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MORPHOLOGICAL AND RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER VARIATION ANALYSIS IN SOME ETHIOPIAN DROUGHT TOLERANT AND SUSCEPTIBLE CHICKPEA (CICER ARIENTINUM *L.) GENOTYPES*

By

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MORPHOLOGICAL AND RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER VARIATION ANALYSIS IN SOME EHIOPIAN DROUGHT TOLERANT AND SUSCEPTIBLE CHICKPEA (CICER ARIENTINUM *L.) GENOTYPES*

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Melese Dadi

A thesis presented to the Graduate Studies Program, Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Science in Biology (*Applied genetics*)

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ABSTRACT

Seventeen chickpea genotypes were planted in Randomized block design with four replications at Debre Zeit Agricultural Research Center during 2003/2004 cropping season. Ten plants were tagged for morphological data collection. Both fifteen quantitative and five qualitative traits were recorded. RAPD study was conducted at the Genetics Research Laboratory, Department of Biology, Addis Ababa University. The study was conducted to assess the genetic diversity of chickpea using both morphological and RAPD data, heritability and correlation of quantitative trait.

Shannon diversity, analyses of variance, correlation coefficient, heritability and genetic advance, phenotypic and genotypic variance and principal component were computed from morphological traits using SPSS and SAS Software Programs. RAPD data generated from bands recorded was used for computing gene diversity, DNA polymorphism and Jaccard's similarity via POPGENE software.

Shannon diversity index showed the availability of variations within chickpea populations. Similarly, high variation within and between chickpea genotypes was recorded for quantitative traits evaluated except for days to flowering and maturity, plant height, canopy width and leaflet/leaf. OPC-02 primer used for RAPD marker showed that RAPD can discriminate chickpea population in diversity analysis of chickpea population.

High heritability and with genetic advance recorded for some traits such as hundred seed weight, biological yield/plant, grain yield/plant, days to maturity and flowering, number of primary and secondary branches/plant, number of seeds and pods/plant, harvest index and root biomass indicated that progress in selection could be high for such traits in chickpea improvement.

Strong and positive significant correlation observed between biological and grain yield, biological yield and number of seeds/plant, biological yield and number of pods, and grain yield with number seeds and pods/plant, number of pods/plant and number of seeds/plant showed that their improvement lead to yield improvement in chickpea. As confirmed by the principal component analysis, these quantitative traits had also high contribution for chickpea variability and hence, can be used as a source of chickpea improvement.

Key words: Chickpea, diversity, RAPD

1. INTRODUCTION

Chickpea (*Cicer arientimum* L.) is one of the first grain legumes domesticated in old world (Van der Maesen, 1987). It has been cultivated for centuries in the Middle East, Asia, India, the Mediterranea and Ethiopia (Westphal, 1974). Chickpea is used as supplies of food for human being (Yetneberk, 1990 and Westphal, 1974) and used in rotation with several cereals like tef or wheat on heavy soils (Westphal, 1974; Debela *et al.* 1987; Bejiga *et al.* 1996).

An average chickpea yield on farmers field is usually below 1t/ha although its potential is more than 5t/ha (Jagdish *et al.* 1995; Bejiga *et al.* 1998). This is resulted from susceptibility of landraces to frost, drought, water logging and poor cultural practices; low or no protection measures against weeds, diseases and insect pests (Tilaye *et al.* 1994; Bejiga *et al.* 1996). Crop's improvement depends on the availability of gene for better agronomic traits, disease resistance, earliness and high yield (Hunter, 1996; Winter, 1997 and Gupta, 2003).

For this, characterization of genetic variability of a population is required since genetic variation within population and between species determines the rate of adaptive evolution and response to traditional crop improvement (Hunter, 1996). Genetic diversity is a raw material for evolution, thus enabling populations of species to survive, evolve and adapt to resist long-term changes in the environment. This is very important in the plant breeding strategies for developing high yielding varieties and for maintaining the productivity of such varieties through introduction of genes for resistance to disease, insect pests and other abiotic factor (Winter, 1997). Genetic diversity of domestic species allows people to act as

agents of selection and develop different forms of the same species for a variety of purposes, including enabling the producers to grow the same species in different environments, each with a different requirement of climate, pathogens, predators, competitors etc. (Hunter, 1996**).** Differences within and between plants can be a strategic value to conservation as they provide a clear justification for protecting a species across its entire geographic range and all the subspecies of major populations.

There are few studies on diversity analysis of Ethiopian chickpea landraces. Feven (2002) studied morphological and isoenzyme diversity of Ethiopian chickpea. Anbessa and Bejiga (2002) had also evaluated and screened 482 chickpea landraces collected from different regions for their tolerance to drought. Similarly, Bejiga and Anbessa (1994) evaluated chickpea genotypes for their drought tolerance. Characterizations of such genotypes via morphological, biochemical and DNA markers for their genetic variation will help plant breeder to utilize them in the chickpea improvement. . However, there is no information on the genetic diversity of these drought tolerant and susceptible chickpea genotypes. Therefore, this study was conducted to fill this gap by evaluating diversity of these genotypes using both morphological and RAPD traits.

1.1 OBJECTIVES

The general objective of the present study is to evaluate the diversity of the drought tolerant and susceptible chickpea genotypes. The specific objectives include:

- to study morphological and RAPD genetic variation of some of Ethiopian drought tolerant and susceptible chickpea genotypes
- to determine the correlation between quantitative traits
- to evaluate heritability and genetic advance of quantitative traits

2. LITERATURE REVIEW

2.1 CHICKPEA

Western Asia and South Europe are probably the origin of chickpeas (Westphal, 1974). It has been extensively cultivated in the Indian subcontinent and the Middle East during the cool season of the year and was introduced to tropical Africa, Central and South America and Australia. Chickpea is found only under cultivation and sometimes escapes from cultivation though unable to colonize successfully without human intervention (Van der Maesen, 1984). In Ethiopia, chickpea is cultivated at an altitude ranging from1400-2300 meter above sea level (m.a.s.l) and requires annual rainfall ranging from 700-2000 mm on vertisol with pH 6.4-7.9 (Debela *et al*. 1987). It is mostly produced in northern and central highlands of Ethiopia, which includes Shoa, Gojam, Tigray, Southwestern Wollo and Gonder (Bejiga, 1980). The crop grows under rain fed conditions after the end of rainy season (Bejiga and Tulu, 1982, Debela *et al.* 1987).

There are two types of chickpea cultivated in the world. These are called *desi*, which have small angular colored seeds and *kabuli* chickpea, which are characterized with ram shaped, white/yellow/pale cream colored seeds. Desi type is cultivated widely in Ethiopia (Singh and Malhotra, 1984; Bejiga *et al*., 1996). Approximately, more than 85% of the area is covered with desi types and the rest 15% is covered with kabuli type (Singh and Malhotra, 1984). The desi chickpea is grown in the Idian sub-continent and Ethiopia while kabuli type is cultivated in the Mediterrean region and Latin America (Singh and Malhotra, 1984). A wide diversity is available in the cultivated chickpea and greater diversity is found in wild species, in which only seed color, size, and anthocyanin content distinguishes

accessions (Van der Maesen, 1984). The greatest amount of diversity of chickpea is found in its areas of origin that is the region adjoining Turkey, Iran, Afganstan and USSR (Singh and Malhotra, 1984).

Chickpea is an annual crop that grows best on heavy soil; rough seed bed and require moderately high temperature (Westphal, 1974). It also grows on poor soils and it cannot tolerate heavy rains. Hence, it fails to grow in wet tropical areas. Several factors, including the length of growing period, mean air temperature, soil drainage, soil reaction, soil depth, occurrence of soil borne diseases etc. determine the adaptation and performance of chickpea (Anbessa, 2003).

Chickpea provides multiple benefits to growers. It is consumed in various ways and plays a substantial role in subsistence farming system. It is a good source of dietary; particularly it supplies protein to poor and thus known as poor man's meat. Seed, young shoots and pods are used for human consumption. Threshed and the dried vines are used as cattle feed. Seeds are eaten raw, cooked or boiled, prepared by splitting the seeds and removing the husk (Westphal, 1974). In Ethiopia, seeds are consumed raw, roasted or in 'wot'. Sometimes, the flour is mixed with other crops for preparing injera and also unleavened bread. Green pods and tender shoots are used as a vegetable. The roasted and salted chickpea is used as snack. It can also be mixed with cereals and root crops as a protein supplement in preparing "fafa" (Yetneberk, 1990). It is also an important legume crop used in rotation with several cereals like tef or wheat on heavy soils (Westphal, 1974; Debela *et al.,* 1987; Bejiga *et al*., 1996).

Chickpea is a good source of carbohydrate and protein and the two together constitutes

about 80% of the total dry seed mass (Geervani, 1990). In addition, chickpea has hypocholchlorestermic and hypolipidemic effect, and is effective in reducing cholesterol levels (Geervani, 1990). It has been found to control diabetics.

Chickpea yield reduction was resulted from several biotic and abiotic factors including susceptibility of landraces to frost, drought, water logging and poor cultural practices; low or no protection measures against weeds, diseases and insect pests and inherently low grain yielding potential of landraces (Tilaye *et al.,* 1994; Bejiga *et al.,* 1996). Jagdish *et al* (1995) showed that diseases and drought were the major causes for yield failure in chickpea. Saxena *et al.* (1993) estimated that there was a yield loss ranging from 30-60% due to drought, depending on geographic, location and climatic conditions during cropping season. There may be also total loss of yield under worst drought year (Singh and Saxena, 1996). Saxena (1987) indicated that water deficiency accounts for nearly 50% of variation in chickpea production caused by both biotic and abiotic factors. In Ethiopia, advancing chickpea planting to early September increased a yield in more than 50 % as compared to the traditional farmers practice (Bejiga and Tulu, 1982), indicating that water stress significantly reduces the yield. Similar research findings were recorded in Western and Northern Asian countries and Semi arid tropical areas (Johasen *et al*., 1986).

2.2 GENETIC DIVERSITY

Genetic diversity is defined as the extent to which heritable material differs within a group of plants (Van Hintum, 1995). Genetic differentiation is the extent to which heritable material differs between groups of plant and it is the result of evolution, including domestication and plant breeding. The processes of natural evolution resulted in a build up

of genetic diversity in natural populations whereas domestication caused further differentiation of small parts of the diversity of wild species, which became adapted to human requirement. Genetic diversity can be assessed at four levels of organization: among species and among populations, within populations and within individuals (Hunter, 1996). Subdividing the variation into its components may assist in genetic conservation and utilization, and establishment of in situ gene conservation (Bekele, 1985; Peceti and Damania, 1996; Tsehaye and Kebebew, 2002).

Species with greater genetic diversity are more likely to be able to evolve in response to a changing environment than those with low diversity while population that lack genetic diversity may experience low fertility, high mortality among offspring, even in the environments that are not changing (Hunter, 1996).

Several research results (Bekele, 1983; Demeke *et al.,* 1992; Demissie and Bjornstrand, 1996; Staub *et al*., 1997) showed that studying the extent and patterns of distribution of genetic variation of a crop species is essential for effective utilization of germplasm in plant breeding programs, devising appropriate sampling procedures for germplasm collection and conservation, obtaining core collections for efficient germplasm management and elucidating the taxonomy, evolution and origin of crop species. Hence, knowledge of genetic diversity and relatedness in germplam is needed for the crop improvement programs, management and evaluation (Bekele, 1983; Demeke *et al.,* 1992; Demissie and Bjornstrand, 1996; Staub *et al.,* 1997). Furthermore, knowledge about genetic diversity and population structure is a good base line for formulating effective conservation plans and can often provide novel conservation- relevant site, where an effective conservation strategy for species can be made only after detailed population genetic information

becomes available (Avise, 1994). Genetic variation can be measured by using different genetic markers.

