0.569 | 0.014 |

*Refer to Table 24 for the abbreviation of characters

Both fertilizer and soil apparent phosphorus uptake efficiencies were not associated with phosphorus harvest index, while phosphorus yield and physiological efficiencies showed strong positive association with phosphorus harvest index (r = 0.50 and 0.65, respectively). Phosphorus physiological efficiency was not improved by increased phosphorus apparent uptakes both from the soil and fertilizer while phosphorus yield efficiency was improved in both cases (Table 30).

#### 8.3.7.2. Relationships between agronomic characters

A number of agronomic components including pod and seed number, shoot and biomass dry weight and physiological components including grain production efficiency, and biomass production and economic growth rates showed highly significant positive correlation coefficients with each other and with grain yield in all cases. Grain yield was positively associated with grain filling period, number of pods and seeds, shoot and biomass dry weight, harvest index, grain production efficiency and biomass and economic growth rates. It is generally assumed that improvements in any one of these traits would result in indirect improvement of grain yield.

It was observed that the shorter the days to flowering and maturity, the higher would be the grain yield as discussed for symbio-agronomic performance earlier. Therefore, at least within a certain limit, the development of earlier and high-yielding chickpea varieties that can fit into a short growing season with residual moisture and double cropping system with crops like wheat and barley would be technically possible. Care must be taken, however, that this result was obtained under a shorter growing period when the genotypes were grown with residual moisture. In another environment, where there is adequate moisture for longer growing period, it could be possible that late-maturing genotypes may more efficiently exploit the moisture resource and better perform than early genotypes as mentioned earlier. For instance, in winter grown chickpea, where moisture use efficiency was better (Singh, 1990), late-maturing varieties gave better yields than early-maturing varieties because of their phenological advantages (Özdemir and Kardavut, 2003).

Table 30. Correlation coefficients (r) between plant tissue phosphorus contents, phosphorus yields and phosphorus use efficiency traits in 155 chickpea genotypes grown on Vertisol with residual moisture in the absence and presence of phosphorus fertilizer in Ethiopia

| Characters | P level | Characters[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GPC | SPC | BMPC | GPY | SPY | BMPY | PHI | APUfs | APUf | APUs | PYE | PPE |
| GPC | $P_0$ | 1.00 | 0.36** | 0.85** | 0.99** | 0.36** | 0.88** | 0.31** | --- | --- | --- | --- | --- |
| | $P_1$ | 1.00 | 0.22** | 0.70** | 0.99** | 0.22** | 0.84** | 0.52** | 0.68** | 0.39** | 0.31** | 0.87** | 0.32** |
| SPC | $P_0$ | | 1.00 | 0.78** | 0.37** | 0.99** | 0.78** | -0.75** | --- | --- | --- | --- | --- |
| | $P_1$ | | 1.00 | 0.59** | 0.23** | 0.99** | 0.71** | -0.68** | 0.58** | 0.32** | 0.29** | 0.17* | -0.39** |
| BMPC | $P_0$ | | | 1.00 | 0.85** | 0.78** | 0.97** | -0.18* | --- | --- | --- | --- | --- |
| | $P_1$ | | | 1.00 | 0.71** | 0.59** | 0.83** | 0.64** | 0.96** | 0.56** | 0.44** | 0.56** | -0.23** |
| GPY | $P_0$ | | | | 1.00 | 0.37** | 0.89** | 0.31** | --- | --- | --- | --- | --- |
| | $P_1$ | | | | 1.00 | 0.23** | 0.85** | 0.51** | 0.68** | 0.39** | 0.31** | 0.87** | 0.31** |
| SPY | $P_0$ | | | | | 1.00 | 0.76** | -0.72** | --- | --- | --- | --- | --- |
| | $P_1$ | | | | | 1.00 | 0.71** | -0.68** | 0.58** | 0.32** | 0.29** | 0.30** | -0.39** |
| BMPY | $P_0$ | | | | | | 1.00 | -0.15 | --- | --- | --- | --- | --- |
| | $P_1$ | | | | | | 1.00 | -0.01 | 0.81** | 0.46** | 0.38** | 0.72** | 0.02 |
| PHI | $P_0$ | | | | | | | 1.00 | --- | --- | --- | --- | --- |
| | $P_1$ | | | | | | | 1.00 | -0.06 | 0.01 | -0.07 | 0.50** | 0.65** |
| APUfs | $P_0$ | | | | | | | | 1.00 | --- | --- | --- | --- |
| | $P_1$ | | | | | | | | 1.00 | 0.56** | 0.47** | 0.57** | -0.22** |
| APUf | $P_0$ | | | | | | | | | 1.00 | --- | --- | --- |
| | $P_1$ | | | | | | | | | 1.00 | -0.46** | 0.39** | -0.02 |
| APUs | $P_0$ | | | | | | | | | | 1.00 | --- | --- |
| | $P_1$ | | | | | | | | | | 1.00 | 0.20** | -0.22** |
| PYE | $P_0$ | | | | | | | | | | | 1.00 | --- |
| | $P_1$ | | | | | | | | | | | 1.00 | 0.60** |
| PPE | $P_0$ | | | | | | | | | | | | 1.00 |
| | $P_1$ | | | | | | | | | | | | 1.00 |

[1]For abbreviation of characters refer to Table 24

Therefore, the importance of improvement in phenological traits may depend on the environment as selection for a long growing duration may also result in yield increments under the moisture-rich environments. Seed size and grain harvest index were either non-significantly or negatively correlated with most of the traits with only a few exceptions. Seed size, for instance, showed significant positive association only with days to flowering and maturity both under phosphorus fertilized and unfertilized conditions and with shoot dry matter weight and economic growth rate under phosphorus fertilized condition.Seed size also negatively correlated with grain filling period but non-significantly with grain yield. Grain harvest index showed consistent and significant positive association with grain filling period, grain production efficiency, economic growth rate and grain yield with and without phosphorus. Improvement of seed size through selection among this gene pool could, therefore, result in a negative selection for traits like pod and seed numbers because of compensatory growth but it may not have any adverse effect on grain yield (Table 31).

#### 8.3.7.2. Relationships between phosphorus and agronomic characters

Days to flowering and maturity revealed significant ($P \leq 0.01$) negative correlation coefficients with grain phosphorus content, phosphorus yield and phosphorus harvest index. However, they showed positive correlation coefficients with shoot phosphorus content and phosphorus yield, no association with apparent use of phosphorus both from the soil and fertilizer and significant negative association with phosphorus yield ($r = -0.34$) and phosphorus physiological ($r = -0.39$) efficiencies. On the other hand, grain filling period was positively associated with both phosphorus yield and physiological efficiencies. This may be an indication that genotypes with the faster developmental switch to reproductive growth earlier in the growing season had better comparative advantage to mobilize and more efficiently use the available phosphorus for reproductive growth as discussed earlier.

Table 31. Correlation coefficients (r) between agronomic traits in 155 chickpea genotypes grown on Vertisol with residual moisture in the absence and presence of phosphorus fertilizer in Ethiopia

| Characters | P level | Characters[1] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DTF | DTM | GFP | NP | NS | SDMW | BMWT | HI | GPE | BPR | EGR | TSW | YLD |
| DTF | $P_0$ | 1.00 | 0.55** | -0.82** | -0.30** | -0.33** | -0.02 | -0.16 | -0.54** | -0.63** | -0.18* | -0.20* | 0.21* | -0.40** |
| | $P_1$ | 1.00 | 0.52** | -0.83** | -0.32** | -0.34** | -0.06 | -0.15 | -0.42** | -0.63** | -0.18* | -0.21* | 0.18* | -0.34** |
| DTM | $P_0$ | | 1.00 | 0.03 | -0.12 | -0.06 | -0.07 | -0.16 | -0.34** | -0.29** | -0.23* | -0.33** | -0.06 | -0.30** |
| | $P_1$ | | 1.00 | 0.05 | -0.04 | -0.03 | 0.05 | 0.01 | -0.31** | -0.18** | -0.11 | -0.19* | -0.03 | -0.17* |
| GFP | $P_0$ | | | 1.00 | 0.27** | 0.35** | -0.02 | 0.09 | 0.41** | 0.55** | 0.06 | 0.01 | -0.29** | 0.27** |
| | $P_1$ | | | 1.00 | 0.34** | 0.38** | 0.11 | 0.17* | 0.28** | 0.62** | 0.14 | -0.01 | -0.23** | 0.29** |
| NP | $P_0$ | | | | 1.00 | 0.83** | 0.61** | 0.66** | 0.09 | 0.63** | 0.66** | 0.60** | -0.53** | 0.65** |
| | $P_1$ | | | | 1.00 | 0.79** | 0.55** | 0.56** | 0.12 | 0.57** | 0.58** | 0.50** | -0.45** | 0.56** |
| NS | $P_0$ | | | | | 1.00 | 0.54** | 0.60** | 0.12 | 0.62** | 0.58** | 0.54** | -0.65** | 0.62** |
| | $P_1$ | | | | | 1.00 | 0.47** | 0.53** | 0.27** | 0.63** | 0.53** | 0.52** | -0.59** | 0.62** |
| SDMW | $P_0$ | | | | | | 1.00 | 0.97** | -0.21* | 0.64** | 0.96** | 0.81** | 0.04 | 0.77** |
| | $P_1$ | | | | | | 1.00 | 0.74** | -0.23* | 0.58** | 0.96** | 0.70** | 0.17* | 0.69** |
| BMWT | $P_0$ | | | | | | | 1.00 | -0.01 | 0.79** | 0.98** | 0.91** | 0.02 | 0.90** |
| | $P_1$ | | | | | | | 1.00 | 0.12 | 0.70** | 0.74** | 0.78** | 0.12 | 0.80** |
| HI | $P_0$ | | | | | | | | 1.00 | 0.47** | -0.06 | 0.27** | -0.09 | 0.37** |
| | $P_1$ | | | | | | | | 1.00 | 0.52** | -0.07 | 0.41** | -0.12 | 0.49** |
| GPE | $P_0$ | | | | | | | | | 1.00 | 0.76** | 0.83** | -0.08 | 0.95** |
| | $P_1$ | | | | | | | | | 1.00 | 0.69** | 0.75** | -0.01 | 0.92** |
| BPR | $P_0$ | | | | | | | | | | 1.00 | 0.90** | 0.05 | 0.88** |
| | $P_1$ | | | | | | | | | | 1.00 | 0.79** | 0.16 | 0.80** |
| EGR | $P_0$ | | | | | | | | | | | 1.00 | 0.08 | 0.96** |
| | $P_1$ | | | | | | | | | | | 1.00 | 0.18* | 0.95** |
| TSW | $P_0$ | | | | | | | | | | | | 1.00 | -0.06 |
| | $P_1$ | | | | | | | | | | | | 1.00 | 0.10 |
| YLD | $P_0$ | | | | | | | | | | | | | 1.00 |
| | $P_1$ | | | | | | | | | | | | | 1.00 |

[1]For abbreviation of characters refer to Table 24

On the other hand, genotypes that lagged behind at flowering might have invested more resources at vegetative growth and exposed relatively a longer part of their reproductive growth to an end-of-season moisture stress as discussed earlier (sub-section 4.2.2.).

Under both fertilized and unfertilized conditions, grain harvest index had shown significant positive association with grain phosphorus content, grain phosphorus yield, phosphorus harvest index and phosphorus yield and physiological efficiencies. On the contrary, grain harvest index showed significant negative associations with shoot phosphorus content and shoot phosphorus yield but no significant associations were observed with biomass phosphorus content and phosphorus yield. Increments in number of pods and seeds, shoot and biomass weight, grain production efficiency, biomass and economic growth rates and grain yield generally increased plant tissue phosphorus contents and phosphorus use efficiency parameters in general in most of the cases.

Seed size did not have a strong association with parameters of plant tissue phosphorus contents, tissue phosphorus yields, and phosphorus harvest index and phosphorus use efficiency except in a few cases when phosphorus was applied from external source.

A number of agronomic and physiological traits including number of pods and seeds, shoot and biomass dry weight, grain production efficiency, biomass and economic growth rates, and grain yield were positively associated with both phosphorus yield and physiological efficiencies but more strongly with the former (Table 32). It should also be noted that all phosphorus-related characters including plant tissue (shoot, grain and biomass) phosphorus contents and phosphorus yields, phosphorus harvest index and attributes of phosphorus uptake and use efficiency, namely apparent use of phosphorus from fertilizer and soil sources, apparent use of phosphorus from fertilizer, apparent use of phosphorus from soil and phosphorus physiological efficiency were positively correlated with phosphorus yield efficiency with no exception (Table 30). This indicated that phosphorus yield efficiency is more important in identifying efficient genotypes than the apparent use of

phosphorus both from the soil and fertilizer and phosphorus physiological efficiency.

It should be noted that the need to perform destructive plant tissue (grain and foliage) analysis, which requires additional labor, time and expense, would complicate breeding for higher tissue (shoot, grain and biomass) phosphorus contents which is, not required to calculate phosphorus yield efficiency.

This study also indicated that improvement in phosphorus content and use efficiency could be achieved through indirect selection for better grain yield and a number of its agronomic and physiological determinants. Economic growth and biomass production rates, grain production efficiency, and shoot and biomass dry weight which showed significant positive correlation coefficients ranging from r = 0.70-0.99 with phosphorus yield efficiency (Table 32).

From this study, it can be understood that growing chickpea with and without phosphorus resulted only in changes of magnitude but not of the direction of correlation coefficients between characters with only a few exceptions. Similar results were reported by Sinebo (2002) who observed a similar pattern of trait association in barley grown with and without nitrogen and phosphorus fertilizers in Ethiopia.

Theoretically, the genetic expression of different traits and the extent and pattern of their relationship with each other may vary with changes in the test environment (Lawes *et al.*, 1983), including with soil fertility levels (Banziger *et al.*, 1997). Particularly when a "crossover" type of genotype by management level interaction revealed, the management levels are considered distinctly different and they do not represent one another in terms of variety development (van Oosterom *et al.*, 1993).

In the present case, fortunately, the same selection criteria can serve to improve phosphorus use efficiency and grain yield both in the presence and absence of phosphorus, indicating that the same sets of genes control each trait under both conditions or there is no independent genetic control between the two traits whether or not the crop is grown with and without phosphorus (Falconer, 1989).

Table 32. Correlation coefficients (r) between plant tissue phosphorus contents, phosphorus yields and phosphorus use efficiency with agronomic traits in 155 chickpea genotypes grown on Vertisol with residual moisture in the absence and presence of phosphorus fertilizer in Ethiopia

| Characters | P level | Characters[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GPC | SPC | BMPC | GPY | SPY | BMPY | PHI | APUfs | APUf | APUs | PYE | PPE |
| DTF | $P_0$ | -0.38** | 0.38** | -0.01 | -0.36** | 0.38** | -0.06 | -0.60** | --- | --- | --- | --- | --- |
| | $P_1$ | -0.32** | 0.27** | -0.01 | -0.31** | 0.27** | -0.08 | -0.47** | -0.01 | -0.01 | 0.02 | -0.34** | -0.39** |
| DTM | $P_0$ | 0.22** | 0.27** | 0.01 | -0.21** | 0.27** | -0.02 | -0.36** | --- | --- | --- | --- | --- |
| | $P_1$ | -0.11 | 0.30** | 0.15 | -0.11 | 0.30** | 0.08 | -0.37** | 0.16 | 0.02 | 0.15 | -0.17* | -0.29** |
| GFP | $P_0$ | 0.29** | -0.27** | 0.02 | 0.29** | -0.27** | 0.07 | 0.47** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.30** | -0.12** | 0.11 | 0.29** | -0.12 | 0.15 | 0.30** | 0.11 | 0.03 | 0.08 | 0.29** | 0.26** |
| NP | $P_0$ | 0.60** | 0.20* | 0.54** | 0.61** | 0.20* | 0.52** | 0.22** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.46** | 0.15 | 0.39** | 0.46** | 0.15 | 0.42** | 0.23** | 0.38** | 0.27** | 0.01 | 0.56** | 0.34** |
| NS | $P_0$ | 0.56** | 0.05 | 0.44** | 0.56** | 0.05 | 0.42** | 0.31** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.51** | 0.07 | 0.34** | 0.51** | 0.07 | 0.41** | 0.33** | 0.30** | 0.20** | 0.10 | 0.62** | 0.45** |
| SDMW | $P_0$ | 0.71** | 0.57** | 0.86** | 0.72** | 0.57** | 0.79** | -0.08 | --- | --- | --- | --- | --- |
| | $P_1$ | 0.58** | 0.57** | 0.56** | 0.58** | 0.57** | 0.73** | -0.05 | 0.58** | 0.45** | 0.14 | 0.70** | 0.22** |
| BMWT | $P_0$ | 0.82** | 0.48** | 0.85** | 0.82** | 0.48** | 0.82** | 0.09 | --- | --- | --- | --- | --- |
| | $P_1$ | 0.65** | 0.33** | 0.80** | 0.30** | 0.33** | 0.65** | 0.16 | 0.80** | 0.55** | 0.27** | 0.80** | 0.22** |
| HI | $P_0$ | 0.30** | -0.29** | -0.09 | 0.30** | -0.29** | 0.07 | 0.56** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.46** | -0.48** | -0.02 | 0.45** | -0.48** | 0.07 | 0.78** | -0.01 | -0.05 | 0.04 | 0.49** | 0.55** |
| GPE | $P_0$ | 0.85** | 0.10 | 0.59** | 0.85** | 0.10 | 0.65** | 0.50** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.82** | 0.07 | 0.48** | 0.81** | 0.06 | 0.62** | 0.54** | 0.49** | 0.30** | 0.20* | 0.92** | 0.58** |
| BPR | $P_0$ | 0.80** | 0.44** | 0.82** | 0.80** | 0.44** | 0.78** | 0.12 | --- | --- | --- | --- | --- |
| | $P_1$ | 0.67** | 0.48** | 0.55** | 0.67** | 0.48** | 0.74** | 0.10 | 0.55** | 0.43** | 0.14 | 0.80** | 0.36** |
| EGR | $P_0$ | 0.85** | 0.31** | 0.71** | 0.85** | 0.31** | 0.75** | 0.28** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.81** | 0.23** | 0.56** | 0.81** | 0.22** | 0.42** | 0.42** | 0.57** | 0.42** | 0.16 | 0.95** | 0.53** |
| TSW | $P_0$ | -0.03 | 0.13 | 0.02 | -0.04 | 0.13 | 0.04 | -0.14 | --- | --- | --- | --- | --- |
| | $P_1$ | 0.12 | 0.17* | 0.12 | 0.11 | 0.17* | 0.17* | -0.09 | 0.15 | 0.08 | 0.09 | 0.10 | -0.05 |
| YLD | $P_0$ | 0.89** | 0.24** | 0.70** | 0.89** | 0.24** | 0.74** | 0.39** | --- | --- | --- | --- | --- |
| | $P_1$ | 0.87** | 0.17* | 0.56** | 0.87** | 0.17* | 0.72** | 0.50** | 0.57** | 0.39** | 0.19* | 0.99** | 0.59** |

[1]For abbreviation of characters refer to Table 24

### 8.3.7. Genetic variation and expected gains from selection

#### 8.3.7.2. Frequency distribution

Considerable differences were observed between the minimum and maximum values for a number of traits both in the presence and absence of phosphorus as revealed from the frequency distributions (Figures 26-29). This indicated the existence of a wealth of genetic variability in the Ethiopian chickpea gene pool for most of the traits including the economically important ones such as phosphorus use efficiency and grain yield.

Both the minimum and the maximum values were increased by the application of phosphorus as expected in almost all the traits, except grain and phosphorus harvest indices which showed no increase, but a tendency to decline with the application of phosphorus. This is because the application of phosphorus resulted in higher relative increase in shoot dry matter weight than in grain yield and in a higher relative increase in the gross amount of phosphorus (but not concentration) in the shoot relative to the seed.

#### 8.3.7.2. Broad sense heritability and genetic gain from selection

In the absence of phosphorus fertilizer, broad sense heritability values ranged from 60-93% while genetic advance values ranged from 4-62%. The corresponding broad sense heritability and genetic advance values in the presence of phosphorus fertilizer ranged from 59-93% and 4-79% in that order. Heritability and genetic advance values did not show a definite trend with the presence or absence of phosphorus. From the available literature, the magnitudes of heritability and genetic advance values under favorable and unfavorable environments do not also appear to follow a simple trend. Some authors reported that favorable environments show higher estimates of heritability and genetic advance values than unfavorable environments (Singh, 2002; Simmonds, 1991; Banziger and Edmeades, 1997).

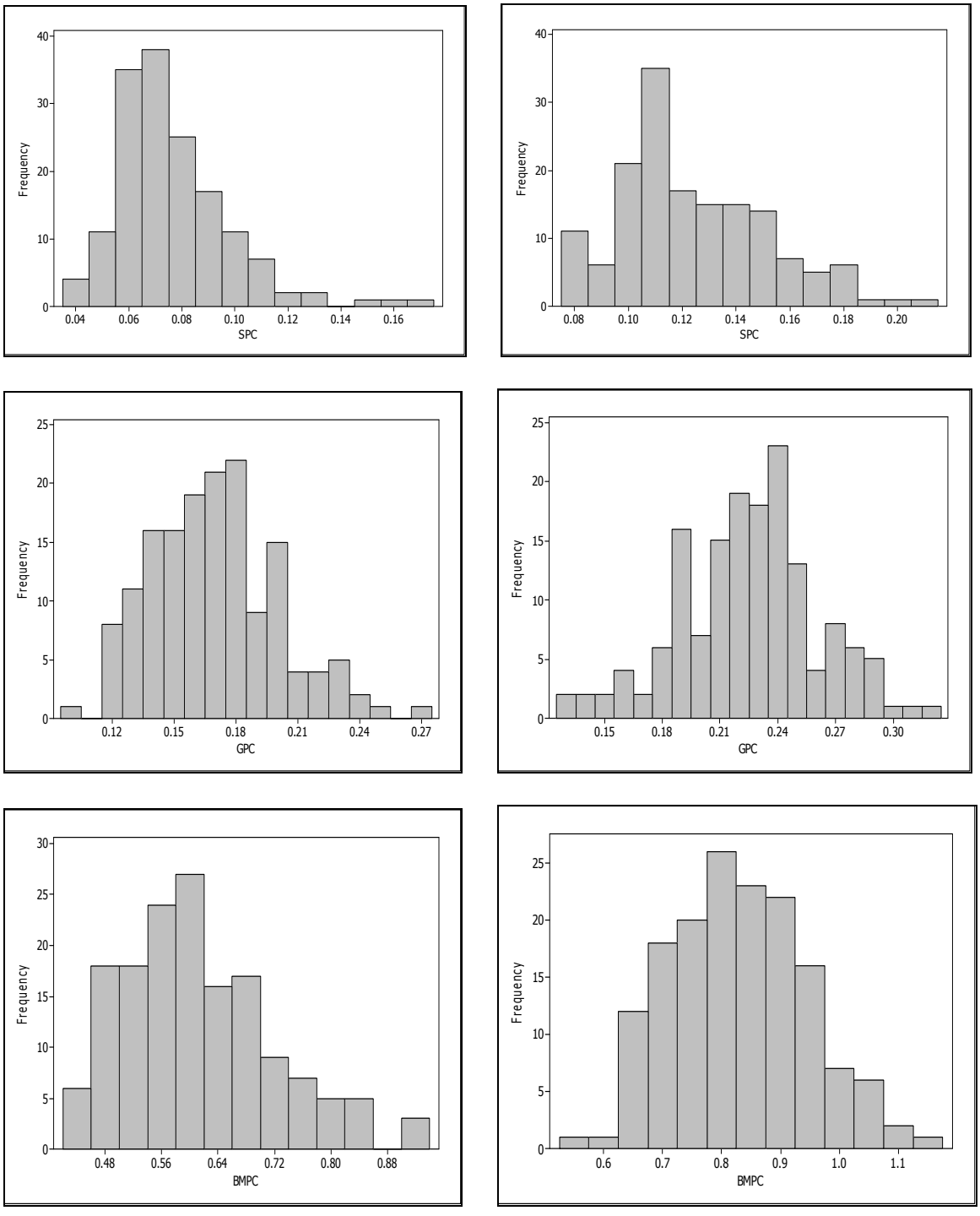



Figure 26. Frequency distributions for plant tissue phosphorus contents in 155 chickpea genotypes grown at in the presence (right) and absence (left) of phosphorus fertilizer on Vertisol with residual moisture in Ethiopia (refer to Table 24 for the abbreviation of characters).

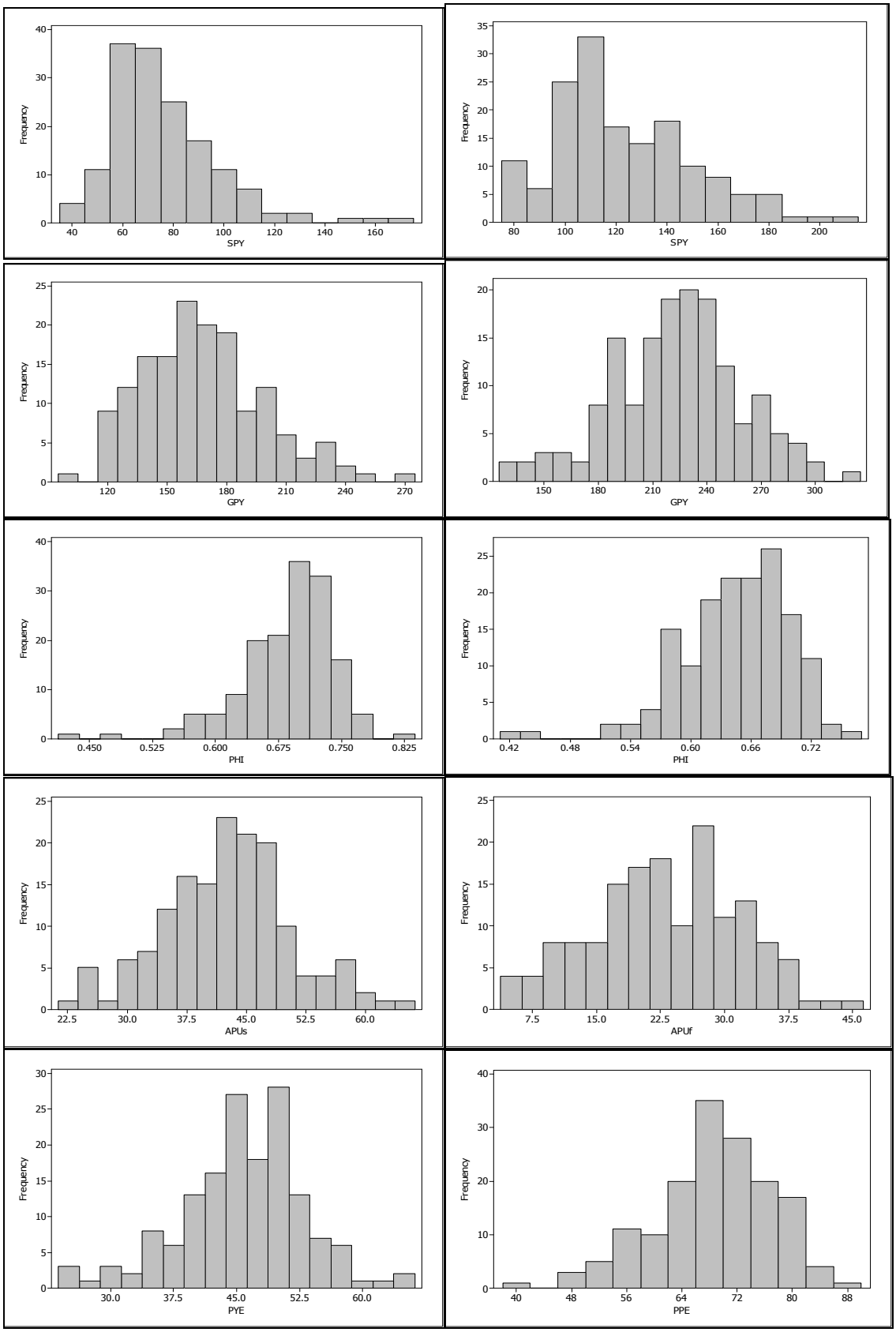



Figure 27. Frequency distributions for plant tissue phosphorus yields and phosphorus uptake and use efficiency in 155 chickpea genotypes grown in the presence (right) and absence (left) of phosphorus fertilizer on Vertisol with residual moisture in Ethiopia (refer to Table 24 for the abbreviation of characters).

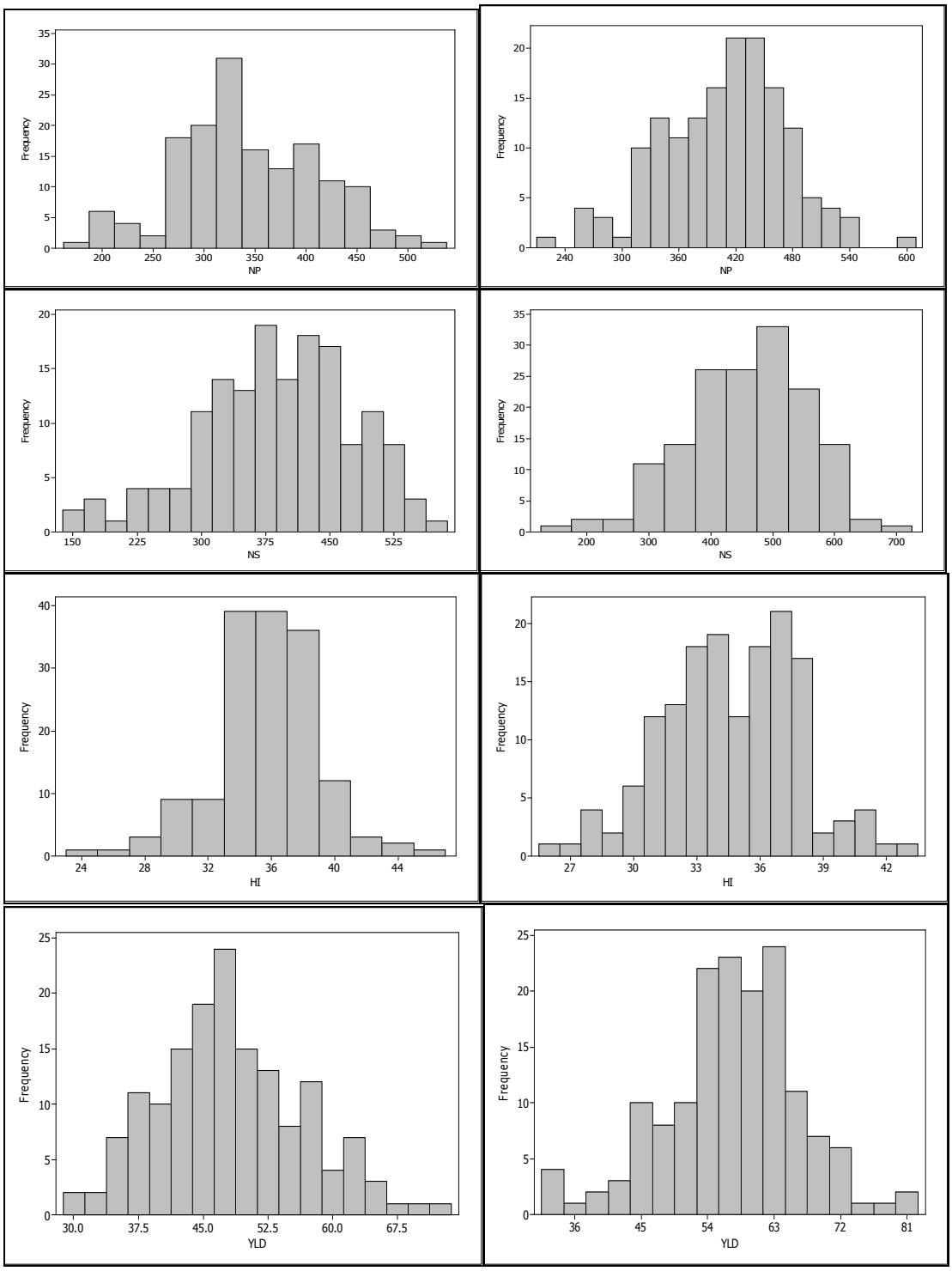



Figure 28. Frequency distributions for grain yield and some agronomic yield components in 155 chickpea genotypes grown at in the presence (right) and absence (left) of phosphorus fertilizer on Vertisol with residual moisture in Ethiopia (refer to Table 24 for the abbreviation of characters)

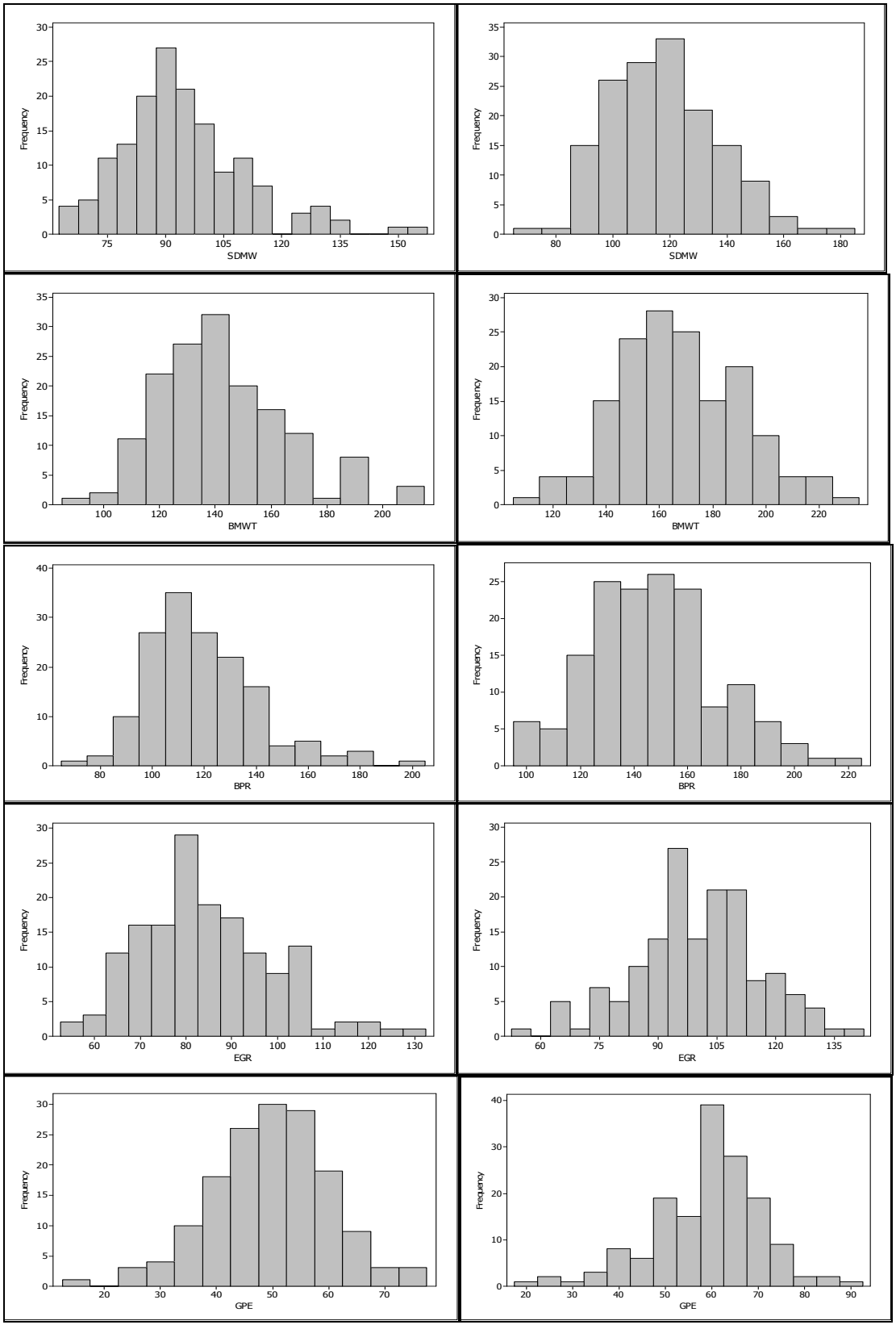



Figure 29. Frequency distributions for some physiological and agronomic components in 155 chickpea genotypes grown at in the presence (right) and absence (left) of phosphorus fertilizer on Vertisol with residual moisture in Ethiopia (refer to Table 24 for the abbreviation of characters)

This is because heritability and genetic advance values may be concealed due to a greater genotype by environment under unfavorable condition (Rosielle and Hamblin, 1981). On the contrary, other reports indicated that there is no interrelationship between the type of the environment (yield level) and the magnitude of heritability and genetic advance values (Ceccarelli and Grando, 1996). The magnitude of heritability and genetic advance values, according to the latter, is rather affected by the nature of the genetic material under consideration than the test environment.

Selection among the present gene pool for most of the traits appeared to be effective both in the presence and absence of phosphorus as they mostly showed favorable interrelationships and medium to high broad sense heritability values, indicating that the phenotype could reflect the genotype (Singh, 2002). Selection for a trait with a high heritability (80% or more) should be fairly easy as there would be a close correspondence between genotype and phenotype (Singh, 2002). Broad sense heritability and genetic advance from selection for phosphorus-related and agronomic traits in the absence and presence of phosphorus are given in Figures 30 and 31.

Linear regressions were fitted between heritability and expected genetic gain values of traits calculated from the same genotypes grown in the presence and absence of phosphorus. There were a strong positive relationship between heritability ($R^2$ = 0.4548) and perfectly positive relationship genetic advance ($R^2$ = 0.9469) values generated with and without phosphorus (Figure 32). This indicated the existence of similar pattern of heritability and genetic gains from selection with and without phosphorus, as genotype by phosphorus level interaction effects were minimal (Table 24 for the traits studied).

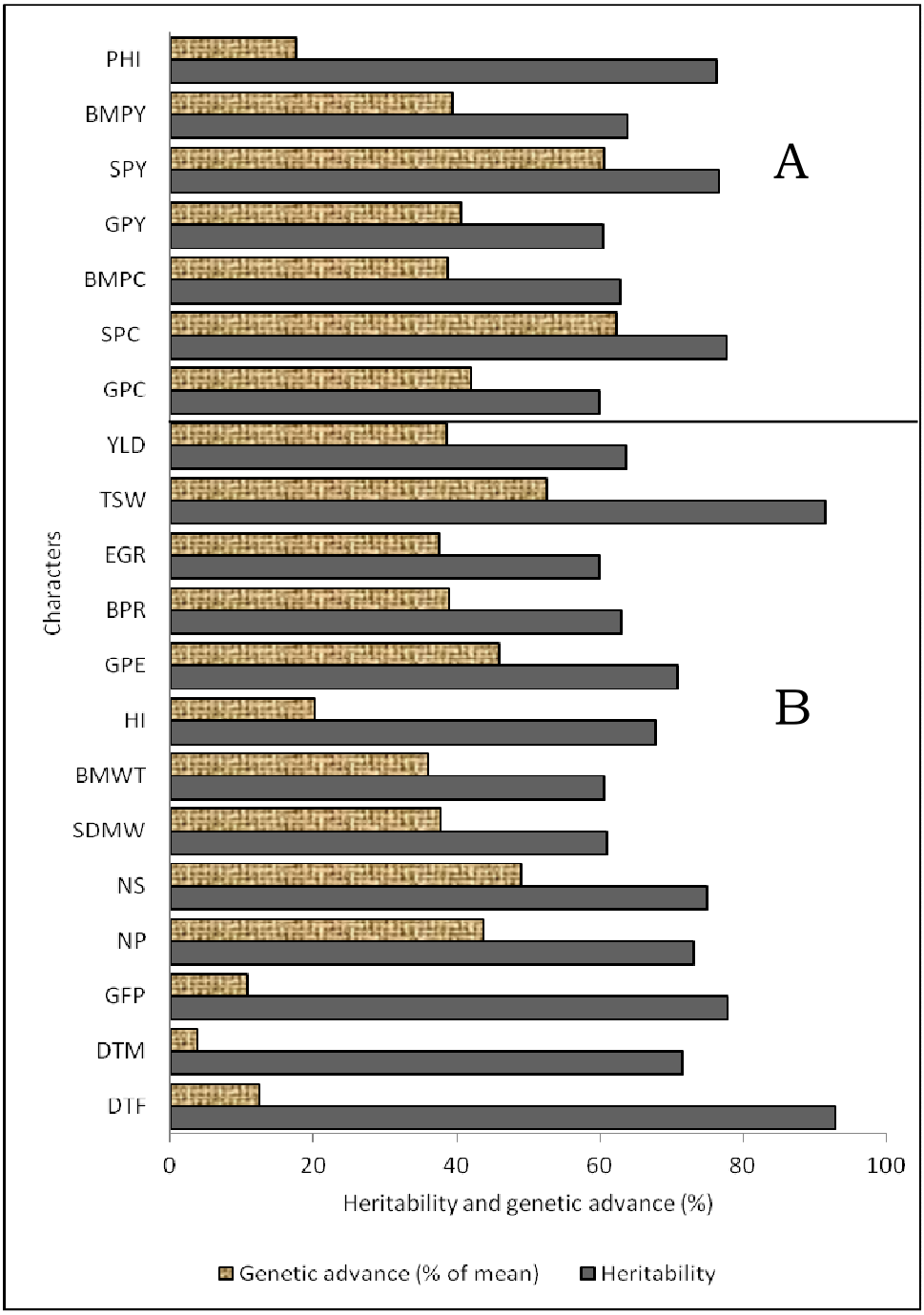



Figure 30. Estimates of broad-sense heritability ($h_b^2$) and expected genetic advance (GA) from selection in 155 chickpea genotypes grown without phosphorus fertilizer for phosphorus and agronomic characters (refer to Table 24 for abbreviations of characters).

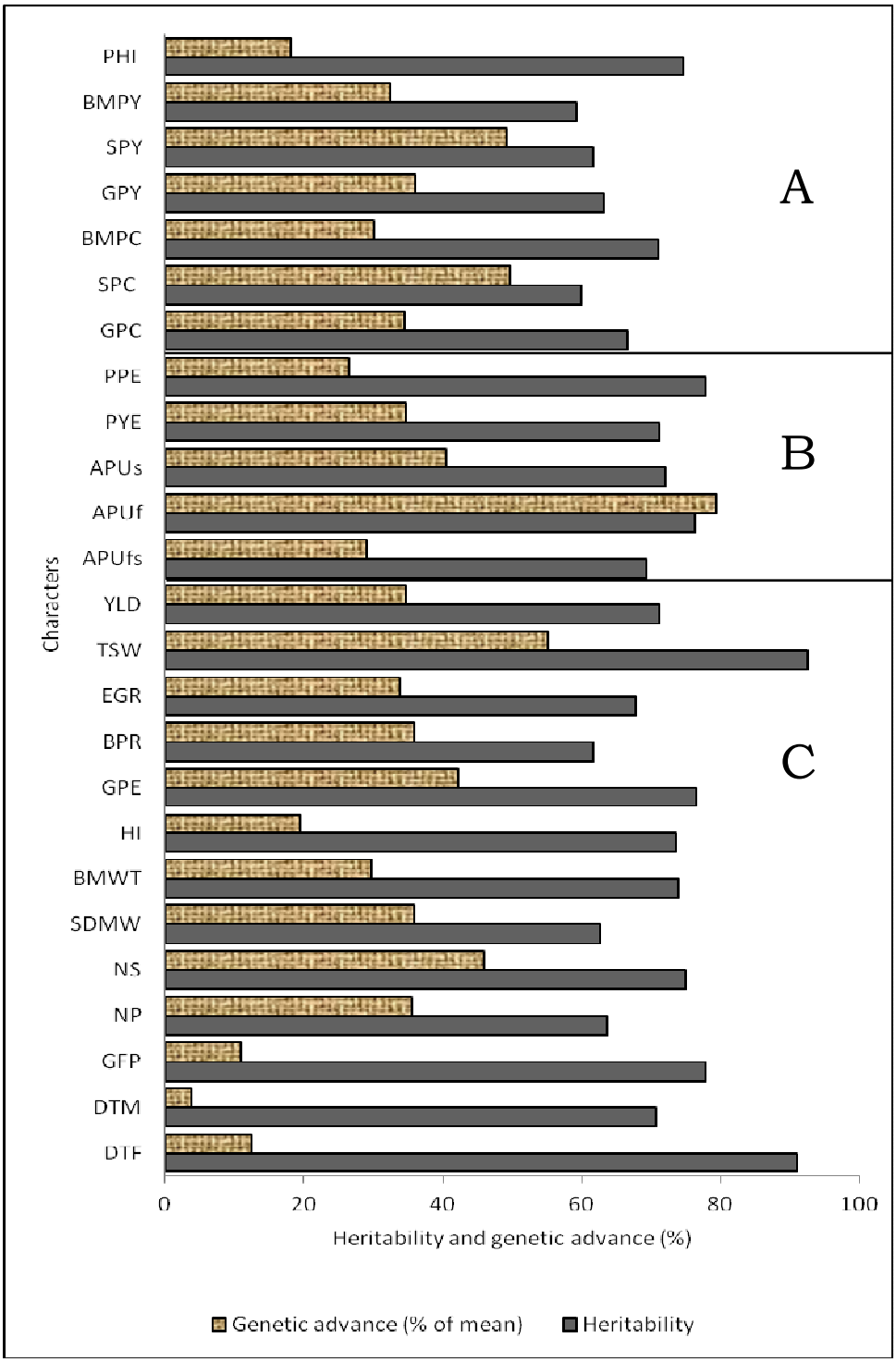



Figure 31. Estimates of broad-sense heritability ($h_b^2$) and expected genetic advance (GA) from selection in 155 chickpea genotypes grown with phosphorus fertilizer for phosphorus and agronomic characters (refer to Table 24 for abbreviations of characters).

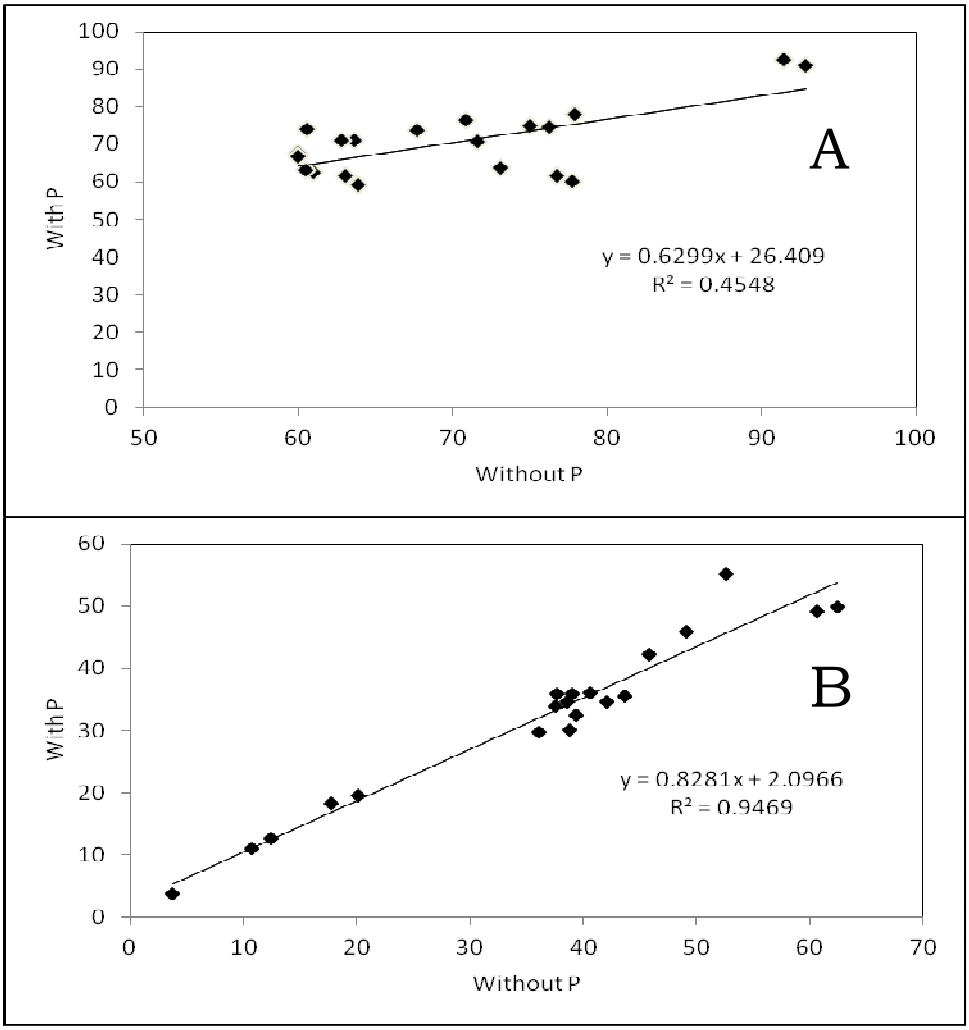



Figure 32. Interelationship between (A) heritability and (B) genetic advance from selection values in 155 chickpea genotypes grown without and with phosphorus fertilizer showing a strong association between genetic parameters of traits calculated in the two environments

#### 8.3.7.3. Comparison of selected genotypes with the original population

The Z-test also showed highly significant differences between means of the selected subsets of the top 5% best genotypes ($\overline{X}$) and the population parameters (μ) for plant tissue phosphorus content, phosphorus yields, phosphorus harvest index, phosphorus use efficiency and all agronomic characters under both fertilized and unfertilized conditions (Table 33).

Comparison of the average performances for respective characters of the selected subsets of the top 5% best genotypes with the average performances of the whole population revealed possibilities for different level of improvements through selection. The least change of 13% was observed in phosphorus harvest index both with and without phosphorus. The highest difference of 90% and 108% was observed for seed size without and with phosphorus, respectively (Table 34). This indicated that the selected genotypes were not true representatives of the population and that almost all characters effectively responded to phenotypic selection both with and without phosphorus (Singh, 2001).

Comparison of the top 5% best genotypes with a recently released variety Natoli also resulted in comparative advantages of 51-72% and 7-18% for biomass phosphorus content in the absence and present of phosphorus, respectively. Similarly, the top 5% best genotypes resulted in comparative advantages of 48-75% and 16-23%, respectively, for biomass phosphorus yield in the absence and presence of phosphorus. The difference for phosphorus harvest index ranged from 17-28% and 14-22% and for biomass dry weight ranged from 42-59% and 1-14 % in the absence and presence of phosphorus, respectively (Table 34).

The top 5% best genotypes superior for parameters of plant tissue phosphorus content, biomass phosphorus yield, phosphorus harvest index and biomass dry weight to Natoli are given in Table 34. The best 5% of the genotypes superior to Natoli for parameters of phosphorus uptake and use efficiency are presented in Table 35. The best genotypes were superior to Natoli by 5-14% for apparent use of phosphorus from fertilizer and soil sources, 30-46% for apparent use of phosphorus from soil, 12-39% for apparent use of phosphorus from fertilizer, 14-32% for phosphorus yield efficiency and 23-32% for phosphorus physiological efficiency. According to Syers *et al.* (2008), on average, only up to 25 percent of the added fertilizer phosphorus may be taken up by the crop and the remainder of the phosphorus must come from soil sources.

Table 33. Comparison of the mean performances of selected subsets ($\overline{X}$) of the 5% best accessions for symbiotic and agronomic characters with the average performances of the whole population (μ) of 155 chickpea genotypes

| Characters[1] | Without phosphorus | | | | | With phosphorus | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean of selected genotypes ($\overline{X}$) | Population parameter (μ) | Change through selection ($\overline{X}$-μ) | Change as % of population parameter (μ) | Z | Mean of selected genotypes ($\overline{X}$) | Population parameter (μ) | Change through selection ($\overline{X}$-μ) | Change as % of population parameter (μ) | Z |
| Plant tissue phosphorus contents and yields | | | | | | | | | | |
| SPC | 0.137 | 0.076 | 0.061 | 80.26 | 7.95** | 0.187 | 0.123 | 0.064 | 52.03 | 6.65** |
| GPC | 0.240 | 0.168 | 0.072 | 42.86 | 6.67** | 0.296 | 0.224 | 0.072 | 32.14 | 5.58** |
| BMPC | 0.874 | 0.612 | 0.262 | 42.81 | 6.90** | 1.076 | 0.828 | 0.248 | 29.95 | 6.03** |
| SPY | 136.538 | 76.305 | 60.233 | 78.94 | 7.86** | 186.738 | 123.369 | 63.369 | 51.37 | 6.58** |
| GPY | 240.188 | 168.089 | 72.099 | 42.89 | 6.69** | 295.750 | 223.395 | 72.355 | 32.39 | 5.63** |
| BMPY | 351.350 | 244.393 | 106.957 | 43.76 | 6.96** | 444.888 | 346.761 | 98.127 | 28.30 | 5.53** |
| PHI | 0.779 | 0.686 | 0.093 | 13.56 | 4.72** | 0.729 | 0.644 | 0.085 | 13.20 | 4.47** |
| Phosphorus uptake and use efficiency | | | | | | | | | | |
| APUfs | --- | --- | --- | --- | --- | 83.59 | 65.749 | 17.841 | 27.14 | 5.75** |
| APUf | --- | --- | --- | --- | --- | 39.74 | 23.151 | 16.589 | 71.66 | 5.58** |
| APUs | --- | --- | --- | --- | --- | 59.59 | 42.574 | 17.016 | 39.97 | 5.86** |
| PYE | --- | --- | --- | --- | --- | 60.13 | 45.797 | 14.333 | 31.30 | 5.53** |
| PPE | --- | --- | --- | --- | --- | 83.03 | 68.519 | 14.511 | 21.18 | 4.79** |
| Agronomic characters | | | | | | | | | | |
| NP | 483.05 | 343.11 | 139.940 | 40.79 | 5.70** | 538.09 | 408.40 | 129.69 | 31.76 | 5.58** |
| NS | 538.18 | 387.52 | 150.660 | 38.88 | 4.81** | 621.90 | 455.35 | 166.55 | 36.58 | 4.82** |
| SDMW | 136.39 | 94.25 | 42.140 | 44.71 | 7.50** | 160.24 | 117.48 | 42.76 | 36.40 | 6.38** |
| BMWT | 199.68 | 142.53 | 57.150 | 40.10 | 7.07** | 217.10 | 167.85 | 49.25 | 29.34 | 6.00** |
| HI | 42.71 | 35.68 | 7.030 | 19.70 | 6.08** | 40.90 | 34.63 | 6.27 | 18.11 | 5.65** |
| GPE | 70.19 | 49.59 | 20.600 | 41.54 | 5.56** | 80.82 | 59.04 | 21.78 | 36.89 | 5.26** |
| BPR | 174.08 | 119.45 | 54.630 | 45.73 | 7.37** | 200.68 | 147.19 | 53.49 | 36.34 | 6.38** |
| EGR | 117.41 | 84.37 | 33.040 | 39.16 | 6.61** | 130.85 | 100.02 | 30.83 | 30.82 | 5.63** |
| TSW | 220.38 | 115.65 | 104.730 | 90.56 | 9.99** | 220.288 | 111.95 | 108.34 | 96.78 | 10.63** |
| YLD | 66.08 | 48.21 | 17.870 | 37.07 | 6.04** | 75.16 | 57.25 | 17.91 | 31.28 | 5.53** |

Refer to Table 24 for abbreviations of characters; ** = highly significant ($P \leq 0.01$) and * = significant ($P \leq 0.05$)

Table 34. Comparison of mean performances of 5% of the genotypes selected for better performances for attributes of phosphorus content, phosphorus yield, phosphorus harvest index and biomass dry weight as compared to Natoli, a recently released variety

| Without phosphorus | | | With phosphorus | | |
|---|---|---|---|---|---|
| Biomass phosphorus content (g/5 plants) | | | | | |
| | Performance | | | Performance | |
| Genotype | Mean | %over Natoli | Genotype | Mean | %over Natoli |
| 209093 | 0.940 | 71.69 | 41066 | 1.135 | 17.92 |
| 209096 | 0.935 | 70.78 | 41215 | 1.112 | 15.53 |
| 207657 | 0.930 | 69.86 | 41103 | 1.092 | 13.45 |
| 207734 | 0.858 | 56.62 | 41275 | 1.072 | 11.38 |
| 41274 | 0.840 | 53.42 | 41015 | 1.070 | 11.17 |
| 41284 | 0.835 | 52.51 | 207763 | 1.058 | 9.92 |
| 41066 | 0.833 | 52.05 | 41053 | 1.038 | 7.84 |
| ICC 19180 | 0.825 | 50.68 | 41274 | 1.033 | 7.32 |
| Mean | 0.875 | 59.70 | Mean | 1.076 | 11.82 |
| Natoli | 0.548 | --- | Natoli | 0.963 | --- |
| Biomass phosphorus yield (mg/5 plants) | | | | | |
| 209093 | 390 | 75.09 | 41275 | 462 | 23.25 |
| 207657 | 385 | 72.85 | 207763 | 455 | 21.25 |
| 209096 | 363 | 63.02 | Habru | 447 | 19.25 |
| 41066 | 339 | 52.15 | 207645 | 445 | 18.50 |
| 41274 | 336 | 50.63 | 41111 | 440 | 17.38 |
| 41111 | 336 | 50.63 | 207734 | 438 | 16.74 |
| 41015 | 332 | 49.01 | ICC 4918 | 436 | 16.32 |
| 231328 | 330 | 48.20 | 41015 | 436 | 16.16 |
| Mean | 351 | 57.70 | Mean | 445 | 18.61 |
| Natoli | 223 | --- | Natoli | 375 | --- |
| Phosphorus harvest index | | | | | |
| 209089 | 0.825 | 27.91 | 207742 | 0.758 | 21.69 |
| 41298 | 0.785 | 21.71 | 41115 | 0.740 | 18.88 |
| ICC 4918 | 0.780 | 20.93 | 209097 | 0.730 | 17.27 |
| 41160 | 0.780 | 20.93 | 207761 | 0.728 | 16.87 |
| 41311 | 0.775 | 20.16 | 207658 | 0.723 | 16.06 |
| 207744 | 0.773 | 19.77 | 41312 | 0.723 | 16.06 |
| 41296 | 0.760 | 17.83 | 207150 | 0.723 | 16.06 |
| 41274 | 0.758 | 17.44 | 207659 | 0.713 | 14.46 |
| Mean | 0.780 | 20.84 | Mean | 0.730 | 17.17 |
| Natoli | 0.645 | --- | Natoli | 0.623 | --- |
| Biomass dry weight (g/5 plants) | | | | | |
| 41284 | 215 | 59.08 | 41053 | 234 | 14.01 |
| 207734 | 213 | 57.82 | 207763 | 223 | 8.32 |
| 41274 | 208 | 53.82 | 41049 | 220 | 7.20 |
| 41272 | 194 | 43.51 | 41019 | 217 | 5.55 |
| 209093 | 193 | 42.99 | 41274 | 215 | 4.62 |
| ICC 19180 | 193 | 42.70 | Habru | 211 | 2.58 |
| 207657 | 193 | 42.70 | 41215 | 209 | 1.90 |
| 209096 | 191 | 41.51 | 41275 | 208 | 0.97 |
| Mean | 200 | 48.02 | Mean | 217 | 5.64 |
| Natoli | 135 | --- | Natoli | 206 | --- |

Table 35. Comparison of mean performances of 5% of the genotypes selected for better performances for attributes of phosphorus uptake and use efficiency parameters* as compared to Natoli, a recently released variety

| | APUfs | | | APUs | | | APUf | | | PYE | | | PPE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | %over | | | %over | | | %over | | | %over | | | %over |
| Genotype | Mean | Natoli | Genotype | Mean | Natoli | Genotype | Mean | Natoli | Genotype | Mean | Natoli | Genotype | Mean | Natoli |
| 41103 | 87.36 | 13.71 | 41015 | 64.29 | 46.45 | Habru | 45.70 | 38.78 | 41053 | 65.65 | 31.83 | 207150 | 86.43 | 31.87 |
| 41015 | 85.70 | 11.54 | 209093 | 62.72 | 42.87 | ICC 4948 | 41.35 | 25.57 | 207763 | 63.97 | 28.45 | 207563 | 84.97 | 29.65 |
| 207763 | 84.49 | 9.97 | 41320 | 59.05 | 34.51 | 225878 | 40.99 | 24.48 | 207742 | 61.40 | 23.29 | 209084 | 83.10 | 26.79 |
| 41215 | 83.42 | 8.58 | 41274 | 58.79 | 33.92 | ICC 4918 | 38.54 | 17.04 | ICC 4918 | 59.64 | 19.76 | 41115 | 82.70 | 26.18 |
| 41053 | 83.10 | 8.16 | 41103 | 58.70 | 33.71 | 41076 | 38.54 | 17.04 | 41274 | 58.63 | 17.73 | 209027 | 82.67 | 26.14 |
| 41274 | 82.78 | 7.74 | 207742 | 58.26 | 32.71 | 207763 | 38.46 | 16.79 | Habru | 57.43 | 15.32 | 41312 | 81.82 | 24.84 |
| 207761 | 81.05 | 5.49 | ICC 19181 | 57.91 | 31.91 | 41053 | 37.63 | 14.27 | 212589 | 57.28 | 15.02 | 41290 | 81.70 | 24.66 |
| 41275 | 80.78 | 5.14 | 41222 | 57.01 | 29.86 | 207734 | 36.73 | 11.54 | 41019 | 57.01 | 14.48 | 219799 | 80.85 | 23.36 |
| Mean | 83.59 | 8.79 | Mean | 59.59 | 35.74 | Mean | 39.74 | 20.69 | Mean | 60.13 | 20.74 | Mean | 83.03 | 26.69 |
| Natoli | 76.83 | --- | Natoli | 43.90 | --- | Natoli | 32.93 | --- | Natoli | 49.80 | --- | Natoli | 65.54 | --- |

*Refer to Table 24 for the abbreviation of characters

A released variety, Habru, and the genotype introduced from ICRISAT, ICC 4918, were found to be among the top 5% best genotypes for apparent use of phosphorus from fertilizer and phosphorus yield efficiency. However, it was observed that the desirable characters were found to distribute among different genotypes and a single genotype combining desirable attributes for phosphorus uptake and use efficiency may be of rare occurrence in this gene pool. Therefore, the utilization of these valuable germplasm particularly in the efforts underway to develop efficient genotypes needs a series of multiple crossing in order to bring the desirable traits into a single genetic background.

