ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY ANALYSIS IN CHICKPEA (*Cicer arietinum* L.) LANDRACES OF ETHIOPIA

A Thesis presented to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science in Biology (Applied Genetics)

By

Feaven Workeye

November, 2002

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This Work is dedicated to my loving husband

Timotios Endale

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Acknowledgments

First and for most I give praise to the almighty God the creator of the heaven and earth, who made everything possible. I express my appreciation to my advisor Prof. Endashaw Bekele for his intellectual advices and scientific guidance and critical reading of the manuscript which contributed much to the success of the work. I would like to thank my co-advisor Dr. Geletu Bejiga for his constructive guidance and all the reference materials which made the writing easy.

I would like to forward my gratitude to the whole staff of the Debre Zeit Agricultural Research Center (DZARC), with special reference to the Pulse Division, for their kindness, support and efforts during the field work. The few names that deserve special thanks are Ato Yadeta Anbesse (Head, Pulse division), and Ato Tessema Shibiru who helped me through the hardship of the field work. My heart felt appreciation and honor goes to Dr. Seid Ahmed (Director, DZARC) for his constant encouragement, concern and follow-up of my research work. Dr Getachew Belay receives my deep appreciation and regard for the moral support, constructive comments and thorough reading of the manuscript. Many thanks go to the Administration and Finance division workers for all their friendly cooperation.

I extend many thanks to the whole staff of the Institute of Biodiversity Conservation and Research (IBCR), for the friendly atmosphere and their kind cooperation. The few names that deserve special thanks are Dr. Abebe Demissie (Director, IBCR) for allowing me to use the laboratory facilities and the seed samples. My deepest appreciation goes to my friend Yemane Tsehay whose concern, encouragement and help in data analysis contributed much to my success. My regards and thanks to Dr. Haile Selassie Yibrah whose cooperation during purchase of chemicals made the isozyme work possible. W/t Eleni Shiferaw deserves many thanks for her concern and devotion for technical help during the laboratory work. W/t Maria Degefu and her colleagues are highly acknowledged for their cooperation in the protein content analysis of my samples.

Many thanks go to the Awassa Agricultural Research Center for allowing me to join the Addis Ababa University and paying my salary during the study period. I thank the Ethiopian Agricultural Research Organization (EARO) and Agricultural Research and Training Project (ARTP) for giving me this opportunity and covering the thesis research cost. I would like to acknowledge the Department of Biology, Addis Ababa University for creating the academic atmosphere for my graduate study. Dr. Kifle Dagne receives my heart felt regard and appreciation for his concern and follow-up during the whole year of my study. My regards and thanks to Dr. Zemede Asfaw for his thorough reading of the paper and valuable comments. The secretaries of the department deserve acknowledgment for their kindness and cooperation in every way.

It gives me pleasure and honor to express my heart felt appreciation to my loving husband Timotios Endale, his concern, encouragement and love is the key to my success. Many thanks goes to my father Ato Workeye Mamo and my mother W/o Tsigie Moges, my brother Jose and my sisters Raye, Merry, Hale and little Betty for their love and support throughout my study year. I also like to thank all those peoples who assisted me in one way or another during my study.

Abstract

Fifty landrace populations (1500 individuals) of chickpea (*Cicer arietinum* L.) collected from ten different chickpea growing regions of Ethiopia were studied for variation of seven qualitative and ten quantitative traits. Shannon and Weaver diversity indices (H') were calculated for the qualitative characters, populations, regions of origin and altitude classes. The highest mean diversity index pooled over characters was observed in collections from Shewa (0.67±0.08) and Arsi-Bale (0.65±0.08) collections and the lowest mean for those from Hararghe-Sidamo and Gamo Gofa (0.59±0.09 and 0.56±0.10, respectively). The over-all mean diversity index for Ethiopia in this study was 0.64±0.08. Relatively higher mean diversity index for traits pooled over the altitude classes were recorded in the higher altitude classes. The analysis of variance for the individual traits shows that much of the variation was within population and altitude groups. Multivariate methods including cluster and principal component analysis were used to asses the pattern of variation using the ten quantitative characters. Cluster analysis using Ward's method grouped the fifty populations sampled from the ten regions into seven clusters, and into another eight clusters using both the qualitative and quantitative characters together. The Mahalanobis's distance (D^2) analysis also revealed the existence of considerable variability among the chickpea populations, and hence parental lines could be selected for hybridization and improvement of the crop. The isozyme test using ten different enzyme systems was not able to reveal any variation between the chickpea accessions, indicating a need for the use of more sensitive genetic markers in future studies. As indicated by the t-test undertaken for crude protein content of the landraces, significant variations were observed which might indicate a niche for improvement.

Key words: Chickpea (*Cicer arietinum),* landraces (farmers' varieties), morphological traits, genetic diversity, isozyme

1. INTRODUCTION

Chickpea (*Cicer arietinum* L) belongs to the genus *Cicer* that consists of about 43 species (Van der Maesen, 1987), in the tribe *Cicereae* Alef. Chickpea, a self-pollinated crop that probably originated in Western Asia or Southern Europe, has been cultivated for centuries in the Middle East, India, the Mediterranean region and Ethiopia (Westphal, 1974). In terms of acreage and production the crop ranks fifteenth among the food crops, third among the pulse crops in the world and first among the food legumes grown in West Asia, North Africa and Central Asia (Singh and Malhotra, 1984).

Chickpea is an important food legume crop in Ethiopia. It is the second crop after faba bean in area coverage and production (Pundir and Mengesha, 1982). The main production areas are Northern and Central Ethiopia, but small plots may also be found elsewhere. In Ethiopia, chickpea is mostly grown for local market and is considered to be one of the major sources of cheap protein for the poor rural and urban community. It is valued for its nutritive seeds with high protein content; on the average 20% protein, 5% fat and 55% carbohydrate (Oplinger *et al.*, 1990).

Ethiopia is the center of diversity for cultivated chickpea. The bulk of chickpeas grown in this country are landraces. For progress in chickpea improvement, more genes for better agronomic traits, disease resistance, earliness, good quality, and higher biological yield are necessary. The availability of such genes partly depends on the identification of sites of concentration for various characters. The identification of these sites is of paramount importance for collection and appropriate *in situ* site selection. The choice of sites for *in situ* conservation may depend on high

diversity estimates based on markers and knowledge of adaptive traits linked to certain ecological conditions (Negassa, 1986; Demissie and Bjørnstad, 1996).

Studying the pattern of genetic variation and their differential distribution over the various microcenters could give an insight into the evolutionary forces responsible for maintaining genetic variability (Bekele, 1983). In addition, prior knowledge of the nature and extent of genetic variation is crucial since successful conservation and utilization of germplasm depends on the proper assessment of variation within and among populations (Bekele, 1984; Negassa, 1986; Tessema and Belay, 1991).

A considerable number of diversity studies have been done by different workers on important crops of the country, notably focusing on cereals. For example, on tef (Bekele, 1983; Assefa *et al.*, 1999: Kefyalew *et al.*, 2000), barley (Demissie, 1996; Asfaw, 1988, 1989), wheat (Bekele, 1984; Negassa, 1986; Belay, 1997; Kibebew *et al.*, 2001) and sorghum (Ayana and Bekele, 1998). However, there was no much effort done to study the genetic diversity of the important pulse crop, chickpea in Ethiopia. This study was, therefore, undertaken to fill this gap and study morphological and biochemical diversity of chickpea landraces collected from various regions of Ethiopia.

1.1 Objectives

The overall objective of the study was to generate information on diversity of chickpea landraces covering various morphological and biochemical characters.

Specific objectives:

- to evaluate the diversity of chickpea landraces with respect to some morphological and biochemical characters.
- to study the distribution of morphological and biochemical characters with respect to altitude and region.
- to identify sites of high genetic diversity for *in-situ* (on-farm) conservation or target collection of the crop.
- to study the association between morphological and biochemical characters.

2. LITERATURE REVIEW

2.1. Chickpea (*Cicer arietinum* L.)

Chickpea is grown in tropical, sub-tropical and temperate regions (Oplinger *et al.*, 1990) on soils of vertic property mostly on residual moisture at elevations between 1400-3000 meters above sea level where the annual rainfall ranges from 600 to 2000 mm (ICRISAT, 1990).

Chickpea (*Cicer arietinum*) is one of the dominant pulse crops. It is an ancient crop, cultivated for its edible seeds in different parts of the world. In Ethiopia it is considered as one of the priority pulse crops for improvement. The crop has wide diversity in the country, and a number of important characteristics such as drought tolerance, short duration maturity, high protein content and nitrogen fixing capacity.

Two types of chickpeas are recognized, desi (small seeded = 1,500 seeds/lb, with angular seeds, rough seed surface and seed coat color varying from yellow to black) and kabuli (large seeded = 800 seeds/lb, ram-head shaped, beige colored) types (Malhotra *et al.*, 1987). Yadav *et al* (2000) suggested that desi and kabuli types may represent different gene pools within cultivated chickpea. Desi cultivars account for about 85% of the world's area and production of chickpea and are grown mostly in South Asia, Iran, Ethiopia and Mexico (ICRISAT, 2000) while the kabuli types are grown in temperate regions (Muehlbauer and Singh, 1987; Malhotra *et al.*, 1987). In Ethiopia, about 90% of chickpea grown are desi type and production is concentrated in the central and northern highlands of the country, whereas kabuli type, mostly found in mixture with desi type chickpeas. Figure 1 shows the seeds of desi and kabuli type chickpeas.

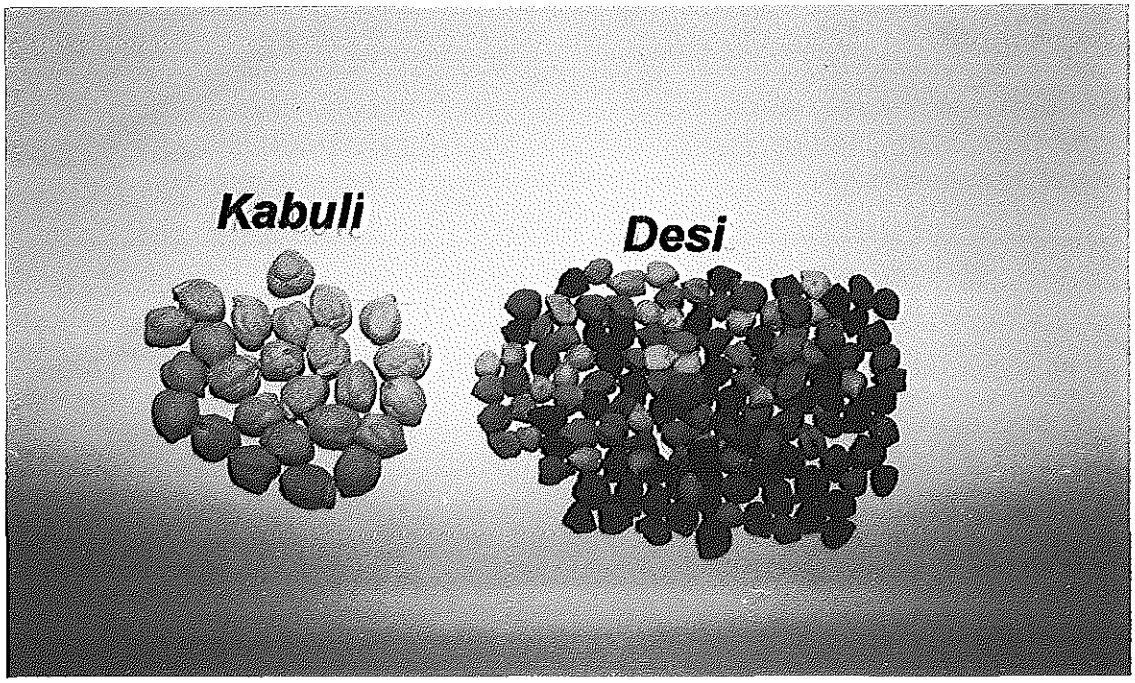

Figure 1. The *desi* and *kabuli* type of chickpea seeds

Ethiopia is the center of diversity for cultivated *Cicer* species. The related wild and weedy species (*Cicer cuneatum*) has been found in the Tigray region (Northern Ethiopia) and in the Shewa uplands in an altitude range of 1000-2200 m, which shows its diversity and rich genetic potential for further improvement programs in the country (Hedberg and Edwards, 1989). There is an abundant genetic variation in chickpea for various qualitative and quantitative morphological traits (Yadav *et al.*, 2000). The extensive variation available in *Cicer* forms a basis for breeding efforts and is virtually important to chickpea improvement i.e. to produce high yielding genotypes with quality attributes that are acceptable to producers and consumers (Muehlbauer and Singh, 1987).

2.1.1. Origin, Taxonomy and Distribution of Chickpea

Chickpea is an ancient crop that has been grown in India, the Middle East and parts of Africa for many years (Oplinger *et al.*, 1990). It is one of the oldest food legume crops, its cultivation date back to about 5450 BC in Hacillar, Turkey (Helbaek, 1970).

Vavilov (1926) designated Southwest Asia and the Mediterranean as primary centers of origin (now centers of diversity), and Ethiopia as a secondary center. Ladizinsky (1976) reported the center of origin to be Southeastern Turkey, which is supported by Van der Maesen (1987) who recognized the southeastern part of Turkey adjoining Syria as the possible center of origin of chickpea based on the presence of the closely related annual species, *C. reticulatum* Ladizinsky and *C. echinospermum* P.H. Davis. The botanical and archeological evidence indicates that chickpeas were first domesticated in the Middle East and were widely cultivated in India, Mediterranean area, the Middle East, and Ethiopia since antiquity. Wild species are most abundant in Turkey, Iran, Afghanistan, and Central Asia (Duke, 1981). The wild species, *Cicer cuneatum,* was also collected from Ethiopia.

The genus *Cicer* L. belongs to the family Leguminosae, subfamily Papilionaceae, tribe Cicereae Alef and comprises 43 species out of which nine are annuals, including the cultivated species *Cicer arietinum* L (Van der Maesen 1987; Ladizinsky *et al* 1988; Muehlbauer, 1993). Chickpea and most of its wild relatives are diploids and have 2n=16 chromosomes. The crop has now several vernacular names in different parts of the world, some of them are: Chickpea (English), Garbanzo (Spanish), Garbanzo bean (USA), Chana (India), Hommos (Arabic), Nakhut (Turkey, Romania, Bulgaria, Iran, Afghanistan and the adjoining areas of the former USSR),

Shimbra/Shumbura (Ethiopia), Garo (Portuguese), Mdengun (Swahili) and Poice cheche in France (Singh and Saxena 1999).

The stems of chickpea plant are branched, glandular pubescent, erect or spreading, 0.2-1 m tall. The root system is robust, up to 2 m deep, because of its deep tap root system chickpea can withstand drought conditions by extracting water from the deeper layers of the soil (Oplinger *et al.,* 1990). The leaves are glandular-pubescent with 3-8 pairs of leaflets and a top leaflet. It is a predominantly self-pollinated crop with reported cross-pollination of only 1 % (Singh, 1987; Smithson *et al.*, 1985). Flowers are borne in groups of two or three and come in purple, white, pink or blue colors depending upon the variety. Each flower produces a short, pubescent pod that appears to be inflated and carry a maximum of three seeds each. The seeds come with either rough or smooth surfaces and can be cream, yellow, brown, black or green in color (Oplinger *et al.,* 1990; Duke, 1981; Van der Maesen, 1987).

The distribution of chickpea cultivation and consumption coincides with the most concentrated pocket of poverty in the world, for example, South Asia that includes, Bangladesh, India, Nepal, Pakistan and Sri Lanka (ICRISAT, 2000), indicating its importance in the developing countries. Referring the intercontinental situation, the distribution of chickpea area and production in the world is very uneven; around 92% of the area and 89% of the production of chickpea are concentrated in Asia, Africa and America, with Africa accounting for nearly 4-5% of the world area and production (Jodha and Subbarao, 1987). In the sub-Saharan Africa, the crop is especially prominent in Ethiopia with smaller amounts grown in Malawi and Tanzania (ICRISAT, 2000).

2.1.2. Production and Importance

The major chickpea growing countries in the world are India, Pakistan, Turkey, Ethiopia, U.S.A., Mexico and Australia (FAO, 1994). Like most pulses, chickpea production has increased only marginally in the last three decades because of the decline in the area under production. However, the productivity of the crop has improved by about 37% in the corresponding period due to the development of high-yielding cultivars and improved technologies (Singh and Saxena, 1999).

Chickpea accounts for 15% of the world's cultivated area and 13% of the world's production of pulses (Jodha and Subbarao, 1987). The total annual grain production is around 5.6 million metric tones. Chickpea was cultivated in 44 countries of the world in 1994 compared to 33 countries 15 years ago, indicating an increase in its expansion (Singh and Saxena, 1999). It is the second most important food legume grown in Africa preceded by faba bean (Halila and Beniwal, 1990). The crop is traditionally grown in several countries in eastern Africa (ICRISAT, 2000). The area covered by the crop, average yield obtained and its production for the years 1997-1999 is given in Table 1.

