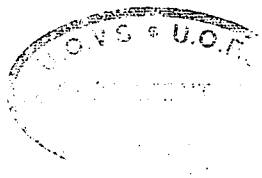

# STUDIES ON GENETIC VARIABILITY, INHERITANCE AND HETEROSIS IN PEPPER (*CAPSICUM ANNUUM* L.)

By

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Submitted in accordance with
the academic requirements for the degree of

**Philosophiae Doctor**

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**November 2003**

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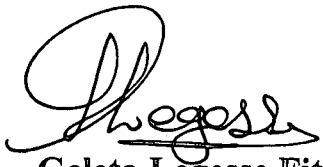
# DECLARATION

I declare that the thesis hereby submitted by me for the **Philosophiae Doctor** degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I further cede copyright of the thesis in favor of the University of the Free State.



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# AKNOWLEDGEMENTS

It is my great pleasure to thank and appreciate my promoter Prof. M.T. Labuschagne for her close supervision, guidance, critical comments, support and hospitality. I would like also to express my gratitude to Dr. C.D. Viljoen, my co-promoter, for his advice and valuable comments.

My leaving allowance, research and other costs were covered by the Agricultural Research and Training Project (ARTP) of the Ethiopian Agricultural Research Organization (EARO), Ethiopia. I wish to acknowledge Drs. Seifu Ketema and Abera Debelo, the former Director General and Deputy Director General of EARO, respectively for their advice and encouragement to pursue for my study. The contribution of Dr. Aberra Deressa, Manager of Melkasa Research Center, through facilitating managerial matters for me when I was preparing to leave for my study is appreciated.

Some of the genotypes were kindly provided by Dr. L.M. Engle, Geneticist and Head, Genetic Resources and Seed Unit, Asian Vegetable Research and Development Center (AVRDC), Taiwan; Dr. S. Norbert, Red Pepper Research and Development Ltd., Department of Szegedch and Development Ltd., Department of Szegedch, Hungary; and Mr. D. Nieuwoudt, MayFord Seeds, South Africa.

I am thankful to Mrs. S. Geldenhuys for her active and efficient accomplishment of all administrative matters on time. I would also like to thank Prof. C.S. Van Deventer for his useful suggestions. The assistance of Ms. E. Koen in AFLP analysis is appreciated.

I thank my wife, Banchewesen Melaku, for her general support. I am very grateful to my daughter, Ada, and son, Na'ol, for their understanding, patience and silence during my study period.

# DEDICATION

*This piece of work is dedicated to my late father Legesse Fite Debela and my mother Daksitu Huluka Geleto. My parents sent me to school and supported me from the small income that they were getting from a subsistence farm.*

## ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| AA | ascorbic acid |
| ABM | aerial biomass |
| ACL | actual |
| AFLP | amplified fragment length polymorphism |
| ANOVA | analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| ARTP | Agricultural Research and Training Project |
| AVRDC | Asian Vegetable Research and Development Center |
| bp | base pair |
| CATE | Centro Agronomico Tropical de Investigacion y Ensenanza |
| cM | centimorgan |
| CC | corolla color |
| CTAB | cetyl triethyl ammonium bromide |
| CV | coefficient of variation |
| CW | canopy width |
| DAF | DNA amplification fingerprinting |
| DC | double cross |
| DF | days to flowering |
| df | degree of freedom |
| DM | days to maturity |
| DNA | deoxyribonucleic acid |
| DNTP | deoxynucleoside triphosphate |
| EARO | Ethiopian Agricultural Research Organization |
| EDTA | ethylenediamin tetra acetic acid |
| *et al.* | et alii |
| etc. | et cetera |
| FAO | Food and Agricultural Organization |
| $F_1P$ | $F_1$ hybrid performance |
| FD | fruit diameter |

| | |
|---|---|
| FL | fruit length |
| FLD | field |
| FMP | fruit maturation period |
| FN | fruit number |
| FP | flower position |
| FWT | fruit weight |
| FY | fruit yield |
| g | gram |
| GCA | general combining ability |
| GD | genetic distance |
| GFAA | green fruit ascorbic acid |
| GFTSS | green fruit total soluble solid |
| GH | greenhouse |
| $g_i$ | GCA effect of inbred i |
| ha | hectare |
| $h^2b$ | heritability in broad-sense |
| $h^2n$ | heritability in narrow-sense |
| $H_2O$ | water |
| HCl | hydrochloric acid |
| HI | harvest index |
| HP | high-parent value |
| HPH | high-parent heterosis |
| i.e. | id est |
| IFC | immature fruit color |
| FSP | fruit shape |
| IPGRI | International Plant Genetic Resources Institute |
| KCl | potassium chloride |
| Kg | kilograms |
| LC | leaf color |
| LSD | least significant difference |
| M | molar |

| | |
|---|---|
| m | meter |
| MFC | mature fruit color |
| mg | milligram |
| $MgCl_2$ | magnesium chloride |
| min | minute |
| ml | milliliter |
| mM | millimolar |
| MP | mid-parent value |
| MPH | mid-parent heterosis |
| MOA | Ministry of Agriculture |
| mol | mole |
| MS | mean square |
| NaCl | sodium chloride |
| NaOH | sodium hydroxide |
| NCSS | number cruncher statistical system |
| ng | nanogram |
| nm | nanometer |
| ns | non-significant |
| PCR | polymerase chain reaction |
| PCT | pericarp thickness |
| PH | plant height |
| PR | predictability ratio |
| PRD | predicted |
| PVMP | pepper veinal mottle virus |
| $r_{cop}$ | cophenetic correlation |
| $r$ | correlation coefficient |
| RDA | recommended daily allowance |
| RAPD | random amplified polymorphic DNA |
| RFAA | red fruit ascorbic acid |
| RFLP | restriction fragment length polymorphism |
| RFTSS | red fruit total soluble solid |

| | |
|---|---|
| rpm | revolutions per minute |
| SC | single cross |
| SCA | specific combining ability |
| SD | standard deviation |
| SDS | sodium dodecyl sulphate |
| sec | second |
| SH | standard heterosis |
| $s_{ij}$ | SCA effect of hybrid ij |
| SSR | simple sequence repeat |
| STS | sequence tagged site |
| TAE | tris, acetic acid and EDTA |
| Taq | thermus aquaticus |
| TE | tris EDTA |
| TSS | total soluble solids |
| TWC | three-way cross |
| UPGMA | unweighted pair-group method with arithmetic averages |
| UPOV | Union de Protection and Obtention Végétable |
| UV | ultraviolet |
| $V_d$ | dominance variance |
| $V_g$ | genotypic variance |
| $V_p$ | phenotypic variance |
| w/w | weight per weight |
| °C | degree Celsius |
| µg | microgram |
| µl | micro liter |
| $\sigma^2A$ | additive variance |
| $\sigma^2D$ | dominance variance |
| $\sigma^2_{GCA}$ | GCA variance |
| $\sigma^2_{SCA}$ | SCA variance |
| % | percent |

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# CHAPTER 1

## General introduction

The genus *Capsicum* originated in the American tropics (Pickersgill, 1997). The genus represents a diverse plant group and includes 27 species, five domesticated and 22 undomesticated species (DeWitt and Bosland, 1993). The domesticated species include *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinenese*, *Capsicum baccatum* and *Capsicum pubescens*. *C. annuum* is the most important species from an agricultural prospective and contains both the larger-fruited bell pepper and the small pungent types. *Capsicum* species, with few exceptions, are diploid (2n = 24, infrequently 2n = 26) and have similar karyotypes (Lippert *et al.*, 1966; Moscone *et al.*, 1993). Chile peppers grow as a perennial shrub in suitable climatic conditions. The *Capsicum* genus has a large set of common names, such as pepper, chili, chile, chilli, aji, and paprika. The word 'chile' is used for the plant and the fruit, whereas 'chili' is used for a specific dish of food (Bosland and Votava, 2000).

Pepper (*Capsicum* sp.) is grown in most countries of the world. By volume, red pepper products, pungent and non-pungent, represent one of the important spice commodities in the world (Bosland and Votava, 2000). A report by the FAO (2000) indicates that the production of pepper for use as spice and as a vegetable has increased by more than 33% between 1991 and 2000. According to this report, the world production of pepper in 2000 was 18 501 000 metric tons, Asia being the largest producer. It is the second most cultivated vegetable species after tomato in the third world (Lefebvre *et al.*, 1995).

Peppers are known to be a versatile crop. They have a wide variety of uses such as flavoring in food manufacturing, adding pungency and color to foods, coloring for cosmetics and imparting heat to medicines. They are also a good source of income. In addition to their use as food, condiment and medicine, peppers are also used as ornamentals in the garden. Ornamental peppers are a unique class of peppers. They are covered with red fruits during the holiday seasons and are often called Christmas peppers.

Ornamental peppers as potted plants are popular in Europe and gaining in popularity in the United States (Bosland *et al.*, 1994).

Peppers provide essential vitamins and minerals. According to Bosland and Votava (2000), pepper consumption is increasing, and may be an important source of vitamins for the world population. The antioxidant vitamins A, C and E are present in high concentrations in various types of peppers and they are good sources of many essential nutrients. A pepper pod from green to red succulent contains enough vitamin C to meet or exceed the adult recommended daily allowance (RDA). The amount of vitamin C obtained from one medium sized pepper fruit is six times as much as that of an orange. One medium green bell pepper (148 g) provides 180% of vitamin C of the RDA and 8% of vitamin A. Vitamin C content diminishes by about 30% in canned and cooked pepper, and nearly vanishes from dried pepper (Bosland and Votava, 2000). In general, ascorbic acid, soluble solids and dry matter content vary with maturity (Niklis *et al.*, 2002). It is not only the nutritional quality that makes pepper an important food crop but it also stimulates the flow of saliva and gastric juices that serve in digestion. It has been said that pepper raises body temperature, relieves cramps, stimulates digestion, improves the complexion, reverses inebriation, cures a hangover, smoothes gout, increases passion, etc.

Pepper is the first major spice crop in Ethiopia. Even though no documented information is available, it was probably introduced to Ethiopia by the Portuguese in the 17$^{th}$ century (Hafnagel, 1961). It has since been grown as an important spice and vegetable crop almost everywhere in the country both under rain-fed and irrigation conditions. It is widely grown in areas with altitudes ranging from 1400 to 2100 m (MOA, 1984).

They are used in different forms based on the fruit characters such as size, pungency (organoleptic sensation of heat) level and color. Pepper powder, a mixture containing ground pepper, oregano, cumin, garlic powder and others, is moderately pungent and used in daily preparation of local dishes in Ethiopia. Chili powder, made from the small-fruited highly pungent types is used to add pungency to certain foods. Pepper is also used as vegetable at the green mature stage. Besides its food value, it is a cash-generating crop

particularly for small-scale farmers in the country. It is also used as a raw material for agro-industries that produce paprika and capsicum oleoresins for the export market. Thus, it plays an essential role in the sustainability of livelihood of smallholder farmers and their families providing both food and income. Due to its economical importance a large area of land is cultivated every year. However, the average national yield is very low, dry fruit production is only 0.41 tons/ha (Jackson, 1987). This is mainly due to the lack of improved high yielding pure lines or hybrid varieties.

In Ethiopia, the demand for pepper is increasing consistently due to an ever-increasing population. On the other hand, there is a sharp decrease in productivity mainly due to the use of unimproved cultivars for yield and other important agronomic characteristics. Besides this, the ratio of farmland to human population is declining at an alarming rate. As a result farmers tend to switch from growing peppers to growing other crops in some parts of the country. This has resulted in decreased supply for both local consumers and agro-industries. Therefore, it is very important to replace the old varieties with improved ones and produce more pepper from less land, with less water, and fewer pesticides.

The study of genetic diversity levels among the available pepper genotypes will increase efficiency of the Ethiopian pepper breeding program. Genetic variability is the bases of genetic improvement. Genetic diversity among and within genera, species, subspecies, populations, and elite breeding materials is of equal interest in plant genetics and breeding. Plant breeding, classification schemes, and evolutionary studies all rely on genetic variability (Prince *et al.*, 1992). Evaluation of genetic diversity levels among adapted, elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure line cultivar development (Manjarrez-Sandoval *et al.*, 1997) and may estimate the degree of heterosis in progeny of some parental combinations (Barbosa-Neto *et al.*, 1996). The studies of levels and patterns of genetic diversity among adapted germplasm of different geographic origin may be useful for identifying diverse parental combinations to create segregating progenies with maximum genetic variability for selection.

Species within the genus *Capsicum* have long been differentiated using morphological, cytogenetical and molecular markers (Conicella *et al.*, 1990; Lefebvre *et al.*, 1993; 2001; Nam *et al.*, 1997; Paran *et al.*, 1998; Pickersgill, 1988; Prince *et al.*, 1992; Yayeh Zewdie and Zeven, 1997). According to Pickersgill (1997), the genetic diversity available within the various domesticated *Capsicum* species has hardly been exploited and has certainly not yet been exhausted. The author further indicated that this diversity should be easy to utilize compared with the problem associated with inter-specific gene transfer. Palloix (1992) also indicated that in *Capsicum* many breeding programs for agronomic traits involve intra-specific crosses between *C. annuum*. The available local and exotic germplasm currently used in the pepper breeding program of Ethiopia have not been analyzed and compared for their genetic divergence.

High pepper yield and quality is an important goal for breeders and producers. The diallel analysis has probably attracted more attention and been the subject of more theoretical and practical application than any other mating design (Wright, 1985). The concept is defined as making all possible crosses among a group of genotypes (Saghroue and Hallauer, 1997). Studies on diallel analyses for yield and component characters in peppers have been reported (Ahmed *et al.*, 1997; Kaul and Sharma, 1988; Kordus, 1991; Legesse, 2000; Mishra *et al.*, 1991; Pandian and Shanmugavelu, 1992; Patel *et al.*, 1998; Stevanovic *et al.*, 1997; Szwadiak and Kordus, 1991; Zecevic and Stevanovic, 1997). The information generated from these studies has contributed significantly to pepper breeding. On the other hand, most of them mainly dealt with genotypes of the same locality and that of similar varietal groups (small-, intermediate-, and large-fruited). Moreover, diallel analysis of any particular character applies only to a particular population under study and environmental conditions under which the study is undertaken. The study of diallel analysis between Ethiopian and exotic genotypes is scanty.

Heterosis has been documented in hot and sweet peppers, and hybrids are increasingly used by farmers throughout the world (Berke, 2000). Bosland and Votava (2000) also indicated that peppers grown from hybrid seeds are highly uniform and usually higher

yielding. Thus, in Ethiopia, the hybrid production system needs to be developed as an important strategy to increase the yield potential of pepper beyond the existing cultivars.

A primary objective of hybrid crop breeding programs is predicting the performance of the hybrids. However, the identification of parental inbred lines that form superior hybrids is the most costly and time-consuming phase in hybrid development. Since *per se* performance does not predict the performance of hybrids for yield (Hallauer and Miranda, 1988), methods that could predict $F_1$ hybrid performance with some accuracy prior to field evaluation are of particular interest. The use of genetic markers to assess genetic divergence among pairs of inbred lines has been suggested as a means to maximizing the probability of predicting hybrid performance by selecting the most divergent parents (Riaz *et al.*, 2001). Thus, characterization of inbred lines by molecular markers and their subsequent use in predicting hybrid performance has been the focus of recent research.

Hybrid varieties are superior to pure line varieties or open-pollinated land-race cultivars. There are different forms of hybrids: single, three-way, double or top crosses. According to Cockerham (1961) the expected genetic variance and yield potential decline from single to three-way to double to top crosses. On the other hand, it is assumed that yield stability is high in three-way and double cross hybrids owing to higher genetic heterogeneity among populations within a cultivar from three-way and double cross hybrids as compared to single cross hybrids. Eberhart *et al.* (1964) found higher genotype-year interactions in single crosses than in three-way crosses.

