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Genetic Variation in Chickpea (Cicer arietinum L.) : Enzyme Marker Loci and Quantitative Traits

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GENETIC VARIATION IN CHICKPEA (CICER ARIETINUM L.):

Enzyme Marker Loci and Quantitative Traits

By

Solomon Tuwafe

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in partial fulfillment of the requirement for the degree

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Dedicated to my wife

Mulumabet Fesseha

and my wonderful children

Negus Solomon and Merbebe Solomon

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Enzyme Marker Loci and Quantitative Traits

Abstract

Solomon Tuwafe

Under the supervision of Dr. Alex L. Kahler and Dr. A. Boe

Chickpea (Cicer arietinum L), due to its nutritive quality, is one of the most important grain legume crop of the temperate and subtropical regions. Average productivity world-wide is about 710 kg/ha. In Central South Dakota, seed production was as high as 2500 kg/ha which is approximately twice the production rate obtained from areas where chickpea is commonly grown.

To improve productivity of chickpea at the international level, the crop has been included in the research mandates of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India; and International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

The objectives of this study were to determine inheritance relationships of isozyme polymorphisms, to compare and contrast allozyme diversity within and among germplasm collections, and to evaluate adaptability of chickpea introductions in South Dakota.

Starch gel electrohporetic methods were used to assay chickpea germplasm and breeding lines. Three enzyme systems, acid phosphatase (ACP), esterase(EST) and malate dehydrogenase (MDH), were monomorphic overall collections, and 3 enzyme systems alcohol dehydrogenase (ADH),

6-phosphogluconate dehydrogenase (PGD) and peroxidase (PRX) were polymorphic among the chickpea collections assayed.

Inheritance studies of polymorphic enzymes showed simple Mendelian segregation for 4 diallelic enzyme loci including Adh1, Pgd1, Pgd2, and Prx1. A total of 12 genotypes were observed among the four loci including Adh1 (4 genotypes), Pgd1 (3 genotypes), Pgd2 (3 genotypes) and Prx1 (2 genotypes).

Estimation of fixation indicies and theoretical inbreeding coefficients supported the notion that chickpea is a highly self-pollinated crop with less than 1% of outcrossing. This result suggested that the mating system and selection are important factors maintaining genetic variability in chickpea.

Genotypic and allelic frequencies demonstrated the presence of appreciable genetic variation in chickpeas. Most of the genetic variability was observed in Middle Eastern (Palestine, Iraq, Lebanon, Syria, Jordan); Asian (Indian, Afghanistan, Pakistan, USSR) and East African (Ethiopia) germplasm. The observation of large amounts of genetic variability within closely situated regions suggests that genetic conservation strategies should stress collection of large numbers of populations in each agroecological zone.

Field trials demonstrated significant differences within and among five quantitative traits. Correlation and path coefficient estimates showed that seed size is an important character to consider when selecting for increased seed yield. Regression studies indicated that chickpea varieties respond to environmental variation. The results

suggested that selection for yield and seed size should be carried out in favorable environments, because of genotype x environment interactions. This work provides information useful for planning efficient sampling strategies, for planning optimum methods of germplasm preservation, and for utilization of existing genetic variability in plant breeding programs.

INTRODUCTION

Chickpea (Cicer arietinum L.) is the fifth most important grain legume crop, after soybeans (Glycine max), ground nuts (Arachis hypogea), dry beans (Phaseolus vulgaris), and dry peas (Pisum sativum) of the temperate and subtropical regions of the world (54). The major production area is the Indian subcontinent which contributes over 80% of the world's total annual production of 6.25 million tons on 10.25 million hectares of land (108).

Two types, Kabuli (large, ram-shaped, and cream-colored) and Desi (small, angular, and dark-colored), of chickpea are grown throughout the world (96). Generally, the large-seeded, cream-colored chickpea (Kabuli) are grown in the Mediterranean region, which includes Southern Europe, Western Asia, and Northern Africa. The small-seeded, dark-colored types (Desi) mainly are grown in Ethiopia and on the Indian subcontinent which includes Bangladesh, Burma, India, and Pakistan.

Popova (86) reported four subspecies, thirteen geographical types, and sixty-four varieties of chickpeas; however, van der Maesen (111) considered Popova's varieties and geographical probes as subraces and races. In 1972, van der Maesen recognized 31 perennial and 8 annual species of Cicer.

Anatolia was considered a possible site of origin for chickpea (111). Vavilov's (112) centers of origin included the Mediterranean, central Asian, the Neareastern and Indian centers, and a secondary center in Ethiopia. These centers of origin are now acknowledged as centers of diversity rather than centers of origin for cultivated chickpea.

Chickpeas commonly are raised on residual soil moisture with relatively minimal precipitation during the growing season. These marginal cultivation conditions, combined with natural selection, may have fixed adaptive gene blocks for specific regions, as observed in other crops by Frankel and Bennet (45). As a result, chickpeas exhibit narrow adaptation, which restricts development of lines for cultivation.

Chickpea varieties from geographically diverse regions generally are preferred for hybridization programs, assuming recovery of promising segregations. Two plant breeding approaches are usually used to produce new varieties. The first approach is to develop varieties with a wide genetic base. This allows adaption to a broad spectrum of environments. These varieties would be expected to show relatively low variability across environments and could easily be identified if selection trials represented the environmental population (62). The second is to develop several varieties, each highly adapted to a specific area with a relatively uniform environment.

Selection of parents, based on genetic divergence, has been used in cultivar development for many crop species. Conventionally, methods of identifying cultivars have been based on phenotypic expressions. However, these expressions are strongly influenced by the enviornment in which the plant is grown. Isozymes are useful markers because usually they are not affected by the environment (4,5).

More than 22 crop species have been studied electrophoretically (105). Most of the the electrophoretic work has been done with barley (Hordeum vulgare), corn (Zea mays), sunflower (Helianthus annus),

potato (Soloanum tuberosum L.) broad beans, (Vica faba), and soybeans (Glycine max). Kahler and Allard (66) have noted that enzyme marker loci are useful tools for studies of the extent of genotypic and allelic variability within and between different populations, and the geographical distribution of such variability. To the present time, enzyme marker loci have not been reported in chickpea. Determining the inheritance of isozyme polymorphism in chickpea was one object of this study.

Presently, genetic conservation is highly emphasized in many cultivated crops. Modern agriculture that deals with pure line breeding enhances extensive erosion of existing genetic resources, creating crops with narrow genetic bases that are susceptible to diseases, insects, and adverse environmental conditions (16, 66). Knowledge of the geographical distribution of genetic diversity may provide means for efficient sampling, preservation, and utilization of existing gene pools in breeding programs. The second objective of this study was to compare and contrast allozyme diversity within and among germplasm from different countries and trace the genetic differentiation across the geographical range of cultivated chickpea.

The third objective was to evaluate adaptability of diverse chickpea cultivars at several locations in South Dakota, with the hope of identifying germplasm adapted to cropping systems in the Northern Great Plains.

LITERATURE REVIEW

Electrophoresis

Electrophoresis began with the work of Tiselius in 1937 while analyzing serum protein in solution (106). Later, the method was improved by using stabilized medium, instead of solution, for separating each protein in components into a zone. Many forms of media, including filter paper, agar gel, starch grain, starch gel, cellulose acetate, and acrylamide gel, were used (85).

In 1955, Smithies (103) described zone electrophoresis using starch gel as supporting medium. In 1957 Hunter and Markert(59) demonstrated that enzymes could be visualized directly on starch gels, when treated with a specific histochemical stain. Since then, based on simplicity and clarity, starch gel electrophoresis has become the most popular technique used to study isozymes (18).

The term, isozyme, was first coined by Markert and Moller (75) to describe different molecular forms of enzymes with the same substrate specificity. More than twenty years have lapsed since the first genetic studies of plant isozymes. During this period, the study of isozymes has provided useful data for a broad range of basic and applied disciplines of plant science (46). In some of the more intensively studied taxa, such as maize, wheat, barley, and tomato, the inheritance has been established for many of the assayable enzyme systems. In addition, many loci have been mapped to specific points on chromosomes. Two enzyme loci, Adh1 and Adh2, in maize have been cloned and sequenced. Numerical procedures for utilizing data derived from isozyme studies in plant populations also have advanced (105).

Isozyme and Genetic Analyses

There is a paucity of information on cultivar identification and genetic control of isozymes in legume species. However, there is a considerable amount of information on the characterization of individual seed protein in soybeans. In this section, enzymes which were used for chickpea are reviewed with particular attention paid to genetic control of isozymes in legume species.

Acid phosphatase (ACP): Many researchers have studied the activity of ACP in legume crops; among those researchers Broue et.al.(21), and Gorman and Kiang (48), reported that ACP zymograms in soybeans are complex, thus comparisons are difficult. However, Gorman and Kiang reported the presence of four ACP isozyme phenotypes in cultivated and wild soybeans. Malek and Singh (74) reported five ACP isozyme bands in normal soybean seed coats but no bands in a black seed coated mutant. Hilderbrand et. al. (57), and Gorman and et. al. (49), using disc electrophoresis, reported the presence of three codominant alleles at a single locus in soybeans. Heterozygotes showed the two parental phenotypes with no intermediate bands, indicating a monomeric structure.

Bassiri and Adams, (14) studied ACP banding patterns in primary leaves, stems and roots of common bean cultivars. They observed 10, 10 and 8 bands for leaves, stems and roots, respectively. All the bands observed in stems and roots were monomorphic for all cultivars studied. Seven of the ten ACP bands in the primary leaves also were monomorphic. Hence, due to the presence of a large number of monomorphic bands in

stems, roots and leaves, they concluded that the ACP enzyme system was not suitable for isozyme analysis in Phaseolus vulgaris.

Cherry and Ory (29) used starch gel electrophoresis to examine anodal ACP from individual peanut seeds grown in five geographic areas of the United States. They observed inter-varietal anodal isozyme variation for ACP activity from the variety, Virginia 56R, grown in Lousiana. The variation within and between most of the cultivars from the other areas were consistent and limited to three phenotypes.

In 1978, de Vienne (34) resolved ACP isozymes using polyacrylamide gels in alfalfa. Quiros (87) also reported the presence of 11 bands of ACP enzyme in leaf tissue of alfalfa.

Alcohol dehydrogenase (ADH): Gorman and Kiang (47) examined seeds from 113 commercial soybean varieties with polyacrylamide slab gel electrophoresis. They reported that distinct variety specific isozyme patterns could be produced for the ADH enzyme system. Thus isozymes are stable varietal characteristics and could be used in varietal identification. They also reported that variant isozyme patterns are simply inherited.

Broue et. al.(21) used ADH to examine 20 populations, representing four soybean species (G. canescens, G. clandestina, G. tabacina, and G. tomentella,) by comparing isoenzyme phenotypes revealed by horizontal starch gel electrophoresis. They observed that three of the four species fit the traditional taxonomic grouping, but one of the populations (G. canescens) did not behave as a homogenous group. Some populations tended to group with G. clandestina whereas, others grouped less readily with G. tomentella.

Cherry and Ory (29) showed no ADH variation between several peanut cultivars, and Quieros (87) reported the presence of strong enzyme activity for ADH in alfalfa.

Esterase (EST): Fottrel (44) reported multiple forms of esterase, using starch gel electrophoresis from soybean root nodules. He reported a change in isozyme patterns during growth of the plant. Two groups of esterases (Est1 and Est2) were detected by Ferrer-Monge (40). He reported that Est1 produces three anodic bands with α and β naphthyl acetate, while Est2 acts only on β naphthyl acetate, exhibiting three cathodic bands.

West and Garber (109) demonstrated the use of crude extracts of cotyledons from germinating seedlings of 15 species of Phaseolus to obtain isozymes of the esterase(EST) and leucine amino peptidase (LAP). They reported that species of Phaseolus could be identified by comparing both EST and LAP zymograms. Bassiri and Rouhani (15), using starch gel electrophoresis, studied differences between esterase isozyme patterns of 40 broad bean cultivars. They concluded that esterase is suitable for varietal identification and various genetic studies of broad beans. Bassiri and Adams (14), in their study of evaluation of common bean cultivar relationships, reported that cultivars could be grouped into different classes based on stem EST patterns.

Cherry and Ory (29) studied isozyme patterns of esterase in individual seeds from serveral peanut cultivars using polyacrylamide and starch gel electrophoresis. They reported that cathodal esterase could be used for identifying differences between varieties.

Malate dehydrogenase (MDH): Reports on MDH activities are limited in legumes. Fottrell (43) observed three cytosol NAD active MDH electrophoretic bands from soybean nodules. Broue et. al.(21) reported the use of MDH zymograms in identification of Glycine species. Later, in 1982, Gorman et al. (49) reported the presence of only one isozyme pattern in the northern soybean cultivar.

Phosphogluconate Dehydrogenase(PGD) : Gorman et al. (49), observed two homozygous PGD isozyme phenotypes in G. max and two additional types in G. soja. They reported that the first phenotype contained two dominant alleles at a single nuclear locus while the third type was the result of recessive null alleles at the same locus.

Peroxidase (PRX) : Buttery and Buzzell (28) observed high and low peroxidase activities in soybean seed coats by polyacrylamide gel electrophoresis. Further, they found from their genetic analysis that high and low peroxidase activities in soybean seed-coats was controlled by a single locus, with a dominant allele producing low activity. In 1969, Brim et. al.(19), reported large differences in peroxidase isozymes among different soybean tissues.

Bassiri and Rouhani (15) studied differences between cathodal peroxidase isozyme patterns in broad beans and concluded that peroxidase is a useful enzyme for studying differences in broad beans.

In alfalfa, Quiros and Morgan (89) identified four loci by using progeny tests and crosses involving plants with different phenotypes. They reported Mendelian segregation for monomeric enzymes and reported linkage relationships between the four loci.

Martin and Jain (76) observed that rose clover populations introduced 30 years ago on the California rangeland showed significant genetic divergence for morphological and allozyme polymorphisms and quantitative traits. They reported that most genetic changes which occurred were shown in allelic frequencies, rather than in the occurence of many unique alleles. They concluded that colonization has produced rapid micro-evolutionary shifts, most likely due to local selective forces.

Broich and Palmer (20), in their studies of allelic frequencies at 10 loci common through out the genus Glycine subgenus Soja, observed that alleles for grey pubescence, low seed coat peroxidase level, and blunt pubescence tip, probably arose as mutations during the domestication of G. max. They reported that the seven loci studied were polymorphic throughout the subgenus, soja. Differences among collections of G. soja seemed to be the result of differing selection pressures. Cluster analysis of their allelic frequencies revealed two distinct groups within the subgenus corresponding to G. soja and G. max. Semi-wild accessions of G. max, while morphologically more similar to cultivated plants, clustered with samples of G. soja. The semi-wild accessions examined are thought to have arisen via hybridization between G. soja and G. max.

The following works on a few cereal crops are reviewed because of electrophoretic inheritance and genetic variability studies in legumes are rather limited and these works are similar to the present study.

Numerous studies involving isozymes in cereals, particularly barley and maize, have been conducted by many researchers. Kahler and Allard (66) and Kahler (64) electrophoretically assayed progeny arrays derived by self-pollinating barley plants which differed in isozyme phenotypes for enzymes EST, PGD, GOT and ACP. Inheritance studies showed that progeny arrays segregated as expected for single loci with codominant alleles. Linkage studies showed that esterase loci Est1, Est2, and Est3 are tightly linked and that Est4, Pgd1, Got1 and Acp1 are inherited independently of each other and of the Est1, Est2, and Est3 linkage group on chromosome 3.

Efron (37) reported the presence of three acid phosphatases AP1, AP2 and AP3 in maize pollen. Later, in 1970, he reported that AP1 isozymes were under the control of locus AP1. El-Metainy and Omar (38) reported the inheritance of a second dimeric acid phosphatase locus AP2. They also reported that loci AP1 and AP2 were not linked.

Kahler (65), using segregating F2 progeny arrays of five self-fertilized single-cross F1 hybrids, reported the inheritance and linkage relationships among 11 enzyme loci of maize. He demonstrated that Acp4 is a monomeric enzyme locus with at least six codominant alleles (allozymes). He also determined linkage relations of enzyme loci Idh2, Got1, Mdh2, Acp1, Prx1, Est1, Est4, Glu1 and Pgd1 with locus Acp4.

Kahler and Allard (66) demonstrated that esterase isozymes in the 30 parents of Barley Composite Cross V were governed by seven loci, and that the allelic status of individual seedlings can be determined precisely for each locus by starch gel electrophoresis. They also

reported that esterase isozymes are useful research tools for studies of the extent of allelic variability within different local populations of barley, and the geographical distribution of such variability.

Brown (24) stated that the wild progenitor of cultivated barley (Hordeum spontaneum Koch) is polymorphic for Adh1 and Adh2 in natural populations in Israel. The polymorphism is markedly differentiated geographically. He also observed that the two loci are tightly linked.

Kahler and Allard (64) studied 1506 accessions of domestic (Hordeum vulgare L.) and wild (H. spontaneum Koch) barley. They reported that, world-wide, the four esterase loci, Est1, Est2, Est3 and Est4, have a minimum of 7, 12, 6 and 7 alleles, respectively. They concluded that there was no genetic difference at these four esterase loci between the domestic and the wild barley collections assayed. Substantial genetic polymorphism and heterozygosity occurred within many of the accessions. Patterns of geographical distribution of alleles at these four loci are not at random over both small and large geographical areas, including differences on a continental scale. Four, among 16 four-locus combinations of alleles, were found in excess and all other combinations were in deficiency on a world-wide basis.

Allard et. al. (9), in study of the relationship between the degree of environmental heterogeneity, and genetic polymorphism, observed monomorphism for different electrophoretically detectable variants in extreme xeric and mesic habitats. This indicated that genetic uniformity is one of the adaptive strategies that has been adopted by A. barbata.

Kahler et. al. (67), assayed 31 populations of A. barbata from Israel for seven enzyme systems, including ACP, EST, GOT, MDH, PGD, PGI and PRX. They concluded that isozyme variability was distributed in mosaic patterns and not related to geographical distances. Further, the mosaic patterns of isozyme variation were found to correspond closely to mosaic patterns of the habitat. This structuring of the genetic variability into multilocus combinations was attributed to the combined effects of directional and diversifying selection.

Brown and Munday (26) assayed allozyme variation at 25 genetic loci in 12 indigenous cultivars (land races) of barley from Iran. The data from an isozyme survey of 12 original samples from barley fields was compared directly with information on natural populations of the wild species, and with two composite crosses or breeders populations. The total allozyme diversity in this collection of land races was intermediate between the moderate levels in composite crosses and the high levels in Hordeum spontaneum from Israel. They concluded that land races are valuable genetic resources for plant breeding.

Bekele (17), in his study of 158 land race populations of cultivated barley for 5 enzyme loci, reported that 44.85% of the total gene diversity was due to genetic variation within localities, 20.13% was due to variation between localities within areas; 18.71% was due to variation between areas within regions; and 16.31% of the total gene diversity was due to genetic variation between regions. He also reported that there was no correspondence between geographical distance and heterozygosity.

Agronomic Field Studies

Quantitative Variability

Singh and Tuwafe (97), in a study of variability for seed size and seed number per pod observed a range of 8.2 to 65.5 g for 100-seed weight and 0.9 to 3.0 for number of seed per pod, in over 3000 Kabuli chickpea germplasm accessions. They reported the importance of these two characters in chickpea improvement research. Large-seeded types bring higher prices both in domestic and foreign markets.

Singh and Tuwafe (98), in a study of collection, evaluation and maintenance of 3400 Kabuli accessions from 29 countries, reported wide variation for 25 characters. For example, they reported a range of 15 to 50 cm in plantheight, 15 to 60 cm in plant spread, 0.1 to 3.1 for seeds per pod, and 23 to 921 g grain yield per plot.

Kumar et. al.(71) observed a large coefficient of variation for biological yield, grain yield, and pods per plant, in that order; and a low coefficient of variation for plant height, days to flowering, and seeds per pod in 205 Desi and 125 Kabuli chickpea collections. They reported that there is appreciable variability for biological yield, grain yield, and pods per plant.

Pandey and Tiwari (82) estimated narrow sense heritability and expected genetic gains for ten characters from selections made in the parents, F1 , F2, BC1 and BC2 generations, of five chickpea crosses. They observed varying results, due to seasonal variation and parental differences, and reported up to 66.8%, 96.0%, 98.1% and 90.5% narrow

sense hertitability estimates for plant height, plant spread, 100-seed weight and seed yield, respectively. Their results indicated that these traits are highly heritable and, perhaps, are controled by few genes.

Jain, et. al. (60) reported that the grouping of chickpea genotypes from different ecogeographical areas in the same cluster, confirmed a lack of parallelism between genetic diversity and geographical distribution. They stated that the pattern of clustering was highly influenced by environment. The cluster patterns of Desi and Kabuli types were different from each other, so crosses made between these two types may provide desirable segregants.