According to the Central Statistical Authority (2000), chickpea production in Ethiopia has a total area of 184,790 hectare and 1,646,270 quintal total production, which is about 2.25 and 1.85%, respectively. Ethiopia is one of the ten countries with the largest area, lead by India (6,665,000ha), Pakistan (1,045,000ha), Turkey (723,000ha) and Iran (648,000ha) (Singh and Saxena, 1999). However, the largest producers are different, reflecting a difference in productivity, for example the highest per hectare productivity goes to countries like Lebanon (2039kg), Jordan (1700kg), Mexico (1597kg), Egypt (1557kg) and Israel (1333kg).

In the 1970s, chickpea occupied 34% of the total pulse area and accounted for 40% of the total pulse production in Ethiopia with about 10,000 tonnes of export per year (Bejiga and Eshete, 1996). They also indicated that, Shewa is the most important chickpea-growing region followed by Gonder, Gojam, Tigray and Wello plus a small area under chickpea in the other regions (Appendix 14).

Chickpea is usually grown as a rain-fed cool-weather crop or as a dry climate crop in semi-arid regions. Optimum conditions include 18-26°C day and 21-29°C night temperatures and annual rainfall of 600-1000 mm (Duke, 1981; Smithson *et al.*, 1985). It is usually grown on heavy black vertisols (pH 5.5-8.6) of the Ethiopian highlands (1400-2300 masl) with the annual rainfall of 950-1800 mm (Bejiga, 1980). Frost, hailstorm, and excessive rains damage the crop. Chickpea may be cultivated as a sole crop, or mixed with barley, grass pea, linseed, mustard, peas, corn, coffee, safflower, potato, sweet potato, sorghum, or wheat and in rotation it often follows wheat, barley, or tef (Van der Maesen, 1972).

Table 1. Area under chickpea, yield kg/ha and total production in 1997-1999 for African countries and the world

Country	Area 1000ha			Yield kg/ha			Production Mt		
	1997	1998	1999	1997	1998	1999	1997	1998	1999
World	10609	11224	12034	776	782	768	8235	8779	9244
Africa	397	485	471	662	677	628	263	328	296
Algeria	31	30	28	524	614	472	16	18	13
Egypt	5	6	9	1776	1790	1730	9	11	15
Ethiopia	119	180	168	996	762	828	118	137	139
Malawi	95	96	96	400	417	427	38	40	41
Morocco	59	70	71	677	831	396	40	58	28
Sudan	6	6	7	1361	1500	1538	8	9	10
Tanzania	60	60	61	350	367	377	21	22	23
Tunisia	15	30	25	539	934	880	8	28	22
Uganda	6	6	6	500	508	516	3	3	3

Source: FAO bulletin of statistics 2000

Like other pulse crops, chickpea has multiple functions in the traditional farming system in many developing countries. It is an important source of human food and animal feed, and also maintains soil fertility, particularly in the dry areas (Singh and Saxena, 1999). In Ethiopia, chickpea is important as part of the cropping system because, its use in rotation with cereals can mitigate the decline of soil fertility. The crop is viewed as indispensable protein and energy source and potential crop to diversify cereal monoculture and supplement to cereal-based diets. The seed of the crop is nutritionally rich in protein (25.3-28.9%), essential amino acids, phosphorus (0.3%), calcium (0.2%) and is relatively high in fiber (3%) (Malhotra *et al.*, 2000). It also has 38-59% carbohydrate, 4.8-5.5% oil, and 3% ash (Hulse, 1991). Digestibility of protein

varies from 76-78% and its carbohydrate from 57-60% (Muehlbauer and Tullu, 1997). It is relatively free from antinutritional factors, and therefore, widely appreciated as a healthy food.

In the country, chickpea seeds are generally used as snacks, side dishes, breakfast and infant foods. They are consumed green (Eshet), cooked, roasted (Kolo) or germinated. The dry seeds are mixed with wheat and/or barley and ground to powder to make "Kiyit Injera". Split chickpea seeds (Kik) and powdered seeds (Shiro) are also used to make 'wot' a type of sauce eaten with injera (Bejiga, 1980; Yetneberk and Wondimu, 1994). The vegetative biomass is highly valued as a fodder in dry areas where grazing vegetation is scarce; it is a good source of protein for feeds, except that the amino acids methionine and cystine are deficient (Oplinger *et al.*, 1990).

The leaf extract is rich in malic acid and is sometimes used for medicinal purposes (ICRISAT, 2000) or used as vinegar. An adhesive may also be prepared; although not water-resistant, it is suitable for plywood. Leaves are said to yield an indigo like dye. Chickpeas also yield 21% starch suitable for textile sizing. Among the food legumes, chickpea is the most hypocholesteremic agent; germinated chickpea was reported to be effective in controlling cholesterol level in rats (Geervani, 1991). Generally, the medicinal applications include use for bronchitis, cholera, constipation, diarrhea, flatulence, snakebite, sunstroke, and warts (Muehlbauer and Tullu, 1997).

2.1.3. Production Constraints

High and more stable yields are the major goals of plant breeding programs. However, the maximum yield of crop cultivars, determined by their genetic potential, is rarely achieved because of several limiting factors such as insufficient water and nutrients, damage by plant

diseases and insect pests, poor traditional agronomic practices by subsistence farmers plus poor input availability, low prices, lack of sustainable marketing policy, lack of mechanization (Singh *et al.,* 1994), and poor extension services (ICRISAT, 1990).

Low and unstable yields are obtained from the chickpea crop. Biotic and abiotic stresses are responsible for the instability of chickpea yield (Halila and Beniwal, 1990).The first step to solve these problems is to detect and identify the major production constraints and set priorities for improvement, which is followed by the appropriate measure such as, evaluation and selection of desirable local landraces. Proper understanding of important stresses in different countries and the genetics of resistance are required for more systematic approach in plant breeding (Singh *et al.*, 1994). Moreover, wild *Cicer* species hold promise and deserve attention in resistance breeding, as they appear to be valuable sources of resistant genes.

A. Biotic Factors

The general estimates of yield losses by biotic factors (pests, diseases or weeds) range from 5-10 % in temperate regions and 50-100% in tropical regions (Van Emden, 1988). Biotic stresses, especially Helicoverpa pod borer insect, Fusarium fungal wilt, Ascochyta blight, and Botrytis gray mold detract significantly from chickpea's potential productivity (ICRISAT, 2000).

Because chickpea leaves, stems and pods are heavily pubescent with glandular hairs that secrete malic and oxalic acid they suffer little direct damage from aphids and other insects (Oplinger *et al.* 1990). However, some of the important pests are: pod borer *(Helicoverpa armigera)* that feeds on leaves and developing seeds (Smithson *et al.*, 1985). Occasionally it causes more than 80% pod damage on early sown chickpea in Ethiopia (Bejiga and Eshete, 1996). Cutworms *(Agrotis*

sp.) groundnut aphid (*Aphis craccivora*), pea aphid (*Acyrthsosiphon pisum*), cowpea bean seed beetle (*Callosobruchus maculatus*), and Adzuki bean seed beetle (*C. chinensis*) are also some of the pests of this crop. Furthermore, storage insects specifically *Bruchid* sp. that lowers seed viability is a serious pest of stored chickpea.

Wilt and root rots caused by *Fusarium oxysporium* f.sp. ciceri, *Rhizoctonia bataticola* and others are the most important chickpea diseases in the Nile valley and Red sea countries (Ethiopia, Egypt, Sudan and Yemen) and North Africa, particularly Tunisia (Bejiga *et al.*, 1988). Generally Ascochyta blight, Rhizoctonia root rot, Pythium rot, Fusarium wilt, white mold, and bacterial blight are disease problems in production fields of chickpea. These are typical diseases that affect other legume crops and they are favoured by periods of high rainfall, humidity and high temperatures. There are also viruses isolated from chickpea that include alfalfa mosaic, pea leaf roll, pea streak, and bean yellow mosaic (Duke, 1981; Smithson *et al.*, 1985).

These biotic constraints are best controlled by using good quality seed, proper crop rotations, proper tillage practices, burying diseased residue and using disease resistant varieties (Oplinger *et al.*, 1990). For the storage pests some practices that have been recommended for control are, dusting with pesticides or fumigation with methyl bromide (Duke, 1981).

B. Abiotic Factors

Among the abiotic factors, drought stands to be the number one problem in major chickpea growing regions because the crop is grown on residual soil moisture and is eventually exposed to terminal drought (Johansen *et al.*, 1994). The effects of drought are heightened, as the soil starts cracking and roots get pruned and the crop-growing period is shortened (Saxena, 1987, Bejiga

and Eshete, 1996). In west Asia and North African countries, low temperature causing freezing injury or death or delayed pod setting reduces yield tremendously (Singh, 1987). Heat and salinity problems are relatively important following drought and cold stresses (Singh *et al.*, 1994). Water logging is a major constraint to August-sown chickpea in Ethiopia in years when rainfall is high; it may even kill the crop. Soil nutrient imbalances are also important in localized areas (ICRISAT, 2000).

Poor agronomic practices are other factors for the reduction of productivity. In East African agriculture, food legumes face "non cultivated culture" where a single ploughing with a pair of oxen or no ploughing is done prior to planting. In Ethiopia, chickpeas are planted in late August or mid September and harvested in late January or mid February with no weeding practice at any stage of crop growth. Seeding rates are also extremely low 40 to 50 kg/ha, while research recommendations are a minimum of 80 to 90 kg/ ha. Fertilizer is not used in chickpea production since it is considered as restorer of soil fertility for the following cereal crops (Telaye, 1988).

2.2. Crop Genetic diversity

The importance of crop genetic resources was first realized by Vavilov (1926) who discovered wide variation among plants of the same species. Crop genetic resources is described as the total of genetic diversity of cultivated species and their wild relatives, which includes commercial varieties, landraces (farmer varieties), special genetic stocks (mutants, breeder's lines etc.) and wild and weedy relatives of potential value to man (Worede, 1988).

Breeders realize that the success in any breeding program is largely dependant upon the availability of wide genetic diversity (Malhotra *et al.*, 1987). Genetic diversity is the amount of genetic variability among individuals of a variety, population or species (Brown, 1983). Nevo and Beiles (1989) express it as the foundation for survival, adaptation and evolution of any species.

Diverse gene pools are the foundation of an effective crop improvement program, since they do not only provide the necessary building blocks for further crop improvement, but also are essential if high levels of productivity are to be sustained. Knowledge of the nature and extent of such genetic variation is crucial for successful conservation and utilization of germplasm, which depends on proper assessment of diversity within and among populations. The most important use made of the germplasm collection has been their exploitation as source for resistance and tolerance to biotic and abiotic stresses, and to improve the nutritional quality of the crop, both for human and animal consumption (Robertson *et al.*, 1996).

Ethiopia is one of the world's richest countries of crop diversity and its genetic resources are of critical value world wide. The Ethiopian region is often referred to as a major Vavilovian center of origin and/or diversity for several domesticated crops and their wild and weedy relatives. It is a center of origin for various crops such as, tef (*Eragrostis tef*), coffee (*Coffea arabica*), noug (*Guizotia abyssinica*), enset (*Ensete ventricosum*) and mustard (*Brassica carinata*). It is a center of diversity for a number of crop species that have been introduced centuries ago and developed in to wide genetic diversity. These include pulse crops such as faba bean, chickpea, lentil, cowpea, grass pea and major cereal crops such as barley, sorghum, durum wheat, finger millet, and some oil crops like linseed, castor bean, sesame and safflower.

2.3. Genetic Erosion

Today there is a great concern over the loss of genetic diversity, partly with the substitution of a diverse set of genetically variable crop landraces with few genetically uniform improved varieties (Brush, 1991; Harlan, 1992). This concern is especially valid in areas of crop domestication and a center of genetic diversity such as Ethiopia, where diversity of several crop species (e.g. durum wheat, barley, sorghum, coffee, etc.) is concentrated. Changes and development in agriculture or land use led towards destructions of the ecosystems. Furthermore, development of hydroelectric or irrigation projects, highways, industrial sites and housing areas aggravated the loss of the wild and weed species of crop plants (Sneep and Hendrikson, 1979).

In Ethiopia, the existing broad range of genetic diversity, particularly the primitive and wild gene pools, are presently subjected to serious genetic erosion and irreversible losses. Genetic erosion in the various food legumes grown in the country is progressive on account of extensive selection where indigenous landrace populations represent the bulk of the material used in breeding programs. The extent to which the displacement of native seeds by exotic/improved materials occurs has not yet been fully documented, it would also vary between regions and crops.

As a single gene or few major genes control most of the desired traits, it is thus obvious that growing uniform cultivars over vast areas is potentially dangerous. The products are frequently vulnerable to changing conditions such as unexpected drought or a particularly virulent virus because of their narrow genetic base (IBPGR, 1981).

The concern of Westphal (1974) specifically to chickpea was that, even though most of the area under chickpea cultivation is still sown to landraces, and contributes to the preservation of much of the genetic diversity, there is a danger of genetic erosion because of plant breeding and the production of new cultivars in many countries. Ahmad (1999) mentioned that the genetic erosion of chickpea resources due to biotic and abiotic stresses as well as economic and strategic reasons is a persistent process. Some of the good examples which indicate this continuous genetic loss of chickpea landraces are the 1968/69 and again the 1978/79 ascochyta blight which developed in an epiphitotic form and killed almost all of the chickpea crop in Punjab province of India and the neighboring Pakistan and the loss caused by drought in Ethiopia during the mid 1970s which reduced chickpea cultivation by half (Sneep and Hendrikson, 1979).

2.4. Genetic Conservation

About 1000 chickpea accessions of indigenous landraces are available in the Institute of Biodiversity Conservation and Research (IBCR). Owing to the limited available indigenous landrace collection, the national chickpea improvement program relied heavily on introductions and approximately over 6000 chickpea lines and segregating populations have been introduced from which several lines were selected for yield and other desirable characteristics (Bejiga and Anbesse, 1994).

There are two strategies of germplasm conservation, *ex-situ* and *in-situ*. Both systems are being followed in Ethiopia's crop genetic resources conservation. However, *in-situ* which is commonly a community-based conservation of farmers' varieties in different agro-ecological regions is not yet rigorously undertaken as that of *ex-situ*.

Efforts to conserve crop diversity to-date have focused on maintaining genetic diversity in static *ex-situ* gene banks. In Ethiopia, the Institute of Biodiversity Conservation and Research (IBCR) has been practicing *ex-situ* conservation system and undertaken systematic crop germplasm exploration and collection operations in the different administrative regions of the country, covering a wide range of agro-ecological conditions. This method has, however, arrested the complex interaction of genetically diverse traditionally cultivated varieties (landraces) with their associated pests, predators, and pathogens. *Ex-situ* conservation also fails to retain traditional farmers knowledge associated with landraces, knowledge that can be instrumental in utilization and development of new crop varieties from farmer's original varieties.

Therefore, there has been increasing interests in recent years on *in situ* conservation with a view to complement the *ex situ* conservation effort. Choice of sites for *in situ* conservation may be based on two types of data, (1) high diversity estimates based on markers, or (2) knowledge of adaptive traits linked to certain ecological conditions. Under appropriate ecological and environmental condition, *in situ* populations of the crop species may not only maintain a large level of variation but also a high frequency of desirable genes (Demissie and Bjørnstad 1996).

2.5 Genetic Markers

For genetic conservation work, determining the magnitude of genetic variability and its pattern of distribution in the different *in situ* crop conservation sites and altitude gradients is essential (Bekele, 1985). Such information could serve as a benchmark for future assessment of genetic erosion (Hammer *et al.*, 1996), and for a proper management and a better exploitation of the existing gene pool (Jain *et al.*, 1975; Bekele, 1984).

The pattern of variation as measured by means of markers, such as morphological and biochemical markers, could give an insight into the evolutionary forces responsible for maintaining genetic variability and may also assist in genetic resources conservation and utilization, by determining the relative contribution of the different levels of variability to the total diversity available in an area of crop domestication, and in a center of genetic diversity (Kebebew *et al.*, 2001). The importance of a hierarchical approach to genetic conservation to quantitatively define the variation and its pattern of distribution in a center of microcenters was discussed by Bekele (1985).

2.5.1. Morphological traits

Morphological characteristics have traditionally been used as a basis for classification since the early days of taxonomy, and overwhelming reliance is still placed on morphological traits to produce practical classifications. Observations and studies of phenotypic traits are the basis for Vavilov's determination of centers of diversity for various crop species. In order for the plant genetic resources collections to be of practical value to plant breeders, characterization for agronomic and morphological traits is imperative (Andersen and Fairbanks, 1990). Hence, morphological expressions of any genetic constituent have been demanded so far for the identification and characterization of plant germplasm.