### 8.4. The role of phosphorus to enhance symbiotic nitrogen fixation

Analysis of variance combined over phosphorus level and locations showed significant differences ($P \leq 0.01$ or $P \leq 0.05$) among the genotypes for all symbiotic characters except, total biomass nitrogen fixation and non-significant differences for genotype by phosphorus interaction effects (Table 36). The consistent non-significant genotype by phosphorus level interaction effects for symbiotic character indicated that selection of better performing genotypes in the absence of phosphorus may enable the identification of genotypes that can repeat nearly the same performances in the presence of phosphorus as well and *vice versa*. Thus, separate evaluations without and with phosphorus may not be necessarily needed as evaluation under any one of the two may serve to identify appropriate genotypes for both conditions.

The comparison of performances for symbiotic traits with and without phosphorus showed that the magnitudes of response to the application of phosphorus varied from character to character. Experiments which did not receive phosphorus suffered, on the average, relative reductions of 35% for nodule number, 27% each for nodule dry weight and shoot nitrogen yield, 23% for biomass nitrogen yield, 22% for shoot nitrogen fixation, 20% for

grain nitrogen yield and 16% for nodulation index as compared to the experiments which received phosphorus (Table 36).

Other characters including shoot and grain protein contents, grain and total biomass nitrogen fixation, fixed nitrogen assimilation efficiency and nitrogen harvest index practically did not respond to the application of phosphorus.

Better symbiotic performance was also reported from the application of phosphorus in chickpea (Shukla and Yadav, 2000) and a number of other legumes (Sanginga *et al.*, 2000; Osman *et al.*, 2002; Singh *et al.*, 2005). Favourable effects of inoculation in chickpea with effective *Rhizobium* for increasing not only symbiotic characters but also phosphorus uptake and use efficiency was reported (Zaidi *et al.*, 2003).

While nitrogen itself is important for the synthesis of amino acids, proteins and nucleic acids (Hubbell and Kidder, 2003; Lindemann and Glover, 2003), legumes also require more phosphorus as an important constituent for the synthesis of adenosine triphosphate (ATP) because they require additional ATP for nodulation and symbiotic nitrogen fixation in particular (Sinclair and Vadez, 2002; Muhammad *et al.*, 2004; Bucher, 2006; Qiao *et al.*, 2007). Therefore, the yield increment due to the application of phosphorus stated earlier (Figure 23 and Table 25) may be in part attributed to the indirect effect of the enhancement in symbiotic nitrogen fixation, indicating the possibility for improving the productivity of chickpea through the integration of effective strains of *Rhizobium* and a proper amount of phosphorus fertilizer.

In the absence of phosphorus fertilizer, the top 5% best fixers of atmospheric nitrogen in their biomass were Acc. Nos. 41021, 41222, 41197, 41026, Arerti, Worku, 207734 and 41129, the amount fixed being 39-42% ($\overline{X}$ = 40.01%). In the presence of phosphorus fertilizer, the top 5% best fixers were 41029, 41074, 41222, 41075, 41320, 41299, 41308 and 41129 with the same range of 39-42% ($\overline{Y}$ = 39.64%).

It is interesting to note that Acc Nos. 41222 and 41129 had showed consistently best performances for biomass nitrogen fixation under both

fertilized and unfertilized conditions. Similarly, in the absence of phosphorus fertilizer, the top 5% best genotypes which accumulated the highest amount of biomass nitrogen yield ranging from 3.45-3.96 g/5 plants ($\bar{X}$ = 3.64 g/5 plants). The accessions were 207734, 41293, 41280, 41272, 41284, 207657, 209093 and 41274. In the presence of phosphorus, the corresponding top 5% best genotypes with the highest amount of biomass nitrogen yield ranged from 4.31-4.94 g/5 plants ($\bar{X}$ = 4.52 g/5 plants) were 41275, 41019, 41049, 41053, 41185, 207763, 207645 and 207742.

The result indicated that the top 5% best genotypes with the highest amount of symbiotic nitrogen fixation in the absence of phosphorus did not necessarily repeat the same performance in the presence of phosphorus despite the non-significant genotype by phosphorus interaction for all symbiotic characters.

Classification of the genotypes based on the amount of total biomass nitrogen fixation and biomass nitrogen yield in the absence and presence of phosphorus fertilizer showed that the best fixers of nitrogen in the absence of phosphorus fertilizer (efficient fixers) may not necessarily be the best fixer in the presence of phosphorus fertilizer (responder fixers) and *vice versa*. However, there were genotypes that were both efficient and responder fixers (Figure 33).

As stated earlier, this may indicate possibilities to develop chickpea varieties that are efficient fixers of atmospheric nitrogen where farmers cannot afford the application of phosphorus fertilizer or responder fixers of atmospheric nitrogen where farmers can apply adequate amount of phosphorus.

Table 36. Mean square of analysis of variance (combined over phosphorus level and locations), genotype by phosphorus interaction, CV (%), range, mean performances of 155 chickpea genotypes for attributes of symbiotic nitrogen fixation relative reductions due to lack of phosphorus at two locations in Ethiopia

| Character[1] | Mean square | | CV (%) | Range | | Mean | | |
|---|---|---|---|---|---|---|---|---|
| | Genotype (G) | G × P | | With P | Without P | With P | Without P | Relative reduction |
| NI* | ** | NS | 22.26 | 0.335-4.033 | 0.243-3.562 | 1.559 | 1.306 | 0.16 |
| NN* | ** | NS | 21.43 | 5.50-44.75 | 2.75-33.75 | 15.974 | 10.413 | 0.35 |
| NDW* | ** | NS | 23.80 | 105.06-1643.71 | 65.04-955.80 | 484.41 | 355.97 | 0.27 |
| SPC | ** | NS | 19.51 | 4.89-11.43 | 5.00-9.43 | 7.68 | 7.03 | 0.08 |
| GPC | ** | NS | 9.94 | 15.53-26.22 | 14.46-25.16 | 22.27 | 21.64 | 0.03 |
| SNF* | ** | NS | 22.28 | 13.76-56.94 | 9.27-45.27 | 33.68 | 26.26 | 0.22 |
| GNF* | * | NS | 19.08 | 26.72-45.73 | 28.40-45.63 | 35.55 | 36.35 | -0.02 |
| BNF* | NS | NS | 16.25 | 26.33-42.20 | 24.48-41.60 | 33.36 | 33.12 | 0.01 |
| FNAE* | ** | NS | 11.07 | 63.06-93.66 | 64.43-92.56 | 78.89 | 81.67 | -0.04 |
| GNY | ** | NS | 22.96 | 0.830-2.873 | 0.667-2.310 | 2.057 | 1.640 | 0.20 |
| SNY | ** | NS | 26.01 | 0.582-2.590 | 0.508-1.700 | 1.443 | 1.058 | 0.27 |
| BNY | ** | NS | 21.41 | 1.412-4.935 | 1.175-3.963 | 3.500 | 2.699 | 0.23 |
| NHI | ** | NS | 10.99 | 0.475-0.692 | 0.475-0.700 | 0.591 | 0.610 | -0.03 |

For abbreviations of characters refer to Table 11; *Excluding non-nodulating references with no value for the corresponding characters

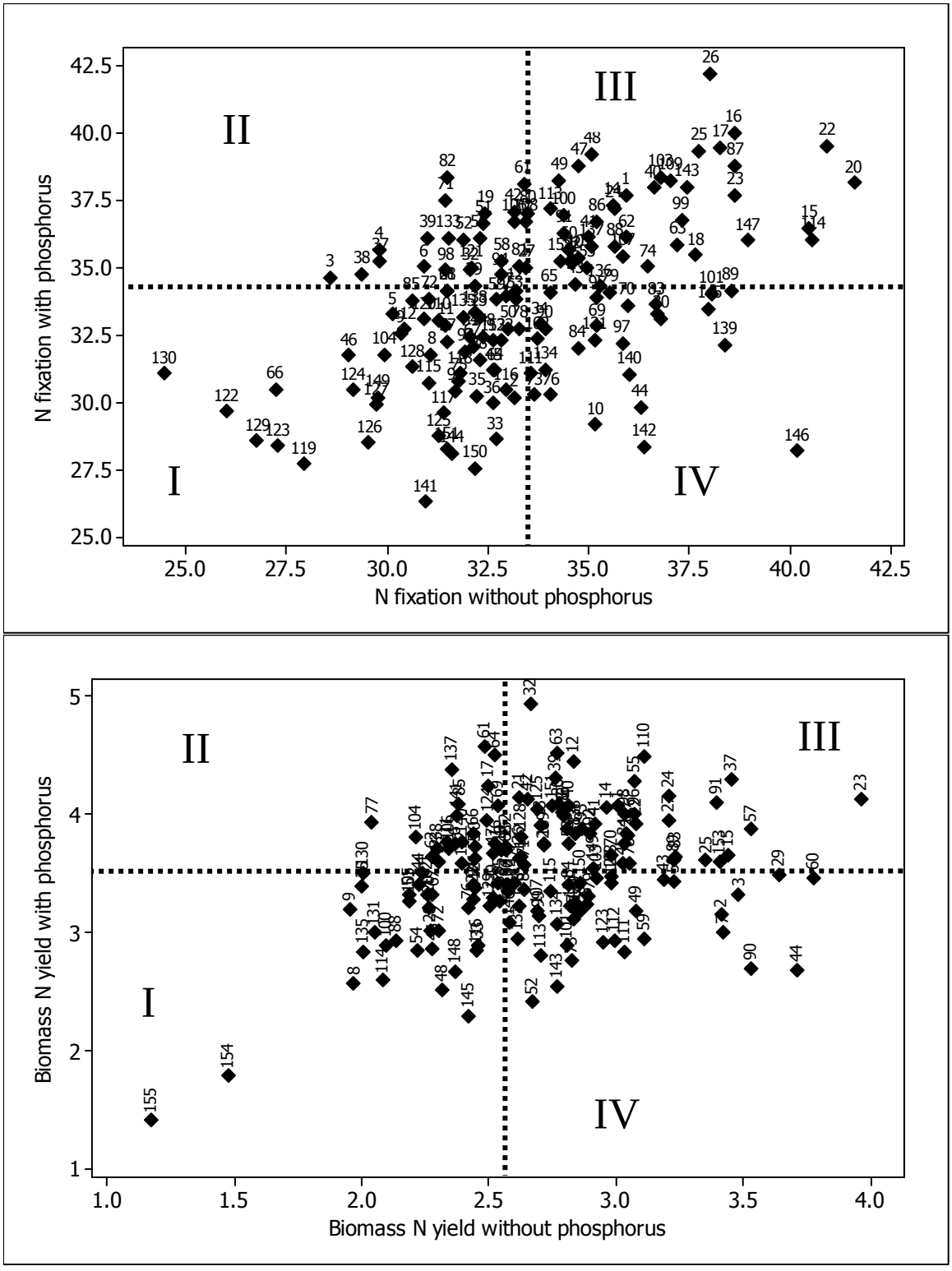



Figure 33. The relationship between (A) total biomass nitrogen fixation and (B) biomass nitrogen yield in 155 chickpea genotypes grown with and without phosphorus at two locations in Ethiopia showing different nitrogen fixation efficiency groups in response to the presence and absence of phosphorus fertilizer: (I) inefficient, non-responder; (II) inefficient, responder; (III) efficient, responder; and (IV) efficient, non-responder. The serial Nos. Marking the data labels stand for genotypes as given in Table 1.



I

## 8.5. Genetic characterization and evaluation for resistance to adzuki bean beetle

### 8.5.7. Performance evaluation

Pooled analyses of variance across the three locations showed statistically significant differences among the locations and genotypes for all the characters recorded except for non-significant differences among locations for seed weight loss for the sum of the first and the second progenies and among the genotypes for the proportion of un-infested seeds, i.e. seeds with no signs of eggs or emergence holes (Table 37).

Table 37. Combined analysis of variance (over locations) for response to infestation by adzuki bean beetle of 130 chickpea genotypes tested at three locations

| | | Mean square | | | CV |
|---|---|---|---|---|---|
| Character | Progeny | Location (L) | Genotype (G) | G x L | (%) |
| No of eggs laid female$^{-1}$ | First | ** | ** | ** | 25.69 |
| Proportion of un-infected seed (%) | First | * | NS | NS | 20.87 |
| Days to adult emergence | First | ** | * | NS | 2.32 |
| | Second | ** | ** | NS | 3.31 |
| | Total | ** | ** | NS | 2.00 |
| No of adults emerged | First | ** | ** | ** | 27.33 |
| | Second | ** | ** | NS | 22.31 |
| | Total | ** | ** | NS | 17.01 |
| Adult recovery (%) | First | ** | ** | ** | 16.89 |
| Seed weight loss (g) | First | ** | ** | NS | 30.77 |
| | Second | ** | ** | NS | 19.89 |
| | Total | NS | ** | NS | 12.35 |
| 1000 seed weight (g) | --- | --- | ** | --- | 19.17 |
| Seed coat weight (%) | --- | --- | ** | --- | 8.85 |

** = highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and NS = non-significant ($P > 0.05$)

### 8.5.8. Effect of genotype

The damage and loss by the second progeny were higher than that incurred by the first progeny in almost all the cases. Number of eggs laid female$^{-1}$ by the first progeny ranged from 22-51, days to adult emergence from 31-33 for the first progeny and from 33-35 for the second progeny. Differences of only one or two days for days to adult emergence were statistically significant, but that much delay of adult emergence may not have any serious practical meaning. This may be due to a lack of sufficient genetic variation among the genotypes for this particular trait. Number of emerged adults ranged from 104-222 for the first progeny and from 455-1136 for the second progeny, with recoveries of 56-85% for the first progeny. Seed weight loss ranged from 2-13 g for the first progeny and from 9-20 g for the second progeny (Appendix 8). Alemayehu (2005) also reported a similar level of seed weight loss for a local variety grown in Gojam.

The higher infestation and loss by the second progeny may be related to the temporal variations in weather parameters, population dynamism and the associated changes in aggressiveness of progenies or there may be a positive founder effect of infestation by the preceding progenies on the successive ones because of elevated initial number (density) of the pest and existence of adequate food sufficient to support both progenies in this specific case. This may not apply when there is a limitation in food supply. Earlier progenies with first access to food may not only consume the lion's share of the limited food, but may also "spoil" the leftover for the successive progenies.

Complete resistance was not observed in any of the current chickpea genotypes studied. Genes for complete resistance to insect pests in general and storage insects in particular are of rare occurrence in cultivated species (Acosta-Gallegos *et al.*, 2008) even if they have often been reported in related wild species of a number of legume crops (Ishimoto *et al.*, 1995; Somta *et al.*, 2007; 2008; Chen *et al.*, 2007). A few cases of complete resistance also have been reported in cultivars or germplasm collections of haricot bean (Ishimoto *et al.*, 1995; 1996; Goossens *et al.*, 2000), field pea (Morton *et al.*, 2000; Clement *et al.*, 2002), cowpea (Redden and McGuire, 1983), black

gram (Dongre *et al.*, 1996) and chickpea (Pacheco *et al.*, 1994; Shaheen *et al.*, 2006).

The improved genotypes showed considerably higher susceptibility particularly in terms of number of eggs female$^{-1}$, adults emerged and seed weight loss. A number of other reports indicate that improved cultivars may be more susceptible compared to landraces (Lale and Kolo, 1998). This may be because improvements associated with seed quality for human consumption might have resulted in more susceptibility of the improved cultivars as compared to local cultivars (Shaheen *et al.*, 2006). The larger the seed size, as is the case for the improved varieties and breeding lines tested here, the more susceptible they may be in terms of number of eggs laid, adult emergence and seed weight loss. Comparable results were observed from a similar study by Yadav *et al.* (2006). Large-seeded cultivars may provide more inter-seed space for easily movement during egg laying. Cultivars with smooth, soft, and thin seed coats maybe preferred for oviposition than those with rough, hard, wrinkled and spiny seed coats (Shaheen *et al.*, 2006) but there are some controversies and doubts to consider these features as universal indicators of resistance (Lale and Kolo, 1998; Somta *et al.*, 2007). The present finding does not concur with the previous findings in Ethiopia which indicated that small-seeded accessions were more preferred in terms of oviposition that resulted in the emergence of larger number of progenies (Lemma, 1990), perhaps because entirely local germplasm accessions which may not adequately vary for seed size were studied in the latter. The impact of past chickpea breeding for seed quality in Ethiopia will be presented in more details latter.

### 8.5.9. Sources of resistance to adzuki bean beetle

Frequency distributions of the 130 genotypes for the attributes showed the existence of high levels of total variation for most of the attributes (Figure 34). A few accessions had a lower extreme of only 22 eggs female$^{-1}$ while a few at the higher extreme had as high 52 eggs. Similarly, the corresponding number of adult emergences ranged between the lowest of 100 to the highest of over 220 for the first progeny. Seed weight loss ranged between 5 g and 13 g for the

first progeny. Attributes of both infestation levels (specifically number of eggs female$^{-1}$ and number of adults emerged) and seed weight loss appeared to be normally distributed for the first progeny.

Distributions appeared more skewed for the second progeny than for the first progeny, particularly for number of adults emerged and seed weight losses (Figure 34). The disparity between the patterns of distribution of responses to the first and second progenies may have been induced during infestation by the first progeny which may somehow be related to differential levels of consumption and spoilage of the seeds that, in turn, might have furthered the differential consumption by the second progeny and skewed the patterns of responses. Therefore, transformation of data may be more necessary for responses to the second progeny than to the first progeny.

It may be difficult to find a region with accessions that combine attributes responsible for resistance in a balanced manner. Accessions identified as best for one attribute did not consistently repeat the same performance for another attribute. Specific accessions for best resistance may be found for one or another attribute of resistance. This may indicate that, in different genetic backgrounds, different mechanism of resistance, starting from deterring oviposition to hindering adult development, may operate (Panda and Khush, 1995).

Despite the inconsistencies, however, some accessions like Acc. Nos. 41320, 41289, 41291, 41134, 41315, 207658, 41103, 41168, 41142, 41174, 41029, 41207, 209087 and 231327 were found to have relatively better resistance. Additional accessions like Acc. Nos. 41161 and 41008 had also low infestation and damage levels except for relatively higher adult emergence in second progeny (Appendix 8). From among the improved genotypes, Worku and Akaki had lower seed weight loss despite the heavy infestation.

### 8.5.10. Effect of location

The location effects were significant for all traits except for total seed weight loss. It was observed that genotype and location effects, as reflected from the level of significance for different characters, are more important than their interaction (Table 37).

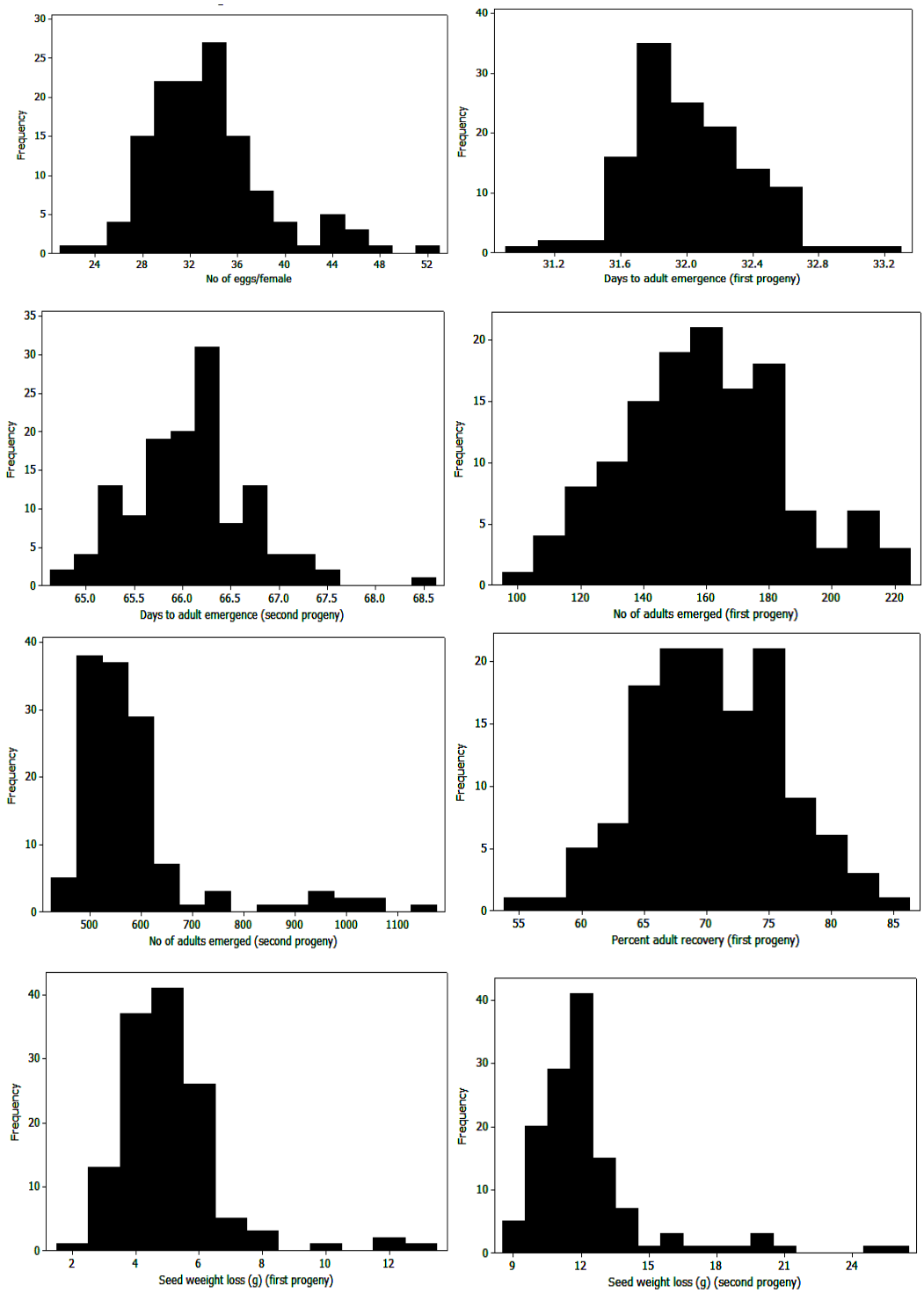



Figure 34. Frequency distributions in 130 chickpea genotypes for ten characters related to response to infestation by adzuki bean beetle

Locations did not exhibit consistent mean values in terms of magnitude of response to infestation by adzuki bean beetle, i.e. a location with the least mean value for one character may not repeat the same for other characters and *vice versa*. Nevertheless, it was interesting to note that in some cases locations where the lower values were recorded for the first progeny turned out to exhibit the higher values for the second progeny. These patterns were observed for days to adult emergence at Holetta, adult emergence at Ambo and seed weight loss at Debre Zeit (Table 38). A comprehensive study with spatial as well as temporal replication may be needed as the present investigation does not provide sufficient insight into the nature and cause of this pattern of changes.

Table 38. Mean performances of 130 chickpea genotypes for response to infestation by adzuki bean beetle over three locations in Ethiopia

| Character | Progeny | Holetta | Ambo | Debre Zeit |
|---|---|---|---|---|
| No of eggs laid female$^{-1}$ | First | 35.02$^{a}$ | 31.48$^{b}$ | 33.63$^{a}$ |
| Proportion of uninfected seed (%) | First | 29.34$^{b}$ | 32.29$^{a}$ | 30.59$^{ab}$ |
| Days to adult emergence | First | 31.40$^{b}$ | 32.62$^{a}$ | ---- |
| | Second | 34.85$^{a}$ | 33.49$^{b}$ | ---- |
| | Total | 65.29$^{b}$ | 66.88$^{a}$ | ---- |
| No of adults emerged | First | 172.75$^{a}$ | 146.82$^{c}$ | 159.09$^{b}$ |
| | Second | 556.93$^{b}$ | 668.82$^{a}$ | 543.40$^{b}$ |
| | Total | 729.68$^{b}$ | 815.38$^{a}$ | 702.49$^{c}$ |
| Adult recovery (%) | First | 73.01$^{a}$ | 68.66$^{b}$ | 69.38$^{b}$ |
| Seed weight loss (g) | First | 5.32$^{a}$ | 5.20$^{a}$ | 4.52$^{b}$ |
| | Second | 6.77$^{b}$ | 7.19$^{a}$ | 7.94$^{a}$ |
| | Total | 12.09$^{a}$ | 12.39$^{a}$ | 12.46$^{a}$ |

*Figures within a row sharing the same letter are statistically non-significant

### 8.5.11. Genotype by location interaction effects

Genotype by location interaction effects, i.e. the differential response of genotypes at different locations or the differential discrimination of the locations or both, were non-significant for all attributes but number of eggs female$^{-1}$, number of adults emerged (first progeny) and adult recovery (%) (Table 37). This indicated that genotypes selected for better resistance at one location may display a similar relative performance at other locations for most of the attributes measured.

If genotypes perform consistently across locations, breeders may be able to effectively evaluate genotypes with a minimum cost in a few locations for an ultimate use for wider areas. When significant, i.e. for number of eggs female$^{-1}$, number of adults emerged and adult recovery, genotype by location interaction effects were "cross-over" types; i.e. interaction associated with frequent rank order changes among genotypes across locations (Figure 35). It may be evident that, for these characters, the genotypes differentially responded across locations or the locations differentially discriminated the genotypes or both to the level of frequent rank order changes.

Cross-over types of interaction may be inevitable for quantitative traits (Smithson and Grisley, 1992), but the nature and cause of such interaction is not clear (Smithson and Grisley, 1992; Annicchiarico, 2002). Under such condition, when cross-over type of interaction reveals, it is not only high average resistance that is important in variety development, but also the stability of resistance across locations.

Variety evaluation should therefore be replicated over locations to adequately capture spatial variations. The appropriateness of varieties for release in terms of performance consistency across a range of physical environments (spatial variability) and years (temporal variability) is usually confirmed by multi-location evaluation of varieties even if it is laborious, costly and time consuming. If not, breeders may be required to go for specific breeding program targeting specific locations in order to identify superior genotypes adapted to specific conditions (Annicchiarico, 2002), but the cost of germplasm evaluation would greatly be escalated.

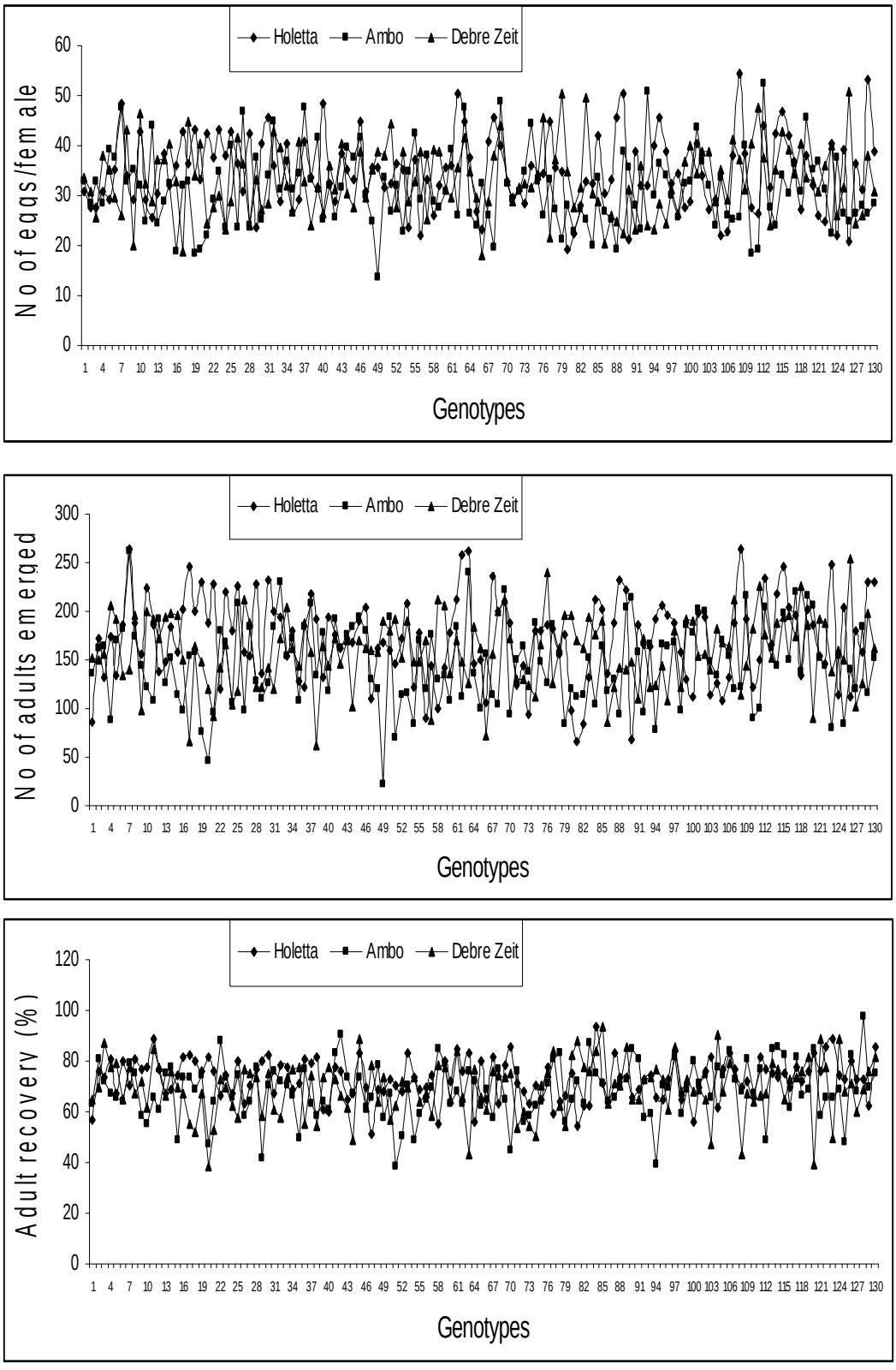



Figure 35. Responses of 130 chickpea genotypes to adzuki bean beetle at three locations showing the existence of crossover type of genotype by location interaction

### 8.5.12. Impact of past breeding on bruchid resistance

Genetic progresses achieved from breeding efforts since the last 15 years were considered with more focus on temporal changes in grain yield and seed quality as associated with changes in infestation levels and seed damage by the adzuki bean beetle. Disaggregated evaluation of the improved cultivars particularly the *Kabulis* which have large seed size were found to be more susceptible to adzuki bean beetle than the *Desi* in general and the local accessions in particular. This may somehow be related to the relatively stable nature of populations as compared to improved genotypes (Bekele, 1985) which have been forced through selection to attain genetic uniformity.

Primitive landraces are usually more stable and flexible to pest attack than the improved varieties as they have coexisted with and constantly exposed to insect pest on an evolutionary time scale (Acosta-Gallegos *et al.*, 2008). In improved varieties, on the other hand, artificial selection might have interfered with and dismantled the equilibrium for harmonious coexistence and the pattern of evolution. Therefore, the genotypes might have been forced to evolve in different direction by artificial selection than it would have been in landrace population (Bekele, 1985; Keneni and Simane; 2003). As stated earlier, improvements associated with grain quality for human consumption may also result in more susceptibility of the improved cultivars as compared to local cultivars (Shaheen *et al.*, 2006).

The tendency that *Kabulis* compared to *Desi* types are more susceptible to insect attacks was also reported by Yadav *et al.* (2006). The fact that *Desi* are more resistant to adzuki bean beetle than the *Kabulis* may be true but it is not ruling out the existence of *Desi* that are as susceptible or even more than the *Kabulis* in specific cases as shown in Figure 36.

A large-seeded *Desi* type released in Ethiopia, Natoli, was also found to be as susceptible as the released *Kabulis*. The *Kabulis* may also be characterized with thinner seed coats than the *Desi* (Figure 37). Nevertheless, complete resistance was not observed in Ethiopian chickpea germplasm accessions so far unlike the reports by other workers in other areas (Pacheco *et al.*, 1994; Shaheen *et al.*, 2006).

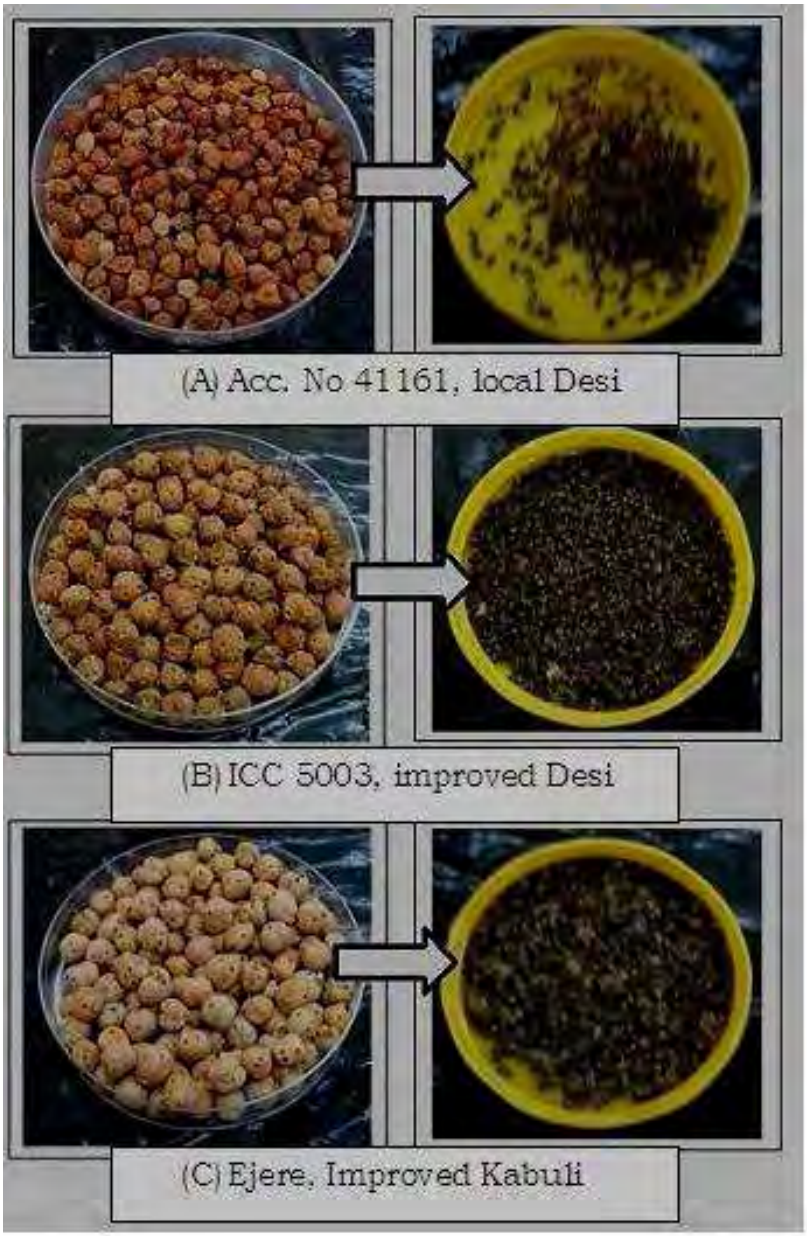



Figure 36. Partial view of the amount of seed damage (left side) and adult emergence (right side) in one of the relatively resistant Ethiopian chickpea germplasm accessions, Acc. No 41161 (A), with improved *Desi*, ICC 5003 (B), and *Kabuli*, Ejere (C), varieties showing more relative susceptibility of the improved cultivars to adzuki bean beetle. This is the emergence from the second progeny of the beetle.

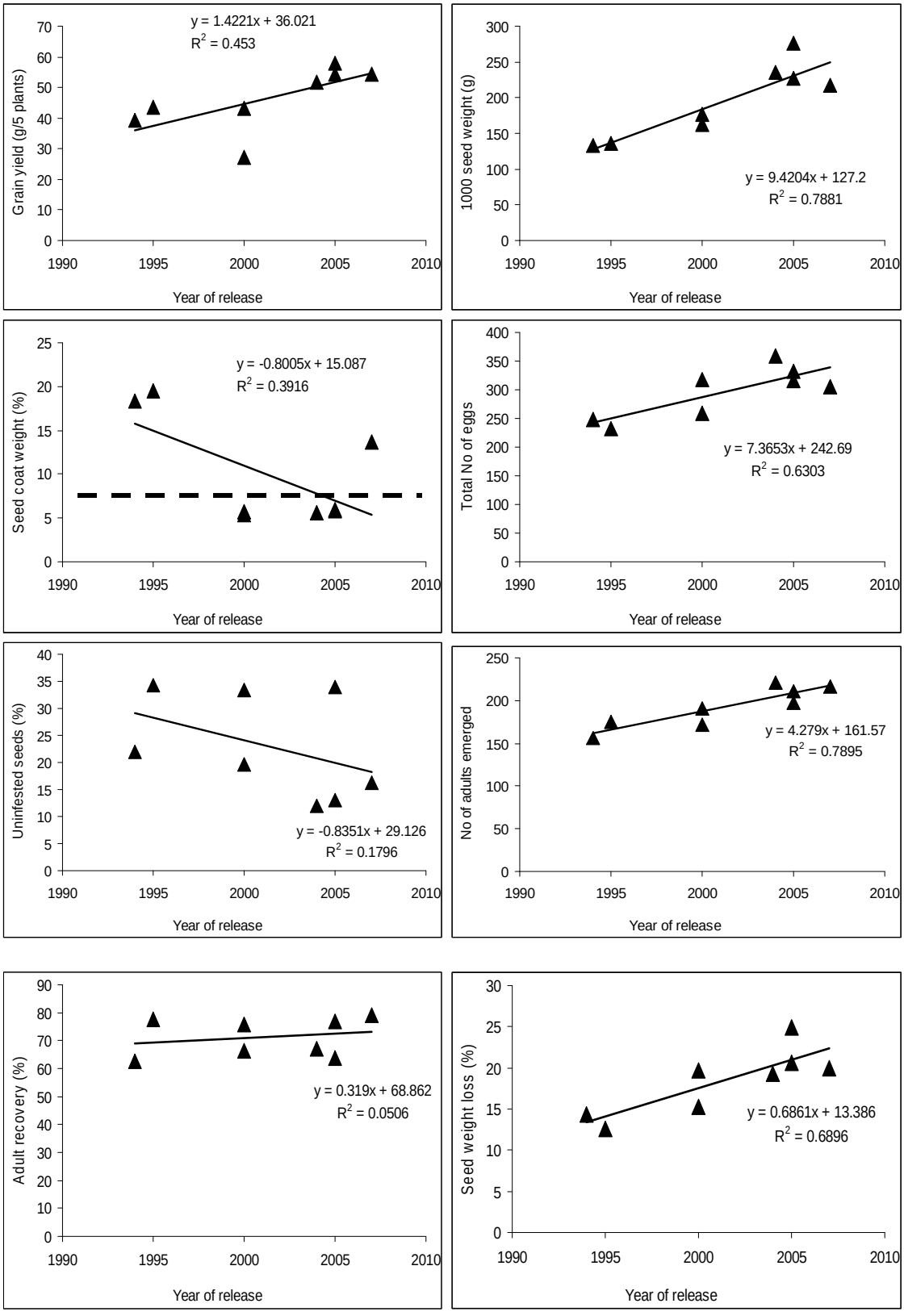



Figure 37. Bi-plots of grain yield and attributes of seed quality and response of chickpea improved varieties to infestation by adzuki bean beetle against years of release using Worku as an oldest base reference showing that improvements in seed quality had resulted in susceptibility to adzuki bean beetle (the *Kabulis* have thinner seed coat weights as shown below the broken horizontal line in the second graph at the left hand side)

Disaggregated comparison of all the released varieties with the oldest Worku showed that the magnitude of temporal increases from breeding progresses in grain yield and seed size varied from variety to variety. This could be attributable to the stage and purpose of variety development. For instance, the *Kabuli* variety Shasho was primarily released for its better relative seed size and white colour as compared to the previously released *Desi*, Worku and Akaki. This variety, though attractive for its seed quality, has become more susceptible to ascochyta blight (*Ascochyta rabiei*) diseases since very recently (own observations).

Disregarding Shasho, which was a low yielder, the temporal yield increments from breeding during the last 15 years ranged between 10% and 49%, the average being 30%. However, the corresponding increments in seed size ranged between 2% and 106%, with an average of 53%. Similar results with more dramatic increments in seed size than in grain yield were also reported from faba bean breeding in Ethiopia (Temesgen, 2008).

On the other hand, the proportion by weight of seed coat to the total seed weight dramatically reduced as a result of breeding, which may be associated not with intentional selection for thin seed coat types as such but with the general move from the *Desi* types to the *Kabulis* and even in *Kabuli* to more large seed size, which are characterized by thin seed coats in response to the market demand. The same was also true for the proportion of uninfected seeds.

The temporal changes in magnitudes of adzuki bean beetle infestation and seed damage, as compared to their magnitudes in Worku as a base reference variety, more or less followed the incremental trends in seed size. On the average, total number of eggs increased by 17%, number of adults emerged by 15%, adult recovery by 9% and seed weight loss by 27% (Table 39). More susceptibility to adzuki bean beetle of the recent varieties may, therefore, be associated with the increment in seed size than it may be associated with the increment in grain yield as discussed in detail latter.

Table 39. Temporal trends in mean performances and their percent increments of seed yield (g/5 plants), seed size, proportion by weight of seed coat (%) and attributes of response to infestation by Adzuki bean beetle in chickpea varieties released during the last 15 years in Ethiopia

| Variety | Seed yield (g 5 plants$^{-1}$) | | Seed size (g/1000) | | Seed coat weight (%) | | Number of eggs | | No of uninfected seeds | | No of adults emerged | | Adult recovery (%) | | Seed weight loss (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | % over Worku | Mean | % over Worku | Mean | % over Worku | Mean | % over Worku | Mean | % over Worku | Mean | % over Worku | Mean | % over Worku | Mean | % over Worku |
| Worku | 39 | --- | 134 | --- | 18 | --- | 258 | --- | 76 | --- | 171 | --- | 66 | --- | 15 | --- |
| Akaki | 43 | 10 | 136 | 2 | 20 | 11 | 249 | -4 | 63 | -17 | 157 | -8 | 76 | 15 | 14 | -1 |
| Shasho | 27 | -31 | 162 | 21 | 5 | -72 | 318 | 23 | 66 | -13 | 190 | 11 | 63 | -5 | 20 | 33 |
| Arerti | 43 | 10 | 177 | 32 | 6 | -67 | 316 | 23 | 78 | 3 | 176 | 3 | 78 | 18 | 13 | -13 |
| Habru | 52 | 33 | 236 | 76 | 6 | -67 | 231 | -11 | 64 | -16 | 198 | 16 | 64 | -3 | 25 | 67 |
| Ejere | 58 | 49 | 276 | 106 | 6 | -67 | 333 | 29 | 77 | 1 | 211 | 23 | 77 | 17 | 21 | 40 |
| Teji | 54 | 39 | 227 | 69 | 6 | -67 | 359 | 39 | 67 | -12 | 222 | 30 | 67 | 2 | 19 | 27 |
| Natoli | 54 | 39 | 217 | 62 | 14 | -22 | 306 | 19 | 79 | 4 | 217 | 27 | 79 | 20 | 20 | 33 |
| Average | 46 | 21(30)* | 196 | 53 | 10 | -50 | 296 | 17 | 71 | -7 | 193 | 15 | 71 | 9 | 18 | 27 |

*Mean value for yield increment in the bracket is excluding Shasho

The genetic progress obtained since 1994, using the average yield performance of the variety Worku as reference base, ranges between negative gains for variety Shasho to close to 50% gains for variety Ejere. The annual rate of genetic progress for grain yield was 1.42 g 5 plants$^{-1}$ year$^{-1}$ or over 21 g 5 plants$^{-1}$ in 15 years ($R^2$ = 0.45). The negative gain of variety Shasho could be attributed to its limited initial yield potential and recent susceptibility to diseases.

Better genetic progress was obtained from breeding for seed size than it was for grain yield. Results from faba bean breeding in Ethiopia showed the same trends (Temesgen, 2008). There are even results elsewhere in other crops showed no yield gain at all from breeding, which was implicated as the attainment of yield "plateus" (Egli, 2008). From the linear regression of seed size against years of release in this study, an annual rate of genetic progress of 9.42 g 1000 seeds$^{-1}$ ($R^2$ = 79) was obtained, i.e. more than 141 g 1000 seeds$^{-1}$ in 15 years. Most recent varieties showed by far higher breeding progress for seed size.

Past breeding efforts have, therefore, resulted in substantial progresses in improving seed size in chickpea. While grain yield is the primary trait of interest and a prime objective in most of the Ethiopian crop breeding programs for the last many decades, seed size also received a special attention since recently and this is happening at international and national level in response to the current move to meet the export-market demand for seed quality particularly for large seed size which fetches high prices in the world market (EARO, 2000).

In chickpea, preferences vary from place to place but larger seeds with lower proportion of husk may mostly be preferable (Singh, 1987). It also appears that there is a slight shift from the *Desi* types that are mostly small-seeded to the *Kabulis* that are large-seeded, which is again emanated from the market demand. The introduced *Kabuli* contributed a lot to the improvement of seed size as all of the released varieties have their origins in introductions from ICRISAT and ICARDA where increasing seed size is the primary breeding target.

It was, nevertheless, observed in this study that the genetic progress in seed size, as an attribute of great economic importance and prime breeding objective, is not immune of some technical shortcomings. Characters for response to adzuki bean beetle including total number of eggs laid, number of adults emerged and seed weight loss showed simultaneous increments with the gains in seed size (Figure 38).

On the contrary, number of uninfected seeds decreased with the increasing seed size over time. This indicated that improvement in seed size had inadvertently increased susceptibility of the improved cultivars to adzuki bean beetle (Table 40 and Figure 37). However, this was not observed in a previous study by Lemma (1990) who found that small-seeded chickpea accessions were more preferred for oviposition and adult emergence than the large-seeded ones. This disagreement could be because he studied almost entirely local germplasm accessions which may not sufficiently vary for seed size as stated earlier.

Why the recent varieties are more susceptible to infestation by adzuki bean beetle may need an independent study but a few logical speculations may be made based on the available evidences and sources. First, it was observed in this study that the proportion by weight of seed coat to the whole weight periodically reduced through breeding for better seed quality (Figure 37). Morphologically, varieties with smooth, soft and thin seed coats may be preferred for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Ahmed *et al.*, 1989; Shaheen *et al.*, 2006). Desroches *et al.* (1995) found that the seed coat in faba bean acted like a physical barrier against penetration by *C. chinensis* and C. *maculatus*.

Secondly, seed size showed significantly positive relationship with response to adzuki bean beetle like the number of eggs laid, the adults emerged and the weight loss thereof. This may have something to do with the physical suitability of large-seeded cultivars in terms of insects' behaviors like existence of more inter-seed space for easily movement during consumption and egg laying. Previous studies also showed that the larger the seed size in chickpea, the thinner the seed coat may be (Singh, 1987), and the more susceptible it would be to storage insects (Yadav *et al.*, 2006).

That the improved varieties with better seed quality may be more susceptible to storage insects as compared to landraces was also reported before (Desroches *et al.*, 1995; Lale and Kolo, 1998) as improvements associated with grain quality for human consumption might also result in more susceptibility of the improved cultivars as compared to local cultivars (Shaheen *et al.*, 2006). Breeding for resistance against storage insect pests using wild relatives as gene sources may improve resistance but often reduces the quality of the produce or may even make it unfit for consumption (Singh, 2002) particularly when the mechanism of resistance in itself reduces seed or chemical resistance when the compounds involved are also toxic to man (Edwards and Singh, 2006). It was generally observed in chickpea itself that the large-seeded chickpeas with thin seed coat are also generally assumed to possess better cooking quality than those with smaller seeds which have thick seed coats (Cubero, 1987).

Table 40. Trends in genetic progresses from breeding chickpea for better grain yield and seed quality during the last 15 years with the associated increments in susceptibility to adzuki bean beetle

| Parameter | MS of regression | P level | Annual rate of breeding progress (b) | Gain in 15 years | $R^2$ |
|---|---|---|---|---|---|
| Grain yield (g/5 plants) | 336 | 0.066 | 1.422 | 21.33 | 0.45 |
| Grain yield (g/5 plants)* | 240 | 0.002 | 1.219 | 18.29 | 0.87 |
| 1000 seed weight (g) | 14510 | 0.003 | 9.420 | 141.30 | 0.79 |
| Seed coat weight (%) | 105 | 0.097 | -0.801 | -12.00 | 0.39 |
| Total No of eggs | 2994 | 0.003 | 7.365 | 110.48 | 0.63 |
| Uninfected seeds (%) | 453 | 0.042 | -0.835 | -12.53 | 0.18 |
| No of adults emerged | 2994 | 0.003 | 4.279 | 64.19 | 0.79 |
| Adult recovery (%) | 17 | 0.592 | 0.319 | 4.79 | 0.05 |
| Seed weight loss (g) | 77 | 0.011 | 0.690 | 10.35 | 0.69 |

*The second grain yield results exclude Shasho as an outlier for lower grain yield

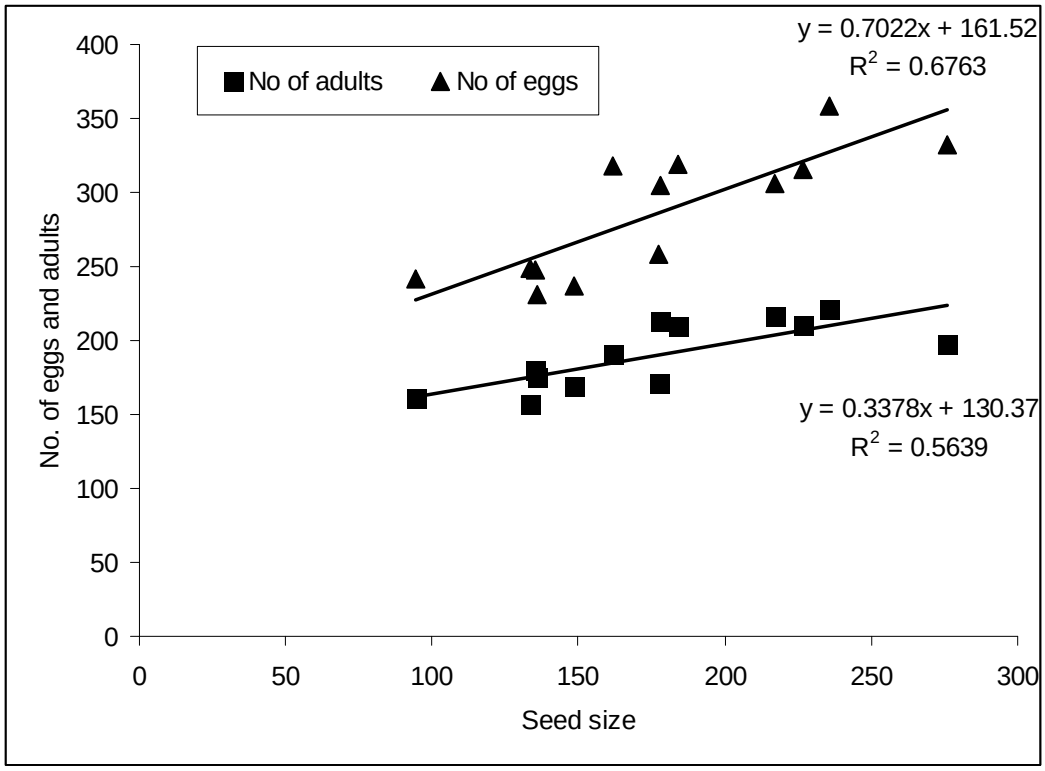



Figure 38. Relationship of seed size (1000 seed weight, g) with number of eggs laid and adults emerged in chickpea improved genotypes showing that improvements in seed size resulted in susceptibility to adzuki bean beetle

### 8.5.13. Phenotypic diversity

#### 8.5.13.2. Cluster and distance analysis

Cluster analysis grouped the 130 chickpea genotypes primarily into three clusters ($C_1$, $C_2$ and $C_3$), based on the mean of 11 response characters to infestation. The number of member genotypes varied from cluster to cluster. Cluster $C_1$, with a total of 46 members or 35% of the total population, totally excluded improved genotypes and entirely constituted local germplasm accessions. Cluster $C_2$ with 71 members or 58% of the total population, entirely constituted local germplasm accessions with only four improved genotypes, namely ICC 4948, ICC 15996, Worku and Akaki, as a few exceptions, all of the latter being interestingly from the improved *Desi* types. Cluster $C_3$, with only nine members or 7% of the total population, totally excluded local accessions and constituted entirely improved genotypes (Table 41 and Figure 39).