Although three-way and double cross hybrids are probably higher yielding, they are heterogeneous compared to single cross hybrids. Crop uniformity is considered a desirable character in modern agriculture because product uniformity is essential in marketing; uniformity in maturity permits crop scheduling; and uniformity in plant structure and maturation permits effective mechanical harvest (Janick, 1999). It is also an essential feature of crop quality especially in horticultural commodity. On the other hand, crop diversity is also considered desirable in some environments and situations because it

is assumed to produce population buffering under stress as diversity spreads risk. In pepper, although single cross hybrids are widely used, there is no report on the merit of producing and growing three-way and double cross hybrids.

Genetic diversity is the foundation of all plant improvement programs. Diallel analysis is used to obtain information on values of varieties as parents, to assess the gene action involved in various characters, and thereby develop appropriate selection procedures and understand heterotic patterns of the progenies at an early stage of the hybridization program. Commercial hybrid cultivars contribute greatly to important agricultural traits such as high yield and environmental adaptability, early maturity, and major disease resistance. In Ethiopia, although a number of pepper landraces are currently grown, there is no improved inbred line or hybrid variety in the production system.

With this view in mind, this study was undertaken with the objectives of:

1. Studying genetic variability among pepper genotypes of different geographical origins based on morphological and amplified fragment length polymorphisms markers.
2. Assessing the heterotic patterns and the relationships between genetic diversity and hybrid performance.
3. Investigating the nature of inheritance and heterosis of yield and other characters in a diallel cross of selected parental lines from diverse genetic backgrounds.
4. Identifying suitable parental lines to use in the breeding programs to develop hybrids and new pure lines of improved yield and yield contributing traits.
5. Investigating and comparing the performance and heterosis of single, double and three-way cross hybrids in pepper.

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# CHAPTER 2

# Literature review

## Genetic diversity

Genetic diversity is derived from wild progenitors, modified in response to cultivation and hence, it is a function of ancestry, geographic separation and adaptation to differing environments (Moll *et al.*, 1965). Genetic variability within a taxon is of great importance for plant genetics, breeders and taxonomists (Prince *et al.*, 1992). Diversity within a given plant population is a product of an interplay of biotic factors, physical environment, artificial selection and plant characters such as size, mating system, mutation, migration and dispersal (Frankel *et al.*, 1995). Genetic distances within crop species are measures of the average genetic divergence between populations or cultivars (Souza and Sorrells, 1991).

Germplasm curators as well as plant breeders have an interest in quantification and classification of genetic diversity. In germplasm collection, such a classification may help designate core collections to enhance efficiency of collection management and utilization (Brown *et al.*, 1987). In general, knowledge of genetic diversity and relationships among sets of germplasm and its potential merit would be beneficial to all phases of crop improvement (Lee, 1995). Evaluation of genetic diversity levels among adapted or elite germplasm provides the estimates of genetic variation among segregating progeny for pure line development (Manjarrez-Sandoval *et al.*, 1997) and the degree of heterosis in the progeny of certain parental combinations (Barbosa-Neto *et al.*, 1997; Cox and Murphy, 1990).

Broad-based plant germplasm resources are imperative for sound and successful crop improvement programs. If the breeding base is narrow, then there will be, on average, fewer genetic differences segregating within breeding populations and, therefore, a reduced genetic distance between the resultant progeny and between those progeny and

their parents (Smith and Smith, 1992). Without a continued source of variability, the ability to create new plateaus of agronomic performance that are based on complex genetic combinations could decline.

Genetic improvement of crops by man can be regarded as directed evolution acting on the existing genetic variability in the germplasm (Melchinger *et al.*, 1999). In order to optimize and accelerate breeding, it is essential to screen and evaluate the genetic variability available in the germplasm. Genetic diversity in domesticated crop species provides a source of variation which is raw material for the improvement of agricultural crops, and is essential to decrease vulnerability to biotic and abiotic stresses and to ensure long-term selection gain in genetic improvement and to promote rational use of genetic resources (Martin *et al.*, 1991; Barrett and Kidwell, 1998; Messmer *et al.*, 1993; Smith and Smith, 1989). Yang *et al.* (1996) also indicated that estimation of genetic diversity in plant species can assist in the evaluation of different germplasm as possible sources of genes that can improve the performance of cultivars. It becomes more important as cropping intensity and monoculture continue to increase in the world.

A complete array of germplasm in a crop consists of (1) wild relatives and landraces in the areas of diversity, (2) unimproved or purified cultivars used earlier in the major production areas that are still used in minor areas, and (3) improved germplasm in commercial production and genetic testers from breeding programs and genetic studies. Information about genetic diversity in the available germplasm is important for the optimal design of breeding programs. Thus, the notion of genetic relationships among lines, populations or species has become an important tool for the effective management of genetic diversity in a given gene pool (Manjarrez-Sandoval *et al.*, 1997).

Trangressive segregation may be more likely to occur when parents in a cross are less similar, allowing different favorable alleles to be combined in the offspring (Cowen and Frey, 1987). The genus *Capsicum* is among the intermediately divergent agricultural crops. Generally out-crossing species such as maize (Smith, 1988), *Brassica* (Figdore *et al.*, 1988) and potato (Gebhardt *et al.*, 1989) show a high level of genetic diversity among

cultivated types. On the other hand, autogamous species like soybean (Apuya *et al.*, 1988), tomato (Miller and Tanksley, 1990) and wheat (Chao *et al.*, 1989) show a relatively low level of polymorphism between cultivars. The intermediate level of polymorphism in *C. annuum* can be related to the reproductive behavior of the domesticated peppers and the way of domestication (Lefebvre *et al.*, 1993). The higher level of outcrossing may help in hybrid breeding in pepper.

## Methods of genetic distance measurements

Genetic distance (GD) is the extent of gene differences between cultivars, as measured by allele frequencies at a sample of loci (Nei, 1987). Genetic relationships among individuals and populations can be measured by similarity of any number of quantitative characters (Souza and Sorells, 1991). The methods include morphological traits, isozymes and DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), etc. Genetic relationships among a large number of cultivars can then be summarized using cluster analysis to place similar genotypes together (Souza and Sorells, 1991).

### *Morphological traits*

In plant populations, variability and relatedness have traditionally been studied based on morphology such as flower color and shape, leaf shape, plant height and usage of the plant (Goodman, 1972; Weier *et al.*, 1982). Morphological description can provide unique identification of cultivated varieties (Molina-Cano and Elena Rossello, 1978). This assumes that differences between characters of the genotypes reflect the genetic divergence of the genotype. When phenotypic estimates are used to represent the degree of genetic relationship between two lines or populations, it is assumed that the similarity in phenotype accurately reflects similarity in genotype (Cox *et al.*, 1985; Van Beuningen and Busch, 1997).

Morphological traits controlled by a single locus can be used as genetic markers if their expression is reproducible over a range of environments. As cited by Melchinger *et al.* (1994) discrete morphological traits are the basis for description of identity and distinctness of cultivars in plant variety protection and registration under the guidelines of the Union de Protection Obtention Végétable (UPOV, 1980).

Morphological traits have been used for diversity analysis in different agricultural crops. These markers have been used since the turn of the 20$^{th}$ century to build genetic maps (Paterson *et al.*, 1991). Morphological traits have long been used to estimate systematic relationship in the genus *Capsicum* (Pickersgill, 1988; Zewdie and Zeven, 1997). The genus *Capsicum* exhibits considerable variation in fruit shape, color, and size, pubescence of leaves and number of followers per node (Walsh and Hoot, 2001). Greenleaf (1986) indicated that the five major cultivated species of *Capsicum* can usually be distinguished by a combination of flower and fruit characteristics. *C. annuum* has white flowers, blue to purple anthers, a toothed calyx, and typically single-fruited nodes with the possible exception of an occasional double-flowered axil in a lower main fork. *C. frutescencs* has greenish flowers, a non-toothed, non-constricted calyx that encloses the fruit base, blue anthers, and mostly single-fruited nodes but with a few double-flowered nodes on each plant, as in Tabasco, unless the plants are stunted. Certain wild forms of *C. frutescens* apparently produce up to five fruits per node. *C. chinense* has white or greenish white flowers, blue anthers, a constricted, toothed calyx, and typically from one to three fruits per node. *C. baccatum* is easily identified from the white flowers with the yellow corolla spots, yellow anthers, and the long, curved, characteristically pendant fruit pedicels and leaf petioles. *C. pubescens* with its larger, showy purple flowers, soft pubescent leaves, yellow-orange fruits, and black seeds is unique. In general the morphological differences between wild and cultivated chiles are easily discerned. All wild forms of chiles have small, red, berry-like fruits with colors and fruits attractive to birds. Domesticated fruits exhibit variable fruit and flower coloration; gigantism of fruits, seeds, flowers, and leaves (Eshbaugh, 1976); and retention of the fruit on the peduncle at maturity (Eshbaugh, 1976; Pickersgill, 1969).

*C. annuum* is the most important species from an agricultural prospective and contains both the larger-fruited bell pepper and the small pungent types. *C. annuum* pod types are usually classified by fruit characteristics, i.e. pungency, color, shape, flavor, size and use (Smith *et al.*, 1987; Bosland, 1992). Bosland and Votava (2000) classified the species pod types as pungent and non-pungent. Non-pungent pepper types include bell, pimento, Cuban and squash. The pungent types include cayenne, New Mexican, jalapeno, serrano, ancho, pacilla, mirasol, de Arbol and piquin.

Numerical taxonomy based on morphological characters of wild, semi-domesticated and domesticated accessions of *C. annuum* showed that domesticated *annuum* peppers appear more heterogeneous than the wild peppers of the same species (Pickersgill *et al.*, 1979). However, there is a plethora of magnificently colored and shaped pepper grown worldwide (Bosland and Votava, 2000). Lefebvre *et al.* (1993) also indicated that compared to other self-pollinated crop species, *C. annuum* is fairly variable.

Categorizing germplasm accessions into morphologically, presumably genetically similar, groups is most useful when: (i) little is known about the crop history, (ii) the population structure in a collection is unknown, or (iii) when new breeding methods are applied to a crop (Souza and Sorrells, 1991). However, the drawback of morphological traits for the study of genetic diversity has been reported by different investigators. Morphological markers may present altered phenotype that interferes with growers' needs.

Evaluation of genetic relationships among germplasm using morphological characteristics are lengthy, costly, and cumbersome (Cooke, 1984; Patterson and Weatherup, 1984). Morphological characters must also be assessed during a fixed vegetative phase of a crop. Smith and Smith (1992) indicated that increased number of genetically related releases by plant breeders have made unique identification more difficult to achieve. The genetic control of many morphological characters is assumed to be complex, often involving epistatic interactions, and has often not been elucidated (Smith and Smith, 1989). Many morphological markers are also recessive and therefore

only expressed in the homologous condition. Most elite cultivated and breeding materials do not abound with any of the readily observable morphological markers, a large number of which have deleterious effects on agronomic performance (Smith, 1986).

Morphological traits can also be influenced by environmental factors. Most morphological attributes are subjected to large genotype-environment interaction effects and they reflect not only the genetic contribution of the cultivar, but also the interaction of the genotype with the environment in which it is expressed (Lin and Binns, 1984; Patterson and Weatherup, 1984; Smith and Smith, 1989; Yee *et al.*, 1999). The fact that such factors may modify a gene's expression of phenotype may limit its usefulness as a genetic marker. Yee *et al.* (1999) also indicated that if the magnitude of environmentally induced variation is large in comparison to genetic variation, diversity estimates based on morphological data may poorly reflect actual levels of genetic diversity among accessions. Hence, morphological appearance cannot adequately describe cultivars without extensive replicated trials and, therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1989). On the other hand, discrete morphological traits are the basis for description of identity and distinctness of cultivars in plant variety protection and registration under the guidelines of the UPOV (UPOV, 1980). Furthermore, morphological traits are almost entirely used for crop diversity analysis in countries like Ethiopia where economy and trained manpower are the limiting factors to establish modern technologies for crop diversity analysis.

### *Isozymes*

Isozymes are protein molecules that are separated electrophoretically based on their charges (Tanksley, 1983a). They are variants of the same enzyme having identical or similar function, but differing in electrophoretic mobility. Isozymes reveal differences in the gene sequence and function as co-dominant markers (Kumar, 1999).

Isozyme data can be used to quantify similarities and differences between genotypes because:

(i) Isozyme surveys represent a basic level of investigation for species that are poorly documented;

(ii) Isozymes are universal in a sense that estimates of the extent of distribution of genetic diversity can be directly compared between individuals, populations, or species; and

(iii) Isozyme methods are appropriate to investigate genetic variation from large samples of individuals because the procedure is quick, simple and inexpensive, and interpretation is relatively easy (Cooke, 1984).

Since enzymes catalyze specific biochemical reactions, it is possible to visualize the location of particular enzymes on gels by supplying the appropriate substrate and cofactors, and involving the product of enzymatic reaction in a color producing reaction. The colored product is deposited on the gel, forming a visible band where a particular enzyme has been electrophoretically localized. Bands visualized from specific enzymes, represent protein products, have a genetic basis, and can provide genetic information as co-dominant markers.

Isozymes have been successfully utilized to characterize the genetic variation in numerous taxonomic and population genetic studies. Isozyme studies have been used in genetic studies and breeding research for many purposes, particularly for measuring genetic variability of populations understanding breeding structures, defining systematic and phylogenetic relationships, and for gene mapping (Tanksley, 1983a). They have been extensively used in studying different crops including the genus *Capsicum* (Biles *et al.*, 1997; Conicella *et al.*, 1990; Loaiza-Fiueroa *et a*l., 1989; McLeod *et al.*, 1983; Pickersgill, 1988; Prince *et al.*, 1993; Tanksley, 1984). Isozyme studies by Conicella *et al.* (1990) and Loaiza-Figueroa *et al.* (1989) show that the GD between domesticated and semi-domesticated pepper forms are lower than among the wild forms. Combined cytogenetical and isozyme studies have also demonstrated to be useful for phylogenetic

studies in *Clarkia* to confirm the effectiveness of enzyme studies when coupled with cytologic data for investigating the evolution within *C. annuum* (Gottlieb, 1977).

Although isozymes have been successfully used in numerous taxonomic and evolutionary studies (Hamrick and Godt, 1997), they often failed in the classification of elite breeding materials due to the limited number of marker loci available and low level of polymorphism. The usefulness of isozymes for obtaining reliable estimates of genetic diversity is generally limited by the insufficient sampling of the genome (Melchinger *et al.*, 1991), small number of loci, and low degree of polymorphism among closely related genotypes (Messmer *et al.*, 1992). Their expression can also be significantly influenced by environmental factors and management practices and by plant developmental stages (Beeching *et al.*, 1993; Bellamy *et al.*, 1996). Melchinger (1999) noted that the development of molecular markers such as RFLPs, RAPDs, SSRs, and AFLPs in recent years removed most of the limitations associated with isozymes. Because the new marker systems reveal differences at the DNA level, they provide an extremely powerful tool for assessment of genetic diversity in cultivated and wild plant species. RFLPs and PCR-based genetic marker assays such as RAPDs, SSRs and AFLPs are the most commonly used techniques (Karp *et al.*, 1996).