2.3 GENETIC MARKERS AND THEIR APPLICATIONS

Genetic marker can be defined as a specific gene that produces a recognizable trait and can be used in family or population studies**.** There are different genetic markers for evaluating genetic variation: morphological, biochemical and DNA markers (Bekele, 1983; Demeke *et al.,* 1992; Demissie and Bjornstrand, 1996; Karl, 1996; Dulloo *et al.,* 1997; Ruiz *et al.,* 1997; Staub *et al.*, 1997; Gwaname *et al.,* 2000). Using such markers, genetic variation studies were conducted on different crops so far, including cereals, pulses, horticultural crops and fruits (Bekele, 1983; Cipriani *et al.,* 1996; Demeke *et al.,* 1992; Gianfranceschi *et al.,* 1994; Shah *et al*., 1994; Weeden *et al*., 1994; Margal'e *et al.,* 1995; Demissie and Bjornstrand, 1996; Hoey *et al*., 1996; Samec and Nasinec. 1996; Spooner *et al*., 1996; Dulloo *et al.,* 1997; Sharman *et al.,* 1997; Staub *et al.,* 1997; Sant *et al.,* 1999; Gwaname *et al.,* 2000).

Morphological traits and statistical analysis of quantitative agro-morphological traits along with ecogeographic information are used in estimating genetic diversity (Dulloo *et al.,* 1997). Morphological studies of Ethiopian chickpea landraces showed that there is considerable variability with and between chickpea population (Feven, 2002). The author indicated that there is a variation of chickpea in different regions and altitude. High coefficient of variation for number of primary and secondary branches, grain yield, number of seeds/plant and biological yields in most of regions and altitude. This report also indicated high significance difference between populations for most of the quantitative

morphological traits except for days to maturity, grain yield/plot, biological yield and harvest index. Mengesha (1975) also indicated high variation of chickpea in Shewa; medium variation in Gonder, Gojam and Tigray. Kumar *et al.* (1981) also reported high coefficient of variation for biological yield followed by grain yield, pods/plant and effective branches. They also indicated low of variation for days to flower, plant height, and seeds/pod. A Research report on the Ethiopian chickpea landraces also showed low variation in traits such as days to flowering and maturity and plant height (Feven, 2002). Some morphological traits are confined to certain areas (Bekele, 1983 and Van der Maesen, 1984) that resulted from homologous mutation (Bekele, 1983). On chickpea there was showing that black seeded chickpeas are mainly confined in Ethiopia and absent in Turkish, Afganstan and Caucasus (Van der Maesen, 1984).

Although morphological markers are technically simple, they have several limitations. Discrete morphological traits, which show high heritability, are limited in number, each being governed by a few genes and hence, cover only a small portion of the genome. Quantitative traits are influenced by environmental factors, implying that these traits show continuous variation. This results in low heritability and high genotype by environment interactions that make it difficult to determine genetic variation accurately. There are various studies on heritability and genetic gain for different morphology traits of chickpea. High value of genetic gain and heritability was for seed weight, number of pods and seed yield (Adhirkari and Pandey, 1982), seed index, number of seeds/plant and time to flowering (Khorgade *et al.* 1985). High heritability coupled with high expected genetic gain may result due to high additive gene effects and thus selection applied on such traits lead to yield improvement (Arora, 1991; Misra, 1991). On the contrary, there was low expected genetic gain for days to flowering and seeds/pod (Adhirkari and Pandey, 1982), plant

height, number of pods, number of pods and yield/plant (Khorgade *et al.* 1985). In such traits, most of the variation is environmental thus leading to low heritability and low expected genetic gains from selections and eventually results in low progress of selection. The usefulness of estimates of heritability and expected genetic advance from selection depends on their repeatability, which is found to vary with methods of estimation, cross, generation, sample size, and the environment (Pandey and Tiwari, 1983). These authors described that for most characters the genetic gain from selection were low that might be due to restricted genetic variation. Association among these morphological traits are useful for selecting genotypes possessing groups of desired characters although such correlation coefficients could vary with genotypes studied and the environment where the test is carried out (Hadjichristoudoulou, 1987). Among the seed yield components effective plant height and effective pods/plant are important for seed yield improvement (Dahiya *et al.* 1980).

Molecular markers (Biochemical and DNA markers) are developed to overcome limitations of morphological data although it does not mean that any of the biochemical or molecular techniques or both replace morphological marker. According to Sharman *et al.* (1997), molecular markers have several advantages over morphological markers. These include numerous markers that can be identified in a single breeding population with relatively large number of alleles available for one marker, most of them exhibiting as co-dominant mode of inheritance. They are generally silent in their effects on phenotypes. The environments do not influence them and they can be scored at a very early development stage allowing early population screening. A number of molecular markers including isozyme, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) etc are available for

different uses at different time. Different research findings showed that these different molecular markers have been reliably used in cultivars identification, diversity analysis, construction of genetic maps, tagging agronomically important genes, prediction of hybrids, germplasm management, assessment of genetic purity of inbred lines and varieties, selection of recurrent parental genome in backcrosses and segregating generations thereby reducing the number of generations required in producing inbred lines, paternity analysis in perennial and tree crops, monitoring genetic stability of germplasm conserved in the form of seeds and tissues and their detection of somaclonal variants, screening of duplicate accessions in gene bank etc. (Demeke *et al.,* 1992; Hadrays *et al.,* 1992; Margal'e *et al.,* 1995; Cipriani *et al.,* 1996; Samec and Nasinec,1996; Dulloo *et al.,* 1997; Winter, 1997; Sant *et al*., 1999). Chickpea breeding, especially resistance breeding will also benefit from the application of molecular markers for development of superior resistant cultivars (Winter, 1997).

Isoenzyme is popular molecular marker that used in diversity analysis of different plants (Dulloo *et al,*. 1997). It possesses several positive attributes for use in genetic analysis: its electrophoresis could provide relatively quicker and easier access to be used as reliable biochemical marker; only small amounts of tissues are required; alleles exhibit simple Mendelian inheritance and co-dominate expression in most cases and comparisons of homologous loci across populations and related species are straightforward (Schaal *et al.,* 1991). Nevertheless, isoenzyme has well known limitations, the most serious of which are highly biased genomic samplings (only genes encoding soluble enzymes are surveyed) and small numbers of loci (maximally about 40) are available for study. Due to low polymorphism detectable with isoenzyme markers and other inherent disadvantages such as low allelic number and the need for specific developmental stage for certain enzymes have

limited its use for gene tagging of resistance particularly in chickpea where little intraspecific polymorphism is available (Sharman *et al.,* 1997). Isoenzyme electrophoresis of chickpea had revealed insufficient polymorphism particularly in cultivated chickpea as a result of a narrow genetic variability (Sharman *et al.* 1997; Feven, 2002). However, as Winter (1997), reviewed by citing Kazan *et al.* (1993), showed that there is already developed map based on the morphological and isoenzyme markers for crosses between *C. arietinum, C. reticulum* and *C. echinospermum*, in to which a DNA marker map could be integrated.

Random amplified polymorphic DNA (RAPD) also represents a source of genetic variation that is easy to access, very quick, efficient in the production of DNA polymorphism (Hadrays *et al.,* 1992; Margal'e *et al.,* 1995; Samec and Nasinec, 1996). It provides supplementary information to morphological or agronomic characters. It can detect misclassification of accessions in collections obtained (Margal'e *et al.,* 1995). Its protocol is ready for use in breeding, for registration, control of distribution of commercial cultivars, the control of seed purity, categorizing accessions in germplasm stock, determining the relation between population and creating subsets of genetically closer populations, reorganizing and comparing germplasm collections characterized by morphological descriptors, creating subsets within the same morphological group and quantifying their variability (Demeke *et al*., 1992; Tingey and Del Tufo, 1993; Whitkus *et al.,* 1994; Margal'e *et al.,* 1995; Cipriani *et al.* 1996; Hoey *et al*., 1996; Samec and Nasinec, 1996; Spooner *et al.,* 1996; Dulloo *et al.,* 1997; Sant *et al.,* 1999). Furthermore, the number of potential RAPD markers is higher in magnitude than for the morphological or isozyme markers, leading to a much higher level of marker saturation on genetic maps. RAPD analysis was used in diversity analysis of different plants such as coffee (Aga *et al*., 2000),

cereals (Ayana, 2000 and Dullo *et al*, 1997), horticulture (Persson *et al.*, 1998; Dullo *et al*.,1997; Gwaname *et al.*, 2000 and Birmata *et al*., 2004). RAPD was also used in study the relationships among annual *Cicer* species and revealed existence of genetic relationships among these species (Ahmad, 1999). However, Hardys *et al.* (1992) and Fritsch and Rieseberg, (1996) identified the following drawback of RAPD. First, there is a problem of dominant allelic expression and the associated problem of having to assume two alleles per locus. As a result, RAPD provides less genetic information on a per locus basis than co-dominant loci when applied to questions of population genetic structure, paternity, outcrossing rates or hybridization. Second, a primer size determines the degree of specificity in genome scanning. A primer having short length may amplify unreasonably large number of sequences and those larger primers will amplify too few sequences to be routinely informative. Increasing primer length beyond certain point may also increase non-specific primer annealing; consequently increasing the probability of random non-reproducible amplification patterns. Third, the assumption of the use of RAPD techniques is that the amplified fragments are unique. Eluting individual PCR products from gels and reprobing the products via southern analysis can easily detect the co-migration. Fourth, the principal limitation of RAPD arises from its sensitivity to the reaction condition and a slight change in the reaction condition may affect the reproducibility of the amplification. Fifth, there is non-Mendelian inheritance indicating that RAPD artifact that may lead to misleading results in some applications. Sixth, unlike other methods, the nature of the sampling regions and differences in band intensity in RAPD is poorly understood. Finally, there is a general prevalence of non-parental artifacual bands that could reduce the utility of RAPD in molecular genetic, especially in paternity studies.

In general, multivariate analysis performed on sets of traits (agro/morphological,

biochemical and agro/morphological plus biochemical traits) showed that the relation between agro/morphological and biochemical descriptors were low. The two markers reflect different patterns of diversity even though neither of them identifies unique phenotypes. Gepts (1995) also pointed out that results from morphological, biochemical and DNA trait studies are not always correlated and this discrepancies may be attributed to possible selective effects that more likely to be associated with morphological traits than molecular markers. Such results imply no single method (biochemical, morphological or DNA markers) is adequate for assessing genetic variation in germplasm as these different methods sample genetic variation at different levels and hence, differ in their power of genetic resolution as well as the quality of information. Because of their high polymorphism and high resolution, DNA markers should be considered only as complementary to morphological and biochemical characterization and analysis (Gepts, 1995; Ruiz *et al.,* 1997). Such results underscore the use of more than one category markers for characterization of genetic diversity.

3. MATERIALS AND METHODS

3.1 MORPHOLOGICAL CHARACTERIZATION

3.1.1 DESCRIPTION OF THE STUDY AREA

The present study was conducted at Debre Zeit Agricultural Research Center (DZARC). DZARC is located 47 km South East of Addis Ababa with 8^{o} 44'N, 38^{o} 58' E. Its altitude is 1860 meter above sea level (m.a.s.l) and it receives an annual rainfall ranging from 412.9 to 926.9 milliliters (ml) with annual mean of 682.08 ml. The temperature of this location ranges from 11.23^{o}C to 25.19^{o}C with mean annual temperature of 19^{o}C. The dominant soil types of DZARC are vertisol, mollisol and alfisols.

3.1.2 EXPERIMENTAL MATERIALS

From 482 chickpea landraces screened for their tolerance to drought (Abbessa and Bejiga, 2002), seventeen chickpea genotypes that are drought tolerant and susceptible were obtained from Debre Zeit Agricultural Research Center and used as experimental materials (Table 1).