Table 41. Clustering of one hundred thirty chickpea genotypes into three clusters using mean of 11 response characters to infestation by adzuki bean beetle

| Cluster | Number of Accessions | Accessions included |
|---|---|---|
| $C_1$ | 46 | Acc. No. 231327, Acc. No. 231328, Acc. No. 209094, Acc. No. 41002, Acc. No. 41074, Acc. No. 41021, Acc. No. 41103, Acc. No. 41320, Acc. No. 41029, Acc. No. 41015, Acc. No. 41276, Acc. No. 41275, Acc. No. 207744, Acc. No. 41298, Acc. No. 41311, Acc. No. 41280, Acc. No. 41315, Acc. No. 41295, Acc. No. 41296, Acc. No. 41289, Acc. No. 41291, Acc. No. 41297, Acc. No. 41293, Acc. No. 41048, Acc. No. 41054, Acc. No. 209087, Acc. No. 41160, Acc. No. 41161, Acc. No. 207665, Acc. No. 41134, Acc. No. 41128, Acc. No. 207658, Acc. No. 41142, Acc. No. 41207, Acc. No. 41215, Acc. No. 41008, Acc. No. 209035, Acc. No. 41174, Acc. No. 41171, Acc. No. 209036, Acc. No. 207563, Acc. No. 207895, Acc. No. 219800, Acc. No. 219803, Acc. No. 212589, Acc. No. 207660, |
| $C_2$ | 75 | Acc. No. 209093, Acc. No. 208829, Acc. No. 209096, Acc. No. 209097, Acc. No. 209098, Acc. No. 207761, Acc. No. 207763, Acc. No. 41026, Acc. No. 41075, Acc. No. 41076, Acc. No. 41027, Acc. No. 207734, Acc. No. 41271, Acc. No. 207745, Acc. No. 41277, Acc. No. 207743, Acc. No. 41273, Acc. No. 41274, Acc. No. 207741, Acc. No. 207742, Acc. No. 41316, Acc. No. 41313, Acc. No. 41312, Acc. No. 41308, Acc. No. 41299, Acc. No. 41046, Acc. No. 41047, Acc. No. 41304, Acc. No. 41303, Acc. No. 41290, Acc. No. 41053, Acc. No. 41052, Acc. No. 209084, Acc. No. 209091, Acc. No. 209088, Acc. No. 209089, Acc. No. 209090, Acc. No. 41159, Acc. No. 207661, Acc. No. 207667, Acc. No. 207666, Acc. No. 41141, Acc. No. 41168, Acc. No. 41129, Acc. No. 41130, Acc. No. 41110, Acc. No. 41111, Acc. No. 41066, Acc. No. 41011, Acc. No. 41007, Acc. No. 41176, Acc. No. 41175, Acc. No. 41170, Acc. No. 41185, Acc. No. 41190, Acc. No. 41195, Acc. No. 207151, Acc. No. 207564, Acc. No. 219797, Acc. No. 219799, Acc. No. 221696, Acc. No. 41114, Acc. No. 41113, Acc. No. 207659, Acc. No. 225878, Acc. No. 225873, Acc. No. 225874, Acc. No. 225877, Acc. No. 207645, Acc. No. 207646, Acc. No. 225876, ICC 4948, ICC 15996, Worku (DZ-10-16-2), Akaki (DZ-10-9-2) |
| $C_3$ | 9 | ICC 5003, ICC 4918, ICC 4973, Shasho (ICCV 93512), Arerti (FLIP 89-84C), Ejere (FLIP-97-263c), Teji (FLIP-97-266c), Habru (FLIP 88-42c), Natoli (ICCX-910112-6) |

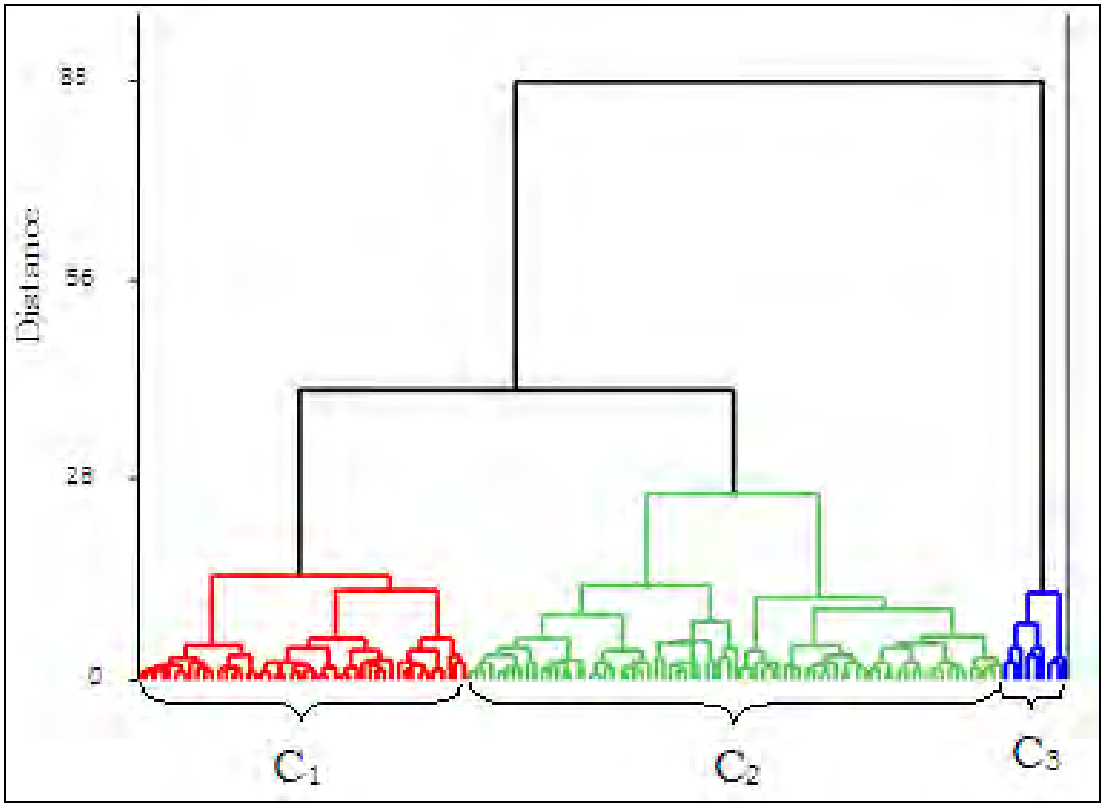



Figure 39. Dendrogram of hundred thirty chickpea genotypes developed by Ward's agglomerative hierarchical classification method based on Euclidian distance using mean of 11 response characters to infestation by adzuki bean beetle

The pairwise generalized squared distances ($D^2$) among the three clusters based on the Mahalanobis's $D^2$ statistics revealed non-significant ($P>0.05$) inter-cluster distance between clusters $C_1$ and $C_2$ ($D^2 = 6.30$). On the other hand, the maximum distances were found between clusters $C_1$ with $C_3$ ($D^2 = 154.03$) and the second largest distance between clusters $C_2$ with $C_3$ ($D^2 = 122.90$) which significantly ($P\leq0.01$) diverged from each other (Figure 40). Members in clusters with non-significant distance were assumed to have more close relationships with each other than they are with those in significantly distant clusters (Singh and Chaudhary, 1985).

Characterization of the clusters based on cluster mean performance showed varying response of member genotypes in each cluster to infestation by adzuki bean beetle. Cluster $C_1$ constituted accessions with the least number of eggs and adults emerged, highest number of uninfected seeds, least percentage of adult recovery and seed weight loss and smallest seed

size with largest proportion of seed coat weight. On the other hand, cluster $C_3$ constituted improved genotypes with the largest number of eggs laid and adults emerged, lowest number of uninfected seeds, largest percentage of adult recovery and seed weight loss and largest seed size with the least proportion of seed coat weight.

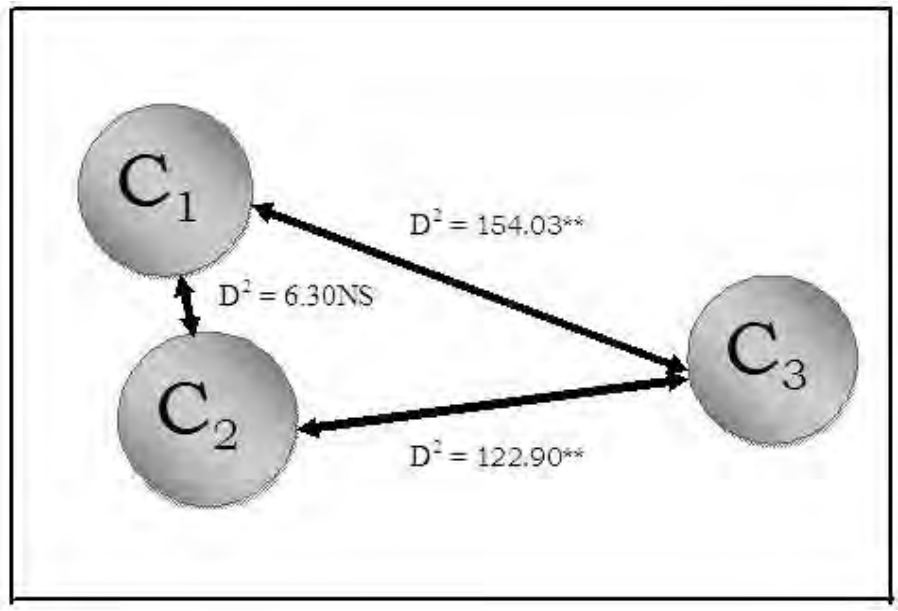



Figure 40. Cluster diagram showing the pair-wise generalized squared distances between three clusters constituting 130 chickpea genotypes ($D^2$ values are not to scale; **highly significant, $p \leq 0.01$)

The total number of eggs laid and adults, seed weight loss and seed size noticeably increased with susceptibility as we move along from cluster $C_1$ to $C_3$. Other characters, namely proportion of seed coat weight and number of uninfected seeds decreased in the same direction. Adult recovery percent was the least in cluster $C_1$ (67%) and almost equal in clusters $C_2$ and $C_3$ (72%). There was practically no difference among the clusters for days to adult emergence. Therefore, it is clear that cluster $C_1$ constituted accessions characterized by better resistance to adzuki bean beetle while cluster $C_3$ constituted improved genotypes mostly of *Kabuli* types that are the most susceptible to adzuki bean beetle.

Among the top 10% best accessions (Acc. Nos. 41320, 41289, 41291, 41134, 41315, 207658, 41103, 41168, 41142, 41174, 41029, 41207, 209087, 231327, 41161 and 41008) we selected for better relative resistance to the same pest based on univariate analysis as discussed earlier (sub-section 4.5.3.), all of them were grouped in cluster $C_1$ except Acc. No. 41168 which fell into cluster $C_2$. The second cluster, cluster $C_2$, was characterized by intermediate values between clusters $C_1$ and $C_3$ in a number of cases. In a practical sense, there was no variation among the clusters as far as days to adult emergence was concerned may be due to lack of sufficient genetic variation among the genotypes for this trait (Table 42).

Table 42. Differences among the three clusters of 130 chickpea genotypes for mean performance of 11 response characters to infestation by adzuki bean beetle

| Character | Cluster | | | Grand mean |
|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_3$ | |
| Total No of eggs | 207.2 | 241.3 | 306.6 | 251.7 |
| No of uninfected seeds (%) | 35.26 | 29.65 | 20.32 | 28.41 |
| Days to adult emergence (1st progeny) | 32.11 | 31.99 | 31.72 | 31.94 |
| Days to adult emergence (2nd progeny) | 34.18 | 34.06 | 33.63 | 33.96 |
| No of adults emerged (1st progeny) | 135.3 | 169.4 | 201.2 | 168.7 |
| No of adults emerged (2nd progeny) | 561.6 | 557.4 | 1002 | 707.2 |
| Adult recovery (%) (1st progeny) | 67.34 | 72.01 | 71.95 | 70.43 |
| Seed weight loss (g) (1st progeny) | 4.06 | 5.23 | 8.07 | 5.79 |
| Seed weight loss (g) (2nd progeny) | 11.13 | 12.15 | 19.72 | 14.33 |
| 1000 seed weight | 103.5 | 109.6 | 199.1 | 137.4 |
| Proportion of seed coat weight (%) | 19.09 | 18.7 | 8.41 | 15.4 |

Although the improved genotypes were found to be the most susceptible to infestation by adzuki bean beetle, a few of them showed a new avoidance mechanism to the pest. Normally, the pest would have been expected to pupate inside the seed and the adult to emerge in a couple of weeks upon

the completion of the developmental stages (Gwinner *et al.* 1990). It was interestingly noted in the improved cultivars, particularly the *Kabuli* types (Shasho, Arerti, Ejere, Teji and Habru), as opposed to the other genotypes, that the young larvae (instead of the adult) prematurely emerged out of the seeds may be before they consumed the seeds to their maximum body size (adult beetles do not feed on stored product). Those young larvae might have alternatively fed on the frass outside the seed in order to grow and develop into full adult. Accurate reasoning for premature emergence of the larvae in these improved cultivars may be the subject of future investigation, but three reasons can be speculated. First, the proportions by weight of the seed coats to the whole seed weights of these improved cultivars were mostly found to be considerably lower than they were in germplasm accessions. It could be expected that the thin seed coats might have allowed more number of ovipositioning and larval penetration as witnessed from larger number of egg counts and adult emergence but they might be too weak to anchor the young larvae after penetration from falling back tight into the holes of the seeds. Secondly, the thin seed coat might somehow be associated with soft cotyledon, due either to gene linkage or pleiotropic effects, particularly in the improved *Kabuli* types which might not only be mechanically but also chemically digested to make a seemingly wider hole at the early stage that cannot again anchor the larvae tight into its position. Thirdly, it is also not clear if there is any sort of toxic substances from the seeds of these improved varieties have been involved to expel the larvae before maturity. This observation, even if it was not systematically recorded and analyzed, from biological point of view, may pave the way towards investigation of a new mechanism of avoidance by the most susceptible genotypes.

#### 8.5.13.3. Geographic pattern of phenotypic diversity

Cluster analysis primarily divided the population into the local accessions and the improved genotypes at the upper hierarchal level (with only a few exceptions) and then extended to re-divide the local accessions into two clusters at the lower hierarchy (Figure 39). A few improved *Desi* types (ICC 4948, ICC 15996, Worku and Akaki) showed exceptional affinity to be

grouped with the local accessions in cluster $C_2$ may be due to the fact that *Desi* types are generally more resistant to bruchids than the *Kabuli* types (Yadav *et al.*, 2006). These exceptionally grouped genotypes produced mean values similar with member genotypes in cluster $C_2$ than those in cluster $C_3$ for a number of characters particularly for total number of eggs ($\overline{X}$=239.67), percent uninfected seeds ($\overline{X}$=27.98), number of adults emerged ($\overline{X}$ for first progeny = 165.61; $\overline{X}$ for second progeny =746.05), seed weight loss ($\overline{X}$ for first progeny = 4.78), 100 seed weight ($\overline{X}$=128.16) and proportion of seed coat weight ($\overline{X}$=18.34). Days to adult emergence and adult recovery percent were not important determinants between clusters $C_2$ and $C_3$.

It could not be certain from this study whether the division into the local and improved genotypes at the upper hierarchy is due to differences associated with the divergence in eco-geographical origins of the two groups or whether it is due to differences in the level of prior genetic manipulation to which the improved genotypes have been subjected or both. This is because the effects of differences in origins and levels of prior genetic manipulation have been confounded as all introduced genotypes are improved and, conversely, all local accessions are not improved. On the other hand, clustering pattern with the local collections apparently revealed no definite relationship with origin as accessions from the same origin fell into both clusters ($C_1$ and $C_2$ ) with non-significant inter-cluster distance and *vice versa* (Table 43). Theoretically, the genetic architecture of a population is believed to be the result of breeding system, gene flow within and between populations, isolation mechanisms and prolonged selection by various natural and artificial forces (Chandel and Joshi, 1983). Populations from areas far separated geographically with mountains and valleys, having complex environments and varied ecological conditions are normally expected to accumulate enormous genetic diversity (Chandel and Joshi, 1983; Singh, 2002) as geographical separation with physical barriers and genetic barriers to inter-crossing are believed to give rise to genetic diversity among genetic materials (Singh, 2002).

Table 43. Clustering pattern of chickpea genotypes from different origins over three clusters based on mean performance of 11 response characters to infestation by adzuki bean beetle

| Origin | Number of accessions | No. of accessions in each cluster | | |
|---|---|---|---|---|
| | | $C_1$ | $C_2$ | $C_3$ |
| Arsi | 11 | 4 | 7 | -- |
| East Gojam | 10 | 5 | 5 | -- |
| West Gojam | 12 | 4 | 8 | -- |
| North Gonder | 13 | 4 | 9 | -- |
| South Gonder | 9 | 7 | 2 | -- |
| West Harerge | 8 | 2 | 6 | -- |
| East Shewa | 13 | 5 | 8 | -- |
| North Shewa | 10 | 5 | 5 | -- |
| West Shewa | 10 | 4 | 6 | -- |
| Tigray | 9 | 4 | 5 | -- |
| South Wello | 12 | 2 | 10 | -- |
| Improved genotypes | 13 | -- | 4 | 9 |
| Total | 130 | 46 | 75 | 9 |

The inter-regional similarity among accessions from Ethiopia may be related to the informal seed interchange between farmers, “founder” effect of original introductions with similar genetic background for resistance to this pest or the nature of selection forces operating under local conditions for this particular pest after introduction may be similar across eco-geographical regions or the combination of all. That almost all of the improved genotypes clustered independently as more susceptible groups is

not beyond expectation as they have not been bred for resistance to adzuki bean beetle. Primitive landraces may be more stable and flexible to pest attack than the improved varieties as they have coexisted with and constantly exposed to insect pest on an evolutionary time scale (Acosta-Gallegos *et al.* 2008).

In improved varieties, on the other hand, artificial selection might have interfered with and dismantled the equilibrium for harmonious coexistence and the pattern of evolution. Therefore, the genotypes might have been forced to evolve in different direction by artificial selection than it would have been in landrace population. On the other hand, the local accessions from all eco-geographical origins distributed over the first two clusters with non-significant distance (Table 43), indicating that accessions from different sources of origin may share similar genetic background as far as response to adzuki bean beetle is concerned. Therefore, accessions may show genetic similarities for seed infestation and damage levels by adzuki bean beetle regardless of differences in eco-geographic origins. That a few improved *Desi* genotypes from exotic sources and the local accessions fell into the same cluster, $C_2$, may indicate that it is not possible to rule out existence of exceptional improved genotypes sharing similar genetic background with the local landraces.

#### 8.5.13.4. Principal component analysis

Principal component (PC) analysis showed that the first five of the eleven principal components ($PC_1$-$PC_5$) accounted for 87% of the total variation among the genotypes, $PC_1$ alone with the largest contribution of nearly 48%. The first two principal components ($PC_1$ and $PC_2$) contributed about 60% of the total variation. Therefore, characters with relatively larger absolute values of eigenvector weights in $PC_1$ had the largest contribution to the differentiation of the genotypes into clusters as it is normally assumed that characters with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). Accordingly, most of the characters individually contributed small effects ($\pm$ 0.07 to 0.39) to the

variation in $PC_1$ and, therefore, the differentiation of the accessions into different clusters was rather dictated by the cumulative effects of a number of characters.

Characters with relatively greater positive weights of eigenvectors in $PC_1$ include seed weight loss, 100 seed weight and number of eggs and adults while proportion of seed coat weight and number of uninfected seeds had relatively large negative weights on this component. Breeding efforts may need to simultaneously focus on genetic manipulation of these characters in order to reduce infestation and seed damage levels by adzuki bean beetle. Two characters, namely adult recovery and days to adult emergence, with smaller negative and positive weights, respectively, contributed the least to the differentiation of the population. Different characters also contributed to the variation in the second and third principal components ($PC_2$ and $PC_3$) but both components accounted for relatively smaller total variations of 12% and 11%, respectively (Table 44).

A pattern analysis based on scatter plots of the genotypes using the first three principal components ($PC_1$, $PC_2$ and $PC_3$) accounted for about 60-70% of the estimated total variation more or less conformed with the pattern of genetic diversity revealed by the cluster analysis of the standardized Mahalanobis's $D^2$ statistics. Clusters $C_1$ and $C_2$ distributed closer to each other around the center of the PC plane while cluster $C_3$ was spatially situated relatively far apart from both clusters $C_1$ and $C_2$ (Figure 41).

### 8.5.8. Interrelationships between characters

Interrelations between the characters revealed strong associations in a number of cases both in positive and negative directions (Table 45). Positively significant associations were observed between seed weight loss and three component characters, namely total number of eggs, number of adults emerged and seed size in both progenies.

Conversely, number of uninfected seeds (%) and proportion of seed coat weight (%) had strong negative correlation with seed weight loss. Seed weight loss could, therefore, be reduced by selection for smaller seed size, least number of eggs and adult emergence or from selection for increased percent

seed coat weight and number of uninfected seeds. In order to reduce seed weight loss, therefore, smaller seed size should be combined with least numbers of eggs, adult emergence and proportion of uninfected seeds with thicker seed coat into a single genotype. Smaller seed size and thicker seed coat could be considered as good secondary traits for use as selection criteria for resistance to adzuki bean beetle as they had also better heritability and expected genetic gain from selection (Figure 42), strongly correlated with seed weight loss and are easily measurable.

Table 44. Percentage and cumulative variances and eigenvectors on the first five principal components for eleven characters in hundred thirty chickpea genotypes

| Parameter | Principal components (PCs) | | | | |
|---|---|---|---|---|---|
| | $PC_1$ | $PC_2$ | $PC_3$ | $PC_4$ | $PC_5$ |
| Variance (%) | 47.48 | 12.31 | 10.74 | 9.12 | 7.21 |
| Cumulative (%) | 47.48 | 59.79 | 70.54 | 79.65 | 86.86 |
| Character | ----------Eigenvectors---------- | | | | |
| Total No of eggs | 0.36 | 0.31 | -0.11 | -0.26 | 0.15 |
| No of uninfected seeds (%) | -0.31 | -0.22 | -0.09 | 0.32 | -0.01 |
| Days to adult emergence (1st progeny) | -0.13 | -0.45 | 0.47 | -0.06 | 0.60 |
| Days to adult emergence (2nd progeny) | -0.09 | 0.30 | -0.42 | 0.58 | 0.59 |
| No of adults emerged (1st progeny) | 0.32 | 0.48 | 0.23 | -0.002 | 0.11 |
| No of adults emerged (2nd progeny) | 0.35 | -0.37 | -0.05 | 0.26 | -0.08 |
| Adult recovery (%) (1st progeny) | 0.07 | 0.26 | 0.70 | 0.51 | -0.17 |
| Seed weight loss (g) (1st progeny) | 0.33 | -0.05 | 0.04 | -0.21 | 0.33 |
| Seed weight loss (g) (2nd progeny) | 0.39 | -0.23 | 0.01 | -0.01 | 0.21 |
| 1000 seed weight | 0.38 | -0.19 | -0.06 | 0.25 | -0.04 |
| Proportion of seed coat weight (%) | -0.35 | 0.21 | 0.18 | -0.26 | 0.28 |

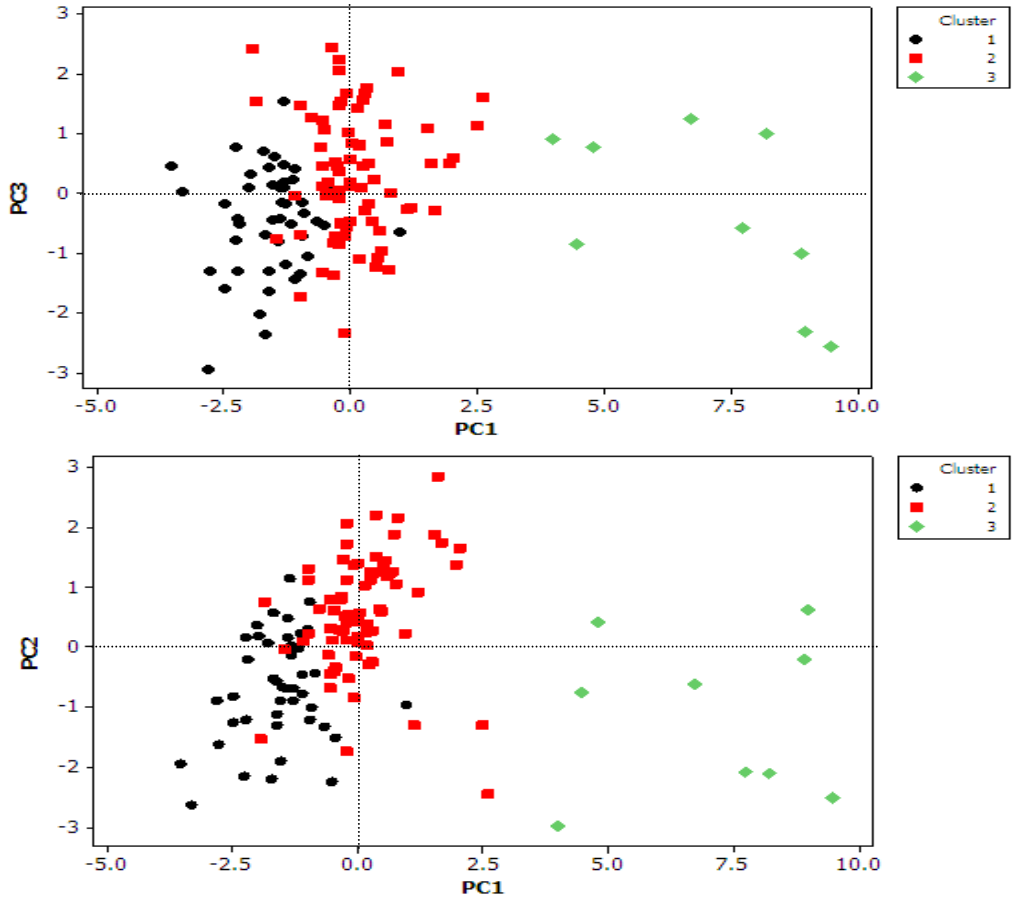



Figure 41. Scatter plots of the first three principal components ($PC_1$, $PC_2$ and $PC_3$) showing the patterns of distribution of hundred thirty chickpea genotypes for 60-70% of the total variation among the genotypes

Other reports indicated that chickpea varieties with smooth, soft and thin seed coats may be preferred for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Ahmed *et al.*, 1989; Shaheen *et al.*, 2006) as stated earlier. Desroches *et al.* (1995) also found that the seed coat in faba bean acted like a physical barrier against penetration by *C. chinensis* and C. *maculatus*.

The effectiveness of selection on seed-based traits as a strategy could be useful for reducing infestation. However, seed size in many legumes including chickpea is an economic trait deserved the second priority next to grain yield as a prime objective of the breeding program in Ethiopia (EARO, 2000).

Unfortunately, improvement in seed size and seed coat thinness could result in a negative selection for resistance to the beetle.

Previous studies also showed that the larger the seed size in chickpea, the thinner the seed coat may be (Singh, 1987), and the more susceptible it would be to storage insects (Yadav *et al.*, 2006).

As increased seed size and decreased seed coat thickness are desirable seed quality traits in chickpea (Singh, 1987), for improving seed size and seed coat thinness through selection, a compromise must be made for resistance to adzuki bean beetle or the breeder must set minimum standards for one trait while selecting for the other. Or else, it is better to follow separate breeding programs for these seed quality attributes and resistance to adzuki bean beetle.

Strong positive associations were also observed among total number of eggs with number of adults emerged and seed size, number of adults emerged with seed size and number of uninfected seeds with the proportional weight of the seed coat. Adult recovery showed strong positive association only in one case with number of adults emerged.

Days to adult emergence was weakly associated with a number of other traits. Strong negative associations were observed among total number of eggs with number of uninfected seeds and percent seed coat weight, number of adults emerged with number of uninfected seeds, number of uninfected seeds with seed size and seed size with percent seed coat weight.

Aslam *et al.* (2006) evaluated varieties of chickpea for response to the same beetle and found significant positive correlation between number of eggs and adults, number of adults and percent weight loss, number of adults and number of holes, number of eggs and weight loss, number of eggs and number of holes, percent weight loss and number of holes. More or less similar patterns of association were also reported in cowpea infested by *C. maculates* (Redden and McGuire, 1983).

Existence of strong association indicated that selection for one trait can indirectly introduce changes in the other trait be it in the positive or negative direction due to either genetic linkage or existence of pleiotropic gene effects. The magnitude of interrelationship between characters was completely

different when the population as a whole was evaluated and when the germplasm accessions and the improved genotypes were disaggregated. For instance, seed size in the germplasm accessions had no definite association with grain yield, seed coat weight, number of eggs and the proportion of uninfected seeds.

On the other hand, strong positive associations were observed between seed size and grain yield, number of eggs laid, adults emerged and the ultimate seed weight loss in the improved genotypes, constituting the varieties released in Ethiopia and a few new introductions from ICRISAT.

Seed size also showed a strong negative association with seed coat weight in improved genotypes, i.e. the larger the seed size the thinner is the seed coat. This kind of relationship was observed since a long period of time (Williams and Singh, 1987). The thinner the seed coat and the higher would also be the number of eggs laid. There was consistent positive association between numbers of eggs with adults emerged and seed weight loss as expected in all categories of genotypes.

Existence of a strong correlation is the indication that the two traits are conditioned by the same set of genes, be it in the positive or negative direction, while absence of strong correlation as in most of the cases for adult recovery with other traits may show independent genetic control between traits (Falconer, 1989). Systematic study may be needed to investigate the nature of the co-variation between these characters, i.e. whether linked genes or pleiotropic gene effects or both are involved.

Significant positive correlation coefficients were also reported between a number of traits for infestation level and seed damage (Aslam *et al.*, 2006). Thus, it could be concluded that the cause-effect interrelationship between the characters did not appear to follow a simple trend in terms of magnitude (but not direction) due only to the differences in the nature of variation between the different categories of the test accessions/genotypes.

Weak association indicates existence of an independent genetic control between the two traits and improvement in any one of the two would not cause significant change on the other (Singh, 2002).

Table 45. Correlation coefficients (r) between response characters to infestation by adzuki bean beetle in Ethiopian chickpea germplasm accessions

| Characters [a] | TNE | DTAE (1st) | DTAE (2nd) | NAE (1st) | NAE (2nd) | NUIS | AR | TSW | SCWT | SWL (1st) | SWL (2nd) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TNE | 1.00 | | | | | | | | | | |
| DTAE (1st progeny) | -0.34** | 1.00 | | | | | | | | | |
| DTAE (2nd progeny) | -0.09NS | -0.11NS | 1.00 | | | | | | | | |
| NAE (1st progeny) | 0.83** | -0.28** | -0.05NS | 1.00 | | | | | | | |
| NAE (2nd progeny) | 0.45** | -0.08NS | -0.17NS | 0.34** | 1.00 | | | | | | |
| NUIS (%) | -0.64** | 0.30** | 0.20* | -0.59** | -0.54** | 1.00 | | | | | |
| AR (%) | -0.06NS | 0.03NS | -0.05NS | 0.40** | 0.08NS | -0.09NS | 1.00 | | | | |
| TSW | 0.60** | -0.18* | -0.12NS | 0.51** | 0.84** | -0.45** | 0.12NS | 1.00 | | | |
| SCW (%) | -0.51** | 0.31** | 0.13NS | -0.38** | -0.80NS | 0.40** | -0.05NS | -0.77** | 1.00 | | |
| SWL (1st progeny) | 0.58** | -0.13NS | -0.16NS | 0.49** | 0.44** | -0.58** | 0.06NS | 0.51** | -0.48** | 1.00 | |
| SWL (2nd progeny) | 0.60** | -0.10NS | -0.19* | 0.47** | 0.78** | -0.55** | 0.09NS | 0.77** | -0.66** | 0.80** | 1.00 |

[a] TNA = total No of eggs, DTAE = days to adult emergence, NAE = No of adults emerged, NUIS = No of uninfected seed, AR = Adult recovery, TSW = 1000 seed weight, SCW = Seed coat weight and SWL = seed weight loss

### 8.5.9. Broad-sense heritability and expected gains from selection

Seed-related traits (seed size, percent seed coat weight and seed weight loss) generally exhibited larger heritable variation, i.e. the proportion of the total variability that is due to genetic causes or the ratio of the genotypic variance to the phenotypic variance, than insect-related traits (total number of eggs, days to adult emergence, number of adults emerged, number of uninfected seed and adult recovery). This was clearly revealed from the higher broad-sense heritability and predicted genetic gains values for seed-related and extremely lower values for insect-related characters (Figure 42). The levels of heritable genetic variation ranged from 43-76% for seed-related traits indicating that the phenotypic values for these traits could better reflect the genotypic values. Heritable genetic variation for insect-related traits, on the other hand, were extremely lower ranging from 0.20-11%.

The corresponding expected genetic gains from selection, i.e. the difference between the mean genotypic values of the selected population over the mean genotypic values of the original population, as percent of respective means varied from 28-42% for seed-related traits. The present study indicated that resistance could either be improved through direct selection for reduced seed weight loss as a primary trait or through indirect selection for small seed size and increased seed coat weight as component characters or both. Progresses that could be expected from selection for the component traits ranged from 2 g for seed weight loss in first progeny to 38 g for seed size. In other words, it is possible to reduce seed weight loss by 31% through direct selection for first progeny or by 42% at second progeny. Likewise, seed size can also be reduced by 33% while seed coat weight can be increased by 28%. Better expected genetic gain from selection for seed size as a seed quality trait of agronomic importance was also reported earlier (Yücel *et al.*, 2006).

It is obvious that genetic gain from selection depends on the extent of genetic variation and on the magnitude of the heritable portion of this variation. Some authors advise that when heritability values are high, selection for such a trait would be effective. This is because there would be a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to the phenotype. Considering

heritability values between 40-80% as moderate, even if selection for insect-based traits would not be warranting, fortunately, infestation could fairly be reduced using seed-based traits. This study showed that targeting seed weight loss for direct selection and use of seed size and seed coat thickness for indirect selection as secondary traits would result in effective selection to increase resistance to adzuki bean beetle in this gene pool. However, we necessarily need to compromise seed size and thinness of seed coat as attributes of seed quality for developing adzuki bean beetle resistant chickpea.

The expected genetic gain from selection for insect-related traits ranged from 0.01-6%, suggesting that, having such extremely low heritability and genetic gain values from selection, indirect selection for these traits to reduce infestation and seed damage would rather be a difficult proposition whatever the level of correlation of these characters with seed weight loss (Figure 42). Selection could be difficult or virtually impractical for a trait with low heritability, say less than 40%, due to the masking effect of the environment on the genotypic effects (Singh, 2002). As environmental and genotype by environment interaction effects are not heritable and, hence, the higher the environmental and the genotype by environment interaction effects, the lesser will be the level of success from selection. Therefore, it appeared that making genetic progresses from selection in this gene pool based on insect-related traits would at least be very difficult. If at all expected, response to selection for insect-related traits may only be possible with the use of larger number of germplasm accessions, greater selection intensity and more precise evaluation to capture the very rare trait of this type.

The explanation for the lower genetic variation for insect-based characters may be related to excessive environmental control of the traits than the genetic control. Other reports also showed that extremely lower heritability in some characters often causes a limited expected success from genetic manipulation of inter-specific parents for resistance to storage insects in legumes (Byrne *et al.*, 2008). There was also a stronger interrelationship between phenotypic and genotypic changes expected from selection in seed-

based characters ($R^2$ = 0.92) than it was from insect-based characters ($R^2$ = 0.35) (Figure 43), which is the indication of better response to selection (Singh, 2002).

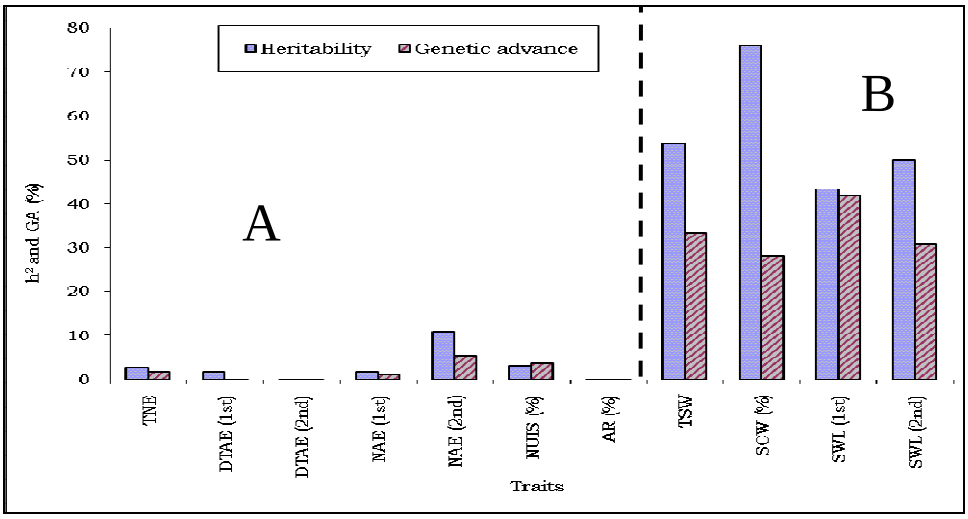



Figure 42. Estimates of broad-sense heritability ($h_b^2$) and expected genetic advance (GA) for insect-based (A) and seed-based (B) response characters to infestation by adzuki bean beetle in 130 chickpea genotypes (insect-based traits are demarked from seed-based traits by the broken vertical line)

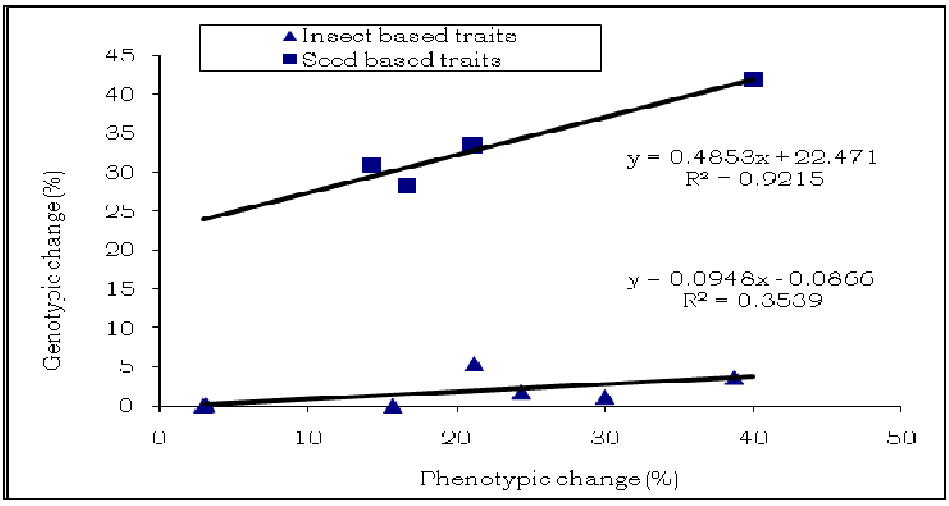



Figure 43. Relationship of phenotypic change from selection of the 5% best genotypes with genotypic change showing that more genetic improvements could be anticipated from selection for seed-based traits than it is for insect-based traits in order to reduce seed weight loss by adzuki bean beetle in Ethiopian chickpea germplasm accessions

### 8.5.10. Comparison of selected genotypes with the original population

Average performances of selected subsets of the top 5% best genotypes for attributes of both infestation levels and seed weight loss are presented in Table 46. Comparison of the average performance for respective characters of the selected subsets of the top 5% best genotypes with the average performances of the whole population revealed possibilities for different level of improvements through selection, as selection for resistant accessions significantly changed the mean of the original population in just one cycle of selection at inter-accession levels (Table 46).

Selected genotypes scoring least number of eggs and adults, adult recovery, seed weight loss and smallest seed size on the one hand and those with delayed adult emergence, highest number of uninfected seeds (%) and largest seed coat weight (%) as compared to the population means were considered as the most resistant.

The Z-test showed significant differences between means of the selected subsets of the top 5% best genotypes ($\overline{X}$) and the population parameters (μ) for different traits except seed weight loss recorded on the second progeny which was not significantly changed through selection. This again indicated that the selected accessions were not true representatives of the population and that almost all characters effectively responded to phenotypic selection (Singh, 2001). Even though differences of a single or two days appeared statistically significant, it would practically be impossible to delay adult developmental period through selection among the populations studied.

Generally, resistance to storage insects in grain crops is of rare occurrence in nature. For instance, only one out of 6000 cowpea (*Vigna unguiculata*) accessions was reported resistant to *C. maculatus* (Redden and McGuire, 1983). From the present study, complete resistance was practically not observed as accessions identified as best for one character may not consistently repeat the same performance for another character.

Despite the inconsistencies, however, some accessions like Acc. No. 41320, Acc. No. 41289, Acc. No. 41291, Acc. No. 41134, Acc. No. 41315 and

Acc. No. 207658 were found to be among the top 5% best sources of resistance based on relative ranking.

Therefore, genetic enhancement of these accessions to increase the frequency of resistance genes through further intra-accession selections would be expected to result in more resistant lines. There is also a need to evaluate more number of local and exotic collections in order to obtain genotypes with a required level of resistance. Interestingly, none of the improved genotypes stood one among the selected subsets of the top 5% best genotypes for any one of the attributes of resistance.

Table 46. Comparison of the mean performances of selected subsets ($\bar{x}$) of the 5% best accessions for response characters to infestation by adzuki bean beetle with the average performances of the whole population (μ) of 130 chickpea genotypes

| Characters | Progeny | Population parameter (μ) | Mean of selected genotypes ($\bar{x}$) | Change through selection ($/\mu - \bar{x}/$) | Change as % of population parameter (μ) | Z |
|---|---|---|---|---|---|---|
| Total No of eggs | First | 234 | 177 | 57 | 24.36 | 3.99** |
| Days to adult emergence | First | 32 | 33 | 1 | 3.13 | 7.00** |
| | Second | 34 | 35 | 1 | 2.94 | 4.45** |
| No of adults emerged | First | 160 | 112 | 48 | 30.00 | 4.52** |
| | Second | 590 | 465 | 125 | 21.19 | 2.37* |
| No of uninfected seed (%) | First | 31 | 43 | 12 | 38.71 | 4.90** |
| Adult recovery (%) | First | 70 | 59 | 11 | 15.71 | 4.49** |
| 1000 seed weight | --- | 114 | 90 | 24 | 21.05 | 2.10* |
| Seed coat weight (%) | --- | 18 | 21 | 3 | 16.67 | 2.45* |
| seed weight loss | First | 5 | 3 | 2 | 40.00 | 2.45* |
| | Second | 7 | 6 | 1 | 14.29 | 1.23NS |

** = highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and NS = non-significant ($P > 0.05$)

# 9. CONCLUSION AND RECOMMENDATIONS

Chickpea plays an important economic and ecological role in the socio-economic lives of the farming communities all over the world. Successes from chickpea breeding efforts, like any other crop, largely depend, among other factors, on the knowledge about germplasm characteristics and genetic relationships. Information on the extent and pattern of variability and diversity present in the population and trait interrelationship is, therefore, absolutely essential for further improvement of the crop. Even though Ethiopia is considered as the secondary center of genetic diversity with a large germplasm holding for chickpea, systematic characterization and evaluation of these germplasm was not made particularly for some attributes of economic and ecological significance like symbiotic nitrogen fixation, phosphorus uptake and use efficiency and adzuki bean beetle resistance.

Molecular characterization of the chickpea genotypes showed the existence of high genetic diversity and a large number of duplications in Ethiopian chickpea germplasm collections. These duplications maybe reduced using strategies such as systematic bulking and the formation of core collections. Future germplasm collection and utilization strategies should take into consideration the magnitude and pattern of genetic diversity established both at genotypic and phenotypic levels by the present investigation. The eco-geographic pattern of distribution of genetic diversity established in this study may also help as a benchmark in selection of in-situ conservation sites (Ford-Lloyd and Jackson, 1986; Chahal and Gosal, 2002; Singh, 2002) and in the study of chickpea evolution (Singh, 2002).

The present study also revealed that the Ethiopian chickpea landraces had considerable significance as sources of genotypes with desirable attributes for symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and resistance to adzuki bean beetle with other agronomic characters. It was possible to identify sources for desirable characters that were superior to both the recently released variety Natoli and to the average performance of all the released varieties. Genetic enhancement or increasing the frequency of desirable genes of accessions identified as better source

materials in this study through purification and selection is likely to produce better genotypes.

Despite the important roles they could have played in chickpea breeding programs, unfortunately, the potential of the Ethiopian chickpea landraces has been under exploited by the hitherto breeding efforts in Ethiopia. The accessions selected here could serve as the bases for formulating efficient scheme of multiple trait selection. It can be expected that individual lines to be developed through selection from among the accessions identified here may result even in better performances than the "mother" accessions themselves.

It was observed that a limited number of accessions maybe uniquely serve as genetic sources for the multiple attributes of economic and ecological importance studied. For instance, three accessions, namely 41029, 41103 and 41320, were found to be among the best sources selected for both symbiotic nitrogen fixation and adzuki bean beetle resistance. However, the challenge is that the desirable characters were found to exist distributed among different accessions and a single group or a single genotype combining desirable attributes of symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and resistance to adzuki bean beetle may be of rare occurrence in this gene pool.

The utilization of these valuable germplasm particularly in the efforts underway to develop efficient genotypes for symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and resistance to adzuki bean beetle warrants a critical assessment. A series of multiple crossing may be required in order to bring desirable traits distributed among multiple parents into a single genetic background for further selection among the progenies. Introductions from exotic sources should also be included in the parents to be developed from the selected accessions particularly in order to exploit complementary genes, e.g. to improving seed size as an economic trait.

It was also suggested that without going far away from the areas of past achievements, the conversion of the otherwise well adapted released varieties through incorporation of the responsible genes for better symbiotic

nitrogen fixation, phosphorus uptake and use efficiency, and adzuki bean beetle resistance using different breeding techniques seem to be the best strategy not only in terms of time saving but also in terms of effectiveness and efficiency.

It may be concluded from this study that improving adzuki bean beetle resistance and symbiotic nitrogen fixation through selection would be more difficult than improving phosphorus uptake and use efficiency. This is because of the existence of sources of only partial resistance to adzuki bean beetle and lack of positively associated traits with higher heritability that is easily observable during selection for symbiotic nitrogen fixation. Beyond relative differences, sources of complete resistance to adzuki bean beetle have not been obtained, maybe because genes for complete resistance to this storage insect are underrepresented or under expressed in Ethiopian chickpea germplasm accessions. The existence of extremely lower heritable variability in insect-related traits could also hamper genetic progresses to be expected from selection for resistance to adzuki bean beetle.

For improving symbiotic nitrogen fixation, it appears that increased nitrogen yield, nitrogen harvest index and fixed nitrogen assimilation efficiency with increased above ground biomass are more important than increasing the *per se* concentration of nitrogen in plant tissue. But as most of these traits are not easily observable and require destructive sampling, there is a need to develop laboratory facilities that enable the analysis of plant tissue nitrogen content.

For improving adzuki bean beetle resistance, more care and more precise evaluation of larger number of germplasm accessions, greater selection intensity and more precise evaluation to capture the very rare gene may be needed. Genetic resources with complete resistance should also be introduced and included into this gene pool. Introduction and introgression of new resistance genes from other sources into the breeding scheme in addition to retaining and maintaining the already available ones at least for integrated use with other cultural, chemical and biological control methods may also be advisable. Alternative approaches like mutation breeding and

introgression of new genes from wild relatives to generate more variation may be necessary.

Negative associations were found between primary attributes of economic importance like seed size and a number of other component characters. That means improvement made in any one of the traits may cause reduction in the other and *vice versa*. Some possible strategies may help overcome this problem. In-depth studies may be needed to investigate whether the genetic nature of this negative relationship is associated with undesirable gene linkage or plieotrophic gene effects. The result would enable to break linkages by producing large number of segregants (Singh, 2002), if linkage is the main cause of negative association. It may also be possible to accept some level of compromise between two negatively related characters in such a way that one character should be kept constant in order to improve the other. It is wise to consider application of separate breeding programs particularly, when the two traits that are associated negatively are controlled by plieotrophic gene effects. A few morpho-agronomic characters in chickpea are under gene linkage and pleiotropic effects as reviewed by Muehlbauer and Singh (1987).

It was demonstrated that Ethiopian chickpea germplasm accessions were more distinctly diverged from the introductions of ICARDA and ICRISAT than they diverged from each other. This may imply that chickpea had given rise to a new and distinct pattern of variation after its introduction to Ethiopia. The distinct grouping of the introduced improved genotypes to a separate cluster may also be somehow related to the level of prior breeding to which they had been subjected at ICRISAT and ICARDA before their introduction to Ethiopia. The relative similarity among Ethiopian collections may also be due to the extensive seed exchange between farmers or to common features of the chickpea original introduction in different regions of Ethiopia. It may also be implicated that the easy access to a wide array of improved cultivars developed by the international institutions supported the broadening of genetic base of chickpea breeding in Ethiopia.

The genotypic variation revealed in this gene pool for attributes of symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and resistance to adzuki bean beetle justifies the need for the initiation of a planned breeding program for improving these attributes in chickpea. However, the mechanisms of efficient symbiotic nitrogen fixation and phosphorus uptake and use, and partial resistance to adzuki bean beetle in the genotypes tested here needs further studies. Generally, even though a number of specific mechanisms in all aspects have been claimed to be investigated in different legumes as discussed earlier, it yet appears that much remains the subject of future investigation in understanding the dynamics and detailed mechanisms underlying symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and resistance to adzuki bean beetle in this crop.

The conventional breeding approach has been playing decisive roles in breeding for symbiotic nitrogen fixation, phosphorus uptake and use efficiency, and resistance to adzuki bean beetle. However, as varietal breakthroughs for these attributes are still far from fruition, the conventional approach should be supported by molecular tools for better efficiency and effectiveness. A comprehensive study to map the associations of the molecular markers revealed here and attributes of symbiotic nitrogen fixation, phosphorus use efficiency, adzuki bean beetle resistance and agronomic traits of economic and ecological importance is required in order to identify key genes controlling these traits and mine the best alleles from these germplasm in chickpea breeding programs.

# REFERENCES

Abaidoo, R.C., George, T., Bohlool, B.B. and Singleton, P.W. (1990). Influence of elevation and applied nitrogen on rhizosphere colonization and competition for nodule occupancy by different rhizobial strains on field-grown soybean and common bean. *Canadian Journal of Microbiology* **36**:92-96.

Abbo, S., Berger, J. and Turner, N.C. (2003). Evolution of cultivated chickpea: Four bottlenecks limit diversity and constrain adaptation. *Functional Plant Biology* **30**:1081–1087.

Acevedo, E. and Fereres, E. (1993). Resistance to abiotic stresses. In: *Plant Breeding: Principles and Prospects*, pp 406-421, Hayward, M.D., Bosemark, N.O. and Romagosa, I., eds.). Chapman and Hall, Great Britain.

Acosta-Gallegos, J.A., Kelly, J.D. and Gepts, P. (2008). Pre-breeding in common bean and use of genetic diversity from wild germplasm. *Crop Science* **48**:3-16.

Adeboye, M.K.A., Iwuafor, E.N.O., Agbenin, J.O. and Kparmwang, T. (2007). Contributions of nitrogen from grain and herbaceous legumes to a succeeding maize crop in the moist savanna of Nigeria. In: *Demand-driven Technologies for Sustainable Maize Production in West and Central Africa,* pp 155-168, (Badu-Apraku, B., Fakorede, M.A.B., Lum, A.F., Menkir, A. and Ouedraogo, M., eds.). Proceedings of the 5th Biennial Regional Maize Workshop, IITA-Cotonou, Bénin, 3–6 May, 2005. WECAMAN/IITA, Ibadan, Nigeria.

Adenew, B. (2009). Competitiveness of the Ethiopian Agriculture with Emphasis on Selected Products: Pulses, Oil crops, Fruits & Vegetables and Flowers. New challenges and opportunities, The National PSD Conference on the Competitiveness of the Ethiopian Private Sector, Addis Ababa Chamber of Commerce and Sectoral Association, Addis Ababa. (www.addischamber.com).

Adgo, E. and Schulze, J. (2002). Nitrogen fixation and assimilation efficiency in Ethiopian and German pea varieties. *Plant and Soil* **239**:291–299.

Adjei-Nsiah, S., Kuyper, T.W., Leeuwis, C., Abekoe, M.K., Cobbinah, J., Sakyi-Dawson, O. and Giller, K.E. 2008. Farmers' agronomic and social evaluation of productivity, yield and N2-fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. *Nutrient Cycle and Agroecosystem* **80**:199–209.

Ae, N., Arihara, J., Okada, K., Yoshihara, T. and Johansen, C. (1990). Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. *Science* **248**:477–480.

AFRNA. 1992. The AFNETA alley farming training manual, Volume 2: Source book for alley farming research. Alley Farming Research Network for Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria (http://www.fao.org/Wairdocs/ILRI/x5546E/x5546e05.htm).

Agegnehu, G., Fikre, A. and Tadesse, A. (2006). Cropping systems, soil fertility and crop management research on cool-season food legumes in the Central Highlands of Ethiopia. In: *Food and Forage Legumes of Ethiopia: Progress and Prospects*, pp. 135-145, (Ali, K., Keneni, G., Ahmed, S., Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M.H., eds). Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria.

Agegnehu, G. and Ghizaw, A. (2004). Effects of drainage and genotype on yield and some yield components of faba bean on a highland vertisol in Ethiopia. *Ethiopian Journal of Natural Resources* **6**:167-187.

Ahlawat, I.P.S., Gangaiah, B. and Zahid, M.A. (2007). Nutrient management in chickpea. In: *Chickpea Breeding and Management*, pp 213-232, (Yadav, S.S., Redden, R. and Sharma, B., eds). Cromwell Press, Trowbdige, UK.

Ahmad, Z., Gill, M.A. and Qureshi, R.H. (2001). Genotypic variations of phosphorus utilization efficiency of crops. *Journal of Plant Nutrition* **24**:1149-1171.

Ahmed, K., Khalique, F., Afzal, M., Tahir, M. and Malik, B.A. (1989). Variability in chickpea (*C. arietinum* L.) genotypes for resistance to *Callosobruchus maculates* F. (Bruchidae). *Journal of Stored Products Research* **25**:91-99.

Alemayehu, M. (2005). Post harvest losses in chickpea caused by bruchids and management of *Callosobruchus chinensis* using botanicals in Enemay *Woreda*. M.Sc thesis, Alemaya University, Alemaya. Ethiopia.

Ali, K. and Habtewold, T. 1994. Research on insect pests of cool-season food legumes. In: *Cool-season Food Legumes of Ethiopia*. pp 367-396, (Tilaye, A., Bejiga, G., Saxena, M.C. and Solh, M.B., eds). Proceedings of the 1st National Cool-season Food Legumes Review Conference, 16-20 December 1993, Addis Ababa, Ethiopia. ICARDA/IAR. ICARDA, Syria.

Ali, M.Y., Krishnamurthy, L., Saxena, N.P., Rupela, O.P. Kumar, J. and Johansen, C. (2002). Scope for genetic manipulation of mineral acquisition in chickpea. *Journal of Agronomy and Crop Science* **191**:464-472.

Allen, F. L., Comstock, R.E. and Rasmuddon, D.C. (1978). Optimal environments for yield testing. *Crop Science* **8**:747-751.

Alloush, G.A., Zeto, S.K. and Clark, R.B. (2000). Phosphorus source, organic matter and arbuscular mycorrhiza effects on growth and mineral acquisition of chickpea grown in acidic soil. *Journal of Plant Nutrition* **23**:1351–1369.

Annicchiarico, P. (2002). Genotype x Environment Interaction: Challenges and Opportunities for Plant Breeding and Cultivar Recommendation. FAO Plant Production and Protection Paper No. 174. Food and Agriculture Organization, Rome.

AOAC. (1970). Official Methods of Analysis, 11th ed., Association of Official Agricultural Chemists (AOAC), Washington, D.C.

Appleby, J.H. and Credland, P.F. (2004). Environmental conditions affect the response of West African *Callosobruchus maculatus* (Coleoptera: Bruchidae) populations to susceptible and resistant cowpeas. *Journal of Stored Products Research* **40**:269-287.