### *DNA markers*

Molecular DNA markers are new tools for genetic improvement of food crops, which can be used in various fields of plant breeding and germplasm management (Thottappilly *et al.*, 2000). Several DNA based marker systems that reveal polymorphism at DNA level (Kumar, 1999) have been developed for measuring genetic similarities in agricultural crops. They have proven to be powerful tools in the assessment of genetic variation within and between plant populations and in the elucidation of genetic relationships among adapted cultivars and accessions (Lee, 1995; Karp *et al.*, 1996). Molecular markers are invaluable for understanding the genetic make-up of agricultural crops and to assess the degree to which a collection's gene pool overlaps with nature or other collections. Like morphological markers, molecular markers are used to observe genetic

differences between two or more individuals. However, molecular markers differ from morphological markers in several ways. Firstly, molecular markers usually occur in greater numbers, secondly, they can be distinguished without relying on complete development of the plant, and, thirdly, their expression is not altered by environment (Tanksley, 1983b).

As it was noted by Melchinger (1999), before 1970, measuring genetic diversity between taxonomic units was based on pedigree analysis and morphological, physiological or cytological markers as well as biometrical analysis of quantitative and qualitative traits, heterosis or segregating variance in crosses. Since then several molecular markers have been developed. Molecular markers have been used in construction of a molecular linkage map, in selection of DNA markers tightly linked to major important traits, and grouping crop germplasm. Because DNA markers can reveal immense numbers of genetic loci, and are phenotypically neutral and not subject to environmental effects, they are especially informative and superior to those revealed by traditional methods such as morphological traits and protein markers in resolving genetic differences.

Two types of DNA markers are available (Karp *et al.*, 1996): firstly, those that rely on hybridization between probe and homologous DNA segments within the genome, restriction fragment length polymorphism (RFLP) (Beckman and Soller, 1983) and secondly, those that use polymerase chain reaction (PCR) (Mullis *et al.*, 1986) to exponentially amplify genome segments between arbitrary or specific oligonucleotide priming sites. PCR is an *in vitro* method of nucleic acid synthesis by which a particular segment of DNA can be specifically replicated (Mullis and Faloona, 1987). The process involves two oligonucleotide primers that flank the DNA fragment of interest. Amplification is achieved by a series of repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences, and extension of the annealed primers with a thermophilic DNA polymerase.

The potential applications of molecular markers in plant breeding are (i) fingerprinting of genotypes for plant variety identification and protection, and (ii) assessing the genetic

similarity among parents for prediction of quantitative-genetic parameters such as heterosis or progeny variance (Bohn *et al.*, 1999). According to Bohn and colleagues molecular markers are highly polymorphic, abundant in numbers and well distributed over the entire genome. Estimation of genetic similarity between genotypes can be obtained directly by measuring their resemblance for biochemical of DNA markers (Smith *et al.*, 1991). However, although DNA marker systems directly measure DNA sequence variation among genotypes, results may be confounded by biased or incomplete coverage, detection of co-migrating non-homologous fragments, or high crossover frequency between markers used in the evaluation and linked genetic material (Barrett and Kidwell, 1998). Tanksley *et al.* (1989) and Bohn *et al.* (1999) indicated some DNA marker techniques also require the use of hazardous radioactive isotopes. In addition, DNA marker techniques are generally labor intensive, time consuming and relatively expensive, so that sample sizes are usually small and the power to test statistical hypothesis is limited (Melchinger *et al.*, 1991). However, studies indicate that different DNA marker techniques have their own merits and demerits.

*Restriction fragment length polymorphisms (RFLPs)*

DNA data as revealed by RFLPs provide taxonomic, genetic, and phylogenetic information (Kirby, 1990). The ability to cleave DNA at specific nucleotide base recognition sequences with restriction endonucleases, coupled with methods to separate, label, hybridize complementary DNA sequences, and reveal the relative position or molecular weight of a DNA fragment following electrophoresis, have together made possible the direct use of variation in DNA sequence as a descriptor (Smith and Smith, 1992). Identification of genomic DNA fragments is made by Southern blotting, a procedure whereby DNA fragments, separated by electrophoresis, are transferred to nitrocellulose or nylon filter (Southern, 1975). Filter-immobilized DNA is allowed to hybridize to radioactively labeled probe DNA. The filter is placed against photographic film, where radioactive disintegrations from the probe result in visible bands. Such bands are visualizations of RFLPs, which are co-dominant markers.

A fragment length polymorphism is generated when a particular recognition site of a restriction enzyme is absent in one individual and present in another, resulting in different sized restriction fragments at that locus. The polymorphic fragments are detected by resolving the DNA fragments using electrophoresis and detection with probes (Southern, 1975).

RFLP-based similarity estimates have proven useful for (i) discrimination of lines from different heterotic groups, (ii) assignment of lines of unknown origin into heterotic groups, and (iii) detection of closely related inbred lines (Melchinger *et al.*, 1991; Messmer *et al.*, 1992). The RFLP technique has been particularly useful in mapping species that display a high level of intra-specific variation.

The technique has been used extensively in different crops to study the genetic diversity within and between a given species. In *Capsicum* research, Tanksley *et al.* (1988) used RFLP to study genetic similarities and differences between *Capsicum* and *Lycopersicon*. The investigators also used RFLP to construct the first RFLP linkage map of pepper, however, a more complete RFLP linkage map of pepper was developed by Prince *et al.* (1993). Livneh *et al.* (1990) applied RFLP to analyze a hybrid cultivar of pepper and distinguish between parental lines and hybrids. Lefebvre *et al.* (1993) demonstrated that RFLPs were more useful than isozymes for mapping and diversity studies in *Capsicum* species. Using RFLPs they found that cultivars of bell pepper (all of European and North American origin) were much more similar to one another than were small-fruited accessions of European, Mexican, Indian and Ethiopian accessions. Prince *et al.* (1992) used RFLPs to study genetic diversity in *Capsicum* and they grouped Mexican accessions into two groups based on species and geographic origin. Prince *et al.* (1995) again used RFLP and differentiated the studied accessions and they suggested that any two accessions could be used as possible parents for RFLP mapping. RFLP and biological tests were also used to map the loci involved in PVMV resistance and to determine if these loci are involved in other potyvirus resistance in *C. annuum* lines (Caranta *et al.*, 1996). A molecular map of pepper totaling 720 cM has been constructed in inter-specific

$F_2$ cross with restriction fragment length polymorphisms and isozymes (Prince *et al.*, 1993).

In spite of its extensive application for diversity studies, RFLP mapping is time consuming, costly and labor intensive. The technique is difficult in some species with large and complex genomes. Van der Beek *et al.* (1992) indicated that RFLPs have limitations in revealing polymorphisms in tomato (*Lycopersicon esculentum*). Detection of RFLPs by Southern blot hybridizations is laborious and incompatible with the high analytical throughput required for many applications (Beckmann, 1988). Furthermore, the technique requires a substantial amount of DNA.

*Random amplified polymorphic DNA (RAPDs)*

RAPD utilizes the polymerase chain reaction (PCR). Polymorphic markers are generated using single primers, which are usually 10 base pairs long (Williams *et al.*, 1990). The technique is simple, sensitive and relatively cheap in comparison to RFLP. Because a single primer allows amplification of multiple loci dispersed throughout the genome, RAPDs provide a rapid assay for nucleotide sequence polymorphism (Tingey *et al.*, 1992). The RAPD markers are well suited for genetic mapping, for plant and animal breeding applications, and for DNA fingerprinting, with particular utility for studies of population genetics (Williams *et al.*, 1990). The primer/linkage complexes are used as substrates for DNA polymerase to copy the genomic sequences 3′ to the primers. Iteration of this process yields a discrete set of amplified DNA products that represent target sequences flanked by opposite oriented primer annealing sites. Amplification products can be separated by electrophoresis on agarose or polyacrylamide gels and visualized by staining with ethidium bromide or silver. The number of DNA fragments amplified is dependent on the sequence of the primer and the size of the genome being used as a template. RAPDs are usually dominant markers with polymorphisms between individuals defined as the presence or absence of a particular RAPD band.

The technique has been used for identification purposes in many crops (Khandka *et al.*, 1996; Iqbal and Rayburn, 1994; Golembiewski *et al.*, 1997; He and Prakash, 1997). The technique has also been used in *Capsicum* to study genetic diversity, linkage and to provide additional molecular markers for mapping. Las Heras Vazquez *et al.* (1996) applied the RAPD technique to fingerprint pepper breeding lines and observed higher genetic diversity in chile cultivars than in the bell types. Ballester and de Vicente (1998) reported that RAPD is an efficient method in pepper $F_1$ hybrid seed purity testing. Lefebvre *et al.* (1997) utilized RAPDs, RFLPs, known function genes, isozymes and phenotypic markers to develop an intra-specific molecular linkage map developed from the $F_1$ hybrid derived from two double haploid *C. annuum* populations. Baoxi *et al.* (2000) determined two RAPD markers linked to major fertility restorer genes in pepper. Although the RAPD techniques have been used for identification purpose, primarily because of its simple and rapid nature, evidence suggests that RAPD is not robust because of its sensitivity to changes in reaction conditions and DNA quality (Ellsworth *et al.*, 1993). As a result, this method is finding less favor now that more reliable methods are available.

*Simple sequence repeats (SSRs)*

DNA sequences with short repeated motifs (2 – 6 bp) are called simple sequence repeats (SSRs) or microsatellites (Hamada *et al.*, 1982; Litt and Luty, 1989; Epplen *et al.*, 1991; Todocoro *et al.*, 1995). They are polymorphic and abundantly present in plant genomes. The fragment polymorphism relates to total sequence length as determined by the number of repeat units and the heterozygote for different fragments in diploid genomes can generally be distinguished. Individual loci corresponding to specific primer pairs are therefore co-dominant and can be multi-allelic. The products generated have been found to be highly reproducible (Jones *et al.*, 1997) and although these markers are usually species specific, costly to develop, and prior sequence information is required, once the primers have been developed the system becomes relatively inexpensive.

The discovery of the existence of poly (dC-dA) and other kinds of short sequence repeats in mammalian genomes (Hamada *et al.*, 1982), combined with the ability to observe repeat length variation by means of PCR (Litt and Luty, 1989), have made SSRs a useful genetic tool. The positive features that characterize SSR, such as the random distribution throughout the genome, the large allelic variation, and the ease of use, have made SSRs the preferred marker for detailed mapping of genomes (Dietrich *et al.*, 1994) and disease genes (Yu *et al.*, 1994) and for population and evolutionary genetic studies (Bowcock *et al.*, 1994).

The presence of SSRs in a wide number of plant species has been well documented (Akkaya *et al.*, 1992; Lagercrantz *et al.*, 1993; Sharma *et al.*, 1995; Taramino and Tingey, 1996).

SSR markers for studies have generally been developed by three routs:

(i) Transfer from closely related species (Provan *et al.*, 1996; White and Powell, 1997)

(ii) Searching sequence databases (Sanwell *et al.*, 2001; Senior and Heun, 1993; Bell and Ecker, 1994), and

(iii) Screening cDNA or small insert libraries with tandemly repeated oligonucleotides and sequencing candidate clones (Powell *et al.*, 1996).

The use of SSRs for variety profiling can provide high discrimination, with excellent reproducibility at less cost than some other marker analysis like for RFLPs.

The SSR loci can be amplified by PCR (Saiki *et al.*, 1988) using primers which are complementary to the regions flanking the repeats. The resulting products, separated electrophoretically, are highly polymorphic and provide co-dominant genetic markers with Mendelian inheritance (Beckmann and Soller, 1990). Microsatellite techniques have also been utilized to analyze the relationships within different crops. Provan *et al.* (1996) used SSR to analyze the relationships within cultivated potato (*Solanum tuberosum*). Smith *et al.* (2000) also utilized simple sequence repeats to study the genetic diversity

among elite sorghum inbred lines. Sanwell *et al.* (2001) studied the development of pepper SSR markers from sequence databases and reported that polymorphisms between *Capsicum* lines can be detected with five of the studied primer pairs.

*Amplified fragment length polymorphisms (AFLPs)*

AFLP is a PCR-based technology for marker-assisted breeding and genotyping. The AFLP technique, developed by Vos *et al.* (1995), is a powerful tool for DNA finger printing of organismal genomes. The technique combines the advantage of the time efficiency of PCR-based markers and the reliability of RFLP markers. It is a reproducible, highly multiplex assay with the ability to generate a large number of polymorphic genetic loci. AFLP represents a significant breakthrough compared to the currently available methods in terms of facility, precision, flexibility and speed. Although the AFLP procedure is more labor intensive and expensive than RAPD analysis, a large number of loci are sampled per reaction (Powell *et al.*, 1996; Yee *et al.*, 1999). The technique enables the generation of thousands of DNA markers from a genome of any complexity and without prior knowledge of the genome's structure or sequence.

Production of AFLPs is based on selective amplification of restriction enzyme-digested DNA fragments (Vos *et al.*, 1995). The technique involves four distinct steps: (i) restriction enzyme digestion of DNA, (ii) ligation of adaptors to the restricted sites, (iii) PCR amplification of restricted fragments with primers that bind to the adaptor sequence and adjacent selective nucleotide, and (iv) acrylamide gel electrophoresis. Usually, restriction enzymes with two different specificities such as *EcoRI* and *MseI* are used to generate a large number of fragments.

PCR amplification with the specific primers ensures reliable and reproducible detection of restricted fragments. A subset of fragments is selectively amplified by PCR primers, which have 2- or 3-base extensions into the restriction fragments. Only those fragments that perfectly match the primer sequences can be amplified by PCR. Therefore the complexity of PCR amplifications is reduced. Relative ease of implementation, large

number of polymorphisms detected per gel, small amount of genomic DNA required and high reproducibility of DNA fingerprint patterns recommended AFLP as an attractive method to study DNA polymorphism in general. As indicated above, AFLP assay requires no prior sequence knowledge but detects at least 10 times more genetic loci than RFLP and RAPD analysis in many crops (Tohme *et al.*, 1996; Maughan *et al.*, 1996; Hill *et al.*, 1996). Therefore, AFLP assay has the ability to detect thousands of independent genetic loci in a short time.

The AFLP technique has been successfully applied for intra- and inter-specific genetic variability studies in different crops such as tea, *Camellia sinensis* L., (Paul *et al.*, 1997); sunflower, *Helianthus annuus* L., (Hongtrakul *et al.*, 1997); wheat, *Triticum aestivum* L., (Barrett and Kidwell, 1998); cassava, *Maninhot* spp., (Roa *et al.*, 1997); tef, *Eragrostis tef* (Zucc) Trotter, (Ayele and Nguyen, 2000; Bai *et al.*, 1999); peanut, *Arachis hypogaea* L., (He and Prakash, 1997); rapeseed, (Lombard *et al.*, 2000; Tohme *et al.*, 1996); *Arabidopsis thaliana* (Miyashita *et al.*, 1999); grapes (Scott *et al.*, 2000) and pepper, *Capsicum* sp., (Lefebvre *et al.*, 2001; Paran *et al.*, 1998). In all of these studies, AFLP has detected a large number of polymorphisms more efficiently. Pierre *et al.* (2000) also identified markers linked to Bs3 and defined a 2.1-cM interval containing the target gene using AFLP in pepper. It has also been used for genetic linkage mapping in crops like rice, *Oryza sativa* L., (Maheswaran *et al.*, 1997). Melchinger (1999) indicated that AFLP provides sufficient accuracy in measuring genetic distance within the breeding materials, because a large number of marker bands can be evaluated in a single assay. This technique has not been utilized in pepper diversity studies between Ethiopian and exotic pepper germplasm that are currently used in Ethiopian pepper breeding programs.