3.1.3. EXPERIMENTAL PROCEDURE

A randomized block design with four replications was used. Each genotype was sown in 2.5m^2 plots area with 1.1m and 1.5 m spacing between plots and blocks, respectively. Four rows were used in each plot. Hundred seeds/genotypes were planted in each plot by using

10 cm spacing between plants. Ten individual plants were tagged randomly from each genotype per plot and they were used for morphological data collection. Recording morphological character was conducted following the procedure described by chickpea descriptor (IBPGR, 1985; IBPGR, ICRISAT & ICARDA, 1993) with minor modifications. The following qualitative and quantitative morphological traits were recorded.

A. **Qualitative morphological traits:**

1. **Growth habit:** Observed growth habit of the plant and rated as 1=erect; 2= semi-erect and 3=spreading
2. **Plant anthocyanin pigment**: Observed anthocyanin pigment of the plant and recorded as 1=absent and 2=present
3. **Plant hairiness:** Observed hairiness of the plant and recorded as 1=Hairs lightly pubescent; 2=Pubescent (moderate) and 3=densely pubescent
4. **Seed surface**: Observed seed coat texture and rated as 1=rough 2=smooth and 3=tuberculated
5. **Seed color(SC):** Observed color of seeds of the plant and rated as 1=Light brown; 2=Dark brown; 3= orange; 4= variegate; 5=Gray; 6=Black; 7= Golden and 8=Yellow

B. **Quantitative morphological traits:**

1. **Root biomass (RB):** Weight of fresh root per plant
2. **Root/shoot biomass (RSB):** Ratio of root biomass to biological yield per plant multiplied by 100 (%)
3. **100 Seed weight (HSW):** Weight (g) of 100 seeds selected randomly per plants
4. **Biological yield (BYD):** Weight (g) of all above ground plant part per plants
5. **Grain yield (GY):** Dried weight (g) of seed per plant at 12% moisture

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6. **Plant height (cm)(PHT):** Height of plants at the end of flowering from ground to the highest part of the plant
7. **Days to 50% flowering (DTF):** Days from sowing to the stages when 50% flower
8. **Days to 90% maturity (DTM**): Days from sowing to the stages when 90% pods mature per genotypes
9. **Number of primary branches (NPB):** Actual counts of primary branches on the main stem per plants
10. **Number secondary branches (NSB):** Actual counts of secondary branches per primer branches on main stems per plants
11 **Number of pods (NPP):** Actual counts of pods per plant
12. **Number of seeds (NSP):** Actual counts of seeds per plant
13. **Number leaflets per leaf (LPL):** Actual counts of number of leaflet/leaf per plant
14. **Plant spread (CAN):** Measure (cm) of spread of the plants at their maximum growth stage
15. **Harvest index (%)(HI):** Ratio of grain yield to biological yield per plant multiplied by 100

Table 1. Lists of drought tolerant and susceptible chickpea genotypes with their respective former admistrative regions and altitudes (m.a.s.l)

Region	Accession number	Drought Tolerance	Awraja	Woreda	Altitude
Shewa	41135	Tolerant	Yerer & Kereyu	Ada/ D/Zeit	1920
	41118	Tolerant	Yerer & Kereyu	Ada/ D/Zeit	1860
	41131	Tolerant	Menagesha	Akakibeseka	2180
	41140	Tolerant	Yerer & Kereyu	Mojo (Lume)	2030
	DZ-10-11	Susceptible	Yerer & Kereyu	Ada	1900
	41174	Susceptible	Jibat &Mecha	Ambo	2120
	41205	Susceptible	Menagesha	Sebeta	2070
Gojam	41078	Susceptible	Mota	Helet eju & inese	2480
	41235	Tolerant	-	Goncha Sinsonesi	2560
	41275	Tolerant	Bahir Dar	Adet	1920
	41244	Susceptible	Bichena	Yeid Wuha	2360
Gonder	41053	Susceptible	Gayint	Lay Gaint	3120
	41299	Tolerant	Gonder	Konga	1920
	41284	Susceptible	Chilga	Anbesame	1900
	41306	Susceptible	Gonder	Denbia	2060
Tigray	207894	Tolerant	Raya & Zebo	Indama hone	-
Hararghe	209090	Tolerant	Habro	Habro	1740

3.2 RAPD DATA ANALYSIS

3.2.1 GERMINATION OF SEEDS FOR DNA EXTRACTION

Healthy seeds with identical dimensions were selected by visual observation and planted in pot for three weeks at Debre Zeit Agricultural Research Center. Watering was done once a day and after three weeks healthy leaves were harvested and transported to Addis Ababa University, Department of biology, Genetics Research Laboratory and stored at –21^{o}c frozen in refrigerator for use for genomic DNA extraction.

3.2.2 DNA EXTRACTION

Total DNA was extracted from three weeks young chickpea leaves following the CTAB procedure (Wang *et al.,* 1996). Six plants per genotypes were used for DNA extraction and only a total of 86 individual plants that had showed a good quality DNA were used for statistical analysis. The leaves were frozen in liquid nitrogen and ground using mortar and pistel. The powder weighing 3.0 gm was collected in 2ml eppendorf tube, and homogenized by adding 750µl of extraction buffer (0.1M Tris pH 7.5, 0.05M EDTA, 0.5M NaCl) and 100µl SDS. The mixture was incubated for 20 minutes at 65^{o}C. After incubation, 250µl of 5M KAc was added to the mixture, kept on ice for at least 30 minutes and centrifuged for 15 minutes at 14000 rpm. The supernatant was transferred to a new eppendorf tube; then after, equal volume of cold iso-propanol was added, kept at room temperature for 5 minutes, and centrifuged for 10 minutes at 14000 rpm. After air-dried, the pellet was dissolved in 250µl of TE (10mM Tris HCl, pH 7.6, and 1mM EDTA), 250µl of CTAB buffer (0.2M Tris pH 7.5, 50mM EDTA, 2M NaCl and 2% CTAB), and incubated for 15 minutes at 65^{o}C. DNA was extracted three times

with equal volume of chloroform and subsequently centrifuged at 14000 rpm for 5 minutes and then transfer the water-phase to a new tube each time.

The final water-phase transferred to a new tube was precipitated with equal volume of chilled iso-propanol and centrifuged at 14000 rpm for 15 minutes. The DNA pellet was washed twice with 70% ethanol with subsequent centrifugation at 14000 rpm for five minutes each time. The DNA pellet was air-dried, dissolved in 50µl of TE, and left overnight. Then, 5µl of 1mg/ ml of RNAse was added, incubated at 37^{o}C for 30 minutes, and kept at –20^{o}C for later use. This DNA was used as stock for preparation of working solution after quality of DNA was checked by electrophoresis in 1% agarose gel and the concentration was estimated by repeated measurements with spectrophotometer at 260 nm optical density.

3.2.3 PRIMER SCREENING

Six primers (Table 2) were screened for chickpea DNA amplification. Only OPC-02 detected polymorphism in chickpea while the others did not amplify the DNA.

Table 2. Lists of primers and their sequence used in primer screening

Name primer	Sequence
OPA-07	ATGAGCCGTC
OPA-08	CTGAGGTGAC
OPA-13	TTGAGGCGTG
OPA-17	GAGAGGCTTC
OPA-20	GTGAGACGTC
OPC-02	GTGAGGCGTC

3.2.4 PCR AMPLIFICATION

The amplification reaction was performed in a total volume of 20µl reaction mixture, containing 1x reaction buffer (75mM Tris HCl, pH8.8, 20mM $(NH_4)_2SO_4$, and 0.01%(v/v) Tween 20), 3.0 mM $MgCl_2$, 14ng primer, 0.4mM dNTPs (100mM each of dATP, dCTP, dGTP and dTTP), 0.5 units of Taq polymerase and 20ng of genomic DNA.

Amplification was carried out in a Hybaid Omnigene thermocycler for one cycle of initial strand separation at 94^{o}C for 3 minutes followed by 45 cycles of 1 minute at 94^{o}C (denaturation), 1 minute at 37^{o}C (annealing), and 2 minutes at 72^{o}C (chain elongation) using the fastest possible transition temperatures. The last cycle was followed by additional extension at 72^{o}C for 10 minutes to ensure that the primer extension reaction was completed. This was followed by hold time at 4^{o} C until samples were collected.

3.2.5 AGAROSE GEL ELECTROPHORESIS

After amplification, 5µl of loading buffer (0.12% bromo-phenol blue and 30% glycerol) was added to each tube containing PCR amplification products, and the products were separated on 1.2% agarose gel containing 0.5µg/ ml ethidium bromide, and run in 1xTAE buffer (40mM Tris, pH 8.0, 1mM EDTA, and 19mM HAc) for 2½ hours at 90 voltage. The gel was visualized under UV trans-illumination and bands recorded visually. Only bands that were visible were used for analysis.

3.3 STATISTICAL ANALYSIS

3.3.1 MORPHOLOHGICA DIVERSITY ANALYSIS

3.3.1.1 ESTIMATION OF DIVERSITY INDEX FOR QUALITATIVE TRAITS

Genetic diversity index was estimated to measure the diversity of each qualitative trait employed in this study. The amount of genetic variation was determined using the Shannon diversity index, which was calculated by the formula described by (Hutchenson, 1970):

$$H' = -\sum Pi \log_e(P_i)$$

Where pi = the proportion of total number of entries in the i^{th} class of an "n" class trait.

$\sum$= summation of entries for the i^{th} "n" phenotypic class trait

The index was standardized to keep its value in range 0 to 1, by dividing H' by $\log_e^n$ as Balakrishnan *et al.* (2000) cited from Yu Li *et al*. (1996).

3.3.1.2 QUANTITATIVE MORPHOLOGICAL DATA ANLYSIS

3.3.1.2.1 ANALYSIS OF VARIANCE

Analysis of variance (ANOVA) was computed for all quantitative traits to detect the variability present among the seventeen chickpea genotypes. The variances were analyzed following the standard procedure applicable to randomized block design as suggested by Gomez and Gomez (1984) using SAS (1999) statistical computer software. From this analysis, expected mean square was estimated.

3.3.1.2.2 ANALYSIS OF GENOTYPIC AND PHENOTYPIC VARIANCES

The variability of each quantitative morphological trait was estimated by simple statistical measures such as mean, range, phenotypic and genotypic variances and coefficient of variations. The phenotypic and genotypic variation and coefficient of variations were calculated following the formula suggested by Singh and Chaundhary (1977) as follows:

$\sigma^2_p = \sigma^2_g + \sigma^2_e$ where, σ^2_p = phenotypic variance

σ^2_g= genotypic variance

σ^2_e = environnemental variance

$\sigma^2_g = (MSg - MS_e)/r$ where, MS_t = mean square of genotypes

MS_e = mean square of Error

r = number of replications

$PCV = (\sqrt{\sigma^2_p} / \bar{x}) \times 100$ where, PCV= phenotypic coefficient of variation

$\bar{X}$= Population mean

$GCV = (\sqrt{\sigma^2_g} / \bar{x}) \times 100$ where, GCV= genotypic coefficient of variation.

3.3.1.2.3 HERITABILITY (IN BROAD SENSE) AND GENTIC ADVANCE

Broad sense heritability was estimated according to Singh and Chaundhary (1977) by dividing genotypic variance by phenotypic variance: H^2= vg/vp.

Expected genetic advance was also computed by the formula:

GA= $i.h^2.\sqrt{vp}$ and GA (% of mean)= (GA⁒m). 100%

where, m= mean value; vp = phenotypic variance; vg= genotypic variance; h^2= heritability in broad sense; i= selection differential that varies depending up on the selection intensity and stands at 2.06 for selecting 5% of the genotypes.

3.3.1.2.4 ESTIMATION OF CORRELATION COEFFICIENT

Correlation coefficient between two variables was estimated using the following formula suggested by Falconer and Mackay (1996).

$$\text{Correlation coefficient (r)} = \frac{\sigma_{pxy}}{\sqrt{\sigma^2_{px} \; X \sigma^2_{py}}}$$

where, σ_{pxy} = co-variance between traits X and Y.