Morphological studies are done by using morphological markers that are highly heritable traits such as seed color, seed size, leaf color, flower color etc. They are inexpensive, simple and rapid to score. However, they have weaknesses since many of the traits are controlled by many genes, phenotype of most morphological markers can only be determined at the whole plant level and

only dominant alleles are visible morphologically (Powell, 1992). Moreover, some changes to genes cannot be observed at a morphological or physiological level because the structural differences in the gene product may not alter its biological activity sufficiently to result in an altered phenotype (Sabir *et al.,* 1992). In addition to that, through characterization of a large collection, particularly for agronomic traits, may be impractical and also these traits have the disadvantage of being influenced by environmental factors (Andersen and Fairbanks, 1990). However, these morphological markers are generally useful in crop improvement programs (Demissie and Bjørnstad, 1996).

The measurement of morphological characters is used to assess diversity, but the environmental effect on these characters renders this measure relatively insensitive, particularly where differences are small. Therefore, morphological markers need to be supported with more sensitive markers especially when measuring diversity between and within populations of a single species (Willicms *et al.*, 1990).

2.5.2 Biochemical markers

Isozymes

Biochemical markers offer a better alternative than morphological markers to characterize and classify germplasm collections, because they are more stable across environments and generally polymorphic (Aradhya *et al.*, 1994). These are widely used techniques, which include seed protein and isozyme. Isozymes are practical and useful genetic and biochemical markers as well as being good estimators of genetic variability in plant populations (Hvid and Nielsen, 1977). They operate at the gene product level, where the environment has very little influence but their

major draw back is the limited number of informative units which can be visualized and the fact that they focus on a very narrow region of the genome (Willicms *et al.*, 1990).

Isozyme offers a unique and simple method for the characterization of genetic variation and is applicable to many differing forms of genetic studies, ranging from the description of the comparative levels of variability, which occur in natural population through to detailed measurement of linkage relationships on the plant genome (Hayward *et al.*, 1995).

Isozyme analysis has contributed to understanding of geographic distribution of genetic variation and the origin, domestication and evolution of crop plants (Doebley, 1989). It is also useful for examining the degree of genetic diversity and the relationship within and among species (Aradhya *et al.*, 1994) as it allows individual character loci to be identified. Furthermore, isozyme has been used extensively for characterization of genetic resources in several plant species (Smith, 1988; Andersen and Fairbanks, 1990; Matus and Hucl, 1999), to study progenitors, loss of genetic variation, center of genetic diversity and relationship between isozyme and morphology (Pasquet *et al.*, 1999). Such data have been used in preparation of key for classification, providing representative phenograms, determining allozyme frequency of different loci, identifying rare alleles, understanding heterozygosity/homozygosity at different loci and the like (Mauria *et al.*, 2000).

Seed proteins

Seed proteins can be classified based on their biological functions. Although seeds contain various types of proteins including metabolic and structural protein, the major function which accounts 50% or more of the total protein in the seed is storage protein (Gepts, 1990). Storage

proteins are defined as any protein accumulated in significant quantity in developing seed, which on storage is rapidly hydrolyzed, and provide sources of reduced nitrogen for the early stage of seedling growth.

The storage proteins of plant seeds are interesting possibilities to perform comparative genetic studies on plant since they posses a high level of polymorphism, generally inherited co-dominantly like isozymes and environmental factors have little or no effect although the amount of protein stored may be affected (Demissie, 1996).

3. MATERIALS AND METHODS

3.1. Morphological study

3.1.1. Description of the study site

This study was conducted at Chefe Donsa Research Site (Altitude, 2400 meters above sea level, 39° 08' E and 08° 58' N) in Gimbichu Wereda of East Shewa Zone. The site is under the supervision of Debre Zeit Agricultural Research Center, which is the coordinating center for national chickpea research in the country.

3.1.2. Plant Material

Fifty chickpea (*Cicer arietinum* L.) populations collected from Shewa, Gonder, Gojam, Arsi, Bale, Tigray, Wello, Gamo Gofa, Hararghe and Sidamo administrative regions (Appendix 13) and covering different agro-ecological zones were used for the study (Table 2). The materials along with their passport data were obtained from the Institute of Biodiversity Conservation and Research (IBCR) of Ethiopia. Selections of the populations were made based on altitude and administrative regions from the available long-term collections of the Institute.

Initially, populations from ten regions were sampled for this particular study. However, to avoid the impact of sample size on the experiment, accessions from regions with sample size less than four were mixed. In doing so, accessions from Arsi were included in Bale (adjacent region) and that of Sidamo (specifically Liben) in to Hararghe (more or less similar in agroecology). Hence, the regions of origin that were originally ten were rounded to eight. The altitude range of the

collection localities was between 1420-3120 masl and is arbitrarily classified into six altitudinal groups (≤ 1500, 1501-1700, 1701-1900, 1901-2100, 2101-2300, and > 2300).

Table 2. List of chickpea populations/accessions with their respective administrative region, district (woreda) and altitude

Administrative Region	Accession Number	Woreda	Altitude masl	Adminstrative Region	Accession Number	Woreda	Altitude masl
Shewa	41007	Debre Libanos	2670		41279	Bahir Dar	1890
	41012	Alem Gena	2150		212686	Kemkem	2050
	41124	Akaki	1970	Arsi	41002	Tenta	2080
	41135	Ada	1900		207761	Tenta	2080
	41138	Lume	1920	Wello	41096	Debre Sina	2510
	41144	Minjar	1850		41113	Kalu	1650
	41163	Debre Zeit	1970		41114	Were Babo	1560
	41189	Wolkite	1870		41115	Adela wedih	2290
	41194	Tulu Bolo	2250		41116	Dese Zuriya	1950
	41209	Deneba	2600	Tigray	219798	Adi Abeyti	2200
Gonder	41018	Wegera	1840		219804	Axum	1700
	41019	Iste	2500		235391	Chercher	1420
	41047	Chilga	2160		235393	Abiy Adi	1500
	41048	Farta	2640		236482	Indabaguna	1690
	41053	Lay Gayint	3120	Gamo Gofa	41037	Hamer	1490
	41295	Fogera	1820		212914	Gardula	1520
	41296	Addis Zemen	1850		212916	Gardula	1860
	41319	Enfraz	2000		212917	Kemba	1580
	207619	Farta	3114	Bale	41034	Sinana	2400
Gojam	41026	Hulet Iju	2280		229955	Nensebo	1940
	41245	Bahir Dar	2450		229956	Nensebo	2570
	41258	Kuye	2650	Hararghe	41052	Mieso	1510
	41268	Mota	1770		41054	Chiro	1500
	41270	Adet	1840		41055	Chiro	1500
	41272	Adet	1960	Sidamo	235963	Liben	1470

3.1.3. Experimental procedures

The experiment was laid out in randomized complete block design (RCBD) with three replications (Figure 2). Each population was sown in a three-meter long single row with 0.6 m and 1.5 m intra- and inter-block distances, respectively. The seeds were sown in 0.1m spacing between plants. Ten individual plants from every accession in a plot, a total of 1500 individual plants were tagged randomly and evaluated for the qualitative and quantitative characters mentioned below using the International Board of Plant Genetic Resources descriptor for chickpea (1985) with minor modifications (Table 3). Days to flowering, days to maturity, grain yield and biological yield were measured on plot basis.

Figure 2. Experimental layout

Table 3. List of qualitative and quantitative characters recorded, their codes and descriptions

Qualitative characters	Code	Description
Plant Anthocyanin	ANT	1=absent, 2=present
Plant hairiness	PHR	1=none-hairy, 2=intermediate, 3=hairy
Flower color	FC	1=light-pink, 2=pink,3=purplish-pink, 4=white
Seed color	SC	1=yellow, 2=variegated, 3=light-brown, 4=dark-brown, 5=black
Seed surface	SS	1=smooth, 2=slightly-wrinkled, 3=coarsely-wrinkled
Seed shape	SSh	1=angular, 2=globular, 3=rounded
Growth habit	GH	1=erect, 2=semi-erect, 3=spreading, 4=prostrate
Quantitative characters		
Days to 50 % flowering	DTF	The number of days from planting up to flowering of 50% of the populations
Days to 90 % maturity	DTM	The number of days from planting up to maturity of 90% of the populations
Number of primary branches	NPB	The actual count of primary branches on the main stem
Number of secondary branches	NSB	The actual count of secondary branches on the primary branches
Plant height at maturity (cm)	PH	The height /distance from the ground level to the tip of the plant
Thousand seed weight (g)	TSW	Weight of 1000 seeds randomly taken from each accession
Biological yield (g)	BIY	The weight of the plant taken above ground
Grain yield per plot (g)	GYPP	The dried weight at 12% moisture content of seeds from the plant
Number of seed per plant	NSPP	The actual count of seeds of the individual plants
Harvest index	HI	The ratio of grain yield to biological yield multiplied by 100

Phenotypic variance (Vp) = Genotype MS/r

Error variance (Ve) =Error MS/r

Genotypic variance (Vg) = Vp-Ve,

Phenotypic coefficient of variation (PCV) = 100 x ($\sqrt{Vp}$)/m

Genotypic coefficient of variation (GCV) = 100 x ($\sqrt{Vg}$)/m

Heritability (h^2) = Vg/Vp

Genetic advance (GA) = (i) (h^2) ($\sqrt{Vp}$)

GA (% of mean) = (GA/m) x 100

Where r = number of replications, MS = mean squares, m = the mean value, i = selection differential (varies depending upon the selection intensity and stands at 2.06 for selecting 5% of the genotypes)

To visualize the association among region through an ordination technique using the qualitative traits, the regions contingency data was assessed in terms of row profiles (regions) and column profiles (traits) using correspondence analysis (MINITAB, 1998). Variability is partitioned as with principal components, but rather than partitioning the total variance, it partitions the Pearson chi-square statistic which is termed as inertia (*chi-square/n*, accounted for each component). Simple correlation analysis between all possible traits was carried out from the population means using SPSS for Windows Release 9, computer program (SPSS, 1999).

The group formation of the accessions was investigated by means of subsequent cluster (CA) and principal component (PCA) analysis using correlation matrix to define the existing pattern of variation both between populations and between their regions of origin. The standardized data were used as the input for cluster analysis. Ward's method, which minimize within cluster variance summed over all variables, appear to give the most satisfactory clustering result with most populations included in clusters of similar size (Sneath and Sokal, 1973). On the bases of the pseudo-t^2 statistics, seven clusters were considered as the optimum partition (SAS, 1999). Within cluster, means of the quantitative traits were computed for ease of interpretation, and the dendrogram was plotted. The relationship among the clusters were assessed by measuring the inter cluster distances using Mahalanobis's distance (D^2). The D^2 values obtained from pair of clusters were considered as the calculated value of chi-square (χ^2) and were tested for significance both at 1% and 5% probability level against the tabulated value of χ^2 for 'P' degree of freedom, where P is the number of characters considered (P=10) (Singh and Chaudhary, 1985). Data were analyzed using SAS Release 8.2 (SAS, 1999) and MINITAB for Windows Release 12.22 (MINITAB, 1998).

In this particular study since no variation is observed between the accessions for the number of enzyme systems applied, the statistical analysis such as average number of alleles per loci (A), percent of polymorphic loci (P) and average heterozygosity (H), genetic distance (D) cluster analysis and Shannon diversity index cannot be performed. The mean protein content of the populations over regions of origin and over altitude groups were calculated. One sample t-test was also performed to analyze the crude protein data obtained from the protein content analysis carried out using the Kjeldahl method.

4. RESULTS AND DISCUSSION

4.1. Morphological diversity

4.1.1. Regional Distribution of characters

The percentage frequencies of the phenotypic classes of each character, population, region and altitude group and respective chi-square values for the seven qualitative characters considered in the present study are shown in Table 4. The variability of chickpea was estimated to be high in Shewa, Gonder, Gojam, Tigray region and medium in Wello, Arsi, Hararghe and Sidamo (Mengesha, 1975).

Absence of anthocyanin pigmentation is the predominant phenotypic class in all geographical regions with very limited (< 10%) presence in Shewa, Gonder, Arsi-Bale and Tigray. However, Pundir and Mengesha (1983), signifying the importance of this character, indicated that chickpea cultivars with higher anthocyanin content are relatively more stable and yield better. Figure 3 shows the anthocyanin pigmentation in chickpea plant. Since most parts of the chickpea plant are hairy or pubescent (Singh and Saxena, 1999), non-hairy populations were not found. However, intermediate plant hairiness was observed in higher frequencies on materials from Wello and Gonder, while hairy populations are more frequent in Gamo Gofa. In some crops this hairiness character is found to relate with disease resistance, for example association of glume hairiness with resistance to kernel bunt in durum wheat (Warham, 1988). In chickpea, Oplinger *et al.* (1990) mentioned that because the leaves, stems and pods are heavily pubescent with glandular hairs that secrete malic and oxalic acid the crop suffers little direct damage from aphids and other insects.

The flowers of desi type chickpeas are variably pigmented. In this study pink flower color is most frequent in all the regions (>50%) except in Gamo Gofa (47%). This is in line with Pundir *et al.* (1985) record of 71% pink flowers evaluating 12,000 accessions of chickpea. Light pink flower is the next dominant flower color which was relatively higher in frequency than the other flower color classes especially in populations from Gamo Gofa (45%) and Wello (40%). Populations with purplish pink flower are observed in Shewa, Gojam and Gonder at a very low frequency.

Seed color in chickpea is one of the most important diagnostic characters which determine the quality and acceptance of cultivars in many chickpea producing countries. The distribution of seed color characters in this study revealed that, light brown was the predominant character observed with higher frequency in all the regions (>50%), except in Arsi-Bale (33%). Yellow seed types are high in frequency in materials from Arsi-Bale (44%), they are also observed in reasonable frequencies in populations from Shewa (29%) and Gojam (23%). Black seed color was rare, reached 20% only in Gamo Gofa and the highly significant chi-square ($\chi 2$) deviation could attribute to the concentration of this character in this region (Table 4). Van der Maesen (1972) indicated that the black seeded chickpeas are mainly confined to Ethiopia while they are absent in the Turkistan, Afghanistan and the Caucasus. Dark brown types were frequent in Tigray and some variegated seeds were also observed in a very small amount in some regions.

The human factor might have played a role in the presence of almost all seed color types in all regions. For example, Belay *et al.* (1995) discussed in wheat the various seed colors are traditionally preferred for specialized consumption purposes. Asfaw (1988) in his study on barley indicated the association of grain type (color, size) and the use of the grain (utility). This can also

be attributed to the lack of serious seed selection which is difficult in other crops unlike maize and sorghum where individual ears are selected in traditional farming practice (Asfaw, 1988).

Chickpea seeds are characteristically beaked, often angular and wrinkled (Singh and Saxena, 1999). Hence, most of the materials in this study were with seeds which are intermediate to coarsely wrinkled, but smooth seeds were also observed in some populations from Shewa, Gonder, Gojam and Tigray. It is also observed that most of the populations show angular type of seed shape a typical seed shape of desi type chickpeas, the highest frequency being in Gamo Gofa (76%) and Gonder (63%) while the globular were frequent in materials from Hararghe-Sidamo and Shewa. There were very rare observations of round shaped seeds in Arsi-Bale and Shewa regions.

Variation in growth habit showed higher frequency of erect type plants in almost all of the regions. This might indicate that erect type growth habit is highly preferred by most farmers in the different regions, which may be due to its suitability for cultivation. Singh and Saxena (1999) mentioned that erect and semi erect growth habits are preferred for mechanical harvesting in different chickpea growing countries. Higher frequency of semi-erect types is observed in Hararghe-Sidamo region. In areas of low rainfall and high evapotranspiration spreading types are preferred (Singh and Saxena, 1999). In this study such type of growth habit were high in populations from Wello and Gonder regions while the prostrate types are more in Shewa and Hararghe-Sidamo populations. As it is shown in Table 4, the Hararghe-Sidamo region shows almost equal distribution of all the four growth habit classes followed by Wello and Shewa.

The difference between regions is only reflected through frequency differences in the states of the characters studied. Generally, most of the morphological traits studied in this experiment were not unique to any single region. As explained by Belay *et al.* (1997), this could be attributed to germplasm exchange (gene flow) between regions, or similar origin of the landraces (founder effect). Human preference for a certain crop type (color, growth habit or the like) also might have played a role. However, the differential frequency representation of the various states of characters studied partly implies the fitness of the various genotypes in the region considered.

Figure 3. Anthocyanin pigmentation

materials. This could be due to germplasm exchange or the impact of similarity in agro-ecology between the neighboring regions.