**Comparison of methods of genetic distance measurements**

Scientists are interested to know which of the available DNA marker technology is most suitable for various applications in plant genetics and breeding. Different marker technologies have been compared in diversity studies of several crops. In an *Avena sterilis* genetic study, neither RFLP nor allozyme variation was highly correlated with

morphological variation (Beer *et al.*, 1993). Comparison of RFLPs and RAPDs in diversity studies with maize (Hahn *et al.*, 1995) and barley (Russell *et al.*, 1997) showed moderate agreement in GD estimates, which was largely attributed to the lower reproducibility of RAPDs (Melchinger, 1999). In the study designated to determine the usefulness of different methods, Gerdes and Tracy (1994) compared RFLPs, morphology, isozymes, and pedigree to accurately assess relatedness and relationships of sweet corn inbreds. They concluded that isozymes and morphology did not accurately group the studied genotypes while RFLP and pedigree grouped the materials into clusters. Yang *et al.* (1996) studied the comparison of DNA marker technologies (RFLPs, RAPDs and ISSR) in characterizing plant genome diversity of Chinese sorghum. They reported that each of these approaches supported equivalent conclusions regarding the relatedness of the studied materials, but that the ISSR technique provided larger and more informative datasets with less effort and expense. Fingerprinting of the genus *Capsicum* was accomplished with 17% of the DNA clones used individually in Southern analysis of *EcoRI*-digested total plant genomic DNA, as well as with 12.5% of the RAPD PCR primers, used in polymerase chain reaction (Prince *et al.*, 1995).

Genetic distance estimates based on RFLPs and AFLPs showed high correlations in studies with several crops such as barley (Russell *et al.*, 1997) and maize (Melchinger *et al.*, 1998). Comparisons of RFLPs with SSRs in maize also revealed high correlations in GD estimates (Smith *et al.*, 1997). Melchinger (1999) indicated that SSR and AFLP markers show great promise to complement or substitute RFLP for many applications in plant breeding and variety protection. The study conducted by Chavarriaga-Aguirre *et al.* (1999) on genetic diversity and redundancy in cassava core collections using microsatellite, isozymes and AFLPs showed that traditional markers have been highly effective at selecting unique genotypes for the core. In a comparative assessment of DNA fingerprinting techniques in tetraploid potato (*Solanum tuberosum* L.) germplasm, AFLPs identified the germplasm better than other techniques (McGregor *et al.*, 2000). Comparison of AFLP and morphological traits in a tef diversity study showed that diversity for the latter is lower than that for the former (Ayele and Nguyen, 2000). He and Prakash (1997) also found AFLP more efficient than DAF in identification of

polymorphic DNA markers in cultivated peanut (*Arachis hypogea* L.). Higher polymorphism was also detected with AFLP than RAPD (Wachira *et al.*, 2001). Paran *et al.* (1998) studied variation in *C. annuum* revealed by RAPD and AFLP markers and concluded that AFLP primers were four times more efficient than RAPD primers in their ability to detect polymorphism in pepper.

**Diallel analysis**

The diallel analysis has probably attracted more attention and has been the subject of more theoretical and practical application than any other mating design (Wright, 1985). The diallel concept is defined as making all possible crosses among a group of genotypes (Saghroue and Hallauer, 1997). Sprague and Tatum (1942) introduced the diallel-crossing concept to the field of plant breeding by making all possible matings among a set of maize inbred lines. The analysis has been used by breeders to obtain information on value of varieties as parents, to assess the gene action involved in various characters, and thereby develop appropriate selection procedures and understand heterotic patterns of the progenies at an early stage of the hybridization program (Egesel *et al.*, 2003; Le Gouis *et al.*, 2002; Saghroue and Hallauer, 1997; Virmani and Edwards, 1983).

***Combining ability***

Combining ability is defined as the performance of a line in hybrid combinations (Kambal and Webster, 1965). Griffing (1956) proposed four methods to analyze combining ability by using the genetic estimates of the parent and hybrid components of a diallel analysis, represented by general combining ability (GCA) and specific combining ability (SCA). GCA is expressed as the average performance of a line in hybrid combinations and SCA is defined as a case in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the line involved (Sprague and Tatum, 1942). Considering the GCA and SCA effects, inferences can be made about additive and non-additive gene effects. The GCA of each

parent ($g_i$) is important to develop superior genotypes while the SCA effect ($s_{ij}$) is important to provide information about hybrid performance (Cruz and Regazzi, 1994).

For self-pollinated crops, it is generally difficult to make breeding, experimental and commercial hybrids. On the other hand, for cross-pollinated crops, it is generally easy to make breeding and experimental hybrids and may be easy to make commercial hybrids. Peppers are considered as a self-pollinated crop (Allard, 1960). However, as noted by Bosland and Votava (2000), the rate of out-crossing is as high as 2 to 90% and several investigators argue that *Capsicum* should be considered as a facultative cross-pollinating species in field research. The amount of out-crossing is highly dependant on the natural pollinators such as bees and ants.

In a diallel study conducted on *Capsicum* by Zhenhui and Ming (1995) capsaicin, ascorbic acid, dry matter and sugar contents showed significant GCA effects. Zewdie *et al.* (2001) also found similar results for different capsaicinoid contents. Significant GCA and SCA were also observed in certain agronomic characters by Szwadiak and Kordus (1991). Moreover, Stevanovic *et al.* (1997) found highly significant differences concerning the GCA for fruit length, fruit width, pericarp thickness, fruit weight, number of fruits per plant and fruit yield. The authors also observed that additive gene effect had a more important role than the non-additive gene effect in inheritance of yield and its components in pepper genotypes.

*GCA:SCA ratio*

GCA:SCA mean square ratio was studied as an indicator of the nature of genetic variability in a diallel analysis (Sayed, 1978; Quick, 1978); a high value of the ratio indicates the prevalence of additive genes while a low value of the ratio indicates the prevalence of non-additive gene effects in determining a particular character. The relative importance of GCA and SCA could also be assessed from the components of variance by expressing them as the $2\sigma^2_{GCA}/(2\sigma^2_{GCA} + \sigma^2_{SCA})$ ratio (Baker, 1978). The closer this ratio to unity, the greater is the magnitude of additive genetic effects.

## *Variance components and heritability*

### *Variance components*

Quantitative genetics is concerned with the inheritance of traits that show continuous variation or quantitative traits (Wricke and Weber, 1986). Falconer and Mackay (1996) indicated that the amount of variation is measured and expressed in terms of variance. As it is noted by Falconer and Mackay (1996), the total variance is the phenotypic variance, or the variance of phenotypic values, and is the sum of the separate components. The partitioning of the variance into its components allows the breeders to estimate the relative importance of the various determinants of the phenotype, in particular the role of heredity versus environment.

The total variance of a given character is its phenotypic variance ($V_P$) and environmental variance ($V_E$) which is that part of the phenotypic variance attributed to environmental conditions (Falconer and Mackay, 1996). The total genetic variance ($V_G$) also known as variance of genotypic value, is the part of phenotypic value which can be attributed to genotypic differences among the phenotypes (Dudley and Moll, 1969). The authors further indicated that the total genetic variance is further portioned into additive genetic variance ($V_A$), dominance genetic variance ($V_D$) and epistatic genetic variance ($V_I$). The additive genetic variance, which is the variance of breeding values, is the important component. It determines the observable genetic properties of the population and the response of the population to selection. A primary goal of any plant breeding program is to develop and identify high yielding transgressive segregants. Kisha *et al.* (1997) indicated that populations with greater genetic variance are expected to produce higher yielding transgressive segregants than populations having lower genetic variance.

*Heritability*

The relative importance of heredity in determining phenotypic values is called the heritability of the character (Falconer and Mackay, 1996). There are two types of heritability, broad sense and narrow sense heritability. Hanson (1963) defined heritability in the broad sense as a consideration of total genetic variability in relation to genotypic variability, and heritability in narrow sense as consideration of only additive portion of the genetic variability in relation to phenotypic variability. Falconer and Mackay (1996) and Wricke and Weber (1986) defined broad-sense heritability as the ratio of genotypic to phenotypic variance, $V_G/V_P$. The ratio of $V_A/V_P$, narrow-sense heritability, expresses the extent to which phenotypes are determined by the genes transmitted from parents (Falconer and Mackay, 1996).

The knowledge of the relative heritabilities of the various traits and their genotypic and phenotypic correlation can aid in the design of efficient breeding systems where many traits need to be improved simultaneously (Jones, 1986). Heritability is not the measure of desirability (Jones, 1986); it determines the degree of resemblance between relatives and is therefore of the greatest importance in breeding programs.

Heritability estimates provide an indication of the expected response to selection in a segregating population and are useful tools in designing an effective breeding program (Burton and DeVane, 1953). Selection is effective when genetic variation in relation to environmental variation is high than when it is low. The net gain from selection depends upon the combined effect of the heritability, the amount of genetic variation present, and the selection intensity (Poehlman, 1987). Heritability in the narrow sense can be useful in making selection progress estimates. Characters with high narrow sense heritability values can be improved more rapidly with less intensive evaluation than those with low values and hence are useful in making selection progress estimates. Since broad-sense heritability includes non-additive effects, it overestimates the response to selection (Dudley and Moll, 1969). Sidwell *et al.* (1976) indicated that estimates of heritability

depend on the method used to estimate them, the population from which the estimates are derived and environmental conditions encountered during the test.

## Heterosis

The term heterosis was coined by Shull (1914). Shull (1952) described heterosis as the increased vigor, size, fruitfulness, speed of development, resistance to diseases and pests, or to climatic vigours of any kind. Rieger *et al.* (1976) defined heterosis as "the superiority of heterozygous genotypes with one or more characters in comparison with the corresponding homozygotes." Heterosis (or hybrid vigor) implies that there is dispersion for dominant alleles between the parents, which may increase or decrease the character. There are two prominent theories of heterosis called the dominance and the overdominance hypothesis (Crow, 1952). Heterosis under the dominance hypothesis is produced by the masking of deleterious recessive in one strain by dominant or partially dominant alleles in the second strain. Wricke and Weber (1986) noted that there are several hypotheses to genetically explain the phenomenon of heterosis: (1) partial dominance of large number of loci, (2) overdominance of several loci, and (3) several types of epistasis. The authors indicated that for hybrid breeding a substantial number of loci should show overdominance. Miranda Filho (1999) indicated that no heterosis can be detected if genes controlling the trait act in a strictly additive way (no dominance). Heterosis under the over-dominance hypothesis is due to heterozygote superiority and, therefore, increased vigor is proportional to the amount of heterosis.

Burton (1968) stated that heterosis results from combined action and interaction of allelic and non-allelic factors and is usually closely and positively correlated with heterozygosity. According to Morgan (1998), heterosis is brought about by bringing together in the $F_1$ the dispersed genes of dominant alleles showing directional dominance and non-allelic interactions, but not by heterozygote superiority or complementary epistasis. However, Coors *et al.* (1999) indicated that dominance and epistasis are the principal genetic factors in the exploitation of heterosis.

Heterosis can be expressed as mid-parent, better-parent and standard heterosis. Mid-parent heterosis or hybrid vigor is defined as the difference between the hybrid and the mean of the two parents (Falconer and Mackay, 1996). Lamkey and Edward (1999) noted that mid-parent heterosis or percentage of mid-parent is difficult to interpret from quantitative genetics point of view. Further, they indicated high-parent heterosis or the performance of $F_1$ hybrid over the better parent is preferred in some circumstances, particularly in self-pollinated crops, for which the goal is to find a better hybrid than either of the parents. From commercial point of view heterosis may be described as the degree of hybrid performance over the best available line variety (standard heterosis) (Virmani and Edwards, 1983).

The commercial exploitation of the phenomenon of heterosis is one of the most important contributions for plant breeding. Heterosis in plants has been used on large scale for the past several years, as carefully selected and produced hybrid cultivars (Duvick, 1999). As noted by Duvick (1999) field crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench.), and sunflower (*Helianthus annus* L.) are produced as hybrids in the entire industrialized world; they also are grown as hybrids in increasing amounts in the developing world. Hybrid rice (*Oryza sativa* L.) is grown extensively in China, and increasingly in India. The authors also noted that many of the commercial vegetable and flower crops are grown almost entirely as hybrids. Heterosis is credited for large increases in production per unit area, thus sparing large amounts of land for other uses.

Vegetable crops can be grouped according to how adaptable they are to hybrid production (Wehner, 1999). Pepper is a self-pollinated crop with high percentage of out-crossing. Dikil *et al.* (1973) reported yield heterosis of 28 to 47% with higher levels occurring when different ecological groups with different growth patterns were crossed. In a study done in Israel where peppers are grown for export, hybrids had a 9% advantage over inbred cultivars for total yield, but a 75% advantage over marketable yield (Shifris and Rylski, 1973). In general, pepper hybrids can exhibit moderately high heterosis for yield.

Hybrid peppers are becoming popular among farmers throughout the world (Berke, 2000). Peppers grown from hybrid seeds are highly uniform and usually higher yielding (Bosland and Votava, 2000). The importance of growing peppers from hybrid seeds can also be judged from the high price of pepper hybrid seeds. Berke (2000) noted that the price of pepper hybrid range from \$300 to \$25 000 $kg^{-1}$ depending on the company and where the seed is sold.

The unique utility and attraction of hybrid peppers is that they allow breeders to assemble, in one cultivar, complementary genes for disease resistance and for other important agronomic traits. Pepper hybrids can also be commercially successful. Expensive means of seed production, such as hand pollination, are feasible with peppers, because pepper is a high value crop, has low seed requirements, and relatively large number of seeds produced per pollination. The other important factor that makes pepper hybrid production feasible in countries like Ethiopia is the low labor cost.

The extent of heterotic response of the $F_1$ hybrid largely depends on the breeding value and genetic diversity of the parents included in crosses, and on the environmental conditions under which hybrids are grown (Bhatt, 1971; Knobel *et al.*, 1997; Jordaan, 1999; Young and Virmani, 1990). Cultivars are known to differ in their ability to combine with others when they are crossed. Identification of those specific combinations of parents is therefore essential in the exploitation of heterosis in agricultural crops (Bhatt, 1971; Jordaan *et al.*, 1999; Virmani and Edwards, 1983). Assessment of the extent of heterotic values for yield and agronomic characters between Ethiopian and exotic germplams is scanty.

### *Genetic diversity and heterosis/hybrid performance*

There are two phases, in general, in the development of improved commercial hybrids: development of lines and choice of those to be tested in hybrid combinations, and comparison of hybrids among the selected lines. In the development and selection of lines

attention is commonly given to genetic diversity in origin, important plant characters, and general combining ability of the lines.

Parental selection is the first step in any plant breeding program. Knowledge of genetic relationships among individuals or populations is essential to breeders for planning crosses in line and hybrid development. According to Link *et al.* (1996), the optimum strategy for dealing with different germplasm pools in a breeding program strongly depends on the type of variety to be developed. In breeding line cultivars, genotypes from different germplasm pools are often used as parents for 'wide crosses' to establish new base populations with a large genetic variance. Thus, germplasm pools are mixed with the final goal of combining favorable alleles from each source in new line cultivars. In contrast, divergent germplasm pools are important in hybrid breeding and are usually bred separately, because heterosis and hybrid performance are generally greater in inter-pool crosses (Melchinger *et al.*, 1992). Messmer *et al.* (1993) also demonstrated that crosses between genetically divergent parents are expected to yield a greater amount of heterosis in hybrids and a larger genetic variance among progenies in subsequent selfing generations than crosses of closely related parents. However, according to Moll *et al.* (1962), there is an optimum genetic distance between pools and exceeding it may actually decrease hybrid performance.