Gowda (52), using simple leaf character as a marker, obtained an estimate of 1.92% outcrossing.

Correlations

Josh (63) determined correlations between yield and yield components. He observed that number of pods, number of seeds, and branch numbers showed a high positive correlation with yield; which suggested that the number of pods per plant was a good selection criterion for yield in chickpea.

Gowda (52) observed that seed yield in chickpea was significantly and positively correlated with number of pods per plant, number of branches, and days to flowering. The correlation with 100- seed weight was low, but positive.

Singh et. al. (96) observed that yield in chickpea was positively correlated with pod number but was negatively correlated with 100-seed weight.

Pandey and Torrie (83) studied seed yield components in seven soybean cultivars, grown at three different seeding rates for three years. They used both correlation and path coefficient analysis to relate the direct and indirect effects of seed components on seed yield. Pods per unit area and seeds per pod had the greatest effect on seed yield in five and four of the nine set treatment combinations, respectively.

Jatasra et. al. (61) stated that yield was positively correlated with pods per plant, number of secondary branches per plant, number of primary branches per plant and seeds per pod in progeny arrays of fifty F2 populations of chickpea. Pods per plant showed positive association with seeds per plant.

Gowda and Pandya (53), in a study involving 49 pure strains of chickpea, found that number of pods per plant and 100-grain weight had larger effects on grain yield than any other component.

Asawa and Tiwari (12) computed correlation path coefficient, multiple regression, and multiple correlations in 10 cultivars and F3 segregating populations of chickpeas. They observed that yield was correlated with plant spread and number of seeds per plant in an F3 bulk population, at both phenotypic and genotypic levels.

As a whole, these studies suggested that factors such as seed weight, pods per plant, flowering period, harvest index, plant height, and plant spread should be taken into account in chickpea improvement programs.

Genotype x Environment Interaction

Although studies of genotype x environment interactions on chickpea have been conducted in the Indian subcontinent literature on these subjects is limited. For this reason, classical examples of self-pollinated crops, such as barley, wheat, and oats, are reviewed for this topic.

Finlay and Wilkinson (42) studied adaptation of 277 barley varieties from the world collection. The varieties were grown in replicated trials for several seasons at three sites. A linear regression of yield on the mean yield for each variety, site, and season, was computed as a measure of variety adaptation. They observed that variation in sensitivity was proportionately less among varieties with higher mean yield. Varieties with highest mean yield exhibited, within very narrow limits, a similar degree of adaptation to all environments over the wide range. They also found that varieties belonging to a given geographical region of the world showed similar adaptation. Their results provide a useful basis for plant introduction.

Frey (41) concluded that non-stress conditions resulted in retention of oat strains with wide adaptation whereas the stress conditions did not.

Krull et. al.(70) obtained yield data from 25 spring wheat cultivars grown in experiments at 16 locations in the Near East (two in Mexico, and one in Colombia). The cultivars highest for yield were identical in the four highest and in the four lowest productivity

experiments. Therefore, they concluded that testing wheat lines for grain yield should be done on the test sites with high fertility and optimum management since the highest yielding cultivars were selected in such an environment.

Roy and Murty (90) compared selections in wheat made under different environmental conditions. They concluded that those made in high yielding environments performed well, even under rainfed conditions, because in the stress environment the high genotype x environment interaction prevented the identification of superior genotypes.

Vela-Cardenas and Frey(113) considered optimum environmental conditions for maximizing heritability and genetic gain from selection. They observed that, with the exception of 100-seed weight, all environments were about equally effective for genetic gain. There was perfect coincidence of heritability and genetic gain in the optimum environments. They also suggested that selection for traits, such as yield in one environment, may have little or no usefulness if the selected genotypes are used in different environments.

Allen et. al. (11), in yield tests under opitimal environments for oats, barley, wheat, soybean, and flax, observed that productivity in yield trial environments differed among unfavorable, intermediate, and favorable environments. Mean yields in the unfavorable environements were less than one half.

Tomar et. al. (107) studied phenotypic stability of chickpea yield and observed that large-seeded types had poor phenotypic stability. They suggested that large-seeded varieties should be grown

under optimum environmental conditions, such as adequate moisture, warm temperature, and heavy soil, to maintain their large seed size. Under adverse environmental conditions, seed size was reduced.

MATERIALS AND METHODS

Genetic Variation Analyses:

This analyses was based on electrophoretic studies conducted during the period 1982 through 1984.

Electrophoresis Methods: The electrophoretic procedures used were based on those used in the laboratory of Dr. A.L. Kahler at the Northern Grain Insects Research Laboratory, Brookings. Starch gel electrophoretic assays were conducted on young seedlings grown in the greenhouse. The seedlings were excised for analysis when they attained 10 to 12 centimeters height between seven to eleven days after germination. Crude enzyme extracts were obtained by crushing stem tissues of seedlings in small petri dishes using plexiglass rods. The extracted juice was absorbed into filter paper wicks (10 x 3 mm, Beckman 319329). The wicks were inserted into gels with dimensions 0.9 cm x 16 cm x 18.5 cm. Gel and tray buffers (Table 1) were prepared in bulk. Materials for two starch gels were first prepared by suspending 80 g of hydrolyzed potato starch in 175 ml of cold buffer which was then mixed with 485 ml boiled buffer. This mixture was shaken vigorously to prevent formation of lumps. The gel mixture was vacuumed, for about 2 minutes, to remove air bubbles. The hot gel was poured into 0.9 x 16 x 18.5 cm molds and covered with plastic plates. Gels were prepared 12 hours prior to use and cooled to 4 degrees centigrade before wicks were inserted in a slot 4 cm from the Cathodal end. By using sponge cloth to provide the bridge between the gel and the tray buffer, electrophoresis was conducted at 4 C with constant current (DC) of 25 MA. After 15

TABLE - 1: Buffers, stains and fixing solutions used in the chickpea electrophoretic study at Brookings, South Dakota during 1982 to 1984

Buffer

System I Gel buffer (g/l) ph 8.2
1.82 g Tris
.43 g Citric acid

Tray buffer (g/l) ph 8.5
18.2 g Tris
3.8 g Citric acid

System II Gel buffer (g/l) ph 5.5
1.01 g L. Histidine
.38 g Citric acid

Tray buffer (g/l) ph 5.8
10.09 g L. Histidine
3.8 g Citric acid

Stains

1 - ADH (Alcohol Dehydrogenase)
.01 g DPN (NAD)
8 ml 1M Tris-HCL ph 7.4 buffer
3 ml 95% Ethanol
1 ml KCN (.005 g/ml)
3 ml MTT (10 mg/ml
1 ml PMS (5 mg/ml)
84 ml Distilled water

2 - Est(+) Esterase - Anodal
.2 g Fast blue RR salt
5 ml 1% α Naphthyl Acetate
2 ml 1% β Napthyl Acetate
10 ml 1M Tris-HCL pH 6.0 buffer
83 ml Distilled water

3 - Est (-) (Esterase-Cathodal)
.2 g Fast Blue RR salt
2 ml 1% α naphthyl acetate
3 ml 1% β naphthyl acetate
10 ml 1M phosphate pH 5.5 buffer
84 ml Distilled water

4 - PRX (Peroxidase - Cathodal)
20 ml Oidianisidine
80 ml Distilled water
1 ml 3% hydrogen peroxide

5 - MDH (Malate Dehydrogenase)
.03 g - DPN
50 ml pH 7.0 MDH buffer
46 ml Distilled water
3 ml MTT
1 ml PMS

6 - PGD (Phosphogluconate dehydrognase)
.025 g 6. phosphogluconic acid
.01 g TPN (NADP)
5 ml -1M Tris-HCl pH 8.0 buffer
1.5 ml MTT
0.5 ml PMS
93.0 ml Distilled water

7 - ACP (Acid Phosphatase)
0.1 g Fast garnet GBC salt
1 ml 1% $MgCl_2$
2 ml sodium acetate (NaAc) pH 4.7
92 ml Distilled water
5 ml 1% Naphthyl acid phosphate

Fixing Solution
40% Ethanol

minutes, electrophoresis was discontinued and wicks were removed from the gel. Electrophoresis was then continued for another 6 hours, for buffer systems I and III. Upon completion of electrophoresis, gels were removed from the trays. Each gel was sliced horizontally into three or four portions and each portion was stained (for one or two hours depending on resolution and intensity of the bands) for a specific enzyme system. Staining recipes also are presented in Table 1. The stained gels were fixed in 40% ethanol.

Enzyme systems which were assayed included: esterase (EST-EC 3.1.1.1), peroxidase (PRX-EC 1.11.1.7), alcohol dehydrogenase (ADH-EC 1.1.1.1), acid phosphatase(ACP-EC 3.1.3.2), 6-phosphogluconate dehydrogenase (PGD-EC 1.1.1.44) and malate dehydrogenase (MDH-EC 1.1.1.37). Enzymes systems EST, PRX, and ADH were examined, using the tris-citric acid gel buffer system. The histidine buffer system was used for ACP, PGD, and MDH. Two zones of activity for PGD and a single zone of activity for ADH and PRX were used to identify genotypes at enzyme loci.

The system of nomenclature followed for enzyme loci, alleles, and genotypes, was identical to that used for maize (65). The first locus to be verified by segregation tests for any enzyme system was designated locus 1, the second locus 2, and so on. Alleles (allozymes) were designated by migration distances of their products (in cm from the origin) on zymograms and were assigned laboratory numbers for computer analyses. For example, the gene symbols, Adh1-11, Adh1-12, and Adh1-22 were assigned to the slow homozygote, intermediate heterozygote, and fast homozygote genotypes, respectively.

Inheritance and linkage:

Parental and F1 seeds of different chickpea lines were supplied by Dr. K.B. Singh, ICARDA (The International Center for Agricultural Research in the Dry Areas), Aleppo, Syria. Crosses between ILC 2678 and ILC 2653 were made at SDSU (South Dakota State University), Brookings, South Dakota. A double hetrozygote for Adh1 and Pgd2 was obtained from populations provided by W-6 Regional Plant Introduction, Pullman, Washington.

The inheritance of isozyme patterns of Adh1 was studied in F1 hybrids and F2 progenies, in matings between ICC 76 (pink flowered) fast banded Adh1-22 and ILC 480 (white flowered), slow-banded Adh1-11 homozygotes. The inheritance pattern of Pgd1 was studied in F1 hybrids and F2 progenies, in matings between FLIP 8272, slow banded Pgd1-11 and ILC 72, fast banded Pgd1-22 homozygotes. The inhertance of Prx1 was studied in F1 hybrids and F2 progenies, in matings between ILC 72, null Prx1-00 and FLIP 7264, banded Prx1-11 homozygotes.

The genetic relationship between Adh1 and Pgd1 was studied in F1 hybrids and F2 progenies, in matings between ILC 2653, slow banded for Adh1 and Pgd1, and ILC 2678, fast banded for both Adh1 and Pgd1. The genetic relationship between Adh1 and Pgd2 was studied on an individual (PI 359259) heterozygoous for Adh1-12 and Pgd2-12. Materials for the inheritance study are presented in Table 2.

Zones of enzyme activity (isozyme bands) are defined in terms of distance migrated (in cm) from the gel origin, which was the point at which the wick was originally inserted. Loci are identified within

TABLE 2: Pedigree of four chickpea crosses and one heterozygote used to study the inheritance patterns of ADH, PGD and PRX enzyme systems at Brookings, South Dakota during 1982 to 1984

Generations	Flower color	Seed color	Loci+			
			Adh1	Pgd1	Pgd2	Prx1
P_1 ICC 76	Pink	Black	22	11	11	11
F_1 ICC 76 x ILC 480	Pink	Black	12	11	11	11
P_2 ILC 480	White	Cream	11	11	11	11
P_1 FLIP 8272	White	Cream	11	11	11	11
F_1 FLIP 8272 x ILC 72	White	Cream	11	12	11	11
P_2 ILC 72	White	Cream	11	22	11	00
P_1 ILC 2653	White	Cream	11	11	11	11
F_1 ILC 2653 x ILC 2678	White	Cream	12	12	11	11
P_2 ILC 2678	White	Cream	22	22	11	11
P_1 ILC 72	White	Cream	11	22	11	00
F_1 ILC 72 x FLIP 7264	White	Cream	11	12	11	11
P_2 FLIP 7264	White	Cream	11	11	11	11
P[359 259 (double Heterozygote)	Pink	Brown	12	11	12	11

+ The 22,12, and 11 designation for Adh1 signifies an Adh1-22 homozygote, Adh1-12 heterozygote and Adh1-11 homozygote, respectively, which normally would be written Adh1-2/Adh1-2, Adh1-1/Adh1-2 and Adh1-2/Adh1-2, respectively. The symbol 00 for Prx1 designate a null (no band) genotype.

zones and alleles (allozymes) are designated by the migration distances of their products from the gel origin (65)

Genetic variation within and among populations:

This study was based on a large number of chickpea germplasm accessions which originated in 25 different coutries (Table 3). Six hundred fifty five accessions, representing 24 countries were received from ICARDA in 1982. During the same year, another 32 entries were obtained from Davis, California and 1500 USDA accessions from Pullman, Washington. Accessions received from Pullman were originally from India and Iran and were designated India-2 and Iran-2 to distinguish them from accessions of similar origin received from ICARDA. A total of 27 populations were established on the basis of origin. In each population, a maximum of 150 seedlings (3 seedlings per accession) were grown in the greenhouse.

Amounts of genetic variability within and between populations were determined using standard gene and genotypic frequency models. Genotype and allele frequencies at each locus were calculated after the number of individuals observed with each genotype had been determined. The models to estimated genotype frequencies (Hedrick) were as follows:

$$\hat{P} = \frac{N_{11}}{N}, \qquad \hat{H} = \frac{N_{12}}{N}, \qquad \hat{Q} = \frac{N_{22}}{N}$$

TABLE - 3: Population number, origin, source, number of accessions per population and sample size per population used to study distribution of allozyme variation in cultivated chickpeas at Brookings, South Dakota, during 1982 - 1984

Population No.	Origin	Population Source	No. of Acc./ Population	Sample Size/pop.
1	Afghanistan	ICARDA	50	150
2	Algeria	ICARDA	14	42
3	Chile	ICARDA	12	36
4	Cyprus	ICARDA	6	18
5	Egypt	ICARDA	40	119
6	Ethiopia	ICARDA	26	78
7	Greece	ICARDA	9	27
8	India	ICARDA	50	150
9	Iran	ICARDA	49	147
10	Iraq	ICARDA	22	66
11	Jordan	ICARDA	31	94
12	Lebanon	ICARDA	20	60
13	Mexico	ICARDA	9	28
14	Morocco	ICARDA	16	48
15	Pakistan	ICARDA	20	60
16	Palestine	ICARDA	31	93
17	Portugal	ICARDA	1	3
18	Spain	ICARDA	50	150
19	Sudan	ICARDA	6	20
20	Syria	ICARDA	50	150
21	Tunisia	ICARDA	32	96
22	Turkey	ICARDA	50	150
23	USA	ICARDA	22	66
24	USSR	ICARDA	39	117
25	Davis	U.C. Davis	32	96
26	India-2	Pullman	361	361
27	Iran-2	Pullman	341	341
Total			1389	2766

where

$\hat{P}$ = frequency of 11 genotypes

$\hat{H}$ = frequency of 12 genotypes

$\hat{Q}$ = frequency of 22 genotypes

N = total number of individuals in the population

N_{11} = number of 11 genotypes in the population

N_{12} = number of 12 genotyes in the population

N_{22} = number of 22 genotypes in the population

The estimated allelic frequency was calculated from the sample as:

$$\hat{p} = \frac{N_{11} + \frac{1}{2}N_{12}}{N}$$

When null genotypes were observed in a population, allele frequencies were estimated following Hedrick's (56) formula for two codominant alleles and one recessive null allele:

$$\hat{p}_1 = 1 - \left(\left(\frac{N_{22} + N_{23} + N_{33}}{N}\right)^{\frac{1}{2}}\right)$$

$$\hat{p}_2 = 1 - \left(\frac{N_{11} + N_{13} + N_{33}}{N}\right)^{\frac{1}{2}}$$

$$\hat{p}_3 = \left(\frac{N_{33}}{N}\right)^{\frac{1}{2}}$$

where

$\hat{p}_1, \hat{p}_2, \hat{p}_3$ are frequencies of the slow(1) fast(2) and null alleles, respectively,

N_{22}, N_{11}, and N_{33} are the number of 22,11 and null genotypes, respectively,

N is total number of individuals in the population, and

N_{23} and N_{13} are the number of heterozygotes with fast/null(23) and slow/null(13) genotypes

When the sum of the allele frequencies did not equal unity, adjusted estimates were calculated by letting the deviation from unity be:

$$d = 1 - (\hat{p}_1 + \hat{p}_2 + \hat{p}_3)$$

this value was then used to obtain new estimates of allele frequencies as:

$$\hat{p}_1' = (1 + 1/2d)\ \hat{p}_1$$

$$\hat{p}_2' = (1 + 1/2d)\ \hat{p}_2$$

$$\hat{p}_3' = (1 + 1/2d)\ (\hat{p}_3 + 1/2d)$$

Intrapopulational variation was measured by the average frequency of heterozygotes per locus and by the proportion of polymorphic loci in the population. Average frequency of the heterozygotes per locus is the expected frequency of heterozygotes that would exist under Hardy-Weinberg equilibrium, and an average of all loci sampled (78).

The proportion of polymorphic loci was used as a measure of heterogeneity in a given population. The proportion of polymorphic loci was calculated using the no criterion limits of Selander et. al. (91); thus, all individuals within the population were considered to determine polymorphism.

To measure genetic differences between populations, Nei's (78) indices of 'genetic identity' (I) and 'genetic distance' (D) were used. Both statistics were calculated for all possible pair-wise comparisons between populations. The genetic identity of Nei's is:

$$I_N = \frac{J_{xy}}{(J_x J_y)^{\frac{1}{2}}}$$

where

$$J_{xy} = \sum_{i=1}^{n} P_{i.x} P_{i.y}$$

$$J_x = \sum_{i=1}^{n} P_{i.x}^2$$

$$J_y = \sum_{i=1}^{n} P_{i.y}^2$$

and Pi.x and Pi.y are the frequencies of the ith allele in population x and population y. The genetic distance between two populations is then defined as

$$D_N = -\ln(I_N)$$
$$= \ln J_{xy} - \tfrac{1}{2}\ln J_y - \tfrac{1}{2}\ln J_x.$$

The 27 populations were clustered using dendrograms on genetic distance (Fig.14). The observed genotypic comparisons, as defined by the four loci, Adh1, Prx1, Pgd1, and Pgd2, were determined and distance matrixes were generated from allelic frequencies, using Nei's distance and identity measures. Dendrograms based on allelic frequencies, are compared and contrasted.

Field studies:

Agronomic characters were studied in replicated field plantings. Materials for the study were supplied by ICARDA. Three trials, CAT (Chickpea Adaptation Trial), CF3YT (Chickpea F3 Yield Trial), and CLYT (Chickpea Large Seeded Yield Trial), consisting of both inbred and segregating populations, were evaluated for yield and other agronomic characters at Brookings, Highmore, and Rapid City, in South Dakota in 1982 and 1983.

CAT included 16 entries of chickpeas which originated from eight different countries, representing a wide range of environments (Table 4). These entries are provided to many countries by ICARDA and ICRISAT (International Crop Research Institute in the Semi-Arid Tropics) for testing widely adapted genotypes over a range of environments. This trial was conducted at Brookings and Highmore in 1982, and at Highmore and Rapid City in 1983.

CF3YT consisted of 14 F3 populations and two varieties in the 1983 trial. The populations included crosses of Aschocyta resistant lines to widely adapted, tall, and high-yielding genotypes. They were screened with the aim of providing early segregating populations to supplement national and regional programs.