Table 5. Ordinate scores, inertia, proportion of variance and cumulative variance of the first two components for chickpea populations in the eight regions

Regions	C1	C2
Shewa	-0.057	0.098
Gonder	0.036	-0.080
Gojam	0.017	-0.024
Arsi-Bale	-0.080	0.284
Tigray	-0.034	-0.125
Wello	-0.066	-0.096
Gamo Gofa	0.360	0.020
Hararghe-Sidamo	-0.175	-0.078
Inertia	0.0221	0.0161
% of total variance	35.70	25.95
% cumulative variance	35.70	61.65

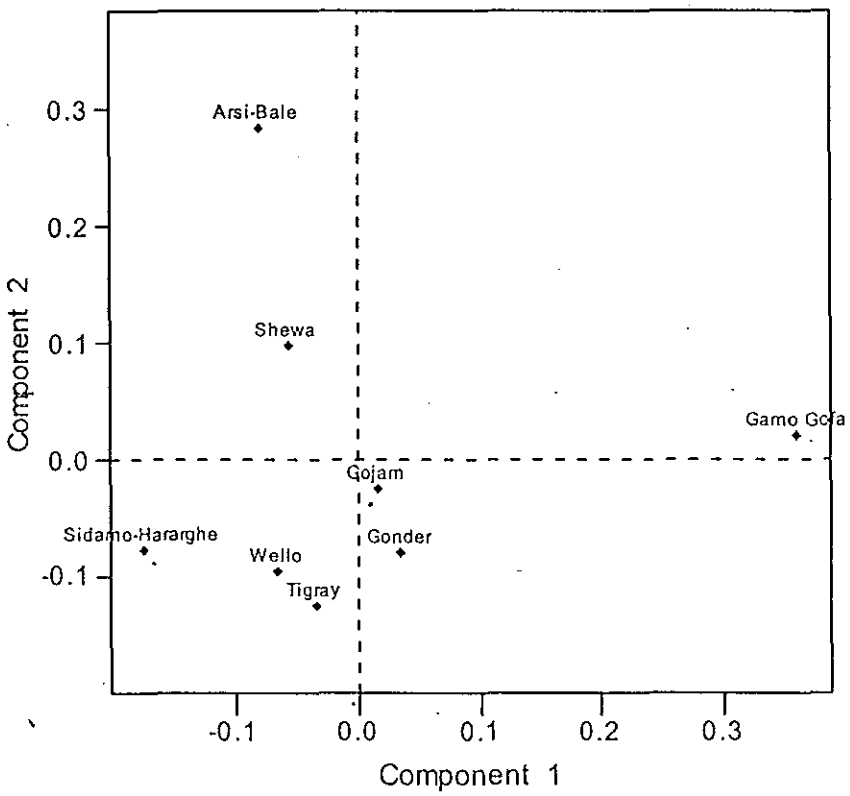

Figure 4. Distribution of the regions of origin of chickpea populations along the two axes of the component variables using qualitative characters

4.1.3. Altitudinal distribution of characters

Table 6 shows the phenotypic frequencies of individual characters across each altitude class and the corresponding chi-square values for the traits evaluated. Higher frequency of absence in anthocyanin pigmentation is observed in all altitude classes with limited frequency of presence in altitude group 1701-1900 and 1901-2100 meters above sea level.

Almost equal distribution of plant hairiness character frequency was observed in all altitude groups with overall mean percentage frequency of 41% intermediately hairy to 59% hairy. Among the flower color characters, the preference in all altitude groups goes to pink color (>50% except in altitude group 2101-2300masl) followed by the light pink and with lower frequency in case of purplish pink.

Light brown seed color is the predominant character in all the altitude groups (>50%) and its frequency shows a clinal variation and tends to decrease with increase in altitude. Yellow color seed frequency varies from 9% (1501-1700masl) to 29% (2101-2300masl) and the data shows higher values in the higher altitude. Most of the variegated seeds lie in the lower altitude group (≤1500). Black seeds are observed in very low frequency in all altitude groups, the highest being 12% (in altitude groups 1501-1700masl). As it is explained by Asfaw (1989) on his work in barley, the concentration of some traits at higher or lower altitudes might have resulted from random or selective accumulation of certain genes at higher or lower elevations which could be a result of partly farmers selection of morphotypes based on their selection criteria of suitability to the prevailing climatic and edaphic conditions of the area.

For seed surface, the wrinkled character is the most frequent in all the altitude groups with the exception of altitude range 2101-2300masl where the intermediately wrinkled ones are higher. Seeds with smooth surface are also observed at higher altitudes (>2100masl). Seed shape is mostly globular in the lower altitude groups, while it is angular in the rest altitude classes. Round seed types are observed in populations from the altitude groups 1901-2100 and >2301meters above sea level.

All the growth habit classes are observed in all the altitude groups with different frequencies. However, the predominant growth habit in all altitude classes was erect type followed by considerable frequencies of semi erect and spreading types. The distribution of the growth habit classes (erect, semi-erect and spreading) is not that much significantly different for most of the altitude classes (Table 6), indicating the existence of variability in growth habit character in different environments. The prostrate type of growth habit is observed in limited frequency ranging from 11% (1701-1900masl) to 18% (≤1500masl).

Table 6. Percentage frequency of phenotypic classes of individual qualitative characters* and chi-square values of chickpea landraces for each altitude groups

Altitudes	Anthocyanin			Plant hairiness				Flower color					Seed color						Seed surface				Seed shape				Growth habit					
	1	2	χ^2	1	2	3	χ^2	1	2	3	4	χ^2	1	2	3	4	5	χ^2	1	2	3	χ^2	1	2	3	χ^2	1	2	3	4	χ^2
≤1500	97	3	0.03	0	29	71	5.04	39	54	6	1	4.75	13	4	63	16	4	7.26	0	42	58	2.99	46	54	0	3.88	36	27	19	18	3.85
1501-1700	97	3	0.03	0	48	52	2.67	37	55	8	0	0.17	9	0	63	16	12	20.71	1	48	51	0.13	61	39	0	1.63	36	19	31	14	1.94
1701-1900	96	4	0.14	0	42	58	0.17	29	60	11	0	3.54	20	1	60	18	1	3.67	3	39	58	0.17	65	35	0	4.11	46	21	22	11	2.75
1901-2100	95	5	0.86	0	42	58	0.17	34	57	9	0	0.62	25	1	58	14	2	2.67	0	45	55	6.7	57	42	1	1.53	38	20	25	17	0.35
2101-2300	97	3	0.03	0	37	63	0.38	46	47	7	0	3.75	29	2	57	11	1	7.57	2	56	42	3.39	52	48	0	0.79	46	17	23	14	2.37
>2300	98	2	0.55	0	42	58	0.17	36	58	6	0	0.76	26	1	53	13	7	3.93	3	39	58	1.17	50	49	1	2.27	31	23	30	16	2.81

* For character description see Table 3

4.1.4. Diversity of characters

4.1.4.1. Estimates and analysis of phenotypic diversity

Estimated values of phenotypic diversity for each of the characters, populations, regions and altitudes are given in Table 7. On regional basis, with the exception of anthocyanin pigmentation, the least diverse character which shows monomorphism in several regions (e.g. Gamo Gofa, Gojam, Wello and Hararghe-Sidamo), all the other characters exhibited an intermediate to high level of phenotypic diversity (H'), ranging from 0.52 for plant height in Gamo Gofa to 0.99 for growth habit in Shewa, Arsi-Bale, Wello and Hararghe-Sidamo. Growth habit was the most diversified character in all the regions and in the entire collection.

The highest mean diversity index for each population over characters (Appendix 1) was exhibited by accession 41018 sampled from Gonder (mean H' = 0.66±0.04). On the contrary, population 212916 from Gamo Gofa showed the least diversity estimate (mean H' = 0.43±0.1). As shown in Table 7, the highest mean diversity index pooled over characters was observed for collections sampled from Shewa (0.67±0.08) and Arsi-Bale (0.65±0.08), while the Hararghe-Sidamo and Gamo Gofa regions showed lower diversity estimates (0.59±0.09 and 0.56±0.10 respectively). On country basis, H' was high for all characters (>0.60) except for anthocyanin pigmentation which was 0.21. The overall mean diversity index for the country in this study was estimated to be 0.64±0.08, indicating the existence of considerable morphological variability of the crop in Ethiopia comparable to what has been obtained in grass pea 0.61 (Tadesse, 1999). Higher estimates were also obtained in other important crops of the country: in wheat 0.87±0.04 (Jain *et al.*, 1975), 0.77±0.09 (Belay *et al.*, 1997) and 0.81±0.03 (Negassa, 1986); in barley 0.71±0.09 (Demissie and Bjørnstad, 1996) and in sorghum 0.90±0.02 (Ayana and Bekele, 1998).

When altitude was used as the classifying variable, the mean diversity index is found to increase with the altitude (Table 7), the maximum H' being in the higher altitudes. With the exception of anthocyanin pigmentation, all the characters exhibited an intermediate to high level of H', which ranges from 0.55 for plant height at ≤1500 masl to 0.98 for growth habit at >2300masl.

The analysis of variance (ANOVA) of H' for individual traits is presented in Table 8. The analysis revealed that the level of diversity of chickpea in Ethiopia is about the same in all the regions and altitude classes. For most of the characters, the variances were attributed to the within population and altitude groups rather than between region and altitude group implying that sampling within populations would catch most of the available variation. Similar results were obtained in durum wheat by Belay *et al.* (1997) and Ayana and Bekele (1998) in sorghum. Growth habit, was the only character that showed significant ($P<0.01$) difference between regions (Table 8).

Hierarchical analysis of variance pooled over characters also revealed that, much of the variance was due to the within population and within altitude class components (Table 9). Negassa (1986) also reported a non-significant variation at the highest hierarchical level of the region ANOVA in wheat. In their work on barley, Demissie and Bjørnstad (1996) explained the situation as the absence of pronounced geographical types, which may be explained in terms of the environmental heterogeneity of regions, irrelevance of administrative and regional boundaries in maintaining diversity and the significant degree of seed migration among regions.

Table 7. Diversity index H' estimates for each character in each region, altitude and the entire collection

Region	ANT	PLH	FC	SC	SCT	SS	GH	MEAN ± SE
Shewa	0.24	0.63	0.68	0.69	0.75	0.68	0.99	0.67±0.08
Gonder	0.24	0.63	0.63	0.62	0.71	0.60	0.88	0.62±0.07
Gojam	0.16	0.59	0.66	0.64	0.66	0.63	0.90	0.61±0.08
Arsi-Bale	0.27	0.59	0.57	0.74	0.63	0.74	0.99	0.65±0.08
Tigray	0.24	0.61	0.67	0.60	0.67	0.63	0.89	0.62±0.07
Wello	0.18	0.63	0.67	0.53	0.62	0.63	0.99	0.61±0.09
Gamo Gofa	0.07	0.52	0.65	0.72	0.57	0.50	0.89	0.56±0.10
Hararghe-Sidamo	0.17	0.63	0.58	0.55	0.59	0.62	0.99	0.59±0.09
Altitude								
<1500	0.21	0.55	0.65	0.69	0.62	0.63	0.97	0.61±0.08
1500-1700	0.21	0.63	0.64	0.65	0.67	0.61	0.96	0.62±0.08
1701-1900	0.23	0.62	0.65	0.62	0.72	0.59	0.90	0.62±0.08
1901-2100	0.27	0.62	0.66	0.66	0.63	0.68	0.97	0.62±0.08
2101-2300	0.18	0.60	0.65	0.66	0.70	0.63	0.92	0.63±0.09
>2301	0.15	0.62	0.62	0.72	0.72	0.65	0.98	0.64±0.08
Total	0.21	0.62	0.65	0.69	0.69	0.65	0.96	0.64±0.08

Table 8. Mean square of total variance for the different levels of grouping involving regions and altitudes from the ANOVA of H' for individual characters

	Between		Between populations within	
Characters	Region (df=7)	Altitude groups (df=5)	Region (df=42)	Altitude groups (df=44)
Anthocyanin	0.021	0.018	0.034	0.035
Plant hairiness	0.006	0.004	0.003	0.004
Flower color	0.013	0.004	0.008	0.009
Seed color	0.023	0.004	0.014	0.017
Seed surface	0.012	0.012	0.016	0.016
Seed shape	0.016	0.015	0.010	0.010
Growth habit	0.031**	0.006	0.007	0.011

**, P<0.01

Table 9. Hierarchical analysis of variance of diversity index (H') over all characters

Source of variation	df	Mean squares
Between		
Regions	7	0.0250 ns
Populations within region	42	0.0128 ns
Altitude	5	0.0090 ns
Populations within altitude	44	0.0152 ns
Characters within population	300	0.0557

df=degree of freedom, ns non significant

4.1.4.2. Mean and coefficient of variance for quantitative characters

Mean and coefficient of variation (descriptive statistics) in agronomic traits are widely used to determine variations within and between populations (Bekele, 1996; Belay, 1997). Summary statistics mean (M) and coefficient of variation (CV) of the morphological characters by region and altitude over the entire individuals of the fifty chickpea landrace populations are presented in Table 10. The result shows that there were differences in coefficient of variations in each trait studied, in all regions and altitude classes. Regions and altitude groups with high CV are associated with high variability for the particular traits considered. In the present study, the highest CV was obtained in number of primary branches, number of secondary branches, grain yield, number of seed per plant and biological yield in most of the regions and altitude classes (Table 10). Kumar *et al.* (1981) in their study using 330 chickpea lines obtained highest CV for biological yield, grain yield and pods per plant. This indicates the presence of appreciable variability for the recorded traits.

Number of secondary branches and number of seed per plant are highly variable characters in all regions showing above 40% CV. Similarly, Tadesse *et al.* (1994) obtained high variability (CV >40%) for number of secondary branches and number of pods per plant in all the regions they considered. The populations also show highest CV for number of primary branches (>35%), grain yield (>29%) and >20% for biological yield, in most of the regions and altitude groups. In the case of harvest index the CV is >20% only for materials from Gonder, Tigray and Hararghe-Sidamo and plant height in all regions, except Gojam and Hararghe-Sidamo. Days to maturity is the least variable character, this could be due to forced maturity by the shortage of water in the season that causes cracking of the soil and exposure of the roots to atmospheric air draft, the

same result in chickpea was obtained and explained by Tadesse *et al.* (1994). Low coefficient of variation was also observed in days to flowering and thousand seed weight in all regions and altitude groups.

Generally, region wise, the highest variation was depicted in Tigray for all traits studied, except days to flowering, thousand seed weight and harvest index, indicating the diversity of each trait in the region which can be related to the selection pressure in the area. In line with this, the lowest altitude group, which includes populations collected from ≤1500masl, showed higher CV for all characters considered except for days to flowering, days to maturity and thousand seed weight. Days to flowering and days to maturity were slightly higher for populations in the altitude groups > 2101masl.

Populations from Wello show highest mean for grain yield, number of seed per plant and harvest index, while the highest mean for days to flowering, plant height and biological yield are obtained from Hararghe-Sidamo. Days to maturity and thousand seed weight are higher in Arsi-Bale, while number of primary branches is in Gonder and number of secondary branches in Shewa. When the quantitative morphological characters are seen in terms of altitude, the highest mean grain yield per plot was obtained from medium altitude groups (1901-2100masl), thousand seed weight and harvest index were also high in this altitude range.

Analysis of variance for the ten quantitative morphological traits over the entire data showed highly significant differences between populations in most of the recorded characters, but not in days to maturity, grain yield per plot, biological yield and harvest index (Table 11).