Genetic development is based on the selection of superior individuals in segregating populations (Barbosa-Neto *et al.*, 1997). Parents that have different alleles for more loci affecting a trait should produce hybrids with greater heterosis, if dominance is present (Falconer, 1981). Genetic progress through selection is related to the amount of variability present in the base population and the quality of genes contributed by the parents (Allard, 1960); as a consequence, the correct choice of parents can maximize genetic progress in a breeding program.

Breeders of authogamous crops every year produce a multitude of potentially useful crosses and evaluate their varietal ability (Gallais, 1979) by testing either selfed progenies or doubled haploid lines in field experiments. If breeders could predict the

prospects of crosses for line development before producing and testing lines derived from them in field trials, this would increase the efficiency of breeding programs by concentrating the efforts on the most promising crosses (Betran *et al.*, 2003; Bohn *et al.*, 1999).

Classical methods for identifying heterosis include the diallel crossing system (Griffing, 1956; Gardner and Eberhart, 1966). Heterosis in $F_1$ progeny has been used as a measure of genetic diversity between parents. Assuming that heterosis is a function of heterozygosity, heterosis should be an increasing function of parental diversity (Smith and Smith, 1989; Martin *et al.*, 1995). The performance of hybrids is associated with the level of heterosis, i.e., to the superiority of hybrids over their inbred parents. To exploit heterosis efficiently, populations are grouped into heterotic groups, where population crosses within and among groups produce low and high levels of heterosis, respectively. A heterotic group is a collection of closely related inbred lines. The co-ancestries within a heterotic group are usually high, whereas the co-ancestries between two heterotic groups comprising a heterotic pattern are usually low. Hybrids are then produced by crossing inbred lines from different heterotic groups. Generally, parents with a higher GCA and a long GD can produce a hybrid with better yield performance (Boppenmaier *et al.*, 1993; Cox and Murphy, 1990; Diers *et al.*, 1996). However, kinship as a measure of genetic diversity has limitations because of the simplifying assumptions of unrelated ancestral parents, no selection, and homozygous parents.

When intra- and inter-group hybrids are compared, inter-group hybrids showed greater genetic distance, mid-parent heterosis, and $F_1$ performance in maize (Dudley *et al.*, 1991). Inter-group hybrids also have the advantage with regard to more favorable ratio of GCA to SCA variances (Melchinger, 1999). Melchinger (1999) further indicated that with predominance of $\sigma^2_{GCA}$ over $\sigma^2_{SCA}$, early testing becomes more effective and promising hybrid can be identified and selected mainly based on their prediction from GCA effects, which makes hybrid breeding more efficient.

The identification of parental inbred lines that form superior hybrids or lines through a diallel crossing system is the most costly and time-consuming phase in hybrid development (Betran *et al.*, 2003). This is because it is necessary to cross the available inbred lines and evaluate the hybrids in extensive yield trials. Moreover, in some cases it is even difficult to evaluate all possible single-cross hybrid combinations between the available inbred lines because the number of possible hybrids is often prohibitive. Thus, because of space limitations only a portion of all the possible hybrids generated from a relatively small number of inbred parents can realistically be evaluated (Bernardo, 1992; Smith, 1986). In addition, trait expression is often highly influenced by environmental factors. *Per se* performance of inbred lines also does not predict the performance of hybrid. For example, maize grain yields (Hallauer and Miranda, 1988).

The development of molecular marker techniques has provided new tools for heterosis prediction and DNA markers have been used extensively in investigating correlations between parental GD and $F_1$ hybrid performance ($F_1P$) or mid-parent heterosis (MPH). Molecular breeding techniques are becoming more and more indispensable in many parts of the world.

Genetic diversity as revealed by molecular markers and its relationship with hybrid performance has been studied in other crops. However, the results were variable. The relationship between hybrid performance and genetic diversity in maize, oilseed rape and rice were significant (Ajmone-Marsan *et al.*, 1998; Bernardo, 1994; Boppenmair *et al.*, 1993; Diers *et al.*, 1996; Riaz *et al.*, 2001; Saghai Maroof *et al.*, 1997; Smith *et al.*, 1990; Zhang *et al.*, 1995). High correlation between GD and heterosis was also reported in sunflower (Cheres *et al.*, 2000). On the other hand, studies in wheat (Barbosa-Neto *et al.*, 1996; Chao *et al.*, 1989; Fabrizius *et al.*, 1998; Liu *et al.*, 1999; Kam-Morgan *et al.*, 1989; Martin *et al.*, 1995), rice (Kwon *et al.*, 2002; Xu *et al.*, 2002; Zhao *et al.*, 1999) and alfalfa (Riday *et al.*, 2003) have shown low correlations between hybrid performance and parental GD. Similar results were also found in soybean (Cerna *et al.*, 1997). It is assumed that this might be the low level of cross pollination in crops like wheat and soybean as compared to maize, rapeseed and rice. Molecular marker heterozygosity

would be predictive of hybrid performance when dominance effects are strong, allele frequencies are negatively correlated between parents, heritabilities are high, and there is linkage between most markers and quantitative trait loci (Bernardo, 1992). Application of such technique would be very important for pepper breeders in order to increase the efficiency of developing hybrids through identification of combination with strong yield heterosis.

Melchinger (1999) indicated that the potential application of DNA markers in hybrid breeding depends very much upon whether divergent heterotic groups have been established or not. He further indicated that if well-established heterotic groups are not available, marker-based GD estimates can be used to avoid producing and testing of crosses between related lines. In such cases DNA markers can be of great help to recognize groups of genetically similar and dissimilar materials.

Melchinger and Gumber (1998) recommended three criteria for choice of heterotic groups and patterns in hybrid breeding: (i) high mean performance and large genetic variance in the hybrid population; (ii) high *per se* performance and good adaptation of the parent populations to the target region(s); and (iii) low inbreeding depression, if the hybrids are produced from inbreds. It was also indicated that with a smaller number of populations evaluation of diallel crosses is a common practice. However, where a large amount of germplasm exists but no established heterotic groups are available, it is important to first identify groups of genetically similar germplasm (Melchinger, 1999). One can then produce and evaluate diallel crosses among the representative genotypes in each group and finally select promising groups as heterotic groups or patters.

According to Poehlman (1987), desirable crosses may be found if the parental varieties do not differ greatly in genotype so that fewer genes and less segregation are involved assuming that both parents are relatively satisfactory already. Thus, a cross between two high yielding varieties normally produce more high yielding segregants than a cross between one or both of the parents that are low yielding. Busch *et al.* (1974) also pointed out that the probability of recovering a superior progeny genotype is greater if both

parents are similar in performance as opposed to one parent being inferior for one or more traits. Genetic diversity between parents, yet, is important to derive transgressive segregants from a cross.

Although GD often fails to correlate with heterosis, it has been successfully used to classify individuals into heterotic groups (Cheres *et al.*, 2000; Sant *et a*l., 1999; Chowdari *et al.*, 1998; Ajmone-Marsan *et al.*, 1998). In either case such a study has not been reported in pepper breeding. Intra-specific crosses have advantages over inter-specific crosses and are widely utilized by many pepper breeding programs. Lefebvre *et al.* (1995) reported that low fertility and recombination rates are found in inter-specific crosses. Predicting the prospects of crosses of different forms of peppers for line development before producing and testing lines derived from the crosses in field trials may increase the efficiency of pepper breeding programs by concentrating the efforts on the most promising crosses.

***Heterosis and performance in multiple cross hybrids***

Hybrids are not always single crosses, but three-way and double cross hybrid varieties also exist. The single-cross hybrid is the product of a cross between two inbred lines. The inbred lines are chosen on the basis of general combining ability tests for their ability to mate and to produce vigorous and productive progeny. On the other hand, the double cross is a cross between two single cross hybrids. Similarly, the three-way cross hybrid is a cross between a single cross and an inbred line. Thus, double and three-way cross hybrids are less uniform than single cross hybrids.

The values of three-way and double crosses can be estimated from single crosses (Wricke and Weber, 1986). In the case of no epistasis the values of three-way and double crosses can be predicted using all the single crosses not directly involved in the three-way and double crosses (Wricke and Weber, 1986) as:

$$T_{ij.l} = \frac{1}{2}(S_{il} + S_{jl})$$

$$D_{ij.lm} = \frac{1}{4}(S_{il} + S_{im} + S_{jl} + S_{jm})$$

According to Wricke and Weber (1986), to develop three-way or double cross hybrids the breeder can use the following selection procedure: (i) selecting parents on general combining ability, (ii) making all $\frac{K(K-1)}{2}$ single crosses between the selected parents, (iii) predicting the values of the three-way or double crosses, and (iv) testing only those three-way or double crosses with high predicted values.

Literature shows that the cost of corn hybrid seeds on inbred lines was prohibitive in the past as the inbred lines are weak and unproductive. Jones (1918) suggested crossing two single-cross hybrids and producing double cross-hybrid seed, which made the production of hybrid seed corn economically feasible because the seed would be produced on vigorous hybrid plants instead of weak inbred plants. However, single or three-way crosses have replaced double crosses for the production of commercial corn hybrid seeds (Poehlman, 1987). The change from double- to single-cross hybrids become possible because, as a result of effective management, modern inbred lines are more vigorous and produce larger and more uniform kernels than earlier inbred lines in corn.

In hybrid breeding programs, the three types of hybrids have their own merits and demerits. Considering all possible hybrids from a given sample of inbred lines, there is a decline expected in genetic variance and consequently in the highest predicted yield potential from single to three-way to double to top crosses (Cockerham, 1961). Thus, the maximum yield performance will be found among single-cross hybrids.

The reverse of the above is true considering yield stability. The degree of uniformity decreases from single cross to double cross hybrids. Uniformity offers an advantage for the farmer. However, there may be a danger that varieties with a high level of uniformity are not as stable and show a larger interaction with locations and years. Experimental

results on maize (Eberhart and Russel, 1969; Weatherspoon, 1970), sorghum (Patanothai and Atkins, 1974) and rye (Becker *et al.*, 1982) have, in general, confirmed this expectation.

Successful new varieties must show high performance for yield and other characters. Moreover, their superiority should be reliable over a wide range of environmental conditions. An ideal variety is the one that combines high yield with stability of performance (Eberhart and Russell, 1966). The use of three-way or double crosses can reduce the risk of failing to detect the best single cross because more than one single cross is used for the prediction of the best three-way or double cross. No study has been undertaken on the comparison of different types of pepper hybrids in Ethiopia. Pepper performance stability is very important in Ethiopia, where environmental conditions vary considerably and the means of modifying the environment are inadequate. Stability of cultivar performance is very important when target environments are different from selection environments. Lack of high yielding and stable varieties is one of the major problems in pepper production in Ethiopia.

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# CHAPTER 3

## Genetic variability as measured by morphological data and amplified fragment length polymorphism markers


### ABSTRACT

Data on genetic diversity levels among available pepper genotypes will increase efficiency of the Ethiopian pepper breeding-program. The objectives of this study were to group pepper (*Capsicum annuum* L.) genotypes into clusters according to their distance as measured by morphological traits and amplified fragment length polymorphisms (AFLP) markers and to assess the relationships between the genetic distances determined by the two methods. Thirty-nine pepper genotypes obtained from different countries were grown in the greenhouse at the University of the Free State, South Africa during 2001 and 2002. The experiments were laid out in randomized complete block design with three replications. A total of 20 different quantitative and qualitative traits and six AFLP primer pairs were used to determine pairwise genetic distances. Both methods showed moderately high genetic distances among the different genotypes indicating high genetic diversity among them. On the other hand, the mean genetic distance among Ethiopian genotypes was lower than that between the Ethiopian and the exotic ones. The dendrogram based on morphological data clustered the genotypes on the basis of fruit size. The same was found with AFLP data, where genotypes with similar fruit sizes clustered together. Significant, positive correlation was observed between the two methods of diversity measurements. Thus, the combination of morphological data and AFLP markers provides a useful measure of genetic distance in peppers. The narrow genetic basis among the Ethiopian cultivars suggests that the pepper breeding-program of Ethiopia should focus on enriching its germplasm through local collections and introductions from other parts of the world.

## INTRODUCTION

The knowledge of genetic similarity and dissimilarity among and within crop cultivars is of vital importance for breeders. Data on genetic diversity levels among germplasm sources can be used to increase the efficiency of breeding efforts to improve crop species. Several methods to measure genetic distances such as phenotypic descriptors and molecular markers have been widely used in crop diversity studies. The importance of using molecular markers as an additional tool for varietal description was emphasized, as the genetic controls of morphological traits are mostly polygenic and their expression depends on environmental factors (Soller and Beckmann, 1983, Smith and Smith, 1989). Molecular markers have proved to be invaluable for understanding the genetic make-up of agricultural crops. They differ from phenotypic traits in that molecular markers usually occur in greater numbers, they can be distinguished without relying on the complete development of the plant and their expression is not altered by the environment (Tanksley, 1983).

Lee (1995) noted that DNA markers can be used in germplasm management, which is a multifaceted endeavour involving acquisition, maintenance, and characterization such that the plant genetic resources are conserved and utilized for crop improvement. DNA markers help to assess the degree to which a collection's gene pool overlaps with the natural population or other collections. Traditionally, this has been accomplished on the basis of morphological variation. However, this becomes more difficult with large collections, where phenotypic differences are not always distinct.

Amplified fragment length polymorphism (AFLP), developed by Vos *et al.* (1995), is a short, rapid, reproducible, multiplex assay with the ability to generate large numbers of polymorphic genetic loci. This technique is being used extensively for genetic mapping and fingerprinting in plants. It has been used to analyze diversity in many crop species (Mackill *et al.*, 1996; Paul *et al.*, 1996). AFLP also allows the retrospective analysis of the consequences of breeding and selection for the production of new lines. The information obtained from AFLPs can also be used to facilitate the strategic planning of

new breeding approaches based on combining and selecting new genotypes to maximize the rate of line improvement (Ellis *et al.*, 1997).

The level of genetic diversity among cultivated crops depends on their reproductive behavior. Among others, out-crossing species such as maize (Smith, 1988), *Brassica* (Figdore *et al.*, 1988) and potato (Gebhardt *et al.*, 1989) show high levels of genetic diversity. Conversely, autogamous species like soybean (Apuya *et al.*, 1988), tomato (Miller and Tanksley, 1989) and wheat (Chao *et al.*, 1989) show a relatively low level of polymorphism between cultivars.

Cultivated peppers are preferably self-pollinated; however in open-field conditions a high rate of allogamy can occur (Tanksley, 1984; Belleti and Quagliotti, 1989). Lefebvre *et al.* (1993) noted that *Capsicum annuum* is fairly variable compared to other self-pollinated crop species. They hypothesized that the intermediate level of variation in *C. annuum* can be related to the reproductive behavior of domesticated pepper and the way in which this has occurred.

Various types of pepper cultivars are grown worldwide. Peppers are classified based on fruit characters as pimento, squash or cheese, ancho, long green chile, cayenne, jalapeno, small hot, cherry and Tabasco (Greenleaf, 1986). Grouping of landraces and improved cultivars is useful for the study of evolutionary relationships of the taxon and consequently the history of the crop. It is also useful for determining unrelated genotypes with a view to breeding new varieties.

The genetic diversity among and between *Capsicum* species has been investigated in previous studies using morphological, cytogenetical and molecular markers such as isozymes, restriction fragment length polymorphism, random amplified polymorphic DNA and AFLP (Conicella *et al.*, 1990; Lefebvre *et al.*, 1993; 2001; Nam *et al.*, 1997; Paran *et al.*, 1998; Pickersgill, 1988; Prince *et al.*, 1992; Zewdie and Zeven, 1997).