TABLE - 4: List of chickpea entries in CAT, CF_3YT and CLYT trials used in the agronomic studies at Brookings, Highmore and Rapid City, South Dakota, during 1982 to 1983

C A T			CF_3YT		CLYT	
Entry	Origin	Seed Type	Entry Cross #	Pedigree	Entry	Origin
ICC 4918	India	Desi	x TH 53	ILC 1929 x ILC 256	ILC 35	Syria
ICC 4948	India	Desi	x TH 56	ILC 1920 x ILC 3279	ILC 76	Spain
ICC 5003	India	Desi	x TH 84	ILC 191 x ILC 262	ILC 83	Spain
ICC 5810	India	Desi	x TH 85	ILC 191 x ILC 237	ILC 112	Spain
ICC 10136	ICRISAT	Desi	x TH 101	ILC 72 x ILC 191	ILC 116	Spain
ICC 11524	ICRISAT	Desi	x TH 104	ILC 72 x ILC 482	ILC 132	Spain
ICC 11529	ICRISAT	Desi	x TH 105	ILC 72 x ILC 484	ILC 134	Spain
			x TH 111	ILC 191 x ILC 202	ILC 135	Spain
ILC 482	Turkey	Kabuli	x TH 112	ILC 191 x ILC 482	ILC 136	Spain
ILC 519	Egypt	Kabuli	x TH 123	ILC 191 x ILC 484	ILC 165	Tunisia
ILC 1919	India	Kabuli	x TH 120	ILC 200 x ILC 484	ILC 171	Tunisia
ILC 1922	Morocco	Kabuli	x TH 125	ILC 202 x ILC 482	ILC 254	Turkey
ILC 1929	Syria	Kabuli	x TH 126	ILC 202 x ILC 484	ILC 451	Turkey
ILC 1931	Turkey	Kabuli	x TH 146	ILC 72 x ILC 73	ILC 464	Turkey
ILC 1932	Jordan	Kabuli			ILC 496	Turkey
ILC 1934	Iran	Kabuli	ILC -482	Acc.No.26780-68	ILC 613	Tunisia
ILC 3256	Cyprus	Kabuli	Loc.Check	SD selection	ILC 620	Moroco
					ILC 629	Tunisia
					ILC 2587	Turkey
					Loc.chekck	SD selection

CAT = Chickpea Adaptation Trial
CF_3YT = Chickpea F_3 Yield Trial
CLYT = Chickpea Large Seeded Yield Trial

TABLE 5 : Climatological observations during 1982 and 1983 crop growing seasons

Month	Brookings				Highmore				Rapid City	
	1982		1983		1982		1983		1983	
	Av. Temp (°F)	Prec (In)	Av. Temp (°F)	Prec (In)	Av. Temp (°F)	Prec (In)	Av.Temp (°F)	Prec. (In)	Av. Temp. (°F)	Prec. (In)
April	40.5	1.43	39.1	1.28	43.9	0.88	41.5	1.13	40.3	0.90
May	47.8	4.31	52.4	1.14	58.1	5.67	53.0	3.08	51.4	3.02
June	60.8	2.25	64.0	4.45	64.0	1.55	65.1	5.75	63.6	0.70
July	71.0	5.55	74.2	3.03	74.8	4.20	76.0	2.13	75.2	1.86
August	67.9	1.92	74.9	4.29	73.0	2.30	80.1	1.60	76.8	2.67
September	57.8	2.74	59.2	2.35	60.8	1.40	63.8	1.60	60.1	0.25
Total		18.19		16.54		16.0		15.0		9.40
Mean	59.3		60.63		62.4		63.26		61.23	

Ninteen large-seeded entries, originating from four different countries (Table 4) and one 1982 South Dakota selection, were included in the CLYT (1983) trial conducted at Brookings and Highmore. This trial was designed to evaluate quality and hence marketability of crop.

All experiments were planted with a 4-row cone planter at a depth of 5 to 8 cm. Entries were planted in a randomized complete block design with four replications for CAT and CLYT and three replications for CF3YT. Plots were 3 m long, with four rows, and inter and intra row spacings of 30 and 10 cm, respectively. Only central rows of each plot were harvested for yield measurements.

In 1982, the CAT trial was planted on May 4 and 22, at Brookings and Highmore, respectively; and was harvested on August 30 at Brookings, and September 15 at Highmore. CAT was again planted in 1983 at Highmore and Rapid City, on April 22 and 31, respectively. The crop was harvested on August 15 at Highmore and on August 22 at Rapid City.

In 1983, the CLYT trial was planted at Highmore on April 22 and at Rapid City, on April 31. Harvesting was done at Highmore and Rapid City on August 15, and 22, respectively.

The CF3YT trial was planted at Brookings on May 10 and at Highmore on April 22, in 1983. The crop was harvested at Highmore on August 16, and at Brookings on September 20.

Data on plant height, spread, stand, seeds per pod, 100-seed weight, and grain yield were recorded. Procedures used to evaluate plant characters were as follows: a) plant height - average distance from soil surface to the top of the canopy at maximum growth; b) plant

spread - horizontal measurement of the canopy at maximum growth; c) plant stand - % of plant population at maturity; d) seeds/pod - the total seeds in 10 randomly chosen pods divided by the number of pods; e) 100-seed weight - weight of 100 randomly chosen seeds in grams.

Statistical Analysis: Means, ranges, and coefficients of variation were calculated for characteristics in each trial. Methods described by Steel and Torrie (104) were utilized for analysis of variance. Estimates of variance components were used to obtain broad sense heritabilities and expected genetic advance (2).

$$\text{Heritability} = \frac{\sigma_G^2}{\sigma_P^2}$$

$$\sigma_P^2 = \sigma_V^2 + \frac{\sigma_{VL}^2}{L} + \frac{\sigma_e^2}{RVL}$$

where

σ_V^2 = Genetic variance due to variety

σ_P^2 = Phenotypic variance

σ_{VL}^2 = Variance due to genetic x location interactions.

σ_e^2 = Error variance

R = Replication

L = Location

Y = Year

$$\text{Genetic advance (GS)} = \frac{\sigma_G^2}{\sigma_P^2} \text{ X } K\sigma p \text{ ,}$$

where $K\sigma p$ is the selection differential in phenotypic standard deviations. K was given values of 2.06 and 1.76, which are expectations 5% and 1% selection differentials, respectively.

Simple correlation coefficients were obtained between all possible combinations of traits related to seed yield. These correlations were analyzed further, using Wright's (110) and Dewey and Lu's (33) methods to obtain direct and indirect path coefficients. Stability indices, linear regression, and deviations from regression(42) on yield and 100-seed weight were estimated for CAT. Estimates of b and s2d values were used as measures of general adaptation and stability, respectively. An entry with b=1.0 is considered to be adapted to all environments, whereas one with b>1.0 is better adapted to high yield environments and those with b<1.0 are better adapted to low yield environments. A stable variety has an s2d that is not significantly different from zero. An ideal variety is one with b=1.0, s2d=0, and is high yielding (81).

RESULTS AND DISCUSSION

MONOMORPHIC ENZYMES

Three enzyme systems, acid phosphatase (ACP), esterase (EST) and malate dehydrogenase (MDH) were monomorphic in all populations studied.

ACP: This enzyme was monomorphic and homogeneous among the populations assayed. At least eight bands were present in each individual. Band number 4 was very strong in intensity, whereas band number 8 was very faint (Figure 1).

EST: Both anodal and cathodal esterases were monomorphic in all cultivars studied. At least six bands could be observed in the anodal esterase. Band number 6 was the strongest in intensity (Figure 2). Cathodal esterase exhibited three bands. Band number 2 was the strongest in intensity (Figure 3).

MDH: MDH exhibited four anodal bands. The second band of the four was the lightest in staining intensity. The remaining three bands (Number one, three, and four) were all very strong in intenstity (Figure 4). MDH also was monomorphic in all cultivars studied.

Dobzhansky (35) noted that adaptedness represents the ability of a population, organism, or genotype to survive and reproduce in a particular environment. If adaptedness is defined as the ability of a population to live and reproduce in a wide spectrum of environments, then the observed fixed enzyme systems in chickpea could be considered to be a genetic mechanism for the existance and reproduction of the crop over a wide range of environments. Because the material for the present

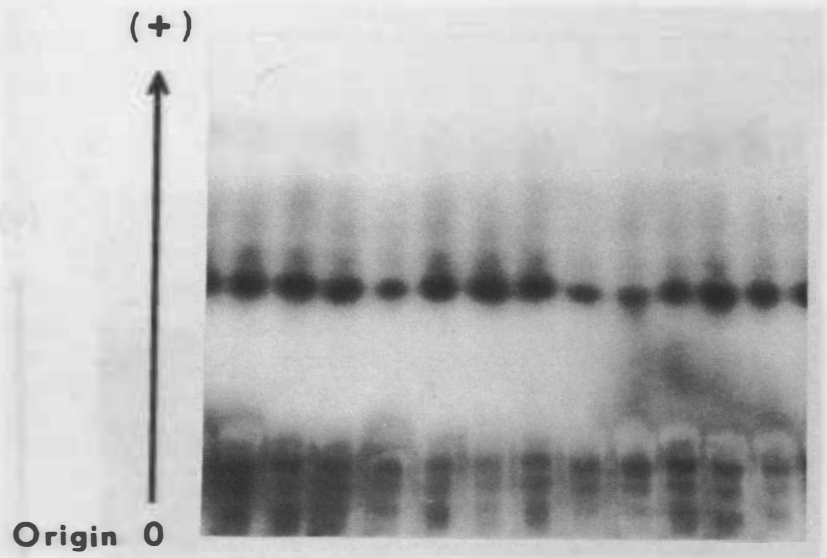

FIGURE 1: Photographic representation of ACP zymogram in Chickpea

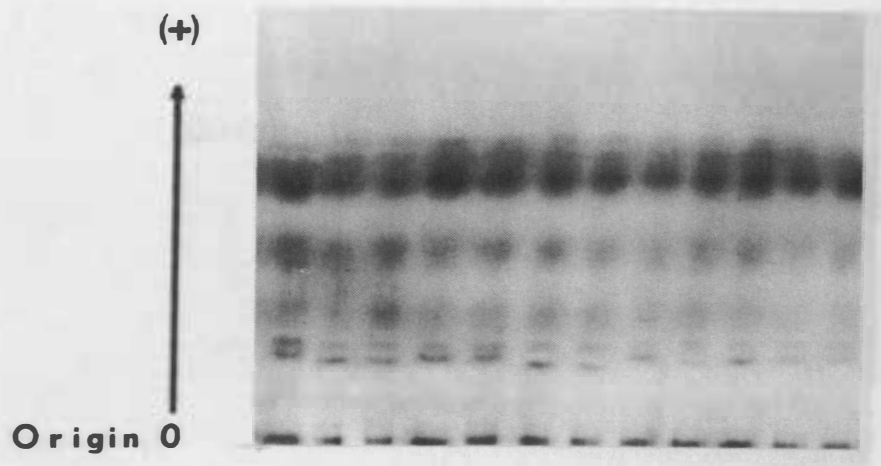

FIGURE 2: Photographic representation of anodal EST zymogram in chickpea

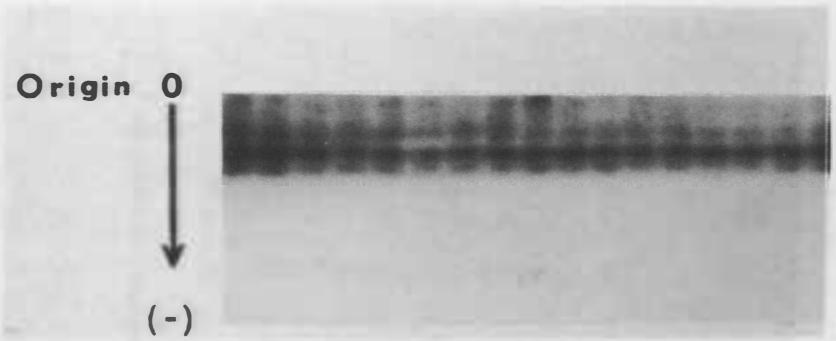

FIGURE 3: Photographic representation of cathodal EST zymogram in chickpea

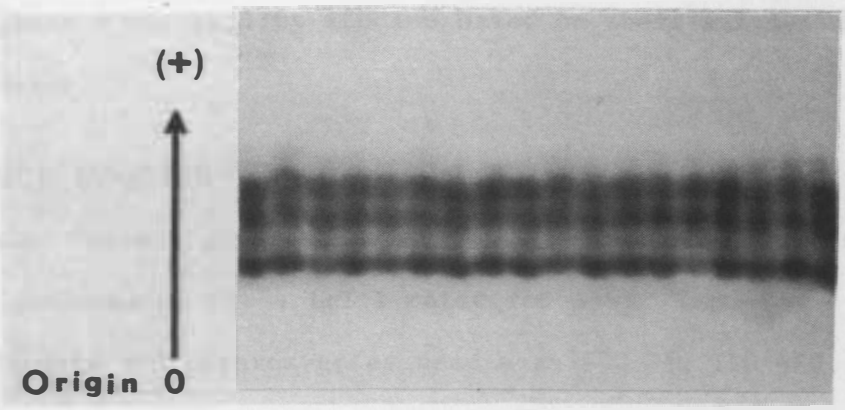

FIGURE 4: Photographic representation of MDH zymogram in chickpea

study was from different ecogeographical regions, the nature of selective forces operating under one ecogeographical region seems to be similar to the other regions. This suggests that these fixed enzymes perhaps are associated with certain morphological and physiological factors common to all varieties.

POLYMORPHIC ENZYMES

Alcohol dehydrogenase (ADH), 6-phosphogluconate dehydrogenase (PGD) and peroxidase (PRX) were found to be polymorphic. Inheritance and other genetic variability studies based on these enzymes are presented below.

INHERITANCE STUDIES

ADH: Table 6 gives observed genotype number and chi-square values for goodness of fit a 1:2:1 ratio for Adh1.

Parents and heterozygotes used were ICC 76, ILC 480, ILC 2653, ILC 2678, ICC 5810, and PI 359295. For gel runnning times of 6 hours, ILC 480 and ILC 2653 parental material exhibited bands at approximately 2.9 cm from the origin, whereas ICC 76 and ILC 2678 exhibited bands at approximately 3.9 cm. Bands observed at approximately 2.9 cm were considered "slow bands" and bands obtained at 3.9 cm were "fast bands". The slow and fast bands were assigned laboratory numbers 1 and 2, respectively.

Hybrids of 11 and 22 homozygotes showed both parental bands and an additional band at intermediate position (3.4 cm) to the parental bands (Figure 5). This 3-banded phenotype was designated 12 (the hybrid

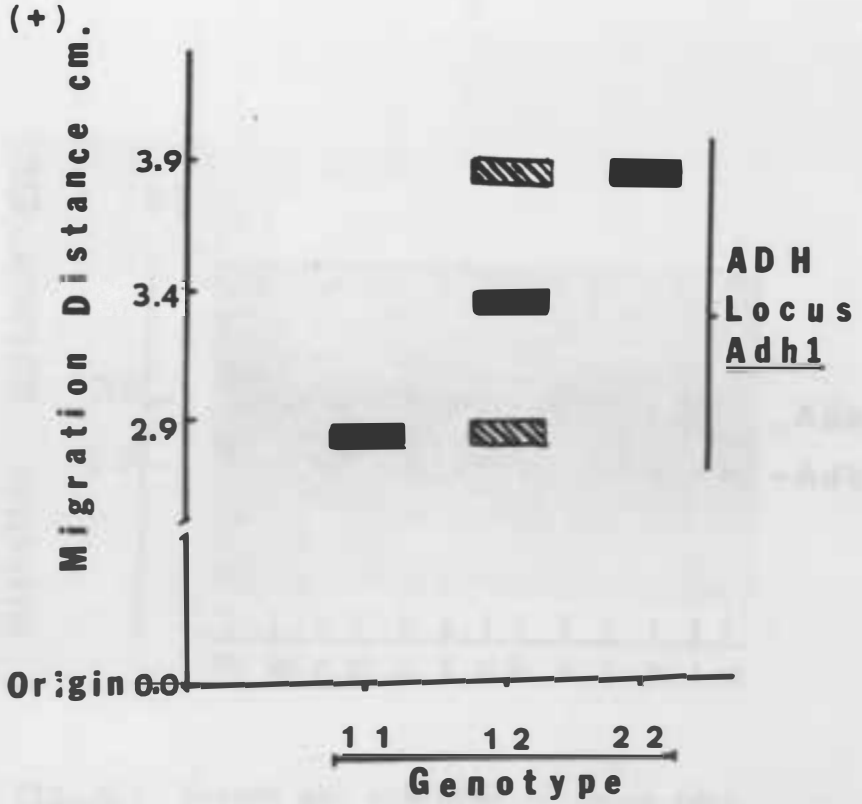

Fig. 5 A schematic representation of Alcohol dehydrogenase isoenzyme genotypes of parent ILC 480 (11), parent ICC 76 (22) and F_1 hybrid (12) in chickpea at Adh1 locus

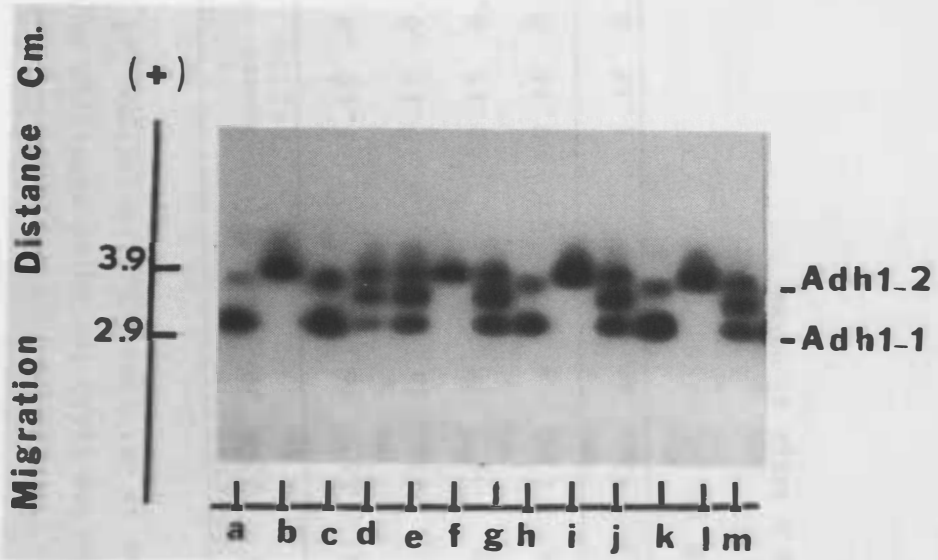

Fig. 6 : Starch gel zymogram of locus Adh1 segregating for allozymes Adh1-1 and Adh1-2 in F_2 progeny of cross ICC 76 X ILC 480. Individuals a,c,h and k are 11 homozygotes; individuals d,e, g,j and m are 12 heterozygotes; and individuals b,f,i and l are 22 homozygotes

TABLE - 6: F_2 segregation ratio for three genotypes at the *Adh1* locus in chickpea (*Cicer arietinum* L.)

Cross/Heterozygotes	Genotype 11	12	22	df	(Chi-square	< P+ < 1:2:1)	
ILC 2653 x ILC 2678	14	16	10	2	40	2.40	0.30-0.50
ICC 76 x ILC 480	10	19	11	2	40	0.15	0.90-0.95
ICC 5810	7	25	16	2	48	3.45	0.10-0.20
PI 359295	8	14	9	2	41	0.36	0.70-0.90
Total	39	74	46	2		1.38	0.50-0.70

Heterogeneity $x^2_{[6]} = 4.98$ $(0.50 < P < 0.70)$

P = probability of obtaining a large x^2 value

phenotype). F2 populations, obtained from selfed hybrids and progenies of selfed heterozygotes (ICC 5810 and PI 359295), segregated in the 1:2:1 ratio expected for a monogenic model (Table 6). These data demonstrated that ADH isozymes in the gel zone 2.9 to 3.9 cm from the gel origin are governed by a single locus with at least 2 codominant alleles. Figure 5 represents segregating individuals of the ICC 76 x ILC 480 hybrid. All parental lines showed single bands and heterozygotes triple bands. This indicates that ADH has dimeric subunit structure in chickpea. The gene symbol Adh1 was assigned to this locus, with alleles Adh1-1 assigned to the slow positioned at 2.9 cm; and Adh1-2 assigned to the fast band, positioned at 3.9 cm.

PGD: Parents, ILC 2653, ILC 2678, ILC 72, and FLIP 8264, were used to study the heritance of PGD isozymes. ILC 2653 and FLIP 8272 displayed bands at approximately 3 cm while ILC 72 and ILC 2678 exhibited bands at 3.6 cm from the origin for gel running times of 6 hours. Hybrids between parents with slow and fast bands produced both parental bands and a hybrid band with intermediate mobility (Figure 7). The heterozygote with a triple-banded phenotype was denoted 12. Eighty progeny from two selfed hybrids were assayed to determine whether PGD isozymes marked alleles at a single locus. The two families segregated according to the expected 1:2:1 monogenic ratio (Table 7). These data indicated that PGD isozymes, 3.0 to 3.6 cm from the origin, were controlled by a single dimeric locus with codominant alleles. The gene symbol, Pgd1, was assigned to this locus, and alleles were designated Pgd1-1 and Pgd1-2, for slow and fast mobilities, respectively.