Table 10. Mean (M) and coefficient of variation (CV) by altitude groups and region of the quantitative characters over the entire data

Altitude		DTF	DTM	NPB	NSB	PH	TSW	BIY	GYPP	NSPP	HI
<1500	M	47.22	137.94	1.82	17.48	31.92	124.89	795.66	258.37	98.14	32.82
	CV	8.24	1.82	47.80	67.84	25.08	7.23	36.80	50.08	104.06	29.64
1501-1700	M	45.71	136.71	1.91	16.01	33.45	123.62	711.75	242.61	105.59	33.67
	CV	5.83	2.53	40.22	53.57	21.13	6.30	31.05	39.71	80.48	21.18
1701-1900	M	47.17	137.33	2.16	18.17	34.21	123.17	757.68	261.45	116.19	35.32
	CV	4.81	1.38	35.33	47.31	20.29	7.03	30.86	29.97	75.50	19.32
1901-2100	M	46.30	137.67	2.05	17.80	32.96	127.07	726.58	270.33	117.70	37.46
	CV	4.37	1.64	37.02	48.54	22.01	9.44	30.63	37.15	74.75	21.13
2101-2300	M	45.83	138.28	2.03	17.87	32.54	121.67	661.49	230.16	99.89	35.64
	CV	5.32	1.96	39.85	51.45	21.90	11.76	31.93	29.20	88.33	19.07
>2300	M	48.09	137.33	2.18	20.45	33.07	124.48	731.14	270.05	119.12	37.31
	CV	8.45	1.93	36.36	46.70	22.28	10.64	29.84	33.49	77.11	16.86
Region											
Shewa	M	47.80	136.70	2.14	20.22	33.92	120.73	726.45	258.11	124.20	36.26
	CV	6.55	1.54	37.83	48.85	20.07	8.48	28.68	29.06	72.57	19.34
Gonder	M	46.87	137.97	2.19	18.36	32.43	124.27	745.58	263.66	113.99	36.40
	CV	6.09	1.63	36.22	47.60	21.85	5.10	34.19	34.21	79.91	20.92
Gojam	M	48.00	137.19	2.23	19.67	34.13	121.10	700.77	258.25	104.75	37.11
	CV	5.69	1.23	30.28	43.75	19.52	10.79	31.42	38.20	75.38	19.74
Arsi-Bale	M	45.33	139.13	1.90	16.09	32.11	135.80	713.01	264.75	111.58	36.85
	CV	4.48	1.84	40.79	52.26	24.27	12.89	25.88	32.18	83.88	14.57
Tigray	M	44.33	137.93	1.55	12.27	29.71	129.80	595.30	191.67	68.13	32.54
	CV	5.14	2.50	45.08	60.26	26.18	7.47	44.40	55.33	91.33	30.37
Wello	M	45.73	136.93	2.08	18.43	33.57	123.40	761.18	291.52	127.43	38.66
	CV	6.82	2.06	36.75	46.94	22.71	4.89	30.89	33.10	76.78	13.80
Gamo Gofa	M	47.25	137.67	1.96	18.09	34.30	119.33	759.34	252.08	108.04	32.69
	CV	6.56	1.98	41.02	55.05	20.79	7.37	26.66	38.11	75.49	14.06
Hararghe-Sidamo	M	48.33	136.83	2.02	19.75	34.98	124.08	899.94	286.07	123.38	31.80
	CV	6.96	2.22	42.63	58.51	19.84	4.88	21.38	35.67	92.53	29.18
Total	M	46.84	137.50	2.05	18.18	33.13	124.33	732.20	258.35	111.53	35.69
	CV	6.65	1.89	38.85	51.81	22.01	9.08	32.00	36.83	81.09	21.18

Table 11. Mean square for the ten quantitative morphological traits of chickpea populations as obtained from the analysis of variance (ANOVA)

Characters*	Mean squares			
	Replication (2)	Landraces (49)	Error (98)	CV (%)
DTF	14.48*	19.36**	4.88	4.72
DTM	2.18	7.36	6.64	1.87
NPB	1.91**	0.22**	0.1	15.15
NSB	190.56**	43.60**	19.91	24.55
PH	18.8	23.37**	10.07	9.58
TSW	4.51	248.74**	70.59	6.76
BIY	71580.03	84383.89	73240.55	37.06
GYPP	2863.58	4996.67	4056.89	37.24
NSPP	491.69	2089.14*	1333.77	32.74
HI	550.39**	108.48	79.96	35.44

*: P< 0.05 **: P< 0.01

* For character codes see Table 3

4.1.5. Heritability (broad sense) and genetic advance

Heritability estimates are important to indicate the genetic gain that may accrue through selection. Estimates of phenotypic (PCV) and genotypic coefficient of variations (GCV), broad sense heritability (h^2) and genetic advance as percent of the mean for the present study are presented in Table 12. Across the ten characters, the phenotypic and genotypic coefficients ranged in that order from about 1.14 – 23.86% and 1.1 – 21.50%, respectively. PCV and GCV values of roughly more than 20% is considered to be high, values less than 10% as low and values in between as medium (Deshmukh *et al.*, 1986). The lowest PCV and GCV were obtained for days to maturity and the highest for grain yield per plot. The lower CV indicates that selection is not effective for the particular character because of narrow genetic variability. For most of the characters studied, phenotypic coefficient of variance was slightly higher than the corresponding genotypic coefficient of variation signifying that genotypic factors exerted reasonable effect in estimating the variation. On the other hand, the relative wide gap between some corresponding PCV and GCV values (22.96% - 8.35% and 23.82% - 12.21%, for biological yield and harvest index respectively) indicate the influence of other factors such as environment, in addition to the genetic factors.

Broad sense heritability (h^2) is an important determinant in the response to selection and breeding programs (Ferguson and Robertson, 1999). In this study, h^2 estimate varied from 13% for biological yield to 90% for days to maturity (Table 12). On the whole, days to maturity, days to flowering and thousand seed weight demonstrated high heritability estimate: 90%, 75% and 72% respectively. Similarly, highest estimates of heritability in chickpea were obtained by Pandey and Tiwari (1983) for maturity (99.1%) and seed weight (98.1%). This indicated that these characters

are less influenced by environmental factors. Tullu (1985) also obtained highest heritability estimate for 100 seed weight, number of seeds per pod and plant height in chickpea. Low heritability were observed for grain yield (h^2= 13%), biological yield (h^2= 19%) and harvest index (h^2= 26%), which clearly indicates the influence of environment on these characters. Assefa *et al.* (1999), citing other works, indicated that relatively lower heritability estimates for grain yield than for other characters were also reported in sorghum, barley and tef. Intermediate h^2 estimates of 36-57% were obtained for the rest of the characters, number of seed per plant, plant height, number of secondary and primary branches in order of increase. The relatively low heritability estimate of characters indicates that selection for these traits would be more difficult than for the other characters.

Estimates of expected genetic advance were not high for most of the characters, and ranged from 2.12% for days to maturity to 23.32% for number of secondary branches (Table 12). These estimates were comparatively low and increased from 2.12% to 12.76% for days to maturity, biological yield, plant height, days to flowering, grain yield, thousand seed weight and harvest index in order of increase. Better estimates of genetic advance were observed in number of primary branches (15.12%) numbers of seed per plant (17.55%) and number of secondary branches (23.32%).

Generally, in this particular study, intermediate to high estimate of PCV and GCV were observed on characters such as number of secondary branches, grain yield, number of seed per plant and biological yield. Of all the characters evaluated in this study, number of secondary branch appears to combine relatively high values of GCV, h^2 and GA (% of the mean), making it one of

the most important characters which can easily be improved by selection for larger number of secondary branches. It should also be taken into consideration that estimates of heritability and expected genetic advance are influenced by the method of estimation, sample size and the environment, and the usefulness of these estimates depends on their repeatability (Pandey and Tiwari, 1983).

Table 12. Summary statistics and estimate of phenotypic (PCV) and genotypic coefficient of variation (GCV), broad sense heritability (h^2) and genetic advance (GA) in the chickpea populations for ten quantitative morphological traits

Characters*	Mean	Vp	Vg	Ve	PCV %	GCV %	h^2 %	GA (% of mean)
DTF	46.84	6.45	4.82	1.63	5.42	4.68	75	8.37
DTM	137.5	2.45	0.24	2.21	1.14	1.10	90	2.12
NPB	2.05	0.07	0.04	0.03	12.68	8.28	57	15.12
NSB	18.18	14.53	7.89	6.64	20.96	15.46	54	23.32
PH	33.13	7.79	4.43	3.36	8.42	5.52	43	7.46
TSW	124.33	82.91	59.38	23.53	7.33	6.20	72	10.87
BIY	730.35	28127.96	3714.44	24413.52	22.96	8.35	13	6.15
GYPP	171.02	1665.33	313.04	1352.29	23.86	21.50	19	9.34
NSPP	111.53	696.38	251.79	444.59	23.66	18.91	36	17.55
HI	25.23	36.16	9.51	26.65	23.82	12.21	26	12.76

Vp= phenotypic variance, Vg= genotypic variance, Ve= error variance

* For character codes see Table 3

4.1.6. Correlation analysis

Character associations are important to measure the similarity in the pattern of geographic variation of characters and to indicate similar response of characters to selection pressures (Bekele, 1996). Correlation coefficients between traits could be used as a measure of trait association. Correlation was calculated for each trait in all accessions and each region and the data is presented in Table 13 and Appendix 2-9, respectively. The strongest correlation ($r=0.67^{**}$) was observed between number of primary branches and number of secondary branches which are more or less complimentary phonological components. These two characters also show highly significant correlation with most of the characters except days to maturity and thousand seed weight.

Plant height shows highly significant positive correlation with most of the characters, like $r=0.55^{**}$ with number of seed per plant and $r=0.45^{**}$ with number of secondary branches, but negative non-significant correlation with days to maturity, harvest index and thousand seed weight. The analysis of correlation coefficients (Table 13) indicated that grain yield had a highly significant positive correlation with all the characters except days to maturity where it is not significant. Its strong positive correlation with biological yield in all accessions and regions indicates that any input in above ground biomass will be reflected in increasing yield, the same correlation matrix was reported in chickpea by Muehlbauer and Singh (1987) and Singh and Saxena (1999). However, Singh and Saxena (1999) further mentioned that biological yield is low in chickpea seldom exceeding 10 t/ha compared to over 20 t/ha in most cereals, indicating a considerable scope for progress. Harvest index has been used as a selection criterion in many crops as it has a strong positive correlation with grain yield, and in this study highly significant

and positive correlation was observed with grain yield, number of primary branches, number of secondary branches, number of seed per plant and negative significant correlation with biological yield (-0.463**) and thousand seed weight (-0.052*).

It is also observed in the correlation analysis of characters by region (Appendix 2-9) that grain yield per plot had significant positive correlation with most of the characters in different regions. However, plant height and number of seeds per plant were the characters with significant positive correlation with grain yield in all the regions. The highest correlation in most of the regions was obtained between number of primary branches and number of secondary branches, where r= 0.71** for Shewa, 0.65** in case of Wello and Gojam and 0.62** for Gonder. Generally, in agreement with Tullu (1985), the results of this study indicated that selection for tall plant height, higher number of primary and secondary branches and number of seeds per plant is useful to develop chickpea plants which are high yielding.

Table 13. Correlation among ten different traits of fifty chickpea populations in Ethiopia

Characters*	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY	0.091**	0.025	0.538**	-0.463**	0.097**	0.115**	0.204**	0.220**	0.285**
DTF		-0.094**	0.125**	-0.017	-0.412**	0.125**	0.214**	0.066**	0.105**
DTM			0.040	0.024	-0.049	-0.041	-0.017	0.037	-0.009
GYPP				0.286**	0.084**	0.248**	0.351**	0.382**	0.352**
HI					-0.052*	0.098**	0.106**	0.094**	-0.015
TSW						-0.049	-0.086**	-0.022	-0.021
NPB							0.671**	0.381**	0.216**
NSB								0.532**	0.449**
NSPP									0.577**

Significance level: ** P< 0.01 * P< 0.05.

* For character codes see Table 3 .

4.1.7. Cluster analysis

Cluster analysis has proven to be a useful tool in studying diversity in germplasm collections (Van de Wouw *et al.*, 1999) and allowed the collection to be clustered successfully in groups with similar characteristics, assisting in future evaluation and use of the collection. The combined qualitative and quantitative traits were used as the input for cluster analysis. Ward's method which minimizes within cluster variance summed over all variables was used in this study. This method appeared to give the most satisfactory clustering result with most populations included in clusters of similar size (Sneath and Sokal, 1973). The dendrogram obtained using this method grouped the original fifty chickpea populations into eight clusters (Figure 5).

Cluster A contains the maximum number of populations (14). Most of them are from Shewa, Gonder and Gojam, which are the main chickpea growing areas in the country. This cluster consisted of all altitude groups, where most of the populations lie between 1701-2300 masl. *Cluster B* had seven populations out of which three are from Gonder the others are from Wello, Shewa, Tigray and Arsi-Bale. Materials from almost all altitude groups are present. *Cluster C* contains ten populations from Wello, Gonder, Gojam, Gamo Gofa and Shewa. Altitude wise, four out of the ten are from altitude range 1701-1900masl and the rest from the other groups except from <1500masl. *Cluster D* had materials from Arsi-Bale, Tigray, Hararghe-Sidamo and Shewa. These materials are from different altitude group except the altitude ranges 1501-1700 and 1701-1900. *Cluster E* had two populations one sampled from Gonder and the other from Shewa, but both are from higher elevations (≥2300 masl). *Cluster F* had seven populations sampled from Hararghe-Sidamo, Gojam, Shewa, Gonder and Wello, representing all altitude groups except altitude range 2101-2300 masl. *Cluster G* was the outlying cluster, which contains a single

population collected from Shewa at higher altitude (2600masl). *Cluster H* contains two populations from the same region (Tigray) but from different altitude group. The distribution of the 50 chickpea populations over the eight clusters by region and altitude groups based on qualitative and quantitative characters are presented in Appendix 10.

The populations were grouped based on quantitative morphological characters, into seven clusters (Figure 6) using the standardized mean of the quantitative characters as an input. The number of populations varied from two populations in cluster C up to twelve populations in cluster E. *Cluster A* contains eight populations, three of which are from Tigray, the other three from Bale and the rest two are from Wello and Gonder. The populations are from all groups of altitude except the altitude range 1701-1900masl. Populations of this cluster are characterized by lateness (days to maturity), smaller number of primary and secondary branches, medium plant height, medium biological yield, grain yield and number of seeds per plant and higher seed weight (Table 14).

Populations in *cluster B* were seven in number out of which three are from Gonder, two from Gojam, one from Gamo Gofa and the other from Shewa. All the materials are collected from altitude class greater than 1701masl. The populations in this cluster are characterized by lateness, medium branch number, plant height and thousand seed weight, lower biological and grain yield plus smaller number of seeds per plant.

Only two populations both from Tigray are grouped in *cluster C*, they are collected from lower altitudinal groups. As it is expected from lower altitudes materials in this cluster are characterized by medium maturity date, very small number of primary and secondary branches, shorter plant

height, lower biological and grain yield and very low number of seed per plant plus higher thousand seed weight.

Members in *cluster D* were eight, collected from divers regions including Shewa, Gonder, Hararghe, Wello and Gojam. Four out of the eight are from higher altitude group (> 2300masl) and the rest are from < 2100masl. The main characters of this cluster as it is expected in higher altitudes are late flowering, higher branch number, longer plant height, very high per plot grain and biological yield and number of seed per plot plus medium seed weight.

Cluster E consisted of the maximum number of populations (12) that were with high number of primary branches, medium plant height, biological and grain yields, higher seed weight, medium number of seeds per plant and short maturity period. The populations were collected from different regions, four are from Shewa, from Gonder (three), and the rest are from Wello, Gojam and Arsi. Four out of these are from altitude group 1701-1900masl and greater that 1901masl except one population from lower altitude.

Cluster F contained six populations from Shewa, Gonder, Hararghe, Wello and Gamo Gofa and they are all from altitude classes less than 2100masl. The populations are with medium branch number, plant height, higher biological and grain yield plus medium seed weight. The last cluster, *cluster G* consisted of seven populations from different regions of the country (Gojam, Gamo Gofa, Shewa, Arsi, Sidamo and Gonder) that were with late flowering and maturity time, higher branch number, longer plant height, medium biological and grain yield per plot plus lower seed weight and harvest index. The populations are collected from varying altitudes, which are greater than 1501masl.

As it is observed in the results of the cluster analysis, genotypes from different regions were grouped in the same cluster and those from the same origin were grouped under different clusters, implying the absence of relationships between geographic origins and genetic diversity of the materials. Similar results were obtained by Ghaderi *et al.* (1984) in faba bean and common bean and Kefene *et al.* (1997) in sunflower.

The above results were further confirmed by the distance analysis (Mahalonobis's distance D^2) among the clusters (Table 15). Distance between clusters A and B, F and E was minimum and not significant ($D^2 = 9.63$ and $D^2 = 13.59$ respectively). Although they are significantly different from each other, the distance between clusters E and B ($D^2 = 18.31$), F and A ($D^2 = 18.51$), F and G ($D^2 = 18.60$) and clusters E and A ($D^2 = 19.97$) were relatively low. Lower inter-cluster distance indicates the similarity of the populations in their genetic makeup, thus crossing between these clusters seems not to produce desirable recombinants. The maximum distance was observed between cluster C and D ($D^2 = 249.19$). In addition, cluster C also showed larger distances with clusters E ($D^2 = 135.56$), F ($D^2 = 109.04$) and G ($D^2 = 144.42$). Cluster C which consists of two materials from Tigray region is found to be unique in that it is very distant from all the other clusters. This is in line with the previous results. As maximum variation in the subsequent generation is expected from crosses, the crossing of parents selected from cluster C with those from cluster D, E, F, and G could produce desirable recombinants. Moreover, the distance between clusters A and C, D; B and C, D were fairly large and significantly different from one another, thus hybridization of parents from these clusters could also produce segregants with moderate to high variation.