σ^2_{px} = variance of trait X.

σ^2_{py} = variance of trait Y.

The correlation coefficients between all possible pairs of traits were tested for their significance.

3.3.1.2.5 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis was computed following the formula described by Noirot *et al.* (1996) by using SPSS (1999) software. Quantitative morphological data was normalized by

subtracting character mean from each value of the respective data and then dividing by the character standard deviation.

3.3.2 RAPD DATA ANALYSIS

3.3.2.1 DATA SCORING

Following Lynch and Milligan (1994) assumptions, each amplified product was treated as an independent locus and assigned numbers in order of decreasing molecular weight. The size of each band was estimated using the DNA molecular weight marker (100 base pair ladder). A band was scored as present (1) if there was band without considering their intensity difference or absent (0) for no bands in the respective locus. Data scored as present (1) was used for statistical analysis of RAPD.

3.3.2.2 ESTIMATION OF GENETIC DIVERSITY

Genetic variation was analyzed using Nei's and Shannon diversity index, which was computed using POPGENE (1997) software that was designed for population genetic analysis.

Average Nei's gene diversity (Hs) was estimated following formula described by Nei's (1973) while Shannon information was estimated following formula described by Lewontin (1972). The gene diversity in total populations and partitioning of genetic diversity was made following the statistical procedures described by Nei (1973, 1975; 1976)

3.3.2.3 ESTIMATION OF COEFICIENT OF SIMILARITY

Pair wise genetic similarity between chickpea genotypes was computed using Jaccard's similarity (Jaccard, 1908) as cited from Msumbuko *et al*. (2003).

$$S_{ij} = a/(a +b+c)$$

where "a" is the total number of bands shared between genotypes "i" and "j"

"b" is the total number of bands present in genotype "i" but absent in "j"

"c" is the total number of bands present in genotype "j" but absent in "i"

4. RESULTS

4.1 MORPHOLOGICAL DIVERSITY

4.1.1 MORPHOLOGICAL DIVERSITY BASED ON QUALITATIVE TRAITS

The overall mean diversity of chickpea genotypes varied from 0.34 ± 0.14 for accession number 41235 to 0.83 ± 0.08 for Acc-207894 with an overall mean of 0.52 ± 0.1 (Table 3). Chickpea genotypes such as Acc-207894, Acc-209090, Acc-41118 and Acc-41284 had greater mean diversity index as compared with the rest genotypes. Accession number 207894 had high diversity ranging from 0.62 to 0.99 with mean of 0.83 ± 0.08 while Acc-209090 had diversity ranging from 0.49 for seed surface to 0.99 for anthocyanin pigment with mean of 0.75 ± 0.08. Acc-41118 had also diversity varying from 0.43 for growth habit to 0.72 for anthocyanin pigment with an average of 0.58 ± 0.05 while Acc-41284 had diversity varied from 0.46 for anthocyanin pigmentation to 0.65 for seed color with an overall mean 0.57 ± 0.03. More than 0.52 mean Shannon diversity index was recorded for seed texture, seed color and plant hairiness.

4.1.2 MORPHOLOGICAL DIVERSITY BASED ON QUANTITATIVE TRAITS

4.1.2.1 ANALYSIS OF VARIANCE

Analysis of variance for quantitative morphological traits computed from the seventeen chickpea genotypes showed significance differences between chickpea genotypes except for canopy width (Table 4). Analysis of variance computed using quantitative traits of drought tolerant (Appendix 4) and drought susceptible genotypes (Appendix 5) showed significant differences in all the traits.

Table 3. Shannon diversity index and mean Shannon diversity with standard errors for chickpea genotypes

Accession number	Anthocyanin pigment	Seed surface	Seed color	plant hairiness	Growth habit	Mean± S.E
41118	0.72	0.60	0.63	0.52	0.43	0.58 ± 0.05
41053	0.29	0.72	0.51	0.10	0.76	0.48 ± 0.13
41131	0.00	0.64	0.68	0.61	0.62	0.51 ± 0.13
41140	0.29	0.38	0.53	0.94	0.58	0.54 ± 0.11
41174	0.00	0.67	0.72	0.67	0.62	0.54 ± 0.14
41205	0.00	0.87	0.62	0.18	0.54	0.44 ± 0.16
41135	0.38	0.62	0.46	0.60	0.18	0.45 ± 0.08
DZ-10-11	0.29	0.64	0.41	0.78	0.56	0.54 ± 0.09
41078	0.00	0.58	0.56	0.52	0.49	0.43 ± 0.01
41235	0.00	0.56	0.54	0.60	0.00	0.34 ± 0.14
41244	0.00	0.63	0.50	0.62	0.00	0.35 ± 0.15
41275	0.29	0.62	0.46	0.87	0.43	0.53 ± 0.10
41299	0.16	0.66	0.64	0.43	0.68	0.51 ± 0.10
41284	0.46	0.54	0.65	0.60	0.62	0.57 ± 0.03
41306	0.00	0.60	0.37	0.49	0.38	0.37 ± 0.10
209090	0.82	0.49	0.66	0.83	0.95	0.75 ± 0.08
207894	0.99	0.62	0.68	0.86	0.98	0.83 ± 0.08
All population	0.56	0.68	0.77	0.82	0.74	0.71 ± 0.05
Mean ± .S.E	0.28 ± 0.08	0.62± 0.02	0.58 ± 0.03	0.61 ± 0.06	0.52 ± 0.05	0.52 ± 0.10

4.1.2.2 MEAN, RANGE AND COEFFICIENT OF VARIATION

Coefficient Variation computed from the seventeen chickpea genotypes showed that there was more than 50% variation for biological yield, grain yield, root biomass, root to shoot biomass ratio, number of seeds/plants and number of pods/plant (Table 6). Similarly, number of branches and harvest index had more than 20% CV while the rest traits had lower than 20% CV. Least variation was record for days to 50% flowering and days to 90% maturity. Similar patterns of variation were also recorded for coefficient of variation computed from the respective drought tolerant except for harvest index (Appendix 2) and susceptible chickpea genotypes except number of pods/plant (Appendix 2).

The range of quantitative traits showed a variation (Table 6). Range for biological yield (4.70-221.70), grain yield (1.4-98.9), number of pods/plant (11.00-465.00) and number of seeds/plants (13.0-743.0) showed the existence of considerable variation. These traits had also showed a considerable mean range in different genotypes of chickpea. (Appendix 1) However, hundred seed weight/plant, root biomass, days to flowering and maturity had relatively low range.

Table 4. Mean square for quantitative[@] morphological traits of chickpea genotypes as obtained from ANOVA

Source of variation	DF	Mean squares														
		HSW	BYD	GY	CAN	PHT	HI	RB	RSB	DTF	DTM	NPB	NSB	NSP	LPL	NPP
Replication	3	11.6**	10408.6**	2703.2**	228.3**	65.4*	1385.8**	49.7**	18.2	704.7**	829.6**	73.0**	1.5	1077**	57.3**	3.10
Genotypes	16	28.9**	2016.3**	717.8**	27.7	98**	1348.4**	13.1**	54.0**	196.5**	666.3**	30.8**	7.9**	3207**	5.4**	7.6**
Error		1.5	26.8	13.0	5.8	4.6	10.80	1.50	4.1	3.1	5.0	2.4	1.3	92.3	1.40	66.4
CV(%)		11.4	56.7	52.8	19.3	13.2	20.20	56.60	65.0	7.0	5.0	28.3	38.8	50.5	11.5	52.0
Mean		13.1	47.3	24.6	29.9	34.7	53.5	2.70	6.4	43.9	100.5	8.5	3.3	182.8	12.0	127.6

Table 5. Principal component analysis of quantitative traits[@] of chickpea genotypes

Principal Components	HSW	BYD	GY	CAN	PHT	HI	RB	RSB	DTF	DTM	NPB	NSB	NSP	LPL	NPP
1	0.01	0.78	0.94	0.19	0.31	0.37	0.32	-0.36	0.21	-0.56	-0.33	0.15	0.94	-0.25	0.98
2	0.27	0.51	0.19	0.19	0.59	-0.36	0.81	0.60	0.83	0.30	0.72	0.79	-0.04	0.10	0.11
3	-0.47	0.002	-0.02	0.86	0.46	-0.60	0.26	0.30	-0.09	0.24	-0.33	0.16	0.10	0.80	0.05
4	0.69	-0.17	0.23	0.01	0.21	0.80	-0.17	-0.07	0.004	0.49	0.02	0.11	0.11	-0.25	0.09

** & *= significant at p<0.01 & p<0.05 respectively; @ = the expanded name for quantitative traits are given on page 15 & 16

4.1.2.3 PHENOTYPIC AND GENOTYPIC VARIANCE

PCV and GCV values <10%, 10-20% and >20% are considered to be low, intermediate and higher respectively (Khorgade *et al.,* 1985). There was a wide range of variation for all characters except number of leaf let per leaf that has got 9.9 % and 15.2% of GCV and PCV, respectively (Table 6). All other traits had medium to high value of PCV and GCV. Canopy width, plant height, days 50% flowering and to 90% maturity had medium value of PCV and GCV. Biological yield, grain yield, root biomass, root/shoot biomass ratio/plant, number of pods per plant, number of seeds per plant and number of secondary branches had more than 54.0% PCV and more than 37.48% GCV.

4.2 HERITABILITY (IN BROAD SENSE) AND GENETIC ADVANCE

All the quantitative traits had high heritability ranging from 31.17% - 86.66% except for canopy width that had the lowest heritability (4.39%) (Table 6). Hundred seed weight (74.90%), harvest index (72.54%), days to 50% flowering (83.21%) and days to maturity (86.7%) had very high heritability. The rest quantitative traits such as biological yield, grain yield, root/shoot biomass, number of seeds and pods/plant, number of secondary branches, number of leaflet/leaf and plant height had higher heritability ranging from 31.17 to 48.26 %.

High heritability and genetic advances (as % mean) was obtained for harvest index and root biomass with more than 54% and followed by number of primary and secondary branches/plant and number of seeds/plant that had more than 40% heritability and genetic gain. Low genetic gain was recorded for canopy width followed by number of leaflet/leaf

followed by number of leaflet/leaf, days to 50% flowering and 90% maturity.

4.3 PRINCIPAL COMPONENT ANALYSIS

The principal component analysis, which is used for data reduction, was summarized and presented for chickpea genotypes presented in Tables 5. By taking eigenvalue greater than unity as a measure of significance for principal component analysis (PCA), four components were extracted from the mean of normalized quantitative traits of genotypes. A gross variance of about 29.3%, 25.4%, 14.6% and 10.8% was extracted from the first to the fourth components, respectively and 80% of total variance was explained by the four components.

The first principal component was associated strongly with traits such number of pods/plant, number of seeds per plant, grain and biological yield/plant and days to maturity in order of their importance. Likewise, days to 50% flowering, root biomass, number of secondary and primary branches, root/shoot biomass/plant and plant height had contributed much of the variation to the second component. Canopy width and number of leaflet/leaf were the major contributor to the variance of the third component while harvest index and hundred seed weight had contributed toward the fourth components.