Araújo, A.P., Antunes, I.F. and Teixeira, M.G. (2005). Inheritance of root traits and phosphorus uptake in common bean (*Phaseolus vulgaris* L.) under limited soil phosphorus supply. *Euphytica* **145**:33–40.

Araújo, A.P., Teixeira, M.G. and de Almeida, D.L. (1998). Variability of traits associated with phosphorus efficiency in wild and cultivated genotypes of common bean. *Plant and Soil* **203**:173–182.

Armstrong, E.L., Heenan, D.P., Pate, J.S. and Unkovich, M.J. (1997). Nitrogen benefits of lupines, field pea and chickpea to wheat production in south-eastern Australia. *Australian Journal of Agricultural Research* **48**:39-47.

Arumuganathan, K. and Earle, E.D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter* **9**:208-219.

Aryal, U.K., Xu, H.L. and Fujita, M. (2003). Rhizobia and AM Fungal Inoculation Improve Growth and Nutrient Uptake of Bean Plants Under Organic Fertilization. *Journal of Sustainable Agriculture* **21**:27-39.

Ascher, J.S., Graham, R.D., Hollamby, G.J., Paull, J., Davies, P., Huang, C., Pallotta, M.A., Howes, N., Khabaz-Saberi, H., Jefferies, S.P. and Moussavi-Nik, M. (2001). Micronutrients. In: *Application of Physiology in Wheat Breeding*, pp 219-240, (Reynolds, M.P., Ortiz-Monasterio, J.I. and McNab, A., eds). Mexico, D.F.: CIMMYT.

Aslam, M. (2004). Pest Status of Stored Chickpea Beetle, *Callosobruchus chinensis* Linnaeus on Chickpea. *Journal of Entomology* **1**:28-33.

Aslam, M., Shaheen, F.A., Abbas, M.A. and Saba, A. (2006). Management of Callosobruchus chinensis Linnaeus through use of resistance in stored chickpea varieties. *World Journal of Agricultural Sciences* **2**:82-84.

Austin, R.B. (1993). Augmenting yield-based selection. In: *Plant Breeding: Principles and Prospects*, pp 391-405, (Hayward, M.D., Bosemark, N.O. and Romagosa, I., eds). Chapman and Hall, Great Britain.

Bagge, M. and Lübberstedt, T. (2008). Functional markers in wheat: technical and economic aspects. *Molecular Breeding* **22**:319–328.

Bahl, G.S. and Pasricha, N.S. (1998). Efficiency of P utilization by pigeonpea and wheat grown in a rotation. *Nutrient Cycling in Agroecosystems* **51**:225–229.

Baldani, J., Caruso, L, Baldani, V.L.D., Goi, S.R. and Döbereiner, J. (1997). Recent advances in BNF with non-legume plants. *Soil Biology and Biochemistry* **29**:911-922.

Bambara, S. and Ndakidemi, P.A. (2010). *Phaseolus vulgaris* response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. *African Journal of Agricultural Research* **5**:1804-1811.

Banziger, M., Betran, F.J. and Lafitte, H.R. (1997). Efficiency of high nitrogen selection environments for improving maize for low-nitrogen target environments. *Crop Science* **37**:1103–1109.

Banziger, M. and Edmeades, G.O. (1997). Predicted productivity gains from breeding maize under stressed vs. non-stressed conditions. IN: *Maize Productivity Gains through Research and Technology Dissemination*, pp 136–140, (Ransom, J.K., Palmer, A.F.E., Zambezi, B.T., Mduruma, Z.O., Waddington, S.R., Pixley, S.R. and Jewell, D.C., eds). Proceedings of the 5th Eastern and Southern Africa Regional Maize Conference, Arusha, Tanzania, 3–7 June 1996. CIMMYT, Addis Ababa, Ethiopia.

Banziger, M. and Lafitte, H.R. (1997). Breeding for N-stressed environments: How useful are N-stressed selection environments and secondary traits? In: *Developing Drought and Low N-tolerant Maize*, pp. 401-404, (Edmeades, G.O., Banzinger, M., Mickelson, H.R. and Pena-Valdivia, C.B., eds). Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico. Mexico D.F., CIMMYT.

Baraket, G., Chatti, K., Saddoud, O., Abdelkarim, A.B., Mars, M., Trifi, M. and Hannachi, A.S. (2011). Comparative assessment of SSR and AFLP markers for evaluation of genetic diversity and conservation of fig, *Ficus carica* L., genetic resources in Tunisia. *Plant Molecular Biology Reporter* **29**:171–184.

Bassam, N.E. (1998). A concept of selection for low input wheat varieties. *Euphytica* **100**:95–100.

Beck, D.P. and Rupela, O.P. (1996). Symbiotic nitrogen fixation in chickpea in WANA and SAT, pp 207-216, In: *Adaptation of Chickpea in the West Asia and North Africa Region*, (Saxena, N.P., Saxena, M.C., Johansen, C. and Harris, H., eds.). ICRISAT: Patancheru, India. ICRDA: Aleppo, Syria.

Beebe, S.E., Rojas-Pierce, M., Yan, X., Blair, M.W., Pedraza, F., Munóz, F., Tohme, J. and Lynch. J.P. (2006). Quantitative Trait Loci for root architecture traits correlated with phosphorus acquisition in Common Bean. *Crop Science* **46**:413–423.

Beem, V. and Smith, M.E. (1997). Variation in nitrogen and phosphorus efficiency and root system size in temperate maize genotypes. In: *Developing Drought and Low N-tolerant Maize*, pp. 241–244, (Edmeades, G.O., Banzinger, M., Mickelson, H.R. and Pena-Valdivia, C.B., eds). Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico. Mexico D.F., CIMMYT.

Bejiga, G. 2004. Current status of food legume production and use of biological nitrogen fixation in Ethiopia. In: *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp. 263-266, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Bejiga, G. and Daba, K. (2006). Breeding chickpea for wide adaptation. In: *Food and Forage Legumes of Ethiopia: Progress and Prospects*, pp. 59-66, (Ali, K., Keneni, G., Ahmed, S., Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M.H., eds). Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria.

Bejiga, G. and van der Maesen, L.J.G. (2006). *Cicer arietinum* L. *Plant Resources of Tropical Africa 1: Cereals and Pulses*, pp. 42-46, (Brink, M. and Belay, G., eds). PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands.

Bekele, E. (1985). The biology of cereal landrace population I. Problems of gene conservation, plant breeding selection schemes and sample size requirement. *Hereditas* **103**:119-134.

Belay, Z. and Assefa, F. (2011). Symbiotic and phenotypic diversity of *Rhizobium leguminosarum* bv. *viciae* from Northern Gondar, Ethiopia. *African Journal of Biotechnology* **10**:4372-4379.

Berger, J., Turner, N.C. and French, R.J. (2003). The role of phenology in adaptation of chickpea to drought: Solutions for a better environment. In: Proceedings of the 11th Australian Agronomy Conference, (Unkovich,M. and O'Leary, G., eds). 2-6 Feb 2003, Geelong, Victoria (http://www.regional. org.au/au/asa/2003/c/4/turner.htm).

Beringer, J.E., Bisseling, T.A. and LaRue, T.A. (1988). Improving symbiotic nitrogen fixation through the genetic manipulation of *Rhizobium* and legume host plants. In: *World Crops: Cool Season Food Legumes*, pp. 691-702, (Summerfield, R.J., ed). Kluwer Academic Publishers, Dordrecht.

Blair, M.W., Fregene, M.A., Beebe, S.E. and Ceballos, H. (2007). Marker-assisted selection in common bean and cassava. In: *Marker-Assisted Selection: Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish,* pp. 81-115, (Ruane, B.D., Sonnino, S.A. and Dargie, J.D., eds). Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Blair, G.J. and Godwin, D.C. (1991). Phosphorus efficiency in pasture species. VII relationships between yield and P uptake and root parameters in 'Itvo accessions of white clover. *Australian Journal of Agricultural Research* **42**:1271-1283.

Bøckman, O.C. (1997). Fertilizers and biological nitrogen fixation as sources of plant nutrients: Perspectives for future agriculture. *Plant and Soil* **194**:11–14.

Bohlool, B.B., Ladha, J.K., Garrity, D.P. and George, T. (1992). Symbiotic nitrogen fixation for sustainable agriculture: A perspective. *Plant and Soil* **141**:1-11.

Borisov, A.Y., Danilova, T.N., Koroleva, T.A., Kuznetsova, E.V., Madsen, L., Mofett, M., Naumkina, T.s., Nemankin, T.A., Ovchinnikova, E.S., Pavlova, Z.B., Petrova, N.E., Pinaev, A.G., Radutoiu, S., Rozov, S.M., Rychagova, T.S., Shtark, O.Y., Solovov, I.I., Stougaard, J., Tikhonovich, I.A., Topunov, A.F., Tsyganov, V.E., Vasil'chikov, A.G., Voroshilova, V.A., Weeden, N.F., Zhernakov, A.I. and Zhukov, V.A. (2007). Regulatory genes of garden pea (*Pisum sativum* L.) controlling the development of nitrogen-fixing nodules and Arbuscular Mycorrhiza: A review of basic and applied aspects. *Applied Biochemistry and Microbiology* **43**:237–243.

Borlaug, N.E. (2000). The green revolution revised and the road ahead. Special 30th Anniversary Lecture, The Norwegian Nobel Institute, Oslo.

Borlaug, N.E. (2002). Agriculture and peace: the role of science and technology in the 21st century. U Thant distinguished lecture series, United Nations University, Tokyo.

Bowen, G.D. and Zapata, F. (1991). Efficiency in uptake and use of nitrogen and phosphorus by plants. In: *Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies*, pp. 349-362, Proceedings of an International Symposium on the use of Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies, 1-5 Oct. 1990, IAEA, Vienna.

Brockwell, J., Gault, R.R., Herridge, D.F., Morthorpe, L.J. and Roughley, R.J. (1988). Studies on alternative means of legume inoculation: Microbiological and agronomic appraisals of commercial procedures for inoculating soybeans with *Bradyrhizobium japonicum*. *Australian Journal of Agricultural Research* **39**:965-972.

Broughton, W.J., Zhang, F., Perret, X. and Staehelin, C. (2003). Signals exchanged between legumes and *Rhizobium*: agricultural uses and Perspectives. *Plant and Soil* **252**:129–137.

Brown, A.H.D. (1989). The case for core collections. *The Use of Plant Genetic Resources*, pp. 136-156, (Brown, A.H.D., Marshal, D.R., Frankel, O.H. and Williams, J.T., eds). Cambridge University Press, Cambridge,

Bucher, M. (2006). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Physiologist* **173**:11–26.

Buddenhagen, I.W. and Richards, R.A. (1988). Breeding cool season food legumes for improved performance in stress environments. In: *World Crops: Cool Season Food Legumes*, pp. 81-95, (Summerfield, R.J., ed). Kluwer Academic Publishers, The Netherlands.

Bunder, J., Loeber, A., Broers, J.E.W. and Havertkort, B. (1996). An integrated approach to biotechnology development. In: *Biotechnology: Building on Farmers' Knowledge*, pp. 201-227, Bunders, J., Haverkort, B. and Hiemstra, W., eds). Macmillan, London and Basingstoke.

Burger, H., Schloen, M., Schmidt, W. and Geiger, H.H. (2008). Quantitative genetic studies on breeding maize for adaptation to organic farming. *Euphytica* **163**:501–510.

Bushara, A.G. (1988). Insect depredation during storage. In *World Crops: Cool Season Food Legumes*, pp. 367-378, (Summerfield, R.J., ed.). Kluwer: Dordrecht, the Netherlands.

Byrne, O.M., Hardie, D.C., Khan, T.N., Speijers, J. and Yan, G. (2008). Genetic analysis of pod and seed resistance to pea weevil (B. pisorum) in a *Pisum sativum* x *P. fulvum* interspecific cross. *Australian Journal of Agricultural Research* **59**:854-862.

Campbell, L.G. and Lafever, H.N. (1980). Effects of locations and years upon relative yields of the soft red wheat region. *Crop Science* **20**:23-28.

Carlsson, G. and Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* **253**:353–372.

Carvalho, M.A. (2004). Germplasm characterization of *Arachis pintoi* Krap. and Greg. (*Leguminosae*). PhD Thesis, University of Florida, USA.

Cassman, K.G., Peng, S., Olk, D.C., Ladha, J.K., Reichardt, W., Dobermann, A. and Singh, U. (1998). Opportunities for increasing nitrogen use efficiency from improved resource management in irrigated rice systems. *Field Crops Research* **56**:7–38.

Cattivelli, L., Rizza, F., Badeck F.W., Mazzucotelli, E., Mastrangelo, A.M., Francia, E., Mare C., Tondelli, A. and Stanca, A.M. (2008). Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field Crops Research* **105**:1-14.

Ceccarelli, S. (1989). Wide adaptation: how wide? *Euphytica* 40:197-205.

Ceccarelli, S. (1994). Specific adaptation and breeding for marginal conditions. *Euphytica* **77**:205-219.

Ceccarelli, S. (1997). Adaptation to low/high input cultivation. *Adaptation in Plant Breeding*, pp. 225-236, (Tigerstedt, P.M.A., ed), Kluwer Academic Publishers, The Netherlands.

Ceccarelli, S. and Grando, S. (1996). Importance of specific adaptation in breeding for marginal conditions. In: *Barley Research in Ethiopia: Past Work and Future Prospects*, pp. 34-58, Gebre, H. and van Leur, J., eds). Proceedings of the 1st Barley Research Review Workshop, 16-19 October 2003, Addis Ababa: IAT/ICARDA. Addis Ababa, Ethiopia.

Ceccarelli S., Grando, S., Baum, M. and Udupa, S.M. (2004). Breeding for drought resistance in a changing climate. In: *Challenges and Strategies for Dryland Agriculture*, pp. 167-190, Crop Science Society of America and American Society of Agronomy. CSSA Special Publication No. 32, USA.

Celucia, S.U., de la Peña, R.C. and Villa, N.O. (2009). Genetic Characterization of *Brassica rapa chinensis* L., *B. rapa parachinensis* (L. H. Bailey) Hanelt, and *B. oleracea alboglabra* (L. H. Bailey) Hanelt Using Simple Sequence Repeat Markers. *Philippine Journal of Science* **138**:141-152.

Chahal, G.S. and Gosal, S.S. (2002). *Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches*. Narosa Publishing House, New Delhi.

Chandel, K.P.S. and Joshi, B.S. (1983). Multivariate analysis in green-seeded pea. *Indian Journal Agricultural Sciences* **53**:198-200.

Chen, H.M., Liv, C.A., Kuo, C.G., Chien, C.M., Sun, H.C., Huang, C.C., Lin, Y.C. and Ku, H.M. (2007). Development of molecular marker for a bruchid (*Callosobruchus chinensis*) resistance gene in mungbean. *Euphytica* **157**:113-122.

Chen, M.M., Yin, H.B., O'Connor, P., Wang, Y.S. and Zhu, Y.G. (2010). C: N: P stoichiometry and specific growth rate of clover colonized by arbuscular mycorrhizal fungi. *Plant Soil* **326**:21–29.

Chen, Q., Zhang, X., Terefework, Z., Kaijalainen, S., Li, D. and Lindström, K. (2000). Diversity and compatibility of peanut (*Arachis hypogaea* L.) bradyrhizobia and their host plants. *Plant and Soil* **255**:605–617.

Clark, K.W., Brockwell, J. and Thompson, J.A. (1988). Role of inoculants in improving nitrogen fixation in legumes. In: *World Crops: Cool Season Food Legumes*, pp. 731-743, Summerfield, R.J., ed). Kluwer Academic Publishers, Dordrecht.

Clement, S.L., Hardie, D.C. and Elberson, L.R. 2002. Variation among accessions of *Pisum fulvum* for resistance to pea weevil. *Crop Science* **42**:2167–2173.

Coffman, W.R. and Smith, M.E. (1991). Roles of public, industry and international research center programs in developing germplasm for sustainable agriculture. In: *Plant Breeding and Sustainable Agriculture: Consideration for Objectives and Methods*, pp. 1-9, (Sleper, D.A., Baker, T.C. Bramel-Cox, P.J. and Francis, C.A., eds). Proceedings of a Symposium in Las Vegas, CSSA Publication No. 18, Crop Science Society of America, Madison.

Collaku, A., Harrison, S.A., Finney, P.L. and van Sanford, D.A. (2002). Clustering of environments of southern soft red winter wheat region for milling and baking quality attributes. *Crop Science* **42**:58–63.

Commodityonline. (2009). India's Green Revolution that bypassed pulses (http://www.commodityonline.com/news/Indias-Green-Revolution-that-bypassed-pulses-15163-3-1.html).

Cox, T.S., Ben-huli, L.S., Stears, R.G. and Martin, T.J. (1988). Genetic improvement in agronomic traits of hard red winter wheat cultivars from 1919 to 1987. *Crop Science* **28**:756-760.

Creste, S., Neto, A.T., de Oliveira Silva, S. and Figueira, A. (2003). Genetic characterization of banana cultivars (*Musa* spp.) from Brazil using microsatellite markers. *Euphytica* **132**:259–268.

Crouch, J.H., Buhariwalla, H.K., Blair, M., Mace, E., Jayashree, B. and Serraj, R. (2004). Biotechnology based contributions to enhancing legume productivity in resource poor areas. In: *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp 47-65, (Serraj, R. ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

CSA. (2011). Area and production of crops in the Meher season. Statistical Bulletin. Central Statistical Agency (CSA), Addis Ababa, Ethiopia.

Cubero J.I. (1987). Morphology of chickpea. In: *The Chickpea*, pp. 35-66, C.A.B. International, Wallingford, Oxon, OX10 8DE, UK.

Dadi, M. (2004). Morphological and RAPD marker variation analysis in some drought tolerant and susceptible chickpea (*Cicer arietinum* L.) genotypes of Ethiopia. M.Sc Thesis, Addis Ababa University, Ethiopia

Dakora, F.D. and Keya, S.O. (2000). Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biology and Biochemistry* **29**:809-817.

Damte, T. and Dawd, M. (2006). Chickpea, Lentil and grass pea insect pest research in Ethiopia: A review. In: *Food and Forage Legumes of Ethiopia: Progress and Prospects*, pp. 260-273, (Ali, K., Keneni, G., Ahmed, S., Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M.H., eds). Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria.

Daoui, K., Karrou, M., Mrabet, R., Fatemi, Z., Draye, X. and Ledent, J. F. (2012). Genotypic variation of phosphorus use efficiency among Moroccan faba bean (*Vicia faba* Major) under rainfed conditions. *Journal of Plant Nutrition* **35**:34–48.

Davis, T.M., Foster, K.W. and Phillips, D.A. (1985). Nodulation mutants in chickpea. *Crop Science* **25**:345–348.

Davis, T.M., Foster, K.W. and Phillips, D.A. (1986). Inheritance and expression of three genes controlling root nodule formation in chickpea. *Crop Science* **26**:719-723.

de Boef, W.S., Berg, T. and Haverkort, B. (1996). Crop genetic resources. In: *Biotechnology: Building on Farmers' Knowledge*, pp 103–128, (Bunders, J., Haverkort, B. and Hiemstra, W., eds). Macmillan, London and Basingstoke.

de Boef, W.S. and Ogliari, J.B. (2008). Participatory crop improvement and informal seed supply: general introduction. In: *Farmers, Seeds and Varieties*, pp 177–185, (Thijssen, M.H., Bishaw, Z., Beshir, A. and de Boef, W.S., eds). Wageningen International, The Netherlands.

Demissie, A. and Bjϕrnstad, A. (1997). Geographical, altitude and agro-ecological differentiation of isozyme and hordein genotypes of landrace barleys from Ethiopia: implications to germplasm conservation. *Genetic Resources and Crop Evolution* **44**: 43-55.

de Oliveira, W.S., Meinhardt, L.W., Sessitsch, A. and Tsai, S.M. (1998). Analysis of *Phaseolus–Rhizobium* interactions in a subsistence farming System. *Plant and Soil* **204**:107–115.

de Vicente, M.C., Guzmán, F.A., Engels, J. and Rao, V.R. (2005). Genetic characterization and its use in decision making for the conservation of crop germplasm: The role of biotechnology. In: *International Workshop the Role of Biotechnology for the Characterization and Conservation of Crop, Forestry, Animal and Fishery Genetic Resources*, pp 121-128, FAO, FOBIOTECH, ECOGENE, SIGA. Turin, Italy, 5-7 March 2005.

Delwiche, C.C. and Wijler, J. (1956). Non-symbiotic nitrogen fixation in soil. *Plant and Soil* **7**:113-119.

Desroches, P., Elshazly, E., Mandon, N., Duc, G. and Huignard, J. (1995). Development of *Callosobruchus chinensis* (L.) and C. *maculatus* (F.) (Coleoptera: Bruchidae) L. in seeds of *Vicia faba* differing in tannin, convicine and vicine Contents. *Journal of Stored Products* **31**:83-89.

Devi, M.J., Sinclair, T.R. and Vadez, V. (2010). Genotypic variability among peanut (Arachis hypogea L.) in sensitivity of nitrogen fixation to soil drying. *Plant Soil* **330**:139–148.

DFID. (2004). Agricultural Sustainability. Department for International Development (DFID) in collaboration with Jules Pretty of the Department of Biological Sciences, University of Essex, UK (http://dfid-agriculture-consultation.nri.org/summaries/wp12.pdf).

Dibabe, A., Bekele, T. and Mamo, T. (2001). Nutrient management in highland vertisols of Ethiopia. *Advances in Verisols Management in Ethiopian Highlands*, pp 81-98, (Dubale, P., Dibabe, A., Zeleke, A., Ayele, G. and Kirub, A., eds.).Proceedings of the International Symposium on Vertisols Management. 28 Nov – 1 Dec, 2001, Debre Zeit, Ethiopia. Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia.

Dita, M.A., Rispail, N., Prats, E., Rubiales, D. and Singh, K.B. (2006). Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. *Euphytica* **147**:1–24.

Dixon, R., Cheng, Q., Shen, G., Day, A. and Dowson-Day, M. (1997). *Nif* gene transfer and expression in chloroplasts: Prospects and problems. *Plant and Soil* **194**:193–203.

Donald, C.M. (1968). The breeding of plant ideotypes. *Euphytica* **17**:385-403.

Dongre, T.K., Pawar, S.E., Thakare, R.G. and Harwalker, M.R. (1996). Identification of resistant sources to cowpea weevil (*Callosobruchus maculatus* (F.)) in *Vigna* spp. and inheritance of their resistance in black gram (*Vigna mungo* var. *mungo*). *Journal of Stored Products Research* **32**: 201-204.

Doughton, J.A., Saffigna, P.G., Vallis, I. and Mayer, R.J. (1995). Nitrogen fixation in chickpea: II. Comparison of $^{15}N$ enrichment and $^{15}N$ natural abundance methods for estimating nitrogen fixation. *Australian Journal of Agricultural Research* **46**: 225-236.

Doyle, J.J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus* **12**:13-15.

Dwevedi, K.K. and Lal, G.M. (2009). Assessment of genetic diversity of cultivated chickpea (*Cicer arietinum* L.). *Asian Journal of Agricultural Sciences* **1**:7-8.

EARO. (2000). National Crop Research Strategy. Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia.

Edde, P.E. and Amatobi, C.A. (2003). Seed coat has no value in protecting cowpea seed against attack by *Callosobruchus maculatus* (F.) *Journal of Stored Products Research* **39**: 1-10.

Edmeades, G.O., Bolanos, J., Banziger, M., Ribaut, J.M., White, J.W., Reynolds, M.P. and Lafitte, H.R. (1998). Improving crop yields under water deficits in the tropics. In: *Crop Productivity and Sustainability – Shaping the Future*, pp. 437-451, (Chopra, V.L., Singh, R.B. and Varma, A., eds). Proc. 2nd Int. Crop Science Congress. Oxford and IBH, Newdelhi.

Edmeades, G.O., Bolanos, J. and Chapman, S.C. (1997). Value of secondary traits in selecting for drought tolerance in tropical maize. In: *Developing Drought and Low N-tolerant Maize*, pp. 222-234, (Edmeades, G.O., Banzinger, M., Mickelson, H.R. and Pena-Valdivia, C.B., eds). Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico. Mexico D.F., CIMMYT.

Edwards, O. and Singh, K.B. (2006). Resistance to insect pests: What do legumes have to offer? *Euphytica* **147**:273–285.

Egli D. B. (2008). Soybean yield trends from 1972-2003 in mid-western USA. *Field Crops Research* **106**:53-59.

El-Sharkawi, H., Honna, T., Yamamoto, S. and Eneji, A. (2007). Biological nitrogen fixation by native microorganisms in a waste-amended paddy soil. *Journal of Sustainable Agriculture* **29**:23-38.

EMA. (1988). National Atlas of Ethiopia. Ethiopian Mapping Authority (EMA), Addis Ababa.

Ehsete, M. (1994). Chickpea and lentil agronomy research. In: *Cool-season Food Legumes of Ethiopia*. pp 230-251, (Tilaye, A., Bejiga, G., Saxena, M.C. and Solh, M.B., eds). Proceedings of the 1st National Cool-season Food Legumes Review Conference, 16-20 December 1993, Addis Ababa, Ethiopia. ICARDA/IAR. ICARDA, Syria.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology* **14**:2611-2620.

Evans, D.E. (1987). Stored Product. In *Integrated Pest Management*, pp. 425-455, (Burn, A.J., Cooker, T.H. and Jepson, P.C., Eds). Academic Press: London, UK.

Evans, J., McNeill, A.M., Unkovich, M.J. Fettell, N.A. and Heenan. D.P. (2001). Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. *Australian Journal of Experimental Agriculture* **41**:347 – 359.

Fageria, N.K., Baligar, V.C. and Li, Y.C. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of Plant Nutrition* **31**:1121–1157.

Falconer, D.S. (1960). *Introduction to Quantitative Genetics*. 2rd ed. Longman, London, England.

Falconer, D.S. (1989) *Introduction to Quantitative Genetics*. 3rd ed. Longman, London, England.

Fan, F., Zhang, F., Song, Y., Sun, J., Bao, X., Guo, T. and Li, L. (2006). Nitrogen fixation of faba bean (*Vicia faba* L.) interacting with a non-legume in two contrasting intercropping systems. *Plant and Soil* **283**:275–286.

FAO. (1984). Fertilizer and plant nutrition guide. Food and Agriculture Organization of the United Nations (FAO). FAO Fertilizer and Plant Nutrition Bulletin 9, Rome.

FAO. (1989). Fertilizers and food production. FAO fertilizer and plant nutrition bulletin 9. Food and Agriculture Organization of the United Nations (FAO), Rome.

FAO. (1996). FAO/WFP crop and food supply assessment mission to Ethiopia (http://www.fao.org/docrep/004/W3787e/W3787e00.HTM).

Commodityonline. (2009). India's Green Revolution that bypassed pulses. (http://www.commodityonline.com/news/Indias-Green-Revolution-that-bypassed-pulses-15163-3-1.html).

Fatou, N., Guene, D., Diouf, A. and Gueye, M. (2003). Nodulation and nitrogen fixation of field grown common bean (*Phaseolus vulgaris*) as influenced by fungicide seed treatment. *African Journal of Biotechnology* **2**:198–201.

Felsenstein, J. (2007). *Theoretical Evolutionary Genetics*. University of Washington, USA.

Fernández-González, M., Mena, A., Izquierdo, P. and Martínez, J. (2007). Genetic characterization of grapevine (*Vitis vinifera* L.) cultivars from Castilla La Mancha (Spain) using microsatellite markers. *Vitis* **46**:126–130.

Fikresilassie, M. (2008). Genetic divergence for morpho-physiological and nodulation traits and their associations in Ethiopian fenugreek (*Trigonella foenum-graecum* L.) landraces. M.Sc Thesis, Haramaya University, Ethiopia.

Fomeg-as, D.Y. (2004). Multi-farm trial of biological nitrogen fixation (BNF) in beans (*Phaseolus vulgaris*) at mountain province. Presented during the Regional Sectoral/Commodity Review, ATI-CAR, BSU cmpd., La Trinidad, Benguet on June 15-17, 2004.

Ford-Lloyd, B. and Jackson, M. (1986). *Plant Genetic Resources: An Introduction to their Conservation and Use*. Edward Arnold, Australia.

Fox, P.N. and Rosielle, A.A. (1982). Reducing the influence of environmental main effects on pattern analysis of plant breeding environments. *Euphytica* **31**: 645–656.

Franco, M.C., Cassini, S.T., Oliveira, V.R., Vieira, C., Tsai, S.M. and Cruz, C.D. (2001). Combining ability for nodulation in common bean (*Phaseolus vulgaris* L.) genotypes from Andean and Middle American gene pools. *Euphytica* **118**:265–270.

Gahoonia, T.S., Ali, R., Malhotra, R.S., Jahoor, A. and Rahman, M.M. (2007). Variation in root morphological and physiological traits and nutrient uptake of chickpea genotypes. *Journal of Plant Nutrition* **30**: 829–841.

Gahoonia, T.S. and Nielsen, N.E. (1996). Variation in acquisition of soil phosphorus among wheat and barley genotypes. *Plant and Soil* **178**:223-230.

Gardner, W.K. and Boundy, K.A. (1983). The acquisition of phosphorus by *Lupinus albus* L. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. *Plant and Soil* **70**:391–402.

Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S. and McCouch, S. (2005). Genetic structure and diversity in *Oryza sativa* L. *Genetics* **169**:1631–1638.

Gerloff, S. (1977). Plant efficiencies in the use of N, P and K. In: *Plant Adaptation to Mineral Stress in Problem Soils*, pp. 161-174, (Wright, M.J., ed). Cornell Univ. Press: New York.

Ghizaw, A., Du Freez, C. C. and Bekele, T. (2005). Effect of phosphorus fertilizer on grain yield of field pea (*Pisum sativum* L.) cultivars. *Ethiopian Journal of Natural Resources* **7**:1-21.

Ghosh, P. K., Bandyopadhyay, K.K., Wanjari, R.H., Manna, M.C., Misra, A.K., Mohanty, M. and Rao, A.S. (2007). Legume effect for enhancing productivity and nutrient use-efficiency in major cropping systems–An Indian perspective: A review. *Journal of Sustainable Agriculture* **30**: (doi:10.1300/J064v30n01_07) (http://jsa.haworthpress.com).

Gilbert, J.E., Lewis, R.V., Wilkinson, M.J. and Caligari, P.D.S. (1999). Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theoretical and Applied Genetics* **98**:1125-1131.

Gill, A.A.S., Sadana, U.S. and Samal, D. (2005). Phosphorus influx and root-shoot relations as indicators of phosphorus efficiency of different crops. *Communications in Soil Science and Plant Analysis* **36**:2315–2327.

Giongo, A., Luciane, M. P., João, P., Freire, R.J. and de Sá, E.L.S. (2007). Genetic diversity and symbiotic efficiency of population of rhizobia of *Phaseolus vulgaris* L. in Brazil. *Biology and Fertility* of *Soils* **43**:593–598.

Goicoechea, N., Antolin, M.C. and Sánchez-Diaz, M. (1997). Influence of arbuscular mycorrhizae and *Rhizobium* on nutrient content and water relations in drought stressed alfalfa. *Plant and Soil* 192: 261–268.

Golley, F., Baudry, J., Berry, R.J., Bornkamm, R., Dahlberg, K., Jansson, A-M., King, J., Lee, J., Lenz, R. and Sharitz, R. (1992). What is the road to sustainability? *INTECOL Bulletin* **20**: 15–20

Gomez, K.A. and A. Gomez. (1984). *Statistical Procedures for Agricultural Research*. John Wiley & Sons. New York.

Goossens, A., Quintero, C., Dillen, W., de Rycke, R., Valor, J.F., de Clercq, J., Montagu, M.V., Cardona, C. and Angenon, G. (2000). Analysis of bruchid resistance in the wild common bean accession G02771: No evidence for the insecticidal activity of arcelin 5. *Journal of Experimental Botany* **51**:1229-1236.

Gorfu, A. 1998. The role of fertilizers and faba bean (*Vicia faba*) in sustaining production of cereals in different crop rotations in the South-eastern Ethiopian Highlands. Doctoral Dissertation, Georg-August-Universität Göttingen, Germany.

Górny, A.G. (2001). Variation in utilization efficiency and tolerance to reduced water and nitrogen supply among wild and cultivated barleys. *Euphytica* **117**:59–66.

Gunes, A. I., Alpaslan, M. and Cakmak, I. (2006). Genotypic variation in phosphorus efficiency between wheat cultivars grown under greenhouse and field conditions. *Soil Science and Plant Nutrition* **52**: (doi: 10.1111/j.1747-0765.2006.00068.x).

Graham, P.H. and Vance, C.P. (2003). Legumes: Importance and Constraints to Greater Use. *Plant Physiology* **131**:872–877.

Graham, R.D. (1984). Breeding for nutritional characteristics in cereals. *Advances in Plant Nutrition* **1**:57–102.

Graham, R.D. (1988). Development of wheats with enhanced nutrient efficiency: Progress and potential. In: *Wheat Production Constraints in Tropical Environments*, pp 305-320, (Klatt, A.R., ed). Mexico, D.F.: CIMMYT.

Graham, R.D., Ascher, J.S. and Hynes, S.C. (1992). Selecting Zn efficient cereal genotypes for soils of low Zn status. *Plant and Soil* 146: 241–250.

Gulden, R.H. and Vessey, J.K. (1998). Low concentrations of ammonium inhibit specific nodulation (nodule number $g^{-1}$ root DW) in soybean (*Glycine max* [L.] Merr.). *Plant and Soil* **198**:127–136.

Gunes, A. I., Alpaslan, M. and Cakmak, I. (2006). Genotypic variation in phosphorus efficiency between wheat cultivars grown under greenhouse and field conditions. *Soil Science and Plant Nutrition* **52**: (doi: 10.1111/j.1747-0765.2006.00068.x).

Gwinner, J., Harnisch, R. and Mück, O. (1990). Manual on the prevention of post-harvest grain losses. Post-harvest Project, Pickhuben, Hamburg, FRG.

Hagedorn, D.J. (1984). *Compendium of Pea Diseases*. The American Phytopathological Society and the University of Wisconsin-Madison, USA.

Haile, A. (2006). On-farm storage studies on sorghum and chickpea in Eritrea. *African Journal of Biotechnology* **5**:1537-1544.

Hailemariam, A. and Tsige, A. (2006). Biological nitrogen fixation research on food legumes in Ethiopia. In: *Food and Forage Legumes of Ethiopia: Progress and Prospects*, pp. 172-176, (Ali, K., Keneni, G., Ahmed, S., Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M.H., eds). Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria.

Hardarson, G. (2004). Enhancement of symbiotic nitrogen fixation in grain legumes: selected results from the FAO/IAEA program. In: *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp 163-171, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Hardarson, G. and Atkins, C. (2003). Optimising biological N2 fixation by legumes in farming systems. *Plant and Soil* **252**: 41–54.

Harris, H.C., Goebel, W. and Cooper, P.J.M. (1987). Crop genotype-environment interaction. In: *Drought Tolerance in Winter Cereals*, pp. 37-53, (Srivastana, J.P., Porceddu, E., Acevedo, E. and Varma, S., eds). Proceedings of an International Workshop, 27-31 Oct. 1985, Capri, Italy.

Hartley, H.O. (1950). The maximum F-ratio as a short cut test for heterogeneity of variances. *Biometrika* **37**:308-312.

Hawtin, G.C. (1982). The genetic improvement of faba bean. *Faba Bean Improvement*, pp. 15-32, (Hawtin, G. and Webb, C., eds). Proceedings of the Faba Bean Conference held in Cairo, Egypt, March 7-11, 1981. Martinus Nijhoff Publishers, The Netherlands.

Hawtin, G.C., Muehlbauer, F.J., Slinkard A.E. and Singh K.B. (1988). Current status of cool season food legume crop improvement: assessment of critical needs. *World Crops: Cool Season Food Legumes*, pp. 67-80, (Summerfield, R.J., ed). Kluwer Academic Publishers, The Netherlands.

Hayward, H.D. and Breese, E.L. (1993). Population structure and variability. In: *Plant Breeding: Principles and Prospects*, pp. 16-29, (Hayward, M.D., Bosemark, N.O. and Romagosa, I., eds). Chapman and Hall, Great Britain.

He, Q., Li, X.W., Liang, G.L., Ji, K., Guo, Q.G., Yuan, W.M., Zhou, G.Z., Chen, K.S., van de Weg, W.E. and Gao, Z.S. (2011). Genetic diversity and identity of Chinese loquat cultivars/accessions (*Eriobotrya japonica*) using apple SSR markers. *Plant Molecular Biology Reporter* **29**:197–208.

Helland, J. (2004). The continuing concern over phosphorus. Information Brief, Minnesota House of Representatives Research Department, 600 State Office Building, St. Paul, MN 55155.

Herridge, D. F., Marcellos, H., Felton, W.L., Turner, G.L. and Peoples, M.B. (2000). Chickpea increases soil-N fertility in cereal systems through nitrate sparing and $N_2$ fixation. *Soil Biology and Biochemistry* **27**:545-551.

Herridge, D.F., Peoples, M.B. and Boddey, R.M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* **311**:1–18.

Herridge, D. and Rose, I. (2000). Breeding for enhanced nitrogen fixation in crop legumes. *Field Crops Research* **65**: 229–248.

Higgins, V.J., Lu, H., Xing, T., Gelli, A. and Blumwald, E. (1999). The gene-for-gene concept and beyond: Interactions and signals. *Canadian Journal of Plant Pathology* **20**:150-157.

Hirsch, A.M. (2009). Brief history of the discovery of nitrogen-fixing organisms (http://www.mcdb.ucla.edu/Research/Hirsch/imagesb/ History DiscoveryN2fixingOrganisms.pdf).

Hoare, J. (1992). Sustainable dryland cropping in southern Australia: a review. *Agriculture, Ecosystems and Environment* **38**:193-204.

Holford, I.C.R. and Crocker, G. J. (1997). A comparison of chickpeas and pasture legumes for sustaining yields and nitrogen status of subsequent wheat. *Austtralian Journal of Agricultural Research* **48**:305-315.

Huang, X., Wolf, M., Ganal, M.W., Orford, S., Koebner, R.M.D. and Röder, M.S. (2007). Did modern plant breeding lead to genetic erosion in European winter wheat varieties? *Crop Science* **47**:343–349.

Hubbell, D.H. and Kidder, G. (2003). Biological nitrogen fixation (http://edis.ifas.ufl.edu/SS180).

Hüttel, B., Winter, P. and Weising, K. (1999). Sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). *Genome* **42**:210–217.

IBPGR, ICRISAT and ICARDA. (1993). Descriptor for chickpea (Cicer arietinum L.). IBPGR, Rome Italy/ ICRISAT, Patancheru, India/ICARDA, Aleppo, Syria.

IFPRI. (2010). Pulses value chain in Ethiopia: Constraints and opportunities for enhancing exports. Working Paper. International Food Policy Research Institute (http://www.ifpri.org/sites/default/files/ publications/ethiopian agsectorwp _pulses.pdf).

Ignacimuthu, S. and Prakash, S. (2006). *Agrobacterium*-mediated transformation of chickpea with α- amylase inhibitor gene for insect resistance. *Journal of Biological Sciences* **31**: 339-345.

Isfan,D., Lamarre, M and D'Avignon, A. (1995). Nitrogen and phosphorus fertilizer recovery in spring wheat and soil as related to the rate and time of application. In: *Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation*, pp. 175-187, Proceedings of a Symposium, 17-21 Oct. 1994, IAEA, Vienna.

Ishimoto, M., Sato, T., Chrispeels, M.J. and Kitamaru, K. (1996). Bruchid resistance of transgenic adzuki bean expressing seed α-amylase inhibitor of common bean. *Entomologia Experimentalis et Applicata* **79**: 309-315.

Ishimoto, M., Suzuki, K., Iwanaga, M., Kikuchi, F. and Kitamaru, K. (1995). Variation of seed α-amylase inhibitors in the common bean. *Theoretical and Applied Genetics* **90**:425-429.

Ishizuka, J. (1992). Trends in biological nitrogen fixation research and application. *Plant and Soil* **141**:197-209.

Jarso, M., G. Keneni and T. Wolabu. (2011). Enhancing the technical relevance of pulses and oilseed crops through target oriented breeding. In: *Oilseeds: Engine for Economic Development*, pp. 45-60, (Terefe, G., Wakjira, A. and Gorfu, D., eds). Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa, Ethiopia.

Javaid, A. (2009). Arbuscular Mycorrhizal mediated nutrition in plants. *Journal of Plant Nutrition* **32**:1595–1618.

Jemo, M., Abaidoo, R.C., Nolte, C., Tchienkoua, M., Sanginga, N. and Horst, W. J. (2006). Phosphorus benefits from grain-legume crops to subsequent maize grown on acid soils of southern Cameroon. *Plant Soil* **284**:385–397.

Jensen, E.S. and Hauggaard-Nielsen, H. (2003). How can increased use of biological N2 fixation in agriculture benefit the environment? *Plant and Soil* **252**:177–186.

Johnston, A.W.B. (1989) Biological nitrogen fixation. In: *A Revolution in Biotechnology*, pp.103-118, (Marx, J.L., ed). Cambridge University Press, Cambridge, New York.

Jomová, K., Benková, M., Žáková, M., Gregová, E. and Kraic, J. (2005). Clustering of chickpea (*Cicer arietinum* L.) accessions. *Genetic Resources and Crop Evolution* **52**:1039–1048.

Jones, J.B. (2003). *Agronomic Handbook: Management of Crops, Soils and their Fertility*. Corner Research Center, USA.

Jørgensen, F.V., Jensen, E.S. and Schjoerring, J.K. (1999). Dinitrogen fixation in white clover grown in pure stand and mixture with ryegrass estimated by the immobilized 15N isotope dilution method. *Plant and Soil* **208**:293–305.

Joshi, A.B. and Dhawan, N.L. (1966). Genetic improvement in yield with special reference to self-fertilizing crops. *Indian Journal of Genetics* **26**:101-103.

Joshi, P.K., Rao, P., Gowda, C.L.L., Jones, R.B., Silim, S.N., Saxena, K.B. and Kumar, J. (2001). *The World Chickpea and Pigeonpea Economies: Facts, Trends and Outlook*. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pardesh, India.

Kaga, A., Ishii, T., Tsukimoto, K., Tokoro, E. and Kamijima, O. (2000). Comparative molecular mapping in *Ceratotropis species* using an inter-specific cross between *V. umbellata* and *V. angularis*. *Theoretical and Applied Genetics* **100**:207-213.

Kashiwaba, K., Tomooka, N., Kaga, A., Han, O.K. and Vaughan, D.A. (2003). Characterization of resistance to three bruchid species (*Callosobruchus* spp., Coleoptera, Bruchidae) in cultivated rice bean (*Vigna umbellata*). *Journal of Economic Entomology* **96**:207-213.

Kassie, M., Shiferaw, B., Asfaw, S., Abate, T., Muricho, G., Ferede, S., Eshete, M. and Assefa, K. (2009). Current situation and future outlooks of the chickpea sub-sector in Ethiopia. ICRISAT and EIAR (http://www.icrisat.org/tropicallegumesII/pdfs/Current_Situation.pdf).

Keatinge, J.D.H., Chapanian, N. and Saxena, M.C. (1998). Effect of improved management of legumes in a legume-cereal rotation on field estimates of crop nitrogen uptake and symbiotic nitrogen fixation in northern Syria. *The Journal of Agricultural Science* **110**:651-659.

Keneni, G. 2007. Concerns on mismatches between environments of selection and production of crop varieties in Ethiopia. *East African Journal of Sciences* **1**:93-103.

Keneni, G., Assefa, F., Imtiaz, M., Bekele, E. and Regassa, H. (2010). Genetic diversity for attributes of biological nitrogen fixation in Abyssinian field pea (*Pisum sativum* var. *Abyssinicum*) germplasm accessions. *Journal of Genetics & Breeding* (in review process).

Keneni, G. and Imtiaz, M. (2010). Demand-driven breeding of food legumes for plant-nutrient relations in the tropics and the sub-tropics: Serving the farmers; not the crops! *Euphytica* **175**:267–282.

Keneni, G. and Simane, B. (2003) Implications of geographic origin and botanical sub-species for parental selection in genetic enhancement of groundnut (*Arachis hypogaea* L.). *Journal of Genetics and Breeding* **57**: 93-100.

Keneni, G. and Wakjira, A. (2004) Genetic uniformity of crop cultivars: challenges and opportunities. In: *SEBIL*. Vol. 10., pp. 1-9, Proceedings of the 10th Annual Conference of the Crop Science Society of Ethiopia. 19-21 June 2001, EARO, Addis Abeba, Ethiopia.

Keyser, H.H. and Li, F. (1992). Potential for increasing biological nitrogen fixation in soybean. *Plant and Soil* **141**:119-135.

Kimani, J.M., Kimani, P.M., Githiri, S.M. and Kimenju, J. W. (2007). Mode of inheritance of common bean (*Phaseolus vulgaris* L.) traits for tolerance to low soil phosphorus (P). *Euphytica* **155**:225–234.

Kimani, J.M. and Tongoona, P. (2008). The mechanism of genetic control for low soil nitrogen (N) tolerance in common beans (*Phaseolus vulgaris* L.). *Euphytica* **162**:193–203.

Kirkegaard, J., Christen, O., Krupinsky, J. and Layzell, D. (2008). Break crop benefits in temperate wheat production. *Field Crops Research* **107**:185–195.

Kong, Q., Li, X., Xiang, C., Wang, H., Song, J. and Zhi, H. (2011). Genetic diversity of radish (*Raphanus sativus* L.) germplasm resources revealed by AFLP and RAPD markers. *Plant Molecular Biology Reporter* **29**:217–223.

Kramer, P.J. (1980). Drought, stress and the origin of adaptation. In: *Adaptation of Plants to Water and High Temperature Stress*, pp. 7-20, (Turner, N.C. and Kramer, P.J., eds). John Wiley & Sons, New York.

Krasilnikoff, G., Gahoonia, T. and Nielsen, N.E. (2003). Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere processes. *Plant and Soil* **251**:83–91.

Ku-Hwan, L., Seung-Keun, J., Sang-II, P., Beom-Heon, S., Yong-Gu, C. and Hong-Sig, K. (2002). Agronomic characteristics of resistant mungbean to *Callosbruchus chinensis*. *Journal of Agricultural Science* **19**:41-52.

Ladizinsky, G. and Adler, A. (1976). The origin of chickpea, *Cicer arietinum* L. *Euphytica* **25**:211-217.

Lale, N.E.S. and Kolo, A.A. (1998). Susceptibility of eight genetically improved local cultivars of cowpea to *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) in Nigeria. *International Journal of Pest Management* **44**:25-27.

Lawes, D.A., Bond, D.A. and Poulsen, M.H. (1983). Classification, origin, breeding methods and objectives. In: *The Faba Bean (Vicia faba L.)*, pp. 23-76, (Hebblethwaite, P.D., ed). The University Press, Cambridge, UK.

Lemma, T. (1990). The biology and control of the adzuki been beetle (*Callosobruchus chinensis* L.) on chickpea (*Cicer arietinum* L.). M.Sc Thesis, Alemaya University, Ethiopia.

Li, L., Tang, C., Rengel, Z. and Zhang, F. (2003a). Chickpea facilitates phosphorus uptake by intercropped wheat from an organic phosphorus source. *Plant and Soil* **248**:297–303.

Li, L., Zhang, F.S., Li, X.L., Christie, P., Yang, S.C. and Tang, C. (2003b). Interspecific facilitation of nutrient uptakes by intercropped maize and faba bean. *Nutrient Cycling in Agroecosystems* **65**:61-71.

Li, M. G., Osaki, M., Rao, I.M. and Tadano, T. (1997). Secretion of phytase from the roots of several plant species under phosphorus–deficient conditions. *Plant and Soil* **195**:161–169.

Li, Y.D., Wang, Y.J., Tong, Y.P., Gao, J.G., Zhang, J.S. and Chen, S.Y. (2005). QTL mapping of phosphorus deficiency tolerance in soybean (Glycine max L. Merr.). *Euphytica* **142**:137–142.

Liao, M., Hocking, P.J., Dong, B., Delhaize, E., Richardson, A. E. and Ryan, P. R. (2008). Variation in early phosphorus-uptake efficiency among wheat genotypes grown on two contrasting Australian soils. *Australian Journal of Agricultural Research* **59**:57–166.

Lim, G. and Burton, J.C. (1982). Nodulation status of the Leguminosae. In: *Nitrogen Fixation. Vol 2. Rhizobium*, pp 1-34, (Broughton, W.J., ed). Oxford University Press, UK.

Lin, C., Chen, C. and Horng, S. (2005). Characterization of resistance to *Callosobruchus maculatus* (Coleoptera: Bruchidae) in mungbean variety VC6089A and its resistance-associated protein VrD1. *Journal of Economic Entomology* **98**:1369–1373.

Lindemann, W.C. and Glover, C.R. (2003). Nitrogen Fixation by Legumes. Cooperative Extension Service, College of Agriculture and Home Economics. New Mexico State University, Mexico.

Little, T.M. and Hills, F.J. (1978). *Agricultural Experimentation: Design and Analysis*. John Wiley and Sons. New York.

Liu, Y., Wu, L., Baddeley, J.A. and Watson, C.A. (2010). Models of biological nitrogen fixation of legumes: A review. In: Agronomy for Sustainable Development. (http://www.agronomy-journal.org/index.php?option=com_article&access=standard&Itemid=129&url=/articles/agro/abs/first/a9189/a9189.html; DOI 10.1051/agro/2010008).

López-Bellido, L., López-Bellido, R.J., Redondo, R. and Benítez, J. (2006). Faba bean nitrogen fixation in a wheat-based rotation under rainfed Mediterranean conditions: Effect of tillage system. *Field Crops Research* **98**:253–260.

López-Bellido, R.J., López-Bellido, L., Castillo, J.E. and López-Bellido, F.J. (2004). Chickpea response to tillage and soil residual nitrogen in a continuous rotation with wheat II. Soil nitrate, N uptake and influence on wheat yield. *Field Crops Research* **88**:201–210.

Lorieux, M. (2005). Comparative QTL mapping for drought tolerance. A Project proposal, IRD/CIAT. Colombia.

Löschenberger, F., Fleck, A., Grausgruber, H., Hetzendorfer, H., Hof, G., Lafferty, J., Marn, M., Neumayer, A., Pfaffinger, G. and Birschitzky, J. (2008). Breeding for organic agriculture: the example of winter wheat in Austria. *Euphytica* **163**:469–480.

Macduff, J.H. and White, R.E. (1984). Components of the nitrogen and phosphorus cycle measure for cropped and grassland. *Soil Plant Systems* **76**:35-47.

Malhotra, R.S. and Singh, K.B. (2004). Classification of chickpea growing environments to control genotype by environment interaction. *Euphytica* **58**:5-12.

Mamo, T., Haque, I. and Kamara, C.S. (1988). Phosphorus status of some Ethiopian Vertisols. In: *Management of Vertisols in Sub-Saharan Africa*, pp. 232-252, Jutzi, S.C., Haque, I., McIntire, J. and Stares, J.E.S., eds). Proceedings of a Conference, 31 August-4 September 1987, ILCA, Addis Ababa, Ethiopia.

Manly, B. F. J. (1986). *Multivariate Statistical Methods: A Primer*. Chapman and Hall. London.

Mayt, S.N. and Bohlool, B.B. (1983). Competition among Rhizobium leguminosarum Strains for Nodulation of Lentils (*Lens esculenta*). *Applied and Environmental Microbiology* **45**:960-965.

McGuire, S.J. and Sperling, L. (2008). Leveraging farmers' strategies for coping with stress: seed aid in Ethiopia. *Global Environmental Change* **18**:679-688.

McKnight Foundation. (2008). Increasing phosphorus efficiency and production of grain legumes in China and Africa (http://mcknight.ccrp.cornell.edu/program_docs/project_documents/SAF_05-780_plegumes/05780_plegumes_yr7_07-08_vweb.pdf;http://mcknight.ccrp.cornell.edu/projects/saf_cop/SAF_plegumes/plegumes_project.html).

Mekibeb, H., Abebe, D. and Abebe, T. (1991). Pulse Crops of Ethiopia. In: *Plant Genetic Resources of Ethiopia*, pp. 328-343, (Engels, J.M.M., Hawkes, J.G. and Worede, M., eds). Cambridge University Press, UK.

Mian, M.A.R., Kang, S., Beil, S.E. and Hammond, R.B. (2008). Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. *Theoretical and Applied Genetics* **117**:955–962.

Miklas, P.N., Kelly, J.D., Beebe, S.E. and Blair, M.W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* **147**:105–131.

MoA. (2010). Crop variety register. Issue No. 13. Ministry of Agriculture (MoA), Addis Ababa, Ethiopia.

Montañez, A. (2000). Overview and case studies on biological nitrogen fixation: Perspectives and limitations. Food and Agriculture Organization of the United Nations (FAO), Rome.

Moreno-González, J. and Cubero, J.I. (1993). Selection strategies and choice of breeding methods. *Plant Breeding: Principles and Prospects*, pp. 281-313, (Hayward, M.D., Bosemark, N.O. and Romagosa, I., eds). Chapman and Hall, UK.

Morton, R.L., Schroeder, H.F., Bateman, K.S., Chrispeels, M.J., Armstrong, E. and Higgins, T.J.V. (2000). Bean α-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proceedings of National Academy of Sciences* **97**:3820–3825.

Muehlbauer, F.J. and Singh, K.B. (1987). Genetics of chickpea. In: *The Chickpea*, pp. 99-125, C.A.B. International, Wallingford, Oxon, OX10 8DE, UK.

Muehlbauer, F.J. and Tullu, A. (1997). *Cicer arietinum* L: New crop fact sheet(http://www.hort.purdue.edu/newcrop/cropfactsheets/chickpea.html#Origin).

Muhammad, D., Gurmani, A. H. and Khan, M. (2004). Effect of phosphorus and *Rhizobium* inoculation on the yield and yield components of mungbean under the rainfed conditions of D.I. Khan. *Sarhad Journal of Agriculture* **20**:575-582.

Mytton, L.R. and SkØt, L. (1993). Breeding for improved symbiotic nitrogen fixation. *Plant Breeding: Principles and Prospects*, pp. 451-472, (Hayward, M.D., Bosemark, N.O. and Romagosa, I., eds). Chapman and Hall, UK.

Nayak, S.N., Zhu, H. and Varghese, N. (2010). Integration of novel SSR and gene base SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. *Theoretical and Applied Genetics* **120**:1415–1441.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from small number of individuals. *Genetics* **89**:583-590.

Nogueira, M. A., Nehls, U., Hampp, R., Poralla, K. and Cardoso, E. J. B. N. (2007). Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. *Plant Soil* **298**:273–284.

Nuruzzaman, M., Lambers, H., Bolland, M.D.A. and Veneklaas, E.J. (2005). Phosphorus uptake by grain legumes and subsequently grown wheat at different level of residual phosphorus fertilizer. *Australian Journal of Agricultural Research* **56**:1041-1047.

Ogoke, I.J., Togun, A.O. and Dashiell, K.E. (2006). Soybean phosphorus-use efficiency in the moist Savanna of West Africa. *Journal of Sustainable Agriculture* **28**:5-18.

Ohri, D. and Pal, M. (1991). The origin of chickpea (*Cicer arietinum* L.): Karyotype and nuclear DNA amount. *Heredity* **66**:367-372.

Ojo, D.K., Bodunde, J.G., Ogunbayo, S.A. and Akinwale, A.F. (2006). Genetic evaluation of phosphorus utilization in tropical cowpea (*Vigna unguiculata* (L) Walp). *African Journal of Biotechnology* **5**:597-602.

Ortiz-Monasterio, J.I., Manske, G.G.B. and van Ginkel, M. (2001). Nitrogen and phosphorus use efficiency. In: *Application of Physiology in Wheat Breeding*, pp. 200-207, (Reynolds, M.P., Ortiz-Monasterio, J.I. and McNab, A., eds). Mexico, D.F.: CIMMYT.

Osman, A.M., Almekinders, C.J.M., Struik, P.C. and van Bueren, E.T.L. (2008). Can conventional breeding programmes provide onion varieties that are suitable for organic farming in the Netherlands? *Euphytica* **163**:511–522.

Osman, E., Muir, J.P. and Elgersma, A. (2002). Effect of Rhizobium inoculation and phosphorus application on native Texas legumes grown in local soil. *Journal of Plant Nutrition* **25**:75–92.

Özdemir, S. and Kardavut, U. (2003). Comparison of the performance of autumn and spring sowing of chickpeas in a temperate region. *Turkey Journal of Agriculture and Forestry* **27**:345-352.

Pacheco, I.A., Bolonhezi, S., Sartori, M.R., Turatti, J.M., Paula, D.C. and de Lourencao, A.L. (1994). Resistance to bruchids, fatty acid composition and grain texture in genotypes of chickpea. *Bragantia* **53**:61-74.

Panda, N. and Khush, G.S. (1995). *Host Plant Resistance to Insects*. CAB International in association with International Rice Research Institute (IRRI). Biddles Ltd., Guildford, UK.