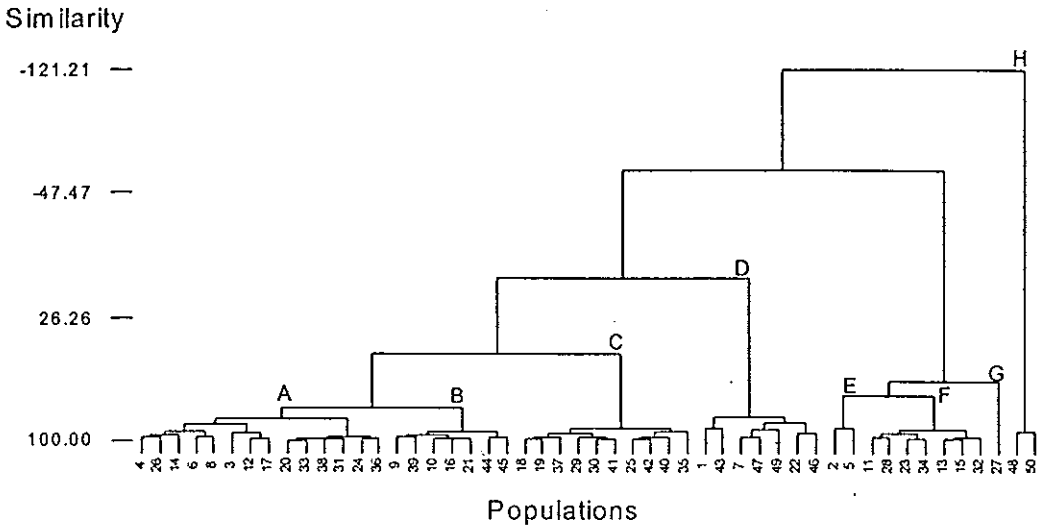

Figure 5. Dendrogram constructed using Ward's method based on quantitative and qualitative morphological classification of 50 chickpea populations

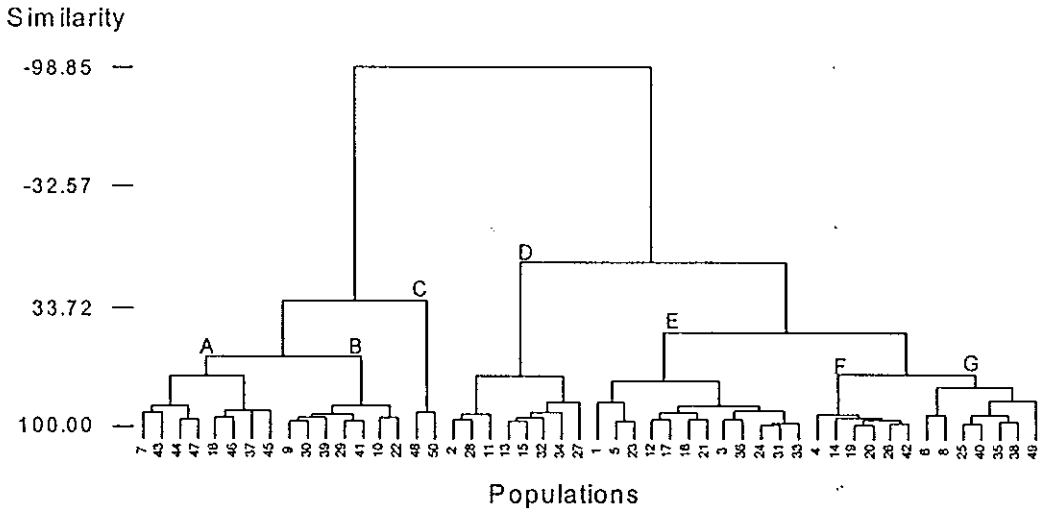

Figure 6. Dendrogram constructed using Ward's method based on quantitative morphological characters of 50 chickpea populations

Table 14. Summary of mean (M) and standard deviation (SD) of qualitative characters* over the seven clusters of chickpea populations

Cluster		No.	DTF	DTM	NPB	NSB	PH	TSW	BIY	GYPP	NSPP	HI
A	M	8	44.33	139.04	1.78	14.62	31.74	132.54	700.5	256.2	101.91	36.66
	SD		0.79	1.37	0.30	1.76	1.57	10.47	101.7	49.1	20.30	3.11
B	M	7	46.57	138.05	1.99	17.08	31.01	120.10	574.8	200.61	92.53	36.31
	SD		1.55	1.04	0.16	1.60	1.58	5.67	57.9	25.80	9.23	4.11
C	M	2	45.33	137.00	1.57	10.08	25.32	125.50	470.0	117.5	40.93	27.69
	SD		1.41	0.47	0.05	0.64	1.34	13.44	138.7	34.8	5.33	1.52
D	M	8	50.92	136.38	2.30	23.27	36.40	120.42	832.1	318.5	142.99	38.20
	SD		1.81	1.45	0.12	2.99	1.35	6.85	43.9	50.8	22.41	4.85
E	M	12	45.75	136.64	2.21	18.69	33.77	127.89	701.8	263.85	120.36	38.15
	SD		1.22	0.78	0.23	2.97	1.92	8.48	83.2	21.47	13.77	3.85
F	M	6	47.17	136.22	2.02	17.92	32.53	125.17	847.4	268.9	110.39	31.66
	SD		1.57	0.50	0.05	1.13	1.25	2.21	45.8	28.1	17.11	3.13
G	M	7	47.33	139.19	2.02	19.17	34.75	116.48	839.9	271.49	111.60	32.63
	SD		2.55	1.09	0.22	2.90	1.04	8.16	115.5	24.05	16.02	3.44

* For character codes see Table 3

Table 15. Mahalonobis's distance (D^2) of the seven clusters of chickpea accessions

Cluster	A	B	C	D	E	F	G
A	-	9.63	77.71**	70.99**	19.97*	18.51*	22.99**
B		-	71.35**	72.24**	18.31*	21.63*	27.49**
C			-	249.19**	135.56**	109.04**	144.42**
D				-	30.61**	35.08**	39.89**
E					-	13.59	29.91**
F						-	18.60*
G							-

Significance level: ** P< 0.01, * P< 0.05.

4.1.8. Principal components analysis

Principal components analysis (PCA) was used as a data reduction tool to summarize the available information. A total of ten principal components were extracted, which accounted for the entire (100%) variability evident among the test genotypes. This was then reduced to four principal components (PC) of the variables, with eigenvalue greater than unity. The first two component scores were plotted to aid visualization of the overall variability among the populations (Figure 7).

The first four principal components explained a cumulative of about 81.4 % of the entire variability apparent among the test genotypes (Table 16). To this effect, the first principal component which alone explained for over one third (about 44.8%) of the total variation has been due chiefly to variation in number of primary branches, number of secondary branches, number of seeds per plant, plant height and grain yield, all have positive loading in this principal component (Table 16). Likewise, 14.8% of the overall variability accounted for by the second component originated primarily from thousand seed weight with significant positive loading and days to flowering with a significant negative loading in this component.

Harvest index (with positive loading) and biological yield (with negative loading) contribute more to the third component. Days to maturity was the only trait contributed more to the fourth principal component. The sign of the loading indicates the direction of the relationship between the components and the variable (Seiler and Stafford, 1985). Plotting the first two principal component scores against the clusters (Figure 7) indicated that the populations that lie in the same cluster have a distinct morphological attribute, overlapped to some degree and grouped

together in the first and second axes. Populations in cluster A and B had negative loading in the first PC, cluster C that contains only two populations from the Tigray region once again come out very distinctively with a significant negative loading in the first PC, while cluster D has a significant positive loading. The rest clusters E, F and G lie in the middle and hence they overlapped to some degree with each other, indicating similarity between the populations that lie in these clusters.

In order to study the regional pattern of variation, principal component analysis was made using the means of region of origin based on the ten quantitative traits and accordingly, ten components were extracted. However, for the particular analysis the first three principal components with eigenvalues greater than unity were considered significant. The first three principal components explained 92% of the total variation and the first two components accounted about 81% of the total variation (Table 17). The first three principal component scores were plotted to aid visualization of region of origin differences (Figure 8). The 3D representation of the eight regions of origin appeared very distinct along the first axis, which separates Tigray with high negative loading in the negative extreme, while Shewa, Gojam, Hararghe-Sidamo and Wello with significant positive loading, in the positive extreme of the first axis.

Apparently the first principal component was associated with lateness, higher grain yield, long stature and large number of primary and secondary branches plus large number of seeds per plant. These characters show significantly high loading in this component. Region gene pools which showed earliness, short in stature and having few numbers of branches and lower grain weight had a negative value in the first component (Figure 8).

Table 16. Eigenvalues, percent of variance and cumulative variance of the first four principal components of the ten quantitative traits of chickpea populations

Characters*	PC 1	PC 2	PC 3	PC 4
DTF	0.318	-0.402	0.045	0.182
DTM	-0.134	0.348	0.026	0.747
NPB	0.367	-0.169	0.111	-0.198
NSB	0.412	-0.194	0.099	-0.045
PH	0.365	0.094	-0.241	-0.097
TSW	-0.154	0.511	-0.063	-0.579
BIY	0.313	0.301	-0.524	0.12
GYPP	0.371	0.423	0.060	0.089
NSPP	0.400	0.165	0.018	0.024
HI	0.151	0.291	0.796	-0.005
Eigenvalue	4.475	1.479	1.176	1.011
% of total variance	44.8	14.8	11.8	10.1
% cumulative variance	44.8	59.6	71.3	81.4

* For character codes see Table 3

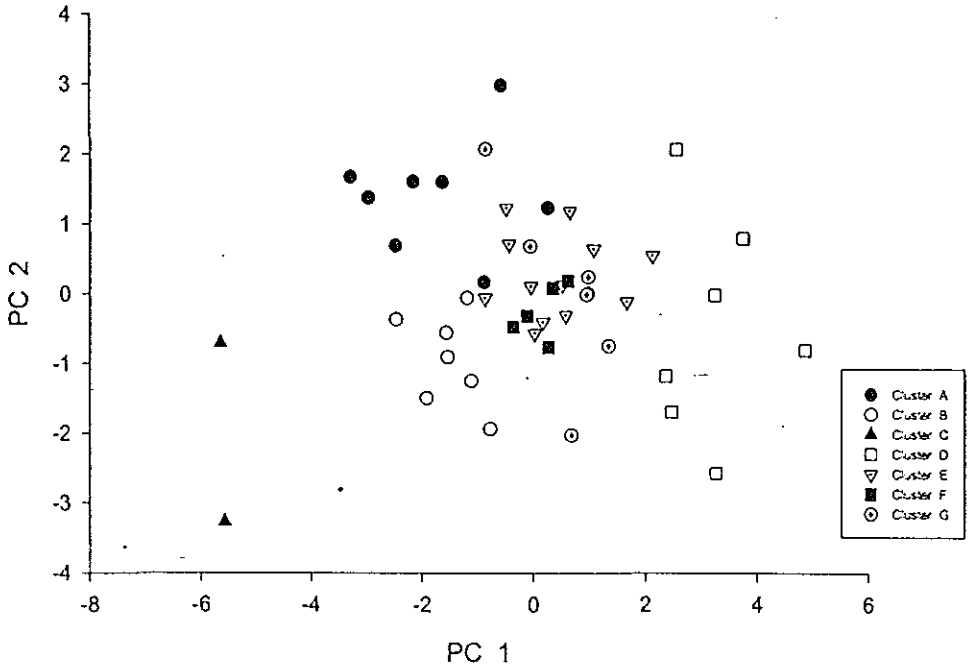

Figure 7. Plots of principal component of the fifty chickpea populations for the ten quantitative characters based on clusters

Table 17. Principal component co-ordinate scores, eigenvalues, total variance and cumulative variance for the first three principal components for the eight regions of the fifty chickpea populations based on mean for the regions

Region	PC 1	PC 2	PC 3
Shewa	1.7151	-0.3125	-0.9531
Gonder	0.3323	0.7361	-0.2517
Gojam	1.2767	-0.0871	-1.3205
Arsi-Bale	-1.9818	2.1870	1.1768
Tigray	-5.3869	-1.2503	-0.3388
Wello	1.2137	1.2769	-0.4393
Gamo Gofa	0.4543	-1.3071	0.3172
Hararghe-Sidamo	2.3767	-1.2431	1.8094
Eigenvalue	6.4223	1.6968	1.1118
% total variance	64.22	16.97	11.12
% Cumulative variance	64.22	81.19	92.31

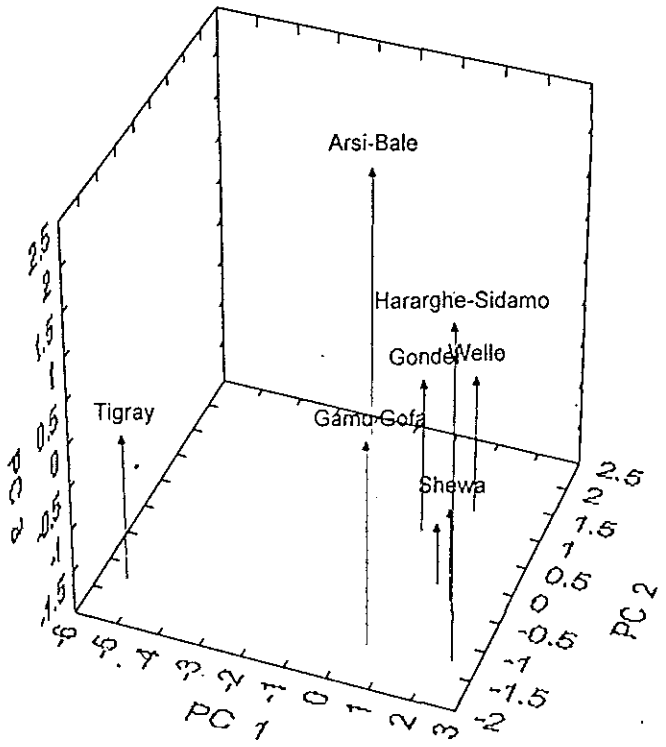

Figure 8. 3D representation of the region of origin resulting from principal components analysis

4.2. Isozyme Analysis

The number of zones of enzyme activity for the enzyme systems used is given in Table 18. Among the ten enzyme systems tested peroxidase (PER) was poorly resolved and only diffused zones were detected in the gels. However, most of the enzyme systems gave bands with variable number of zones. Gels for some of the enzyme systems are shown in Figures 9-12.

Aspartate aminotransferase (AAT) gel revealed a well detectable and very clear single band, which is homogenous in all tested individuals. Aconitase (ACO) and glucose-6-phospate dehydrogenase (G-6-PDH) enzymes gave similar single bands which are very faint in case of aconitase enzyme on the starch gel. Gels stained for the enzymes shikimate dehydrogenase (SDH) and 6-phosphogluconate dehydrogenase (6-PGD) showed similar single bands which are dense. The enzyme isocitrate dehydrogenase (IDH) revealed two clear zones (Idh-1 and Idh-2) which were found to be homogenous in all the individuals analyzed. One of the bands, the fast moving Idh-1, was very thick or denser than band Idh-2. In the enzyme phosphoglucose mutase (PGM), in spite of its diffused zones, one locus which is homogenous in all the individuals was detected. Phosphoglucoisomerase (PGI) showed two presumed loci which are homogenous for the individuals tested. However, one of the bands was very faint. Esterase (EST) is the only enzyme which was found to give more than two bands (four loci were detected) Est-1, Est-2, Est-3 and Est-4, but with no polymorphism within the genotypes studied.

Table 18. Enzyme systems used in the study and the number of loci observed

Enzyme system	Code	loci	Description
Aspartate aminotransferase	AAT	1	good resolution
Esterase	EST	4	good resolution
Isocitrate dehydrogenase	IDH	2	good resolution
Shikimate dehydrogenase	SDH	1	possibly 2 loci, good resolution
Phosphoglucoisomerase	PGI	1-2	poor resolution / faint bands
Phosphoglucose mutase	PGM	1	poor resolution / easily dispersed
6-Phosphogluconate dehydrogenase	6-PGD	1	very clear and dense single band
Aconitase	ACO	1	very faint band
Glucose-6-phospate dehydrogenase	G-6-PDH	2	faint bands

Similar isozyme works done in other countries reported minimal polymorphism in cultivated chickpea germplasm. Studying a large number of cultivated chickpea germplasm accessions from 25 countries, Tuwafe *et al.* (1988) found only four polymorphic isozyme loci among the enzyme systems assayed. Gaur and Slinkard (1990) did not find genetic variation in the cultivated chickpea. Using 95 accessions of cultivated chickpea and nine wild species, Kazan and Muehlbauer (1991) found a total of seven polymorphic isozyme loci among 30 isozymes assayed. Labdi *et al.* (1996) studied isozyme coding for 14 loci for a set of 139 germplasm accessions of *Cicer* out of which 36 were cultivated and found only two loci to be polymorphic and all

accessions of the cultivated species to be less polymorphic than the wild species for the 10 enzyme systems assayed. From survey of isozyme for a set of 20 accessions of diverse geographical origin, Oram *et al.* (1987) found only four polymorphic loci. Similarly, in the present study on Ethiopian chickpea landrace populations under consideration, no polymorphism was obtained in any of the ten enzyme systems assayed.

This low polymorphism of isozymes in chickpea accessions can be explained in different ways. As quoted by Triest and Kabir (2000), Hamrik and Godt (1989) reviewed the allozyme diversity in plant species and found out that populations of selfing species (chickpea is one member) had lowest proportion of polymorphic loci and fewer number of alleles per locus. On the other hand, it is suggested that the reduced variability found in the isozyme profile of cultivated chickpea is probably the reflection of the founder effect suggested by Ladizinsky (1985).