Table 6. Mean, range, Genotypic (GCV) and phenotypic (PCV) coefficient of variation, Genotypic (GV), Phenotypic (PV) and environmental (EV) variance, heritability (H^2) and genetic advance (GA as % of mean) of quantitative traits of the chickpea genotypes

Traits*	Mean	Range	CV	GV	PV	EV	GCV	PCV	H^2	GA
HSW	13.12	3.9-18.6	11.39	6.68	8.92	2.24	19.70	22.77	74.90	35.19
BYD	47.26	4.7-221.7	56.67	324.78	1042.0	717.19	38.13	68.30	31.17	44.10
GY	24.64	1.4-98.9	52.78	137.17	306.28	169.11	47.53	71.03	44.79	65.80
PHT	34.69	21.0-50.0	13.17	19.27	40.14	20.87	12.66	18.26	48.01	18.06
CAN	29.94	15.0-47.0	19.29	-1.40	32.00	33.35	12.36	18.88	4.39	1.70
HI	53.48	10.0-97.2	20.19	307.94	424.53	116.5	32.81	38.53	72.54	57.91
RB	2.68	0.1-10.1	56.63	2.71	5.00	2.29	61.37	83.39	54.15	92.81
RSB	6.38	0.4-53.8	64.00	9.34	26.00	16.65	47.90	79.90	35.93	59.27
DTF	43.86	34.0-57.0	7.00	46.77	56.21	9.44	15.59	17.09	83.21	29.23
DTM	100.48	84.0-116.0	4.94	160.41	185.1	24.69	12.60	13.54	86.66	24.26
NPB	8.47	2.0-18.0	28.3	6.27	12.02	5.75	29.57	40.94	52.17	43.85
NSB	3.33	0.2-10.8	38.76	1.56	3.23	1.67	37.48	54.00	48.26	53.37
NSP	182.87	13.0-743.0	50.51	5888.25	14410.5	8522	42.00	65.68	40.86	55.48
NPP	127.6	11.0-465.0	52.02	2416.5	6821.5	4405	38.53	64.73	35.42	47.20
LPL	12.03	8.30-17.3	11.50	1.42	3.33	1.91	9.90	15.17	42.64	13.47

* The expanded names for the traits are given on page 15 & 16

4.4 CORRELATION ANALYSIS OF QUANTITAITIVE CHARACTERS

High and positively significant correlation were observed between biological and grain yield (r=0.89**), biological yield and number of seeds/plant (r=0.80**), biological yield and number of pods (r=0.78**), grain yield with number seeds/plant(r=0.89**), number of pods/plant (r= 0.87**) and between number of pods/plant and number of seeds/plant (r=0.87**) (Table 7).

Root biomass had also showed a significant positive correlation with biological yield (r=0.59**), grain yield (r= 0.54**), root/shoot biomass ratio (r=0.38**), number of seeds/plant (r=0.46**) and with number of pods/plant (r=0.51**). But it had negative significant correlation with harvest index (r= -0.13**). Harvest index was negatively correlated with most of the traits particularly it had significant and negative correlation with days to maturity (r=-0.1**), biological yield/plant (r= -0.22**) and root biomass (r=-0.13**). However, it had significant and positive correlation with root/shoot biomass ratio (r=0.38**), number of seeds/plant (r= 0.16**), pods per plant (r = 0.13**), grain yield (r = 0.16**) and hundred seed weight (r= 0.1**).

Table 7. Correlation among fifteen quantitative traits[@] of chickpea genotypes in Ethiopia

	HSW	BYD	GY	PHT	CAN	HI	RB	RSB	DTF	DTM	NPB	NSB	NSP	NPP	LPL
HSW	1.00														
BYD	0.22**	1.00													
GY	0.22**	0.89**	1.00												
PHT	0.04	0.14**	0.15**	1.00											
CAN	-0.02	-0.01	-0.001	0.3**	1.00										
HI	0.10**	-0.22**	0.16**	0.05	0.00	1.00									
RB	0.14**	0.59**	0.54**	0.13**	-0.003	-0.13**	1.00								
RSB	-0.12**	-0.28**	-0.27**	0.02	-0.02	0.38**	0.38**	1.00							
DTF	0.01	0.03	-0.02	0.06	-0.01	-0.10*	0.05	0.04	1.00						
DTM	0.15**	0.23**	0.20**	0.18**	-0.02	-0.06	0.2**	-0.01	0.29**	1.00					
NPB	0.08*	0.08*	0.07	0.09*	-0.07	-0.04	0.10**	-0.004	-0.03	0.10*	1.00				
NSB	0.03	0.08*	0.05	0.09*	0.04	-0.01	0.19**	0.09*	0.07	0.07	-0.004	1.00			
NSP	0.06	0.80**	0.89**	0.13**	-0.01	0.16**	0.46**	0.26**	-0.04	0.15**	0.05	0.04	1.00		
NPP	0.08*	0.78**	0.87**	0.13**	-0.04	0.13**	0.51**	-0.19**	-0.03	0.15**	0.03	0.05	0.87**	1.00	
LPL	-0.10**	-0.05	-0.03	0.03	0.03	-0.01	-0.003	0.05	-0.09*	0.03	-0.02	0.03	-0.01	-0.01	1.00

**= P<0.01; *= P<0.05; otherwise non-significant; @ = the expanded name for traits are given on page 15 & 16

4.2 RAPD DATA ANALYSIS

Only one primer (OPC-02) from six primers showed polymorphism and hence the result obtained is not sufficient for diversity analysis of chickpea. A total of eight reproducible polymorphic bands were obtained for 86 individual samples assayed from seventeen chickpea genotypes.

There was a significance differences for quantitative traits as revealed from analysis of variance. Feven (2002) also reported high significance difference between populations for most of the traits except for days to maturity, grain yield/plot, biological yield and harvest index. Thus, there is variability among studied chickpea population.

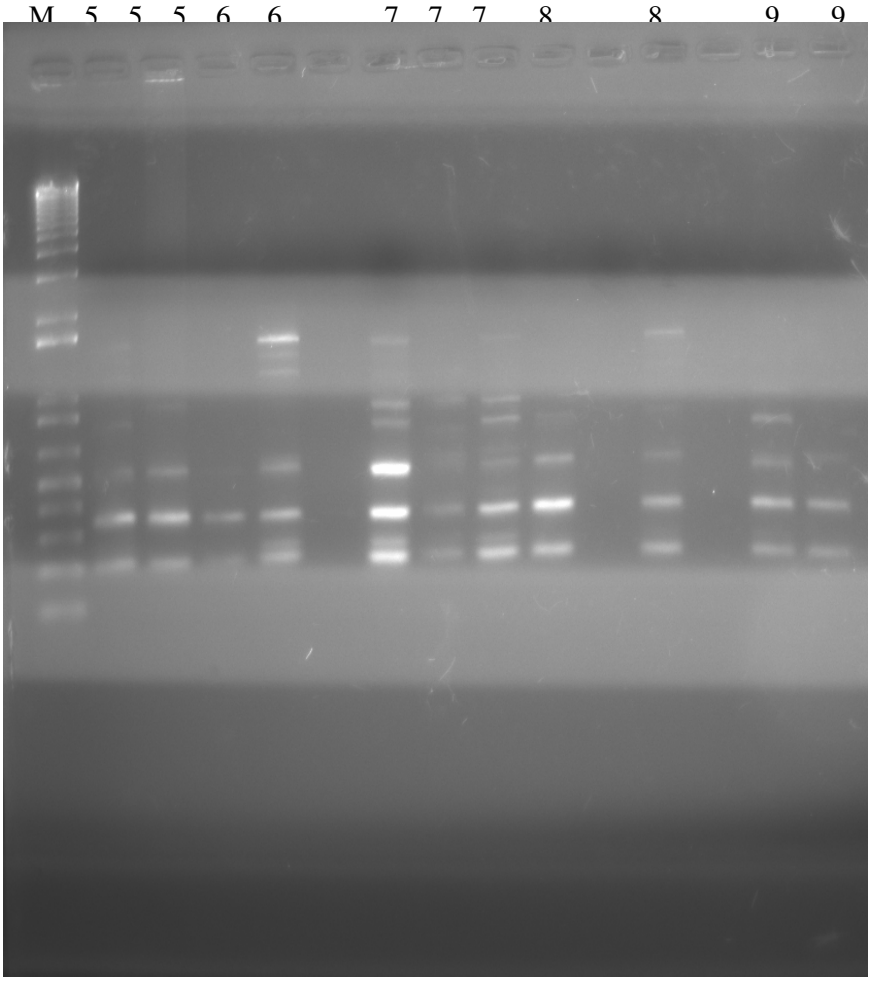
The proportion of DNA polymorphism computed ranged from no polymorphism for Acc-41174 to 87.5% polymorphism to acc-207894, acc-41131, acc-41235, acc-41275 and acc-41078 (Table 8). More than 75 % polymorphism was also recorded for other accessions such as acc-412999, acc-41306, acc-41118 and acc-41053.

Nei's unbiased genetic diversity estimated from seventeen chickpea genotypes varied from 0.0 to 0.37 with mean of 0.25 ± 0.02 while that of Shannon information index varied from 0.0 to 0.52 with mean of 0.37 ± 0.03, implying that there was low variation in chickpea genotypes. The genetic diversity estimated from overall entire data pooled together was 0.6 and 0.41 for Shannon and Nei's diversity, respectively. Nei's and Shannon information index was relatively high for acc-41284, acc-41235 and acc-207894 as compared to other chickpea populations, implying that there was variability in these accessions. Drought

tolerant and susceptible chickpea genotypes had about 0.396 and 0.340 Shannon genetic diversity, respectively while it is 0.269 and 0.233 Nei's gene diversity, respectively (Table 9). Thus, the genetic diversity obtained from the two genetic diversity indexes is high for drought tolerant chickpea genotypes where as the genetic diversity calculated from their respective data showed about the same value for the Shannon information index (Table 9).

Partitioning of genetic diversity of seventeen chickpea populations in to between and within populations was 38.5% and 61.5% for both diversity indexes (Table 9). Partitioning genetic diversity of drought tolerant and susceptible chickpea genotypes in to within and between populations also showed that genetic diversity within the population was high for both Nei's and Shannon diversity than diversity between populations of chickpea genotypes.

Jaccard's coefficient similarity among genotypes varied from 0.14 to 0.85 with mean 0.51 (Table 10). This indicated an average gene similarity among chickpea population.
Relatively high Jaccard's similarity was recorded for acc-41284 with acc-41299, acc-41235, acc-41205, acc-41131, acc-4105, and acc-4053 with acc-41118, DZ-10-11, acc-207894. On other hand there was low coefficient of similarity between acc-41118 and acc-41244, acc-412444 and acc-41205, acc-207894 and acc-41205, showing low genetic similarity between them.



where,
M= Molecular marker; 5= Acc-41205; 6= Acc-41299; 7= Acc-41284; 8= Acc-41053; 9= Acc=209090

Fig. 1. Bands of chickpea genotypes from RAPD markers

Table 8. Number and proportion of polymorphic bands and mean genetic diversity within populations with their standard error of mean for the seventeen chickpea genotypes

Accession number	Number Polymorphic bands	Polymorphic bands (%)	Shannon index ± S.E	Nei's genetic diversity ± S.E
ACC-41205	4.0	50.0	0.31 ± 0.04	0.21 ± 0.03
ACC-209090	4.0	50.0	0.20 ± 0.04	0.17 ± 0.03
ACC-41299	6.0	75.0	0.42 ± 0.04	0.29 ± 0.03
ACC-207894	7.0	87.5	0.52 ± 0.03	0.36 ± 0.02
ACC-41235	7.0	87.5	0.52 ± 0.04	0.35 ± 0.02
ACC-41135	4.0	50.0	0.29 ± 0.04	0.20 ± 0.03
ACC-41174	0.0	0.00	0.00	0.00
ACC-41053	6.0	75.0	0.43 ± 0.04	0.30 ± 0.03
ACC-41244	4.0	37.5	0.18 ± 0.03	0.11± 0.02
ACC-41140	2.0	25.0	0.16 ± 0.04	0.11± 0.03
ACC-41284	6.0	75.0	0.51± 0.04	0.37 ± 0.03
ACC-41131	7.0	87.5	0.49 ± 0.03	0.33 ± 0.02
ACC-41118	6.0	75.0	0.44 ± 0.04	0.30 ± 0.03
ACC-41275	7.0	87.5	0.47 ± 0.03	0.31 ± 0.02
ACC-41306	6.0	75.0	0.43 ± 0.04	0.30 ± 0.03
DZ-10-11	5.0	62.5	0.38 ± 0.04	0.26 ± 0.03
ACC-41078	7.0	87.5	0.48 ± 0.03	0.32 ± 0.02
Mean ± S.E	4.8 ± 0.39	64.0 ± 4.8	0.37 ± 0.03	0.25 ± 0.02
All population	8.0	100	0.60± 0.03	0.41 ± 0.01