Parker, M.A. (2008). Symbiotic relationships of legumes and nodule bacteria on Barro Colorado Island, Panama: A review. *Microbial Ecology* **55**:662–672.

Parr, J.F., Paperndick, I. and Youngberg, I.G. (1983). Organic farming in the United Sate: Principles and perspectives. *Agroecosystem* **8**:183-201.

Peakall, R. and Smouse, P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**:288–295.

Pearson, C.J., Norman, D.W. and Dixon, J. (1995). Sustainable dryland cropping in relation to soil productivity - FAO soils bulletin 72. Food and Agriculture Organization of the United Nations (FAO). Rome.

Pimratch, S., Jogloy, S., Vorasoot, N., Toomsan, B., Patanothai, A. and Holbrook, C.C. (2008). Relationship between biomass production and nitrogen fixation under drought-stress conditions in peanut genotypes with different levels of drought resistance. *Journal of Agronomy and Crop Science* **194**:15-25.

Pineda, J.A., Kipe-Nolt, J.A. and Rojas, E. (1994). *Rhizobium* inoculation increases of bean and maize yields in intercrops on farms in the Peruvian sierra. *Experimental Agriculture* **30**:311–318.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**:945–959.

Provorov, N.A. and Tikhonovich, I.A. (2004). Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. *Genetic Resources and Crop Evolution* **50**:89-99.

Purcell, L.C., de Silva, M., King, C.A. and Kim, W.H. (1997). Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits. *Plant and Soil* **196**:101–113.

Qiao, Y., Tang, C., Han, X. and Miao, S. (2007). Phosphorus deficiency delays the onset of nodule function in soybean. *Journal of Plant Nutrition* **30**:1341–1353.

Quinones, M.A., Borlaug, N.E. and Dowswell, C.R. (1997). A fertilizer based green revolution for Africa. In: *Replenishing soil fertility in Africa*, pp. 81–95, SSSA Special Publication No 51. Agronomy and Soil Science Society of America, 677 S. Segoe Rd., Madison, Wl, 53711, USA.

Radhika, P., Gowda, S.J.M., Kadoo, N.Y., Mhase, L.B., Jamadagni, B.M., Sainani, M.N., Chandra, S. and Gupta, V.S. (2007). Development of an integrated map of chickpea (*Cicer arietinum* L.) using two recombinant inbred line populations. *Theoretical and Applied Genetics* **115**:209-216.

Ransom, J.K., Palmer, A.F.E., Short, K. and Waggingto, S. (1993). Improving productivity of maize in stress environments. In: *Proceedings of the First National Maize Workshop of Ethiopia*, pp. 30-33, (Tolessa, B. and Ransom, J.K., eds). 5-7 May, 1992, Institute of Agricultural Research (IAR) and the International Center for Maize and Wheat Research (CIMMYT), Addis Ababa, Ethiopia.

Rao, D.L.N., Natarajan, T., Ilamurugu, K., Raut, R.S. and Rawat, A.K. (2004). *Rhizobium* inoculation of leguminous oilseeds – results of on-farm and farmers' field demonstrations in the ICAR coordinated project on BNF. In: *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp. 301-309, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Redden, R.J. (1983). The inheritance of seed resistance to *Callosobruchus rnaculatus* F. in cowpea *(Vigna unguiculata* L. Walp.). II. Analyses of percentage emergence and emergence periods of bruchids in F4 seed generation of two reciprocal crosses. *Australian Journal of Agricultural Research* **34**:697-705.

Redden, R.J., Dobie, P. and Gatehouse, A.M.R. (1983). The inheritance of seed resistance to *Callosobruchus rnaculatus* F. in cowpea *(Vigna unguiculata* L. Walp.). I. Analyses of parental, $F_1$, $F_2$, $F_3$ and backcross seed generations. *Australian Journal of Agricultural Research* **34**:681-695.

Redden, R.J. and McGuire, J. (1983). The genetic evaluation of bruchid resistance in seed of cowpea. *Australian Journal of Agricultural Research* **34**:707-715.

Reijntjes, C., Haverkort, B. and Waters-Bayer, A. (1992). *Farming for the Future: An Introduction to Low-External-Input and Sustainable Agriculture*. Macmillan, London.

Rengel, Z. (2002). Breeding for better symbiosis. *Plant and Soil* 245:147–162.

Richards, R.A. (1987). Physiology and the breeding of winter-grown cereals for dry areas. In: *Drought Tolerance in Winter Cereals*, pp. 171-190, (Srivastana, J.P., Porceddu, E., Acevedo, E. and Varma, S., eds). Proceedings of an international workshop 27-31 Oct. 1985, Capri, Italy.

Richards, R.A. (1991). Crop improvement for temperate Australia: Future opportunities. *Field Crops Research* **26**:141-169.

Rincón, A., Arenal, F., González, I., Manrique, E., Lucas, M.M. and Pueyo, J.J. (2008). Diversity of rhizobial bacteria isolated from nodules of the *Gypsophyte tridentata* L. growing in Spanish soils. *Microbial Ecology* **56**:223–233.

Rivas, R., Peix, A., Mateos, P.F., Trujillo, M.E., Martínez-Molina, E. and Velá zquez, E. (2006). Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. *Plant and Soil* **287**:23–33.

Roldan-Ruiz, I., Calsyn, E., Gilliand, T.J., Coll, R., Vaneijk, M.J.T. and de Loose, M. (2000). Estimating genetic conformity between related ryegrass (*Lolium*) varieties: AFLP characterization. *Molecular Breeding* **6**:593-602.

Romagosa, I., Ullrich, S.E., Han, F. and Hayes, P.M. (1996). Use of the additive main effects and multiplicative interaction model in QTL mapping for adaptation in barley. *Theoretical and Applied Genetics* **93**:30–37.

Romdhane, S.B., Aouani, M.E., Trabelsi, M., de Lajudie, P. and Mhamdi, R. (2008). Selection of High Nitrogen-Fixing Rhizobia Nodulating Chickpea (*Cicer arietinum*) for Semi-Arid Tunisia. *Journal of Agronomy and Crop Science* **194**:413–420.

Romeis, J., Sharma, H.C., Sharma, K.K., Das, S. and Sarmah, B.K. (2004). The potential of transgenic chickpeas for pest control and possible effects on non-target arthropods. *Crop Protection* **23**:923-938.

Römer, W. and Schenk, H. (1998). Influence of genotype on phosphate uptake and utilization efficiencies in Spring Barley. *European Journal of Agronomy* **8**:215-224.

Rosielle, A.A. and Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science* **21**:943-946.

Rosswall, T. and Paustain, K. (1984). Cycling of nitrogen and phosphorus in modern agricultural systems. *Plant and Soil* **76**:3-21.

Roy, R.N. (1995). FAO soil fertility and integrated plant nutrition management programs. In: *Nuclear Techniques in Soil Plant Studies for Sustainable Agriculture and Environmental Preservation*, pp. 107–129, Proceedings of a symposium, 17–21 Oct 1994, IAEA, Vienna.

Rubenstein, D.K., Heisey, P., Shoemaker, R., Sullivan, J. and Frisvold, G. (2005). Crop genetic resources: an economic appraisal. United States Department of Agriculture (USDA). Economic Information Bulletin No. 2. (www.ers.usda.gov).

Rupela, O.P. (1992). Natural occurrence and salient characters of non-nodulating chickpea plants. *Crop Science* **32**:349–352.

Sadowsky, M.J. and Bohlool, B.B. (1985). Differential expression of the pea symbiotic plasmid pJB5JI in genetically dissimilar backgrounds. *Symbiosis* **1**:125-138.

Saeed, A., Hovsepyan, H., Darvishzadeh, R., Imtiaz, M., Panguluri, S.K. and Nazaryan, R. (2011). Genetic diversity of Iranian accessions, improved lines of chickpea (*Cicer arietinum* L.) and their wild relatives by using simple sequence repeats. *Plant Mol Biology Reporter*: DOI 10.1007/s11105-011-0294-5.

Sanchez, P.A. (2002). Soil fertility and hunger in Africa. *Science* **295**:2019–2020.

Sanginga, N. (2003). Role of biological nitrogen fixation in legume based cropping systems: A case study of West Africa farming systems. *Plant and Soil* **252**:25–39.

Sanginga, N., Dashiell, K., Okogun, J.A. and Thottappilly, G. (1997). Nitrogen fixation and N contribution by promiscuous nodulating soybeans in the southern Guinea savanna of Nigeria. *Plant and Soil* **195**:257–266.

Sanginga, N., Lyasse, O. and Singh, B.B. (2000). Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant and Soil* **220**:119–128.

Sanginga, N., Vanlauwe, B. and Danso, S.K.A. (1995). Management of biological $N_2$ fixation in alley cropping system: Estmation and contribution of $N_2$ balance. *Plant and Soil* **174**:119–141.

Sarıkamış, G., Yaşar, F., Bakır, M., Kazan, K. and Ergül, A. (2009). Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey. *Genetics and Molecular Research* **8**:880-887.

Sarmah, B.K., Moore, A., Tate, W., Molvig, L., Morton, R.L., Rees, D.P., Chiaiese, P., Chrispeels, M.J., Tabe, L.M. and Higgins, T.J.V. (2004). Transgenic chickpea seeds expressing high levels of a bean α-amylase Inhibitor. *Molecular Breeding* **14**:73-82.

SAS Institute. (1996). SAS/STAT guide for personal computers, version 6.12 edition. Cary, NC: SAS Institute Inc.

Saxena, R. and Chandra, A. (2010). Isozyme, ISSR and RAPD profiling of genotypes in marvel grass (*Dichanthium annulatum*). *Journal of Environmental Biology* **31**:883-890.

Schmidt, E.L. (1988). Competition for legume nodule occupancy: a down-to-earth limitation on nitrogen fixation. In: *World Crops: Cool Season Food Legumes*, pp. 663-674, (Summerfield, R.J., ed). Kluwer Academic Publishers, Dordrecht.

Sedgley, R.H. (1991). An appraisal of the Donald ideotype after 21 years. *Field Crops Research* **26**:221-226.

Serraj, R. and Sinclair, T.R. (1998). Soybean cultivar variability for nodule formation and growth under drought. *Plant and Soil* **202**:159–166.

Serraj, R., Adu-Gyamfi, Rupela, O.P. and Drevon, J.J. (2004). Improvement of legume productivity and role of symbiotic nitrogen fixation in cropping systems: overcoming the physiological and agronomic limitations. In: *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp 67-97, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Sethy, N.K., Shokeen, B. and Bhatia, S. (2006). Isolation and characterization of sequence tagged-microsatellite markers in chickpea (*Cicer arietinum* L.). *Molecular Ecology Notes* **3**:428–430.

Shaheen, F. A., Khaliq, A. and Aslam, M. (2006). Resistance of chickpea (*Cicer arietinum* L.) cultivars against pulse beetle. *Pakistan Journal of Botany* **38**:1237-1244.

Shantharam, S. and Mattoo, A.K. (1997). Enhancing biological nitrogen fixation: An appraisal of current and alternative technologies for N input into plants. *Plant and Soil* **194**:205–216.

Sharma, R. and Behera, U.K. (2009). Recycling of legume residues for nitrogen economy and higher productivity in maize (*Zea mays*)–wheat (*Triticum aestivum*) cropping system. *Nutrient Cycling in Agroecosystems* **83**:197–210.

Sharma, S.K. and Mehta, H. (1990). Manifestation of genetic diversity for physiological traits in soybean under two cropping systems. *Tropical Agriculture* **68**:202-206.

Sharma, S.S., Negi, M.S., Sinha, P., Kumar, K. and Tripathi, S.B. (2011). Assessment of genetic diversity of biodiesel species *Pongamia pinnata* accessions using AFLP and three endonuclease-AFLP. *Plant Molecular Biology Reporter* **29**:12–18.

Sharpley, A.N., Daniel, T., Sims, T., Lemunyon, J., Stevens, R. and Parry, R. (2003). *Agricultural Phosphorus and Eutrophication*. Second Edition. United States Department of Agriculture (USDA), Agricultural Research Service, ARS-149, USA.

Shiferaw, B., Bantilan, M.C.S. and Serraj, R. (2004). Harnessing the Potentials of BNF for poor farmers: Technological, policy and institutional constraints and research needs. IN: *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp 3-27, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Shoko, M.D., Tagwira, F. and Zhou, M. (2007). The potential of reducing nitrogen fertilizer rates using a soyabean-sugarcane production system in the South Eastern Lowland of Zimbabwe. *African Journal of Agricultural Research* **2**:475-480.

Shukla, U.C. and Yadav, O.P. (2000). Effect of phosphorus and zinc on nodulation and nitrogen fixation in chickpea (*Cicer arietinum* L.). *Plant and Soil* **65**:239-248.

Simmonds, N.W. (1991). Selection for local adaptation in a plant breeding program. *Theoretical and Applied Genetics* **82**:363-367.

Simpson, R.D. and Sedjo, R.A. (1998). The value of genetic resources for use in agricultural improvement. *Agricultural Values of Plant Genetic Resources*, pp. 55-66, (Evenson, R.E., Gollin, D. and Santaniello, V., eds). CABI Publishing, Wallingford, UK.

Sinclair, T.R. and Vadez, V. (2002). Physiological traits for crop yield improvement in low N and P Environments. *Plant and Soil* 245:1–15.

Sinebo, W. (2002). Yield relationships of barley grown in a tropical highland environment. *Crop Science* **42**:428-437.

Sinebo, W. and Yirga, C. (2002). Participatory client orientation of research in low-input cropping systems of Ethiopia: a view point. In: *Towards Farmers' Participatory Research: Attempts and Achievements in the Central Highlands of Ethiopia,* pp. 27-43, (Keneni, G., Gojam, Y., Bedane, K., Yirga C. and Dibabe, A., eds). Proceedings of Client-Oriented Research Evaluation Workshop, 16-18 October 2001, Holetta Agricultural Research Center, Holetta, Ethiopia.

Singh, B.D. (2002). *Plant Breeding: Principles and Methods*. Kalyani Publishers, New Delhi-Ludhiana.

Singh, B. and Usha, K. (2003). Nodulation and symbiotic nitrogen fixation of cowpea genotypes as affected by fertilizer nitrogen. *Journal of Plant Nutrition* **26**:463–473.

Singh, K.B. (1987). Chickpea breeding. In: *The Chickpea*, pp. 127-162, C.A.B. International, Wallingford, Oxon, OX10 8DE, UK.

Singh, K.B. (1990). Prospects of developing new genetic material and breeding methodologies for chickpea improvement. *CIHEAM–Options Mediterraneennes* **9**:43-50.

Singh, K.K., Srinivasarao, C. and Ali, M. (2005). Root growth, nodulation, grain yield, and phosphorus use efficiency of lentil as influenced by phosphorus, irrigation, and inoculation. *Communications in Soil Science and Plant Analysis* **36**:1919–1929.

Singh, O. and Rupela, O.P. (1998). A new gene that controls root nodulation in chickpea. *Crop Science* 38:360-362.

Singh, P. (2001). *Numerical Problems in Plant Breeding and Genetics*. Kalyani Publishers, New Delhi, India.

Singh, R.B. (2000). Environmental consequences of agricultural development: a case study from the green revolution state of Haryana, India. *Agricultural Ecosystems and Environment* **82**:97–103.

Singh, R.K. and Chaudhary, B.D. (1985). *Biometrical Methods in Quantitative Genetic Analysis.* Kalyani Publishers, New Delhi-Ludhiana.

Sinha, S.K. (1987). Drought resistance in crop plants: a critical physiological and biochemical assessment. In: *Drought Tolerance in Winter Cereals*, pp. 349-364, (Srivastana, J.P., Porceddu, E., Acevedo, E. and Varma, S., eds). Proceedings of an international workshop 27-31 Oct. 1985, Capri, Italy.

Sithanantham, S. and Rao, G.V.R. (2006). Research needs in pest management for improved productivity and sustainability of food legume crops in Eastern Africa. In: *Food and Forage Legumes of Ethiopia: Progress and Prospects*, pp. 238-246, (Ali, K., Keneni, G., Ahmed, S., Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M.H., eds). Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria.

Slater, A., Scott, A. and Fowler, M. (2003). *Plant Biotechnology: The Genetic Manipulation of Plants*. Oxford University Press, New York.

Smith, A., Elmes, A.E., Howard, R.S. and Franklin, M.F. (1984). The uptake of soil and fertilizer-Phosphorus by barley growing under Scottish climatic condition. *Plant and Soil* **76**:49-57.

Smith, A.P., Chen, D. and Chalk, P.M. (2009). N2 fixation by faba bean (*Vicia faba* L.) in a gypsum-amended sodic soil. *Nutrient Cycling in Agroecosystems* **45**:329–333.

Smith, E.L. (1987). A review of plant breeding strategies for rainfed areas. In: *Drought Tolerance in Winter Cereals*, pp. 79-88, (Srivastana, J.P., Porceddu, E., Acevedo, E. and Varma, S., eds). Proceedings of an international workshop 27-31 Oct. 1985, Capri, Italy.

Smithson, J.B. and Grisley, W. (1992). First African bean yield and adaptation nursery (AFBYAN I). Part II. Performance across environments. Occasional Publication Series No. 3B. CIAT, Dar es Salaam, Tanzania.

Sneller, C.H., Nelson, R.L., Carter Jr., T.E. and Cui, Z. (2005). Genetic diversity in crop improvement: The soybean experience. In: *Genetic and Production Innovations in Field Crop Technology: New Developments in Theory and Practice*, pp. 103-144, (Kang, M.S., ed). International Book Distributing Co., Delhi, India.

Sokal, R.R. and Rohlf, F.J. (1997). *Biometry: The Principles and Practice of Statistics in Biological Research*. Third Edition. W.H. Freeman and Company, New York.

Somasegaran, P. and Hoben, H. J. (1985). Methods in Legume-Rhizobium Technology.Department of Agronomy and Soil Science, University of Hawaii, USA.

Somta, P., Ammaranan, C., Ooi, P.A.C and Srinives, P. (2007). Inheritance of seed resistance of bruchids in cultivated mungbean (*Vigna radiata* L. Wilezek). *Euphytica* **155**:47-55.

Somta, P., Kaga, A., Tomooka, N., Isemura, T., Vaughan, D.A. and Srivines, P. (2008). Mapping of quantitative trait loci for a new source of resistance to bruchids in the wild species *Vigna nepalens* Tateishi & Maxted (*Vigna* subgenus *Ceratotropis*). *Theoretical and Applied Genetics* **117**:621–628.

Somta, P., Talekar, N.S. and Srinives, P. (2006). Characterization of *Callosobruchus chinensis* (L.) resistance in *Vigna umbellata* (Thunb.) Ohwi & Ohashi. *Journal of Stored Products Research* **42**:313– 471.

Spagnoletti, P.L. and Qualset, C.O. (1987). Geographical diversity for quantitative spike characters in a world collection of durum wheat. *Crop Science* **27**:235-241.

Srinivasan, T. and Durairaj, C. (2007). Biochemical basis of resistance to in rice bean *Vigna umbellata* Thunb. (Ohwi and Ohashi) against *Callasobruchus maculatus* F. *Journal of Entomology* **4**:371-378.

Srinivasarao, C., Ganeshamurthy, A.N., Ali M. and Venkateswarlu, B. (2006). Phosphorus and micronutrient nutrition of chickpea genotypes in a multi-nutrient deficient typic ustochrept. *Journal of Plant Nutrition* **29**:747–763.

Sutton, W.D. (1983). Nodule development and senescence. In: *Nitrogen Fixation*. Volume 3. Legumes, pp. 144-112, (Broughton, W.J., ed). Oxford University Press, New York.

Syers, J.K., Johnston, A.E. and Curtin, D. (2008). Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soil phosphorus behaviour with agronomic information. FAO Fertilizer and plant nutrition bulletin 18. Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00153 Rome, Italy.

TAC. (1988). Sustainable Agricultural Production: Implications for International Agricultural Research. FAO, TAC Secretariat, Rome.

Talebi, R., Fayaz, R., Mardi, M., Pirsyed, S.M. and Naji, A.M. (2008). Genetic relationships among chickpea (*Cicer arietinum*) elite lines based on RAPD and agronomic markers. *International Journal of Agriculture and Biology* **10**:301-305.

Tanno, K., and Willcox, G. (2006). The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L.: early finds from Tell el-Kerkh, north-west Syria, late 10th millennium B.P. *Vegetation History Archives* **15**:197-204.

Tanto, T. and Tefera, E. (2006). Collection, conservation, characterization and sustainable utilization of grain legumes in Ethiopia. In: *Food and Forage Legumes of Ethiopia: Progress and Prospects*, pp. 15-22, (Ali, K., Keneni, G., Ahmed, S., Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M.H., eds). Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria.

Tate, R.L. (2000). *Soil Microbiology*. Second Edition. John Wiley & Sons, Inc., New York.

Tilaye, A., Demtsu, B. and Getachew, T. (1994). Role of cool-season food legumes and their production constraints in Ethiopian agriculture. In: *Cool-Season Food Legumes of Ethiopia*, pp. 3-18, (Tilaye, A., Bejiga, G., Saxena, M.C. and Solh, M.B., eds). Proceeding of the first national cool-season food legumes review conference, 16-20 December 1993, Addis Ababa, Ethiopia. ICARDA/IAR. ICARDA, Syria.

Temesgen T. (2008). Genetic gain and morpho-agronomic basis of genetic improvement in grain yield potential achieved by faba bean *(Vicia faba* L.*)* breeding in Ethiopia. MSc Thesis, Hawassa University, Ethiopia.

The Worldwide Gourmet. (2010). Chickpea: All about legumes on the Worldwide Gourmet. http://www.theworldwidegourmet.com/products/vegetables/chickpea/.

Thies, J. E., Bohlool, B.B. and Singleton, P.W. (1991). Subgroups of the cowpea miscellany: Symbiotic specificity within *Bradyrhizobium* spp. for *Vigna unguiculata*, *Phaseolus lunatus*, *Arachis hypogaea*, and *Macroptilium atropurpureumt*. *Applied and Environmental Microbiology* **57**:1540-1545.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature* **418**:671-677.

Toker, C., Canci, H. and Ceylan, F.O. (2006). Estimation of outcrossing rate in chickpea (*Cicer arietinum* L.) sown in autumn. *Euphytica* **151**:201–205.

Toker, C., Canci, H. and Yildirim, T. (2007). Evaluation of perennial wild *Cicer* species for drought resistance. *Genetic Resources and Crop Evolution* (DOI 10.1007/s10722-006-9197-y).

Tomooka, N., Kashiwaba, K., Vaugham, D.A., Ishimoto, M. and Egawa, Y. (2000). The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus *Ceratotropis*. *Euphytica* **115**:27-41.

Tomooka, N., Lairungreang, C., Nakeeraks, P., Egawa, Y. and Thauarasooks, C. (1992). Development of bruchid resistant mungbean line using wild mungbean germplasm in Thailand. *Plant Breeding* **109**:60-66.

Tsai, S.M., Nodari, R.O., Moon, D.H., Camargo, L.E.A., Vencovsky, R. and Gepts, P. (1998). QTL mapping for nodule number and common bacterial blight in *Phaseolus vulgaris* L. *Plant and Soil* **204**:135–145.

Tsegaye, T. (1992). Vertisols of the Centeral Highlands, Ethiopia – characterization, classification and evaluation of the phosphorus status. M.Sc Thesis, Alemaya University of Agriculture, Ethiopia.

Twomlow, S. (2004). Increasing the role of legumes in smallholder farming systems – the future challenge. In: Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture, pp 29-46, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

U.S. Environmental Protection Agency. (1996). Environmental indicators of water quality in the United States. EPA 841–R–96–002.

Upadhyaya, H.D., Dwivedi, S.L., Baum, M., Varshney, R.K., Udupa, S.M., Gowda, C.L.L., Hoisington, D. and Singh, S. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biology* **8**:doi:10.1186/1471-2229-8-106.

Upadhyaya, H.D., Dwivedi, S.L., Gowda, C.L.L. and Singh, S. (2007). Identification of diverse germplasm lines for agronomic traits in a chickpea (*Cicer arietinum* L.) core collection for use in crop improvement. *Field Crops Research* **100**:320–326.

Upadhyaya, H.D., Furman, B.J., Dwivedi, S.L., Udupa, S.M., Gowda, S.L.L., Baum, M., Crouch, J.H., Buhariwalla, H.K. and Singh, S. (2006). Development of a composite collection for mining germplasm possessing allelic variation for beneficial traits in chickpea. *Plant Genetic Resources* **4**:3–19.

van der Maesen, L.J.G. (1987). Origin, history and taxonomy of chickpea. In: *The Chickpea*, pp 11-34, (Saxena, M.C. and Singh, K.B., eds.). C.A.B International, Wallingford, UK.

van Oosterom, E.J., Kleijn, D., Ceccarelli, S. and Nachit, M.M. (1993). Genotype by environment interaction of barley in the Mediterranean Region. *Crop Science* **33**:669–674.

Vance, C.P., Egli, M.A., Griffith, S. and MandMiller, S. S. (1988). Plant regulated aspects of nodulation and N2 fixation. *Plant, Cell and Environment* **11**:413–427.

Velayutham, A. and Kalpana, R. (2004). Phosphorus and seed rate requirement for bold seeded Kabuli chickpea (*Cicer arietinum* L.). *Madras Agricultural Journal* **91**:315-317.

Veneklaas, E.J., Stevens, J., Cawthray, G.R., Turner, S., Grigg, A.M. and Lambers, H. (2003). Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. *Plant and Soil* **248**:187–197.

Vesterager, J.M. Nielsen, N.E. and Høgh-Jensen, H. (2006). Variation in Phosphorus Uptake and Use Efficiencies Between Pigeonpea Genotypes and Cowpea. *Journal of Plant Nutrition* **29**:1869–1888.

Vesterager, J.M. Nielsen, N.E. and Høgh-Jensen, H. (2007). Nitrogen budgets in crop sequences with or without phosphorus-fertilised cowpea in the maize-based cropping systems of semi-arid eastern Africa. *African Journal of Agricultural Research* **2**:261-268.

Waddington, S.R., Karigwindi, J. and Chifamba, J. (2007). The sustainability of a groundnut plus maize rotation over 12 years on smallholder farms in the sub-humid zone of Zimbabwe. *African Journal of Agricultural Research* **2**: 342-348.

Wallace, D.H. and Yan, W. (1998). *Plant Breeding and Whole-System Crop Physiology*. University Press, Cambridge, UK.

Walley, F. L., Kyei-Boahen, S., Hnatowich, G. and Stevenson, C. (2005). Nitrogen and phosphorus fertility management for desi and kabuli chickpea. *Canadian Journal of Plant Science* **85**:73–79.

Wang, L., Liao, H., Yan, X., Zhuang, B. and Dong, Y. (2004). Genetic variability for root hair traits as related to phosphorus status in Soybean. *Plant and Soil* **261**:77–84.

Wani, P.A., Khan, M.S. and Zaidi, A. (2007). Impact of heavy metal toxicity on plant growth, symbiosis, seed yield and nitrogen and metal uptake in chickpea. *Australian Journal of Experimental Agriculture* **47**:712–720.

Warburton, M. and Crossa, J. (2002). Data analysis in the CIMMYT Applied Biotechnology Center. Second Edition. Mexico, D.F.: CIMMYT (http://apps.cimmyt.org/english/docs/manual/protocols/dataAnalysis.pdf.

Ward, J.H. (1963). Hierarchical grouping to optimize an objective function. *Journal of American Statistical Association* **58**:236-244.

Welsh, J.R. (1981). *Fundamentals of Plant Genetics and Breeding*. John Wiley & Sons, New York.

White, P.J., Broadley, M.R., Greenwood, D.J. and Hammond, J.P. (2005). Genetic modifications to improve phosphorus acquisition by roots. Proceedings 568. Paper presented to the International Fertiliser Society at a Conference in Cambridge, on 15th December 2005, The International Fertiliser Society, UK.

Willems, A. (2006). The taxonomy of rhizobia: An overview. *Plant and Soil* **287**:3–14.

Williams, L.B. and Schultz, J.J. (1990). A fertilizer supply strategy for Sub-Saharan Africa. In: Proceedings of Workshop Feeding the Future: Agricultural Development Strategies for Africa. 1989, Sasakawa Africa Assoc., Mexico City. pp. 125-153.

Williams, P.C. and Singh, U. (1987). Nutritional quality and the evaluation of quality in breeding programs. In: *The Chickpea*, pp. 329-356, C.A.B. International, Wallingford, Oxon, OX10 8DE, UK.

Winter, P., Benko-Iseppon, A.M., Hüttel, B., Ratnaparkhe, M., Tullu, A., Sonnante, G., Pfaf, T., Tekeoglu, M., Santra, D., Sant, V.J., Rajesh, P.N., Kahl, G. and Muehlbauer, F.J. (2000). A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. Arietinum* x *C. Reticulatum* cross: localization of resistance genes for fusarium wilt races 4 and 5. *Theoretical and Applied Genetetics* **101**:1155-1163.

Winter, P., Pfaf, T. and Udupa, S.M. (1999). Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Molecular Genomics and Genetics* **262**:90–101.

Winter, P., Staginnus, C., Huettel, B., Jungmann, R., Pfaff, T., Benko-Iseppon, A-M., Rakshit, S., Pinkert, S., Baum, M. and Kahl, G. (2004). Architecture and maps of the chickpea genome: a basis for understanding plant-rhizobium interactions. *Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture*, pp 201-222, (Serraj, R., ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Witty, J.F., Rennie, R.J. and Atkins, C.A. (1988). $^{15}N$ addition methods for assessing $N_2$ fixation in legumes. *World Crops: Cool Season Food Legumes*, pp. 715-730, (Summerfield, R.J., ed). Kluwer Academic Publishers, Dordrecht.

Wolde-meskel, E. (2007). Genetic diversity of rhizobia in Ethiopian soils: Their potential to enhance biological nitrogen fixation (BNF) and soil fertility for sustainable agriculture. *Ethiopian Journal of Biological Sciences* **6**:77-95.

Wolde-meskel, E., Terefework, Z., Frostegard, A. and Lindstrom, K. (2005). Genetic diversity and phylogeny of Rhizobia isolated from agroforestry legume species in Southern Ethiopia. *International Journal of Systematic and Evolutionary Microbiology* **55**:1439-1452.

Wolde-meskel, E., Terefework, Z., Lindstrom, K. and Frostegard, A. (2004). Metabolic and genomic diversity of Rhizobia isolated from field standing native and exotic woody legumes in Southern Ethiopia. *Systematic and Applied Microbiology* **27**:603-611.

Wolfe, M.S., Baresel, J.P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Østergård H. and van Bueren, E.T.L. (2008). Developments in breeding cereals for organic agriculture. *Euphytica* **163**:323–346.

Workeye, F. (2002). Morphological and biochemical diversity analysis in chickpea (*Cicer arietinum*) landraces of Ethiopia. M.Sc Thesis, School of Graduate Studies, Addis Ababa University.

World Phosphate Institute. (2006). Annual Report. Casablanca, Morocco.

Wricke,G. and Weber, W.E. (1986). *Quantitative Genetics and Selection in Plant Breeding*. Walter de Gruter, Berlin.

Yadav, S.S., Kumar, J., Yadav, S.K., Singh, S., Yadav, V.S., Turner, N.C. and Redden, R. (2006). Evaluation of *Helicoverpa* and drought resistance in Desi and Kabuli chickpea. *Plant Genetic Resources* **4**:198–203.

Yadeta, K. (2004). On farm analysis of improved chickpea production technologies in East Shoa, Ethiopia. In: *Sebil,* pp. 216-223, Proceedings of the 10th Annual Conference of the Crop Science Society of Ethiopia. 19-21, June 2001, Addis Ababa, Ethiopia.

Yamada, T., Ikeda, M., Kobayashi, M. and Hattori, K. (2003). Cloning and expression of a cDNA encoding larval α-amylase of adzuki bean weevil, *Callosobruchus chinensis*. *Journal of Insect Biotechnology and Sericology* **72**:139-148.

Yau, S.K., G. Ortiz-Ferrara and J.P. Srivastava. (1991). Classification of diverse bread wheat growing environments based on differential yield responses. *Crop Science* **31**: 571–576.

Yücel, D.O., Anlarsal, A.E. and Yücel, C. (2006). Genetic variability, correlation and path analysis of yield and yield components in chickpea (*Cicer arietinum* L.). *Turkey Journal of Agriculture and Forestry* **30**:183-188.

Yusuf, A.A., Iwuafor, E. N. O., Abaidoo, R. C., Olufajo, O. O. and Sanginga, N. (2009). Grain legume rotation benefits to maize in the northern Guinea savannah of Nigeria: fixed-nitrogen versus other rotation effects. *Nutrient Cycling in Agroecosystems* **84**:129–139.

Yusuf, A.A., Iwuafor, E.N.O., Olufajo, O.O., Abaidoo, R. and Sanginga, N. (2007). Residual benefits of soybean genotypes and natural fallow to subsequent maize in the northern Guinea savanna of Nigeria. In: *Demand-Driven Technologies for Sustainable Maize Production in West and Central Africa*, pp. 138-154, (Badu-Apraku, B., Fakorede, M.A.B., Lum, A.F., Menkir, A. and Ouedraogo, M., eds). Proceedings of the 5th Biennial Regional Maize Workshop, IITA-Cotonou, Bénin, 3–6 May, 2005. WECAMAN/IITA, Ibadan, Nigeria.

Zaidi, A., Khan, M.S. and Amil, M. (2003). Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *European Journal of Agronomy* **19**:15-21.

Zapata, F. (1990). Field experimentation in isotope-aided studies. In: *Use of Nuclear Techniques in Soil-Plant Relationships*, pp. 61-127, (Hardarson, G., ed), Training course series No 2, IAEA, Vienna.

Zapata, F. and C. Hera. (1995). Enhancing nutrient management through use of isotope technique. In: *Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation*, pp. 83-105, Proceedings of a Symposium, 17-21 Oct. 1994, IAEA, Vienna.

Zhang, D., Liu, C., Cheng, H., Kan, G., Cui, S., Meng, Q., Gai, J. and Yu, D. (2010). Quantitative trait loci associated with soybean tolerance to low phosphorus stress based on flower and pod abscission. *Plant Breeding* **129**:243-249.

Zhu, J. (1996). Analysis methods for seed models with genotype x environment interactions. *Acta Genetica Sinica* **23**:56-68.

Appendix 1. Estimated probabilistic memberships of chickpea populations of individual chickpea genotypes from different geographic origins over the five clusters

| Origin | | Distribution over inferred clusters | | | | |
|---|---|---|---|---|---|---|
| | Entries | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ |
| Arsi | Acc. No. 231327 | 0.550 | 0.410 | 0.009 | 0.008 | 0.024 |
| | Acc. No. 231328 | 0.377 | 0.588 | 0.008 | 0.008 | 0.019 |
| | Acc. No. 209093 | 0.986 | 0.002 | 0.003 | 0.002 | 0.007 |
| | Acc. No. 208829 | 0.987 | 0.004 | 0.004 | 0.004 | 0.001 |
| | Acc. No. 209094 | 0.875 | 0.005 | 0.015 | 0.098 | 0.006 |
| | Acc. No. 209092 | 0.995 | 0.002 | 0.001 | 0.001 | 0.001 |
| | Acc. No. 209096 | 0.994 | 0.002 | 0.001 | 0.001 | 0.001 |
| | Acc. No. 209097 | 0.995 | 0.001 | 0.001 | 0.001 | 0.001 |
| | Acc. No. 209098 | 0.995 | 0.002 | 0.001 | 0.001 | 0.001 |
| | Acc. No. 41002 | 0.936 | 0.053 | 0.005 | 0.004 | 0.002 |
| | Acc. No. 207761 | 0.902 | 0.055 | 0.002 | 0.002 | 0.039 |
| | Acc. No. 207763 | 0.372 | 0.613 | 0.009 | 0.004 | 0.003 |
| | Acc. No. 207764 | 0.307 | 0.064 | 0.497 | 0.127 | 0.005 |
| E. Gojam | Acc. No. 41268 | 0.224 | 0.731 | 0.015 | 0.026 | 0.004 |
| | Acc. No. 41026 | 0.007 | 0.982 | 0.005 | 0.005 | 0.002 |
| | Acc. No. 41074 | 0.004 | 0.988 | 0.003 | 0.004 | 0.002 |
| | Acc. No. 41075 | 0.004 | 0.988 | 0.003 | 0.003 | 0.002 |
| | Acc. No. 41073 | 0.097 | 0.865 | 0.030 | 0.006 | 0.002 |
| | Acc. No. 41076 | 0.009 | 0.804 | 0.172 | 0.010 | 0.005 |
| | Acc. No. 41021 | 0.094 | 0.889 | 0.004 | 0.010 | 0.002 |
| | Acc. No. 41027 | 0.004 | 0.986 | 0.005 | 0.004 | 0.001 |
| | Acc. No. 41222 | 0.157 | 0.728 | 0.023 | 0.018 | 0.074 |
| | Acc. No. 207734 | 0.006 | 0.813 | 0.029 | 0.150 | 0.003 |
| | Acc. No. 41103 | 0.009 | 0.971 | 0.005 | 0.006 | 0.008 |
| | Acc. No. 41320 | 0.004 | 0.900 | 0.028 | 0.006 | 0.062 |
| | Acc. No. 41029 | 0.002 | 0.941 | 0.004 | 0.042 | 0.010 |
| W. Gojam | Acc. No. 41015 | 0.005 | 0.726 | 0.203 | 0.038 | 0.028 |
| | Acc. No. 41271 | 0.018 | 0.308 | 0.664 | 0.004 | 0.006 |
| | Acc. No. 41272 | 0.002 | 0.915 | 0.064 | 0.015 | 0.005 |
| | Acc. No. 41276 | 0.003 | 0.643 | 0.337 | 0.015 | 0.002 |
| | Acc. No. 207745 | 0.001 | 0.865 | 0.118 | 0.008 | 0.008 |
| | Acc. No. 41275 | 0.047 | 0.924 | 0.019 | 0.004 | 0.006 |
| | Acc. No. 41277 | 0.002 | 0.932 | 0.007 | 0.006 | 0.053 |
| | Acc. No. 207743 | 0.003 | 0.938 | 0.036 | 0.016 | 0.008 |
| | Acc. No. 207744 | 0.145 | 0.806 | 0.019 | 0.023 | 0.008 |
| | Acc. No. 41273 | 0.002 | 0.982 | 0.010 | 0.003 | 0.003 |
| | Acc. No. 41274 | 0.002 | 0.989 | 0.004 | 0.004 | 0.001 |
| | Acc. No. 207741 | 0.005 | 0.984 | 0.004 | 0.002 | 0.005 |
| | Acc. No. 207742 | 0.063 | 0.605 | 0.007 | 0.106 | 0.220 |
| N. Gonder | Acc. No. 41316 | 0.009 | 0.556 | 0.380 | 0.050 | 0.005 |
| | Acc. No. 41298 | 0.004 | 0.959 | 0.018 | 0.015 | 0.004 |
| | Acc. No. 41311 | 0.002 | 0.983 | 0.011 | 0.003 | 0.002 |
| | Acc. No. 41313 | 0.009 | 0.979 | 0.005 | 0.005 | 0.002 |
| | Acc. No. 41280 | 0.002 | 0.976 | 0.005 | 0.015 | 0.002 |
| | Acc. No. 41312 | 0.007 | 0.976 | 0.004 | 0.007 | 0.005 |
| | Acc. No. 41315 | 0.002 | 0.888 | 0.020 | 0.053 | 0.037 |
| | Acc. No. 41308 | 0.002 | 0.975 | 0.010 | 0.011 | 0.002 |
| | Acc. No. 41299 | 0.006 | 0.971 | 0.016 | 0.004 | 0.002 |
| | Acc. No. 41046 | 0.007 | 0.980 | 0.006 | 0.004 | 0.003 |
| | Acc. No. 41047 | 0.013 | 0.972 | 0.010 | 0.003 | 0.002 |
| | Acc. No. 41304 | 0.020 | 0.767 | 0.127 | 0.004 | 0.081 |
| | Acc. No. 41303 | 0.143 | 0.732 | 0.019 | 0.008 | 0.098 |
| S. Gonder | Acc. No. 41295 | 0.003 | 0.028 | 0.732 | 0.234 | 0.003 |
| | Acc. No. 41296 | 0.006 | 0.700 | 0.023 | 0.040 | 0.231 |
| | Acc. No. 41289 | 0.006 | 0.370 | 0.500 | 0.007 | 0.117 |
| | Acc. No. 41290 | 0.011 | 0.697 | 0.284 | 0.005 | 0.003 |
| | Acc. No. 41284 | 0.002 | 0.976 | 0.007 | 0.007 | 0.007 |
| | Acc. No. 41291 | 0.005 | 0.812 | 0.090 | 0.004 | 0.088 |
| | Acc. No. 41297 | 0.007 | 0.850 | 0.137 | 0.004 | 0.002 |
| | Acc. No. 41293 | 0.100 | 0.183 | 0.569 | 0.120 | 0.029 |
| | Acc. No. 41019 | 0.038 | 0.623 | 0.060 | 0.011 | 0.268 |
| | Acc. No. 41048 | 0.060 | 0.703 | 0.024 | 0.134 | 0.079 |
| | Acc. No. 41049 | 0.114 | 0.098 | 0.564 | 0.221 | 0.002 |
| | Acc. No. 41053 | 0.298 | 0.074 | 0.601 | 0.014 | 0.012 |
| W. Haragie | Acc. No. 41054 | 0.198 | 0.013 | 0.678 | 0.100 | 0.011 |
| | Acc. No. 41052 | 0.238 | 0.228 | 0.520 | 0.012 | 0.002 |
| | Acc. No. 209082 | 0.003 | 0.715 | 0.211 | 0.007 | 0.064 |
| | Acc. No. 209083 | 0.020 | 0.777 | 0.017 | 0.044 | 0.143 |
| | Acc. No. 209084 | 0.003 | 0.103 | 0.847 | 0.044 | 0.003 |
| | Acc. No. 209091 | 0.296 | 0.021 | 0.666 | 0.011 | 0.005 |
| | Acc. No. 209087 | 0.004 | 0.251 | 0.620 | 0.123 | 0.003 |
| | Acc. No. 209088 | 0.190 | 0.312 | 0.491 | 0.005 | 0.003 |
| | Acc. No. 209089 | 0.004 | 0.061 | 0.883 | 0.051 | 0.002 |
| | Acc. No. 209090 | 0.025 | 0.016 | 0.946 | 0.006 | 0.007 |
| | Acc. No. 209081 | 0.131 | 0.009 | 0.846 | 0.010 | 0.004 |

Appendix 1. Continued

| Origin | Entries | Distribution over inferred clusters | | | | |
|---|---|---|---|---|---|---|
| | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ |
| E. Shewa | Acc. No. 41159 | 0.006 | 0.015 | 0.538 | 0.440 | 0.002 |
| | Acc. No. 41160 | 0.002 | 0.008 | 0.973 | 0.013 | 0.003 |
| | Acc. No. 41161 | 0.015 | 0.200 | 0.759 | 0.019 | 0.007 |
| | Acc. No. 207661 | 0.005 | 0.024 | 0.957 | 0.012 | 0.002 |
| | Acc. No. 207667 | 0.003 | 0.030 | 0.813 | 0.152 | 0.002 |
| | Acc. No. 207666 | 0.012 | 0.008 | 0.527 | 0.449 | 0.004 |
| | Acc. No. 41141 | 0.002 | 0.005 | 0.983 | 0.006 | 0.004 |
| | Acc. No. 207665 | 0.080 | 0.038 | 0.823 | 0.057 | 0.002 |
| | Acc. No. 41134 | 0.003 | 0.013 | 0.977 | 0.005 | 0.002 |
| | Acc. No. 41128 | 0.028 | 0.317 | 0.634 | 0.017 | 0.004 |
| | Acc. No. 41168 | 0.015 | 0.018 | 0.954 | 0.010 | 0.003 |
| | Acc. No. 41129 | 0.003 | 0.004 | 0.896 | 0.088 | 0.009 |
| | Acc. No. 41130 | 0.003 | 0.004 | 0.908 | 0.078 | 0.008 |
| N. Shewa | Acc. No. 41110 | 0.002 | 0.005 | 0.987 | 0.005 | 0.002 |
| | Acc. No. 207657 | 0.003 | 0.006 | 0.978 | 0.005 | 0.008 |
| | Acc. No. 41111 | 0.003 | 0.005 | 0.715 | 0.273 | 0.004 |
| | Acc. No. 41106 | 0.006 | 0.012 | 0.971 | 0.006 | 0.005 |
| | Acc. No. 207658 | 0.003 | 0.011 | 0.968 | 0.010 | 0.008 |
| | Acc. No. 41142 | 0.049 | 0.216 | 0.711 | 0.009 | 0.015 |
| | Acc. No. 41207 | 0.005 | 0.022 | 0.947 | 0.016 | 0.010 |
| | Acc. No. 41215 | 0.040 | 0.041 | 0.835 | 0.038 | 0.046 |
| | Acc. No. 41216 | 0.005 | 0.977 | 0.006 | 0.005 | 0.006 |
| | Acc. No. 41066 | 0.174 | 0.611 | 0.003 | 0.200 | 0.011 |
| | Acc. No. 41011 | 0.009 | 0.678 | 0.007 | 0.303 | 0.003 |
| | Acc. No. 41007 | 0.053 | 0.737 | 0.003 | 0.201 | 0.005 |
| | Acc. No. 41008 | 0.038 | 0.625 | 0.003 | 0.322 | 0.013 |
| W. Shewa | Acc. No. 41186 | 0.005 | 0.593 | 0.251 | 0.046 | 0.106 |
| | Acc. No. 209035 | 0.007 | 0.981 | 0.003 | 0.004 | 0.006 |
| | Acc. No. 41176 | 0.018 | 0.012 | 0.003 | 0.638 | 0.330 |
| | Acc. No. 41175 | 0.008 | 0.426 | 0.013 | 0.475 | 0.077 |
| | Acc. No. 41174 | 0.005 | 0.451 | 0.008 | 0.527 | 0.009 |
| | Acc. No. 209027 | 0.002 | 0.003 | 0.004 | 0.980 | 0.011 |
| | Acc. No. 41170 | 0.003 | 0.085 | 0.007 | 0.481 | 0.424 |
| | Acc. No. 41171 | 0.002 | 0.005 | 0.005 | 0.967 | 0.021 |
| | Acc. No. 41185 | 0.002 | 0.555 | 0.141 | 0.301 | 0.002 |
| | Acc. No. 209036 | 0.005 | 0.126 | 0.004 | 0.863 | 0.002 |
| | Acc. No. 41190 | 0.006 | 0.560 | 0.004 | 0.424 | 0.006 |
| | Acc. No. 41195 | 0.002 | 0.151 | 0.019 | 0.810 | 0.018 |
| | Acc. No. 41197 | 0.002 | 0.008 | 0.004 | 0.879 | 0.108 |
| Tigray | Acc. No. 207150 | 0.003 | 0.603 | 0.005 | 0.377 | 0.012 |
| | Acc. No. 207151 | 0.052 | 0.150 | 0.007 | 0.783 | 0.006 |
| | Acc. No. 207563 | 0.006 | 0.120 | 0.005 | 0.867 | 0.002 |
| | Acc. No. 207564 | 0.016 | 0.016 | 0.007 | 0.958 | 0.003 |
| | Acc. No. 207894 | 0.019 | 0.007 | 0.002 | 0.968 | 0.003 |
| | Acc. No. 207895 | 0.003 | 0.100 | 0.003 | 0.891 | 0.002 |
| | Acc. No. 213224 | 0.002 | 0.490 | 0.004 | 0.501 | 0.004 |
| | Acc. No. 219797 | 0.003 | 0.103 | 0.057 | 0.832 | 0.003 |
| | Acc. No. 219799 | 0.011 | 0.009 | 0.004 | 0.975 | 0.002 |
| | Acc. No. 219800 | 0.022 | 0.085 | 0.071 | 0.820 | 0.003 |
| | Acc. No. 219803 | 0.007 | 0.016 | 0.224 | 0.744 | 0.009 |
| | Acc. No. 221696 | 0.009 | 0.041 | 0.011 | 0.929 | 0.009 |
| S. Wello | Acc. No. 41114 | 0.002 | 0.006 | 0.005 | 0.986 | 0.002 |
| | Acc. No. 212589 | 0.113 | 0.011 | 0.011 | 0.855 | 0.009 |
| | Acc. No. 41113 | 0.108 | 0.006 | 0.005 | 0.880 | 0.002 |
| | Acc. No. 207659 | 0.002 | 0.006 | 0.003 | 0.987 | 0.002 |
| | Acc. No. 207660 | 0.001 | 0.007 | 0.244 | 0.726 | 0.022 |
| | Acc. No. 41115 | 0.004 | 0.006 | 0.390 | 0.594 | 0.006 |
| | Acc. No. 225878 | 0.003 | 0.007 | 0.022 | 0.965 | 0.002 |
| | Acc. No. 225873 | 0.004 | 0.015 | 0.039 | 0.939 | 0.002 |
| | Acc. No. 225874 | 0.005 | 0.016 | 0.020 | 0.956 | 0.002 |
| | Acc. No. 225877 | 0.129 | 0.006 | 0.027 | 0.825 | 0.013 |
| | Acc. No. 207645 | 0.098 | 0.005 | 0.027 | 0.857 | 0.012 |
| | Acc. No. 207646 | 0.211 | 0.006 | 0.008 | 0.768 | 0.007 |
| | Acc. No. 225876 | 0.007 | 0.019 | 0.434 | 0.536 | 0.004 |
| Improved | ICC 5003* | 0.111 | 0.029 | 0.036 | 0.117 | 0.707 |
| | ICC 4918 | 0.006 | 0.248 | 0.053 | 0.073 | 0.621 |
| | ICC 4948 | 0.131 | 0.010 | 0.013 | 0.061 | 0.785 |
| | ICC 4973 | 0.242 | 0.002 | 0.003 | 0.005 | 0.748 |
| | ICC 15996 | 0.023 | 0.004 | 0.004 | 0.003 | 0.967 |
| | Shasho (ICCV 93512) | 0.003 | 0.002 | 0.003 | 0.003 | 0.990 |
| | Arerti (FLIP 89-84C) | 0.011 | 0.005 | 0.005 | 0.014 | 0.964 |
| | Worku (DZ-10-16-2) | 0.009 | 0.124 | 0.011 | 0.029 | 0.827 |
| | Akaki (DZ-10-9-2) | 0.008 | 0.020 | 0.021 | 0.012 | 0.940 |
| | Ejere (FLIP-97-263C) | 0.002 | 0.006 | 0.008 | 0.007 | 0.976 |
| | Teji (FLI 97-266C) | 0.012 | 0.078 | 0.007 | 0.019 | 0.883 |
| | Habru (FLIP 88-42c) | 0.018 | 0.005 | 0.002 | 0.002 | 0.972 |
| | Natoli (ICCX-910112-6) | 0.004 | 0.008 | 0.023 | 0.113 | 0.853 |
| | ICC 19180 | 0.003 | 0.014 | 0.066 | 0.015 | 0.901 |
| | ICC 19181 | 0.050 | 0.062 | 0.056 | 0.015 | 0.817 |
| | PM 233 (155) | 0.064 | 0.013 | 0.004 | 0.008 | 0.913 |

Appendix 2. Average performances of the 155 chickpea genotypes for symbiotic nitrogen fixation and the component characters*

| Genotype** | NI | NN | NDW | SNC | SNF | GNC | GNF | BMNF | BNC | FNAE | GNY | SNY | BNY | NHI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.32ew | 6.75pu | 339hu | 1.21dw | 32.10bv | 3.71an | 39.89am | 36.81ap | 4.92br | 81.76ap | 2.20ah | 1.34ao | 3.54ai | 0.63ap |
| 2 | 0.47uw | 11.25eu | 172qu | 1.17ew | 31.49bv | 3.37ku | 32.93ju | 31.67hy | 4.54jv | 75.35lr | 2.13ai | 1.15do | 3.28am | 0.66ae |
| 3 | 1.60bw | 9.88hu | 432eu | 1.24cu | 35.15ar | 3.37ku | 32.66lu | 31.61hy | 4.61hv | 70.61qr | 2.09al | 1.31ao | 3.40al | 0.62ap |
| 4 | 0.39vw | 5.00tu | 183ou | 1.12gx | 27.83fx | 3.53bu | 35.49bu | 32.74dy | 4.65hv | 81.73ap | 1.74bq | 1.14do | 2.88bm | 0.61av |
| 5 | 0.67pw | 7.50mu | 216nu | 1.13gx | 28.78fx | 3.42gu | 33.48hu | 31.70hy | 4.55jv | 75.58jr | 1.97ap | 1.09fp | 3.06am | 0.65ah |
| 6 | 0.64qw | 8.25ku | 242lu | 1.23cu | 34.08at | 3.45du | 34.83cu | 32.96dy | 4.67fv | 77.43fr | 1.84ap | 1.22co | 3.06am | 0.61av |
| 7 | 0.74nw | 7.00ou | 222mu | 1.15gx | 31.16cv | 3.58bu | 37.28au | 34.99av | 4.73dv | 80.40aq | 1.85ap | 1.37ao | 3.21am | 0.58ez |
| 8 | 1.58bw | 14.00bt | 437eu | 1.05ny | 23.73lx | 3.40hu | 34.22eu | 31.42jy | 4.45kv | 83.53ao | 1.45lq | 0.83op | 2.27mn | 0.64am |
| 9 | 1.65av | 13.13ct | 568cu | 1.08kx | 25.19hx | 3.39ku | 34.11eu | 31.45iy | 4.46kv | 82.50ap | 1.54iq | 1.04ip | 2.58en | 0.60bx |
| 10 | 2.21ar | 15.75bt | 701am | 1.18ew | 30.49cv | 3.48du | 34.36eu | 32.17ey | 4.66gv | 75.41kr | 1.90ap | 1.06ip | 2.96am | 0.65ah |
| 11 | 1.35ew | 13.38ct | 380fu | 1.06my | 23.63lx | 3.47du | 35.25cu | 32.16ey | 4.53jv | 84.41am | 1.90ap | 1.16do | 3.05am | 0.62at |
| 12 | 3.00ad | 17.38bs | 737ak | 1.09jx | 26.43gx | 3.52cu | 36.42au | 33.66ay | 4.61hv | 82.65ap | 2.33ac | 1.32ao | 3.65af | 0.64ai |
| 13 | 1.66av | 12.63dt | 479eu | 1.15gx | 27.68fx | 3.47du | 35.80au | 32.72dy | 4.62hv | 82.99ap | 2.09al | 1.22co | 3.31am | 0.65ag |
| 14 | 2.46aj | 20.13bk | 569ct | 1.17ew | 30.79cv | 3.71ao | 39.81an | 36.46as | 4.88bt | 83.24ap | 2.23af | 1.30ao | 3.51aj | 0.64am |
| 15 | 2.37an | 16.75bt | 603ar | 1.57ab | 47.29ao | 3.84ag | 41.57ag | 38.46ag | 5.41ab | 76.56hr | 1.79bq | 1.77ac | 3.55ai | 0.50z |
| 16 | 1.26ew | 16.00bt | 366fu | 1.49ad | 46.05ac | 3.88ad | 42.48ad | 39.31ad | 5.37ac | 78.05er | 1.63eq | 1.51al | 3.14am | 0.52xz |
| 17 | 1.56cw | 22.38bf | 463eu | 1.46ae | 45.04ae | 3.84ag | 41.75af | 38.85ae | 5.30ae | 77.74fr | 1.79bq | 1.58aj | 3.37am | 0.53vz |
| 18 | 2.00av | 13.75bt | 592as | 1.33an | 37.40am | 3.69aq | 39.57ap | 36.56aq | 5.02bm | 79.87ar | 1.95ap | 1.44am | 3.39al | 0.59by |
| 19 | 0.84jw | 17.75br | 387fu | 1.30bq | 35.65ap | 3.56bu | 37.42au | 34.74av | 4.86bt | 79.74ar | 1.75bq | 1.38ao | 3.13am | 0.56iz |
| 20 | 2.32ao | 22.13bg | 552ct | 1.28cr | 36.57an | 3.96ab | 43.54a | 39.86ac | 5.24ag | 82.84ap | 1.90ap | 1.33ao | 3.23am | 0.59bx |
| 21 | 1.00hw | 16.13bt | 305hu | 1.06my | 23.20lx | 3.52cu | 36.00au | 32.81dy | 4.58iv | 83.89an | 2.00ap | 1.38ao | 3.38al | 0.62ap |
| 22 | 1.78av | 16.88bt | 447eu | 1.59a | 48.67a | 4.02a | 42.79ac | 40.20a | 5.61a | 75.09lr | 1.86ap | 1.65ah | 3.50aj | 0.53uz |
| 23 | 2.16at | 16.75bt | 537dt | 1.21dw | 31.13cv | 3.84ah | 42.05ae | 38.15ai | 5.04bk | 84.23am | 2.33ab | 1.71ad | 4.05a | 0.59by |
| 24 | 1.48cw | 14.88bt | 350hu | 1.50ac | 45.63ad | 3.66ar | 38.82ar | 36.41as | 5.16ai | 75.70ir | 1.87ap | 1.82ab | 3.69ad | 0.50z |
| 25 | 0.69ow | 13.13ct | 234lu | 1.45af | 42.11ag | 3.87af | 42.07ae | 38.54ag | 5.32ad | 79.82ar | 1.82ap | 1.67ag | 3.48aj | 0.55pz |
| 26 | 1.44cw | 20.38bj | 562ct | 1.37ak | 41.24ah | 3.95ac | 43.41ab | 40.11ab | 5.32ad | 80.37ar | 2.02ao | 1.42an | 3.44al | 0.59by |
| 27 | 3.03ac | 15.88bt | 625aq | 1.20ew | 31.30cv | 3.52cu | 36.78au | 34.22ay | 4.72ev | 80.66aq | 2.06an | 1.52ak | 3.58ai | 0.58dz |
| 28 | 2.07au | 18.25bq | 579bt | 1.20ew | 31.19cv | 3.37ku | 34.08eu | 31.94gy | 4.57iv | 79.21br | 1.57gq | 1.08hp | 2.65dm | 0.61av |
| 29 | 2.46aj | 21.00bh | 716al | 1.23cv | 34.21at | 3.47du | 34.96cu | 33.25cy | 4.69fv | 77.04gr | 2.07am | 1.49am | 3.56ai | 0.59by |
| 30 | 1.85av | 25.38b | 1035ab | 1.09jx | 25.63hx | 3.65as | 38.64ar | 34.96av | 4.74dv | 85.79al | 2.07am | 1.37ao | 3.44al | 0.62as |
| 31 | 1.07hw | 11.88du | 310hu | 1.17ew | 30.55cv | 3.62at | 37.69au | 34.88av | 4.79cv | 81.42aq | 2.12aj | 1.23co | 3.34am | 0.64am |
| 32 | 2.30ap | 19.50bm | 1005ac | 1.33an | 38.57al | 3.46du | 35.35cu | 33.49by | 4.79dv | 76.28hr | 1.97ap | 1.83a | 3.80ac | 0.54qz |
| 33 | 1.46cw | 23.63bd | 565ct | 0.99ry | 18.97rx | 3.39iu | 34.23eu | 30.66ny | 4.39ov | 88.10af | 1.71bq | 0.97kp | 2.67dm | 0.63ao |
| 34 | 2.37an | 24.88bc | 666ao | 1.07lx | 24.50kx | 3.51du | 36.52au | 33.35cy | 4.58iv | 84.38am | 2.06am | 1.33ao | 3.39al | 0.61au |
| 35 | 0.64qw | 19.63bl | 212nu | 1.15gx | 25.11hx | 3.42gu | 34.62du | 31.24jy | 4.56jv | 84.06an | 1.58fq | 1.11ep | 2.70cm | 0.62ap |
| 36 | 2.28aq | 19.25bn | 841af | 1.06my | 20.99nx | 3.44eu | 33.72fu | 31.30jy | 4.50jv | 80.69aq | 1.71bq | 1.24bo | 2.95am | 0.62at |
| 37 | 0.78lw | 23.63bd | 388fu | 1.03oy | 20.33ox | 3.51du | 36.23au | 32.53ey | 4.54jv | 86.44aj | 2.46a | 1.42an | 3.87ab | 0.64al |
| 38 | 1.55cw | 14.75bt | 547ct | 1.06my | 24.07lx | 3.46du | 35.07cu | 32.06fy | 4.52jv | 83.82an | 1.93ap | 1.08gp | 3.01am | 0.66ad |
| 39 | 0.63rw | 7.125ou | 169qu | 1.03oy | 23.28lx | 3.52cu | 36.68au | 33.54ay | 4.55jv | 84.69am | 2.29ad | 1.24bo | 3.53ai | 0.65ah |
| 40 | 1.50cw | 11.13eu | 589as | 1.15gx | 29.78dw | 3.76am | 40.81ak | 37.29an | 4.91bs | 84.36am | 2.22ag | 1.23co | 3.45ak | 0.64ai |
| 41 | 1.32ew | 12.13dt | 396eu | 1.15gx | 29.20ew | 3.66ar | 38.89ar | 35.56at | 4.81cu | 83.50ao | 2.09al | 1.33ao | 3.42al | 0.62au |
| 42 | 1.51cw | 15.00bt | 374fu | 1.29bq | 36.49ao | 3.61au | 37.70au | 35.11av | 4.90bt | 78.79cr | 1.88ap | 1.51al | 3.39al | 0.56hz |