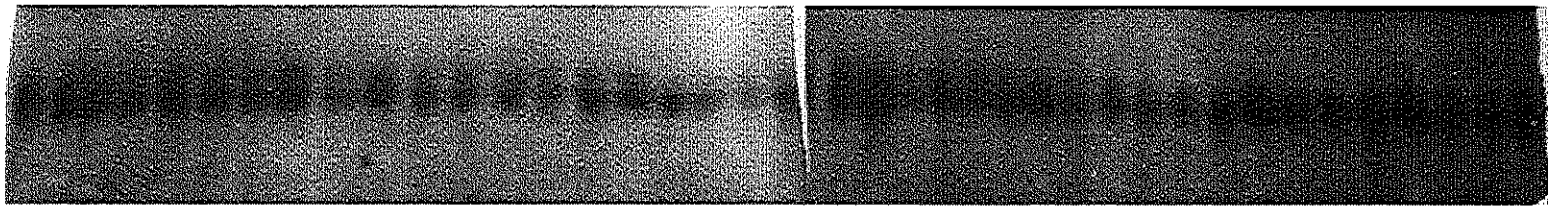

Figure 9. Shikimate dehydrogenase

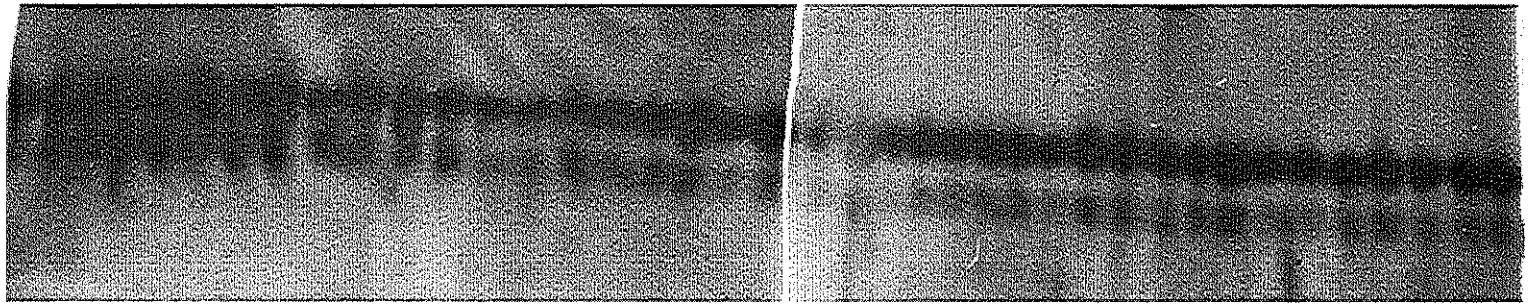

Figure 10. Isocitrate dehydrogenase

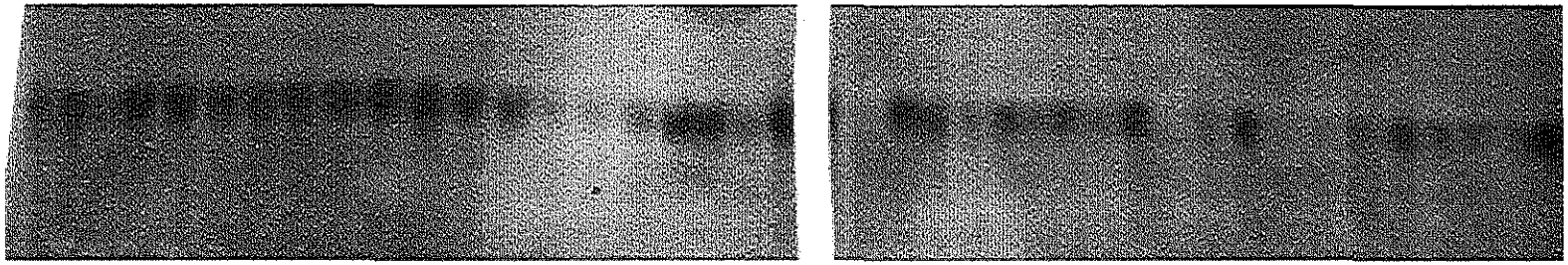

Figure 11. 6-Phosphogluconate dehydrogenase

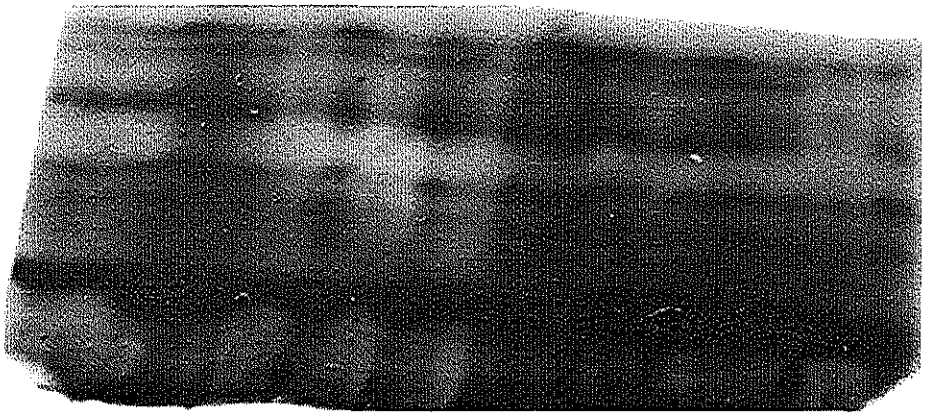

Figure 12. Esterase

4.3. Protein Content

Determination of crude protein content for the 50 chickpea populations collected from eight regions was performed using Kjeldahl method and the results of each accession are shown in Appendix 12. The amount of protein content of the populations ranged between 15 and 22 percent with the regional and altitude means indicated in Table 19. Citing literature covering the chemical composition of chickpea seed, Williams and Singh (1987) stated that, the crude protein content of the seed varies from 17 to 24% with a mean of 23% which is lower than other legumes. However, chickpea protein has high digestibility (Williams and Singh, 1987) and is rich in minerals (like Phosphorus, Calcium and Magnesium) and vitamins (Vitamin A, Thiamine and Riboflavin).

The difference in crude protein content of the chickpea populations was examined using a one-sample t-test (Table 20). The results revealed that there are significant differences between populations and between regions of origin. This is in agreement with Singh and Saxena (1999) who reported the existence of great variation in the protein content of chickpea seed depending on genotype, location and season of growth.

On population bases (Appendix 12) the population collected from Gonder (41018) showed the highest crude protein content (22.09%) followed by population from Tigray, 219804 (20.98%) and two populations from Shewa, 41163 and 41194 (20.69 and 20.66%, respectively). On the other hand the highest regional mean protein score was observed on population from Shewa (18.88%) followed by Gamo Gofa (18.69%), Arsi-Bale (18.46%) and Gonder (18.32%) indicating that most of the populations collected from these regions have better protein content,

while the lowest mean crude protein was scored on Tigray (17.70%) (Table 19). The altitude group 1701-1900 meters above sea level gave the highest mean crude protein score (18.63%) followed by altitude group 1500-1700masl (18.62%) and 1901-2100 (18.59%) while the lower altitude class (>1500masl) gave the lowest mean crude protein content (17.41%) presented in Table 19.

Table 19. Mean protein content of chickpea populations over regions and over altitude groups

Region	Mean	SE
Shewa	18.88	0.34
Gonder	18.32	0.50
Gojam	17.76	0.38
Arsi-Bale	18.46	0.22
Tigray	17.70	0.89
Wello	17.93	0.42
Gamo Gofa	18.69	0.49
Hararghe-Sidamo	18.09	0.59
Altitude		
< 1500	17.41	0.34
1501-1700	18.62	0.62
1701-1900	18.63	0.45
1901-2100	18.59	0.29
2101-2300	17.88	0.57
> 2301	18.15	0.32
Total	18.28	0.18

Table 20. One-Sample t-test for % protein content of the fifty chickpea populations

Source	t	Mean	df	sig (2-tailed)
Altitude	89.54	18.21	5	.000
Region	119.47	18.23	7	.000
Accession	104.46	18.28	49	.000

5. CONCLUSION

From the results of the present study it can be recognized that there is considerable variability within the studied chickpea landrace populations, suggesting a need to look for ways of maintaining and improving this variability. Analysis of variance of quantitative characters showed that differences were observed between populations in the characters recorded. The variation of each population, region, altitude group and the entire sample was quantified using the Shannon-Weaver diversity index. It is difficult to compare the overall diversity (mean H'= 0.64±0.08) obtained in this study with other studies, since studies which have tried to investigate the morphological diversity of the crop in the country were lacking. However, the overall diversity index is considerable when compared with higher diversity estimates obtained in other important crops in the country.

The analysis of variance for H', showed no significant variation between the regions which can be explained in terms of environmental heterogeneity of regions, irrelevance of regional boundaries in maintaining diversity and seed migration among regions. Similarly, the lack of significant difference between the altitude groups indicates the less pronounced role of altitude in maintaining diversity. The observed diversity is found with in the regions and altitude classes.

Cluster analysis also demonstrates the close resemblance between populations from different regions implying that there were no relationships between geographic origins and genetic diversity of the materials studied. The Mahalanobis's distance (D^2) analysis confirmed the existence of variability among the chickpea population, and hence parental lines could be selected for hybridization and improvement of the crop.

As low or no isozyme polymorphism was obtained in the cultivated chickpea by various workers in other countries (Oram *et al.*, 1987, Gaur and slinkard, 1990, Kazan and Muehlbauer, 1991, Labdi *et al.*, 1996, Tuwafe *et al.*, 1998), there was no polymorphism in the isozyme assay using different enzyme systems for the chickpea populations under study in Ethiopia. It is hard to discriminate between accessions studied based on the results of the isozyme study, indicating the failure of the allozyme frequencies to differ among the landrace populations of chickpea. The results of the present study might also indicate that the morphological traits are better indicator of the existing diversity in the studied landrace chickpea populations. However, it was not possible to see the association between the morphological and biochemical characters to meet the last objective, due to lack of isozyme polymorphism.

In summary, a systematic and detailed collection of the chickpea landraces in the country (covering various areas) followed by a wider diversity study with the inclusion of a more sensitive molecular methods and ethnobotanical study will allow to provide the complete picture of existing variability of the chickpea crop in Ethiopia.

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Appendices

Appendix 1. Diversity index for each population over characters

Acc. No	Acc. code	ANT	PLH	FC	SC	SCT	SS	GH	MEAN ± SE
1	41002	0.35	0.56	0.61	0.42	0.63	0.90	0.79	0.61 ± 0.073
2	41007	0.47	0.60	0.39	0.68	0.36	0.60	0.94	0.58 ± 0.075
3	41012	0.21	0.50	0.64	0.83	0.57	0.61	0.84	0.60 ± 0.081
4	41018	0.57	0.58	0.74	0.70	0.61	0.60	0.81	0.66 ± 0.035
5	41019	0.21	0.61	0.49	0.44	0.58	0.62	0.88	0.55 ± 0.077
6	41026	0.21	0.60	0.64	0.42	0.41	0.60	0.87	0.54 ± 0.079
7	41034	0.00	0.60	0.49	0.45	0.62	0.60	0.99	0.54 ± 0.111
8	41037	0.21	0.46	0.68	0.62	0.60	0.56	0.86	0.57 ± 0.076
9	41047	0.21	0.63	0.49	0.58	0.61	0.41	0.80	0.53 ± 0.071
10	41048	0.35	0.58	0.64	0.74	0.50	0.56	0.88	0.61± 0.064
11	41052	0.00	0.56	0.54	0.43	0.58	0.62	0.96	0.53 ± 0.108
12	41053	0.21	0.63	0.64	0.48	0.62	0.60	0.78	0.57 ± 0.068
13	41054	0.00	0.56	0.72	0.57	0.60	0.62	0.97	0.58 ± 0.110
14	41055	0.47	0.56	0.48	0.50	0.61	0.62	0.95	0.60 ± 0.063
15	41096	0.00	0.61	0.68	0.63	0.36	0.50	0.99	0.54 ± 0.116
16	41113	0.35	0.61	0.67	0.57	0.60	0.62	0.99	0.63 ± 0.071
17	41114	0.35	0.61	0.69	0.40	0.63	0.63	0.83	0.59 ± 0.063
18	41115	0.00	0.58	0.64	0.20	0.58	0.63	0.93	0.51 ± 0.117
19	41116	0.00	0.56	0.64	0.28	0.62	0.53	0.95	0.51 ± 0.113
20	41124	0.35	0.63	0.68	0.60	0.61	0.62	0.97	0.64 ± 0.069
21	41135	0.47	0.63	0.70	0.29	0.62	0.62	0.99	0.62 ± 0.081
22	41138	0.00	0.63	0.76	0.64	0.63	0.58	0.85	0.58 ± 0.104
23	41144	0.00	0.61	0.65	0.48	0.60	0.53	0.94	0.54 ± 0.106
24	41163	0.35	0.60	0.67	0.61	0.60	0.62	0.98	0.63 ± 0.070
25	41189	0.00	0.61	0.66	0.69	0.63	0.60	0.92	0.59 ± 0.106

Appendix 1 continued

26	41194	0.21	0.60	0.68	0.58	0.41	0.56	0.93	0.57 ± 0.084
27	41209	0.00	0.61	0.65	0.48	0.82	0.86	0.90	0.62 ± 0.118
28	41245	0.00	0.63	0.58	0.43	0.50	0.63	0.93	0.53 ± 0.106
29	41258	0.00	0.53	0.66	0.48	0.85	0.58	0.81	0.56 ± 0.107
30	41268	0.00	0.58	0.65	0.55	0.63	0.61	0.59	0.52 ± 0.087
31	41270	0.47	0.36	0.55	0.43	0.62	0.41	0.80	0.52 ± 0.057
32	41272	0.00	0.56	0.72	0.48	0.62	0.61	0.85	0.55 ± 0.102
33	41279	0.21	0.62	0.66	0.54	0.61	0.50	0.96	0.59 ± 0.084
34	41295	0.00	0.58	0.39	0.40	0.58	0.53	0.87	0.48 ± 0.100
35	41296	0.00	0.62	0.68	0.63	0.94	0.58	0.80	0.61 ± 0.112
36	41319	0.21	0.63	0.35	0.65	0.56	0.61	0.88	0.56 ± 0.082
37	207619	0.00	0.53	0.61	0.46	0.61	0.62	0.91	0.53 ± 0.104
38	207761	0.47	0.60	0.58	0.61	0.63	0.50	0.94	0.62 ± 0.058
39	212686	0.35	0.62	0.64	0.35	0.56	0.53	0.80	0.55 ± 0.061
40	212914	0.00	0.62	0.72	0.66	0.61	0.58	0.90	0.58 ± 0.106
41	212916	0.00	0.50	0.59	0.63	0.30	0.22	0.80	0.43 ± 0.104
42	212917	0.00	0.41	0.59	0.62	0.61	0.53	0.91	0.52 ± 0.104
43	219798	0.21	0.61	0.72	0.46	0.63	0.63	0.78	0.58 ± 0.072
44	219804	0.35	0.63	0.58	0.48	0.58	0.63	0.84	0.58 ± 0.057
45	229955	0.35	0.58	0.54	0.59	0.62	0.63	0.94	0.61 ± 0.066
46	229956	0.00	0.60	0.56	0.62	0.61	0.73	0.98	0.59 ± 0.112
47	235391	0.00	0.41	0.73	0.65	0.62	0.60	0.91	0.56 ± 0.109
48	235393	0.35	0.58	0.64	0.59	0.58	0.61	0.58	0.56 ± 0.036
49	235963	0.00	0.63	0.48	0.55	0.13	0.53	0.97	0.47 ± 0.122
50	236482	0.21	0.62	0.59	0.49	0.73	0.36	0.55	0.51 ± 0.066

Appendix 2. Correlation among different characters of chickpea in Shewa region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		0.148*	-0.138*	0.688**	-0.502**	0.047	0.222**	0.284**	0.278**	0.206**
DTF			-0.207**	0.306**	-0.067	-0.391**	0.173**	0.277**	0.162**	0.068
DTM				0.036	0.222**	0.009	-0.025	0.044	0.067	0.064
GYPP					0.106	0.025	0.242**	0.367**	0.435**	0.305**
HI						0.057	-0.048	-0.024	0.037	-0.023
TSW							0.133*	-0.078	0.009	0.020
NPB								0.710**	0.387**	0.216**
NSB									0.538**	0.404**
NSPP										0.550**
PH										

Appendix 3. Correlation among different characters of chickpea in Gonder region.