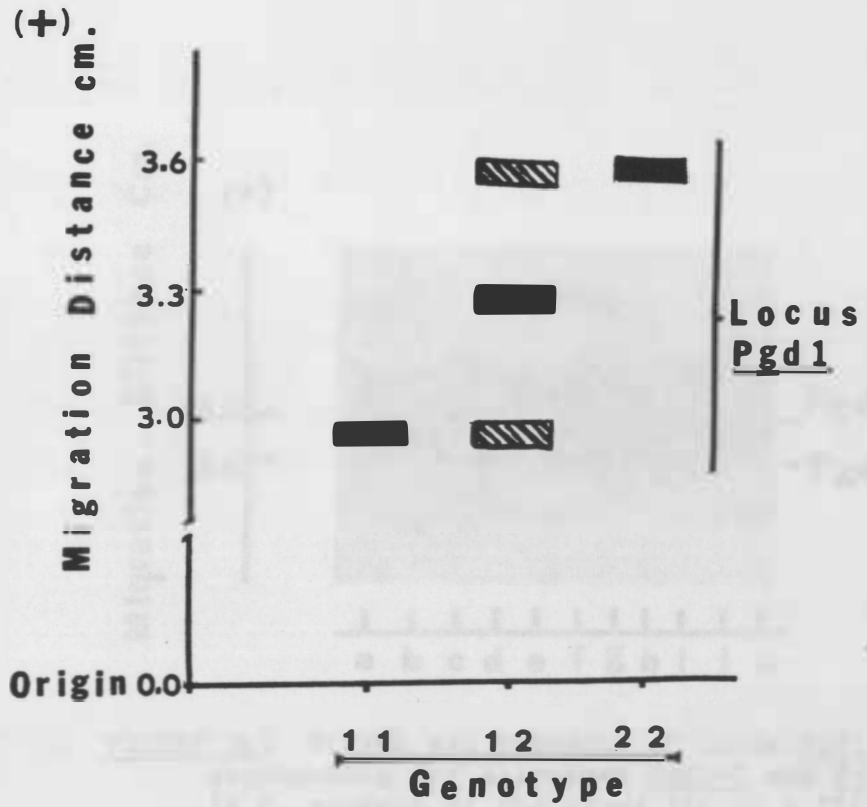

Fig. 7: A schematic representation of Pgd1 isoenzyme genotypes of parent FLIP 8272 (11), parent ILC 72(22) and F_1 hybrid (12) in chickpea

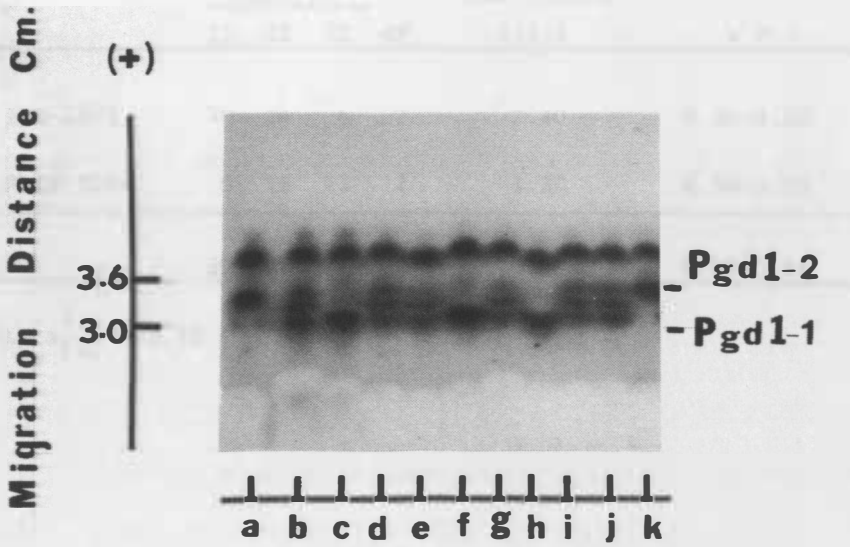

FIGURE 8: Starch gel zymogram of locus Pgd1 segregating for allozymes Pgd1-1 and Pgd1-2 in F_2 progeny of the cross ILC 72 X ILC 8272. Individuals a and k are 22 homozygotes; individuals b,d,e,g,i, and j are 12 heterozygotes; and individuals c, f and h are 11 homozygotes.

TABLE 7: F_2 segregation ratio for three genotypes at the Pgd1 locus in chickpea (Cicer arietinum L.)

Cross	Genotype 11	12	22	df	Chi-Square 1:2:1	< P <
ILC 2653 x ILC 2678	10	24	6	2	2.40	0.30-0.50
ILC 72 x FLIP 8264	9	18	13	2	1.20	0.50-0.70
Total	19	42	19	2	0.20	0.90-0.95

Heterogeneity $x^2_{[2]}$ = 3.20 (0.20 < P < 0.30)

TABLE 8: F_2 segregation ratio for three genotypes at the Pgd2 locus in chickpea (Cicer arietinum L.)

Heterozygote	Genotype 11	12	22	df	Chi-square 1:2:1	< P <
PI 359295	8	15	8	2	.032	0.70-0.90

When accession PI 359295 was selfed, the progeny segregated according to the expected 1:2:1 monogenic ratio (Table 8). The band displayed at 4.2 cm was denoted 1, and the band displayed at 5.0 cm was denoted 2. Heterozygous individuals were triple-banded like the original heterozygote. This triple-banded phenotype was designated 12 (Figure 9). In segregation tests, parental types showed single bands and heterozygotes triple bands, which indicated dimeric subunit structure. The symbol, Pgd2, was assigned to this locus with alleles, Pgd2-1, (4.2 cm band) Pgd2-2 (5.0 cm band).

PRX: Parent FLIP 8264 was banded at position 2.0 cm from the origin and parent ILC 72 was null(no band) at the cathodal region of the gel. In crosses between these banded and bandless parents, the F1 hybrid was banded at 2.0 cm from the origin. Progenies from this F1 hybrid segregated in a 3:1 ratio (Table 9) indicating that the banding for this zone is governed by a single locus with at least one dominant allele (desginated 1), and one recessive (designated 0) null allele (Figure 11). The gene symbol, Prx1, was assigned to this locus. One parental line and heterozygotes exhibited a single band. Hence, progeny testing was necessary to identify homozygotes from the heterozygotes.

LINKAGE STUDIES

Linkage between Adh1 and Pgd1 was determined in an F2 family, derived from a selfed F1 hybrid obtained by crossing variety ILC 2653 and ILC 2678. Linkage between Adh1 and Pgd2 was determined from progenies obtained from selfing a double heterozygote, PI 359295. Genotypes of the two parents, ILC 2653 and ILC 2678 were Adh1-11 and

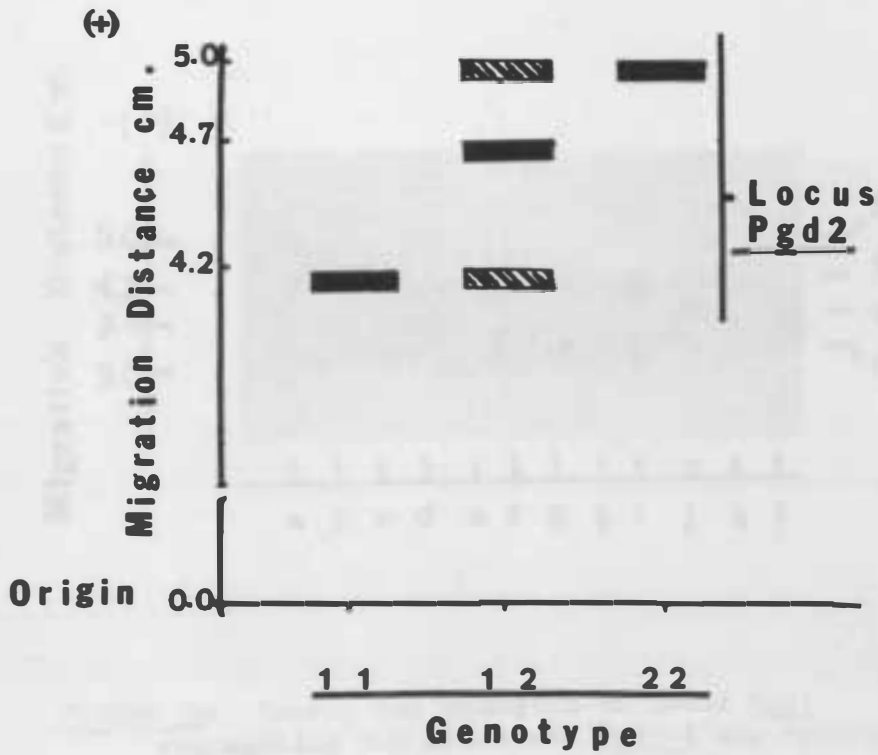

Fig.9 A schematic representation of Pgd2 genotypes (11 and 22 parental types and 12 a heterozygote), obtained from PI 359295

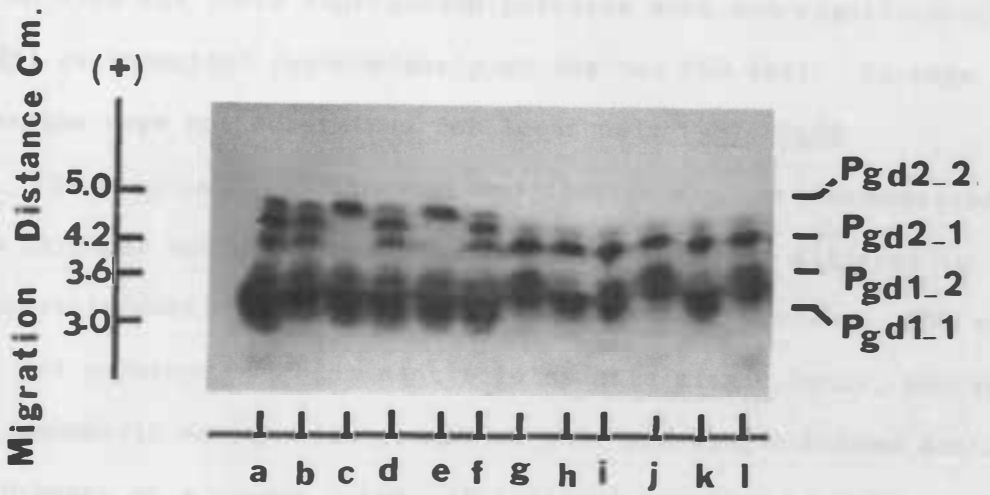

FIGURE 10: Starch gel zymogram of locus Pgd1 segregating for allozymes Pgd1-1 and Pgd1-2; and locus Pgd2 segregating for allozymes Pgd2-1 and Pgd2-2 in progeny of selfed heterozygote, PI 359295. At Pgd2 locus, individuals c and e are 22 homozygotes; individuals g,h,i,j,k and l are 11 homozygotes; and a,b,d,and f are 12 heterozygotes

Adh1-22, respectively. The genotype of the double heterozygote, PI 359295, was Adh1-12 and Pgd2-12. Linkage relationships of Pgd1 and Pgd2, with Adh1, were determined by chi-square test of goodness-of-fit to a 1:2:1:2:4:2:1:2:1 ratio. Table 10 presents observed numbers of two locus genotypes for locus-pairs and Chi-square values for each family assayed. The Chi-square value for locus pair Adh1, Pgd1 was 5.6 ($0.60 < P < 0.70$) and Adh1, Pgd2 was 4.4 ($0.90 < P < 0.95$). Chi-square values calculated from the locus segregation patterns were non-significant; so locus Adh1 is inherited independantly of the two PGD loci. Linkage relationships were not determined for locus pair Pgd1, Pgd2.

The results of inheritance and linkage studies demonstrated that the chickpea varieties assayed, and their crosses, differed in electrophoretic mobilities for at least three enzyme systems. ADH and PGD each are governed by codominant alleles at a single locus; whereas PRX is a monomeric enzyme with recessive null and single-banded dominant alleles present at a single locus. Individual seedlings can be evaluated for all three enzyme systems simultaneously, so that each individual can be assigned to a specific genotype at each locus. The electrophoretic methods used for this study provide a rapid and inexpensive method of screening a large number of individuals in a limited space with few personnel.

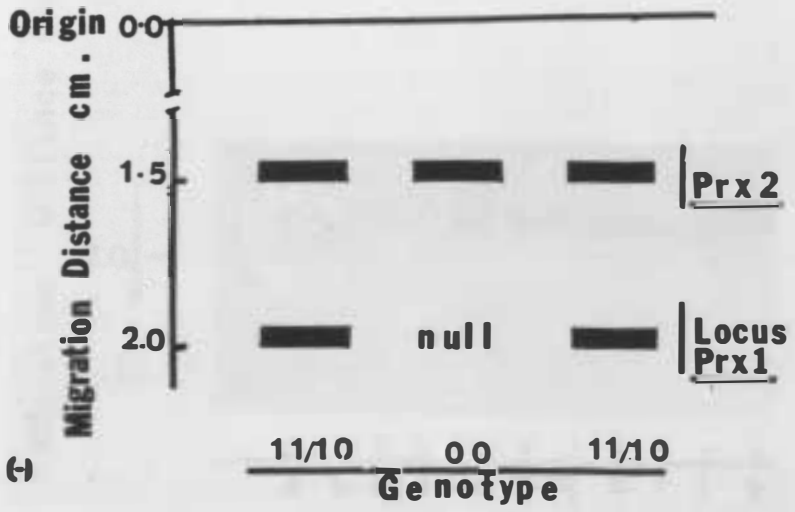

Fig. 11: A schematic representation of Prx1 genotypes of parent ILC 72(00), parent FLIP 7264 (11) and F_1 hybrid(10) in chickpea

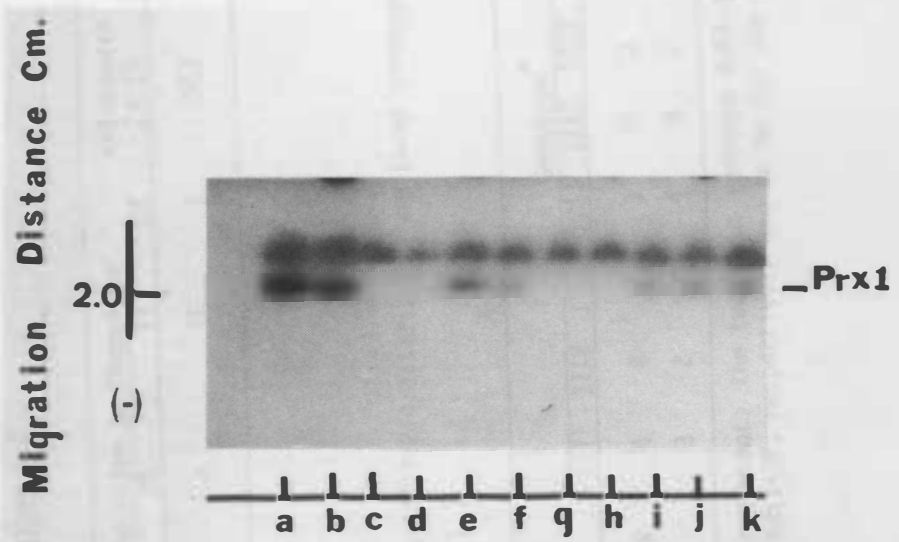

FIGURE 12: Starch gel zymogram of locus Prx1 segregating for Prx1-0(null) and Prx1-1 in F_2 progeny of the cross ILC 72 X FLIP 8264. Individuals a,b,e,f,i,j, and k are either 11 homozygotes or 10 heterozygotes; individuals c,d,q, and h are Prx1-0 homozygotes

Table 9 F_2 segregation ratio for two genotypes and/or phenotypes at the Prx1 locus in chickpea (Cicer arietinum L.)

Cross	Genotype or Phenotype 00	11	df	Chi-square (3:1)	< P <
ILC 72 x FLIP 7264	4	16	1	.067	0.70-0.90

Table 10 F_2 segregation ratio 1:2:1:2:4:2:1:2:1 for two locus genotypes in chickpea (Cicer arietinum L.)

Locus pair	Cross hetro-zygote	Genotype+ 1111	1112	1122	1211	1212	1222	2211	2212	2222	X^2	< P <
Adh1, Pgd1	ILC 2653xILC 2678	4	8	2	3	10	3	3	6	1	5.6	0.60-0.70
Adh1, Pgd2	PI 359295	3	2	3	4	8	2	1	5	3	4.4	0.90-0.95

+Genotype 1111,1112 and 1122 represents the two locus genotype Adh1-11/Pgd1-11, Adh1-11/Pgd1-12 and Adh1-11/Adh1-22, respectively and so on for the rest of the genotypes

Genetic Variability

Genotypic Frequencies: Figure 13 gives a schematic representation of genotypes observed at each locus for each of the four polymorpic enzyme systems. A total of 11 genotypes were observed for the four polymorphic loci. Locus Adh1 had four, Pgd1 and Pgd2 each had three, and Prx1 had two genotypes. Genotypic frequencies for each locus are presented in Table 11.

Adh1: Overall frequencies of 0.888, 0.006, 0.103, and 0.004 were observed for genotypes Adh1-11, Adh1-12, Adh1-22, and Adh1-00 (null), respectively (Table 11). Genotype Adh1-11 was observed at very high frequencies in all populations studied. The lowest frequency (0.58) for Adh1-11 was exhibited by a population from Iraq. Populations from Cyprus, Greece, Mexico, Morocco, Portugal, Sudan, and Tunisia were all monomorphic for this genotype. Individuals with Adh1-12 genotypes were very rare among populations. The highest frequency (0.03) was obtained in populations from Ethiopia and India-2; the lowest frequency was 0.01 in the USSR population. As a whole, heterozygotes were very rare within populations and were observed in only 30% of the populations studied. The third genotype, Adh1-22 was found in relatively high frequencies (0.42, 0.32 and 0.37) in populations from Iraq, Afghanistan, and Ethiopia, respectively. This homozygous genotype was not present in populations from Cyprus, Greece, Mexico, Portugal, Sudan, and Tunisia; and the remaining populations had relatively low frequencies. The fourth genotype was a recessive null (no band). Only three populations, Ethiopia, India and Palestine, exhibited frequencies of 0.04, 0.03 and

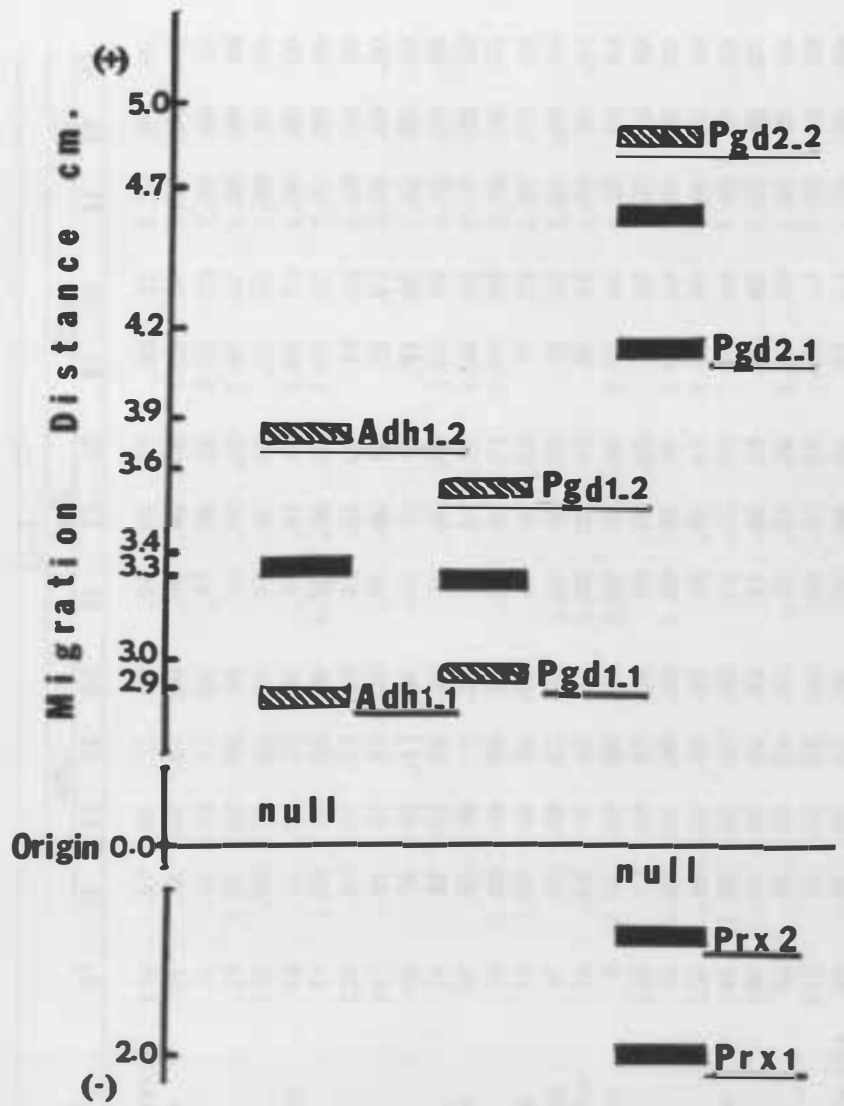

Fig. 13 A schematic representation of verified Adh1, Pgd1, Pgd2 and Prx1 allozymes observed in cultivated ch ckpeas