Appendix 2. Continued

| Genotype | NI | NN | NDW | SNC | SNF | GNC | GNF | BMNF | BNC | FNAE | GNY | SNY | BNY | NHI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 | 2.05au | 21.13bh | 491dt | 1.08kx | 25.52hx | 3.57bu | 37.79at | 34.53aw | 4.65gv | 84.31am | 2.12aj | 1.20co | 3.32am | 0.63ao |
| 44 | 3.19ab | 15.50bt | 869ae | 1.23cu | 34.76as | 3.43fu | 34.71du | 33.05dy | 4.66gv | 76.97gr | 1.86ap | 1.34ao | 3.20am | 0.58ez |
| 45 | 1.83av | 11.13eu | 381fu | 1.29bq | 37.80am | 3.32mu | 32.90ku | 31.91gy | 4.61hv | 74.24mr | 1.85ap | 1.47am | 3.31am | 0.55mz |
| 46 | 2.48aj | 19.25bn | 510dt | 1.30bp | 36.18ap | 3.26pu | 31.14ru | 30.39py | 4.55jv | 73.13nr | 1.35pq | 1.22co | 2.57en | 0.54sz |
| 47 | 1.67av | 7.00ou | 279iu | 1.38aj | 40.99aj | 3.71ao | 39.41aq | 36.78ap | 5.08aj | 77.70fr | 1.71bq | 1.39ao | 3.09am | 0.56iz |
| 48 | 2.49ai | 16.88bt | 593as | 1.16gx | 28.92fx | 3.79al | 40.99aj | 37.14ao | 4.94bq | 85.26al | 1.47jq | 0.95kp | 2.42jn | 0.61av |
| 49 | 1.69av | 17.13bs | 450eu | 1.20ew | 31.55bv | 3.72am | 39.55ap | 36.25as | 4.91br | 83.25ap | 1.90ap | 1.24bo | 3.13am | 0.61av |
| 50 | 0.87iw | 16.63bt | 363fu | 1.18ew | 31.10cv | 3.47du | 35.45bu | 32.88dy | 4.66gv | 81.36aq | 1.85ap | 1.48am | 3.33am | 0.56kz |
| 51 | 1.93av | 13.88bt | 394eu | 1.41ag | 40.47ak | 3.55bu | 37.11au | 34.51ax | 4.95bp | 77.41fr | 1.54iq | 1.22co | 2.76cm | 0.55nz |
| 52 | 2.41am | 19.75bl | 530dt | 1.25ct | 32.72bu | 3.53bu | 36.49au | 33.96ay | 4.77dv | 79.74ar | 1.41nq | 1.13do | 2.54fn | 0.54rz |
| 53 | 1.95av | 11.38eu | 542ct | 1.14gx | 27.66fx | 3.51du | 36.61au | 33.53by | 4.65gv | 83.13ap | 1.98ap | 1.30ao | 3.28am | 0.62at |
| 54 | 3.24a | 17.13bs | 838ag | 1.14gx | 28.93fx | 3.40hu | 34.47du | 32.25ey | 4.54jv | 80.35ar | 1.54iq | 0.99jp | 2.53hn | 0.64ak |
| 55 | 2.45ak | 11.13eu | 604ar | 1.39ai | 39.10al | 3.62au | 37.99at | 34.99av | 5.00bn | 78.77dr | 1.98ap | 1.70ae | 3.68ae | 0.55oz |
| 56 | 2.82ae | 16.13bt | 1042a | 1.09jx | 25.02ix | 3.58bu | 37.73at | 34.19ay | 4.67gv | 85.24al | 1.83ap | 1.17do | 2.99am | 0.62at |
| 57 | 2.62ah | 15.63bt | 954ad | 1.18ew | 28.69fx | 3.42gu | 34.65du | 31.88gy | 4.59hv | 81.91ap | 2.03an | 1.67af | 3.70ad | 0.56jz |
| 58 | 2.20ar | 14.00bt | 741aj | 1.26cs | 35.58ap | 3.50du | 36.17au | 34.05ay | 4.75dv | 78.32dr | 1.76bq | 1.27ao | 3.02am | 0.59by |
| 59 | 2.17as | 13.25ct | 544ct | 1.13gx | 28.08fx | 3.49du | 36.27au | 33.26cy | 4.63hv | 82.82ap | 1.87ap | 1.16do | 3.03am | 0.63ap |
| 60 | 1.40cw | 8.38ju | 419eu | 1.19ew | 30.55cv | 3.62at | 38.31ar | 35.09av | 4.81cu | 82.80ap | 2.20ah | 1.42am | 3.62ah | 0.62at |
| 61 | 1.41cw | 8.88iu | 345hu | 1.12gx | 27.72fx | 3.68ar | 39.06ar | 35.75as | 4.80cu | 83.78an | 2.20ah | 1.34ao | 3.53ai | 0.63ap |
| 62 | 1.27ew | 15.88bt | 369fu | 1.26cr | 35.68ap | 3.65as | 38.88ar | 36.04as | 4.91br | 80.42aq | 1.76bq | 1.20co | 2.96am | 0.59by |
| 63 | 1.28ew | 11.38eu | 279iu | 1.36al | 40.94aj | 3.65as | 38.76ar | 36.53ar | 5.01bn | 77.19fr | 2.04an | 1.61ai | 3.64ag | 0.56iz |
| 64 | 1.93av | 14.00bt | 529dt | 1.16gx | 28.19fx | 3.42gu | 34.80cu | 31.92gy | 4.58iv | 82.49ap | 2.15ai | 1.37ao | 3.51aj | 0.60bx |
| 65 | 2.21ar | 12.13dt | 476eu | 1.21dw | 31.08cv | 3.55bu | 36.91au | 34.06ay | 4.76dv | 81.14aq | 1.77bq | 1.26ao | 3.04am | 0.59by |
| 66 | 1.22ew | 8.63ju | 336hu | 1.07lx | 23.24lx | 3.24ru | 31.39qu | 28.85uy | 4.31tv | 83.01ap | 1.93ap | 1.21co | 3.14am | 0.62as |
| 67 | 1.00hw | 6.25qu | 290hu | 1.13gx | 28.34fx | 3.32iu | 34.33eu | 32.09fy | 4.52jv | 80.50aq | 1.79bq | 1.02jp | 2.80bm | 0.63ao |
| 68 | 1.59bw | 15.13bt | 412eu | 1.38aj | 42.01ag | 3.37ku | 33.66gu | 32.83dy | 4.75dv | 72.68or | 1.72bq | 1.42an | 3.14am | 0.56lz |
| 69 | 1.17fw | 8.50ju | 320hu | 1.20dw | 32.55bu | 3.51du | 36.40au | 34.03ay | 4.71fv | 79.92ar | 2.03an | 1.27ao | 3.30am | 0.63ap |
| 70 | 1.03hw | 8.38ju | 311hu | 1.11hx | 26.35gx | 3.61au | 38.32ar | 34.78av | 4.72ev | 85.42al | 2.03an | 1.29ao | 3.32am | 0.61av |
| 71 | 0.88iw | 19.00bo | 245lu | 1.16fw | 30.17cv | 3.62au | 37.28au | 34.47ax | 4.78dv | 81.78ap | 1.94ap | 1.17do | 3.11am | 0.62ar |
| 72 | 1.71av | 13.13ct | 449eu | 1.16gx | 26.70fx | 3.46du | 35.63au | 32.44ey | 4.62hv | 83.34ao | 1.62fq | 1.05ip | 2.66dm | 0.60ax |
| 73 | 1.73av | 16.25bt | 449eu | 1.12gx | 26.52gx | 3.42gu | 34.58du | 31.98gy | 4.53jv | 81.76ap | 1.72bq | 1.08gp | 2.80bm | 0.62au |
| 74 | 2.37an | 14.88bt | 613ar | 1.16gx | 28.92fx | 3.67ar | 39.38aq | 35.75as | 4.83ct | 84.85am | 1.84ap | 1.22co | 3.05am | 0.61aw |
| 75 | 1.13gw | 9.75hu | 454eu | 1.14gx | 27.13fx | 3.38ju | 34.08eu | 31.29jy | 4.52jv | 82.46ap | 1.77bq | 1.13do | 2.90bm | 0.62as |
| 76 | 2.49ai | 20.88bi | 644aq | 1.19ew | 30.77cv | 3.41gu | 34.75cu | 32.17ey | 4.61hv | 81.36aq | 1.70bq | 1.12eo | 2.82bm | 0.61av |
| 77 | 0.95iw | 7.25nu | 281iu | 1.11hx | 26.28gx | 3.52cu | 36.76au | 33.46by | 4.63hv | 84.45am | 1.70bq | 1.29ao | 2.99am | 0.59bx |
| 78 | 1.34ew | 9.25hu | 332hu | 1.10jx | 25.63hx | 3.49du | 36.18au | 33.01dy | 4.59iv | 84.00an | 2.09al | 1.23co | 3.32am | 0.64an |
| 79 | 1.74av | 13.00ct | 368fu | 1.28cr | 33.91at | 3.59au | 38.01at | 34.81av | 4.87bt | 81.51ap | 1.85ap | 1.25ao | 3.10am | 0.60bx |
| 80 | 2.72ag | 15.38bt | 669an | 1.32ao | 38.94al | 3.58bu | 37.47au | 35.25av | 4.89bt | 77.87er | 1.75bq | 1.34ao | 3.09am | 0.56jz |
| 81 | 0.64qw | 8.00lu | 199nu | 1.17ew | 31.59bv | 3.54bu | 36.70au | 34.19ay | 4.71fv | 80.78aq | 1.91ap | 1.10fp | 3.01am | 0.63ap |
| 82 | 0.97iw | 9.50hu | 301hu | 1.34am | 38.88al | 3.57bu | 37.30au | 34.91av | 4.91bs | 78.01er | 1.90ap | 1.44am | 3.34am | 0.57fz |
| 83 | 0.71ow | 9.88hu | 335hu | 1.18ew | 32.00bv | 3.57bu | 37.73at | 35.15av | 4.75dv | 80.91aq | 2.03an | 1.41ao | 3.44al | 0.59bx |
| 84 | 1.98av | 14.75bt | 629aq | 1.18ew | 30.93cv | 3.52cu | 36.05au | 33.38cy | 4.70fv | 81.15aq | 1.85ap | 1.26ao | 3.11am | 0.60bx |
| 85 | 1.58bw | 17.25bs | 323hu | 1.11gx | 27.04fx | 3.45du | 35.01cu | 32.20ey | 4.57iv | 81.83ap | 1.89ap | 1.35ao | 3.24am | 0.59bx |

Appendix 2. Continued

| Genotype | NI | NN | NDW | SNC | SNF | GNC | GNF | BMNF | BNC | FNAE | GNY | SNY | BNY | NHI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 86 | 2.36an | 21.13bh | 596as | 1.27cr | 36.23ap | 3.64at | 38.53ar | 35.96as | 4.90bt | 79.53br | 1.92ap | 1.49am | 3.41al | 0.56hx |
| 87 | 1.54cw | 9.50hu | 361fu | 1.39ai | 41.25ah | 3.87ae | 42.04ae | 38.70af | 5.26af | 79.97ar | 1.68cq | 1.29ao | 2.98am | 0.57fz |
| 88 | 2.50ai | 14.13bt | 663ap | 1.25cs | 35.34aq | 3.61au | 38.30as | 35.74at | 4.86bt | 79.87ar | 1.51iq | 1.02ip | 2.54gn | 0.60bx |
| 89 | 1.33ew | 7.38nu | 439eu | 1.16fx | 31.44bv | 3.65ar | 39.10ap | 36.35as | 4.81cu | 81.64ap | 2.16ai | 1.26ao | 3.42al | 0.63ao |
| 90 | 1.08hw | 9.88hu | 423eu | 1.15gx | 29.91dw | 3.53bu | 35.19cu | 33.34cy | 4.68fv | 79.02cr | 1.97ap | 1.14do | 3.12am | 0.62aq |
| 91 | 0.80lw | 6.25qu | 203nu | 1.19ew | 32.16bv | 3.60au | 38.01at | 35.34av | 4.79dv | 81.08aq | 2.34ab | 1.41an | 3.75ad | 0.62ap |
| 92 | 1.05hw | 17.88br | 476eu | 1.26cs | 35.53ap | 3.56bu | 37.38au | 34.95av | 4.82cu | 79.69ar | 2.02ao | 1.35ao | 3.37am | 0.61av |
| 93 | 2.20ar | 9.38hu | 410eu | 1.12gx | 26.47gx | 3.43gu | 34.74du | 31.91gy | 4.55jv | 82.67ap | 2.12aj | 1.25ao | 3.37am | 0.64al |
| 94 | 1.84av | 15.25bt | 568ct | 1.18ew | 30.66cv | 3.51du | 36.15au | 33.78ay | 4.69fv | 80.42aq | 1.79bq | 1.13do | 2.92bm | 0.61av |
| 95 | 1.25ew | 7.13ou | 292hu | 1.24cu | 33.48at | 3.55bu | 37.24au | 34.55av | 4.79dv | 80.76aq | 1.71bq | 1.27ao | 2.98am | 0.59by |
| 96 | 1.53cw | 13.13ct | 501dt | 1.16fx | 30.57cv | 3.31nu | 32.47lu | 31.06jy | 4.47kv | 77.20fr | 2.10al | 1.44am | 3.54ai | 0.59by |
| 97 | 1.52cw | 11.25eu | 516dt | 1.25cs | 36.74an | 3.45du | 35.58au | 34.03ay | 4.70fv | 76.74hr | 1.64eq | 1.35ao | 2.99am | 0.55nz |
| 98 | 0.65qw | 6.63pu | 201nu | 1.20ew | 31.55bv | 3.46du | 35.58au | 33.17dy | 4.65gv | 80.15ar | 2.03an | 1.49am | 3.52aj | 0.59by |
| 99 | 1.30ew | 8.63ju | 363fu | 1.29bq | 37.90am | 3.71an | 39.68ao | 37.05ap | 5.01bn | 79.41br | 1.67dq | 1.25bo | 2.92bm | 0.58cz |
| 100 | 1.83av | 11.25eu | 395eu | 1.24cu | 34.69as | 3.61au | 38.22as | 35.66at | 4.84bt | 80.06ar | 1.47kq | 1.03ip | 2.49in | 0.58dz |
| 101 | 0.90iw | 15.63bt | 295hu | 1.25cs | 35.15ar | 3.64at | 38.60ar | 36.04as | 4.89bt | 79.84ar | 1.60fq | 1.25ao | 2.85bm | 0.57gz |
| 102 | 1.40dw | 9.63hu | 486dt | 1.07lx | 25.26hx | 3.47du | 35.90au | 33.05dy | 4.54jv | 83.37ao | 1.78bq | 1.02ip | 2.79bm | 0.63ap |
| 103 | 2.35an | 15.00bt | 766ah | 1.21dw | 32.39bu | 3.83ai | 41.28ai | 37.58al | 5.04bl | 83.85an | 1.97ap | 1.22co | 3.19am | 0.62at |
| 104 | 0.95iw | 11.13eu | 311hu | 1.09kx | 24.84jx | 3.35lu | 33.40hu | 30.85my | 4.43mv | 82.32ap | 1.76bq | 1.26ao | 3.01am | 0.60bx |
| 105 | 2.42al | 19.63bl | 744ai | 1.24cu | 34.46at | 3.56bu | 37.62au | 35.08av | 4.80cu | 80.01ar | 1.70bq | 1.04ip | 2.73cm | 0.63ap |
| 106 | 1.83av | 13.63bt | 462eu | 1.16fx | 30.92cv | 3.57bu | 37.65au | 34.94av | 4.73ev | 81.55ap | 1.88ap | 1.15do | 3.03am | 0.63ap |
| 107 | 2.79af | 14.38bt | 714al | 1.11hx | 26.69fx | 3.67ar | 39.33aq | 35.64at | 4.77dv | 85.77al | 1.92ap | 1.01jp | 2.94bm | 0.65ag |
| 108 | 2.27aq | 18.50bp | 745ai | 1.24cu | 33.24at | 3.60au | 38.07at | 35.06av | 4.84bt | 81.18aq | 1.97ap | 1.26ao | 3.23am | 0.61av |
| 109 | 1.69av | 16.00bt | 514dt | 1.17ew | 29.26ew | 3.80ak | 41.64ag | 37.64ak | 4.98bo | 85.39al | 1.97ap | 1.27ao | 3.23am | 0.62ap |
| 110 | 0.94iw | 11.50eu | 375fu | 1.23cv | 32.21bv | 3.44eu | 34.57au | 32.15ey | 4.67gv | 79.27br | 2.11ak | 1.69ae | 3.80ac | 0.57fz |
| 111 | 1.11gw | 11.00fu | 295hu | 1.01py | 20.66nx | 3.47du | 35.90au | 32.33ey | 4.48kv | 87.02ah | 1.93ap | 1.01jp | 2.94bm | 0.66ae |
| 112 | 0.98iw | 8.00lu | 276iu | 1.02oy | 22.03mx | 3.42gu | 34.57du | 31.56iy | 4.44lv | 84.59am | 1.86ap | 1.11ep | 2.97am | 0.63ap |
| 113 | 1.19ew | 12.25dt | 243lu | 1.10ix | 26.04gx | 3.69ap | 39.49ap | 35.60at | 4.79cv | 86.33ak | 1.76bq | 0.99jp | 2.75cm | 0.64ai |
| 114 | 0.77nw | 9.88hu | 236lu | 1.18ew | 32.20bv | 3.82aj | 41.68ag | 38.31ah | 5.00bn | 83.47ao | 1.43mq | 0.91mp | 2.34ln | 0.61au |
| 115 | 0.53tw | 10.13gu | 221mu | 0.95uy | 16.73ux | 3.41gu | 34.67du | 30.89ly | 4.36pv | 88.74ae | 2.04an | 1.01jp | 3.05am | 0.67ab |
| 116 | 0.41vw | 5.88ru | 114su | 1.13gx | 27.86fx | 3.44eu | 34.28eu | 31.70hy | 4.57iv | 82.71ap | 1.58fq | 1.08hp | 2.65dm | 0.58bz |
| 117 | 1.67av | 13.38ct | 444eu | 1.04ny | 23.03lx | 3.34mu | 33.26iu | 30.50oy | 4.38pv | 83.87an | 2.11ak | 1.33ao | 3.44al | 0.62at |
| 118 | 1.26ew | 6.75pu | 409eu | 1.09jx | 24.65kx | 3.41gu | 34.51du | 31.44iy | 4.50jv | 84.25am | 1.80bq | 1.12eo | 2.93bm | 0.62ar |
| 119 | 1.28ew | 10.63fu | 379fu | 1.00qy | 18.29tx | 3.24ru | 31.12ru | 27.84wy | 4.24uv | 87.10ah | 1.92ap | 1.10fp | 3.02am | 0.64ai |
| 120 | 1.00hw | 11.25eu | 359gu | 1.03oy | 21.03nx | 3.49du | 35.33cu | 32.00fy | 4.51jv | 85.24al | 1.80bq | 0.93kp | 2.74cm | 0.66ac |
| 121 | 0.76nw | 10.13gu | 180pu | 1.36al | 41.12ai | 3.46du | 34.54du | 33.54ay | 4.81cu | 72.33pr | 1.59fq | 1.43am | 3.03am | 0.53tz |
| 122 | 0.81kw | 11.38eu | 257ku | 1.16gx | 26.97fx | 3.18u | 29.66u | 27.84wy | 4.34rv | 79.35br | 1.71bq | 1.15do | 2.86bm | 0.59by |
| 123 | 0.37vw | 8.13ku | 134ru | 0.94wy | 14.02wx | 3.30nu | 31.64ou | 27.82xy | 4.23uv | 89.03ad | 1.94ap | 1.00jp | 2.94bm | 0.66ab |
| 124 | 0.91iw | 14.00bt | 314hu | 1.09jx | 25.14hx | 3.34mu | 32.38lu | 29.80sy | 4.43mv | 81.85ap | 2.05an | 1.18co | 3.22am | 0.64al |
| 125 | 0.65qw | 12.25dt | 297hu | 1.18ew | 29.56dw | 3.33mu | 32.11mu | 30.02qy | 4.51jv | 79.76ar | 1.92ap | 1.45am | 3.37am | 0.59by |
| 126 | 0.45uw | 8.38ju | 102tu | 1.10ix | 26.94fx | 3.21su | 30.25su | 29.02ty | 4.32sv | 76.47hr | 1.80bq | 1.11ep | 2.91bm | 0.61av |
| 127 | 0.65qw | 16.88bt | 206nu | 1.11hx | 26.98fx | 3.25qu | 31.57pu | 29.81ry | 4.36qv | 79.53br | 1.99ap | 1.21cp | 3.20am | 0.63ap |
| 128 | 1.28ew | 14.50bt | 318hu | 1.06my | 24.00lx | 3.37ku | 33.47hu | 30.98ky | 4.43mv | 82.18ap | 2.09al | 1.13do | 3.22am | 0.65af |
| 129 | 0.52tw | 7.75lu | 133ru | 0.97sy | 16.06vx | 3.27ou | 31.13ru | 27.67y | 4.24uv | 87.79ag | 1.89ap | 0.98kp | 2.87bm | 0.66ae |
| 130 | 0.60rw | 11.88du | 203nu | 0.92wy | 13.01x | 3.28nu | 31.76nu | 27.77y | 4.20v | 89.74ac | 1.80bq | 0.95kp | 2.75cm | 0.66ab |
| 131 | 1.46cw | 10.13gu | 351hu | 1.15gx | 27.34fx | 3.55bu | 37.29au | 33.74ay | 4.70fv | 84.79am | 1.60fq | 0.93lp | 2.53hn | 0.64al |

Appendix 2. Continued

| Genotype | NI | NN | NDW | SNC | SNF | GNC | GNF | BMNF | BNC | FNAE | GNY | SNY | BNY | NHI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 132 | 0.78mw | 10.38fu | 265iu | 1.01py | 18.69sx | 3.56bu | 36.98au | 32.55ey | 4.58iv | 90.15ab | 2.07am | 0.92lp | 2.99am | 0.69a |
| 133 | 0.98iw | 15.50bt | 322hu | 1.16fw | 31.10cv | 3.51du | 36.34au | 33.80ay | 4.67gv | 81.37aq | 1.62fq | 1.16do | 2.78bm | 0.59by |
| 134 | 0.99hw | 11.50eu | 285hu | 1.00qy | 20.10px | 3.48du | 36.11au | 32.58ey | 4.48kv | 86.57ai | 1.90ap | 1.02ip | 2.92bm | 0.65af |
| 135 | 1.02hw | 13.63bt | 305hu | 1.15gx | 29.36ew | 3.45du | 34.84cu | 32.53ey | 4.60hv | 80.45aq | 1.46kq | 0.96kp | 2.42jn | 0.60bx |
| 136 | 0.87iw | 13.88bt | 272iu | 1.13gx | 28.62fx | 3.59au | 37.87at | 34.80av | 4.72ev | 83.58ao | 1.84ap | 1.29ao | 3.12am | 0.59by |
| 137 | 0.60rw | 9.63hu | 242lu | 1.21dw | 31.66bv | 3.65as | 38.70ar | 35.44au | 4.85bt | 82.44ap | 1.99ap | 1.38ao | 3.37am | 0.60bx |
| 138 | 0.77nw | 8.63ju | 226mu | 1.14gx | 28.15fx | 3.45du | 35.34cu | 32.75dy | 4.59iv | 81.86ap | 2.06an | 1.29ao | 3.34am | 0.61au |
| 139 | 0.82kw | 6.88pu | 261ju | 1.15gx | 30.01cw | 3.60au | 38.17at | 35.25av | 4.75dv | 82.65ap | 1.79bq | 1.12eo | 2.90bm | 0.61av |
| 140 | 2.50ai | 39.25a | 711al | 1.39ai | 42.80af | 3.49du | 34.15eu | 33.53by | 4.88bt | 69.60r | 1.58fq | 1.50al | 3.08am | 0.51yz |
| 141 | 0.53sw | 8.00lu | 204nu | 1.14gx | 27.97fx | 3.20tu | 30.12tu | 28.65vy | 4.34rv | 78.47dr | 1.92ap | 1.26ao | 3.18am | 0.61av |
| 142 | 0.62rw | 5.63su | 211nu | 1.21dw | 33.96at | 3.37ku | 33.82fu | 32.36ey | 4.58iv | 76.92gr | 1.66dq | 1.47am | 3.14am | 0.53vz |
| 143 | 0.94iw | 23.13be | 279iu | 1.40ah | 42.85af | 3.78al | 40.31al | 37.71aj | 5.18ah | 77.23fr | 1.38oq | 1.29ao | 2.66dm | 0.52wz |
| 144 | 1.17fw | 12.88dt | 346hu | 1.34an | 36.10ap | 3.26pu | 31.60pu | 29.86qy | 4.60hv | 76.42hr | 1.56hq | 1.25ao | 2.81bm | 0.56lz |
| 145 | 0.70ow | 11.88du | 264iu | 1.30bp | 37.25am | 3.61au | 38.37ar | 35.74at | 4.91bs | 79.67ar | 1.17qr | 1.19co | 2.36kn | 0.50z |
| 146 | 0.77nw | 10.50fu | 241lu | 1.21dw | 33.38at | 3.55bu | 36.51au | 34.20ay | 4.77dv | 79.55br | 1.52iq | 1.31ao | 2.83bm | 0.53tz |
| 147 | 1.36ew | 19.75bl | 388fu | 1.20dw | 30.84cv | 3.82aj | 41.44ah | 37.49am | 5.02bm | 85.12am | 1.78bq | 1.24bo | 3.01am | 0.60bx |
| 148 | 0.91iw | 7.375nu | 391fu | 1.04ny | 22.00mx | 3.50du | 35.92au | 32.41ey | 4.54jv | 86.82ah | 1.57gq | 0.95kp | 2.52hn | 0.62ap |
| 149 | 0.62rw | 10.13gu | 293hu | 1.14gx | 28.37fx | 3.28nu | 31.93mu | 29.98qy | 4.42nv | 79.94ar | 1.86ap | 1.37ao | 3.23am | 0.57gz |
| 150 | 1.40cw | 11.38eu | 464eu | 1.11hx | 25.47hx | 3.29nu | 32.39lu | 29.86qy | 4.39ov | 82.46ap | 1.85ap | 1.29ao | 3.14am | 0.59by |
| 151 | 0.76nw | 10.38fu | 339hu | 1.00py | 19.30qx | 3.32mu | 33.02ju | 29.88qy | 4.32rv | 85.89a-l | 2.01ao | 1.39ao | 3.41al | 0.59bx |
| 152 | 1.31ew | 15.13bt | 549ct | 1.18ew | 31.66bv | 3.38ju | 34.26eu | 32.45ey | 4.56jv | 78.53d-r | 1.81ap | 1.33ao | 3.15am | 0.58dz |
| 153 | 0.68ow | 7.00ou | 365fu | 0.96ty | 16.21vx | 3.68aq | 39.33aq | 34.76av | 4.63hv | 90.61a | 2.28ae | 1.22co | 3.50aj | 0.64aj |
| 154 | 0.00w | 0.00u | 0.00u | 0.87xy | 0.00y | 2.44v | 0.00v | 0.00z | 3.31w | 0.00s | 0.81r | 0.83np | 1.64no | 0.49z |
| 155 | 0.00w | 0.00u | 0.00u | 0.79y | 0.00y | 2.426v | 0.00v | 0.00z | 3.217w | 0.00s | 0.75r | 0.55p | 1.29o | 0.58cz |
| Mean | 1.43 | 13.21 | 420 | 1.18 | 29.97 | 3.51 | 35.95 | 33.24 | 4.69 | 80.28 | 1.85 | 1.25 | 3.10 | 0.60 |

*See Table 11 for abbreviations of the characters; ** The serial stand for genotypes as given in Table 1

Appendix 3. Average performances of the 155 chickpea genotypes for agronomic characters*

| Genotype** | SDWF | DTF | DTM | SFD | NP | NS | SDWM | BMWT | HI | GPE | BPR | EGR | TSW | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 33cn | 55km | 112de | 57bm | 423ao | 507al | 114ao | 163bm | 40.27ae | 61.15as | 145bn | 103an | 103qz | 58.79ao |
| 2 | 42bn | 55km | 111de | 57bm | 381bv | 476ap | 100cp | 154bn | 42.88a | 66.44ah | 138bo | 113ae | 100rz | 63.62ae |
| 3 | 42bn | 55km | 114be | 59ai | 368bx | 333pz | 108bp | 159bn | 42.58ab | 67.70af | 140bo | 105am | 118iz | 61.84ai |
| 4 | 40bn | 55km | 114be | 59ai | 353dy | 364hz | 101cp | 137dn | 36.38at | 53.27az | 120do | 83dw | 122iv | 49.04cv |
| 5 | 44bl | 55km | 112de | 57bm | 345ey | 409dy | 98dp | 147bn | 42.07ac | 60.40at | 132bo | 102ap | 124hs | 58.04ap |
| 6 | 45ak | 56il | 113ce | 57bm | 324iz | 394ez | 100cp | 148bn | 36.95ar | 54.64az | 131bo | 93bv | 123it | 52.82bt |
| 7 | 34bn | 57hk | 112de | 55ho | 408ap | 381fz | 118ao | 168am | 32.80gw | 50.35dz | 150am | 93bv | 122iu | 51.26bu |
| 8 | 34bn | 55km | 112de | 57bm | 257vz | 279xz | 79np | 115ln | 36.74as | 43.98oz | 103ko | 75lw | 130gp | 42.68ox |
| 9 | 35bn | 57hk | 113ce | 56dn | 399as | 411cy | 94fp | 136dn | 33.50dw | 45.03mz | 121do | 81fw | 105pz | 45.23ix |
| 10 | 39bn | 55km | 112de | 57bm | 308nz | 296uz | 91fp | 139dn | 40.41ad | 56.92az | 124co | 96au | 141fi | 54.45as |
| 11 | 41bn | 55km | 112de | 57bm | 413ap | 424bx | 111bo | 169am | 34.14dw | 56.37az | 152am | 95au | 128gq | 54.15at |
| 12 | 32en | 55km | 113ce | 58ak | 436ao | 514ak | 119an | 178ah | 37.72an | 70.65a | 158ai | 114ac | 101qz | 66.05ab |
| 13 | 41bn | 55km | 112de | 57bm | 440am | 535af | 105bp | 157bn | 40.12af | 62.83ao | 141bo | 106al | 104pz | 60.24an |
| 14 | 34cn | 58gi | 112de | 54lp | 397at | 440aw | 110bo | 163bm | 38.04am | 55.17az | 146bn | 113ad | 117iz | 59.87an |
| 15 | 35bn | 62de | 112de | 50qs | 425ao | 449av | 113ao | 153bn | 30.22qx | 38.60yz | 137bo | 93bv | 89z | 46.10gx |
| 16 | 33cn | 58gi | 116bc | 58ak | 335gy | 394ez | 100cp | 136dn | 31.10mx | 41.27uz | 117go | 72qw | 94xz | 41.51px |
| 17 | 32en | 58gi | 117b | 59ai | 367bx | 459as | 107bp | 142cn | 33.39ew | 47.49iz | 121do | 79gw | 95vz | 46.76ex |
| 18 | 33cn | 57hk | 116bc | 60af | 358cx | 442aw | 105bp | 149bn | 37.06aq | 55.97az | 128co | 88cw | 108lz | 52.55bu |
| 19 | 52ab | 58gi | 116bc | 58ak | 374bw | 460as | 106bp | 149bn | 32.96gw | 49.32fz | 128co | 85cw | 98rz | 49.00cv |
| 20 | 31en | 60fg | 117b | 58ak | 380bv | 412cy | 104bp | 145cn | 33.32fw | 45.89kz | 123co | 83dw | 94wz | 47.60dw |
| 21 | 36bn | 57hk | 116bc | 60af | 414ap | 464as | 120am | 174al | 35.43cu | 58.79av | 148bn | 93bv | 105oz | 55.71as |
| 22 | 34bn | 60fg | 116bc | 56dn | 377bw | 426bx | 104bp | 142cn | 31.89jx | 43.80oz | 122do | 83dw | 82z | 46.38fx |
| 23 | 41bn | 57hk | 116bc | 60af | 444am | 468aq | 145ab | 199ac | 33.01gw | 65.15aj | 171ad | 103an | 109kz | 61.46aj |
| 24 | 35bn | 61ef | 117b | 56dn | 437an | 533af | 120an | 163bm | 29.99sx | 46.35jz | 139bo | 89bv | 88z | 50.22bu |
| 25 | 41bn | 59gh | 117b | 59ai | 390au | 446av | 109bp | 150bn | 31.91jx | 47.16iz | 127co | 80gw | 102qz | 46.89ex |
| 26 | 43bm | 60fg | 117b | 58ak | 403ar | 459as | 102cp | 145cn | 35.50cu | 49.61ez | 123co | 90bv | 107lz | 51.35bu |
| 27 | 31fn | 57hk | 114be | 57bm | 471ae | 532af | 127ah | 177ai | 32.86gw | 59.89au | 155aj | 102aq | 95uz | 58.48ao |
| 28 | 36bn | 55km | 113ce | 58ak | 332hy | 345nz | 87hp | 128fn | 36.68as | 49.39ez | 114go | 81fw | 120ix | 46.69ex |
| 29 | 37bn | 56hl | 115bd | 59ai | 398as | 456as | 120an | 174al | 33.98dw | 63.22an | 152am | 100aq | 116iz | 59.10ao |
| 30 | 42bn | 55km | 113ce | 58ak | 386av | 413cy | 124aj | 181ag | 34.53dw | 59.59au | 160ah | 98at | 117iz | 56.10ar |
| 31 | 38bn | 55km | 114be | 59ai | 447ak | 518al | 107bp | 155bn | 34.95dv | 62.49ap | 136bo | 99as | 99rz | 58.42ap |
| 32 | 49ae | 55km | 114be | 59ai | 426ao | 523ag | 136ae | 194ad | 31.39lx | 60.85at | 170ae | 96au | 99rz | 56.81aq |
| 33 | 46ai | 55km | 115bd | 60af | 368bx | 445aw | 98dp | 143cn | 33.74dw | 55.47az | 123co | 84dw | 107mz | 50.45bu |
| 34 | 46ai | 55km | 113ce | 58ak | 444al | 483ap | 126ai | 183ag | 34.77dw | 62.08ap | 162ag | 101aq | 109kz | 58.48ao |
| 35 | 45al | 55km | 113ce | 58ak | 340fy | 309rz | 87hp | 127fn | 36.23at | 48.12gz | 113go | 79gw | 108lz | 45.75gx |
| 36 | 40bn | 55km | 113ce | 58ak | 346ey | 359kz | 109bp | 160bn | 35.28cu | 52.28az | 142bo | 85cw | 117iz | 49.28bv |
| 37 | 48ae | 57hk | 113ce | 56dn | 497ab | 556ad | 138ad | 204ab | 36.36at | 69.71ac | 182ab | 125a | 125hr | 70.10a |
| 38 | 38bn | 55km | 114be | 59ai | 375bw | 418cx | 96ep | 143cn | 40.08af | 59.01av | 126co | 94bv | 115jz | 55.25as |
| 39 | 33cn | 55km | 114be | 59ai | 376bw | 502am | 119an | 178ah | 37.17ap | 69.14ad | 157ai | 109ag | 119iy | 64.22ad |
| 40 | 42bn | 54lm | 113ce | 59ai | 411ap | 449av | 108bp | 157bn | 37.68an | 65.41ai | 139bo | 100aq | 107lz | 59.13ao |
| 41 | 39bn | 57hk | 113ce | 56dn | 397as | 469aq | 113ao | 161bn | 35.02dv | 55.39az | 143bn | 102aq | 104pz | 56.26ar |
| 42 | 32en | 56hl | 113ce | 57bm | 396at | 465ar | 113ao | 159bn | 34.07dw | 51.86az | 141bo | 91bv | 125hr | 51.35bu |
| 43 | 36bn | 55km | 114be | 59ai | 456ah | 470aq | 114ao | 167am | 35.67ct | 63.92al | 148bn | 101aq | 111kz | 59.18ao |
| 44 | 36bn | 54lm | 113ce | 59ai | 378bw | 474aq | 109bp | 157bn | 34.93dv | 59.28av | 139bo | 91bv | 101qz | 53.78at |
| 45 | 36bn | 57hk | 115bd | 58ak | 424ao | 519ah | 116ao | 168am | 33.83dw | 58.29aw | 147bn | 96at | 99rz | 55.78as |
| 46 | 36bn | 55km | 113ce | 58ak | 313lz | 364hz | 92fp | 125fn | 30.38px | 42.76sz | 110ho | 70rw | 86z | 40.62qx |
| 47 | 27ln | 57hk | 114be | 57bm | 365cx | 373gz | 101cp | 140cn | 31.51kx | 47.03iz | 123do | 81fw | 106nz | 46.39fx |

Appendix 3. Continued

| Genotype | SDWF | DTF | DTM | SFD | NP | NS | SDWM | BMWT | HI | GPE | BPR | EGR | TSW | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 48 | 40bn | 55km | 114be | 59ai | 325iz | 295vz | 83jp | 118in | 32.82gw | 42.29tz | 103ko | 66uw | 119iy | 39.15sx |
| 49 | 35bn | 57hk | 113ce | 56dn | 374bx | 445av | 104bp | 149bn | 35.65cu | 52.79az | 132bo | 92bv | 104pz | 52.15bu |
| 50 | 47ah | 57hk | 114be | 57bm | 432ao | 469aq | 127ah | 177aj | 32.99gw | 56.65az | 154ak | 93bv | 106mz | 53.90at |
| 51 | 29hn | 55km | 112de | 57bm | 320jz | 388ez | 87hp | 126fn | 34.03dw | 44.50nz | 112go | 77kw | 97tz | 43.59mx |
| 52 | 36bn | 55km | 113ce | 58ak | 350dy | 373gz | 93fp | 129fn | 30.06rx | 40.64vz | 114go | 70sw | 94wz | 39.56rx |
| 53 | 31en | 55km | 113ce | 58ak | 437an | 480ap | 112bo | 160bn | 35.71ct | 59.72au | 142bo | 97as | 101qz | 56.37ar |
| 54 | 35bn | 56hl | 112de | 56dn | 371bx | 393ez | 90fp | 141cn | 34.03dw | 45.35lz | 127co | 82ew | 111kz | 45.52hx |
| 55 | 29hn | 56hl | 110e | 55ho | 515a | 505al | 119an | 169am | 35.68ct | 53.35az | 153al | 100ar | 93xz | 54.28at |
| 56 | 39bn | 57hk | 114be | 57bm | 413ap | 456at | 106bp | 152bn | 34.99dv | 53.47az | 133bo | 88cw | 102qz | 50.86bu |
| 57 | 43bm | 57hk | 112de | 55ho | 485ac | 517aj | 153a | 224a | 30.79nx | 59.03av | 199a | 109ah | 106mz | 59.96an |
| 58 | 42bn | 57hk | 112de | 55ho | 351dy | 424bx | 100cp | 143cn | 35.32cu | 48.99fz | 128co | 92bv | 101qz | 50.40bu |
| 59 | 40bn | 57hk | 112de | 55ho | 423ao | 481ap | 103cp | 148bn | 37.73an | 52.35az | 131bo | 98as | 100rz | 53.83at |
| 60 | 37bn | 57hk | 112de | 55ho | 449aj | 522ag | 118ao | 171al | 36.46as | 59.20av | 153al | 112ae | 103qz | 61.16al |
| 61 | 38bn | 57hk | 112de | 55ho | 344ey | 427bx | 116ao | 167bm | 35.81bt | 58.04ax | 150am | 107ak | 110kz | 58.71ao |
| 62 | 39bn | 56jm | 112de | 56dn | 347ey | 381fz | 95ep | 137dn | 34.84dv | 48.69gz | 123co | 87cw | 109kz | 48.43cw |
| 63 | 38bn | 57hk | 115bd | 56dn | 452ai | 490ao | 119an | 168am | 33.79dw | 57.19ay | 146bn | 100as | 102qz | 56.51aq |
| 64 | 43bn | 55km | 115bd | 60af | 380bv | 442aw | 121am | 178ah | 36.38at | 68.21ae | 155ak | 105al | 116iz | 62.59ag |
| 65 | 28jn | 55km | 115bd | 60af | 433ao | 486ap | 102cp | 148bn | 35.61cu | 54.00az | 129co | 85cw | 92yz | 49.91bv |
| 66 | 33cn | 55km | 115bd | 60af | 388au | 493an | 110bp | 158bn | 36.92as | 64.49ak | 138bo | 100as | 99rz | 59.03ao |
| 67 | 38bn | 55km | 115bd | 60af | 368bx | 412cy | 91fp | 133en | 37.15aq | 57.15ay | 116go | 89cv | 104pz | 52.38bu |
| 68 | 37bn | 55km | 115bd | 60af | 378bw | 407dy | 102cp | 145bn | 35.78bt | 56.13az | 127co | 86cw | 108lz | 51.31bu |
| 69 | 41bn | 55km | 115bd | 60af | 395au | 535af | 104bp | 152bn | 36.59as | 62.84ao | 132bo | 98as | 96uz | 57.83ap |
| 70 | 41bn | 55km | 115bd | 60af | 480ad | 525ag | 120an | 174al | 34.39dw | 61.78ar | 152al | 96au | 104pz | 56.65aq |
| 71 | 38bn | 55km | 115bd | 60af | 367bx | 468aq | 103cp | 149bn | 36.11at | 59.14av | 130bo | 92bv | 98rz | 54.31at |
| 72 | 34bn | 55km | 115bd | 60af | 343ey | 393ez | 90fp | 129fn | 35.83bt | 50.87cz | 112go | 79hw | 115iz | 46.69ex |
| 73 | 45al | 55km | 115bd | 60af | 380bv | 437bw | 97dp | 139dn | 36.19at | 54.25az | 121do | 85cw | 100rz | 50.14bu |
| 74 | 39bn | 55km | 115bd | 60af | 405aq | 490ao | 105bp | 149bn | 33.69dw | 55.02az | 130co | 85cw | 89z | 50.51bu |
| 75 | 45ak | 59gh | 115bd | 56dn | 377bw | 459as | 97dp | 142cn | 36.57as | 50.19ez | 124co | 93bv | 101qz | 51.91bu |
| 76 | 36bn | 55km | 115bd | 60af | 328hy | 454at | 93fp | 137dn | 36.31at | 54.61az | 119do | 82dw | 100rz | 49.37bv |
| 77 | 32en | 55km | 116bc | 61ac | 422ao | 472aq | 113ao | 169am | 32.35hx | 53.76az | 146bn | 80gw | 100rz | 48.30cw |
| 78 | 33cn | 55km | 116bc | 61ac | 387av | 503am | 110bp | 162bn | 37.29ap | 65.51ai | 140bo | 100as | 101qz | 59.70an |
| 79 | 40bn | 55km | 115bd | 60af | 386av | 456at | 98dp | 142cn | 36.55as | 55.53az | 124co | 87cw | 98rz | 51.21bu |
| 80 | 37bn | 55km | 115bd | 60af | 383bv | 417cx | 102cp | 144cn | 33.43ew | 53.06az | 125co | 83dw | 101qz | 48.93cv |
| 81 | 41bn | 56hl | 115bd | 59ai | 370bx | 427bx | 95ep | 141cn | 37.80am | 58.18ax | 124co | 94bv | 109kz | 54.68as |
| 82 | 42bn | 57hk | 116bc | 59ai | 423ao | 446av | 107bp | 151bn | 33.09gw | 56.41az | 130bo | 90bv | 104pz | 53.12bt |
| 83 | 47ag | 57hk | 115bd | 58ak | 422ao | 516aj | 119an | 170al | 33.71dw | 59.91au | 148bn | 98as | 101qz | 56.98aq |
| 84 | 40bn | 55km | 117b | 62a | 355cx | 421bx | 107bp | 154bn | 32.87gw | 58.86av | 132bo | 85cw | 98rz | 51.99bu |
| 85 | 34cn | 55km | 116bc | 61ab | 424ao | 523ag | 124ak | 176ak | 33.32fw | 59.09av | 153al | 93bv | 96tz | 54.72as |
| 86 | 30fn | 55km | 116bc | 61ab | 390au | 481ap | 115ao | 161bn | 32.43hx | 56.86az | 140bo | 87cw | 94wz | 51.87bu |
| 87 | 31en | 56hl | 116bc | 60af | 353dy | 415cx | 92fp | 129fn | 34.32dw | 47.36iz | 111go | 74nw | 92z | 44.19lx |
| 88 | 36bn | 57hk | 116bc | 60af | 309nz | 364hz | 83lp | 119hn | 33.39ew | 44.07oz | 103ko | 72pw | 100rz | 42.33ox |
| 89 | 47ah | 55km | 116bc | 61ab | 418ao | 499an | 109bp | 160bn | 36.79as | 66.68ag | 137bo | 96au | 104pz | 59.02ao |
| 90 | 47ah | 55km | 114be | 59aj | 425ao | 432bx | 102cp | 152bn | 38.00am | 58.12ax | 133bo | 92bv | 111kz | 54.15at |
| 91 | 28kn | 55km | 114be | 59aj | 424ao | 595a | 119an | 176ak | 37.76am | 70.35ab | 155ak | 110af | 97rz | 65.16ac |

Appendix 3. Continued

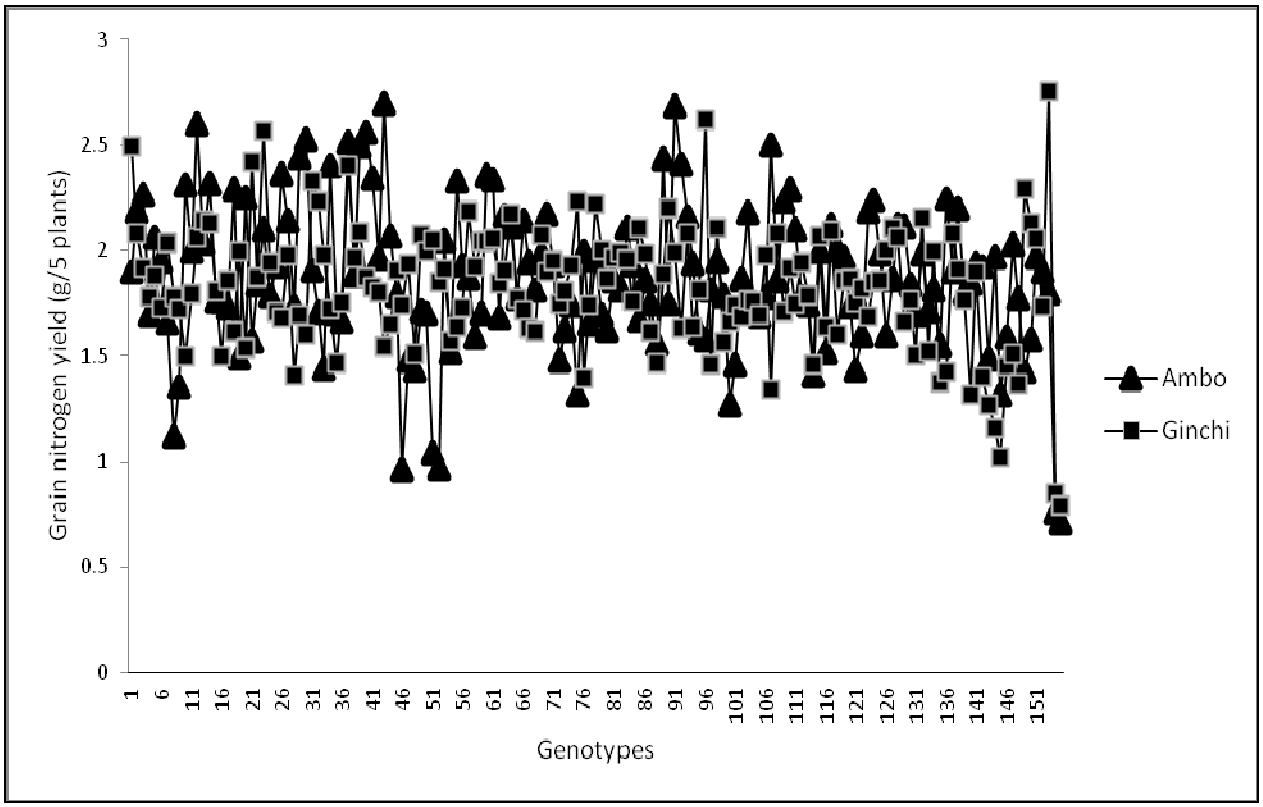
| Genotype | SDWF | DTF | DTM | SFD | NP | NS | SDWM | BMWT | HI | GPE | BPR | EGR | TSW | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 92 | 39bn | 55km | 114be | 59aj | 407ap | 403dy | 103bp | 153bn | 36.87as | 60.91at | 135bo | 96au | 132go | 56.41ar |
| 93 | 32dn | 54lm | 114be | 60af | 429ao | 575a | 108bp | 162bn | 38.00am | 67.57af | 142bo | 103ao | 93xz | 61.04al |
| 94 | 34cn | 58gj | 113ce | 55ho | 328hy | 380fz | 95ep | 137dn | 36.22at | 47.57iz | 122do | 92bv | 112kz | 50.10bu |
| 95 | 25n | 58gj | 114be | 56dn | 343ey | 399ez | 100cp | 141cn | 34.62dw | 46.12kz | 124co | 88cw | 107lz | 48.22cw |
| 96 | 50ad | 55km | 114be | 58ak | 486ac | 556ad | 124aj | 167am | 32.07ix | 65.47ai | 148bn | 108aj | 99rz | 62.33ah |
| 97 | 43bl | 55km | 113ce | 58ak | 382bv | 399ez | 106bp | 144cn | 35.81bt | 50.68dz | 128co | 83dw | 109kz | 47.99dw |
| 98 | 34bn | 56hl | 115bd | 59ai | 403ar | 449av | 122al | 174al | 34.97dv | 62.32ap | 151am | 101aq | 120ix | 58.94ao |
| 99 | 35bn | 58gj | 113ce | 55ho | 345ey | 381fz | 97dp | 134en | 32.63gx | 43.03rz | 118eo | 84cw | 104pz | 45.42hx |
| 100 | 39bn | 58gj | 114be | 56dn | 317kz | 348mz | 83kp | 116kn | 33.88dw | 38.58yz | 103ko | 73ow | 94wz | 40.27qx |
| 101 | 33cn | 57hk | 115bd | 58ak | 308nz | 361jz | 98dp | 133en | 33.79dw | 45.04mz | 116go | 76lw | 98rz | 44.00mx |
| 102 | 36bn | 55km | 113ce | 58ak | 413ap | 427bx | 97ep | 139dn | 35.26cu | 53.49az | 123co | 88cw | 100rz | 50.83bu |
| 103 | 35bn | 55km | 115bd | 60af | 410ap | 438bw | 102cp | 146bn | 35.92bt | 57.22ay | 127co | 86cw | 102qz | 51.65bu |
| 104 | 42bn | 57hk | 113ce | 56dn | 370bx | 297uz | 113ao | 158bn | 34.44dw | 50.79dz | 140bo | 94bv | 152fg | 52.06bu |
| 105 | 39bn | 55km | 113ce | 58ak | 313mz | 320qz | 83jp | 124gn | 38.48aj | 49.97ez | 110ho | 83dw | 139gj | 47.57dw |
| 106 | 38bn | 57hk | 113ce | 56dn | 346ey | 452au | 98dp | 143cn | 37.49ao | 52.45az | 126co | 94bv | 102qz | 52.65bu |
| 107 | 38bn | 55km | 113ce | 58ak | 316kz | 441aw | 92fp | 137dn | 37.54ao | 55.92az | 122do | 90bv | 102qz | 52.14bu |
| 108 | 37bn | 55km | 114be | 59ai | 397at | 427bx | 102cp | 149bn | 36.68as | 58.46av | 131bo | 94bv | 110kz | 54.81as |
| 109 | 32dn | 55km | 115bd | 60af | 389au | 445av | 103cp | 147bn | 35.41cu | 57.24ay | 128co | 86cw | 101qz | 51.60bu |
| 110 | 41bn | 58gj | 114be | 56dn | 445ak | 566ac | 130af | 184af | 33.91dw | 58.95av | 161ah | 108ai | 97sz | 60.44am |
| 111 | 34bn | 57hk | 115bd | 58ak | 411ap | 482ap | 101cp | 148bn | 37.56ao | 56.97az | 128co | 95bv | 98rz | 55.37as |
| 112 | 34bn | 55km | 113ce | 58ak | 416ao | 389ez | 109bp | 158bn | 36.06at | 56.97az | 140bo | 95bv | 113jz | 54.39at |
| 113 | 33cn | 55km | 113ce | 58ak | 339fy | 356lz | 89gp | 129fn | 38.94ai | 50.35dz | 115go | 84cw | 113jz | 48.06dw |
| 114 | 39bn | 55km | 113ce | 58ak | 305oz | 361lz | 77op | 110mn | 34.29dw | 39.42xz | 97no | 65vw | 93xz | 37.50tx |
| 115 | 50ac | 55km | 113ce | 58ak | 406aq | 465ar | 106bp | 158bn | 38.44ak | 61.94aq | 140bo | 102aq | 113jz | 58.83ao |
| 116 | 35bn | 55km | 113ce | 58ak | 355cx | 488ap | 98dp | 138dn | 33.25fw | 49.38ez | 122do | 80fw | 101qz | 46.58fx |
| 117 | 31en | 55km | 113ce | 58ak | 466af | 501an | 129ag | 183ag | 35.48cu | 66.56ah | 162ag | 109ag | 108lz | 63.10af |
| 118 | 36bn | 57hk | 113ce | 56dn | 415ao | 422bx | 100cp | 144cn | 36.46as | 51.63bz | 128co | 95bu | 106mz | 52.74bu |
| 119 | 31en | 55km | 114be | 59ai | 359cx | 521ag | 107bp | 163bm | 38.24al | 62.97an | 143bn | 100as | 112kz | 58.55ao |
| 120 | 43bm | 55km | 114be | 59ai | 360cx | 424bx | 91fp | 134en | 37.21ap | 55.41az | 118fo | 89bv | 115iz | 51.91bu |
| 121 | 28ln | 56hl | 115bd | 59ai | 385av | 413cy | 105bp | 142cn | 33.57dw | 52.57az | 124co | 80fw | 92yz | 45.93gx |
| 122 | 35bn | 55km | 113ce | 58ak | 441am | 462as | 101cp | 146bn | 36.14at | 56.25az | 130bo | 93bv | 105oz | 53.42at |
| 123 | 36bn | 55km | 115bd | 60af | 412ap | 467aq | 105bp | 157bn | 38.05al | 64.63ak | 136bo | 97at | 113jz | 58.07ap |
| 124 | 43bm | 55km | 115bd | 60af | 336fy | 442aw | 105bp | 154bn | 39.23ah | 66.70ag | 133bo | 100aq | 111kz | 60.21an |
| 125 | 44al | 56hl | 115bd | 59ai | 403ar | 442aw | 118ao | 171al | 34.81dv | 61.63ar | 149bn | 99as | 115jz | 57.78ap |
| 126 | 31en | 55km | 114be | 59ai | 408ap | 449av | 102cp | 147bn | 35.44cu | 59.54au | 129co | 95bu | 108lz | 55.72as |
| 127 | 32en | 55km | 113ce | 58ak | 464ag | 483ap | 110bp | 163bm | 38.77aj | 64.39ak | 145bn | 107ak | 110kz | 61.33ak |
| 128 | 38bn | 55km | 113ce | 58ak | 406aq | 490ao | 106bp | 158bn | 39.43ag | 65.52ai | 141bo | 109ah | 102qz | 62.52ag |
| 129 | 35bn | 55km | 115bd | 60af | 373bx | 461as | 100cp | 148bn | 38.56aj | 62.57ap | 128co | 94bv | 106mz | 56.44ar |
| 130 | 32en | 55km | 114be | 59ai | 411ap | 422bx | 100cp | 146bn | 38.86ai | 57.99ax | 128co | 93bv | 108lz | 54.34at |
| 131 | 35bn | 55km | 113ce | 58ak | 321iz | 385ez | 81mp | 118jn | 38.47aj | 47.71hz | 104jo | 79iw | 106mz | 45.27ix |
| 132 | 36bn | 55km | 115bd | 60af | 378bw | 409dy | 93fp | 141cn | 39.47ag | 62.52ap | 123do | 97at | 110kz | 57.59ap |
| 133 | 40bn | 55km | 113ce | 58ak | 344ey | 318qz | 100cp | 141cn | 33.89dw | 49.38ez | 125co | 81fw | 110kz | 46.65ex |
| 134 | 34cn | 55km | 114be | 59ai | 406aq | 450av | 102cp | 147bn | 37.71an | 58.70av | 130co | 94bv | 110kz | 54.85as |
| 135 | 41bn | 57hk | 113ce | 56dn | 391au | 334oz | 85ip | 121hn | 35.75bt | 42.60sz | 107io | 78jw | 116iz | 43.26nx |
| 136 | 34bn | 57hk | 114be | 57bm | 381bv | 359kz | 120an | 167bm | 31.90jx | 50.50dz | 147bn | 90bv | 132gn | 50.65bu |
| 137 | 39bn | 55km | 115bd | 60af | 371bx | 383fz | 111bo | 155bn | 35.44cu | 59.27av | 135bo | 93bv | 107lz | 54.78as |