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		0.248**	0.210**	0.435**	-0.686**	0.043	0.109	0.166**	0.149**	0.263**
DTF			-0.027	-0.223**	-0.322**	-0.449**	-0.023	0.033	-0.072	-0.042
DTM				0.111	-0.048	-0.018	-0.034	-0.114*	0.029	-0.055
GYPP					0.248**	0.227**	0.191**	0.266**	0.330**	0.380**
HI						0.121*	0.027	0.045	0.060	-.0.030
TSW							0.048	0.066	-0.018	-0.043
NPB								0.619**	0.315**	0.182**
NSB									0.460**	0.421**
NSPP										0.630**
PH										

Appendix 4. Correlation among different characters of chickpea in Gojam region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		0.047	-0.130	0.648**	-0.574**	0.508**	0.078	0.175*	0.223**	0.395**
DTF			-0.260**	0.130	0.088	-0.248**	-0.008	0.145*	0.057	0.197**
DTM				-0.158*	-0.051	-0.027	-0.014	0.126	-0.062	-0.066
GYPP					0.116	0.628**	0.013	0.119	0.272**	0.385**
HI						-0.031	-0.076	-0.092	-0.023	-0.122
TSW							-0.084	-0.029	0.198**	0.162*
NPB								0.555**	0.291**	0.092
NSB									0.429**	0.351**
NSPP										0.534**
PH										

Appendix 5. Correlation among different characters of chickpea in Arsi-Bale region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		-0.163*	-0.029	0.580**	-0.433**	-0.189*	0.024	0.113	0.212**	0.189*
DTF			-0.047	-0.457**	-0.311**	-0.265**	0.115	0.060	-0.084	0.036
DTM				-0.009	-0.005	-0.489**	0.027	-0.013	0.113	-0.040
GYPP					0.470**	0.041	0.160	0.386**	0.257**	0.266**
HI						0.270**	0.179*	0.308**	0.041	0.066
TSW							-0.010	0.074	-0.172*	0.018
NPB								0.616**	0.402**	0.244**
NSB									0.517**	0.549**
NSPP										0.573**
PH										

Appendix 6. Correlation among different characters of chickpea in Tigray region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		-0.368**	-0.125	0.695**	-0.260**	0.442**	0.055	0.222**	0.418**	0.302**
DTF			0.422**	-0.234**	0.119	-0.748**	0.065	-0.050	-0.144	-0.232
DTM				0.058	0.210*	-0.211**	-0.018	-0.037	0.039	0.155
GYPP					0.379**	0.262**	0.200*	0.413**	0.587**	0.357**
HI						-0.120	0.131	0.296**	0.222**	0.044
TSW							0.005	0.055	0.160	0.130
NPB								0.625**	0.353**	0.194*
NSB									0.564**	0.481**
NSPP										0.657**
PH										

Appendix 7. Correlation among different characters of chickpea in Wello region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		0.285**	0.043	0.592**	-0.741**	0.059	0.060	0.057	0.150	0.352**
DTF			-0.017	0.426**	-0.070	-0.244**	0.110	0.134	0.108	0.329**
DTM				0.455**	-0.039	-0.443**	0.117	0.141	0.174*	0.041
GYPP					-0.072	-0.416**	0.121	0.116	0.251**	0.352**
HI						-0.204*	0.017	-0.055	-0.033	0.347**
TSW							0.005	0.040	-0.119	-0.197*
NPB								0.649**	0.392**	-0.076
NSB									0.595**	0.198*
NSPP										0.437**
PH										0.548**

Appendix 8. Correlation among different characters of chickpea in Gamo Gofa region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		-0.151	0.060	0.647**	-0.550**	0.279**	0.200*	0.377**	0.138	0.161
DTF			0.537**	-0.310**	-0.077	-0.654**	-0.084	0.213*	-0.062	-0.019
DTM				0.134	0.244**	-0.380**	-0.079	0.302**	0.066	0.048
GYPP					0.243**	0.491**	0.130	0.261**	0.204*	0.206*
HI						0.011	-0.076	-0.084	0.057	0.023
TSW							0.035	-0.228*	0.084	0.063
NPB								0.638**	0.266**	0.117
NSB									0.485**	0.437**
NSPP										0.593**
PH										

Appendix 9. Correlation among different characters of chickpea in Hararghe-Sidamo region

	BIY	DTF	DTM	GYPP	HI	TSW	NPB	NSB	NSPP	PH
BIY		-0.494**	0.731**	-0.027	-0.518**	0.319**	-0.105	-0.138	0.061	0.101
DTF			-0.455**	0.235**	0.449**	-0.191*	0.062	0.149	0.041	-0.087
DTM				0.090	-0.313**	0.133	-0.089	-0.076	0.058	0.049
GYPP					0.848**	-0.186*	0.428**	0.508**	0.402**	0.293**
HI						-0.222*	0.402**	0.493**	0.292**	0.191*
TSW							-0.142	-0.024	0.001	-0.043
NPB								0.811**	0.522**	0.297**
NSB									0.618**	0.439**
NSPP										0.544
PH										

Appendix 10. Distribution of landrace chickpea populations over the eight clusters by region and altitude based on qualitative and quantitative characters.

Regions	Altitude class	Clusters								Altitude total	Region total
		A	B	C	D	E	F	G	H		
Shewa	1										10
	2										
	3		1	1						2	
	4	2			1		1			4	
	5	2								2	
	6					1		1		2	
Gonder	1										9
	2										
	3	1		1			1			3	
	4	1								1	
	5		1							1	
	6	1	1	1		1				4	
Gojam	1										8
	2										
	3	2		1						3	
	4		1				1			2	
	5	1								1	
	6			1			1			2	
Arsi-Bale	1										5
	2										
	3										
	4	1	1		1					3	
	5										
	6				2					2	

Appendix 10 continued												
Tigray	1				1				1	2	5	
	2		1						1	2		
	3											
	4											
	5				1					1		
	6											
Wello	1										5	
	2	1	1							2		
	3											
	4			1						1		
	5			1						1		
	6						1			1		
Gamo Gofa	1	1								1	4	
	2			2						2		
	3			1						1		
	4											
	5											
	6											
Hararghe-Sidamo	1	1			1		1			3	4	
	2						1			1		
	3											
	4											
	5											
	6											
Total		14	7	10	7	2	7	1	2	50	50	

Appendix 11. Total mean of the quantitative characters of the fifty chickpea populations

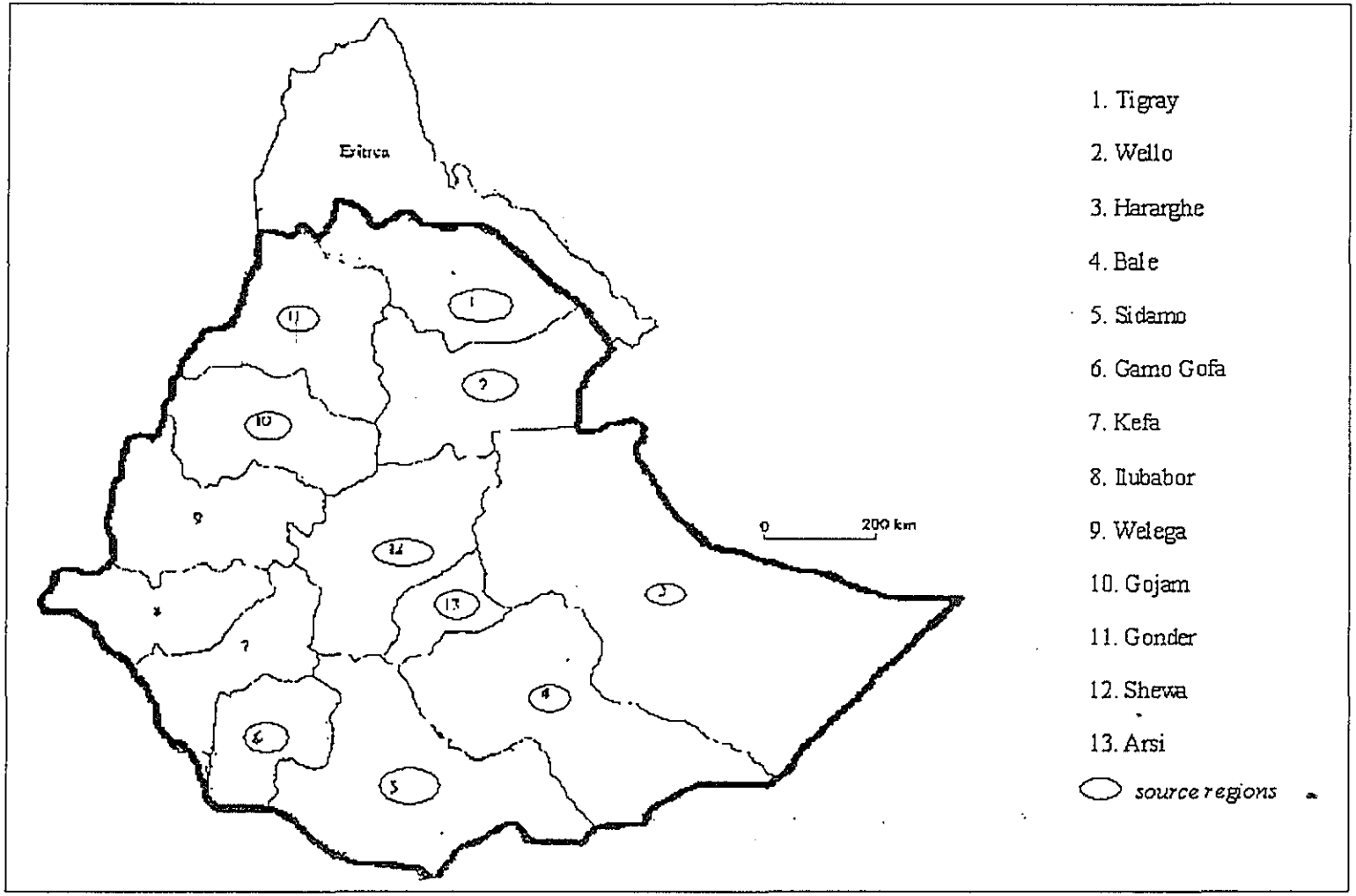
Acc. No	Acc. code	DTF	DTM	NPB	NSB	PLH	TSW	BIY	GYPP	NSPP	HI
1	41002	46	138	2	20	34	151	637	187	101	29
2	41007	53	136	2	25	36	109	823	200	142	24
3	41012	46	137	2	19	35	119	537	155	113	28
4	41018	49	136	2	18	31	128	695	164	84	27
5	41019	45	137	3	23	36	127	848	210	128	25
6	41026	48	139	2	23	34	104	664	133	87	21
7	41034	45	138	2	14	33	155	636	145	82	23
8	41037	52	139	2	22	34	111	920	138	98	17
9	41047	48	139	2	17	29	122	541	133	92	28
10	41048	48	139	2	17	29	117	508	165	96	36
11	41052	50	133	2	21	37	123	823	214	131	26
12	41053	46	136	2	19	30	128	586	212	111	36
13	41054	51	137	2	25	34	122	836	224	133	26
14	41055	49	136	2	17	32	122	988	148	126	14
15	41096	51	137	2	22	37	123	817	224	139	29
16	41113	45	140	2	16	33	118	637	190	123	30
17	41114	44	136	2	19	34	129	853	212	122	26
18	41115	43	137	2	17	31	123	672	184	132	37
19	41116	45	136	2	18	33	124	759	166	121	22
20	41124	47	137	2	19	32	124	920	169	125	18
21	41135	47	136	2	13	33	119	517	163	114	33
22	41138	48	137	2	16	30	116	463	152	98	44
23	41144	47	137	3	24	36	134	786	205	134	27
24	41163	47	136	2	18	34	128	799	172	103	21
25	41189	46	137	2	20	36	120	788	187	127	23
26	41194	46	137	2	19	33	125	725	150	97	22
27	41209	52	137	3	29	35	114	867	270	190	31

Appendix 11 continued

28	41245	53	136	2	23	37	121	744	197	127	26
29	41258	48	137	2	20	32	114	587	126	79	29
30	41268	46	139	2	18	31	126	586	140	83	25
31	41270	46	136	2	17	34	125	816	149	115	20
32	41272	48	138	2	21	36	132	854	253	121	30
33	41279	47	136	2	15	34	126	704	198	121	28
34	41295	49	138	2	19	38	118	822	228	162	29
35	41296	49	140	2	19	34	119	967	179	117	21
36	41319	46	138	2	20	35	126	698	213	149	31
37	207619	45	139	2	18	30	128	721	171	108	26
38	207761	48	140	2	18	34	111	791	162	131	21
39	212686	44	138	2	15	32	129	499	129	93	28
40	212914	46	139	2	17	35	122	879	200	118	23
41	212916	46	137	2	17	34	117	635	144	106	23
42	212917	46	135	2	16	35	127	888	169	110	19
43	219798	44	141	2	13	33	137	617	122	78	20
44	219804	43	138	2	13	34	130	697	160	98	22
45	229955	44	140	2	13	31	129	900	177	134	20
46	229956	44	140	2	15	29	133	679	172	109	26
47	235391	44	137	2	16	31	130	713	123	83	27
48	235393	44	137	2	10	24	135	451	70	45	18
49	235963	44	141	2	15	36	128	1298	148	103	12
50	236482	46	137	2	11	26	116	330	51	37	15

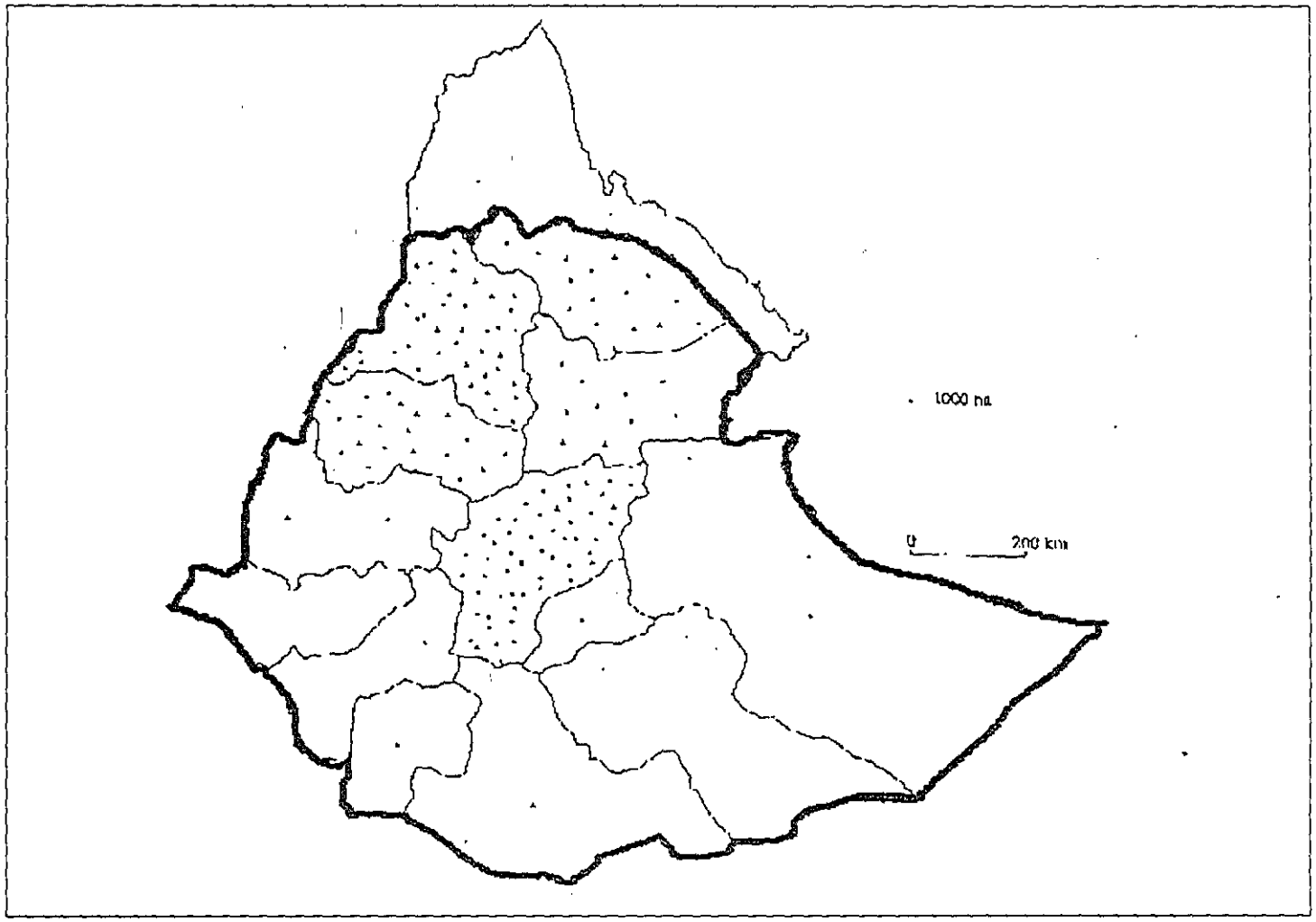
Appendix 12. Protein content of the fifty chickpea landrace populations

Accession code	% Protein	Accession code	% Protein
41002	18.13	41194	20.66
41007	18.11	41209	19.52
41012	17.71	41245	18.28
41018	22.09	41258	16.45
41019	16.71	41268	19.06
41026	17.13	41270	17.51
41034	18.29	41272	18.89
41037	17.30	41279	16.97
41047	16.95	41295	19.02
41048	17.51	41296	17.39
41052	19.42	41319	19.37
41053	17.56	207619	18.32
41054	16.98	207761	18.18
41055	18.74	212686	18.28
41096	19.51	212914	18.82
41113	17.95	212916	19.01
41114	17.64	212917	19.62
41115	17.42	219798	17.43
41116	17.15	219804	20.98
41124	18.37	229955	18.37
41135	18.87	229956	19.34
41138	18.47	235391	16.28
41144	18.00	235393	17.89
41163	20.69	235963	17.24
41189	18.42	236482	15.92



Appendix 13. Former administrative boundaries of Ethiopia

Source: Bejiga and Eshete, 1996



Appendix 14. Chickpea distribution in Ethiopia

Source: Bejiga and Eshete, 1996