TABLE 11: Genotype frequencies at loci *Adh1*, *Pgd1*, *Prx1* and *Pgd2* in 27 populations of chickpea

Population	N+	Loci											
		Adh1				Pgd1			Prx1		Pgd2		
		11	12	22	00	11	12	22	11	00	11	12	22
Afghanistan	150	.67	.01	.32	.00	.94	.01	.05	.85	.15	1.00	.00	.00
Algeria	42	.86	.00	.14	.00	.00	.00	.00	1.00	.00	1.00	.00	.00
Chile	36	.83	.00	.17	.00	.92	.00	.08	1.00	.00	1.00	.00	.00
Cyprus	18	1.00	.00	.00	.00	.83	.00	.17	1.00	.00	1.00	.00	.00
Egypt	119	.98	.00	.02	.00	.93	.00	.07	.95	.05	1.00	.00	.00
Ethiopia	78	.66	.03	.27	.04	.59	.01	.40	1.00	.05	1.00	.00	.00
Greece	27	1.00	.00	.00	.00	1.00	.00	.00	1.00	.00	1.00	.00	.00
India	150	.83	.01	.13	.03	.93	.01	.06	.97	.03	1.00	.00	.00
Iran	147	.91	.02	.07	.00	.94	.00	.06	.98	.02	1.00	.00	.00
Iraq	66	.58	.00	.42	.00	.44	.05	.51	.91	.09	1.00	.00	.00
Jordan	94	.82	.02	.16	.00	.55	.00	.45	1.00	.00	1.00	.00	.00
Lebanon	60	.85	.00	.15	.00	.28	.02	.70	1.00	.00	1.00	.00	.00
Mexico	28	1.00	.00	.00	.00	.89	.00	.11	1.00	.00	1.00	.00	.00
Morocco	48	1.00	.00	.00	.00	.88	.00	.12	.75	.25	1.00	.00	.00
Pakistan	60	.98	.00	.02	.00	1.00	.00	.00	.78	.22	1.00	.00	.00
Palestine	93	.98	.00	.09	.03	1.00	.00	.00	.86	.14	1.00	.00	.00
Portugal	3	1.00	.00	.00	.00	1.00	.00	.00	1.00	.00	1.00	.00	.00
Spain	150	.93	.00	.97	.00	.98	.00	.02	.98	.02	1.00	.00	.00
Sudan	20	1.00	.00	.00	.00	1.00	.00	.00	1.00	.00	1.00	.00	.00
Syria	150	.80	.00	.20	.00	.38	.00	.62	1.00	.00	1.00	.00	.00
Tunisia	96	1.00	.00	.00	.00	.97	.00	.03	1.00	.00	1.00	.00	.00
Turkey	150	.94	.00	.06	.00	.95	.00	.05	.98	.02	1.00	.00	.00
USA	66	.94	.00	.06	.00	.80	.00	.20	1.00	.00	1.00	.00	.00
USSR	117	.94	.01	.05	.00	.89	.02	.09	..80	.20	1.00	.00	.00
Davis, Ca. USA	96	.89	.00	.11	.00	.94	.00	.06	.97	.03	1.00	.00	.00
India-2	361	.83	.03	.14	.00	.98	.01	.01	.87	.13	.97	.01	.02
Iran-2	341	.86	.02	.12	.00	.87	.01	.12	.97	.03	1.00	.00	.00
Overall	2766	.888	.006	.103	.004	.847	.005	.147	.949	.051	.99	.004	.003

+ = total number of plants assayed per population

0.03 respectively. Therefore, null alleles were very rare at this locus.

Pgd1: Overall frequencies of 0.847, 0.005 and 0.147 were observed for Pgd1-11, Pgd1-12 and Pgd1-22 genotypes (Table 11). Homozygote Pgd1-11 was the most frequent genotype observed. Algeria, Greece, Pakistan, Palestine, Portugal, and Sudan were fixed monomorphic for this genotype. Populations from Lebanon, Iraq, Jordan and Ethiopia had frequencies of 0.28, 0.44, 0.55 and 0.59, respectively. All remaining populations exhibited much higher frequencies of the Pgd1-11 genotype. The Pgd1-12 heterozygote was rare (0.01-0.02) in populations from Afghanistan, Ethiopia, India, India-2, and Iran-2, Lebanon, and USSR. The highest frequency of this genotype was 0.05 and was observed in the population from Iraq.

Pgd2: Only India-2 was polymorphic for this locus (Table 11). Frequencies of 0.97, 0.01 and 0.02 were observed for Pgd2-11, Pgd2-12 and Pgd2-22 genotypes, respectively, in the India-2 population. All other populations were monomorphic for the Pgd2-11 genotype.

Prx1: Locus Prx1 had two phenotypes a dominant banded type, desginated Prx1-11, and a recessive null (no band) (Table 11). Overall frequencies for Prx1-11 and null were 0.949 and 0.051, respectively. Thirteen populations were monomorphic for Prx1-11 and the remaining populations showed very high frequencies of the Prx1-11 phenotype. Heterozygotes could not be detected because banded types were dominant to the null types. Recessive null genotypes were observed at relatively high frequencies, 0.25 and 0.22, in populations from Morocco and

Pakistan, respectively. Populations from Afghanistan, Palestine and India-2 showed null genotypes with frequencies of 0.15, 0.14 and 0.13, respectively.

Allelic Variability and Distribution Patterns: Alleles and their migrational ranges for the four polymorpic loci are presented in Table 12. A schematic representation (Figure 13) exhibited three alleles (2.9 cm, 3.9 cm and null) at locus Adh1, two alleles (3.0 cm and 3.6 cm) at locus Pgd1, two alleles (2.0 cm and null) at locus Prx1, and two alleles (4.2 cm and 5.0 cm) at locus Pgd2. Allele frequencies at each of the four variable loci are presented in Table 13. A total of nine alleles were found at Adh1, Pgd1, Pgd2 and Prx1 loci in the 27 chickpea populations. Six codominant and two recessive null alleles were observed. The null alleles were exhibited only by Adh1 and Prx1 loci.

Table 13 shows that allele Adh1-1, at locus Adh1, was found in all populations studied. The other two alleles (Adh1-0 and Adh1-2) at locus Adh1 were rare. The overall frequency of allele Adh1-1 was 0.85, while the frequency of allele Adh1-2 was 0.10. Allele Adh1-0 was rare, with a frequency of 0.05. The null allele (Adh1-0) was observed in only three populations, Ethiopia, 0.55; India, 0.48; and Palestine, 0.48.

At locus Pgd1, allele Pgd1-1 was the most frequent, 0.85, overall populations. Populations Syria, Iraq, and Jordan (22%) were fixed for this allele.

Thirteen (48%) populations were fixed for allele Prx1-1 at locus Prx1. The overall frequency of the allele was 0.85. No population was fixed for the null allele.

Allele Pgd2-1 was fixed monomorphic in all populations, except India-2. In this population the frequency of allele Pgd2-2 was 0.02.

Table- 12: Alleles and their migrational ranges for the four polymorphic loci in Chickpea

Locus	Allele number	Isozyme position(cm)	Migration range (cm)
Adh1	0	0.0	null
	1	2.9	2.7-3.1
	2	3.9	3.7-4.1
Pgd1	1	3.0	2.8-3.2
	2	3.6	3.4-3.8
Pgd2	1	4.2	4.0-4.4
	2	5.0	4.8-5.2
Prx1	0	0.0	null
	1	2.0	1.8-2.2

Isozyme position measurements were taken on unfixed gels

TABLE - 13: Allelic frequencies at loci Adh1, Pgd1, Prx1 and Pgd2 in 27 populations of chickpea

Population	N+	Loci								
		Adh1			Pgd1		Prx1		Pgd2	
		1	2	0	1	2	1	0	1	2
Afghanistan	150	.68	.32	.00	.94	.06	.62	.38	.00	.00
Algeria	42	.86	.14	.00	1.00	.00	1.00	.00	1.00	.00
Chile	36	.83	.17	.00	.92	.08	1.00	.00	1.00	.00
Cyprus	18	1.00	.00	.00	.83	.17	1.00	.00	1.00	.00
Egypt	119	.98	.02	.00	.93	.97	.78	.22	1.00	.00
Ethiopia	78	.33	.12	.55	.61	.39	1.00	.00	1.00	.00
Greece	27	1.00	.00	.00	1.00	.00	1.00	.00	1.00	.00
India	150	.46	.06	.48	.93	.07	.83	.17	1.00	.00
Iran	147	.92	.08	.00	.94	.06	.86	.14	1.00	.00
Iraq	66	.58	.42	.00	.46	.54	.70	.30	1.00	.00
Jordan	94	.83	.17	.00	.55	.45	1.00	.00	1.00	.00
Lebanon	60	.89	.11	.00	.29	.71	1.00	.00	1.00	.00
Mexico	28	1.00	.00	.00	.89	.11	1.00	.00	1.00	.00
Morocco	48	1.00	.00	.00	.88	.12	.50	.50	1.00	.00
Pakistan	60	.98	.02	.00	1.00	.00	.53	.47	1.00	.00
Palestine	93	.52	.00	.48	1.00	.00	.63	.37	1.00	.00
Portugal	3	1.00	.00	.00	1.00	.00	1.00	.00	1.00	.00
Spain	150	.93	.07	.00	.98	.02	.86	.14	1.00	.00
Sudan	20	1.00	.00	.00	1.00	.00	1.00	.00	1.00	.00
Syria	150	.80	.20	.00	.38	.62	1.00	.00	1.00	.00
Tunisia	96	1.00	.00	.00	.97	.03	1.00	.00	1.00	.00
Turkey	150	.94	.06	.00	.95	.05	.86	.14	1.00	.00
USA	66	.94	.06	.00	.80	.20	1.00	.00	1.00	.00
USSR	117	.94	.06	.00	.90	.10	.55	.45	1.00	.00
Davis	96	.89	.11	.00	.94	.06	.83	.17	1.00	.00
India-2	361	.84	.16	.00	.98	.02	.64	.36	0.98	.02
Iran-2	341	.87	.13	.00	.88	.12	.82	.18	1.00	.00
Overall	2766	0.85	0.10	0.05	0.85	0.15	0.85	0.15	1.00	0.00

+ = total number of plants assayed per population

Among the 9 alleles observed at the four loci, the most frequent alleles, over all countries, were Adh1-1, Pgd1-1, Prx1-1 and Pgd2-1. The pattern of distribution of alleles indicated that populations from Middle Eastern countries had a high frequency of allele Pgd1-1. Asian countries had a high frequency of allele Prx1-1. The mean number of alleles per locus (Table 15) ranged from 1.0, in populations from Greece, Portugal, and Sudan, to 2.0, in India and India-2.

The identification of populations rich in alleles would be useful for conserving germplasm resources and for certain selection programs (17). It would facilitate selection for high levels of recombination and thus, the ability to generate new and more highly adapted genotypes. The information on the number of alleles also is important for choosing genetically contrasting populations, in order to study the physiological and ecological consequences of different degrees of genetic variability. Regions with large numbers of alleles are of interest because they indicate high genetic diverisity. Regions with unique or rare alleles also are of interest from the point of view of genetic conservation (17). Observed differences in allelic composition between close geographically situated regions suggests that genetic conservation strategies should stress sampling large numbers of populations from each agroecological zone.

Genetic Polymorphism: Table 14 shows which loci were polymorphic versus monomorphic in each of the 27 populations assayed. For example, populations from Greece, Portugal and Sudan were monomorphic over all loci assayed. All populations except India-2, were monomorphic for locus Pgd2. Hence, India-2 exhibited the maximum polymorphism, based on the four loci studied. Populations from Afghanistan, Egypt, India, Iran, Iraq, Spain, Turkey, USSR, Davis and Iran-2 were polymorphic for three loci. Single locus polymorphism (minimum polymorphism) was exhibited by populations from Algeria, Cyprus, Mexico and Tunisia; while the remaining populations were polymorphic for at least two loci. Over all loci, eighty nine per cent of the populations were polymorphic.

Polymorphism seemed to be highest in Asian and Middle Eastern countries. This is not surprising because these areas are considered centers of origin and diversity. Thus, nearly all countries which are considered to be in the centers of origin or diversity, such as India, Turkey, USSR, and Afghanistan, showed high percentages of loci polymorphic. Those centers which were monomorphic (Greece, Portugal, Sudan and Tunisia) are far from these centers. The introduction of a genotype to a new environment usually involves natural selection favoring the introduced genotype. Founder effects of this type might be one reason for low levels of polymorphism with in and among populations.

TABLE - 14: Summary of polymorphic(P) verses monomorphic(M) loci in 27 populations of chickpea

Population	N*	Locus				
		Adh1	Pgd1	Pgd2	Prx	Overall
Afghanistan	150	P	P	M	P	P
Algeria	42	P	M	M	M	P
Chile	36	P	P	M	M	P
Cyprus	18	M	P	M	M	P
Egypt	119	P	P	M	P	P
Ethiopia	78	P	P	M	M	P
Greece	27	M	M	M	M	M
India	150	P	P	M	P	P
Iran	147	P	P	M	P	P
Iraq	66	P	P	M	P	P
Jordan	94	P	P	M	M	P
Lebanon	60	P	P	M	M	P
Mexico	28	M	P	M	M	P
Morocco	48	M	P	M	P	P
Pakistan	60	P	M	M	P	P
Palestine	93	P	M	M	P	P
Portugal	3	M	M	M	M	M
Spain	150	P	P	M	P	P
Sudan	20	M	M	M	M	M
Syria	150	P	P	M	M	P
Tunisia	96	M	P	M	M	P
Turkey	150	P	P	M	P	P
USA	66	P	P	M	M	P
USSR	117	P	P	M	P	P
Davis	96	P	P	M	P	P
India-2	361	P	P	P	P	P
Iran-2	341	P	P	M	P	P
% of populations polymorphic		74	78	4	48	89

* number of plants sampled per population

Heterozygosity: Estimates of the proportion of heterozygotes obtained in this study are presented in Table 11. In general, proportions of heterozygotes were very low. The overall frequency of heterozygotes at the Adh1 locus was only 0.006. Populations from Ethiopia and India showed maximum heterozygote frequencies of 0.03 each. Iran and Iran-2 populations had heterozygote frequencies of 0.02; whereas Afghanistan, India, and USSR exhibited frequencies of 0.01.

The overall frequency of heterozygotes for Pgd1 was 0.005. The highest frequency (0.05) was obtained in the Iraq population. Populations from Lebanon and USSR had heterozygote frequencies of 0.02; Whereas heterozygote frequencies in populations from Ethiopia, India, India-2 and Iran-2 were 0.01 at this locus.

Population India-2 had a heterozygote frequency of 0.01 at locus Pgd2, and all other populations were devoid of heterozygotes at this locus. Ten populations (37%) had mean heterozygosity levels greater than zero. For example, mean heterozygosity levels over all loci were 0.012, 0.011 and 0.010, in populations from India-2, Iraq and Ethiopia, respectively (Table 15). USSR and Iran-2 populations each had a mean heterozygosity of 0.006, whereas Afghanistan and India populations showed 0.005 heterozygosity. To determine whether heterozygotes are in excess or deficient, the theoretical single-locus inbreeding coefficients (Fn) must be computed. This assumes that only the mating system affects the relationship between gene and genotypic frequencies. Fn values are then compared with fixation indices ($\hat{F}$), which are computed from observed genotypic frequencies (5). In order to compute

TABLE 15: Mean number of alleles per locus, percentage of loci polymorphic and mean heterozygosity in 27 populations of chickpea

Population	Sample size	Mean Number of Alleles per Locus(SE)	Percentage of Loci Polymorphic	Mean Hetrogyzosity (Direct count)
Afghanistan	150	1.8(0.3)	75	.005
Alegeria	42	1.3(0.3)	25	.000
Chile	36	1.5(0.3)	50	.000
Cyprus	18	1.3(0.3)	25	.000
Egypt	119	1.8(0.3)	50	.000
Ethiopia	79	1.8(0.5)	50	.010
Greece	27	1.0(0.0)	0	.000
India	150	2.0(0.4)	75	.005
Iran	147	1.8(0.3)	75	.003
Iraq	66	1.8(0.3)	75	.011
Jordan	94	1.5(0.3)	50	.005
Lebanon	60	1.5(0.3)	50	.004
Mexico	28	1.3(0.3)	25	.000
Morocco	48	1.5(0.3)	50	.000
Pakistan	60	1.5(0.3)	25	.000
Palestine	93	1.5(0.3)	50	.000
Portugal	3	1.0(0.0)	0	.000
Spain	150	1.8(0.3)	50	.000
Sudan	20	1.0(0.0)	0	.000
Syria	150	1.5(0.3)	50	.000
Tunisia	96	1.3(0.3)	0	.000
Turkey	150	1.8(0.3)	75	.000
USA	66	1.5(0.3)	50	.000
USSR	117	1.8(0.3)	75	.006
Davis	96	1.8(0.3)	75	.000
India-2	361	2.0(0.0)	50	.012
Iran-2	341	1.8(0.3)	75	.006

theoretical inbreeding coefficients, precise estimates of the proportion of selfing versus out crossing are required. In chickpea, such estimates are limited. However, Gowda (51) reported a 1.92% outcrossing rate based on morphological studies. Generally it is expected that chickpea is a highly self-pollintated crop; perhaps over 99 percent self-fertilized. Assuming 2% or less outcrossing rates, a range of theoretical inbreeding coefficients (Fn) and estimated fixation indices (F) for Adh1, Pgd1 and Pgd2 were calculated (Table 16). The overall fixation indicies for Adh1 (0.976) and Pgd1 (0.980) were similar to the theoretical inbreeding coefficient of 0.980 expected for 1% outcrossing. These results generally support the notion that chickpea populations are predominantly self fertilizing. More precise estimates of a mating system are needed before estimates of selection can be obtained. It is likely that the mating system and selection are important factors involved in the maintenance of variability in chickpea.

TABLE 16: Theoretical Inbreeding Coefficients (F_n) and Observed Fixation Indices ($\hat{F}$) at loci Adh1, Pgd1 and Pgd2 in 27 populations of chickpea

Population	Observed Fixation Indices ($\hat{F}$)			Theoretical Inbreeding Coefficients (F_n)
	Adh1	Pgd1	Pgd2	
Afghanistan	0.977	0.911	1.000	
Algeria	1.000	1.000	1.000	
Chile	1.000	1.000	1.000	
Cyprus	1.000	1.000	1.000	
Egypt	1.000	1.000	1.000	.01 = 0.980
Ethiopia	0.932	0.979	1.000	.02 = 0.961
Greece	1.000	1.000	1.000	.03 = 0.942
India	0.980	0.923	1.000	.04 = 0.923
Iran	0.864	1.000	1.000	.05 = 0.905
Iraq	1.000	0.889	1.000	.06 = 0.887
Jordan	0.929	1.000	1.000	.07 = 0.869
Lebanon	1.000	0.951	1.000	.08 = 0.852
Mexico	1.000	1.000	1.000	.09 - 0.835
Morocco	1.000	1.000	1.000	.10 = 0.820
Pakistan	1.000	1.000	1.000	.11 = 0.802
Palestine	1.000	1.000	1.000	.12 = 0.786
Portugal	1.000	1.000	1.000	.13 = 0.770
Spain	1.000	1.000	1.000	.14 = 0.754
Sudan	1.000	1.000	1.000	.15 = 0.739
Syria	1.000	1.000	1.000	.16 = 0.724
Tunisia	1.000	1,000	1.000	.17 = 0.709
Turkey	1.000	1.000	1.000	.18 = 0.695
USA	1.000	1.000	1.000	.19 = 0.681
USSR	0.911	0.889	1.000	.20 = 0.667
Davis	1.000	1.000	1.000	-
India-2	0.889	0.745	0.745	-
Iran-2	0.912	0.953	1.000	.95 = 0.026
Overall	0.976	0.980	0.276	

$$F_n = \frac{1 - \hat{t}}{1 + \hat{t}}$$

$$\hat{F} = 1 - \frac{Hij}{2p_i q_j}$$

where F_n = theoretical inbreeding coefficient

$\hat{F}$ = observed fixation indices, H = observed heterozygotes

2pq = Hardy-Weinberg heterozygotes, $\hat{t}$=outcrossing rate

Genetic Identity and Distance Analyses

Table 17 gives a matrix of four locus genetic identity (78) and genetic distance measures for the 27 populations studied. Mean Genetic Distance (D) values ranged from 0.034 (Iran-2) to 0.151 (Ethiopia). The highest absolute value of (0.296) was observed between Lebanon and Palestine populations. Genetic distance values were near zero for country pairs such as Greece and Portugal, Iran and Spain, Portugal and Sudan, and Spain and Turkey.