Appendix 3. Continued

| Genotype | SDWF | DTF | DTM | SFD | NP | NS | SDWM | BMWT | HI | GPE | BPR | EGR | TSW | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 138 | 35bn | 55km | 113ce | 58ak | 370bx | 396ez | 112bo | 159bn | 37.09aq | 61.93aq | 141bo | 102ap | 121iw | 58.91ao |
| 139 | 35bn | 57hk | 113ce | 56dn | 339fy | 354lz | 99cp | 141cn | 35.37cu | 49.24fz | 125co | 90bv | 116iz | 50.26bu |
| 140 | 33cn | 68b | 117b | 49qs | 248wz | 226z | 107bp | 148bn | 30.70ox | 33.90z | 125co | 90bv | 184d | 44.61jx |
| 141 | 37bn | 55km | 115bd | 60af | 343ey | 318qz | 110bo | 163bm | 37.24ap | 65.89ai | 142bo | 100aq | 178de | 60.21an |
| 142 | 30gn | 66bc | 116bc | 50pr | 388au | 541ae | 126ah | 170al | 28.75ux | 39.60wz | 146bn | 99as | 95vz | 50.01v |
| 143 | 29hn | 68b | 116bc | 48rs | 266tz | 195z | 90fp | 118in | 28.15vx | 26.15ab | 101lo | 75mw | 136gk | 35.97ux |
| 144 | 35bn | 66c | 114be | 48rs | 271sz | 309rz | 98dp | 139cn | 35.01dv | 38.25z | 123do | 99as | 149fh | 48.84cv |
| 145 | 41bn | 67bc | 115bd | 48rs | 225yz | 174z | 93fp | 119hn | 27.94wx | 23.68ab | 104jo | 67tw | 162ef | 32.31x |
| 146 | 34bn | 66c | 114be | 48rs | 275qz | 289wz | 111bo | 149bn | 29.52tx | 33.51ab | 131bo | 92bv | 191d | 44.38kx |
| 147 | 32cn | 66c | 114be | 48rs | 273rz | 295vz | 101cp | 140cn | 33.12gw | 35.39z | 123do | 96au | 134gl | 46.41fx |
| 148 | 34bn | 63d | 114be | 51pr | 269sz | 307sz | 91fp | 133en | 33.90dw | 36.33z | 117go | 89bv | 136gk | 44.95ix |
| 149 | 48af | 54m | 114be | 61ac | 201z | 164z | 118ao | 169am | 33.42ew | 63.50am | 148bn | 92bv | 288a | 55.88as |
| 150 | 42bn | 54lm | 115bd | 61ac | 226yz | 195z | 117ao | 167bm | 32.42hx | 63.08an | 146bn | 95bv | 252b | 56.56aq |
| 151 | 43bn | 56hl | 113ce | 56dn | 272sz | 261yz | 140ac | 191ae | 32.17ix | 60.92at | 170af | 107aj | 236bc | 60.22an |
| 152 | 44bl | 66c | 117b | 52or | 265uz | 243z | 116ao | 169am | 33.09gw | 43.16qz | 144bn | 105al | 217c | 53.61at |
| 153 | 61a | 55km | 108f | 53mq | 274rz | 302tz | 128ah | 189ae | 32.92gw | 58.23aw | 175ac | 119ab | 215c | 61.44aj |
| 154 | 25mn | 82a | 128a | 46s | 283pz | 241z | 96ep | 128fn | 26.22x | 18.53b | 100mo | 74nw | 133gm | 33.47vx |
| 155 | 29in | 61ef | 114be | 53mq | 245xz | 249z | 69p | 103n | 33.60dw | 26.96ab | 91o | 59w | 114jz | 31.04x |
| Mean | 37 | 57 | 114 | 58 | 376 | 422 | 106 | 152 | 35.15 | 54.12 | 133 | 92 | 114 | 53 |

*See Table 11 for abbreviations of the characters; ** The serial stand for genotypes as given in Table 1

Appendix 4. G x L interaction (crossover) for nitrogen yield



Appendix 5. Average performances of the 155 chickpea genotypes for plant tissue (shoot, grain and biomass) phosphorus contents

| Genotype* | Shoot P content (g/5 plants) | | | Grain P content (g/5 plants) | | | Biomass P content (g/5 plants) | | |
|---|---|---|---|---|---|---|---|---|---|
| | With P | Without P | Combined | With P | Without P | Combined | With P | Without P | Combined |
| 1 | 0.1287a-o | 0.0615f-p | 0.09513f-v | 0.2350a-n | 0.1875a-i | 0.2112a-m | 0.9000a-o | 0.6200a-l | 0.7600a-m |
| 2 | 0.1152b-o | 0.0943c-p | 0.1047d-v | 0.2400a-m | 0.2350a-c | 0.2375a-g | 0.7950b-p | 0.7650a-l | 0.7800a-l |
| 3 | 0.1100c-o | 0.1618ab | 0.1359b-f | 0.2500a-k | 0.2275a-d | 0.2387a-g | 0.8375a-p | 0.9400a | 0.8888a-g |
| 4 | 0.1345a-o | 0.0903d-p | 0.1124c-u | 0.2400a-m | 0.1500c-i | 0.1950a-o | 0.9475a-n | 0.6000b-l | 0.7738a-m |
| 5 | 0.1013e-o | 0.0973c-o | 0.0993d-v | 0.2525a-k | 0.2250a-e | 0.2387a-g | 0.8275a-p | 0.7625a-l | 0.7950a-l |
| 6 | 0.1293a-o | 0.0640f-p | 0.0966e-v | 0.2675a-h | 0.1600b-i | 0.2138a-m | 0.8950a-o | 0.5300d-l | 0.7125a-m |
| 7 | 0.1037d-o | 0.1475a-c | 0.1256b-k | 0.2525a-k | 0.2175a-h | 0.2350a-h | 0.7350e-p | 0.9350ab | 0.8350a-j |
| 8 | 0.0760o | 0.0585g-p | 0.0673t-v | 0.2250a-p | 0.1275d-i | 0.1763d-o | 0.7400e-p | 0.4550kl | 0.5975i-m |
| 9 | 0.1450a-o | 0.0575g-p | 0.1013d-v | 0.2100b-p | 0.1200g-i | 0.1650e-o | 0.8825a-o | 0.4425kl | 0.6625b-m |
| 10 | 0.1105c-o | 0.0675f-p | 0.0890f-v | 0.2675a-h | 0.1975a-i | 0.2325a-h | 0.8750a-o | 0.6300a-l | 0.7525a-m |
| 11 | 0.1112c-o | 0.0643f-p | 0.0878g-v | 0.2900a-d | 0.1700b-i | 0.2300a-h | 1.0130a-i | 0.6025a-l | 0.8075a-l |
| 12 | 0.1678a-j | 0.0675f-p | 0.1176b-r | 0.2850a-e | 0.1725a-i | 0.2288a-i | 1.0580a-e | 0.5775d-l | 0.8175a-k |
| 13 | 0.1350a-o | 0.0650f-p | 0.1000d-v | 0.2750a-g | 0.1750a-i | 0.2250a-i | 0.9700a-m | 0.5775d-l | 0.7738a-m |
| 14 | 0.1092c-o | 0.0723f-p | 0.0908f-v | 0.2250a-p | 0.1650b-i | 0.1950a-o | 0.8025b-p | 0.5675d-l | 0.6850b-m |
| 15 | 0.1530a-o | 0.0928c-p | 0.1229b-n | 0.1900d-p | 0.1575b-i | 0.1737e-o | 0.8725a-o | 0.6725a-l | 0.7725a-m |
| 16 | 0.1390a-o | 0.0850d-p | 0.1120c-u | 0.1900d-p | 0.1225f-i | 0.1563g-o | 0.8450a-p | 0.5375d-l | 0.6913b-m |
| 17 | 0.1245a-o | 0.0733f-p | 0.0989d-v | 0.2300a-n | 0.0975i | 0.1637e-o | 0.9100a-o | 0.4675i-l | 0.6888b-m |
| 18 | 0.1620a-n | 0.0763d-p | 0.1191b-p | 0.2525a-k | 0.1475c-i | 0.2000a-o | 0.7050h-p | 0.5525d-l | 0.6288f-m |
| 19 | 0.1170b-o | 0.0880d-p | 0.1025d-v | 0.2300a-n | 0.1425c-i | 0.1863a-o | 0.9100a-o | 0.5700d-l | 0.7400a-m |
| 20 | 0.0993f-o | 0.0720f-p | 0.0856h-v | 0.2300a-n | 0.1300d-i | 0.1800c-o | 0.9000a-o | 0.5025e-l | 0.7013b-m |
| 21 | 0.1625a-m | 0.0735f-p | 0.1180b-q | 0.2725a-g | 0.1775a-i | 0.2250a-i | 0.9075a-o | 0.6200a-l | 0.7638a-m |
| 22 | 0.1493a-o | 0.1055c-l | 0.1274b-k | 0.1875e-p | 0.1725a-i | 0.1800c-o | 0.8600a-o | 0.7100a-l | 0.7850a-l |
| 23 | 0.1825a-e | 0.1093b-k | 0.1459b-d | 0.2525a-k | 0.2025a-h | 0.2275a-i | 0.9500a-n | 0.8575a-d | 0.9038a-f |
| 24 | 0.1528a-o | 0.1045c-l | 0.1286b-j | 0.2600a-j | 0.1750a-i | 0.2175a-l | 1.0920a-c | 0.7325a-l | 0.9125a-e |
| 25 | 0.1480a-o | 0.1112b-i | 0.1296b-i | 0.2050b-p | 0.1800a-i | 0.1925a-o | 0.9100a-o | 0.7375a-l | 0.8238a-k |
| 26 | 0.1130c-o | 0.1118b-i | 0.1124c-u | 0.2200a-p | 0.1650b-i | 0.1925a-o | 0.8200a-p | 0.6550a-l | 0.7375a-m |
| 27 | 0.1905a-c | 0.1332a-d | 0.1619ab | 0.2450a-l | 0.2000a-h | 0.2225a-j | 1.0700a-d | 0.8050a-i | 0.9375ab |
| 28 | 0.1262a-o | 0.0610f-p | 0.0936f-v | 0.1925c-p | 0.1425c-i | 0.1675e-o | 0.7675d-p | 0.4850h-l | 0.6263g-m |
| 29 | 0.1143b-o | 0.1090b-k | 0.1116c-u | 0.1950c-p | 0.2050a-h | 0.2000a-o | 0.7750c-p | 0.8050a-i | 0.7900a-l |
| 30 | 0.1503a-o | 0.0665f-p | 0.1084c-v | 0.2350a-n | 0.1725a-i | 0.2037a-o | 0.7350c-p | 0.5850d-l | 0.6600c-m |
| 31 | 0.1035d-o | 0.0518k-p | 0.0776l-v | 0.2600a-j | 0.1700b-i | 0.2150a-m | 0.9200a-o | 0.5675d-l | 0.7437a-m |
| 32 | 0.1955ab | 0.0580g-p | 0.1268b-k | 0.2650a-i | 0.1800a-i | 0.2225a-j | 1.0720a-d | 0.6125a-l | 0.8425a-j |
| 33 | 0.0830l-o | 0.0565h-p | 0.0698r-v | 0.1925c-p | 0.1500c-i | 0.1713e-o | 0.6900i-p | 0.5300d-l | 0.6100h-m |
| 34 | 0.1178b-o | 0.0795d-p | 0.0986d-v | 0.2000c-p | 0.2000a-h | 0.2000a-o | 0.8250a-p | 0.6700a-l | 0.7475a-m |
| 35 | 0.0918i-o | 0.0410op | 0.0664uv | 0.1950c-p | 0.1350c-i | 0.1650e-o | 0.7150f-p | 0.4450kl | 0.5800i-m |
| 36 | 0.1447a-o | 0.0453m-p | 0.0950f-v | 0.1875e-p | 0.1400c-i | 0.1637e-o | 0.6725j-p | 0.4450kl | 0.5587k-m |
| 37 | 0.1353a-o | 0.0840d-p | 0.1096c-v | 0.2825a-f | 0.2525ab | 0.2675a | 1.0330a-g | 0.8400a-e | 0.9362ab |
| 38 | 0.1055d-o | 0.0615f-p | 0.0835h-v | 0.2400a-m | 0.1650b-i | 0.2025a-o | 0.6275n-p | 0.5900d-l | 0.6087h-m |
| 39 | 0.1025d-o | 0.0845d-p | 0.0935f-v | 0.3175a | 0.2150a-h | 0.2662ab | 0.9725a-m | 0.7275a-l | 0.8500a-i |
| 40 | 0.1045d-o | 0.0638f-p | 0.0841h-v | 0.2275a-o | 0.1575b-i | 0.1925a-o | 0.8075b-p | 0.5450d-l | 0.6762b-m |
| 41 | 0.1410a-o | 0.0478l-p | 0.0941f-v | 0.2050b-p | 0.1650b-i | 0.1850a-o | 0.8575a-p | 0.5375d-l | 0.6975b-m |
| 42 | 0.1350a-o | 0.0480l-p | 0.0915f-v | 0.2150b-p | 0.1775a-i | 0.1963a-o | 0.7750c-p | 0.5750d-l | 0.6750b-m |
| 43 | 0.1055d-o | 0.0825d-p | 0.0940f-v | 0.2275a-o | 0.1725a-i | 0.2000a-o | 0.8200a-p | 0.6250a-l | 0.7225a-m |
| 44 | 0.0998f-o | 0.0873d-p | 0.0935f-v | 0.1650i-p | 0.2325a-c | 0.1988a-o | 0.6650l-p | 0.8000a-j | 0.7325a-m |
| 45 | 0.0795o | 0.0800d-p | 0.0798k-v | 0.2425a-m | 0.1725a-i | 0.2075a-o | 0.6825j-p | 0.6775a-l | 0.6800b-m |
| 46 | 0.1315a-o | 0.0665f-p | 0.0990d-v | 0.1575k-p | 0.1575b-i | 0.1575g-o | 0.7400e-p | 0.6025a-l | 0.6712b-m |
| 47 | 0.0953h-o | 0.0655f-p | 0.0804k-v | 0.1925c-p | 0.1250e-i | 0.1587g-o | 0.7675d-p | 0.4950f-l | 0.6313f-m |
| 48 | 0.0800no | 0.0538i-p | 0.0669t-v | 0.1275op | 0.1275d-i | 0.1275no | 0.5350p | 0.4625j-l | 0.4988m |
| 49 | 0.0993f-o | 0.0763d-p | 0.0878g-v | 0.1800g-p | 0.2000a-h | 0.1900a-o | 0.7100g-p | 0.6925a-l | 0.7013b-m |
| 50 | 0.1385a-o | 0.0925c-p | 0.1155c-s | 0.2175a-p | 0.2050a-h | 0.2112a-m | 0.8850a-o | 0.7800a-l | 0.8325a-k |
| 51 | 0.1047d-o | 0.0655f-p | 0.0851h-v | 0.2100b-p | 0.1300d-i | 0.1700e-o | 0.7175f-p | 0.4925g-l | 0.6050h-m |
| 52 | 0.1047d-o | 0.0753e-p | 0.0900f-v | 0.1350n-p | 0.1800a-i | 0.1575g-o | 0.6275n-p | 0.6700a-l | 0.6488d-m |
| 53 | 0.1213b-o | 0.0683f-p | 0.0948f-v | 0.2425a-m | 0.1775a-i | 0.2100a-n | 0.9175a-o | 0.6100a-l | 0.7638a-m |
| 54 | 0.0990f-o | 0.0615f-p | 0.0803k-v | 0.1950c-p | 0.1725a-i | 0.1838b-o | 0.7125g-p | 0.5800d-l | 0.6463d-m |
| 55 | 0.1445a-o | 0.0923c-p | 0.1184b-q | 0.2425a-m | 0.1950a-i | 0.2188a-k | 0.8000b-p | 0.7100a-l | 0.7550a-m |
| 56 | 0.0805m-o | 0.0608f-p | 0.0706q-v | 0.1800g-p | 0.1625b-i | 0.1713e-o | 0.6625m-p | 0.5600d-l | 0.6112h-m |
| 57 | 0.1240a-o | 0.1007c-m | 0.1124c-u | 0.2125b-p | 0.1975a-i | 0.2050a-o | 0.6875j-p | 0.8350a-e | 0.7613a-m |
| 58 | 0.1135b-o | 0.0640f-p | 0.0888f-v | 0.1875e-p | 0.1575b-i | 0.1725e-o | 0.7325f-p | 0.5525d-l | 0.6425e-m |
| 59 | 0.0890i-o | 0.0785d-p | 0.0838h-v | 0.1825f-p | 0.1725a-i | 0.1775d-o | 0.6575m-p | 0.6325a-l | 0.6450e-m |
| 60 | 0.1000f-o | 0.1000c-n | 0.1000d-v | 0.2050b-p | 0.1850a-i | 0.1950a-o | 0.7450e-p | 0.6900a-l | 0.7175a-m |
| 61 | 0.1235b-o | 0.0548i-p | 0.0889f-v | 0.2400a-m | 0.1525b-i | 0.1963a-o | 0.9150a-o | 0.5175e-l | 0.7163a-m |
| 62 | 0.1107c-o | 0.0545i-p | 0.0826i-v | 0.2200a-p | 0.1350c-i | 0.1775d-o | 0.8100b-p | 0.4775i-l | 0.6438e-m |
| 63 | 0.1565a-o | 0.0675f-p | 0.1120c-u | 0.2325a-n | 0.1475c-i | 0.1900a-o | 0.9750a-m | 0.5350d-l | 0.7550a-m |
| 64 | 0.1225b-o | 0.0670f-p | 0.0948f-v | 0.3025ab | 0.1650b-i | 0.2338a-h | 1.038a-f | 0.5700d-l | 0.8038a-l |
| 65 | 0.1148b-o | 0.0588g-p | 0.0868g-v | 0.1975c-p | 0.1550b-i | 0.1762d-o | 0.7700c-p | 0.5325d-l | 0.6513d-m |
| 66 | 0.1287a-o | 0.0558h-p | 0.0923f-v | 0.2400a-m | 0.1575b-i | 0.1988a-o | 0.8725a-o | 0.5300d-l | 0.7013b-m |
| 67 | 0.0998f-o | 0.0500l-p | 0.0749o-v | 0.1900d-p | 0.1425c-i | 0.1663e-o | 0.6825j-p | 0.4825h-l | 0.5825i-m |
| 68 | 0.1230b-o | 0.0513k-p | 0.0871g-v | 0.2075b-p | 0.1500c-i | 0.1788d-o | 0.8375a-p | 0.4950f-l | 0.6662b-m |
| 69 | 0.1032d-o | 0.0560h-p | 0.0796k-v | 0.2375a-m | 0.1500c-i | 0.1937a-o | 0.8400a-p | 0.5075e-l | 0.6737b-m |
| 70 | 0.1225b-o | 0.0708f-p | 0.0966e-v | 0.2375a-m | 0.1800a-i | 0.2087a-o | 0.7750c-p | 0.6250a-l | 0.7000b-m |
| 71 | 0.0968g-o | 0.0738f-p | 0.0853h-v | 0.2425a-m | 0.1725a-i | 0.2075a-o | 0.8500a-p | 0.6150a-l | 0.7325a-m |
| 72 | 0.1038d-o | 0.0758d-p | 0.0898f-v | 0.2225a-p | 0.1525b-i | 0.1875a-o | 0.7950b-p | 0.5575d-l | 0.6762b-m |
| 73 | 0.0955h-o | 0.0410op | 0.0683s-v | 0.1850e-p | 0.1775a-i | 0.1813c-o | 0.6675k-p | 0.5875d-l | 0.6275g-m |
| 74 | 0.1013e-o | 0.0668f-p | 0.0840h-v | 0.2075b-p | 0.1825a-i | 0.1950a-o | 0.7800c-p | 0.6675a-l | 0.7237a-m |
| 75 | 0.1107c-o | 0.0685f-p | 0.0896f-v | 0.2450a-l | 0.1700b-i | 0.2075a-o | 0.7125g-p | 0.5925c-l | 0.6525c-m |
| 76 | 0.1143b-o | 0.0825d-p | 0.0984e-v | 0.2175a-p | 0.1475c-i | 0.1825c-o | 0.8000b-p | 0.5475d-l | 0.6737b-m |
| 77 | 0.1803a-f | 0.0378p | 0.1090c-v | 0.2075b-p | 0.1350c-i | 0.1713e-o | 0.7375e-p | 0.4350l | 0.5863i-m |

Appendix 5. Continued

| Genotype* | Shoot P content (g/5 plants) | | | Grain P content (g/5 plants) | | | Biomass P content (g/5 plants) | | |
|---|---|---|---|---|---|---|---|---|---|
| | With P | Without P | Combined | With P | Without P | Combined | With P | Without P | Combined |
| 78 | 0.1037d-o | 0.0833d-p | 0.0935f-v | 0.2475a-l | 0.2225a-f | 0.2350a-h | 0.8700a-o | 0.7400a-l | 0.8050a-l |
| 79 | 0.1247a-o | 0.0750e-p | 0.0999d-v | 0.2175a-p | 0.1650b-i | 0.1912a-o | 0.8375a-p | 0.5875d-l | 0.7125a-m |
| 80 | 0.1433a-o | 0.0620f-p | 0.1026d-v | 0.2200a-p | 0.1575b-i | 0.1887a-o | 0.8925a-o | 0.5625d-l | 0.7275a-m |
| 81 | 0.0993f-o | 0.0668f-p | 0.0830i-v | 0.2350a-n | 0.1575b-i | 0.1963a-o | 0.8075b-p | 0.5525d-l | 0.6800b-m |
| 82 | 0.1640a-l | 0.0630f-p | 0.1135c-u | 0.2275a-o | 0.1625b-i | 0.1950a-o | 0.9500a-n | 0.5975b-l | 0.7738a-m |
| 83 | 0.1415a-o | 0.1028c-m | 0.1221b-o | 0.2400a-m | 0.1725a-i | 0.2062a-o | 0.9675a-m | 0.6600a-l | 0.8137a-l |
| 84 | 0.1110c-o | 0.0990c-o | 0.1050d-v | 0.2275a-o | 0.1825a-i | 0.2050a-o | 0.8450a-p | 0.7700a-l | 0.8075a-l |
| 85 | 0.1447a-o | 0.0620f-p | 0.1034d-v | 0.2475a-l | 0.1550b-i | 0.2013a-o | 0.7100g-p | 0.5650d-l | 0.6375e-m |
| 86 | 0.1657a-k | 0.0755e-p | 0.1206b-o | 0.2175a-p | 0.1800a-i | 0.1988a-o | 0.9450a-n | 0.6750a-l | 0.8100a-l |
| 87 | 0.1312a-o | 0.0650f-p | 0.0981e-v | 0.1700h-p | 0.1625b-i | 0.1663e-o | 0.7275f-p | 0.5900d-l | 0.6587c-m |
| 88 | 0.1427a-o | 0.0640f-p | 0.1034d-v | 0.1575k-p | 0.1350c-i | 0.1462i-o | 0.7225f-p | 0.4925g-l | 0.6075h-m |
| 89 | 0.1013e-o | 0.0678f-p | 0.0845h-v | 0.2475a-l | 0.1875a-i | 0.2175a-l | 0.8600a-o | 0.6325a-l | 0.7462a-m |
| 90 | 0.0760o | 0.1130b-h | 0.0945f-v | 0.1925c-p | 0.2725a | 0.2325a-h | 0.6525m-p | 0.9300a-c | 0.7912a-l |
| 91 | 0.1493a-o | 0.0993c-o | 0.1243b-m | 0.2925a-c | 0.2325a-c | 0.2625a-c | 1.0250a-h | 0.8175a-h | 0.9212a-d |
| 92 | 0.1395a-o | 0.0870d-p | 0.1133c-u | 0.2925a-c | 0.1700b-i | 0.2313a-h | 0.9150a-o | 0.6325a-l | 0.7738a-m |
| 93 | 0.1085c-o | 0.0733f-p | 0.0909f-v | 0.2725a-g | 0.2200a-g | 0.2463a-e | 0.9475a-n | 0.6925a-l | 0.8200a-k |
| 94 | 0.1260a-o | 0.0780d-p | 0.1020d-v | 0.2500a-k | 0.1625b-i | 0.2062a-o | 0.9050a-o | 0.6000b-l | 0.7525a-m |
| 95 | 0.1335a-o | 0.0858d-p | 0.1096c-v | 0.2225a-p | 0.1750a-i | 0.1988a-o | 0.8900a-o | 0.6575a-l | 0.7738a-m |
| 96 | 0.1500a-o | 0.1005c-m | 0.1252b-l | 0.2700a-h | 0.2025a-h | 0.2362a-g | 1.112ab | 0.7425a-l | 0.9275a-c |
| 97 | 0.1560a-o | 0.0838d-p | 0.1199b-o | 0.2300a-n | 0.1775a-i | 0.2037a-o | 0.9400a-n | 0.6700a-l | 0.8050a-l |
| 98 | 0.1490a-o | 0.1042c-l | 0.1266b-k | 0.2825a-f | 0.2350a-c | 0.2587a-d | 1.135a | 0.8325a-f | 0.9837a |
| 99 | 0.1210b-o | 0.0593g-p | 0.0901f-v | 0.1625j-p | 0.1500c-i | 0.1563g-o | 0.7375e-p | 0.5550d-l | 0.6463d-m |
| 100 | 0.1213b-o | 0.0565h-p | 0.0889f-v | 0.1850e-p | 0.1400c-i | 0.1625f-o | 0.7550d-p | 0.5050e-l | 0.6300f-m |
| 101 | 0.1048d-o | 0.0675f-p | 0.0861h-v | 0.1775g-p | 0.1650b-i | 0.1713e-o | 0.7675d-p | 0.5750d-l | 0.6712b-m |
| 102 | 0.1065d-o | 0.0565h-p | 0.0815j-v | 0.2175a-p | 0.1175h-i | 0.1675e-o | 0.7900b-p | 0.4475kl | 0.6187g-m |
| 103 | 0.1130c-o | 0.0858d-p | 0.0994d-v | 0.2400a-m | 0.1950a-i | 0.2175a-l | 0.8650a-o | 0.7125a-l | 0.7887a-l |
| 104 | 0.1460a-o | 0.0688f-p | 0.1074d-v | 0.2275a-o | 0.1450c-i | 0.1863a-o | 0.9325a-o | 0.5325d-l | 0.7325a-m |
| 105 | 0.1103c-o | 0.0605f-p | 0.0854h-v | 0.2225a-p | 0.1400c-i | 0.1813c-o | 0.7925b-p | 0.4750i-l | 0.6338f-m |
| 106 | 0.1050d-o | 0.0610f-p | 0.0830i-v | 0.2375a-m | 0.1625b-i | 0.2000a-o | 0.6975i-p | 0.5425d-l | 0.6200g-m |
| 107 | 0.0920i-o | 0.0675f-p | 0.0798k-v | 0.1925c-p | 0.1500c-i | 0.1713e-o | 0.6675k-p | 0.5225d-l | 0.5950i-m |
| 108 | 0.1070d-o | 0.0703f-p | 0.0886f-v | 0.2125b-p | 0.1925a-i | 0.2025a-o | 0.7825c-p | 0.6425a-l | 0.7125a-m |
| 109 | 0.1433a-o | 0.0605f-p | 0.1019d-v | 0.1900d-p | 0.1625b-i | 0.1762d-o | 0.8100b-p | 0.5575d-l | 0.6838b-m |
| 110 | 0.1748a-h | 0.0875d-p | 0.1311b-h | 0.2325a-n | 0.1950a-i | 0.2138a-m | 0.9175a-o | 0.7050a-l | 0.8112a-l |
| 111 | 0.0855j-o | 0.0643f-p | 0.0749o-v | 0.1850e-p | 0.1850a-i | 0.1850a-o | 0.6550m-p | 0.6175a-l | 0.6363f-m |
| 112 | 0.1075d-o | 0.0905d-p | 0.0990d-v | 0.2150b-p | 0.2125a-h | 0.2138a-m | 0.7775c-p | 0.7725a-l | 0.7750a-l |
| 113 | 0.1003e-o | 0.0800d-p | 0.0901f-v | 0.2100b-p | 0.1825a-i | 0.1962a-o | 0.7425e-p | 0.6150a-l | 0.6787b-m |
| 114 | 0.0830l-o | 0.0605f-p | 0.0718p-v | 0.1825f-p | 0.1225f-i | 0.1525h-o | 0.6725j-p | 0.4700i-l | 0.5713j-m |
| 115 | 0.0838k-o | 0.0690f-p | 0.0764m-v | 0.2300a-n | 0.1750a-i | 0.2025a-o | 0.7500d-p | 0.6050a-l | 0.6775b-m |
| 116 | 0.1128c-o | 0.0593g-p | 0.0860h-v | 0.1800g-p | 0.1375c-i | 0.1587g-o | 0.7350e-p | 0.5025e-l | 0.6187g-m |
| 117 | 0.1108c-o | 0.0843d-p | 0.0975e-v | 0.2525a-k | 0.1900a-i | 0.2212a-j | 0.8325a-p | 0.6900a-l | 0.7613a-m |
| 118 | 0.1260a-o | 0.0630f-p | 0.0945f-v | 0.2275a-o | 0.1750a-i | 0.2013a-o | 0.8675a-o | 0.5900d-l | 0.7287a-m |
| 119 | 0.0975g-o | 0.0703f-p | 0.0839h-v | 0.2200a-p | 0.1975a-i | 0.2088a-o | 0.7825c-p | 0.6200a-l | 0.7013b-m |
| 120 | 0.0843k-o | 0.0528j-p | 0.0685s-v | 0.2075b-p | 0.1450c-i | 0.1762d-o | 0.7325f-p | 0.4800h-l | 0.6062h-m |
| 121 | 0.1513a-o | 0.0925c-p | 0.1219b-o | 0.2050b-p | 0.1850a-i | 0.1950a-o | 0.9500a-n | 0.6825a-l | 0.8163a-k |
| 122 | 0.1178b-o | 0.0573g-p | 0.0875g-v | 0.2175a-p | 0.1825a-i | 0.2000a-o | 0.8275a-p | 0.5900d-l | 0.7088b-m |
| 123 | 0.0878j-o | 0.0723f-p | 0.0800k-v | 0.2075b-p | 0.2025a-h | 0.2050a-o | 0.7025h-p | 0.6825a-l | 0.6925b-m |
| 124 | 0.1295a-o | 0.0650f-p | 0.0973e-v | 0.3050ab | 0.1800a-i | 0.2425a-f | 0.9275a-o | 0.5950c-l | 0.7612a-m |
| 125 | 0.1430a-o | 0.0833d-p | 0.1131c-u | 0.2850a-e | 0.1625b-i | 0.2237a-j | 0.8500a-p | 0.6075a-l | 0.7287a-m |
| 126 | 0.1190b-o | 0.0910d-p | 0.1050d-v | 0.2825a-f | 0.1525b-i | 0.2175a-l | 0.9750a-m | 0.6000b-l | 0.7875a-l |
| 127 | 0.1128c-o | 0.0720f-p | 0.0924f-v | 0.2300a-n | 0.2075a-h | 0.2188a-k | 0.8275a-p | 0.6650a-l | 0.7462a-m |
| 128 | 0.1160b-o | 0.0613f-p | 0.0886f-v | 0.2675a-h | 0.1750a-i | 0.2212a-j | 0.9175a-o | 0.5650d-l | 0.7412a-m |
| 129 | 0.1090c-o | 0.0663f-p | 0.0876g-v | 0.2350a-n | 0.1950a-i | 0.2150a-m | 0.6975i-p | 0.6325a-l | 0.6650b-m |
| 130 | 0.1098c-o | 0.0695f-p | 0.0896f-v | 0.2600a-j | 0.1500c-i | 0.2050a-o | 0.9075a-o | 0.5275d-l | 0.7175a-m |
| 131 | 0.1095c-o | 0.0668f-p | 0.0881f-v | 0.2375a-m | 0.1275d-i | 0.1825c-o | 0.8250a-p | 0.4600kl | 0.6425e-m |
| 132 | 0.0760o | 0.0475l-p | 0.0618v | 0.2350a-n | 0.1575b-i | 0.1963a-o | 0.7650d-p | 0.4875g-l | 0.6263g-m |
| 133 | 0.1135b-o | 0.0818d-p | 0.0976e-v | 0.1975c-p | 0.1800a-i | 0.1887a-o | 0.8000b-p | 0.6600a-l | 0.7300a-m |
| 134 | 0.1147b-o | 0.0830d-p | 0.0989d-v | 0.2225a-p | 0.1875a-i | 0.2050a-o | 0.8150a-p | 0.6550a-l | 0.7350a-m |
| 135 | 0.0875j-o | 0.0625f-p | 0.0750n-v | 0.1900d-p | 0.1300d-i | 0.1600f-o | 0.6925i-p | 0.4800h-l | 0.5863i-m |
| 136 | 0.1425a-o | 0.0640f-p | 0.1032d-v | 0.2175a-p | 0.1650b-i | 0.1912a-o | 0.7950b-p | 0.6000b-l | 0.6975b-m |
| 137 | 0.1840a-d | 0.0595g-p | 0.1217b-o | 0.2600a-j | 0.1450c-i | 0.2025a-o | 0.9500a-n | 0.5125e-l | 0.7312a-m |
| 138 | 0.1143b-o | 0.0795d-p | 0.0969e-v | 0.2775a-g | 0.1975a-i | 0.2375a-g | 0.9900a-k | 0.6800a-l | 0.8350a-j |
| 139 | 0.1285a-o | 0.0840d-p | 0.1062d-v | 0.2350a-n | 0.1350c-i | 0.1850a-o | 0.8725a-o | 0.5450d-l | 0.7088b-m |
| 140 | 0.1645a-l | 0.0690f-p | 0.1168b-r | 0.2075b-p | 0.1300d-i | 0.1688e-o | 0.7850c-p | 0.5200d-l | 0.6525c-m |
| 141 | 0.1625a-m | 0.0418n-p | 0.1021d-v | 0.2750a-g | 0.1575b-i | 0.2163a-l | 0.9750a-m | 0.4950f-l | 0.7350a-m |
| 142 | 0.1675a-j | 0.0865d-p | 0.1270b-k | 0.2425a-m | 0.1275d-i | 0.1850a-o | 0.9325a-o | 0.6075a-l | 0.7700a-m |
| 143 | 0.1175b-o | 0.1110b-j | 0.1143c-u | 0.1250p | 0.1275d-i | 0.1262o | 0.6300n-p | 0.6275a-l | 0.6288f-m |
| 144 | 0.1560a-o | 0.0718f-p | 0.1139c-u | 0.2225a-p | 0.1225f-i | 0.1725e-o | 0.8850a-o | 0.4850h-l | 0.6850b-m |
| 145 | 0.1060d-o | 0.1152b-g | 0.1106c-u | 0.1475l-p | 0.1225f-i | 0.1350l-o | 0.6875j-p | 0.6375a-l | 0.6625b-m |
| 146 | 0.1115c-o | 0.0768d-p | 0.0941f-v | 0.2150b-p | 0.1375c-i | 0.1762d-o | 0.7600d-p | 0.6050a-l | 0.6825b-m |
| 147 | 0.1135b-o | 0.0720f-p | 0.0928f-v | 0.1950c-p | 0.1350c-i | 0.1650e-o | 0.8000b-p | 0.5325d-l | 0.6662b-m |
| 148 | 0.0843k-o | 0.0668f-p | 0.0755n-v | 0.1550k-p | 0.1275d-i | 0.1412j-o | 0.6100op | 0.4700i-l | 0.5400lm |
| 149 | 0.1285a-o | 0.0973c-o | 0.1129c-u | 0.2250a-p | 0.1925a-i | 0.2087a-o | 0.8825a-o | 0.7100a-l | 0.7962a-l |
| 150 | 0.1702a-i | 0.1180b-f | 0.1441b-e | 0.2350a-n | 0.1975a-i | 0.2163a-l | 0.9875a-l | 0.7675a-l | 0.8775a-h |
| 151 | 0.1782a-g | 0.0908d-p | 0.1345b-g | 0.2675a-h | 0.1375c-i | 0.2025a-o | 0.9950a-j | 0.5775d-l | 0.7862a-l |
| 152 | 0.1420a-o | 0.0798d-p | 0.1109c-u | 0.2325a-n | 0.1425c-i | 0.1875a-o | 0.9625a-m | 0.5475d-l | 0.7550a-m |
| 153 | 0.1335a-o | 0.0960c-p | 0.1147c-t | 0.2450a-l | 0.2325a-c | 0.2387a-g | 0.7950b-p | 0.8250a-g | 0.8100a-l |
| 154 | 0.2055a | 0.1720a | 0.1887a | 0.1525k-p | 0.1225f-i | 0.1375k-o | 0.9475a-n | 0.7250a-l | 0.8363a-j |
| 155 | 0.1775a-h | 0.1322a-e | 0.1549a-c | 0.1425m-p | 0.1225f-i | 0.1325m-o | 0.7150f-p | 0.5850d-l | 0.6500d-m |

* The serial stand for genotypes as given in Table 1

Appendix 6. Average performances of the 155 chickpea genotypes for plant tissue (shoot, grain and biomass) phosphorus yield

| Genotype* | Shoot P yield (mg/5 plants) | | | Grain P yield (mg/5 plants) | | | Biomass P yield (mg/5 plants) | | |
|---|---|---|---|---|---|---|---|---|---|
| | With P | Without P | Combined | With P | Without P | Combined | With P | Without P | Combined |
| 1 | 128.9a-r | 61.72f-r | 95.31d-m | 232.6a-s | 185.7a-k | 209.2a-n | 361.5a-q | 247.5c-k | 304.5a-j |
| 2 | 115.4c-r | 94.43c-r | 104.9c-m | 238.2a-r | 235.8a-c | 237.0a-g | 353.6a-q | 330.2a-g | 341.9a-h |
| 3 | 110.0d-r | 161.5ab | 135.7b-f | 250.5a-o | 228.7a-f | 239.6a-g | 360.5a-q | 390.1a | 375.3a-d |
| 4 | 134.4a-r | 90.11d-r | 112.2b-m | 239.5a-r | 151.9c-k | 195.7a-n | 373.9a-p | 242.0c-k | 307.9a-j |
| 5 | 101.2g-r | 97.39c-q | 99.27c-m | 249.6a-p | 225.7a-g | 237.7a-g | 350.8a-q | 323.1a-i | 336.9a-i |
| 6 | 129.3a-r | 63.83f-r | 96.54d-m | 270.3a-k | 159.0b-k | 214.6a-m | 399.5a-p | 222.8d-k | 311.2a-j |
| 7 | 103.8e-r | 147.5a-c | 125.7b-j | 250.8a-o | 215.7a-i | 233.3a-h | 354.6a-q | 363.2a-c | 358.9a-f |
| 8 | 76.01r | 58.83g-r | 67.42lm | 221.8a-t | 126.3h-k | 174.1e-n | 297.8b-q | 185.2jk | 241.5e-j |
| 9 | 144.7a-r | 57.51h-r | 101.1c-m | 210.8a-t | 120.9i-j | 165.9e-n | 355.5a-q | 178.5k | 267.0a-j |
| 10 | 110.6d-r | 67.69f-r | 89.16e-m | 263.9a-l | 197.6a-j | 230.8a-i | 374.5a-p | 265.3a-k | 319.9a-i |
| 11 | 111.1d-r | 64.03f-r | 87.56e-m | 289.8a-e | 168.1b-k | 228.9a-i | 400.9a-o | 232.1c-k | 316.5a-j |
| 12 | 167.8a-k | 67.78f-r | 117.8b-m | 286.9a-f | 170.3b-k | 228.6a-i | 454.8ab | 238.1c-k | 346.4a-g |
| 13 | 135.2a-r | 64.94f-r | 100.1c-m | 274.1a-i | 174.0b-k | 224.0a-j | 409.3a-m | 238.9c-k | 324.1a-i |
| 14 | 109.3d-r | 72.34f-r | 90.81d-m | 224.7a-t | 163.9b-k | 194.3a-n | 334.0a-q | 236.2c-k | 285.1a-j |
| 15 | 152.9a-r | 92.67d-r | 122.8b-l | 191.8d-t | 158.0b-k | 174.9e-n | 344.7a-q | 250.7c-k | 297.7a-j |
| 16 | 139.0a-r | 85.02d-r | 112.0b-m | 188.7d-t | 124.8h-k | 156.7g-n | 327.7 a-q | 209.8d-k | 268.8a-j |
| 17 | 124.5b-r | 73.61f-r | 99.03c-m | 230.6a-t | 99.18k | 164.9e-n | 355.0 a-q | 172.8k | 263.9c-j |
| 18 | 162.1a-p | 76.00e-r | 119.0b-l | 253.6a-o | 148.0c-k | 200.8a-n | 415.7a-l | 224.0d-k | 319.8a-i |
| 19 | 117.1b-r | 87.89d-r | 102.5c-m | 231.3a-t | 141.4c-k | 186.3a-n | 348.3 a-q | 229.3c-k | 288.8a-j |
| 20 | 99.42g-r | 72.04f-r | 85.74f-m | 230.2a-t | 129.3g-k | 179.7d-n | 329.6 a-q | 201.3e-k | 265.5b-j |
| 21 | 162.7a-n | 73.76f-r | 118.2b-m | 270.7a-k | 178.3a-k | 224.5a-i | 433.4a-h | 252.1c-k | 342.8a-h |
| 22 | 149.5a-r | 105.5c-m | 127.5b-h | 188.9d-t | 174.5b-k | 181.7b-n | 338.4 a-q | 280.0a-k | 309.2a-j |
| 23 | 182.4a-e | 109.4c-k | 145.9a-d | 255.4a-o | 203.9a-j | 229.7a-i | 437.9a-f | 313.3a-j | 375.6a-d |
| 24 | 152.9a-r | 104.5c-n | 128.7b-g | 259.6a-n | 174.3b-k | 217.0a-l | 412.5a-l | 278.7a-k | 345.6a-h |
| 25 | 148.0a-r | 111.2b-j | 129.6b-g | 204.4b-t | 181.7a-k | 193.0a-n | 352.4 a-q | 292.9a-k | 322.6a-i |
| 26 | 112.9c-r | 111.8b-i | 112.4b-m | 218.8a-t | 162.3b-k | 190.6a-n | 331.8 a-q | 274.1a-k | 303.0a-j |
| 27 | 190.4a-c | 133.3a-d | 161.9ab | 245.3a-q | 198.7a-j | 222.0a-k | 435.7a-g | 332.0a-f | 383.8a-c |
| 28 | 126.1b-r | 60.85g-r | 93.50d-m | 194.1d-t | 141.1c-k | 167.6e-n | 320.2a-q | 202.0e-k | 261.1d-j |
| 29 | 114.3c-r | 108.7c-l | 111.5b-m | 194.3d-t | 206.6a-j | 200.4a-n | 308.6a-q | 315.3a-j | 312.0a-j |
| 30 | 150.3a-r | 66.60f-r | 108.5b-m | 238.0a-r | 173.5b-k | 205.7a-n | 388.3a-p | 240.1c-k | 314.2a-j |
| 31 | 103.5e-r | 51.82l-r | 77.64g-m | 259.1a-o | 170.6b-k | 214.8a-m | 362.5 a-q | 222.4d-k | 292.5a-j |
| 32 | 195.7ab | 57.99h-r | 126.9b-i | 266.6a-l | 180.7a-k | 223.7a-j | 462.3a | 238.7c-k | 350.5a-g |
| 33 | 83.15p-r | 56.48h-r | 69.82j-m | 192.0d-t | 148.4c-k | 170.2e-n | 275.2g-q | 204.9d-k | 240.1e-j |
| 34 | 117.9b-r | 79.46d-r | 98.67d-m | 198.9c-t | 199.1a-j | 199.0a-n | 316.8a-q | 278.5a-k | 297.7a-j |
| 35 | 91.73j-r | 41.28qr | 66.50lm | 197.0c-t | 135.5e-k | 166.3e-n | 288.8c-d | 176.7k | 232.8g-j |
| 36 | 144.7a-r | 45.32p-r | 94.99d-m | 188.2d-t | 139.3c-k | 163.7e-n | 332.8a-q | 184.6jk | 258.7d-j |
| 37 | 135.4a-r | 83.83d-r | 109.6b-m | 283.3a-h | 251.8ab | 267.5a | 418.7a-l | 335.6a-e | 377.2a-d |
| 38 | 105.3d-r | 61.37f-r | 83.36f-m | 239.2a-r | 164.8b-k | 202.0a-n | 344.5a-q | 226.2d-k | 285.3a-j |
| 39 | 102.5f-r | 84.34d-r | 93.41d-m | 315.3 A | 212.8a-j | 264.1ab | 417.8a-l | 297.1a-k | 357.5a-f |
| 40 | 104.5e-r | 63.74f-r | 84.11f-m | 226.4a-t | 156.6b-k | 191.5a-n | 330.9a-q | 220.3d-k | 275.63a-j |
| 41 | 141.0a-r | 47.20o-r | 94.11d-m | 206.2b-t | 164.8b-k | 185.5a-n | 347.2a-q | 212.0d-k | 279.63a-j |
| 42 | 135.1a-r | 47.86n-r | 91.47d-m | 215.6a-t | 174.8b-k | 195.2a-n | 350.7a-q | 222.7d-k | 286.73a-j |
| 43 | 105.3d-r | 82.40d-r | 93.85d-m | 228.6a-t | 170.1b-k | 199.4a-n | 333.9a-q | 252.5b-k | 293.23a-j |
| 44 | 99.64g-r | 87.53d-r | 93.58d-m | 166.3k-t | 232.3a-e | 199.3a-n | 266.0j-q | 319.8a-i | 292.93a-j |
| 45 | 79.38qr | 80.04d-r | 79.71f-m | 241.1a-r | 174.5b-k | 207.8a-n | 320.5a-q | 254.5b-k | 287.53a-j |
| 46 | 131.9a-r | 66.45f-r | 99.16c-m | 158.7m-t | 156.9b-k | 157.8f-n | 290.6c-q | 223.4d-k | 257.0d-j |
| 47 | 95.25j-r | 65.25f-r | 80.25f-m | 192.3d-t | 124.4h-k | 158.3f-n | 287.5c-q | 189.6i-k | 238.6f-j |
| 48 | 79.96qr | 53.97k-r | 66.97lm | 128.2st | 127.7g-k | 128.0n | 208.2q | 181.7jk | 194.9j |
| 49 | 99.41g-r | 76.09e-r | 87.75e-m | 182.4f-t | 200.2a-j | 191.3a-n | 281.8e-q | 276.3a-k | 279.03a-j |
| 50 | 138.6a-r | 92.58d-r | 115.6b-m | 215.7a-t | 205.1a-j | 210.4a-n | 354.3a-q | 297.7a-k | 326.0a-i |
| 51 | 105.1d-r | 65.48f-r | 85.28f-m | 209.9b-t | 131.0f-k | 170.5e-n | 315.0a-q | 196.5g-k | 255.7d-j |
| 52 | 104.7e-r | 75.12f-r | 89.94d-m | 137.2r-t | 180.3a-k | 158.7f-n | 241.9o-q | 255.4b-k | 248.7e-j |
| 53 | 121.2b-r | 68.18f-r | 94.69d-m | 241.4a-r | 177.2a-k | 209.3a-n | 362.6a-q | 245.4c-k | 304.03a-j |
| 54 | 99.17g-r | 61.55f-r | 80.36f-m | 195.8d-t | 172.4b-k | 184.1a-n | 294.9b-q | 233.9c-k | 264.4c-j |
| 55 | 144.5a-r | 92.31d-r | 118.4b-m | 242.2a-r | 191.8a-k | 217.0a-l | 386.6a-p | 284.1a-k | 335.4a-i |
| 56 | 80.52qr | 60.52g-r | 70.52i-m | 179.2h-t | 160.7b-k | 169.9e-n | 259.7l-q | 221.2d-k | 240.5e-j |
| 57 | 123.8b-r | 100.8c-p | 112.3b-m | 211.8a-t | 197.9a-j | 204.8a-n | 335.7a-q | 298.6a-k | 317.1a-i |
| 58 | 113.4c-r | 64.15f-r | 88.78e-m | 186.1e-t | 156.2b-k | 171.2e-n | 299.5b-q | 220.3d-k | 259.9d-j |
| 59 | 88.89k-r | 78.29d-r | 83.59f-m | 184.7f-t | 173.3b-k | 179.0d-n | 273.6h-q | 251.6c-k | 262.6c-j |
| 60 | 100.1g-r | 99.88c-p | 99.97c-m | 207.5b-t | 184.2a-k | 195.9a-n | 307.6a-q | 284.1a-k | 295.83a-j |
| 61 | 123.4b-r | 54.38j-r | 88.90e-m | 241.8a-r | 153.1c-k | 197.4a-n | 365.2a-q | 207.4d-k | 286.33a-j |
| 62 | 110.9d-r | 54.45j-r | 82.68f-m | 218.6a-t | 135.4e-k | 177.0d-n | 329.5a-q | 189.9i-k | 259.7d-j |
| 63 | 156.2a-q | 67.47f-r | 111.8b-m | 234.4a-r | 146.0c-k | 190.2a-n | 390.6a-p | 213.5d-k | 302.13a-j |
| 64 | 122.5b-r | 66.99f-r | 94.73d-m | 301.5a-c | 162.2b-k | 231.8a-i | 424.0a-k | 229.2c-k | 326.6a-i |
| 65 | 114.7c-r | 58.87g-r | 86.78f-m | 198.3c-t | 153.8b-k | 176.0d-n | 313.0a-q | 212.6d-k | 262.8c-j |