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Table 9. Partitioning of genetic variation in to within and between populations and regions of origin of chickpea genotypes

Genotypes	Diversity index	Chickpea population				
		Ht	Hs	Dst	Dm	Gst
Entire chickpea	Nei	0.409	0.252	0.158	0.615	0.385
	Shannon	0.600	0.369	0.231	0.615	0.385
Drought tolerant	Nei	0.400	0.269	0.131	0.672	0.323
	Shannon	0.588	0.396	0.192	0.673	0.327
Drought susceptible	Nei	0.410	0.233	0.177	0.568	0.432
	Shannon	0.602	0.340	0.262	0.565	0.435

Where: Ht = total gene diversity in the population or regions; Hs = Mean gene diversity within region or population; Dst = Gene diversity between population or region; Dm = proportion of gene diversity within population or region and Dst = proportion of gene diversity between population or region

Table 10. Jaccard's coefficient of similarity among seventeen chickpea genotypes

Pop ID*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.59	0.72	0.14	0.76	0.55	0.29	0.65	0.16	0.54	0.71	0.68	0.84	0.32	0.74	0.82	0.42
2		0.50	0.47	0.38	0.72	0.61	0.44	0.63	0.32	0.38	0.50	0.35	0.42	0.46	0.40	0.35
3			0.72	0.63	0.52	0.44	0.72	0.20	0.47	0.85	0.75	0.58	0.40	0.72	0.66	0.50
4				0.71	0.45	0.28	0.65	0.19	0.53	0.70	0.72	0.79	0.38	0.84	0.84	0.39
5					0.52	0.33	0.63	0.21	0.55	0.65	0.80	0.85	0.40	0.75	0.78	0.48
6						0.65	0.48	0.38	0.41	0.44	0.60	0.44	0.50	0.54	0.36	0.52
7							0.28	0.29	0.21	0.50	0.41	0.29	0.33	0.34	0.27	0.40
8								0.25	0.58	0.62	0.82	0.68	0.40	0.73	0.61	0.39
9									0.35	0.17	0.23	0.18	0.50	0.23	0.18	0.35
10										0.45	0.54	0.52	0.32	0.65	0.47	0.26
11											0.72	0.68	0.35	0.70	0.68	0.45
12												0.67	0.46	0.83	0.68	0.56
13													0.36	0.71	0.84	0.45
14														0.44	0.34	0.65
15															0.75	0.43
16																0.35

* 1= ACC-41205; 2= ACC-209090; 3= ACC-41299; 4= ACC-207894; 5= ACC-41235; 6= ACC-41135; 7= ACC-41174; 8= ACC-41053; 9= ACC-41244; 10= ACC-41140; 11= ACC-41284; 12= ACC-41131; 13= ACC-41118; 14= ACC-41275; 15= ACC-41306; 16=DZ-10-11; 17= ACC-41078

5. DISCUSIONS

5.1. MORPHOLOGICAL DIVERSITY

Shannon diversity index of chickpea population varied from 0.34 to 0.83 with an over all mean of 0.52 and the diversity index estimated from all data pulled together varied from 0.56 to 0.82 with an overall mean 0.71. This implied the existence of variability within the studied chickpea population. There was also variability in chickpea population from studies of 50 Ethiopian chickpea landraces and it was found that Shannon diversity varied from 0.43 to 0.66 for chickpea population and diversity index estimated for an over all population ranged from 0.21 to 0.96 with mean of 0.64 (Feven, 2002).

There was a significance differences for quantitative traits as revealed from analysis of variance. Feven (2002) also reported high significance difference between populations for most of the traits except for days to maturity, grain yield/plot, biological yield and harvest index. Thus, there is variability among studied chickpea population.

Mean, range and coefficient of variation in agronomic traits were widely used to determine variations available in populations. Most quantitative traits showed a variation as revealed from coefficient of variation. Generally, this result indicated there is variability within chickpea populations except for days to 50% flowering and 90% maturity.

Similarly, high coefficient of variation for number of primary and secondary branches, grain yield, number of seeds/plant and biological yields for chickpea population (Feven,

2002). Kumar *et al.* (1981) also reported high coefficient of variation for biological yield, followed by grain yield, pods/plant and effective branches. They also indicated low of variation for days to flower, plant height, and seeds/pod. This is also was also reported on Ethiopian chickpea landraces for traits such as days to flowering and maturity (Feven, 2002).

Range recorded for quantitative traits showed there is variation among chickpea population. The range of quantitative traits for biological yield grain yield, number of pods/plant and number of seeds/plants showed the existence of considerable variation. However, hundred seed weight/plant, root biomass, days to flowering and maturity had relatively low range, implying that they had relatively low variability as compared to other quantitative traits. Similar considerable variation for chickpea population was also by Pundir *et al*. (1991)

5.2 PHENOTYPIC AND GENOTYPIC VARIANCE

The effectiveness of selection in any crop depends on the extent and nature of phenotypic and genotypic variability present in different agronomic traits of population (Arora, 1991). Generally, genetic parameters including genotypic coefficient of variation, heritability and genetic advance are prerequisite for genetic improvement of crops (Khorgade *et al.* 1985). High genotypic coefficient of variation indicates availability of high genetic variation. The lower value of variation indicates that

selection is not effective for particular character because of the narrow genetic variability (Pandey and Tiwari, 1983; Arora, 1991).

Most of the traits had medium to high variation implying that there is genetic differentiation among chickpea population. High genotypic and phenotypic coefficient recorded for biological yield, grain yield, root biomass, root/shoot biomass ratio/plant, number of pods per plant, number of seeds per plant and number of secondary branches indicated chickpea genotypes had genetic differentiation for such traits. Thus, there is genetic variation among chickpea genotypes and selection could be effective for such traits (Khorgade *et al.* 1985; Pandey and Tiwari, 1983; Arora, 1991).

Arora's (1991) research result showed high PCV and GCV for pods/plant, 100 seed weight, seed yield/plant and moderately high genotypic variability for plant height, canopy width, primary and secondary branches/plant, seeds/pod and harvest index. The author indicated that moderately high PCV with low GCV suggests a relatively high environmental influence for such traits. High PCV for seed yield, number of pods/plant, pods/secondary branches, seed weight and secondary branches (Adhirkari and Pandey, 1982), number of seeds/pod, seed index (Khorgade *et al.* 1985), biological yield (Mandal, 1983) was recorded. On the other hand, low PCV was reported for plant height (Khorgade *et al.* 1985) and harvest index (Mandal, 1983).

This study showed that PCV value was higher than the corresponding GCV for most traits implying that in addition to genetic factors, other factors such as environments influence the variation. On the other hand, for traits such as hundred seed weight, plant

height, number of secondary branches, harvest index, days to 50% flowering and 90% maturity of the genotypes, the PCV value was slightly higher than the corresponding GCV signifying that genotypic factors exerted reasonable effect in estimating the variation.

5.3 HERITABILITY (IN BROAD SENSE) AND GENETIC ADVANCE

Information on heritability and genetic advance of yield attributing traits and their association with seed helps plant breeder to identify characters for effective selection (Misra, 1991). Heritability is an important factor to determine the response of selection and breeding program. Its estimations are important aspect of inheritance of quantitative traits as they indicate the genetic gains that may be gained through selection (Pandey and Tiwari, 1983).

The present study indicated that hundred seed weight; harvest index, days to 50% flowering and days to maturity had very high heritability, indicating that genotypic variance was high and thus, selection based on these traits is very successful. For quantitative traits with high heritability including hundred seed weight, harvest index, root traits, days to 50%flowering and 90% maturity and number of primary branches had high genotypic variance and less affected environmentally.

High heritability and genetic advances (as % mean) was recorded for harvest index and root biomass and followed by number of primary and secondary branches/plant and number of seeds/plant indicated that there is high genotypic variance resulted from

additive gene (Arora, 1991; Misra, 1991). Thus, selection progress will be expected to be high for those traits. According to Arora (1991) and Misra (1991), high heritability and intermediate genetic gain recorded for days to flowering and maturity indicated that the high genotypic variance resulted from non-additive gene. This implied that lower progress would be gained from selection from such traits.

Feven (2002) reported 90%, 75% and 72% heritability for days to maturity, days to flowering and thousand seed weight, in that order, in the Ethiopian chickpea landraces. Abebe (1985) also reported highest heritability for hundred seed weight, number of seeds/pod and plant height in chickpea. Similarly, there were reports showing high heritability for days to flowering and maturity, seed size (Pandey and Tiwari, 1983; Misra, 1991), seed weight, number of pods and seed yield (Adhirkari and Pandey, 1982; Arora, 1991; Misra, 1991), plant height, number of branches/plant, seed index and number of seeds/pod (Khorgade *et al.* 1985). Arora (1991) reported high heritability ranging from 48.22% for secondary branches/plant to 91.15% for 100 seed weight, which showed that all quantitative traits evaluated had high heritability. Generally, those traits with high heritability were less affected by environmental factors.

On the other hand, there was a report showing low heritability for grain yield, biological yield and harvest index (Feven, 2002). Pandey and Tiwari (1983) described the inconsistence of heritability for growth characters such as plant height, spread and branches number, seed yield and its components, pod and seed numbers. Abebe (1985) also confirmed the fluctuation of heritability in years and location for some traits. For instance, pod number and yield showed wide range of heritability, because their

heritability estimates become high in good productive environments and relatively low in poor environments. Therefore, traits with high heritability could be utilized in the breeding program as high heritability signifies the proportion of total variability due to genetic make up of plants.

High value of genetic and heritability was also reported for seed weight, number of pods and seed yield (Adhirkari and Pandey, 1982), seed index, number of seeds/plant, time to flowering (Khorgade *et al.* 1985). High heritability coupled with high expected genetic gain may result due to high additive gene effects and thus selection applied on such traits lead to yield improvement (Arora, 1991; Misra, 1991). On the contrary, days to flowering and maturity had high heritability and low genetic gain from selection. There was also low expected genetic gain for days to flowering and seeds/pod (Adhirkari and Pandey, 1982), plant height, number of pods, number of pods and yield/plant (Khorgade *et al.* 1985). This revealed the presence of nonadditive gene action, which is responsible for selection to be less effective for those traits (Arora, 1991; Misra, 1991). In such traits, most of the variation is environmental thus leading to low heritability and low expected genetic gains from selections and eventually results in low progress of selection. The usefulness of estimates of heritability and expected genetic advance from selection depends on their repeatability, which is found to vary with methods of estimation, cross, generation, sample size, and the environment (Pandey and Tiwari, 1983). These authors described that for most characters the genetic gain from selection were low that might be due to restricted genetic variation.

5.4 PRINCIPAL COMPONENT ANALYSIS

Four principal components were extracted from quantitative traits of genotypes and a gross variance of 29.3%, 25.4%, 14.6% and 10.8% was extracted from the first to the fourth components, respectively and 80% of total variance was explained by four components. Similarly, Feven (2002) showed four principal components extracted from quantitative data of Ethiopian chickpea landraces by taking eigenvalue greater than unity, which explained about 81% total variance.