Overall, the results showed that D was not related to geographical distances. Thus, two populations which were geographically separated, like Greece and Sudan, were genetically similar. On the other hand, populations which were not separated geographically, like Palestine and Lebanon, were gentically different from each other. Kahler et. al. (67) in their studies of associations between isozyme phenotypes and environment, observed that isozyme variability in the slender wild oats was distributed in mosaic pattern and was not related to geographical distances. The same pattern also was observed by Bekele (17) in his study of genotypic composition and genetic distance between Ethiopian barley land races.

A dendrogram, Figure 14, based on the unweighted pair group method was applied to genetic distance and genetic similarity measures given in Table 17. The 27 populations were assigned to four major groups (clusters), based on their overall mean D value. Ethiopia, India and Palestine populations were clustered into one group. Lebanon, Iraq, Jordan and Syria were assigned to a second group; and Afghanistan,

TABLE 17: Matrix of four - locus genetic distance(below) and Identity (above) coefficients

Pop.	Afg	Alg	Chi	Cyp	Egy	Eth	Gre	Ind	Ira	Irq	Jor	Leb	Mex	Mor	Pale	Pal	Por	Spa	Sud	Syr	Tun	Tur	USA	USS	Dav	Ind.2	Ira-2
Afg		.954	.952	.930	968	.837	.939	.929	.968	.917	.898	.812	.935	.964	.972	.941	.939	.968	.939	.849	.938	.966	.934	.978	.976	.992	.976
Alg	.048		.996	.989	983	.875	.996	.938	.994	.873	.945	.861	992	922	.937	912	.996	.994	.996	.892	.995	993	.989	.941	.992	.966	.990
Chi	.050	.005		.993	.980	.899	.990	.937	.992	.911	.972	905	.993	921	.928	.904	.990	.989	.990	.932	.991	.990	.996	.939	.990	.960	.991
Cyp	.074	.013	.008		.986	.877	.995	.917	.991	.889	.972	.917	1.00	.932	.934	.890	.995	989	.995	.933	.997	.991	1.00	.944	.988	.953	.989
Egy	.033	.010	.021	.015		.843	988	.925	.998	.886	.938	.867	.988	977	.982	.927	.988	.997	.988	.888	.989	.998	.982	.985	.998	.989	.996
Eth	.180	.135	.108	.133	.173		.856	.953	.863	.881	.911	.874	.870	.776	.772	.895	.856	855	.856	.898	.860	.858	.889	.798	.862	.822	.872
Gre	.064	.005	.011	.007	.012	.157		.925	995	.853	.941	865	998	933	.945	905	1.00	995	1.00	.888	1.00	.995	.991	.947	.991	.963	.988
Ind	.075	.065	.067	.088	.078	.050	.079		.935	.854	.885	801	921	.883	.891	.985	.925	.935	.925	.836	.924	.933	.921	.902	.937	.927	.936
Ira	.033	.007	.010	.010	003	.149	.006	.068		.892	940	.874	.994	.960	.968	.926	.995	1.00	.995	.898	.995	1.00	.990	.973	1.00	.984	.999
Irq	.089	.138	.096	.120	.123	.130	.161	.160	.117		.953	.939	.877	.879	.854	.828	.853	.879	.853	.958	.860	885	.905	.890	.898	.890	.915
Jor	.108	.058	.030	.030	.065	.095	.061	.123	.054	.051		981	.962	.883	.868	.836	.941	939	.941	.992	.948	.945	.980	.896	.946	.902	.956
Leb	.210	.150	.101	.089	.144	.131	.146	.223	.136	.066	.021		.899	.821	.789	.745	.865	.859	.865	997	.875	.870	.926	.827	.870	.817	.887
Mex	.069	.008	.008	.001	.013	.140	.003	.083	.007	.134	.040	.108		.933	.939	.896	.998	.992	998	.918	.999	.994	.998	.946	.990	.958	.989
Mor	.038	.083	.084	.073	.024	.256	.071	.126	.042	.133	.126	.199	.070		.997	.920	.933	.960	933	.836	.933	.961	.927	1.00	.965	.984	.965
Pak	.030	.066	.076	.070	.019	.260	.058	.110	.034	.160	.142	.238	.064	.005		.933	.945	.970	.945	.811	.943	.969	.928	.998	.972	.992	.969
Pal	.062	.093	.103	.119	.077	.113	.101	.016	.078	.191	.181	.296	.111	.086	.071		905	.928	905	.719	.903	.925	.889	.931	.931	.944	.927
Por	.064	.005	.011	.007	.012	.157	0.00	.079	.006	.161	.061	.146	.003	.071	.058	.101		.995	1.00	.888	1.00	.995	.991	.947	.991	.963	.988
Spa	.033	.007	.012	.013	.003	.158	.005	.068	0.00	.131	.064	.152	.008	.042	.032	.076	.005		.995	.885	.995	1.00	.986	.972	1.00	.987	.997
Sud	.064	.005	.011	.007	.012	.157	0.00	.079	.006	.161	.061	.146	.003	.071	.058	.101	0.00	.005		.888	1.00	.995	.991	.947	.991	.963	.988
Syr	.165	.115	.072	.071	.120	.109	.119	.180	.108	.046	.009	.005	.087	.180	.210	.252	.119	.122	.119		.897	.894	.944	.847	.896	.847	.912
Tun	.065	.005	.010	.004	.012	.152	0.00	.079	.006	.152	.054	.139	.001	.070	.059	.103	0.00	.005	0.00	.109		.995	.993	.947	.992	.962	.989
Tur	.035	.008	.011	.010	.002	.155	.005	.070	0.00	.124	.058	.140	.007	.041	.032	.079	.005	0.00	.005	.113	.005		.989	.973	1.00	.984	.998
USA	.069	.012	.005	.001	.019	.119	.010	.084	.011	.102	.022	.078	.003	.078	.076	.119	.010	.014	.010	.059	.007	.012		.940	.987	.951	.989
USS	.023	.062	.064	.060	.016	.221	.055	.104	.029	.119	.111	.191	.057	.002	.003	.072	.055	029	.055	.167	.055	.028	.063		.977	.993	.977
Dav	.026	.008	.011	.014	.003	.150	.009	.066	0.00	.110	.057	.141	.011	.037	.029	.073	.009	001	.009	.111	.009	.001	.014	.024		.989	1.00
Ind2	.008	.035	.042	.050	.012	.197	.038	.077	.016	.119	.104	.203	.044	.018	.009	.059	.038	.015	.038	.167	.039	.017	.051	.008	.012		.987
Ira2	.025	.011	.009	.013	.004	.138	.013	.066	.002	.091	.045	.121	.011	.037	.033	.077	.013	.003	.013	.093	.012	.003	.012	.024	.001	.014	

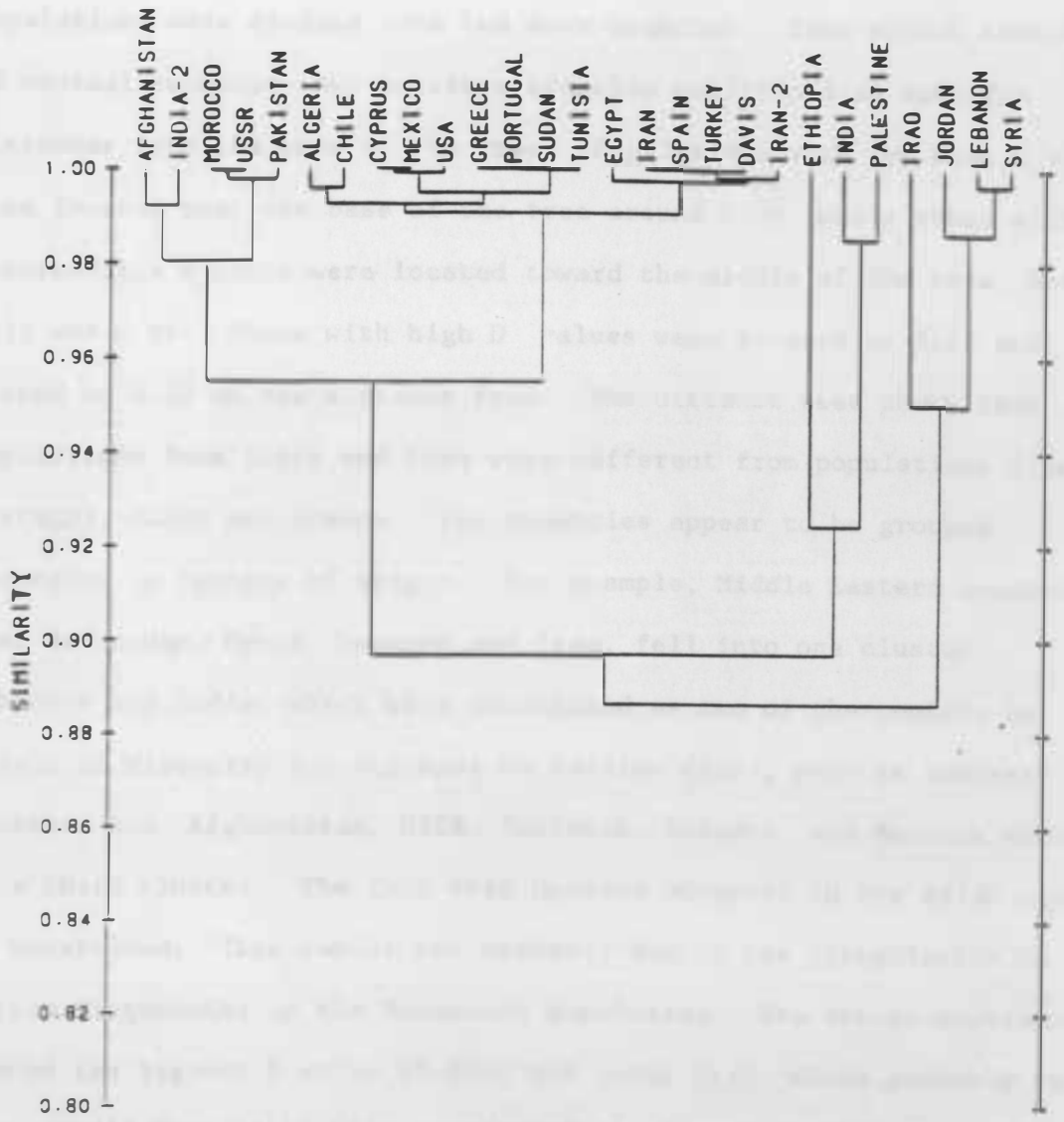

Figure 14: Dendrogram showing genetic similarity relationships among 27 populations based on allele frequency distributions of four enzyme loci (Adh1, Pgd1, Pgd2 and Prx1)

India-2, Morocco, USSR and Pakistan were assigned to a third group. All other populations were clustered into a fourth group. Figure 15 gives a dendrogram of the relative genetic distance between populations. The populations were divided into two main branches. Each branch consisted of several secondary and tertiary branches positioned at specific distances from the base of the tree. Populations with low mean D values were located near the base of the tree around 0.00, while those with intermediate D value were located toward the middle of the tree, between 0.13 and 0.19. Those with high D values were located at 0.19 and extend to 0.32 on the distance tree. The distance tree shows that populations from India and Iraq were different from populations from Portugal, Sudan and Greece. The countries appear to be grouped according to centers of origin. For example, Middle Eastern countries, such as Jordan, Syria, Lebanon and Iraq, fell into one cluster. Ethiopia and India, which were considered as two of the centers of origin or diversity for chickpea by Vavilov (112), were in another cluster; and Afghanistan, USSR, Pakistan, India-2, and Morocco occurred in a third cluster. The fact that Morocco occurred in the Asian cluster is unexpected. This result was probably due to the irregularity in allele frequencies in the Moroccoan population. The latter population showed the highest D value (0.230) for locus Prx1, which probably raised the overall D value of this population to a level similar to the Asian population.

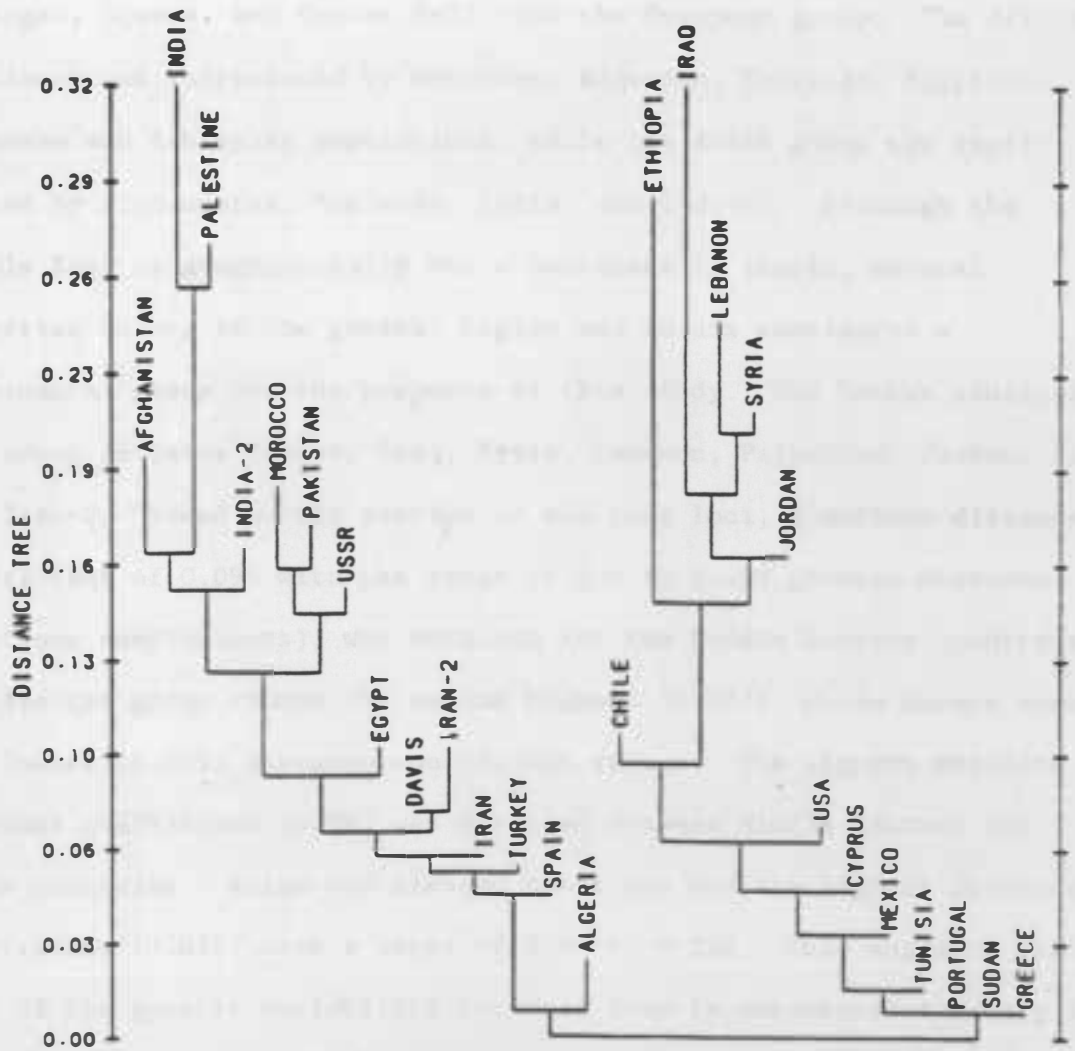

Figure 15: Dendrogram showing genetic distance relationships among the 27 populations based on allele frequency distributions of four enzyme loci (Adh1, Pgd1, Pgd2 and Prx1)

The populations from different countries seemed to fall into five major continental groups (Table 18). Four populations, Davis, USA, Mexico, and Chile represent the American group. Populations from Spain, Portugal, Greece, and Cyprus fall into the European group. The African continent was represented by Moroccan, Algerian, Tunisian, Egyptian, Sudanese and Ethiopian populations; while the Asian group was represented by Afghanistan, Pakistan, India, and India-2. Although the Middle East is geographically not a continent in itself, several countries belong to the general region and so are considered a continental group for the purposes of this study. The latter continental group includes Turkey, Iraq, Syria, Lebanon, Palestine, Jordan, Iran and Iran-2. Based on the average of all four loci, a maximum distance coefficient of 0.090 with the range of 0.0 to 0.295 genetic distance (distance coefficients), was obtained for the Middle Eastern countries. The African group showed the second highest (0.077), while Europe showed the lowest (0.005) distance coefficient values. The highest absolute distance coefficient (0.99) was observed between Middle Eastern and Asian countries. Asian and African countries had the highest distance coefficient (0.070) with a range of 0.00 to 0.258. This suggests that most of the genetic variability for this crop is concentrated mainly in African, Asian and Middle Eastern regions. Table 19 shows that genetic distance coefficients for American and European groups were relatively low (0.007) and for African, Asian and Middle Eastern countries were relatively high (0.083).

TABLE -18: Matrix of distance coefficients averaged by continent

Continent	No. of Individuals	No. of Populations	America	Europe	Africa	Asia	Middle East
America	226	4	0.007 (0.002-0.013)				
Europe	198	4	0.007 (0.000-0.014)	0.005 (0.000-0.011)			
Africa	403	6	0.038 (0.001-0.148)	0.039 (0.000-0.157)	0.077 (0.000-0.254)		
Asia	838	5	0.055 (0.011-0.083)	0.054 (0.015-0.087)	0.070 (0.0C -0.258)	0.044 (0.002-0.109)	
Middle/E	1101	8	0.056 (0.000-0.140)	0.069 (0.000-0.159)	0.086 (0.002-0.197)	0.099 (0.013-0.237)	0.090 (0.000-0.295)

Ranges of distance coefficients within a particular continent and/or continents are given in brackets.

TABLE 19: Matrix of distance coefficients averaged by hemisphere

Hemisphere	No. of Individuals	No. of Populations	American and European	African, Asian and Middle Eastern
America and Europe	424	8	0.007 (0.000-0.014)	
Africa Asia and Middle East	2342	19	0.053 (0.000-0.159)	0.083 (0.000-0.295)

The data appears to offer evidence for the presence of considerable genetic divergence between populations.

Field Trials

Results

Chickpea International F3 Yield Trial (CIF3YT): Means, ranges, and coefficients of variation for plant height, plant spread, plant stand, 100-seed weight, and number of seeds/pod are presented in Table 20. For all characters except plant stand, means were higher and ranges were greater at Brookings than at Highmore. Plant height means of 65 cm (range of 45-81 cm) and 49 cm (range of 37-57cm) were observed for Brookings, and Highmore, respectively. Mean plant spread was 42 cm at Brookings compared to 34 cm at Highmore. Similar plant stand was observed at both locations; however, the range (28-90%) was greater at Highmore. Means for seed size at Highmore and Brookings were 29 and 27g/100-seed weight, respectively. Mean number of seeds/pod recorded at Brookings was 1.1, with a range of 0.9-1.3 . Overall means of 1831 kg/ha, 57 cm, 38 cm and 28g were obtained for seed yield, plant height, plant spread and 100-seed weight respectively. Coefficients of variation were within generally acceptable limits, revealing 20, 17, 20 and 7 for yield, height, spread, and 100-seed weight, respectively.

Table 21 presents seed yield means for each entry in the F3 Yield Trial. Mean seed yields of 2441 and 1222 kg/ha were observed for Brookings and Highmore, respectively. The former also showed a range of 305 kg/ha (check) to 3263 kg/ha (x81TH 111), compared to a range of 716 kg/ha (check) to 1460 kg/ha (x81TH 112) at Highmore. The overall highest mean seed yield (averaged across locations) was 2281kg/ha for x81TH 111. This cross ranked 1st and 5th at Brookings and Highmore,

TABLE 20: Means, ranges, and coefficients of variation for plant height, plant spread, plant stand, 100-seed weight, and number of seeds/pod in F_3YT at Brooking and Highmore, South Dakota in 1983

	Brookings			Highmore		
Character	Mean	Range	C.V.	Mean	Range	C.V.
Plant height (cm)	65	45-81	20	49	37.55	11
Plant spread (cm)	42	30-50	21	34	27-40	18
Plant stand (%)	72	40-83	13	72	28-90	16
100-seed weight (g)	29	14-34	7	27	23-33	7
Number of seeds/pod	1.1	0.9-1.3	15			

TABLE - 21: Seed yield for 16 F_3 entries of chickpea (F_3YT) grown at Brookings and Highmore, South Dakota in 1983

Cross #	Pedigree	Brookings	Highmore	Overall
		kg/ha		
x 81TH 053	ILC 1920 x ILC 2506	2237	1277	1757
x 81TH 056	ILC 1920 x ILC 3279	2831	1332	2082
x 81TH 084	ILC 191 x ILC 262	2547	116	1857
x 81TH 085	ILC 191 x ILC 237	3164	1282	2223
x 81TH 101	ILC 72xx ILC 191	3058	1188	2123
x 81TH 104	ILC 72 x ILC 482	2647	1227	1937
x 81TH 111	ILC 191 x ILC 202	3263	1299	2281
x 81TH 112	ILC 191 x ILC 482	2708	1460	2084
x 81TH 112	ILC 191 x ILC 482	2708	1460	2084
x 81TH 120	ILC 191 x ILC 484	2087	1460	1774
x 81TH 113	ILC 200 x ILC 484	2631	1210	1921
x 81TH 125	ILC 202 x ILC 482	2642	1110	1876
x 81TH 126	ILC 202 x ILC 484	3075	1210	2143
x 81TH 146	ILC 72 x ILC 73	1893	960	1427
ILC-482		1576	1299	1438
Loc. check		305	716	511
LSD(0.05)		707	258	376

respectively. The least significant difference (LSD) revealed that 11 out of 14 F3 populations at Brookings exceeded the best check (ILC 482) by a significant margin. Yields at Highmore were low and there was no significant difference between F3 populations and the best check. However, significant differences were observed between entries and the local check.