Appendix 6. Continued

| Genotype | Shoot P yield (mg/5 plants) | | | Grain P yield (mg/5 plants) | | | Biomass P yield (mg/5 plants) | | |
|---|---|---|---|---|---|---|---|---|---|
| | With P | Without P | Combined | With P | Without P | Combined | With P | Without P | Combined |
| 66 | 128.9a-r | 55.74i-r | 92.33d-m | 240.0a-r | 155.4b-k | 197.7a-n | 368.9a-q | 211.2d-k | 290.03a-j |
| 67 | 99.84g-r | 50.08m-r | 74.96g-m | 189.7d-t | 143.1c-k | 166.4e-n | 289.5c-q | 193.2h-k | 241.4e-j |
| 68 | 123.3b-r | 51.15m-r | 87.25f-m | 207.0b-t | 150.2c-k | 178.6d-n | 330.3a-q | 201.4e-k | 265.9b-j |
| 69 | 103.4f-r | 56.29h-r | 79.83f-m | 239.8a-r | 145.7c-k | 192.8a-n | 343.2a-q | 202.0e-k | 272.63a-j |
| 70 | 122.6b-r | 70.58f-r | 96.60d-m | 238.2a-r | 182.5a-k | 210.4a-n | 360.9a-q | 253.1b-k | 307.03a-j |
| 71 | 96.75i-r | 73.82f-r | 85.28f-m | 244.0a-q | 173.0b-k | 208.5a-n | 340.8a-q | 246.8c-k | 293.83a-j |
| 72 | 103.5e-r | 75.57f-r | 89.51d-m | 221.5a-t | 152.7c-k | 187.1a-n | 324.9a-q | 228.2d-k | 276.63a-j |
| 73 | 95.37j-r | 40.88qr | 68.12k-m | 183.5f-t | 176.7a-k | 180.1c-n | 278.9f-q | 217.6d-k | 248.2e-j |
| 74 | 101.3f-r | 66.75f-r | 84.01f-m | 205.5b-t | 185.0a-k | 195.2a-n | 306.7a-q | 251.7c-k | 279.23a-j |
| 75 | 110.6d-r | 68.54f-r | 89.59d-m | 245.1a-q | 168.3b-k | 206.7a-n | 355.7a-q | 236.9c-k | 296.33a-j |
| 76 | 114.2c-r | 82.33d-r | 98.26d-m | 216.9a-t | 147.3c-k | 182.1b-n | 331.1a-q | 229.6c-k | 280.43a-j |
| 77 | 180.3a-f | 37.67r | 109.0b-m | 207.1b-t | 134.0e-k | 170.6e-n | 387.4a-p | 171.7k | 279.63a-j |
| 78 | 103.8e-r | 83.19d-r | 93.47d-m | 247.4a-p | 219.6a-h | 233.5a-h | 351.2a-q | 302.8a-k | 327.0a-i |
| 79 | 124.7b-r | 75.15f-r | 99.92c-m | 216.5a-t | 166.3b-k | 191.4a-n | 341.2a-q | 241.4c-k | 291.33a-j |
| 80 | 143.0a-r | 62.07f-r | 102.5c-m | 219.3a-t | 155.9b-k | 187.6a-n | 362.3a-q | 218.0d-k | 290.13a-j |
| 81 | 99.05h-r | 66.71f-r | 82.88f-m | 238.0a-r | 159.5b-k | 198.7a-n | 337.1a-q | 226.2d-k | 281.63a-j |
| 82 | 164.1a-m | 62.85f-r | 113.5b-m | 226.5a-t | 161.6b-k | 194.1a-n | 390.6a-p | 224.5d-k | 307.63a-j |
| 83 | 141.5a-r | 102.7c-o | 122.1b-l | 241.1a-r | 170.3b-k | 205.7a-n | 382.6a-p | 273.0a-k | 327.8a-i |
| 84 | 110.7d-r | 99.17c-p | 105.0c-m | 227.9a-t | 183.4a-k | 205.6a-n | 338.6a-q | 282.5a-k | 310.63a-j |
| 85 | 144.7a-r | 62.28f-r | 103.5c-m | 247.2a-p | 153.0c-k | 200.1a-n | 391.9a-p | 215.2d-k | 303.63a-j |
| 86 | 165.9a-l | 75.38f-r | 120.6b-l | 215.4a-t | 181.1a-k | 198.3a-n | 381.3a-p | 256.5b-k | 318.9a-i |
| 87 | 131.3a-r | 65.09f-r | 98.21d-m | 168.7j-t | 163.6b-k | 166.1e-n | 300.0b-q | 228.7d-k | 264.3c-j |
| 88 | 142.5a-r | 64.08f-r | 103.3c-m | 157.8m-t | 138.3c-k | 148.0i-n | 300.3b-q | 202.4e-k | 251.3e-j |
| 89 | 101.3f-r | 67.80f-r | 84.57f-m | 245.0a-q | 186.5a-k | 215.7a-m | 346.3a-q | 254.3b-k | 300.33a-j |
| 90 | 75.98r | 112.8b-h | 94.39d-m | 191.3d-t | 272.3 A | 231.8a-i | 267.3j-q | 385.1ab | 326.2a-i |
| 91 | 149.3a-r | 99.24c-p | 124.3b-k | 291.0a-e | 236.4a-c | 263.7a-c | 440.3a-e | 335.6a-e | 387.9a |
| 92 | 139.5a-r | 87.19d-r | 113.3b-m | 291.9a-d | 169.8b-k | 230.8a-i | 431.4a-i | 257.0b-k | 344.2a-h |
| 93 | 108.2d-r | 73.19f-r | 90.70d-m | 272.9a-j | 217.5a-i | 245.2a-e | 381.1a-p | 290.7a-k | 335.9a-i |
| 94 | 125.8b-r | 78.00d-r | 101.9c-m | 248.4a-p | 162.0b-k | 205.2a-n | 374.2a-p | 240.0c-k | 307.13a-j |
| 95 | 133.5a-r | 85.99d-r | 109.8b-m | 223.9a-t | 174.1b-k | 199.0a-n | 357.4a-q | 260.1a-k | 308.73a-j |
| 96 | 150.2a-r | 100.5c-p | 125.4b-j | 272.2a-j | 200.8a-j | 236.5a-g | 422.3a-k | 301.3a-k | 361.8a-e |
| 97 | 155.9a-q | 83.78d-r | 119.8b-l | 230.2a-t | 176.2a-k | 203.2a-n | 386.1a-p | 260.0a-k | 323.1a-i |
| 98 | 149.0a-r | 104.2c-o | 126.6b-i | 284.8a-g | 234.9a-d | 259.8a-d | 433.8a-h | 339.0a-d | 386.4ab |
| 99 | 121.1b-r | 59.41g-r | 90.24d-m | 164.3l-t | 149.1c-k | 156.7g-n | 285.4d-q | 208.5d-k | 246.9e-j |
| 100 | 121.2b-r | 56.57h-r | 88.88e-m | 184.1f-t | 140.0c-k | 162.0e-n | 305.3a-q | 196.6g-k | 250.9e-j |
| 101 | 104.8e-r | 67.46f-r | 86.15f-m | 177.1i-t | 166.4b-k | 171.7e-n | 281.9e-q | 233.8c-k | 257.9d-j |
| 102 | 106.8d-r | 56.40h-r | 81.58f-m | 216.9a-t | 117.1jk | 167.0e-n | 323.6a-q | 173.5k | 248.5e-j |
| 103 | 113.0c-r | 85.79d-r | 99.40c-m | 239.4a-r | 196.0a-k | 217.7a-k | 352.4a-q | 281.8a-k | 317.1a-i |
| 104 | 146.0a-r | 68.64f-r | 107.3b-m | 226.6a-t | 143.7c-k | 185.1a-n | 372.5a-p | 212.3d-k | 292.43a-j |
| 105 | 110.3d-r | 60.50g-r | 85.39f-m | 222.6a-t | 139.9c-k | 181.3b-n | 332.9a-q | 200.4f-k | 266.73a-j |
| 106 | 104.9e-r | 61.21f-r | 83.03f-m | 233.5a-r | 161.8b-k | 197.7a-n | 338.4a-q | 223.0d-k | 280.73a-j |
| 107 | 92.09j-r | 67.25f-r | 79.67f-m | 190.8d-t | 148.8c-k | 169.8e-n | 282.9e-q | 216.1d-k | 249.5e-j |
| 108 | 106.9d-r | 70.38f-r | 88.65e-m | 210.2b-t | 193.0a-k | 201.6a-n | 317.1a-q | 263.4a-k | 290.23a-j |
| 109 | 143.2a-r | 60.74g-r | 102.0c-m | 190.0d-t | 164.5b-k | 177.2d-n | 333.2a-q | 225.2d-k | 279.23a-j |
| 110 | 174.9a-i | 87.69d-r | 131.3b-g | 231.6a-t | 194.2a-k | 212.9a-m | 406.5a-m | 281.9a-k | 344.2a-h |
| 111 | 85.35m-r | 64.43f-r | 74.89g-m | 187.1d-t | 184.1a-k | 185.6a-n | 272.4i-q | 248.6c-k | 260.5d-j |
| 112 | 107.5d-r | 90.52d-r | 99.01c-m | 213.0a-t | 210.7a-j | 211.9a-n | 320.5a-q | 301.3a-k | 310.93a-j |
| 113 | 100.2g-r | 79.90d-r | 90.03d-m | 209.9b-t | 183.3a-k | 196.6a-n | 310.1a-q | 263.2a-k | 286.63a-j |
| 114 | 83.13p-r | 60.27g-r | 71.70h-m | 180.5g-t | 123.7h-k | 152.1h-n | 263.7k-q | 184.0jk | 223.8h-j |
| 115 | 83.61o-r | 68.96f-r | 76.28g-m | 230.5a-t | 175.6a-k | 203.1a-n | 314.2a-q | 244.6c-k | 279.4a-j |
| 116 | 112.9c-r | 59.14g-r | 86.00f-m | 179.6h-t | 136.0e-k | 157.8f-n | 292.5c-q | 195.2h-k | 243.8e-j |
| 117 | 110.8d-r | 84.37d-r | 97.60d-m | 250.5a-o | 191.4a-k | 221.0a-k | 361.3a-q | 275.8a-k | 318.6a-i |
| 118 | 126.1b-r | 62.77f-r | 94.43d-m | 227.1a-t | 174.5b-k | 200.8a-n | 353.1a-q | 237.3c-k | 295.23a-j |
| 119 | 97.41i-r | 70.51f-r | 83.96f-m | 218.9a-t | 196.7a-k | 207.8a-n | 316.3a-q | 267.2a-k | 291.83a-j |
| 120 | 84.06n-r | 52.60k-r | 68.33k-m | 207.3a-t | 145.5c-k | 176.4d-n | 291.3c-q | 198.1f-k | 244.7e-j |
| 121 | 151.2a-r | 92.28d-r | 121.7b-l | 204.5b-t | 183.2a-k | 193.8a-n | 355.7a-q | 275.5a-k | 315.63a-j |
| 122 | 118.0b-r | 57.33h-r | 87.68e-m | 215.3a-t | 183.3a-k | 199.3a-n | 333.3a-q | 240.6c-k | 286.93a-j |
| 123 | 87.61l-r | 72.17f-r | 79.89f-m | 206.6b-t | 205.2a-j | 205.9a-n | 294.2b-q | 277.4a-k | 285.83a-j |
| 124 | 129.6a-r | 64.89f-r | 97.24d-m | 304.8ab | 177.3a-k | 241.1a-f | 434.4a-h | 242.2c-k | 338.3a-h |
| 125 | 143.0a-r | 83.40d-r | 113.2b-m | 282.1a-i | 161.1b-k | 221.6a-k | 425.1a-j | 244.5c-k | 334.8a-i |
| 126 | 119.0b-r | 91.15d-r | 105.1c-m | 283.4a-h | 150.8c-k | 217.1a-l | 402.4a-o | 242.0c-k | 322.2a-i |
| 127 | 112.9c-r | 71.85f-r | 92.36d-m | 226.8a-t | 206.3a-j | 216.5a-l | 339.6a-q | 278.1a-k | 308.93a-j |
| 128 | 115.8c-r | 61.44f-r | 88.61e-m | 266.7a-l | 176.5a-k | 221.6a-k | 382.4a-p | 237.9c-k | 310.23a-j |
| 129 | 109.0d-r | 66.22f-r | 87.61e-m | 233.8a-r | 195.6a-k | 214.7a-m | 342.8a-q | 261.8a-k | 302.33a-j |

Appendix 6. Continued

| Genotype | Shoot P yield (mg/5 plants) | | | Grain P yield (mg/5 plants) | | | Biomass P yield (mg/5 plants) | | |
|---|---|---|---|---|---|---|---|---|---|
| | With P | Without P | Combined | With P | Without P | Combined | With P | Without P | Combined |
| 130 | 109.7d-r | 69.39f-r | 89.53d-m | 260.3a-m | 148.8c-k | 204.6a-n | 370.0a-p | 218.2d-k | 294.13a-j |
| 131 | 109.3d-r | 66.98f-r | 88.15e-m | 237.5a-r | 126.9h-k | 182.2b-n | 346.8a-q | 193.9h-k | 270.43a-j |
| 132 | 76.08r | 47.37o-r | 61.73m | 232.7a-s | 158.5b-k | 195.6a-n | 308.7a-q | 205.9d-k | 257.3d-j |
| 133 | 113.4c-r | 81.69d-r | 97.53d-m | 199.2c-t | 180.4a-k | 189.8a-n | 312.6a-q | 262.0a-k | 287.33a-j |
| 134 | 114.8c-r | 82.91d-r | 98.85c-m | 222.5a-t | 187.2a-k | 204.9a-n | 337.3a-q | 270.1a-k | 303.73a-j |
| 135 | 87.50l-r | 62.46f-r | 74.98g-m | 190.0d-t | 129.8g-k | 159.9f-n | 277.5f-q | 192.3h-k | 234.9g-j |
| 136 | 142.5a-r | 63.99f-r | 103.2c-m | 216.3a-t | 162.7b-k | 189.5 a-n | 358.8a-q | 226.7d-k | 292.83a-j |
| 137 | 184.0a-d | 59.73g-r | 121.9b-l | 260.5a-m | 143.3c-k | 201.9a-n | 444.5a-d | 203.0e-k | 323.7a-i |
| 138 | 114.3c-r | 79.38d-r | 96.86d-m | 280.5a-i | 196.9a-k | 238.7a-g | 394.8a-p | 276.3a-k | 335.6a-i |
| 139 | 128.6a-r | 83.70d-r | 106.1c-m | 235.8a-r | 134.3e-k | 185.1a-n | 364.4a-q | 218.0d-k | 291.23a-j |
| 140 | 164.8a-l | 69.29f-r | 117.0b-m | 208.7b-t | 128.3g-k | 168.5e-n | 373.4a-p | 197.6f-k | 285.53a-j |
| 141 | 162.5a-o | 41.85qr | 102.2c-m | 273.8a-j | 156.5b-k | 215.1a-m | 436.3a-f | 198.4f-k | 317.3a-i |
| 142 | 167.4a-k | 86.58d-r | 127.0b-m | 242.4a-q | 126.4h-k | 184.4a-n | 409.8a-m | 213.0d-k | 311.43a-j |
| 143 | 117.8b-r | 111.0b-j | 114.4b-m | 127.3t | 130.2g-k | 128.8n | 245.1n-q | 241.2c-k | 243.2e-j |
| 144 | 155.8a-q | 71.66f-r | 113.8b-m | 222.2a-t | 120.5i-k | 171.4e-n | 378.1a-p | 192.2h-k | 285.13a-j |
| 145 | 105.7d-r | 115.0b-g | 110.4b-m | 145.2p-t | 122.1h-k | 133.6l-n | 251.0m-n | 237.0c-k | 244.0e-j |
| 146 | 111.6c-r | 76.88e-r | 94.24d-m | 212.4a-t | 137.3d-k | 174.9e-n | 324.0a-q | 214.2d-k | 269.13a-j |
| 147 | 113.3c-r | 72.29f-r | 92.81d-m | 195.1d-t | 136.2e-k | 165.6e-n | 308.4a-q | 208.4d-k | 258.4d-j |
| 148 | 84.01n-r | 66.49f-r | 75.25g-m | 154.9n-t | 125.9h-k | 140.4j-n | 238.9pq | 192.4h-k | 215.7ij |
| 149 | 128.8a-r | 97.18c-q | 113.0b-m | 225.7a-t | 189.7a-k | 207.7a-n | 354.5a-q | 286.9a-k | 320.7a-i |
| 150 | 170.2a-j | 118.0b-f | 144.1a-e | 235.3a-r | 197.1a-k | 216.2a-m | 405.5a-n | 315.1a-j | 360.3a-f |
| 151 | 178.1a-g | 90.64d-r | 134.4b-f | 269.2a-l | 138.6c-k | 203.9a-n | 447.3a-c | 229.3c-k | 338.3a-h |
| 152 | 141.9a-r | 79.75d-r | 110.8b-m | 233.1a-r | 143.0c-k | 188.1a-n | 375.1a-p | 222.8d-k | 298.93a-j |
| 153 | 133.6a-r | 95.93c-q | 114.8b-m | 248.0a-p | 229.3a-f | 238.6a-g | 381.6a-p | 325.2a-h | 353.4a-g |
| 154 | 205.4a | 172.1a | 188.7a | 154.5o-t | 123.1h-k | 138.8k-n | 359.9a-q | 295.2a-k | 327.5a-i |
| 155 | 177.6a-h | 132.1a-e | 154.9a-c | 141.2q-t | 123.4h-k | 132.3mn | 318.8a-q | 255.6b-k | 287.23a-j |

*The serial stand for genotypes as given in Table 1

Appendix 7. Average performances of the 155 chickpea genotypes for some important characters of phosphorus uptake and use efficiencies

| Genotypes | P uptake and use efficiency* | | | | |
|---|---|---|---|---|---|
| | APUfs (%) | APUs (%) | APUf (%) | PYE | PPE |
| Acc. No. 231327 | 71.84a-r | 49.46a-l | 22.38b-s | 73.50a-t | 51.24a-m |
| Acc. No. 231328 | 63.59a-s | 35.91c-o | 27.69a-r | 78.93a-k | 50.32a-n |
| Acc. No. 209093 | 66.94a-s | 62.72ab | 4.218s | 54.14t-z | 45.00a-r |
| Acc. No. 208829 | 75.86a-p | 47.92a-o | 27.94a-r | 58.51l-z | 44.08b-r |
| Acc. No. 209094 | 66.32a-s | 43.37a-0 | 22.94a-s | 70.38a-w | 46.92a-p |
| Acc. No. 209092 | 71.64a-r | 42.41a-o | 29.22a-p | 67.83a-z | 48.74a-n |
| Acc. No. 209096 | 58.75f-s | 52.60a-j | 6.148p-s | 62.39e-z | 42.34c-r |
| Acc. No. 209097 | 59.30e-s | 36.52c-o | 22.78a-s | 65.90b-z | 38.86d-r |
| Acc. No. 209098 | 70.38a-r | 35.43c-o | 34.95a-j | 61.40g-z | 42.36c-r |
| Acc. No. 41002 | 70.00a-r | 36.94c-o | 33.06a-l | 70.15a-x | 49.15a-n |
| Acc. No. 207761 | 81.05a-g | 48.09a-o | 32.96a-l | 67.35a-z | 53.56a-l |
| Acc. No. 207763 | 84.49a-c | 46.03a-o | 38.46a-d | 76.46a-p | 63.97ab |
| Acc. No. 207764 | 77.71a-l | 46.16a-o | 31.55a-n | 71.11a-w | 54.77a-j |
| Acc. No. 41268 | 64.25a-s | 45.65a-o | 18.60b-s | 77.04a-n | 53.28a-l |
| Acc. No. 41026 | 69.81a-r | 48.18a-o | 21.63b-s | 51.92v-z | 36.09g-r |
| Acc. No. 41074 | 67.65a-r | 42.82a-o | 24.83a-s | 56.50o-z | 38.04e-r |
| Acc. No. 41075 | 72.74a-q | 37.69c-o | 35.06a-i | 64.71c-z | 46.47a-r |
| Acc. No. 41073 | 56.43i-s | 37.14c-o | 19.29b-s | 76.66a-o | 46.94a-p |
| Acc. No. 41076 | 62.37b-s | 23.83no | 38.54a-d | 62.41e-z | 44.02b-r |
| Acc. No. 41021 | 71.90a-r | 40.14a-o | 31.76a-m | 61.74e-z | 44.47b-r |
| Acc. No. 41027 | 72.77a-q | 49.51a-l | 23.25a-s | 71.97a-v | 50.82a-m |
| Acc. No. 41222 | 68.63a-r | 57.01a-g | 11.62k-s | 54.74r-z | 36.55f-r |
| Acc. No. 207734 | 75.95a-p | 39.22b-o | 36.73a-f | 66.59a-z | 50.10a-n |
| Acc. No. 41103 | 87.36a | 58.70a-d | 28.66a-p | 52.25u-z | 45.25a-r |
| Acc. No. 41320 | 72.87a-q | 59.05a-c | 13.82f-s | 51.47w-z | 36.22g-r |
| Acc. No. 41029 | 65.52a-s | 32.23g-o | 33.29a-l | 69.45a-x | 45.01a-r |
| Acc. No. 41015 | 85.70ab | 64.29a | 21.41b-s | 57.31n-z | 49.31a-n |
| Acc. No. 41271 | 61.35c-s | 38.79b-o | 22.56b-s | 66.33a-z | 40.11d-r |
| Acc. No. 41272 | 61.97b-s | 49.97a-l | 12.00i-s | 65.50b-z | 41.22c-r |
| Acc. No. 41276 | 58.74f-s | 38.33b-o | 20.41b-s | 78.20a-l | 50.75a-m |
| Acc. No. 207745 | 73.40a-q | 45.56a-o | 27.84a-r | 71.15a-w | 52.02a-l |
| Acc. No. 41275 | 80.78a-h | 42.37a-o | 36.56a-f | 61.62f-z | 52.64a-l |
| Acc. No. 41277 | 55.07k-s | 37.22c-o | 17.86c-s | 79.75a-i | 43.66b-r |
| Acc. No. 207743 | 65.82a-s | 48.00a-o | 17.82d-s | 73.04a-t | 49.09a-n |
| Acc. No. 207744 | 57.32g-s | 35.74c-o | 21.58b-s | 73.27a-t | 42.21c-r |
| Acc. No. 41273 | 53.90l-s | 25.91k-o | 27.99a-r | 59.85h-z | 42.31c-r |
| Acc. No. 41274 | 82.78a-f | 58.79a-d | 23.99a-s | 71.47a-w | 58.63a-e |
| Acc. No. 207741 | 50.31q-s | 44.00a-o | 6.309p-s | 74.32a-s | 51.02a-m |
| Acc. No. 207742 | 77.90a-l | 58.26a-e | 19.64b-s | 79.29a-j | 61.40a-c |
| Acc. No. 41316 | 64.41a-s | 36.15c-o | 28.27a-q | 63.79c-z | 54.27a-k |
| Acc. No. 41298 | 68.54a-r | 32.36g-o | 36.18a-g | 69.49a-x | 47.85a-n |
| Acc. No. 41311 | 61.93b-s | 46.05a-o | 15.89d-s | 74.35a-s | 45.47a-r |
| Acc. No. 41313 | 65.52a-s | 44.23a-o | 21.29b-s | 75.53a-q | 49.53a-n |
| Acc. No. 41280 | 53.41m-s | 43.01a-o | 10.40l-s | 65.96b-z | 35.22i-r |
| Acc. No. 41312 | 54.41k-s | 34.44c-o | 19.96b-s | 81.82a-e | 48.75a-n |
| Acc. No. 41315 | 59.05e-s | 48.15a-o | 10.91l-s | 52.04v-z | 30.34m-r |
| Acc. No. 41308 | 61.61c-s | 31.83h-o | 29.78a-o | 67.53a-z | 40.31d-r |
| Acc. No. 41299 | 42.95s | 23.99m-o | 18.97b-s | 72.57a-t | 30.32m-r |
| Acc. No. 41046 | 56.71i-s | 34.33c-o | 22.38b-s | 59.08k-z | 38.62d-r |
| Acc. No. 41047 | 70.73a-r | 50.65a-k | 20.08b-s | 64.41c-z | 45.83a-r |
| Acc. No. 41304 | 57.29g-s | 39.46a-o | 17.84d-s | 67.94a-z | 39.98d-r |
| Acc. No. 41303 | 50.21q-s | 44.93a-o | 5.280q-s | 55.96q-z | 26.17p-r |
| Acc. No. 41295 | 73.42a-q | 37.94b-o | 35.48a-h | 66.85a-z | 49.33a-n |

Appendix 7. Continued

| Genotypes | P uptake and use efficiency* | | | | |
|---|---|---|---|---|---|
| | APUfs (%) | APUs (%) | APUf (%) | PYE | PPE |
| Acc. No. 41296 | 56.91h-s | 37.80b-o | 19.11b-s | 72.23a-u | 40.46c-r |
| Acc. No. 41289 | 63.93a-s | 41.63a-o | 22.30b-s | 80.72a-g | 51.30a-m |
| Acc. No. 41290 | 53.26n-s | 40.00a-o | 13.26g-s | 81.70a-f | 43.91b-r |
| Acc. No. 41284 | 55.09k-s | 37.21c-o | 17.88c-s | 78.49a-l | 46.67a-q |
| Acc. No. 41291 | 58.74f-s | 44.12a-o | 14.62e-s | 67.23a-z | 39.34d-r |
| Acc. No. 41297 | 52.87o-s | 43.32a-o | 9.554m-s | 71.12a-w | 40.84c-r |
| Acc. No. 41293 | 59.65d-s | 46.37a-o | 13.28g-s | 80.43a-g | 47.69a-n |
| Acc. No. 41019 | 73.20a-q | 41.59a-o | 31.62a-m | 69.15a-x | 57.01a-g |
| Acc. No. 41048 | 64.81a-s | 38.24b-o | 26.56a-s | 73.67a-t | 46.86a-p |
| Acc. No. 41049 | 78.01a-k | 42.80a-o | 35.22a-h | 72.60a-t | 55.71a-i |
| Acc. No. 41053 | 83.10a-e | 45.48a-o | 37.63a-e | 78.76a-k | 65.65a |
| Acc. No. 41054 | 61.61c-s | 42.56a-o | 19.05b-s | 76.26a-p | 45.53a-r |
| Acc. No. 41052 | 69.87a-r | 42.33a-o | 27.55a-r | 78.75a-k | 55.79a-i |
| Acc. No. 209082 | 54.74k-s | 38.60b-o | 16.15d-s | 79.35a-j | 44.57b-r |
| Acc. No. 209083 | 66.90a-s | 39.53a-o | 27.36a-r | 66.69a-z | 44.01b-r |
| Acc. No. 209084 | 67.22a-r | 40.56a-o | 26.65a-s | 83.10a-c | 55.53a-i |
| Acc. No. 209091 | 61.95b-s | 44.60a-o | 17.35d-s | 80.78a-g | 49.26a-n |
| Acc. No. 209087 | 68.14a-r | 49.15a-l | 18.98b-s | 70.15a-x | 47.22a-o |
| Acc. No. 209088 | 63.69a-s | 44.36a-o | 19.33b-s | 65.14b-z | 41.74c-r |
| Acc. No. 209089 | 53.34n-s | 47.08a-o | 6.262p-s | 75.65a-q | 40.73c-r |
| Acc. No. 209090 | 62.33b-s | 31.23i-o | 31.10a-n | 70.14a-x | 43.68b-r |
| Acc. No. 209081 | 56.92h-s | 47.50a-o | 9.418m-s | 77.52a-m | 48.33a-n |
| Acc. No. 41159 | 63.97a-s | 43.74a-o | 20.23b-s | 72.05a-v | 45.58a-r |
| Acc. No. 41160 | 59.17e-s | 34.69c-o | 24.47a-s | 71.75a-v | 45.79a-r |
| Acc. No. 41161 | 69.70a-r | 51.03a-j | 18.67b-s | 72.04a-v | 49.02a-n |
| Acc. No. 207661 | 67.01a-r | 39.60a-o | 27.41a-r | 67.19a-z | 43.21b-r |
| Acc. No. 207667 | 71.57a-r | 45.05a-o | 26.52a-s | 63.95c-z | 45.22a-r |
| Acc. No. 207666 | 76.11a-p | 41.87a-o | 31.43a-n | 63.69c-z | 48.60a-n |
| Acc. No. 41141 | 75.72a-p | 47.88a-o | 27.84a-r | 60.97g-z | 45.38a-r |
| Acc. No. 207665 | 77.24a-n | 52.66a-j | 24.58a-s | 63.03c-z | 48.63a-n |
| Acc. No. 41134 | 67.37a-r | 41.27a-o | 26.09a-s | 63.62c-z | 43.18b-r |
| Acc. No. 41128 | 56.90h-s | 30.31i-o | 26.59a-s | 79.97a-h | 52.82a-l |
| Acc. No. 41168 | 75.63a-p | 54.03a-i | 21.60b-s | 60.81g-z | 45.63a-r |
| Acc. No. 41129 | 58.19g-s | 42.70a-o | 15.49d-s | 62.01e-z | 34.95i-r |
| Acc. No. 41130 | 57.76g-s | 39.42a-o | 18.34b-s | 57.50m-z | 33.53k-r |
| Acc. No. 41110 | 68.75a-r | 50.39a-l | 18.36b-s | 74.94a-q | 50.88a-m |
| Acc. No. 207657 | 52.11p-s | 45.27a-o | 6.845o-s | 70.73a-w | 36.47f-r |
| Acc. No. 41111 | 71.02a-r | 54.43a-i | 16.60d-s | 69.55a-x | 55.86a-i |
| Acc. No. 41106 | 73.28a-q | 50.62a-k | 22.66a-s | 66.52a-z | 51.51a-l |
| Acc. No. 207658 | 64.20a-s | 39.88a-o | 20.57b-s | 75.10a-q | 52.80a-l |
| Acc. No. 41142 | 62.12b-s | 30.96i-o | 34.46a-k | 67.73a-z | 46.66a-q |
| Acc. No. 41207 | 57.51g-s | 39.63a-o | 18.60b-s | 64.62c-z | 42.64c-r |
| Acc. No. 41215 | 83.42a-d | 45.29a-o | 29.51a-o | 54.70r-s | 49.36a-n |
| Acc. No. 41216 | 65.55a-s | 41.52a-o | 33.27a-l | 63.80c-z | 44.91a-r |
| Acc. No. 41066 | 77.47a-m | 53.60a-i | 23.93a-s | 57.47m-z | 49.40a-n |
| Acc. No. 41011 | 58.79f-s | 37.53c-o | 21.26b-s | 59.57j-z | 34.36j-r |
| Acc. No. 41007 | 60.51c-s | 33.34e-o | 27.17a-s | 59.72i-z | 35.56h-r |
| Acc. No. 41008 | 61.38c-s | 38.98b-o | 22.39b-s | 54.25s-z | 32.97l-r |
| Acc. No. 41186 | 63.05b-s | 35.75c-o | 27.30a-s | 78.69a-k | 49.26a-n |
| Acc. No. 209035 | 69.14a-r | 41.50a-o | 27.65a-r | 62.88d-z | 43.13b-r |
| Acc. No. 41176 | 74.64a-p | 42.69a-o | 31.95a-m | 68.38a-x | 50.01a-n |
| Acc. No. 41175 | 63.38a-s | 37.86b-o | 25.52a-s | 70.43a-w | 44.28b-r |
| Acc. No. 41174 | 56.01i-s | 43.29a-o | 12.72h-s | 77.42a-n | 49.00a-n |
| Acc. No. 209027 | 53.49m-s | 33.85d-o | 19.64b-s | 82.67a-d | 44.62b-r |
| Acc. No. 41170 | 62.51b-s | 35.34c-o | 27.17a-s | 68.83a-x | 43.69b-r |

Appendix 7. Continued

| Genotypes | P uptake and use efficiency* | | | | |
|---|---|---|---|---|---|
| | APUfs (%) | APUs (%) | APUf (%) | PYE | PPE |
| Acc. No. 41171 | 64.66a-s | 32.99f-o | 31.67a-m | 73.21a-t | 44.33b-r |
| Acc. No. 41185 | 73.69a-q | 56.66a-h | 17.03d-s | 69.30a-x | 50.85a-m |
| Acc. No. 209036 | 52.52p-s | 47.45a-o | 5.063rs | 77.31a-n | 43.15b-r |
| Acc. No. 41190 | 62.16b-s | 48.16a-o | 13.99f-s | 67.51a-z | 42.18c-r |
| Acc. No. 41195 | 59.47d-s | 33.19f-o | 26.28a-s | 67.32a-z | 39.01d-r |
| Acc. No. 41197 | 54.00k-s | 30.52i-o | 23.48a-s | 66.99a-z | 34.86i-r |
| Acc. No. 207150 | 60.08d-s | 41.26a-o | 18.82b-s | 86.43a | 51.79a-l |
| Acc. No. 207151 | 58.92f-s | 30.35i-o | 28.56a-p | 67.04a-z | 39.53d-r |
| Acc. No. 207563 | 66.70a-s | 48.95a-m | 17.75d-s | 84.97ab | 56.45a-h |
| Acc. No. 207564 | 69.51a-r | 47.13a-o | 22.38b-s | 64.42c-z | 44.58b-r |
| Acc. No. 207894 | 62.78b-s | 49.69a-l | 13.09g-s | 79.20a-k | 49.41a-n |
| Acc. No. 207895 | 58.55g-s | 28.17j-o | 30.38a-n | 75.99a-q | 43.78b-r |
| Acc. No. 213224 | 75.82a-p | 46.12a-o | 29.70a-o | 48.27yz | 35.82h-r |
| Acc. No. 219797 | 66.16a-s | 47.27a-o | 18.89b-s | 68.04a-y | 44.92a-r |
| Acc. No. 219799 | 56.26i-s | 47.33a-o | 8.928m-s | 80.85a-g | 45.37a-r |
| Acc. No. 219800 | 74.14a-q | 47.70a-o | 26.44a-s | 76.56a-o | 56.99a-g |
| Acc. No. 219803 | 68.06a-r | 48.51a-n | 19.55b-s | 76.52a-o | 52.24a-l |
| Acc. No. 221696 | 77.96a-l | 47.98a-o | 29.97a-o | 67.53a-z | 52.53a-l |
| Acc. No. 41114 | 66.09a-s | 44.19a-o | 21.90b-s | 68.31a-y | 51.05a-m |
| Acc. No. 212589 | 73.17a-q | 45.33a-o | 27.84a-r | 71.43a-w | 57.28a-f |
| Acc. No. 41113 | 55.71i-s | 47.30a-o | 8.416n-s | 74.75a-r | 47.65a-n |
| Acc. No. 207659 | 72.65a-q | 42.18a-o | 30.46a-n | 74.85a-r | 52.72a-l |
| Acc. No. 207660 | 65.98a-s | 36.69c-o | 29.29a-p | 67.10a-z | 44.31b-r |
| Acc. No. 41115 | 61.18c-s | 29.83i-o | 31.35a-n | 82.70a-d | 50.06a-n |
| Acc. No. 225878 | 64.16a-s | 23.17o | 40.99a-c | 57.94m-z | 37.26f-r |
| Acc. No. 225873 | 65.22a-s | 52.41a-j | 12.81h-s | 72.80a-t | 47.16a-o |
| Acc. No. 225874 | 55.43j-s | 25.46l-o | 29.98a-o | 72.83a-t | 39.50d-r |
| Acc. No. 225877 | 63.81a-s | 47.84a-o | 15.97d-s | 72.35a-u | 45.98a-r |
| Acc. No. 207645 | 76.00a-p | 40.84a-o | 35.16a-i | 71.99a-u | 55.02a-j |
| Acc. No. 207646 | 79.36a-j | 54.69a-i | 24.67a-s | 65.52b-z | 52.27a-l |
| Acc. No. 225876 | 69.82a-r | 43.71a-o | 26.11a-s | 69.42a-x | 48.74a-n |
| ICC 5003 | 62.91b-s | 41.68a-o | 21.24b-s | 67.40a-z | 42.71c-r |
| ICC 4918 | 78.01a-k | 39.46a-o | 38.54a-d | 76.42a-p | 59.64a-d |
| ICC 4948 | 74.76a-p | 33.40e-o | 41.35ab | 67.36a-z | 50.55a-m |
| ICC 4973 | 50.53q-s | 38.69b-o | 11.84j-s | 50.21x-z | 25.60r |
| ICC 15996 | 70.82a-r | 38.82b-o | 32.00a-m | 68.16a-y | 48.94a-n |
| Shasho | 55.03k-s | 45.59a-o | 9.447m-s | 47.41z | 25.84qr |
| Arerti | 60.89c-s | 25.77k-o | 35.12a-i | 68.68a-x | 41.40c-r |
| Worku | 63.99a-s | 34.25c-o | 29.74a-o | 66.77a-z | 40.56c-r |
| Akaki | 48.58rs | 37.59c-o | 10.99l-s | 75.23a-q | 36.58f-r |
| Ejere | 70.54a-r | 56.79a-h | 13.75f-s | 66.76a-z | 48.40a-n |
| Teji | 79.30a-j | 53.89a-i | 25.41a-s | 56.33p-z | 46.61a-q |
| Habru | 79.62a-i | 33.92d-o | 45.70a | 71.89a-v | 57.43a-f |
| Natoli | 76.83a-o | 43.90a-o | 32.93a-l | 65.54b-z | 49.80a-n |
| ICC 19180 | 63.62a-s | 45.86a-o | 17.76d-s | 66.54a-z | 50.07a-n |
| ICC 19181 | 75.92a-p | 57.91a-f | 18.01c-s | 38.16z | 29.30n-r |
| PM 233 | 57.38g-s | 46.92a-o | 10.46l-s | 47.93z | 26.58o-r |
| Mean | 65.75 | 42.57 | 23.15 | 68.52 | 45.80 |

*APUfs (%) = apparent use of P from fertilizer and soil, APUf (%) = apparent use of P from fertilizer, APUs (%) = apparent use of P from soil, PYE = phosphorus yield efficiency (GY/P applied, g/g) and PPE = phosphorus physiological efficiency (GY/P in plant, g/g).

Appendix 8. Mean number of eggs laid by each female, days to adult emergence, number of adults emerged, adult recovery (%), seed weight loss and seed size in 130 chickpea genotypes after infestation by the adzuki bean beetle at three locations in Ethiopia

| Genotype | No of eggs/ female | Days to adult emergence* | | No of adult emerged | | Adult recovery (%) | Seed weight loss (g) | | 1000 swt (g) |
|---|---|---|---|---|---|---|---|---|---|
| | | FP | SP | FP | SP | | FP | SP | |
| Acc. No. 231327 | 31a-e | 32ab | 34ab | 123b-g | 545e-h | 62e-m | 5 d-l | 12k-t | 103g-k |
| Acc. No. 231328 | 28b-e | 32ab | 35ab | 156a-g | 577e-h | 76a-k | 3 g-l | 10 o-t | 112f-k |
| Acc. No. 209093 | 29b-e | 32ab | 34ab | 151a-g | 523f-h | 78a-i | 6 d-l | 12 j-t | 119f-k |
| Acc. No. 208829 | 33a-e | 32ab | 34ab | 156a-g | 557e-h | 75a-l | 5 d-l | 12 i-t | 102g-k |
| Acc. No. 209094 | 34a-e | 32ab | 34ab | 161a-g | 509f-h | 71a-m | 4 g-l | 10 n-t | 124f-k |
| Acc. No. 209096 | 35a-e | 32ab | 35ab | 169a-g | 530f-h | 70a-m | 6 d-l | 13 g-t | 122f-k |
| Acc. No. 209097 | 41a-e | 32ab | 34ab | 221a | 576e-h | 76a-k | 5 d-l | 12 i-t | 130e-k |
| Acc. No. 209098 | 38a-e | 32ab | 34ab | 190a-g | 502f-h | 74a-l | 10 a-c | 16 c-h | 105g-k |
| Acc. No. 41002 | 29b-e | 33a | 34ab | 136a-g | 612d-h | 69a-m | 5 d-l | 13 g-t | 141d-i |
| Acc. No. 207761 | 40a-e | 32ab | 35ab | 186a-g | 569e-h | 65c-m | 5 d-l | 12 i-t | 128e-k |
| Acc. No. 207763 | 27c-e | 32ab | 34ab | 158a-g | 584e-h | 80a-d | 4 g-l | 11 l-t | 100g-k |
| Acc. No. 41026 | 34a-e | 32ab | 34ab | 166a-g | 562e-h | 72a-m | 6 d-l | 13 g-t | 89i-k |
| Acc. No. 41074 | 30b-e | 32ab | 34ab | 152a-g | 550e-h | 70a-m | 5 d-l | 11 l-t | 94i-k |
| Acc. No. 41075 | 34a-e | 32ab | 34ab | 172a-g | 492f-h | 74a-l | 5 d-l | 11 l-t | 94i-k |
| Acc. No. 41076 | 33a-e | 32ab | 34ab | 151a-g | 561e-h | 65c-m | 3 i-l | 10 n-t | 97h-k |
| Acc. No. 41021 | 27b-e | 32ab | 35ab | 144a-g | 513f-h | 75a-l | 4 g-l | 10 n-t | 94i-k |
| Acc. No. 41027 | 31b-e | 31b | 34ab | 152a-g | 586e-h | 71a-m | 5 d-l | 12 i-t | 105g-k |
| Acc. No. 207734 | 36a-e | 32ab | 34ab | 170a-g | 530f-h | 67b-m | 5 d-l | 11 l-t | 109g-k |
| Acc. No. 41103 | 30b-e | 32ab | 34ab | 147a-g | 464gh | 73a-m | 4 g-l | 10 n-t | 88jk |
| Acc. No. 41320 | 29b-e | 32ab | 35ab | 112fg | 511f-h | 56m | 3 i-l | 9 st | 102g-k |
| Acc. No. 41029 | 28b-e | 32ab | 34ab | 133a-g | 533f-h | 64c-m | 3 i-l | 10 n-t | 107g-k |
| Acc. No. 41015 | 30b-e | 32ab | 35ab | 142a-g | 549e-h | 76a-k | 5 d-l | 11 l-t | 95i-k |
| Acc. No. 41271 | 34a-e | 32ab | 35ab | 179a-g | 520f-h | 73a-m | 4 g-l | 10 n-t | 120f-k |
| Acc. No. 41276 | 29b-e | 32ab | 34ab | 129a-g | 641d-h | 65c-m | 4 g-l | 13 g-t | 117f-k |
| Acc. No. 207745 | 39a-e | 32ab | 34ab | 181a-g | 504f-h | 71a-m | 5 d-l | 11 l-t | 99h-k |
| Acc. No. 41275 | 33a-e | 32ab | 35ab | 154a-g | 609d-h | 66b-m | 4 g-l | 11 l-t | 99h-k |
| Acc. No. 41277 | 34a-e | 32ab | 35ab | 174a-g | 665d-h | 70a-m | 4 g-l | 12 i-t | 107g-k |
| Acc. No. 207743 | 30b-e | 33a | 34ab | 161a-g | 573e-h | 76a-k | 6 d-l | 13 g-t | 109g-k |
| Acc. No. 207744 | 31a-e | 32ab | 33b | 123b-g | 576e-h | 60i-m | 6 d-l | 14 g-n | 108g-k |
| Acc. No. 41273 | 30b-e | 32ab | 34ab | 164a-g | 551e-h | 76a-k | 5 d-l | 12 i-t | 117f-k |
| Acc. No. 41274 | 36a-e | 32ab | 34ab | 167a-g | 561e-h | 68a-m | 5 d-l | 12 i-t | 125f-k |
| Acc. No. 207741 | 41a-e | 32ab | 34ab | 198a-g | 495f-h | 69a-m | 6 d-l | 12 i-t | 119f-k |
| Acc. No. 207742 | 33a-e | 32ab | 34ab | 170a-g | 526f-h | 74a-m | 5 d-l | 12 i-t | 119f-k |
| Acc. No. 41316 | 36a-e | 32ab | 34ab | 170a-g | 519f-h | 71a-m | 6 d-l | 13 g-t | 107g-k |
| Acc. No. 41298 | 34a-e | 32ab | 34ab | 134a-g | 630d-h | 66c-m | 5 d-l | 20 bc | 104g-k |
| Acc. No. 41311 | 34a-e | 32ab | 34ab | 162a-g | 562e-h | 71a-m | 4 g-l | 10 n-t | 110f-k |
| Acc. No. 41313 | 39a-e | 32ab | 34ab | 191a-g | 523f-h | 72a-m | 4 g-l | 10 n-t | 111f-k |
| Acc. No. 41280 | 30b-e | 32ab | 33b | 127a-g | 588e-h | 65c-m | 4 g-l | 11 l-t | 101g-k |
| Acc. No. 41312 | 34a-e | 32ab | 34ab | 155a-g | 512f-h | 66b-m | 6 d-l | 12 i-t | 99h-k |
| Acc. No. 41315 | 31a-e | 32ab | 35ab | 145a-g | 479f-h | 67b-m | 4 g-l | 9 r-t | 85k |
| Acc. No. 41308 | 33a-e | 32ab | 34ab | 179a-g | 592d-h | 78a-h | 6 d-l | 13 g-t | 105g-k |
| Acc. No. 41299 | 28b-e | 32ab | 34ab | 154a-g | 576e-h | 78a-h | 6 d-l | 13 g-t | 119f-k |
| Acc. No. 41046 | 35a-e | 32ab | 34ab | 168a-g | 501f-h | 67b-m | 4 g-l | 10 n-t | 104g-k |
| Acc. No. 41047 | 35a-e | 32ab | 35ab | 148a-g | 573e-h | 61f-m | 5 d-l | 12 i-t | 106g-k |
| Acc. No. 41304 | 32a-e | 32ab | 34ab | 182a-g | 561e-h | 82a-c | 5 d-l | 12 i-t | 96h-k |
| Acc. No. 41303 | 40a-e | 32ab | 34ab | 181a-g | 586e-h | 64d-m | 5 d-l | 12 i-t | 100g-k |

Appendix 8. Continued

| Genotype | No of eggs/ female | Days to adult emergence* | | No of adult emerged | | Adult recovery (%) | Seed weight loss (g) | | 1000 swt (g) |
|---|---|---|---|---|---|---|---|---|---|
| | | FP | SP | FP | SP | | FP | SP | |
| Acc. No. 41295 | 30b-e | 32ab | 34ab | 129a-g | 557e-h | 65c-m | 5 d-l | 11 l-t | 101g-k |
| Acc. No. 41296 | 30b-e | 32ab | 34ab | 139a-g | 557e-h | 71a-m | 3 i-l | 9 r-t | 110f-k |
| Acc. No. 41289 | 27c-e | 33a | 34ab | 121c-g | 495f-h | 67b-m | 3 i-l | 9 t | 93i-k |
| Acc. No. 41290 | 34a-e | 32ab | 35ab | 176a-g | 565e-h | 66c-m | 7 c-h | 14 g-n | 102g-k |
| Acc. No. 41291 | 33a-e | 33a | 35ab | 131a-g | 501f-h | 58lm | 4 g-l | 10 n-t | 101g-k |
| Acc. No. 41297 | 31a-e | 32ab | 34ab | 143a-g | 598d-h | 64d-m | 5 d-l | 12 i-t | 100g-k |
| Acc. No. 41293 | 31b-e | 32ab | 35ab | 165a-g | 559e-h | 75a-l | 4 g-l | 10 n-t | 103g-k |
| Acc. No. 41048 | 30b-e | 32ab | 34ab | 117d-g | 572e-h | 65c-m | 5 d-l | 13 g-t | 109g-k |
| Acc. No. 41053 | 37a-e | 32ab | 34ab | 164a-g | 568e-h | 64d-m | 5 d-l | 12 i-t | 116f-k |
| Acc. No. 41054 | 29b-e | 33a | 33ab | 122b-g | 593d-h | 67b-m | 3 i-l | 10 n-t | 92i-k |
| Acc. No. 41052 | 31b-e | 32ab | 33ab | 133a-g | 525f-h | 68a-m | 6 d-l | 12 i-t | 99h-k |
| Acc. No. 209084 | 30b-e | 33a | 33ab | 141a-g | 513f-h | 73a-m | 4 g-l | 10 n-t | 96i-k |
| Acc. No. 209091 | 32a-e | 32ab | 34ab | 158a-g | 570e-h | 79a-e | 6 d-l | 14 g-n | 104g-k |
| Acc. No. 209087 | 31b-e | 32ab | 34ab | 136a-g | 517f-h | 67b-m | 5 d-l | 11 l-t | 98h-k |
| Acc. No. 209088 | 33a-e | 33a | 34ab | 184a-g | 502f-h | 79a-f | 5 d-l | 11 l-t | 115f-k |
| Acc. No. 209089 | 36a-e | 32ab | 34ab | 167a-g | 468gh | 68a-m | 5 d-l | 10 n-t | 100g-k |
| Acc. No. 209090 | 43a-d | 32ab | 34ab | 205a-f | 498f-h | 67b-m | 5 d-l | 12 i-t | 89i-k |
| Acc. No. 41159 | 32a-e | 32ab | 33b | 152a-g | 500f-h | 68 a-m | 6 d-l | 12 i-t | 100 g-k |
| Acc. No. 41160 | 26c-e | 32ab | 35ab | 135a-g | 573e-h | 69 a-m | 4 g-l | 12 i-t | 100 g-k |
| Acc. No. 41161 | 22e | 32ab | 33b | 104g | 614d-h | 65c-m | 2 l | 9 t | 106g-k |
| Acc. No. 207661 | 35a-e | 32ab | 35ab | 164a-g | 510f-h | 72a-m | 6 d-l | 12 i-t | 98h-k |
| Acc. No. 207667 | 34a-e | 32ab | 34ab | 166a-g | 521f-h | 72a-m | 7 c-h | 14 g-n | 103g-k |
| Acc. No. 207666 | 44a-d | 32ab | 34ab | 212a-d | 473gh | 72a-m | 6 d-l | 13 g-t | 109g-k |
| Acc. No. 41141 | 32a-e | 33a | 35ab | 149a-g | 605d-h | 69a-m | 5 d-l | 12 i-t | 109g-k |
| Acc. No. 207665 | 31b-e | 32ab | 34ab | 131a-g | 577e-h | 67b-m | 5 d-l | 11 l-t | 101g-k |
| Acc. No. 41134 | 30b-e | 32ab | 35ab | 142a-g | 490f-h | 61e-m | 4 d-l | 11 l-t | 98h-k |
| Acc. No. 41128 | 31b-e | 32ab | 34ab | 115e-g | 578e-h | 59k-m | 5 d-l | 11 l-t | 96i-k |
| Acc. No. 41168 | 36a-e | 32ab | 34ab | 156a-g | 455h | 61e-m | 5 d-l | 11 l-t | 94i-k |
| Acc. No. 41129 | 32a-e | 32ab | 34ab | 161a-g | 532f-h | 66b-m | 4 g-l | 11 l-t | 97h-k |
| Acc. No. 41130 | 34a-e | 32ab | 34ab | 181a-g | 462gh | 75a-l | 6 d-l | 12 i-t | 100g-k |
| Acc. No. 41110 | 32a-e | 32ab | 34ab | 161a-g | 590d-h | 75a-l | 5 d-l | 12 i-t | 104g-k |
| Acc. No. 41111 | 33a-e | 32ab | 34ab | 155a-g | 520f-h | 70a-m | 7 c-h | 12 i-t | 97h-k |
| Acc. No. 207658 | 33a-e | 32ab | 35ab | 144a-g | 503f-h | 59 j-m | 4 g-l | 10 n-t | 93i-k |
| Acc. No. 41142 | 27c-e | 32ab | 35ab | 135a-g | 504f-h | 74a-l | 5 d-l | 12 i-t | 112f-k |
| Acc. No. 41207 | 26c-e | 32ab | 34ab | 113fg | 545e-h | 72a-m | 4 g-l | 11 l-t | 107g-k |
| Acc. No. 41215 | 27c-e | 33a | 34ab | 117d-g | 606d-h | 68a-m | 4 g-l | 12 i-t | 99h-k |
| Acc. No. 41066 | 35a-e | 32ab | 34ab | 155a-g | 521f-h | 75a-k | 5 d-l | 11 l-t | 120f-k |
| Acc. No. 41011 | 26c-e | 32ab | 35ab | 158a-g | 603d-h | 85a | 3 i-l | 10 n-t | 104g-k |
| Acc. No. 41007 | 35a-e | 32ab | 34ab | 184a-g | 525f-h | 79a-g | 6 d-l | 12 i-t | 94i-k |
| Acc. No. 41008 | 24d-e | 32ab | 34ab | 110fg | 623d-h | 64d-m | 3 i-l | 11 l-t | 98h-k |
| Acc. No. 209035 | 27c-e | 32ab | 34ab | 142a-g | 571e-h | 73a-m | 4 g-l | 11 l-t | 102g-k |
| Acc. No. 41176 | 36a-e | 33a | 34ab | 175a-g | 833b-e | 71a-m | 8 b-d | 18 b-f | 152d-g |
| Acc. No. 41175 | 36a-e | 32ab | 34ab | 186a-g | 551e-h | 77a-i | 4 g-l | 11 l-t | 139d-j |
| Acc. No. 41174 | 28b-e | 32ab | 35ab | 139a-g | 509f-h | 72a-m | 5 d-l | 11 l-t | 102g-k |
| Acc. No. 41170 | 30b-e | 33a | 33ab | 149a-g | 593d-h | 72a-m | 7 c-h | 14 g-n | 110g-k |
| Acc. No. 41171 | 29b-e | 32ab | 34ab | 139a-g | 588e-h | 68a-m | 4 d-l | 11 l-t | 101g-k |
| Acc. No. 41185 | 34a-e | 32ab | 34ab | 146a-g | 516f-h | 69a-m | 5 d-l | 11 l-t | 97h-k |
| Acc. No. 209036 | 29b-e | 32ab | 34ab | 125b-g | 553e-h | 61g-m | 3 i-l | 18 b-f | 98h-k |
| Acc. No. 41190 | 36a-e | 32ab | 34ab | 169a-g | 524f-h | 69a-m | 5 d-l | 11 l-t | 113f-k |

Appendix 8. Continued

| Genotype | No of eggs/ female | Days to adult emergence* | | No of adult emerged | | Adult recovery (%) | Seed weight loss (g) | | 1000 swt (g) |
|---|---|---|---|---|---|---|---|---|---|
| | | FP | SP | FP | SP | | FP | SP | |
| Acc. No. 41195 | 32a-e | 32ab | 35ab | 154a-g | 504f-h | 68a-m | 6 d-l | 11 l-t | 113f-k |
| Acc. No. 207151 | 31b-e | 32ab | 34ab | 176a-g | 536e-h | 84ab | 6 d-l | 14 g-n | 100g-k |
| Acc. No. 207563 | 28b-e | 32ab | 34ab | 124b-g | 642d-h | 64d-m | 4 g-l | 11 l-t | 108g-k |
| Acc. No. 207564 | 31b-e | 32ab | 37a | 166a-g | 630d-h | 71a-m | 4 g-l | 11 l-t | 106g-k |
| Acc. No. 207895 | 32a-e | 32ab | 35ab | 155a-g | 508f-h | 68a-m | 4 g-l | 18 b-f | 115f-k |
| Acc. No. 219797 | 38a-e | 32ab | 35ab | 179a-g | 555e-h | 70a-m | 5 d-l | 11 l-t | 108g-k |
| Acc. No. 219799 | 37a-e | 32ab | 35ab | 181a-g | 577e-h | 72a-m | 4 g-l | 11 l-t | 113f-k |
| Acc. No. 219800 | 32 a-e | 32ab | 34ab | 132a-g | 623d-h | 65c-m | 5 d-l | 14 g-n | 111f-k |
| Acc. No. 219803 | 27c-e | 32ab | 35ab | 146a-g | 628d-h | 77a-k | 5 d-l | 11 l-t | 112f-k |
| Acc. No. 221696 | 32a-e | 32ab | 33b | 144a-g | 584e-h | 73a-m | 4 g-l | 11 l-t | 108g-k |
| Acc. No. 41114 | 29b-e | 32ab | 34ab | 147a-g | 535f-h | 82a-c | 5 d-l | 18 b-f | 110f-k |
| Acc. No. 212589 | 32a-e | 32ab | 34ab | 171a-g | 543e-h | 74a-l | 4 d-l | 11 l-t | 102g-k |
| Acc. No. 41113 | 38a-e | 32ab | 34ab | 163a-g | 522f-h | 60h-m | 6 d-l | 11 l-t | 108g-k |
| Acc. No. 207659 | 36a-e | 32ab | 33b | 181a-g | 497f-h | 73a-m | 6 d-l | 14 g-n | 108g-k |
| Acc. No. 207660 | 28b-e | 33a | 34ab | 127a-g | 656d-h | 66b-m | 4 g-l | 11 l-t | 106g-k |
| Acc. No. 225878 | 35a-e | 32ab | 35ab | 155a-g | 523f-h | 75a-l | 4 d-l | 11 l-t | 110f-k |
| Acc. No. 225873 | 45a-d | 32ab | 34ab | 205a-f | 594d-h | 64d-m | 5 d-l | 18 b-f | 110f-k |
| Acc. No. 225874 | 27c-e | 32ab | 34ab | 158a-g | 570e-h | 80a-d | 5 d-l | 11 l-t | 112f-k |
| Acc. No. 225877 | 33a-e | 32ab | 34ab | 182a-g | 591d-h | 79a-g | 6 d-l | 11 l-t | 132d-k |
| Acc. No. 207645 | 42a-e | 32ab | 34ab | 211a-d | 487f-h | 74a-l | 6 d-l | 14 g-n | 107g-k |
| Acc. No. 207646 | 37a-e | 32ab | 34ab | 181a-g | 554e-h | 68a-m | 6 d-l | 11 l-t | 121f-k |
| Acc. No. 225876 | 34a-e | 32ab | 34ab | 191a-g | 607d-h | 78a-i | 3 i-l | 11 l-t | 116f-k |
| ICC 5003 | 46a-c | 33a | 34ab | 210a-e | 1024ab | 69a-m | 12 ab | 18 b-f | 184b-d |
| ICC 4918 | 43a-d | 32ab | 34ab | 213a-c | 886a-d | 75a-l | 5 d-l | 11 l-t | 178c-e |
| ICC 4948 | 35a-e | 32ab | 34ab | 161a-g | 703c-h | 69a-m | 6 d-l | 11 l-t | 95i-k |
| ICC 4973 | 35a-e | 31b | 34ab | 179a-g | 965a-c | 74a-l | 5 d-l | 14 g-n | 136d-k |
| ICC 15996 | 34a-e | 33a | 34ab | 169a-g | 772b-f | 76a-k | 7 c-h | 11 l-t | 149d-h |
| Shasho | 45a-c | 32ab | 33b | 190a-g | 1001ab | 66b-m | 13 al | 11 l-t | 162d-f |
| Arerti | 37a-e | 32ab | 33b | 171a-g | 969a-c | 76a-k | 4 g-l | 18 b-f | 177c-e |
| Worku | 36a-e | 32ab | 34ab | 157a-g | 751b-h | 63d-m | 4 g-l | 11 l-t | 134d-k |
| Akaki | 33a-e | 32ab | 35ab | 176a-g | 758b-g | 78a-i | 3 i-l | 11 l-t | 136d-k |
| Ejere | 48ab | 31b | 34ab | 198a-g | 1035ab | 64d-m | 12 ab | 14 g-n | 276a |
| Teji | 45a-c | 31b | 34ab | 211a-d | 1136a | 77a-j | 8 b-d | 11 l-t | 227a-c |
| Habru | 51a | 31b | 34ab | 222a | 964a-c | 67b-m | 8 b-d | 11 l-t | 236ab |
| Natoli | 44a-d | 32ab | 34ab | 217ab | 1042ab | 79a-f | 7 c-h | 18 b-f | 217bc |
| Mean | 33 | 32 | 34 | 160 | 590 | 70 | 5 | 12 | 114 |

*Figures sharing no similar letters within columns are statistically significant; FP = first progeny; SP = second progeny