Although the production and utilization of different forms of pepper cultivars as spice and vegetable crop in Ethiopia has been in effect since time immemorial, the advance of pepper breeding in the country is still in its infant stage. Only a limited number of accessions have been collected from some regions of the country and from some international research institutes for crossing programs and have not yet been analyzed and compared using molecular markers for their genetic divergence. The objectives of this study were thus (i) to analyze the genetic divergence of locally available and exotic pepper genotypes using morphological data and AFLP markers and (ii) to assess the relationship between genetic distances based on morphological traits and AFLP markers.

## MATERIALS AND METHODS

### Plant materials

Thirty-nine pepper cultivars of different geographical origins (Table 3.1) were used. The collection comprised four varietal groups that were classified based on fruit shape, size and pungency, namely cherry, pungent elongated-fruit, bell pepper and paprika.

Four of the genotypes, namely Mareko Fana, Bakko Local, Mareko Shote and Mareko Dube were Ethiopian cultivars that are at present widely grown for local consumption and agro-industries. Papri King, Papri Queen and Caloras PS are grown for commercial paprika production. PBC 142A, PBC 375, PBC 223, PBC 602, PBC 612, 9852-90 and 9852-91 have been obtained from the Asian Vegetable Research and Development Center (AVRDC) and are being used as breeding lines. Further, 25 genotypes were obtained from different geographical regions.

The seedlings of the 39 accessions were raised in seedling trays with 200 cone-shaped cavities filled with growing medium. At about 50 days after sowing, the seedlings were transplanted into 20 cm polythene pot filled with pseudo duplex soil type in the greenhouse during 2001 and 2002 at the University of the Free State, South Africa. Each

pot had 19 and 24 cm base and top diameter, respectively. The experiments were planted in a randomized complete block design with three replications. Two plants were grown per pot and were fertilized with hydroponic nutrient.

Table 3.1. List of pepper genotypes used for the variability studies.

| Acc. No. | Genotype | Type | Origin/Source |
|---|---|---|---|
| 1 | C00916 | Cherry | Hungary |
| 2 | Bakko Local | Pungent elongated-fruit | Ethiopia |
| 3 | Mareko Shote | Pungent elongated-fruit | Ethiopia |
| 4 | Mareko Dubbe | Pungent elongated-fruit | Ethiopia |
| 5 | Kalocsai "A" Cseresznye | Cherry | Hungary |
| 6 | Mareko Fana | Pungent elongated-fruit | Ethiopia |
| 7 | Szegedi 20 | Pungent elongated-fruit | Hungary |
| 8 | PBC 142A | Pungent elongated-fruit | India |
| 9 | C01994 | Pungent elongated-fruit | Turkey |
| 10 | C03018 | Pungent elongated-fruit | Turkey |
| 11 | Pepper 1972 | Bell | Israel |
| 12 | Pepper1976 | Bell | Israel |
| 13 | Joyang | Pungent elongated-fruit | South Africa |
| 14 | Fire Bomb | Pungent elongated-fruit | Korea |
| 15 | C05809 | Pungent elongated-fruit | USA |
| 16 | PBC 375 | Pungent elongated-fruit | Indonesia |
| 17 | C05692 | Pungent elongated-fruit | Indonesia |
| 18 | PBC 602 | Pungent elongated-fruit | AVRDC |
| 19 | PBC 223 | Pungent elongated-fruit | AVRDC |
| 20 | PBC 612 | Pungent elongated-fruit | AVRDC |
| 21 | 9852-90 | Pungent elongated-fruit | AVRDC |
| 22 | 9852-91 | Pungent elongated-fruit | AVRDC |
| 23 | Quick Set | Paprika | South Africa |
| 24 | Brin III | Paprika | South Africa |
| 25 | Papri King | Paprika | Ethiopia |
| 26 | Papri Queen | Paprika | Ethiopia |
| 27 | Caloras PS | Paprika | Ethiopia |
| 28 | Grande Rico 66 | Bell | South Africa |
| 29 | Florid RG | Bell | South Africa |
| 30 | C01613 | Bell | China |
| 31 | C03796 | Bell | China |
| 32 | C03804 | Bell | China |
| 33 | C03810 | Bell | China |
| 34 | C01132 | Bell | Czechoslovakian |
| 35 | Pepper 1038 | Bell | Israel |
| 36 | Kalocsai V-2 | Pungent elongated-fruit | Hungary |
| 37 | Szegedi 178 | Pungent elongated-fruit | Hungary |
| 38 | Kalocsai 801 | Pungent elongated-fruit | Hungary |
| 39 | Kalocsai "M" Cseresznye | Cherry | Hungary |

## Morphological characterization

The following 20 quantitative and qualitative traits were measured in each plot based on IPGRI *et al.* (1995):

1. *Days to flowering*: number of days from sowing to the first open flower
2. *Corolla color*: recorded at full blooming: 1 (white), 2 (purple)
3. *Flower position*: recorded at anthesis: 3 (Pendant), 5 (Intermediate), 7 (Erect)
4. *Fruit color at intermediate stage*: recorded before the ripening stage: 1 (Light yellow), 2 (Light green), 3 (Green), 4 (Deep green)
5. *Mature fruit color*: 1 (Light red), 2 (Red), 3 (Dark red), 4 (Brown)
6. *Days to maturity*: number of days from sowing to first mature fruit
7. *Fruit maturation period*: number of days from flowering to maturity
8. *Leaf color:* 1 (Green), 2 (Dark green), 3 (Dark purple)
9. *Fruit shape*: 1 (Elongate), 2 (Almost round), 3 (Blocky)
10. *Fruit length* (cm): recorded at the second harvest
11. *Fruit width* (cm): measured at point of maximum width
12. *Fruit weight* (g): measured at the second harvest
13. *Pericarp thickness* (mm): recorded at the second harvest
14. *Pedicel length* (cm): the length of pedicel from its tip to its attachment to the fruit
15. *Plant height* (cm): measured immediately after first harvest
16. *Canopy width* (cm): measured immediately after first harvest, at widest point
17. *Arial biomass* (g): fresh plant weight above soil level taken after the last harvest
18. *Fruit number*: total fruit number per plant from first harvest to last harvest
19. *Fruit yield* (g/plant): total fresh fruit yield from first harvest to last harvest
20. *Harvest index* (%): calculated as $HI = \frac{FY}{ABM + FY} \times 100$, where, FY = fruit yield, and ABM = aerial biomass

## Statistical analyses

Combined analyses of variances for the quantitative traits for the total and the four varietal groups were separately done, and the means and the least significant differences of the combined analysis of variance over the two years were determined for each quantitative character using AGROBASE 2000 software (Agrobase, 2000).

In developing the binary data (one for present and zero for absent), the method of Gerdes and Tracy (1994) was followed. Based on the results obtained from the analysis of variance for the quantitative traits, the genotypes were compared pairwise for each trait, and if two inbreds were not significantly different from each other, they were given a score of one for that trait. Likewise, if the two inbreds were significantly different they were given a score of zero. For qualitative characters, scores of one or zero were given to the genotypes depending on the presence or absence of that trait, respectively. Genetic distances and the resulting dendrogram were determined via the unweighted pair group mean (UPGMA) method using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998).

## AFLP marker assay

### *DNA extraction*

DNA was extracted from approximately 1 g of young fresh leaves collected on ice following the modified monocot extraction protocol (Edwards *et al.*, 1991). The plant material was ground to a fine powder with a pestle and mortar in liquid nitrogen. Thereafter, 10 ml pre-heated (65°C) extraction buffer (1 ml 5M NaCl, 2 ml 0.5M Tris-HCl pH 8.0, 2 ml 0.25M EDTA, 0.625 ml 20% SDS and 4.2 g Urea), 1 ml Cetyl triethyl ammonium bromide (CTAB) buffer (0.2 ml 1M Tris-HCl pH 8.0, 0.2 ml 0.25M EDTA and 0.1 g CTAB) and 2 ml 5M NaCl were added to the macerated tissue, which was mixed and incubated at 65°C for one hour with periodic vortexing every 10 to 20 minutes. The amount of urea was as determined by Prince *et al.* (1997) for the

preparation of genomic DNA from *Capsicum* sp. After incubation, 10 ml chloroform-isoamyl alcohol (24:1 v/v) was added followed by centrifugation for 15 min at 10 000 rpm at room temperature. The DNA was precipitated overnight at 4°C after adding 100% cold ethanol in a 1:2-ratio. The precipitate was spooled, washed in 70% ethanol three times and re-suspended in 0.5 to 1 ml sterile distilled water and stored at -20°C.

*DNA concentration determination and purity assessment*

The concentration and purity of genomic DNA were determined using spectrophotometry at 260 and 280 nm. The concentration was calculated as:

$$[\text{DNA}] = \text{Optical density } (OD_{260}) \times \text{dilution} \times \text{constant } (50\ \mu g/ml)$$

The quality of genomic DNA was assessed by 1% agarose electrophoresis in 0.5× TAE buffer (2.42 g Tris, 0.57 ml acetate and 0.15 g EDTA pH 8.0) for approximately 60 min at 80 volts and visualized under UV light by the inclusion of ethidium bromide in the gel. The integrity and concentration of DNA was visualized and confirmed against the standard DNA Marker III.

***DNA digestion and ligation***

The DNA (250 ng) was double digested in 5 µl 5× reaction buffer (50 mM Tris HCl pH 7.5, 50 mM Mg-Acetate and 250 mM K-Acetate), 2 µl *EcoRI* and *MseI* endonuclease and sterile distilled water to make the total volume 25 µl. The reaction was centrifuged and incubated at 37°C for 2 hours. Thereafter, the mixture was incubated for 15 min at 70°C to inactivate the reaction endonuclease. The digested DNA fragments were ligated to *EcoRI* and *MseI* adaptors (Table 3.2) by mixing 25 µl double digest DNA, 24 µl adapter ligation solution, and 1 µl T4 DNA ligase [(1 unit/µl in 10 mM Tris-HCl pH 7.5, 1 mM DTT, 50 mM KCl and 50% glycerol (v/v)] and incubated at 20°C for 2 hours. A 1:10 dilution of the ligation mixture was made in TE (10 mM Tris-HCl pH 8 and 0.1 mM EDTA) and stored at -20°C.

### *PCR amplification reaction*

AFLP amplification was performed in two steps, namely pre-selective and selective amplification reactions (Vos *et al.*, 1995). Pre-selective reactions were performed in reactions containing 5 µl diluted 1:10 template DNA, 40 µl pre-amplification primer mix, 5 µl 10× PCR buffer $MgCl_2$ and 1 unit of Taq polymerase. The product of pre-selective reactions was (1:50) with TE. For selective amplification, 5 µl pre-selective template DNA, 5.5 µl consisting of 6.7 ng/µl *MseI* primer (containing dNTPs) and *EcoRI* primer (Table 3.2) and 9.5 µl containing 1.9 µl 10× PCR buffer (200 mM Tris-HCl pH 8.4, 15 mM $MgCl_2$ and 500 mM KCl) and 0.1 µl Taq polymerase were mixed and gently centrifuged. Selective PCR amplification was carried out for 35 cycles at 94°C for 30 sec, 65°C for 60 sec (with a temperature reduction of 0.7°C per cycle for 12 cycles to 56°C) followed by 72°C for 120 sec. Following selective amplification, 5 µl was added to 24 µl formamide and 1 µl Rox standard size marker, denatured at 94°C for 10 min, quick cooled using ice slush and resolved on a Perkin Elmer ABI Prism 310 Automated capillary sequencer (PE Biosystems).

Table 3.2. AFLP adapter and primer sequence used for ligation, pre-selective and selective amplification reactions.

| Adapter/Primer | Sequence |
|---|---|
| Adapter | |
| *EcoRI* | 5'-CTCGTAGACTGCGTACC-3' |
| | 3'-CATCTGACGCATGGTTAA-5' |
| *MseI* | 5'-GACGATGAGTCCTGAG-3' |
| | 3'-TACTCAGGACTCAT-5' |
| Primer | Sequence (5'-3') |
| *MseI* | GATGAGTCCTGAGTAA |
| M-CAA | GATGAGTCCTGAGTAACAA |
| M-CTG | GATGAGTCCTGAGTAACTG |
| M-CTA | GATGAGTCCTGAGTAACTA |
| *EcoRI* | GATCTGCGTACCAATTC |
| E-ACA | GATCTGCGTACCAATTCACA (FAM labeled) |
| E-AAC | GATCTGCGTACCAATTCAAC (NED labeled) |

**Data collections and statistical analyses**

The AFLP fragments were analyzed using GeneScan® software (PE Biosystems). Only clear and unambiguous bands were coded as present (1) or absent (0) for all the genotypes. A matrix of binary data was constructed with columns equal to genotypes and rows equal to molecular marker distances. The body of the matrix was filled in with zeros and ones, for absence and presence of the fragments for each genotype, respectively. Every fragment detected was treated as an independent character. All monomorphic loci were excluded from the analyses.

Euclidean distances were calculated from a pairwise comparison of taxa in the data matrix to represent the genetic distances using the NCSS 2000 statistical package as well

as cluster analysis using agglomerate hierarchal clustering (Hintze, 1998). The goodness of fit of the denderogram was confirmed by cophenetic correlation.

Correlations between genetic distance values generated from morphological and AFLP data for the total and the four varietal groups were determined using AGROBASE 2000 software (Agrobase, 2000).

## RESULTS AND DISCUSSION

### Morphological traits

The combined analyses of variances for 14 quantitative characters are presented in Table 3.3. The genotypes showed statistically significant differences for all characters indicating a high level of genetic variability in the genotypes studied. Year and genotype × year interaction generally had significant effects on all measured characters indicating that the performance of the genotypes vary with the environments. Table 3.4 shows the mean performance of the studied genotypes over two years (2001 and 2002). Kalocsai "M" Cseresznye, Kalocsai V-2, Szegedi 20 and Mareko Dube had significantly lower number of days for traits of earliness (DF, DM and FMP). Pepper 1038 and Pepper 1972 had wider FD, high FWT, thicker pericarp and high FY. The longest fruits were observed in Papri King, Papri Queen and C03018. Genotype PBC 142A had the largest FN per plant. On the contrary, several genotypes showed significantly low FL, FWT, FD and PCT indicating that the studied population was diverse enough to develop genotypes of required characters (low or high) through crossing programs. Pictures of some genotypes included in the study are shown in Fig. 3.1.