Analysis of variance of each trait in the F3 segregating entries is presented in Table 22. There was a significant difference between locations for all characters studied . Entries were significantly different for all traits except plant spread. Location x cross interaction were significant for yield and 100-seed weight.

Table 23 presents the correlation matrix for characters studied in the F3 yield trial. In general, all characters except number of seeds/pod, exhibited positive significant correlations with yield. Seeds/pod exhibited nonsignificant negative associations with seed yield and 100-seed weight. Plant height gave the highest correlation (r=0.60) with seed yield. Highly significant positive correlations also were observed between plant spread and yield (r=0.49), and between 100-seed weight and yield (r=0.52).

Path analysis (Table 24) exhibited high coefficient values of 0.51, 0.37, and 0.54 for direct effects for seed yield vs plant height, plant spread and 100-seed weight, respectively. All indirect effects were low compared to direct effects.

TABLE 22: Analysis of variance of four agronomic traits in 16 F_3 populations grown at Highmore and Brookings, South Dakota in 1983.

Source	df	Mean squares			
		Yield	Plant height	Plant spread	100-seed weight
Location(L)	1	35610971**	6257**	1320**	61**
Cross(C)	15	1102109**	340**	45	65**
L x C	15	620395**	62	98	18**
Replications within L	4	1000187**	882**	43	23*

*,** significant at 0.05 and 0.01 levels, respectively

TABLE 23: Phenotypic correlations among four agronomic traits in 16 F_3 populations grown at Brookings and Highmore, South Dakota, in 1983

Traits	Plant height	Plant spread	100-seed weight	Number of seeds/pod
Yield	0.60**	0.49**	0.52**	-0.04
Height		0.24*	0.38**	0.09
Spread			0.27**	0.04
100-seed weight				-0.21

*,** significant at the 0.05 and 0.01 level, respectively

TABLE 24: Path coefficient analysis of influences of plant height, plant spread, 100-seed weight and number of seeds/pod upon seed yield in the Chickpea F_3 Yield Trial

Type of effect		Coefficients
Seed yield vs plant height	r= 0.60	
Direct effect		0.51
Indirect effect via plant spread		0.09
Seed yield vs plant spread	r= 0.49	
Direct effect		0.37
Indirect effect via plant height		0.13
Seed yield vs 100-seed weight	r= 0.53	
Direct effect		0.54
Indirect effect via number of seeds/ pod		0.02
Seed yield vs number of seeds/pod	r=-0.04	
Direct effect		0.08
Indirect effect via 100-seeds weight		-0.11

Chickpea International Large-seeded Trial (CILYT): Means for seed yield, plant height, plant spread, plant stand, and 100-seed weight for varieties in the large-seeded trial are presented in Table 25. Seed yield ranged from 1350 kg/ha for ILC 629 to 2661 kg/ha for ILC 134. Ten entries exhibited higher mean seed yields than the check. Plant height ranged from 32 cm for ILC 35 to 45 cm for ILC 112 and ILC 254. ILC 165 and ILC 464 had the largest plant spread of 46 cm each. The range in plant spread was from 36 to 46 cm, but no significant differences were found among varieties for plant spread. In general, plant stand in the large-seeded trial was poor. The highest stand of 61% was exhibited by ILC 451, while the lowest stand of 24% was observed for ILC 629. Seed size was largest in ILC 112, with a mean of 46g/100-seed weight. The lowest seed size of 20 g /100-seed weight was exhibited by the local check. Twelve varieties exhibited over 40 g/100 seed weight.

Table 26 presents mean squares for varieties, location means and coefficients of variation for seed yield, plant height, plant spread, plant stand, and 100-seed weight in the chickpea large seeded yield trial. All characters studied, except plant spread, exhibited significant differences among entries at both locations. The highest location mean seed yield of 2328 kg/ha was at Rapid City, whereas mean yield at Highmore was 1990 kg/ha. At Rapid City, means of 57% and 41g were obtained for plant stand and 100-seed weight, respectively. Means of 39% and 39g were obtained for stand and 100-seed weight, respectively, at Highmore. Compared to 34 and 36cm at Rapid City, higher means of 47 and 46cm at Highmore were exhibited for plant height and plant spread, respectively.

TABLE 25: Means for seed yield, plant height, plant spread, plant stand, and 100-seed weight of 19 entries of chickpeas grown at Rapid City and Highmore, South Dakota in 1983

Variety	Origin	Seed Yield kg/ha	Plant height cm	Plant spread cm	Plant stand %	100-seed weight g
ILC 35	Syria	1554	32	38	29	38
ILC 76	Spain	2552	44	45	49	41
ILC 83	Spain	2610	44	36	54	43
ILC 112	Spain	2060	45	44	49	46
ILC 132	Spain	2559	43	41	56	43
ILC 134	Spain	2661	41	38	54	43
ILC 135	Spain	2408	39	36	51	42
ILC 136	Spain	2211	44	39	56	41
ILC 165	Tunisia	1921	41	46	44	40
ILC 171	Tunisia	1775	39	38	42	40
ILC 254	Turkey	2381	45	43	54	42
ILC 451	Turkey	2339	35	36	61	36
ILC 464	Turkey	2407	43	46	48	43
ILC 496	Turkey	2070	40	43	43	42
ILC 613	Tunsia	2205	36	41	53	40
ILC 620	Morocco	2181	39	40	49	42
ILC 629	Tunisia	1350	41	44	24	41
ILC 2587	Turkey	1505	39	41	38	39
Loc. check	SD sel.	2194	41	37	58	20
LSD 0.05		928	7	14	29	3

TABLE 26: Variety mean squares, location mean and coefficients of variation of five agronomic traits in 19 large-seeded chickpea lines grown at Highmore and Rapid City, South Dakota in 1983

	Highmore				Rapid City				Overall	
Traits	df	Mean square	Mean	C.V.	df	Mean square	Mean	C.V.	Mean	C.V.
Yield(kg/ha)	18	34348**	1990	28	19	19390**	2328	18	2255	23
Plant height(cm)	18	61*	47	11	19	66**	34	13	41	12
Plant spread(cm)	18	125*	46	18	19	41	36	17	41	18
Plant stand(%)	18	612*	39	40	19	519**	57	19	48	28
100-seed weight(g)	18	110**	39	6	19	111**	41	6	40	6

*, ** significant at 0.05 and 0.01 levels, respectively

Coefficients of variation of 18, 13, 17, 19 and 6% were observed for yield, plant height, plant spread, plant stand, and 100-seed weight, respectively, at Rapid City. Coefficients of variation for plant stand (40%) and yield (28%) were relatively high at Highmore.

Overall means of 2255 kg/ha, 41 cm, 41cm, 48%, and 40g were exhibited for seed yield, plant height, plant spread, plant stand, and 100-seed weight, respectively. Overall coefficients of variation for stand, seed yield, plant height, plant spread and 100-seed weight were 28, 23, 12, 18 and 6%, respectively.

Table 27 presents analyses of variance for characters studied in the large-seeded trial. Significant differences were found between locations and among varieties for all characters studied. Location x Variety interactions were significant for all characters except plant height and spread.

Table 28 presents broad sense heritabilities(H) for yield, plant height, plant spread, and 100-seed weight. The highest heritability estimate (97%) was obtained for seed size (100-seed weight). Plant height ranked second with a heritability of 83%. Seed yield and plant spread were third and fourth, showing 80 and 67%, respectively.

Correlations between seed size and other traits studied are given in Table 29. A low (r=0.20), but highly significant positive correlation was observed between seed size and seed yield. All remaining characters, plant spread, plant height, and number of seeds/pod, exhibited nonsignificant negative associations with yield. A relatively high positive correlation (r=0.578) was observed between

TABLE 27: Analysis of variance of five agronomic traits in the large-seeded trial grown at Rapid City and Highmore, South Dakota in 1983

Source	df	Mean Squares				
		Yield	Plant height	Plant spread	Plant stand	100-seed weight
Location (L)	1	4171382**	5936**	4085**	13173**	89**
Variety (V)	18	1180759**	98*	93**	75**	218**
L x V	18	503201**	33	76	410**	8*
Replication within location	6	961402	52*	75	639**	61**

*, ** significant at 0.05 and 0.01 levels, respectively

TABLE - 28: Estimates of variety (V), variety x location(V x L) interaction, error (e) variances and broad sense heritability for four traits in the cickpea large-seeded trial

Traits	σ^2_V	$\sigma^2_{(V \times L)}$	σ^2_e	H %
Yield	1180759	503201	245593.0	80
Plant height	98	33	23.2	83
Plant spread	93	73	52.2	67
100-seed weight	218	8	5.4	97

TABLE 29: Phenotypic correlations of four agronomic traits in the large-seeded trial grown at Highmore and Rapid City, South Dakota in 1983

Traits	Plant spread	Plant height	Seed yield	No. of seed/pod
100-seed weight	-0.022	-0.030	0.200**	-0.076
Plant spread		0.578**	-0.183**	0.130
Plant height			-0.068	-0.173
Seed yield				-0.080

** significant at 0.01 level

plant height and plant spread. A negative association was exhibited between seed yield and plant height.

Path coefficient analysis of the direct and indirect influences of plant height, plant spread, number of seeds per pod and seed yield upon seed size (100-seed weight) in the large seeded chickpea trial is given in Table 30. Seed size had the largest direct effect on seed yield; followed by plant spread. The observed negative correlation of seed size with plant spread was mainly due to indirect effects through plant height, number of seeds per pod and seed yield.

TABLE 30: Path coefficients analysis of the direct and indirect influences of plant height, plant spread, 100-seed weight and seeds/pod upon seed yield in the Large-seeded Chickpea Trial

Type of effect		Coefficients
Seed yield vs plant height	r=-0.07	
Direct effect		0.052
Indirect effect via plant spread		-0.122
Seed yield vs plant spread	r=-0.18	
Direct effect		-0.21
Indirect effect via plant height		0.03
Seed yield vs 100-seed weight	r= 0.2	
Direct effect		0.20
Indirect effect via seeds/pod		0.01
Seed yield vs seeds/pods	r=-0.08	
Direct effect		-0.06
Indirect effect via 100-seed weight		-0.02

Chickpea Adaptation Trial (CAT): Means, ranges and coefficients of variation for agronomic traits are presented in Table 31.

Table 32 presents variety and location means and ranks at Brookings 1982, Highmore 1982, Rapid City 1983, and Highmore 1983. The highest location mean , 3214 kg/ha, was recorded for Highmore 1982, followed by 2588, 1174, and 899 kg/ha for Rapid City 1983, Highmore 1983, and Brookings 1982, respectively.Ranges of 220-2447 kg/ha, 1484-5192 kg/ha, 1822-3376 kg/ha and 615-1938 kg/ha for yield were observed at Brookings 1982, Highmore 1982, Rapid City 1983, and Highmore 1983, respectively.

Variety means for each location and across locations for each character studied are presented in Table 32. ILC 482 was the highest yielder at Brookings and Highmore in 1982 with mean seed yield of 2447 and 5191 kg/ha, respectively. This variety exhibited the third and the seventh high yields of 2901 and 1214 kg/ha at Rapid City and Highmore,respectively. ICC 5003, brown-seeded variety, was the second best yielder ranking second, first and first at Highmore 1982, Rapid City 1983, and Highmore 1983, respectively. ICC 5003 showed relatively low yield at Brookings 1982. Highest individual location means of 2939 kg/ha for seed yield, 41cm for plant height, 41cm for plant spread, 82% for plant stand, and 31g/100-seed weight for seed size were recorded for ILC 482, ILC 1934 ICC 5810, ICC 5003 and ILC 482, respectively. ILC 1934, exhibited 31g/100-seed weight which was similar to ILC 482 (Table 33). ILC 482 was the only variety that significantly exceeded the overall varietal mean yield of 1966 kg/ha. Variety ILC 3256 was the lowest yielder with an overall mean of 1202 kg/ha.

TABLE 31: Mean, ranges, and coefficients of variation for yield, plant height, plant spread, plant stand, and 100-seed weight in the chickpea adaptation trial at Brookings, Highmore, and Rapid City, South Dakota during 1982 and 1983

	Brookings, 1982			Highmore, 1982			Rapid City, 1983			Highmore, 1983		
Traits	Mean	Range	C.V.	Mean	Range	C.V.	Mean	Range	C.V.	Mean	Range	C.V.
Yield(kg/ha)	889	220-2447	31	3214	1484-5192	22	2588	1822-3376	16	1175	615-1938	14
Plant height(cm)	35	29-43	13	42	37-49	10	28	23-35	18	40	33-47	14
Plant spread(cm)	39	28-50	16	37	28-53	8	30	20-40	19	38	26-52	20
Plant stand(%)	70	41-89	12	76	40-90	13	69	55-80	11	51	18-84	28
100-seed wt.(g)	17	11-27	14	29	14-43	5	23	13-36	7	24	14-31	6

TABLE - 32: Means and ranks for yield for 16 entries in the Chickpea Adaptation Trial (CAT) grown at four locations, South Dakota, during 1982 and 1983.

Variety	Origin	Brookings 1982		Highmore 1982		Rapid City 1983		Highmore 1083		Varietal	Overall
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
ICC 4918	India	995	5	3914	5	2554	10	1475	3	2234	5
ICC 4948	India	1139	4	4095	4	2672	7	1185	8	2273	4
Icc 5003	India	743	9	4565	2	3376	1	1938	1	2657	2
ICC 5810	India	587	11	2713	9	1822	16	817	14	1485	13
ICC 10136	ICRISAT	778	8	3313	7	2489	11	1453	4	2008	7
ICC 11524	ICRISAT	1516	2	3491	6	2720	6	973	12	2175	6
ICC 11529	ICRISAT	992	6	4242	3	2803	4	1544	2	2395	3
ILC 482	Turkey	2447	1	5192	1	2901	3	1214	7	2939	1
ILC 519	Egypt	915	7	2861	8	2946	2	916	13	1910	8
ILC 1919	India	1413	3	2596	11	1970	15	1253	5	1808	9
ILC 1922	Morocco	310	15	2647	10	2259	14	1174	10	1598	12
ILC 1929	Syria	220	16	2396	13	2578	9	615	16	1452	14
ILC 1931	Turkey	391	12	-	-	2270	13	1166	11	1276	15
ILC 1932	Jordan	356	13	2405	12	2725	5	1178	9	1666	11
ILC 1934	Iran	592	10	2292	14	2625	8	1249	5	1670	10
ILC 3256	Cyprus	327	14	1484	15	2374	12	620	15	1202	16
Loc. Mean		899		3214		2588		1174		1966	
Rank		4		1		2		3			

TABLE 33: Means for seed yield, plant height, plant spread, plant stand, and 100-seed weight for 16 entries in the Chickpea Adaptation Trial grown at four locations, South Dakota, during 1982 and 1983

Variety	Origin	N	Yield kg/ha	Plant Height (cm)	Plant Spread (cm)	Plant Stand %	100-seed
ICC 4918	India	16	2234	36	28	74	23
ICC 4948	India	16	2273	33	34	74	14
ICC 5003	India	16	2657	36	32	82	23
ICC 5810	India	16	1485	39	41	73	14
ICC 10136	ICRISAT	16	2008	34	30	80	13
ICC 11524	ICRISAT	16	2175	32	33	75	14
ICC 482	Turkey	16	2939	39	40	54	31
ILC 519	Egypt	16	1910	39	37	66	20
ILC 1919	India	16	1808	38	38	59	23
ILC 1922	Morocco	16	1598	37	38	51	29
ILC 1932	Jordan	16	1666	37	40	59	26
ILC 1934	Iran	16	1670	41	37	71	31
ILC 3256	Cyprus	16	1202	35	39	53	30
LSD 0.05			737	7	10	20	6

Analysis of variance for each trait at each location for the varieties included in the Chickpea Adaptation Trial is presented in Table 34. There were significant differences among varieties for seed yield, plant height, plant spread, plant stand, and 100 seed weight at all locations. Analysis of variance (Table 35) revealed significant differences among locations, and varieties, and significant location x variety interactions for all traits studied.

Estimates of heritability and genetic advance expressed in percent of the mean obtained from the combined analysis of data for locations and years are presented in Table 36. In general, heritabilities for all characters were relatively high. Seeds/pod and 100-seed weight exhibited heritabilities of 98% each. Seed yield had a heritability of 94%, while plant height and plant spread exhibited 87% and 88% heritabilities, respectively.

Table 37 presents a correlation matrix between characters studied in the Chickpea Adaptation Trial. A highly significant correlation coefficient (r=0.31) was observed between plant yield and 100-seed weight, while a significant negative correlation (r=0.18) was obtained between yield and plant spread. Positive association was observed between plant height and yield, while a highly significant correlation was exhibited between spread and height. There also was a highly significant positive correlation (r=0.30), between plant height and 100-seed weight. A highly significant negative correlation, (r=0.57) was recorded between 100-seed weight and number of seeds/pod.

TABLE - 34: Variety mean squares for five agronomic traits in CAT grown at four different locations, South Dakota, during 1982 and 1983.

Traits	Brookings(1982)		Highmore(1982)		Rapid City(1983)		Highmore(1983)	
	df	MS	df	MS	df	MS	df	MS
Yield	15	43358**	14	]33468**	15	18750**	15	15213**
Plant Height	15	80**	14	36*	15	65**	15	54*
Plant Spread	15	154**	14	64**	15	120**	15	227**
Plant Stand	15	1328**	14	626**	15	238**	15	1398**
100-seed wt.	15	71**	14	478**	15	193**	15	176**

*, ** significant at 0.05 and 0.01 levels respectively

TABEL 35: Analysis of variance over all locations of five agronomic traits in 16 entries of CAT, grown in South Dakota during 1982 and 1983

Source	df	MS				
		Yield (kg/ha)	Plant Height (cm)	Plant Spread (cm)	Plant Stand %	100-seed wt.(g)
Location	3	2422451**	2272**	1296**	7396	1605**
Variety	14	119754**	93**	227**	1948	718**
Loc. x var.	42	31175**	49**	115**	602	69**
Rep. within Loc.	12	9082	29	81**	291	5

*, ** significant at 0.05 and 0.01 levels respectively

TABLE 36: Estimates of genotypic, genotype x location, error variances, broad senses heritability, means and expected genetic advances under 5 and 10% selection intensities for various traits

Trait	σ^2_G	$\sigma^2_{(G \times loc)}$	σ^2_e	H	$\bar{x}$	Expected Genetic Advance 5%	Expected Genetic Advance 10%
Yield	3688728.70	9602555.26	225827.34	94	1966.0	47	40
Plant Height	92.53	49.32	23.37	87	36.4	24	20
Plant spread	226.96	115.06	34.64	88	35.8	30	25
100-seeds weight	718.04	68.94	3.15	98	23.0	31	26
Seeds/pod	0.18238	0.004	0.0307	98	1.3	16	13

Heritability (H) = $\frac{\sigma^2_V}{\sigma^2_P}$ = $\frac{\text{Genotypic variance}}{\text{Phenotypic variance}}$

where, $\sigma^2_P = \sigma^2_V + \frac{\sigma^2_{VL}}{L} + \frac{\sigma^2_e}{RVL}$

σ^2_V = variance due to the genotype

σ^2_{GL} = variance due to genotype and location

σ^2_e = total error vairance

L =location, R =replication V = genotype

Genetic advance(GS) =(k) (σP)(H)

Gs = expected genetic advance

σ_P = phenotypic standard deviation

k = selection differential which varied with selection intensity values of k used 2.06(5%) & 1.76(10%)

TABLE - 37: Phenotypic correlations for five agronomic traits in CAT grown at Brookings, Highmore, Rapid City, South Dakota during 1982 and 1983

Traits	Plant Height	Plant Spread	100-seeds weight	No. of seeds/ pod
Plant yield	0.02	-0.18*	0.31 **	-0.10
Plant height		0.43**	0.30 **	-0.03
Plant spread			0.06	-0.16
100-seeds wt.				-0.57**

*,** significant at 0.05 and 0.01 levels, respectively

Path analysis indicated that 100-seed weight had a strong direct effect while number of seeds per pod showed a weak direct effect on yield (Table 38). Seed weight also contributed to seed yield through indirect effects of plant height and plant spread. Plant height and plant spread also have exhibited contribution to yield through indirect effects of seeds per pod.