Number of pods/plant, number of seeds per plant, grain and biological yield/plant and days to maturity high contribution in the first component; days to 50% flowering, root biomass, number of secondary and primary branches, root/shoot biomass/plant and plant height had contributed much of the variation to the second component; Canopy width and number of leaflet/leaf contributor to the third component while harvest index and hundred seed weight had contributed toward the fourth components. This indicated that such traits had high contribution toward chickpea variation (Noirot *et al*., 1996). Thus, different quantitative traits had contribution toward chickpea variation with varying degree. Principal components from previous studies of Ethiopian chickpea landraces indicated that primary and secondary branches, number of seeds/plant, plant height and grain yield contributed to the first components while thousand seed weight and days to flowering effect to the second, harvest index and biological yield to the third, and days to maturity contributed much to the fourth component (Feven 2002).

5.5 CORRELATION ANALYSIS OF QUANTITAITIVE CHARACTERS

Association among traits are useful for selecting genotypes possessing groups of desired characters although such correlation coefficients could vary with genotypes studied and the environment where the test is carried out (Hadjichristoudoulou, 1987).

This study indicated that high and positively significant correlation were observed between biological and grain yield, biological yield and number of seeds/plant, biological yield and number of pods, grain yield with number seeds/plant, number of pods/plant and between number of pods/plant and number of seeds/plant.

Similar result was reported by Feven (2002) except for thousands seed weight. Plant height had significant and positive correlation with most quantitative traits except hundred seed weight, harvest index, days to flowering, number of leaflet/leaf and root/shoot biomass. Similar result was also reported for positive correlation of plant height, primary and secondary branches /plant with most of the traits (Feven, 2002). The high correlation of number of seeds/plant and number of pods per plant will contribute more effectively in increasing seed yield in chickpea improvement program, implying that they can be used as a basis for chickpea selection criteria (Dahiya *et al.* 1980). Previous study showed that high number of secondary number was also recommended for selecting high yielding lines in chickpea improvement (Islam *et al.* 1984).

On the other hand, previous research reports showing that association between traits varied with location and years (Van der Maesen, 1984; Abebe, 1985). From the

description of Abebe (1985), yield and yield component associations showed differences in different seasons, environments and locations, which is signified by the variation of association observed between grain yield and 100 seed weight, plant height and primary branches, seeds per pod and number of pods per plant. Van der Measen(1972) also suggested that seeds/pod may not be always associated strongly and positively with yield of chickpea. Generally, as depicted from such results, the correlation coefficients may reveal differences in magnitude over location and seasons that might be attributable to the environmental conditions prevailing at different locations and seasons. This implies the need for determining the association among important traits over broad arrays of environment and seasons for identifying consistent association among traits that could be used for conducting effective breeding program.

5.6 RAPD DATA ANALYSIS

The proportion of polymorphic loci and average hetrozygosity (gene diversity) per locus are used for measuring genetic variation of a population (Nei, 1975). As the author arbitrarily defined a locus is polymorphic if the frequency of the commonest allele is equal to or less than 0.99.

Nei's unbiased genetic diversity estimated from entire chickpea genotypes varied was 0.25 ± 0.02 while that of Shannon information index was 0.37 ± 0.03. Percentage of DNA polymorphism of chickpea genotypes varied from 0 to 87.5% with mean 64.0 ± 4.8. Thus, there is a genomic DNA variation in the studied chickpea population.

Partitioning of genetic diversity in to within and between chickpea populations showed that genetic diversity within the population was high for both Nei's and Shannon diversity than diversity between populations of chickpea genotypes.

Generally, this result indicated that RAPD markers could be used for discriminating chickpea population for analysis of chickpea diversity.

6. CONCLUSIONS

From the present studies of chickpea genotypes, the following conclusions are made.

Shannon diversity index from qualitative and coefficient of variation from quantitative morphological traits indicated the availability of variation within chickpea genotypes. Similarly, phenotypic and genotypic coefficient of variation, range of quantitative traits and analysis of variance confirmed the existence of variability between chickpea genotypes.

High heritability and genetic advances recorded for quantitative traits indicated that there is high genotypic variance in chickpea population. There fore, selection progress could be expected to be high for such traits and hence, they are important as a source of improvements in chickpea population. The strong and positive significant correlation recorded between biological and grain yield, biological yield and number of seeds/plant, biological yield and number of pods, and grain yield with number seeds and pods/plant, number of pods/plant and number of seeds/plant indicated that the improvements of one trait will lead to yield improvement of chickpea.

From genetic information obtained from OPC-02 primer, RAPD markers can be used in discriminating chickpea population and can complement the genetic information generated from the morphological traits.

7. RECOMMENDATIONS

All the above conclusions were derived from results of studies conducted at one location and one primer used for RAPD marker. So, the followings are recommended for further studies.

Further studies of chickpea genotypes with larger sample size in broad environments and seasons can give additional information on chickpea variability. In addition, a further study of RAPD marker by using large number of primers is recommended in order to give confirmative results. There is no evidence showing studies conducted on Ethiopian chickpea landraces by other molecular markers including cytogenetic profile studies. Thus additional studies using other molecular markers can give more information about chickpea genotype to be used effectively for breeding and conservation programme.

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9. APPENDICES

Appendix 1. Mean, Maximum, minimum and standard deviation (sd) of quantitative traits** of seventeen chickpea genotypes

Accession number		HSW	BYD	GY	PHT	CAN	HI	RB	RSB	DTF	DTM	NPB	NSB	NSP	NPP	LPL
Acc-41118	**Mean**	12.6	57.6	30.0	34.0	30.0	52.5	2.6	4.9	42.7	102.6	7.7	3.2	227.6	159.20	11.5
	Minimum	9.4	15.9	7.2	23.0	20.0	22.3	.60	1.4	34.0	97.09	2.0	1.2	60.0	40.0	8.30
	Maximum	14.6	199.4	83.9	50.0	46.0	71.6	7.7	9.5	47.0	107.09	14.0	7.2	594.0	417.0	13.70
	Sd	1.2	32.9	17.6	6.1	5.5	14.8	1.4	1.9	3.4	2.9	2.7	1.3	128.9	94.83	1.42
Acc-41053	**Mean**	12.6	48.5	25.8	34.8	30.6	53.0	3.1	6.3	41.7	98.7	7.1	3.5	196.2	135.6	12.9
	Minimum	3.9	21.4	2.8	23.0	19.0	13.1	.20	.9	34.0	89.0	3.0	.8	28.0	49.0	9.30
	Maximum	15.9	100.4	45.8	45.0	45.0	69.5	10.1	22.0	48.0	109.0	10.0	7.3	411.0	280.0	15.30
	Sd	2.0	19.4	10.8	4.8	6.0	11.9	1.9	4.1	4.5	4.9	1.9	1.3	85.3	50.3	1.3
Acc-41131	**Mean**	12.7	43.1	25.1	33.7	30.0	59.2	2.4	6.5	44.5	96.0	8.2	3.2	192.1	133.9	11.8
	Minimum	9.8	11.6	7.3	25.0	20.0	35.2	.7	2.6	37.0	88.00	3.0	.8	60.0	41.0	9.00
	Maximum	14.9	106.2	66.6	44.0	44.0	68.0	5.4	14.4	52.0	110.00	14.0	5.8	498.0	378.0	14.3
	Sd	1.3	25.6	14.1	4.0	6.7	5.9	1.2	2.9	4.1	6.51	2.3	1.2	98.9	74.0	1.5
Acc-41205	**Mean**	12.4	46.6	28.0	35.8	30.8	59.6	2.5	5.9	44.0	100.1	7.5	3.1	216.6	142.4	12.6
	Minimum	11.0	17.1	11.3	26.0	21.0	41.4	.6	.80	39.0	89.00	2.00	.6	93.0	58.0	9.7
	Maximum	14.6	90.5	56.80	48.0	43.0	69.6	5.6	12.3	49.0	110.00	12.0	6.5	425.0	317.0	15.3
	Sd	.9	18.5	11.1	5.3	5.8	6.3	1.2	2.3	2.5	5.22	2.3	1.3	89.1	62.6	1.5
Acc-41140	**Mean**	12.7	38.2	22.9	33.7	30.3	61.3	2.1	5.7	42.2	96.18	9.4	3.1	188.0	119.8	11.8
	Minimum	10.50	4.7	1.9	21.0	22.0	40.4	.50	1.2	36.0	85.0	3.0	1.0	16.0	11.0	8.7
	Maximum	15.90	106.6	49.1	49.0	38.0	90.0	6.70	15.3	46.0	109.0	16.0	5.6	395.0	255.0	14.7

	Sd	1.2	21.8	11.6	4.4	4.4	9.4	1.4	3.0	2.5	6.2	2.9	1.1	105.2	62.5	1.4
Acc-41174	**Mean**	12.6	43.1	22.9	34.2	30.9	57.1	1.9	5.6	40.3	94.1	6.7	2.6	179.9	124.2	12.1
	Minimum	9.9	9.9	3.7	27.0	20.0	11.7	.3	1.2	34.0	84.0	4.0	.6	29.00	20.0	9.3
	Maximum	14.8	221.7	98.9	40.0	45.0	75.5	7.0	21.3	46.0	105.0	10.0	4.6	743.00	448.0	15.0
	Sd	1.4	39.0	17.3	3.7	5.8	13.6	1.3	3.8	3.6	7.0	1.7	1.0	132.51	80.8	1.3
Acc-41135	**Mean**	13.2	49.9	29.7	33.3	28.9	57.2	2.6	5.3	45.3	104.7	8.7	2.5	212.56	145.3	11.7
	Minimum	9.1	18.0	9.2	27.0	15.00	16.0	.50	1.4	39.0	95.0	4.0	.6	72.0	37.0	8.7
	Maximum	15.1	110.7	84.5	46.0	38.00	69.7	6.40	11.4	53.0	115.0	16.0	6.0	382.0	302.0	14.0
	Sd	1.2	23.1	15.1	3.9	5.03	10.8	1.6	2.5	3.5	6.1	2.4	1.2	90.7	68.7	1.4
DZ-10-11	**Mean**	13.0	38.5	19.3	31.6	28.7	50.0	2.9	8.6	42.8	94.7	8.3	3.3	147.7	104.2	11.5
	Minimum	7.8	10.5	3.3	22.0	18.0	23.6	0.9	2.4	37.0	85.0	5.0	0.2	43.0	30.0	9.7
	Maximum	18.3	115.9	62.1	40.0	43.0	67.0	6.9	25.7	48.0	109.0	14.0	6.2	448.0	370.0	14.3
	Sd	2.1	22.10	12.3	4.4	5.6	11.0	1.5	4.6	2.8	6.7	2.0	1.4	89.0	63.2	1.1
Acc-41275	**Mean**	14.1	44.3	24.5	34.5	28.3	58.0	1.9	4.9	42.8	100.9	8.6	3.2	179.0	125.5	11.5
	Minimum	11.0	4.80	2.8	29.0	19.0	26.2	0.1	0.6	35.0	95.0	3.0	0.80	18.0	18.0	9.7
	Maximum	16.00	120.3	56.4	43.0	40.0	97.2	6.2	17.3	51.0	110.0	16.0	5.70	418.0	334.0	14.0
	Sd	1.0	23.3	11.3	3.8	5.7	12.6	1.2	3.4	4.8	3.7	2.8	1.3	82.0	62.3	1.2
Acc-41078	**Mean**	13.5	57.3	30.6	36.3	31.2	55.4	3.9	7.9	44.1	102.5	8.9	4.3	221.6	150.1	12.0
	Minimum	10.10	18.00	10.90	26.00	17.00	36.70	0.80	2.70	40.00	93.00	4.00	2.20	73.00	62.00	9.70
	Maximum	16.00	148.20	75.20	45.00	47.00	70.40	9.30	28.30	47.00	110.00	18.00	10.80	439.00	351.00	14.70