Table 3.3. Combined analyses of variance for quantitative characters in diverse pepper genotypes.

| Source | df | DF | DM | FMP | PH | CW | FL | FD | FWT | PCT | PL | FN | FY | ABM | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year (Y) | 1 | 22882.9** | 3963.1** | 7800.0** | 27592.7** | 4765.5** | 11.3** | 7.8** | 6554.0** | 8.0** | 18.6** | 306.9* | 355664.4** | 95210.9** | 35.0 |
| Genotype (G) | 38 | 520.8** | 825.3** | 231.9** | 1061.2** | 515.4** | 80.5** | 28.8** | 13259.8** | 22.3** | 4.7** | 2877.2** | 21464.0** | 3893.5** | 764.2** |
| G×Y | 38 | 75.9** | 153.3** | 166.6** | 168.0** | 131.5* | 3.9** | 0.5** | 757.5** | 1.0** | 1.5** | 275.0** | 9225.6** | 1732.0** | 104.4** |
| Error | 152 | 22.3 | 44.2 | 52.1 | 101.0 | 84.3 | 1.7 | 0.2 | 245.3 | 0.4 | 0.7 | 81.3 | 2650.5 | 410.0 | 50.5 |

DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, CW = canopy width, FL = fruit length, FD = fruit diameter, FWT = fruit weight, PCT = pericarp thickness, PL= pedicel length, FN = fruit number, FY = fruit yield, ABM = aerial fresh biomass, HI = harvest index, $^{*}P < 0.05$, $^{**}P < 0.01$

Table 3.4. Mean performance of diverse pepper accessions tested over two years.

| Acc. No.+ | CC | FP | LC | IFC | MFC | FSP | FY | DF | DM | FMP | PH | CW | FL | FD | FWT | PCT | PL | FN | ABM | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 7 | 1 | 4 | 3 | 2 | 139.0 | 86 | 146 | 60 | 86.5 | 41.8 | 3.0 | 2.0 | 4.1 | 3.2 | 2.5 | 51 | 134.4 | 48.0 |
| 2 | 1 | 3 | 1 | 3 | 2 | 1 | 144.5 | 94 | 181 | 87 | 84.7 | 40.3 | 11.5 | 2.1 | 14.8 | 2.6 | 5.2 | 13 | 105.5 | 57.5 |
| 3 | 1 | 3 | 1 | 3 | 4 | 1 | 174.6 | 84 | 158 | 74 | 84.2 | 54.8 | 12.4 | 2.5 | 19.6 | 3.1 | 6.3 | 11 | 90.1 | 64.8 |
| 4 | 1 | 3 | 1 | 3 | 4 | 1 | 257.2 | 69 | 136 | 67 | 81.7 | 59.2 | 14.4 | 2.2 | 19.5 | 2.4 | 5.7 | 15 | 165.3 | 62.8 |
| 5 | 1 | 3 | 1 | 4 | 3 | 2 | 73.0 | 74 | 142 | 68 | 70.2 | 42.0 | 3.2 | 2.4 | 7.4 | 3.5 | 3.7 | 13 | 71.7 | 49.5 |
| 6 | 1 | 5 | 1 | 3 | 4 | 1 | 169.8 | 84 | 152 | 68 | 79.7 | 45.5 | 11.2 | 2.6 | 16.6 | 2.2 | 5.8 | 13 | 84.5 | 65.0 |
| 7 | 1 | 5 | 1 | 3 | 3 | 1 | 108.9 | 69 | 137 | 68 | 58.7 | 39.5 | 11.1 | 2.4 | 24.3 | 3.0 | 7.0 | 5 | 60.6 | 64.0 |
| 8 | 1 | 3 | 1 | 2 | 1 | 1 | 97.7 | 99 | 164 | 65 | 83.8 | 54.2 | 4.1 | 0.9 | 1.3 | 0.8 | 3.7 | 107 | 117.6 | 45.0 |
| 9 | 1 | 3 | 1 | 2 | 1 | 1 | 178.7 | 76 | 144 | 68 | 66.8 | 44.2 | 11.1 | 3.5 | 27.4 | 2.4 | 4.5 | 9 | 120.2 | 59.7 |
| 10 | 1 | 3 | 1 | 2 | 2 | 1 | 150.3 | 74 | 144 | 70 | 70.7 | 46.8 | 16.2 | 2.1 | 16.8 | 1.8 | 4.8 | 14 | 73.7 | 66.9 |
| 11 | 1 | 5 | 2 | 4 | 3 | 3 | 261.0 | 82 | 154 | 72 | 69.3 | 33.8 | 11.2 | 7.7 | 142.3 | 7.0 | 5.3 | 3 | 89.7 | 72.9 |
| 12 | 1 | 5 | 2 | 3 | 3 | 3 | 250.8 | 81 | 149 | 68 | 71.5 | 41.2 | 12.4 | 7.7 | 146.2 | 7.9 | 6.4 | 2 | 99.8 | 71.3 |
| 13 | 1 | 3 | 1 | 3 | 2 | 1 | 102.6 | 80 | 158 | 78 | 83.2 | 58.0 | 9.7 | 1.8 | 10.7 | 2.0 | 5.1 | 16 | 79.4 | 55.3 |
| 14 | 1 | 3 | 1 | 4 | 2 | 1 | 106.3 | 99 | 175 | 76 | 95.7 | 66.7 | 8.8 | 1.3 | 3.4 | 1.8 | 5.5 | 53 | 109.0 | 47.6 |
| 15 | 2 | 7 | 3 | 5 | 2 | 1 | 33.9 | 90 | 157 | 67 | 53.3 | 27.7 | 1.7 | 1.0 | 1.1 | 1.0 | 3.6 | 50 | 81.8 | 26.6 |
| 16 | 1 | 3 | 2 | 4 | 2 | 1 | 118.6 | 83 | 157 | 73 | 74.2 | 58.8 | 9.0 | 1.4 | 5.9 | 1.8 | 3.9 | 32 | 98.6 | 53.1 |
| 17 | 1 | 3 | 2 | 3 | 2 | 1 | 124.6 | 83 | 157 | 74 | 78.5 | 56.2 | 9.9 | 1.9 | 7.8 | 1.6 | 4.6 | 22 | 91.5 | 56.4 |
| 18 | 1 | 3 | 1 | 3 | 2 | 1 | 139.1 | 87 | 173 | 87 | 76.0 | 52.8 | 7.8 | 1.1 | 4.9 | 1.5 | 5.1 | 28 | 114.3 | 53.2 |
| 19 | 1 | 3 | 1 | 3 | 1 | 1 | 168.7 | 92 | 163 | 71 | 82.7 | 54.3 | 10.0 | 1.4 | 8.2 | 2.2 | 4.7 | 37 | 151.2 | 52.2 |
| 20 | 1 | 3 | 1 | 3 | 2 | 1 | 69.8 | 117 | 177 | 60 | 114.5 | 45.2 | 4.4 | 0.9 | 1.7 | 1.1 | 4.1 | 69 | 165.5 | 29.0 |
| 21 | 1 | 3 | 1 | 4 | 2 | 3 | 152.1 | 81 | 150 | 69 | 74.0 | 36.0 | 9.4 | 1.4 | 9.6 | 1.0 | 5.5 | 31 | 97.8 | 60.9 |
| 22 | 1 | 7 | 1 | 3 | 2 | 1 | 153.8 | 89 | 163 | 74 | 72.3 | 49.8 | 9.3 | 2.0 | 11.6 | 2.1 | 5.5 | 19 | 91.6 | 58.4 |
| 23 | 1 | 3 | 1 | 3 | 2 | 1 | 167.2 | 80 | 147 | 68 | 74.8 | 53.0 | 14.2 | 2.9 | 28.0 | 2.8 | 5.5 | 8 | 90.0 | 63.9 |
| 24 | 1 | 3 | 1 | 3 | 3 | 1 | 143.6 | 77 | 150 | 73 | 72.0 | 56.8 | 14.8 | 3.1 | 30.3 | 2.3 | 4.8 | 6 | 101.0 | 58.1 |
| 25 | 1 | 3 | 1 | 3 | 3 | 1 | 150.5 | 84 | 161 | 77 | 80.5 | 51.3 | 16.6 | 2.7 | 29.1 | 2.7 | 4.5 | 7 | 76.9 | 65.7 |
| 26 | 1 | 3 | 1 | 3 | 3 | 1 | 186.2 | 81 | 149 | 68 | 71.3 | 48.5 | 16.6 | 2.9 | 36.3 | 2.8 | 5.1 | 7 | 94.6 | 67.0 |
| 27 | 1 | 5 | 2 | 1 | 1 | 1 | 134.7 | 79 | 141 | 62 | 58.3 | 46.2 | 7.6 | 2.7 | 16.0 | 2.6 | 4.3 | 11 | 88.9 | 60.7 |
| 28 | 1 | 5 | 1 | 3 | 3 | 3 | 206.0 | 82 | 149 | 67 | 49.2 | 32.5 | 11.3 | 6.9 | 107.4 | 6.4 | 4.0 | 2 | 68.2 | 73.9 |
| 29 | 1 | 5 | 2 | 3 | 2 | 3 | 151.1 | 90 | 151 | 61 | 61.3 | 30.8 | 10.2 | 6.0 | 82.7 | 5.3 | 4.9 | 3 | 81.2 | 66.9 |
| 30 | 1 | 7 | 2 | 2 | 2 | 3 | 198.5 | 75 | 146 | 71 | 56.0 | 41.0 | 9.5 | 5.3 | 57.2 | 3.3 | 4.3 | 4 | 85.9 | 68.9 |
| 31 | 1 | 5 | 1 | 3 | 2 | 3 | 230.3 | 84 | 148 | 65 | 55.3 | 34.2 | 11.5 | 6.9 | 106.5 | 6.1 | 4.1 | 3 | 83.7 | 71.9 |
| 32 | 1 | 5 | 2 | 2 | 2 | 3 | 233.8 | 84 | 149 | 65 | 56.3 | 38.5 | 12.3 | 6.8 | 112.8 | 5.8 | 4.3 | 3 | 91.9 | 71.1 |
| 33 | 1 | 5 | 1 | 3 | 2 | 3 | 259.7 | 81 | 150 | 79 | 51.7 | 35.0 | 11.8 | 7.0 | 101.3 | 6.2 | 3.9 | 4 | 76.3 | 75.7 |
| 34 | 1 | 7 | 2 | 2 | 2 | 3 | 204.2 | 76 | 143 | 67 | 60.0 | 36.3 | 11.0 | 5.8 | 60.6 | 4.2 | 4.2 | 4 | 93.0 | 66.5 |
| 35 | 1 | 7 | 2 | 3 | 3 | 3 | 300.2 | 83 | 156 | 73 | 57.8 | 36.8 | 12.8 | 7.6 | 182.7 | 8.3 | 5.1 | 3 | 73.0 | 80.2 |
| 36 | 1 | 3 | 1 | 3 | 3 | 1 | 117.9 | 72 | 131 | 60 | 68.7 | 44.1 | 9.1 | 2.2 | 17.8 | 2.4 | 5.6 | 9 | 59.3 | 66.8 |
| 37 | 1 | 3 | 1 | 3 | 2 | 1 | 120.9 | 73 | 139 | 65 | 70.5 | 46.2 | 9.2 | 1.9 | 13.4 | 2.7 | 4.2 | 11 | 71.3 | 62.3 |
| 38 | 1 | 3 | 1 | 3 | 3 | 1 | 108.6 | 74 | 139 | 66 | 60.5 | 34.0 | 11.9 | 3.0 | 22.2 | 2.8 | 5.6 | 7 | 62.1 | 62.5 |
| 39 | 1 | 3 | 1 | 3 | 3 | 2 | 85.3 | 72 | 134 | 63 | 61.5 | 47.5 | 3.6 | 2.8 | 11.5 | 3.6 | 4.3 | 13 | 84.9 | 51.1 |
| Mean | - | - | - | - | - | - | 158.3 | 82 | 152 | 69 | 71.2 | 45.2 | 10.2 | 3.3 | 39.0 | 3.2 | 4.8 | 18 | 94.2 | 60.3 |
| $LSD_{0.05}$ | - | - | - | - | - | - | 49.2 | 5 | 6 | 7 | 9.6 | 8.8 | 1.2 | 0.4 | 15.0 | 0.6 | 0.8 | 9.0 | 19.3 | 6.8 |
| CV (%) | - | - | - | - | - | - | 32.5 | 5.7 | 4.4 | 10.4 | 14.1 | 20.3 | 12.8 | 13.5 | 40.1 | 19.4 | 17.2 | 49.2 | 21.5 | 11.8 |

+ Name of accessions is given in Table 3.1. CC = corolla color, FP = flower position, LC = leaf color, IMC = immature fruit color, MFC = mature fruit color, FSP = fruit shape, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, CW = canopy width, FL = fruit length, FD = fruit diameter, FWT = mean fruit weight, PCT = pericarp thickness, PL = pedicel length, FN = fruit number, FY = fruit yield, ABM = aerial biomass, HI = harvest index

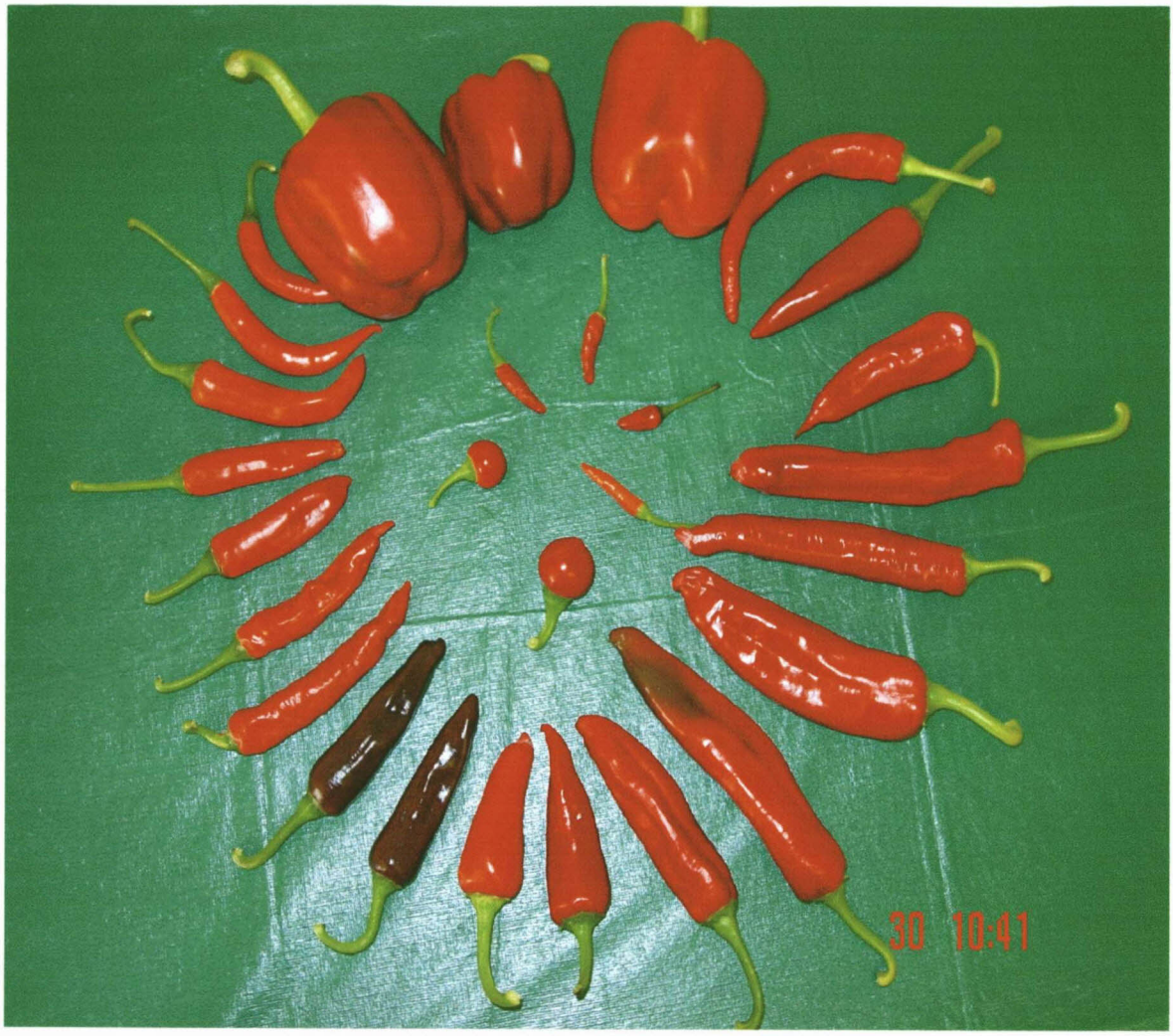


Fig. 3.1. Picture depicting representative of the 39 pepper genotypes analyzed for genetic variability using morphological traits and AFLPs.