Stability parameters for seed yield and 100 seed weight are given in Table 39. Regression cofficients for seed yield ranged from 0.64 to 1.38 and 0.56 to 1.36 for seed size.Varieties ILC 482, ICC 5003, ICC 10136, ICC 11524, and ILC 519 showed regression cofficients of 1.39, 1.38, 1.02, 1.07 and 0.98, respectively. Variety ILC 3256 exhibited the lowest mean seed yield (1202 kg/ha) and a regression coefficient of 0.64.

TABLE 38: Path coefficient analysis of the direct and indirect influecnes of plant height, plant spread, 100-seed weight and number of seeds/pod upon seed yield in the Chickpea Adaptation Trial (CAT)

Type of effects		Coefficients
Seed yield vs plant height	r= 0.02	
Direct effect		0.12
Indirect effect via plant spread		-0.10
Seed yield vs plant spread	r=-0.018	
Direct effect		-0.23
Indirect effect via plant height		0.05
Seed yield vs 100-seed weight	r= 0.31	
Direct effect		0.38
Indirect effect via seeds/pod		-0.07
Seed yield vs number of seeds/pod	r=-0.10	
Direct effect		0.11
Indirect effect via 100-seed weight		-0.21

TABLE 39: Means ($\bar{x}$), regression coefficients (b_1), variances accounted for by regression (R^2), and deviation mean squares (s^2) for yield and 100-seed weight in 15 entries of CAT evaluated at four location

Variety	Origin	Seed Color	Stability Parameters							
			Yield				100-seed weight			
			$\bar{x}$	b_i	R^2	S^2di	$\bar{x}$	bi	R^2	S^2di
ICC 4918	India	Brown	2234	1.13	0.853	87	23	1.01	0.985	958
ICC 4948	India	Brown	2273	1.14	0.822	69	14	0.61	0.995	2787
ICC 5003	India	Brown	2657	1.38	0.879	109	23	1.01	0.957	331
ICC 5810	India	Black	1485	0.76	0.806	62	14	0.60	0.990	1449
ICC 10136	ICRISAT	Brown	2008	1.02	0.855	88	13	0.56	0.990	1551
ICC 11524	ICRISAT	Brown	2175	1.07	0.820	68	14	0.61	0.982	825
ICC 11529	ICRISAT	Brown	2395	1.22	0.858	90	23	0.99	0.992	1938
ILC 482	Turkey	Cream	2939	1.39	0.764	48	31	1.34	0.977	641
ILC 519	Egypt	Cream	1910	0.98	0.868	98	20	0.86	0.988	1210
ILC 1919	India	Cream	1808	0.87	0.756	46	23	0.97	0.981	769
ILC 1922	Moroco	Cream	1598	0.85	0.866	97	29	1.28	0.973	530
ILC 1929	Syria	Cream	1542	0.79	0.819	68	30	1.34	0.947	270
ILC 1932	Jordan	Cream	1666	0.89	0.905	142	26	1.14	0.984	948
ILC 1934	Iran	Cream	1670	0.88	0.909	149	31	1.36	0.978	678
ILC 3256	Cyprus	Cream	1202	0.64	0.843	80	30	1.33	0.978	6655

Discussion

Cicer arietinum, a crop of considerable antiquity, is native to South-west Asia and the Mediterranean regions, while Ethiopia is considered a secondary centre of diversity (112). van der Maesen (111) has conluded that high yields of chickpea realized in Central Asia, Iran, Afghanistan, Turkey, and the Caucasian region of Russia would indicate the spring and summer periods around the Mediterranean and the winter period in South Asia. Although contrasting in several meterological variables, these areas are suitable for high productivity of chickpea. An optimum combination of such variables is yet to be worked out in this crop. The contrasting climatological and cultural factors of chickpea cultivation in India compared to Iran and the Mediterranean regions, successful summer cultivation in Iran versus traditional winter cultivation in India, and the dominant role of natural selection show that existing cultivars are adapted to specific environmental conditions, and therefore will have restricted adaptation.

The phenotypic variability observed in chickpea germplasm for several morphological characters, moderate estimates of broad sense heritability for characters such as seed size, seed yield, seeds per pod, plant height, and lack of any relationship between maturity and yields emphasize the role played by natural selection in shaping the diversity found in present day cultivated types.

This study was designed to evaluate a large number of germplasm collections and segregating populations with the hope of identifying genotypes adapted to South Dakota environments. Three

independent trials F3YT (F3 Yield Trial), LYT (Large-seeded Yield Trial) and CAT (Chickpea Adaptation Trial) indicate the adaptiveness of chickpea to South Dakota environments. The F3 populations were derived from crosses involving parents possessing resistance to ascochyta blight, high yield, and wide adaptation. The large-seeded trial entries were derived from germplasm collections maintained at ICARDA and have shown superior performance either in regional or international trials conducted by ICARDA. The Chickpea Adaptation Trial included land races widely grown in different countries of the Middle East. It also consisted of other entries developed through hybridization and pure line selection in India and Egypt (54).

The observed high means for seed yield, plant height, and plant spread in the F3 populations, especially at Brookings, were attributed to genetic heterogeneity. The F3 populations have Ascochyta resistance from their tall Russian parents and are less affected by high humidity and moisture than Ascochyta susceptable lines, such as the local check. As a result, seed yield and vegetative growth were highest for the F3 entries at Brookings in response to abundant moisture (Table 5). The data showed that eleven F3 populations were significantly better yielders than the best check entry, ILC 482. Under drier conditions at Highmore, no significant differences were observed between ILC 482 and F3 populations. Since most of the resistant parents of these populations were tall and relatively late in maturity their progenies also have exhibited tallness and late maturity. To be productive in South Dakota, it is essential that these populations be

planted as early as possible in the Spring so they will mature before high moisture coupled with cool temperature extend the maturity by encouraging indeterminant growth which results in lower yields, nonuniform seed size, and poor seed quality. Similarly, both LYT and CAT trials demonstrated wide variability among traits studied; except that LYT and CAT exhibited higher means and variation under dry conditions (Highmore and Rapid City). Means of 2328 kg/ha and 41g at Rapid City, compared to 999 kg/ha and 29g at Highmore for seed yield and 100-seed weight, respectively, were resulted in the LYT trial. Moreover, the LYT trial has exhibited wide ranges and moderate coefficients of variation for all characters studied. Seed size ranged from 20g/100-seed weight in the check to 46g/100-seed weight in ILC 112. Although, 65.5g/100-seed weight has been reported Singh & Tuwafe, (97), the observed 42 g/100-seed weight with mean yield of 2060 kg/ha by ILC 112 should categorize this entry into the large-seeded, group with a reasonably high yield. Seed size of chickpeas, in addition to its importance as a seed yield component, influences the commercial value of the crop (84). In addition Pinthus (84) reported that the yield obtained following planting of larger seeds (within the same variety) graded markedly and significantly higher than that obtained from smaller seeds. Likewise, the average yield of large-seeded types consistently and conspicuously exceeded the yield of small-seeded types.

The only difference between the F3YT and the other two trials (LYT) and (CAT), was that the LYT and CAT trials did not have disease resistance genes in their genetic background. Therefore, means from

these two trials (LYT and CAT) for yield and other characters were very low at Brookings and very high at Highmore and Rapid City. The later two locations are more favorable environments for growing chickpea due to their drier and warmer conditions. However, a significant amount of variation for all traits studied was obtained in all locations.

In general, the results provide ample evidence for the presence of extensive genetic variability among and within the germplasm collections studied. The South Dakota results are in agreement with results for 25 morphological characters in 3400 kabuli accessions of chickpea reported by Singh and Tuwafe (98). In general, heritability estimates were high for all traits. This is not surprising because in most cases varieties responded similarly at Highmore and Rapid City, reducing genotype x environment interactions. Singh and Auckland (95) reported similar broad sense heritabilities for plant height and seed size, and Pandey and Tiwari (82) reported narrow sense heritabilities for plant height, plant spread, 100-seed weight, and yield, which are in line with findings of this study. This indicates that breeding for plant height and seed size is possible due to their high heritability.

Highly significant positive correlations of yield with plant height and plant spread were found in F3YT. Whereas in LYT and CAT negative associations of yield with plant height and spread were observed. The positive correlations in F3 populations are expected since the parental lines were originally chosen to consist of tall genotypes with the hope of obtaining tall segregants suitable for mechanized harvest. Moreover, F3 is an early generation and it is not

surprising that it behaved differently from homozygous varieties in the LYT and CAT trials. The significant positive correlations of yield with plant height, plant spread, and 100-seed weight, plus the direct contribution of seed size to yield, in early generations is very encouraging. Populations could be advanced in a particular desired direction without appreciable loss in expression of other desired characters.

The positive relationship of seed size with yield in all three trials suggests that emphasis should be made in regard to seed size while selecting for seed yield. Gawda and Pandya (53) reported a negative correlation between grain yield and plant height, and a positive correlation between grain yield and 100-seed weight. This is in agreement with the present findings in LYT. The exhibited significant positive correlation between seed yield and 100-seed weight is useful because it may be possible to select genotypes with high yield and large seed.

Path coefficient analysis, which facilitates separation of correlation coefficients into direct and indirect effects, gave a better picture of relationships between pairs of traits. Studies on direct and indirect effects (Table 24) revealed that the direct effect of plant height on seed yield was positive and highest thus was true for plant spread and 100-seed weight. This indicated that plant height and plant spread do influence seed yield, while 100-seed weight is an important component of seed yield. The observed negative correlation of yield with seeds/pod was caused by a large negative indirect effect via 100

seed- weight, otherwise the direct effect of seeds/pod was low but positive. Path analysis confirmed that seed size was a major contributor to seed yield in both LYT and CAT trials. Positive direct effects for 100-seed weight and seeds/pod in chickpea also were reported by Gowda and Pandya (53) and Jatasra et. al. (61) Path analysis further confirmed that emphasis should be given to seed size while selecting for seed yeild.

Stability parameter analyses showed that regression coefficients for yield ranged from 0.64 to 1.38. This indicated that the genotypes have very different environmental responses. Varieties ICC 10136 and ICC 11524 with mean yields of 2008 kg/ha and 2175 kg/ha, respectively, showed regression coefficients of 1.02 and 1.07. These yields and regression coefficients nearly equal to 1.0 indicated general adaptability for these two varieties. ILC 519 showed a regression coefficient of 0.98, (approximately 1.0) and low yield; this indicated poor adaptability to all environments. Varieties ILC 482 and ICC 5003 with mean yields of 2939 kg/ha and 2656 kg/ha exhibited regression coefficients of 1.39 and 1.38, respectively. This indicated high adaptability to high yielding environments (favorable environments). The top yielding variety, ILC 482, due to its relative resistance to diseases, exhibited high yield even in the unfavorable environment (Brookings). Much higher yields were obtained at Highmore in 1982, and Rapid City in 1983, which confirmed its superiority under favorable rather than unfavorable environments. The yield reduction by all entries at Highmore in 1983 was attributed to a weed problem rather than

to general environmental conditions. On the other hand, variety ICC 5003 which was the top yielder at Highmore and Rapid City during 1983, and second best yielder at Highmore during 1982 was the 9th yielder at Brookings during the 1982 crop seasons. These results confirmed that this variety was much better adapted to favorable environments.

Stability parameter analyses indicated that regression coefficients for 100-seed weight ranged from 0.56 to 1.36 which showed that varieties had different environmental responses for seed size (Table 39). Varieties ICC 4918, ICC 5003, ICC 11529 and ILC 1919 exhibited regression coefficients of 1.01, 1.01, 0.99 and 0.97 respectively with means of 23g/100-seed weight each. These values indicated that the environmental effect on seed size was similar for these varieties. Since these varieties are small-seeded types, seed size is not expected to deviate much from 23g/100-seeds. On the other hand, varieties ILC 1922, ILC 1929, ILC 1934, ILC 3256 and ILC 482, (large-seeded types) which showed mean seed sizes of 29, 30, 31, 30 and 31g/100-seed weight, respectively, exhibited regression coefficients ranging from 1.28 to 1.36. This indicated that these varieties might produce larger seed in favorable environments.

Varieties ILC 482 and ICC 5003 exhibited high means and steep regression lines for yield indicating positive response to favorable environments. ICC 5003, especially, expressed its sensitivity to change of environment by showing above average yields under favorable, and below average yields under unfavorable environments. Figure 16 provides a generalized interpretation where each variety is represented by a

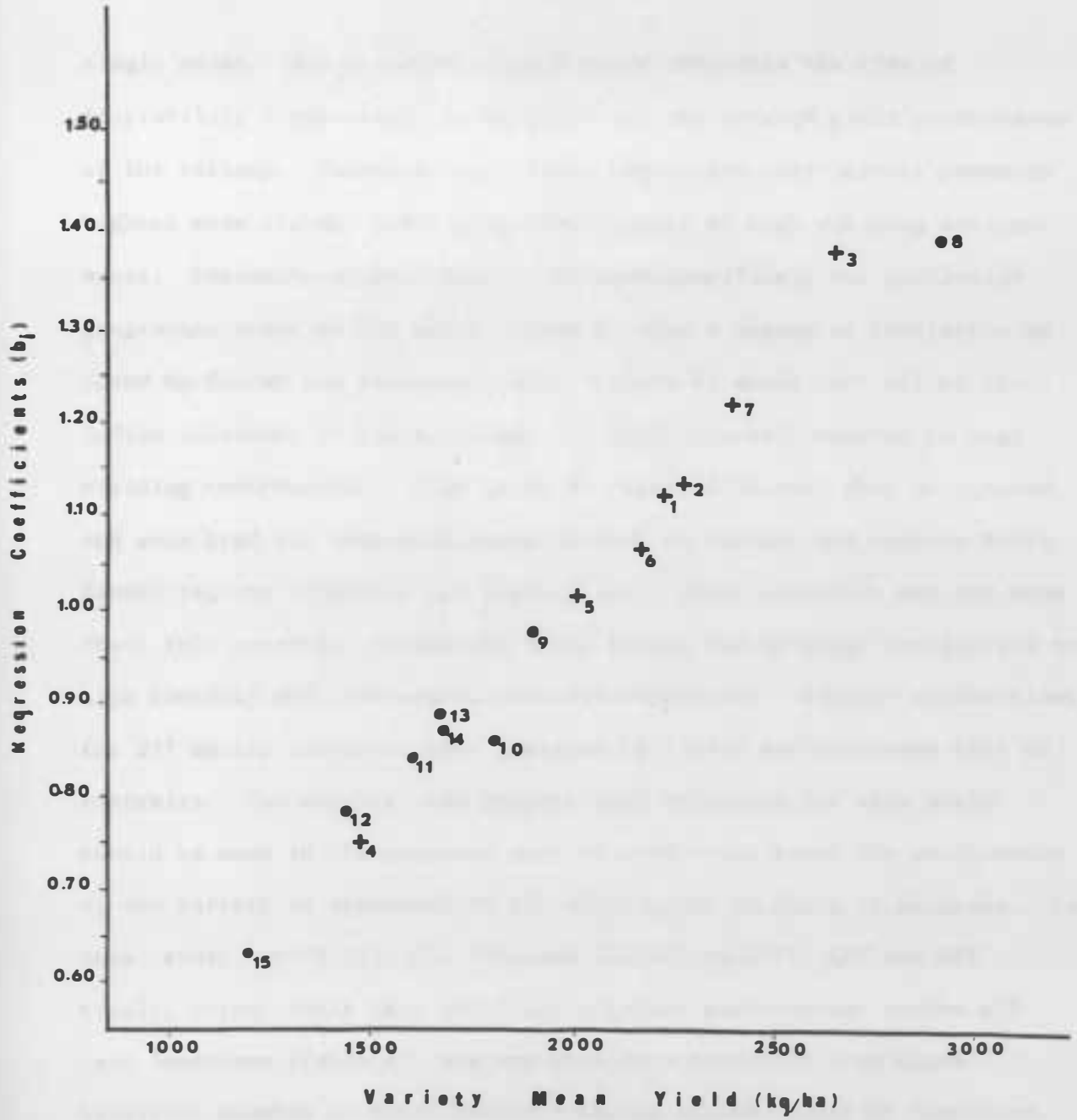

Figure 16: The relationship of variety adaptation (regression coefficient) and variety mean yield for 15 chickpea varieties (1=ICC 4918, 2=ICC 4948, 3=ICC 5003, 4=ICC 5810, 5=ICC 10136, 6=ICC 11524, 7=ICC 11529, 8=ILC 482, 9=ILC 519, 10=ILC 1919, 11=ILC 1922, 12=ILC 1929, 13=ILC 1932, 14=ILC 1934, 15=ILC 3256

single point. The position of each point indicates the type of adaptability (regression coefficient) and the average yield performance of the variety. Varieties with large regression coefficients produced highest mean yields, indicating adaptability to high yielding environments. Varieties originating in, or bred specificaly for particular geographic areas of the world tended to have a degree of similarity as found by Finlay and Wilkinson (42). Figure 16 shows that all of the Indian cultivars (+ signs) except ICC 5810, are well adapted to high yielding environments. This is to be expected because they originated and were bred for semi-arid areas similar to central and western South Dakota regions (Highmore and Rapid City). These varieties may not show their full potential in eastern South Dakota due to their sensitivity to high humidity and late-season moisture conditions. Similar observations for 277 barley varieties were obtained by Finlay and Wilkinson (42) in Australia. The results also suggest that selection for seed yield should be made in the proposed area of production since the performance of any variety is dependent on the environment in which it is grown. In this study, x81TH 111, ILC 134, and ILC 482 in F3YT, LYT and CAT trials, respectively have exhibited superior performances across all test locations (Table 40) and may provide a reservoir from which cultivars adapted to South Dakota cropping systems could be developed.

TABLE 40: Summary of data from F_3YT, LYT, and CAT trials

	F_3YT	LYT	CAT
Mean (kg/ha)	1831	2255	1966
Range (kg/ha)	305-3263	1350-2661	221-5192
C.V. (%)	20	23	21
Number of testing sites	2	2	4
Number of test entries	16	19	16
Top yielding entry	x81TH 111	ILC 134	ILC 482

CONCLUSION

In this study a large number of germplasm accessions and breeding lines of chickpeas were evaluated electrophoretically and in field trials from 1982 to 1984. Electrophoretic assays showed monomorphism for acid phosphatase (ACP), esterase (EST) and malate dehydrogenase (MDH) and polymorphism for alcohol dehydrogenase (ADH), 6-phosphogluconate dehydrogenase (PGD) and peroxidase (PRX) enzyme systems among the chickpea collections assayed.

Inheritance studies of the polymorphic enzymes showed simple Mendelian segregation for 4 enzyme loci including Adh1, Pgd1, Pgd2, and Prx1. A total of 12 genotypes were observed among the four loci including Adh1 (4 genotypes), Pgd1 (3 genotypes), Pgd2 (3genotypes) and Prx1 (2 genotypes).

Estimation of fixation indicies and theoretical inbreeding coefficients supported the notation that chickpea is a highly self-pollinated crop with less than 1% of outcrossing. This result suggests that the mating system and selection are important factors maintaining genetic variability in chickpea.

Genotypic and allelic frequencies demonstrated the presence of appreciable genetic variation in chickpeas. Most of the genetic variability was observed in Middle Eastern (Palestine, Iraq, Lebanon, Syria, Jordan), Asian (Indian, Afghanistan, Pakistan, USSR) and East African (Ethiopia) countries.

The observation of large amounts of genetic variability within closely situated regions suggests that genetic conservation strategies

should stress collection of large numbers of populations in each agroecological zone.

Field trials demonstrated significant differences within and among quantitative traits. High means and variations for charaters studied indicated that there is high genetic variability in the materials tested. The exhibited high heritability values in LYT and CAT for seed yield demonstrated low genotype x environment interaction. This indicated that it is possible to breed suitable varieties for centeral and western South Dakota. Lines such as ILC 482, ICC 5003 and ICC 11529 in CAT; ILC 83 ILC 112, ILC 132 and ILC 134 in LYT; and X81TH 056, X81TH 085, and X81TH 111 in F3YT, that showed good performances in the tests, should be given more consideration for further exploitation. For example, varieties such as ILC 482 must be increased and evaluated in multiple locations within the region.

Correlation and path coefficient estimates showed that seed size is an important character to consider when selecting for increased seed yield. This is confirmed by high significant positive correlations between seed yield and seed size observed in all the three trials. This suggests improvement of seed yield and seed quality both at the same time is possible.

Regression studies indicated that chickpea varieties respond to environmental variation, and selection for yield and seed size should be carried out in favorable environments.

As a whole, the field study has shown that chickpea has potential in South Dakota agriculture. However, like any other major

crop chickpea also does require proper breeding methodologies: introduction, screening, hybridization, selection, evaluation including cultural practice studies to achieve the expected goal.

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