	Sd	1.20	32.59	15.08	4.41	7.32	8.52	2.25	5.28	2.00	3.70	2.85	1.85	100.81	73.06	1.31
Acc-41235	**Mean**	13.69	43.71	22.79	37.03	30.75	55.17	2.92	8.61	46.78	105.38	9.10	3.71	159.20	120.28	12.03
	Minimum	9.00	6.50	2.80	30.00	19.00	29.30	0.30	1.60	40.00	99.00	6.00	0.80	21.00	16.00	9.30
	Maximum	17.80	172.80	60.20	44.00	47.00	72.10	8.80	53.80	53.00	112.00	13.00	6.80	401.00	450.00	15.00
	Sd	1.46	28.84	12.04	3.77	6.72	10.15	1.64	8.67	2.95	3.45	1.75	1.40	78.86	74.59	1.39
Acc-41244	**Mean**	12.79	50.05	22.61	34.35	30.00	45.84	3.34	7.12	45.8750	104.25	10.30	3.43	167.53	113.78	12.31
	Minimum	9.80	7.20	2.80	23.00	20.00	13.40	1.20	1.60	38.00	95.00	3.00	.70	24.00	18.00	9.70
	Maximum	16.20	132.40	50.90	47.00	42.00	63.00	8.40	20.10	51.00	113.00	17.00	7.00	477.00	306.00	14.70
	Sd	1.50	29.59	13.31	5.07	4.89	11.25	1.67	3.86	3.2359	4.71	2.73	1.27	97.8769	70.63	1.32
Acc-41299	**Mean**	13.61	55.30	27.50	35.50	29.43	51.23	3.03	6.24	46.58	105.25	9.23	3.89	197.20	141.58	11.60
	Sd	0.28	5.49	2.69	0.78	1.00	1.89	0.3040	0.57	0.69	0.88	0.38	0.21	16.88	11.94	0.19
	Minimum	9.00	9.90	4.90	26.00	17.00	15.80	0.10	0.40	39.00	94.00	3.00	0.20	49.00	39.00	8.70
	Maximum	16.50	137.80	70.50	47.00	41.00	74.40	8.50	23.20	57.00	114.00	14.00	6.40	510.00	320.00	13.30
	Sd	1.75	34.75	17.01	4.91	6.17	11.94	1.92	3.63	4.38	5.58	2.41	1.32	106.74	75.50	1.21
acc-41306	**Mean**	13.90	53.47	27.40	37.38	29.40	54.12	3.00	6.17	45.35	101.80	8.43	3.54	176.35	136.30	11.94
	Minimum	10.40	8.40	5.00	25.00	20.00	28.40	.60	1.10	41.00	98.00	4.00	.40	48.00	34.00	8.30
	Maximum	16.60	148.60	76.00	50.00	43.00	69.60	9.20	11.80	49.00	109.00	16.00	6.00	382.00	465.00	16.70
	Sd	1.55	33.23	14.25	5.60	6.13	9.3586	1.94	2.84	1.81	3.24	2.66	1.17	74.59	73.73	1.63
Acc-41284	**Mean**	11.04	40.02	15.39	34.63	30.18	38.03	2.42	7.10	42.78	96.38	8.78	3.13	136.18	95.48	12.85
	Minimum	6.00	11.80	1.40	25.00	20.00	10.40	.90	1.70	34.00	88.00	3.00	1.30	13.00	13.00	9.70
	Maximum	13.90	101.00	44.20	50.00	45.00	60.50	7.00	17.50	48.00	108.00	17.00	7.30	337.00	231.00	17.30
	Sd	1.9	20.12	10.3161	5.02	5.52	14.8122	1.17	4.02	4.55	6.53	2.7315	1.30	80.48	56.43	1.46
Acc-	**Mean**	14.03	57.24	25.9300	36.48	30.40	47.3450	3.08	6.3275	47.83	106.75	8.8750	3.61	176.33	130.33	12.29

209090	**Minimum**	7.80	6.90	3.20	29.00	22.00	25.50	.20	.70	43.00	95.00	3.00	1.60	29.00	27.00	9.30
	Maximum	16.40	147.00	59.80	47.00	44.00	68.30	9.40	29.80	57.00	116.00	15.00	6.50	380.00	253.00	15.30
	Sd	1.94	33.81	13.68	3.81	5. 50	9.94	2.09	5.1	4.51	5.59	2.700	1.31	79.58	55.91	1.33
Acc-207894	**Mean**	14.63	36.59	18.39	32.55	29.38	54.19	1.75	5.18	40.23	98.30	8.35	2.90	133. 00	91.40	12.04
	Minimum	12.10	13.70	7.80	22.00	15.00	26.90	.50	1.20	34.00	88.00	4.00	1.20	42.00	37.00	8.70
	Maximum	18.60	82.00	34.70	40.00	42.00	71.70	4.40	10.70	49.00	108.00	14.00	5.50	336.00	190.00	15.00
	Sd	1.40	17.0	7.0406	4.33	6.11	11.2158	1.00	2.44	3.17	5.84	2.48	1.07	60.54	36.73	1.61
Total	**Mean**	13.12	47.26	24.64	34.69	29.94	53.48	2.68	6.38	43.86	100.49	8.47	3.33	182.76	127.60	12.03
	Minimum	3.90	4.70	1.40	21.00	15.00	10.40	.10	.40	34.00	84.00	2.00	.20	13.00	11.00	8.30
	Maximum	18.60	221.70	98.90	50.00	47.00	97.20	10.10	53.80	57.00	116.00	18.00	10.80	743.00	465.00	17.30
	Sd	1.7045	28.12	13.90	4.78	5.84	12.30	1.66	4.19	4.11	6.59	2.5759	1.35	97.55	69.62	1.43

** the expanded names for quantitative traits are given page 15 & 16

Appendix 2. Mean square for quantitative morphological traits of drought tolerant chickpea population as obtained from ANOVA

Source	DF	Mean squares														
		HSW	BYD	GY	CAN	PHT	HI	RB	RSB	DTF	DTM	NPB	NSB	NSP	LPL	NPP
Replication	3	3.1	2716.3*	1003.4**	140.9**	6.0	1653.5**	15.4**	3.8	732.5**	281*	14.0**	0.1 43	125.4**	2.6	17424.8*
Genotypes	16	17.6**	2555.6**	533.6**	24.8	90.8**	755.1**	9.9**	90.6**	253.2**	523.9**	42.6**	7.1**	31360**	2.8	14717.7**
Error		2.1	27.1	13.4	5.7	4.4	10.4	1.5	6.0	2.8	8.7	2.5	1.3	92.7	1.4	68.1
CV(%)		15.6	57.3	53.0	19.3	12.7	18.8	60.1	98.2	6.4	8.6	28.6	38.0	50.1	11.8	52.5
Mean		13.6	47.3	25.2	29.7	34.5	55.1	9.9	6.1	44.3	101.2	8.7	3.1	185.0	11.8	129.7

Appendix 3. Mean square for quantitative[+] morphological traits of drought susceptible chickpea population as obtained from ANOVA

Source	DF	Mean squares														
		HSW	BYD	GY	CAN	PHT	HI	RB	RSB	DTF	DTM	NPB	NSB	NSP	LPL	NPP
Replication	3	3.6	9277.8**	1958.2**	82.0*	260.7**	333.2*	40.1**	13.4	134.7**	943.7**	26.6**	4.3	77045.0**	0.8	43022.1**
Genotypes	***16***	***32.7****	***1391.1****	***993.8****	***54.9****	***15.0****	***2224.0****	***17.7****	***26.1****	***157.6****	***706.8****	***61.6****	***11.5****	***34999.0****	***7.7****	***13959 ****
Error		1.5	26.8	12.6	4.8	5.7	11.1	1.5	3.8	3.1	6.4	2.4	1.3	92.0	1.6	64.3
CV(%)		12.1	55.3	51.1	13.6	18.9	21..3	53.4	58.3	7.2	6.4	29.2	39.2	49.8	12.6	50.1
Mean		12.7	48.4	24.7	35.3	30.4	51.9	2.9	6.6	43.4	99.4	8.2	3.4	185.0	12.3	128.3

**= p<0.01; *=p<0.05 ; + = the expanded names for quantitative traits are given page 15 & 16

Appendix 4. Mean, Genotypic (GCV) and phenotypic (PCV) coefficient of variation, Genotypic (GV), Phenotypic (PV) and environmental (EV) variance, heritability(H^2) and genetic advance(GA as % of mean) of quantitative traits of drought tolerant chickpea landraces

Traits*	Mean	CV	GV	PV	EV	GCV	PCV	H^2	GA (% as mean)
HSW	13.6	15.6	3.300	7.800	4.5	13.4	20.5	65.4	27.0
BYD	47.3	57.3	455.3	1189.9	734.6	45.1	72.9	61.9	93.0
GY	25.2	53.0	88.90	267.1	178.2	37.4	64.9	57.6	77.0
PHT	34.5	12.8	17.90	37.30	19.40	12.3	17.7	69.5	25.3
CAN	29.7	19.3	2.03	24.80	30.80	4.80	16.8	28.6	9.9
HI	55.1	18.8	161.9	269.5	107.6	23.1	29.8	77.5	47.6
RB	2.50	60.1	1.900	4.200	2.300	55.1	82.0	67.2	1.9
RSB	6.10	98.2	13.71	21.76	15.80	60.7	76.47	63.0	4.8
DTF	44.3	6.40	61.30	69.30	8.000	17.7	18.8	94.1	36.6
DTM	101.2	8.60	112.0	187.9	75.90	10.5	13.5	77.8	21.7
NPB	8.70	1.00	2.000	8.100	6.100	16.3	32.7	49.8	33.6
NSB	3.10	38.0	1.400	3.000	1.600	38.2	55.9	68.3	78.6
NSP	185	50.1	5690.6	14288.1	8597.2	40.8	64.6	63.2	84.1
NPP	129.7	52.5	2520.6	7156.1	4635.5	38.7	65.2	59.4	79.8
LPL	11.8	11.8	0.9	2.800	1.900	8.00	14.2	56.3	16.4

*= the expanded names for quantitative traits are given page 15 & 16

Appendix 5. Mean, Genotypic (GCV) and phenotypic (PCV) coefficient of variation, Genotypic (GV), Phenotypic (PV) and Environmental (EV) variance, heritability(H^2) and genetic advance(GA as % of mean) of quantitative traits of drought susceptible chickpea landraces

Traits*	Mean	CV	GV	PV	EV	GCV	PCV	H^2	GA
HSW	12.7	12.1	7.60	9.90	2.300	21.7	24.8	87.5	44.7
BYD	48.4	55.3	168.2	886.5	718.3	26.8	61.5	43.6	55.3
GY	24.7	51.1	208.5	367.5	159.0	58.5	77.6	75.4	29.8
PHT	35.3	13.6	8.00	31.10	23.10	8.00	15.8	50.6	16.5
CAN	30.4	18.9	4.4	28.40	32.90	6.90	17.5	39.4	14.2
HI	51.9	21.3	525.4	647.7	122.3	44.2	49.0	90.2	91.1
RB	2.90	53.4	3.800	6.200	2.400	67.2	85.9	78.2	4.0
RSB	6.60	58.3	2.800	17.60	14.80	25.4	63.6	39.9	52.2
DTF	43.4	7.20	37.00	46.80	9.800	14.0	15.8	88.6	28.8
DTM	99.4	6.40	166.5	207.3	40.80	13.0	14.5	89.7	26.8
NPB	8.20	29.2	14.00	19.80	5.800	45.6	54.3	84.0	93.9
NSB	3.40	39.2	2.500	4.200	1.700	46.5	60.0	77.5	96.2
NSP	184.9	49.8	6632.8	15100.7	8467.9	44.0	66.5	66.2	90.6
NPP	128.3	50.1	2456.4	6590	4133.6	38.6	63.3	61.0	79.5
LPL	12.3	12.6	1.300	3.700	2.400	9.30	15.6	59.6	19.0

*= the expanded names for quantitative traits are given page 15 & 16