The combined analyses of variance for the four varietal groups indicated that there were significant differences among the groups for all measured characters except for ABM (Table 3.5) demonstrating the groups are distinctly different for these characters. Year and genotype × year interaction had significant effects on DF, ABM, FD, FWT, PCT, FY and HI. On the other hand, environment had a small effect on characters such as FL and FN.

Table 3.5. Combined analyses of variance for quantitative characters in the four groups of pepper genotypes.

| Source | df | DF | DM | FMP | FL | FD | FWT | PCT | PL | PH | CW | FN | FY | ABM | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year (Y) | 1 | 2110.0** | 461.4** | 598.0** | 0.4 | 0.9** | 609.2** | 0.8** | 2.0** | 2825.8** | 513.8** | 60.4 | 30598.5** | 10049.9** | 0.0 |
| Genotype (G) | 3 | 53.0** | 206.1** | 57.2** | 130.0** | 29.7** | 13647.6** | 19.1** | 2.8** | 368.2** | 272.6** | 902.4** | 18354.2** | 278.5 | 564.0** |
| G×Y | 3 | 7.4 | 78.7** | 62.1** | 0.1 | 0.2** | 516.6** | 0.7** | 0.6** | 27.2 | 32.1 | 48.0 | 7192.3** | 106.2 | 67.8* |
| Error | 12 | | 7.9 | 6.4 | 0.3 | 0.04 | 20.0 | 0.1 | 0.1 | 18.4 | 19.9 | 23.9 | 779.4 | 136.3 | 15.8 |

DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, CW = canopy width, FL = fruit length, FD = fruit diameter, FWT = mean fruit weight, PCT = pericarp thickness, PL = pedicel length, FN = fruit number, FY = fruit yield, ABM = aerial biomass, HI = harvest index, $^{*}P < 0.05$ $^{**}P < 0.01$.

Table 3.6 illustrates the comparison of mean performance of the four varietal groups. Significantly lower number of days from sowing to flowering, first fruit maturity and fruit maturation period was observed in the cherry group. Pungent elongated-fruit and paprika groups had significantly high FN per plant and FL, respectively. The high CV for fruit number per plant was probably due to the close spacing between the pots that to some extent reduced fruit number per plant in the middle rows. Shorter genotypes were more affected. The highest FD, FWT, PCT and FY were recorded in bell peppers. These results further indicated the availability of moderately high genetic variability within and between the varietal groups.

A wide range of values for all measured characters was observed in the genotypes (Table 3.7). Generally, from the lowest to the highest ranges for measured characters were observed in the groups, the widest range being in the total genotypes. For example, the average fruit number and yield per plant ranged from two to 107 and 33.9 to 300.2 g, respectively.

The measures of spread for the quantitative characters were revealed by their respective standard deviations. FL, FD, FWT, FN and FY appeared to have a higher variation within total genotypes. Similarly, FWT appeared to have a higher variation in the bell pepper varietal group. The highest standard deviations for DF, DM, FN, HI and FY were expressed by the pungent elongated-fruit group indicating that the group had more diverse pepper genotypes as compared to any other varietal group. Within group variations for FL, FN and PCT were low in pungent elongated-fruit types and the majority of the genotypes grouped here were similar for these characters. A few variations for the majority of measured characters were also observed within bell pepper and paprika groups. Similar values of standard deviation for some characters were also observed in the same varietal groups.

Table 3.6. Mean performance of the four pepper groups averaged over two years for measured quantitative characters, 2001/02.

| Group[♣] | DF | DM | FMP | PH | CW | FL | FD | FWT | PCT | PL | FN | FY | ABM | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| II | 77 | 141 | 64 | 72.7 | 43.8 | 3.3 | 2.4 | 7.7 | 3.4 | 3.5 | 26 | 99.1 | 97.0 | 49.5 |
| III | 84 | 155 | 71 | 76.9 | 48.3 | 9.7 | 1.9 | 12.4 | 2.0 | 5.0 | 27 | 133.3 | 99.6 | 55.7 |
| IV | 82 | 149 | 68 | 58.9 | 36.0 | 11.4 | 6.8 | 110.0 | 6.1 | 4.7 | 3 | 229.6 | 84.3 | 71.9 |
| V | 80 | 150 | 70 | 72.3 | 51.6 | 14.2 | 2.9 | 28.9 | 3.4 | 4.9 | 8 | 159.7 | 91.0 | 63.4 |
| Mean | 81 | 149 | 68 | 70.2 | 44.9 | 9.6 | 3.5 | 39.7 | 3.5 | 4.5 | 16 | 155.4 | 92.9 | 60.1 |
| $LSD_{0.05}$ | 2 | 3 | 3 | 4.4 | 4.6 | 0.6 | 0.2 | 4.6 | 0.3 | 0.3 | 5 | 28.7 | ns | 4.1 |
| CV (%) | 2.5 | 1.9 | 3.7 | 6.1 | 9.9 | 6.0 | 6.0 | 11.3 | 9.0 | 7.1 | 30.8 | 18.0 | 12.6 | 6.6 |
| $h^2b$ | 0.86 | 0.62 | - | 0.93 | 0.88 | 0.999 | 0.99 | 0.96 | 0.96 | 0.79 | 0.95 | 0.61 | 0.62 | 0.88 |

[♣] II = cherry group, III = pungent elongate-fruited group, IV = bell pepper group, V = paprika group; DF = days to flowering, DM = days to maturity, FMP=fruit maturation period, PH=plant height, CW=canopy width, FL=fruit length, FD=fruit diameter, FWT=mean fruit weight, PCT=pericarp thickness, PL = pedicel length, FN = fruit number, FY = fruit yield, ABM = aerial biomass, HI = harvest index, $h^2b$ = heritability in broad sense

Table 3.7. Mean, minimum, maximum, range and standard deviation (SD) of quantitative traits for the total and the four groups of pepper genotypes averaged over two years.

| | Varietal group[+] | DF | DM | FMP | FL | FD | FWT | PCT | PL | PH | CW | FN | FY | ABM | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | | | | | | | | | | | | | |
| Mean | | 83 | 152 | 70 | 10.1 | 3.3 | 39.0 | 3.2 | 4.8 | 71.2 | 45.2 | 18 | 158.3 | 94.3 | 60.3 |
| Min. | | 69 | 131 | 60 | 1.7 | 0.9 | 1.1 | 0.8 | 2.5 | 49.2 | 27.7 | 2 | 33.9 | 59.3 | 26.6 |
| Max. | | 117 | 181 | 87 | 16.6 | 7.7 | 182.7 | 8.3 | 7.0 | 114.5 | 66.7 | 107 | 300.2 | 165.5 | 80.2 |
| Range | | 48 | 50 | 27 | 14.9 | 6.8 | 181.6 | 7.5 | 4.5 | 65.3 | 39.0 | 105 | 266.3 | 106.2 | 53.6 |
| SD | | 9.4 | 11.9 | 6.5 | 3.7 | 2.2 | 47.7 | 2.0 | 0.9 | 1353 | 9.4 | 22.1 | 60.6 | 25.8 | 11.4 |
| | II | | | | | | | | | | | | | | |
| Mean | | 77 | 141 | 64 | 3.3 | 2.4 | 7.7 | 3.4 | 3.5 | 72.7 | 43.8 | 26 | 99.1 | 97.0 | 49.5 |
| Min. | | 71 | 134 | 60 | 3.0 | 2.0 | 4.1 | 3.2 | 2.5 | 61.5 | 41.8 | 13 | 73.0 | 71.7 | 48.0 |
| Max. | | 86 | 146 | 68 | 3.6 | 2.8 | 11.5 | 3.6 | 4.3 | 86.5 | 47.5 | 51 | 139.0 | 134.4 | 51.1 |
| Range | | 15 | 12 | 8 | 0.6 | 0.8 | 7.4 | 0.4 | 1.8 | 25.0 | 5.7 | 39 | 66.0 | 62.7 | 3.1 |
| SD | | 9.7 | 7.5 | 5.0 | 0.4 | 0.5 | 4.5 | 0.3 | 1.1 | 15.5 | 4.0 | 27.2 | 43.0 | 40.5 | 1.9 |
| | III | | | | | | | | | | | | | | |
| Mean | | 84 | 155 | 71 | 9.7 | 1.9 | 12.4 | 2.0 | 5.0 | 76.9 | 48.3 | 27.1 | 133.3 | 99.6 | 55.7 |
| Min. | | 69 | 131 | 60 | 1.7 | 0.7 | 1.1 | 0.8 | 3.6 | 53.3 | 27.7 | 5.0 | 33.9 | 26.6 | 26.6 |
| Max. | | 117 | 181 | 87 | 16.2 | 3.5 | 27.4 | 3.1 | 7.0 | 114.5 | 66.7 | 107.0 | 257.2 | 66.9 | 66.9 |
| Range | | 48 | 50 | 27 | 14.5 | 2.8 | 26.3 | 2.3 | 3.4 | 61.2 | 39.0 | 102.0 | 223.3 | 40.3 | 40.3 |
| SD | | 12.1 | 14.8 | 7.3 | 3.3 | 0.7 | 8.1 | 0.7 | 0.9 | 13.5 | 9.8 | 25.5 | 46.8 | 32.1 | 11.3 |
| | IV | | | | | | | | | | | | | | |
| Mean | | 82 | 150 | 68 | 11.4 | 6.8 | 110.0 | 6.1 | 4.7 | 58.8 | 36.0 | 3 | 229.0 | 84.3 | 71.9 |
| Min. | | 75 | 143 | 61 | 9.5 | 5.3 | 57.2 | 3.8 | 3.9 | 49.2 | 30.8 | 2 | 151.1 | 68.2 | 66.5 |
| Max. | | 90 | 156 | 73 | 12.8 | 7.7 | 182.7 | 8.3 | 6.4 | 71.5 | 41.2 | 4 | 300.0 | 99.8 | 80.2 |
| Range | | 15 | 13 | 12 | 3.3 | 2.4 | 125.5 | 4.5 | 2.5 | 22.3 | 10.4 | 2 | 149.1 | 31.6 | 13.7 |
| SD | | 4.4 | 3.9 | 3.8 | 1.1 | 0.9 | 41.2 | 1.5 | 0.8 | 3.8 | 7.4 | 0.8 | 44.4 | 10.3 | 4.3 |
| | V | | | | | | | | | | | | | | |
| Mean | | 80 | 150 | 70 | 14.0 | 2.9 | 28.3 | 2.6 | 4.8 | 71.4 | 50.2 | 8.0 | 156.4 | 90.3 | 63.1 |
| Min. | | 77 | 141 | 62 | 7.6 | 2.7 | 16.0 | 2.3 | 4.3 | 58.3 | 41.2 | 6.0 | 134.7 | 76.9 | 57.1 |
| Max. | | 84 | 161 | 77 | 16.6 | 3.1 | 36.3 | 2.8 | 5.5 | 80.5 | 56.8 | 11.0 | 186.2 | 101.0 | 67.0 |
| Range | | 7 | 20 | 15 | 9.0 | 0.4 | 20.3 | 0.5 | 1.2 | 22.2 | 15.6 | 5.0 | 51.5 | 24.1 | 8.9 |
| SD | | 2.9 | 8.1 | 6.4 | 4.2 | 0.2 | 8.3 | 0.2 | 0.5 | 9.1 | 2.1 | 2.1 | 22.9 | 9.9 | 4.1 |

[+] I = total genotypes, II = cherry group, III = pungent elongate-fruited group, IV = bell pepper group, V = paprika group, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, CW = canopy width, FL = fruit length, FD = fruit diameter, FWT = mean fruit weight, PCT = pericarp thickness, PL = pedicel length, FN = fruit number, FY = fruit yield, ABM = aerial biomass, HI = harvest index

### *Genetic distance and cluster analysis*

Estimates of GD based on quantitative and qualitative characters for all pairwise combinations of (39 × 38)/2 = 741 for the 39 pepper genotypes is presented in Table 3.8. From low to high GDs were observed in the pairwise combinations indicating that the genotypes were quite diverse for the morphological traits measured. The lowest and the highest GDs were observed within and between the varietal groups, respectively. The minimum GD of 0.24 was recorded between Quick Set and Papri Queen, and C03796 and C03804. Quick Set and Papri Queen were paprika varieties. Similarly, C03796 and C03804 were bell peppers that originated from China. On the other hand, the highest GD of 0.58 was recorded between PBC 142A and Pepper 1976, C01613, C01132, and Pepper 1038. All these combinations were between different varietal groups and were also different in geographic origins. Genotype C05809 showed the highest genetic dissimilarity coefficient (mean value is 0.54) and appeared as the most divergent genotype, whereas the least mean dissimilarity coefficient (0.38) was recorded in Kalocsai V-2.

The genetic dissimilarities among the four Ethiopian genotypes ranged from 0.34 (Mareko Shote vs. Mareko Fana) to 0.46 (Bakko Local vs. Mareko Fana) with an average of 0.40. The mean genetic dissimilarity coefficient (0.44) between Bakko Local and the Mareko types (Mareko Shote, Mareko Dube and Mareko Fana) was higher than that within the Mareko types (0.37), indicating the Mareko types are genetically closely related. The average genetic dissimilarity coefficient between the Ethiopian and exotic genotypes was 0.47 and greater than that among the Ethiopian genotypes, revealing that the exotic genotypes included in this study could be valuable sources of genetic variability for the pepper improvement programs in Ethiopia.

Table 3.8. Estimates of genetic distance based on morphological (upper diagonal) and AFLP (lower diagonal) for all pair-wise comparisons of 39 pepper genotypes.

| Acc. No.* | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 0.54 | 0.52 | 0.55 | 0.44 | 0.52 | 0.50 | 0.47 | 0.50 | 0.54 | 0.54 | 0.52 | 0.52 | 0.46 | 0.52 | 0.52 | 0.52 | 0.52 | 0.55 | 0.45 | 0.52 | 0.52 | 0.53 | 0.50 | 0.52 | 0.52 | 0.52 | 0.52 | 0.54 | 0.52 | 0.50 | 0.52 | 0.54 | 0.55 | 0.52 | 0.48 | 0.48 | 0.50 | 0.44 |
| 2 | 0.61 | | 0.42 | 0.44 | 0.48 | 0.46 | 0.44 | 0.52 | 0.46 | 0.44 | 0.54 | 0.54 | 0.39 | 0.44 | 0.54 | 0.44 | 0.43 | 0.36 | 0.43 | 0.48 | 0.46 | 0.42 | 0.43 | 0.43 | 0.45 | 0.44 | 0.48 | 0.50 | 0.52 | 0.56 | 0.48 | 0.54 | 0.50 | 0.52 | 0.54 | 0.42 | 0.39 | 0.39 | 0.50 |
| 3 | 0.58 | 0.56 | | 0.37 | 0.44 | 0.34 | 0.44 | 0.49 | 0.44 | 0.42 | 0.54 | 0.52 | 0.42 | 0.52 | 0.54 | 0.48 | 0.44 | 0.44 | 0.39 | 0.52 | 0.46 | 0.42 | 0.37 | 0.46 | 0.34 | 0.42 | 0.48 | 0.52 | 0.52 | 0.52 | 0.50 | 0.52 | 0.48 | 0.54 | 0.50 | 0.44 | 0.44 | 0.46 | 0.48 |
| 4 | 0.62 | 0.61 | 0.55 | | 0.46 | 0.39 | 0.42 | 0.51 | 0.46 | 0.42 | 0.54 | 0.54 | 0.42 | 0.50 | 0.55 | 0.46 | 0.50 | 0.48 | 0.42 | 0.50 | 0.48 | 0.46 | 0.41 | 0.44 | 0.46 | 0.46 | 0.50 | 0.52 | 0.55 | 0.55 | 0.54 | 0.57 | 0.50 | 0.55 | 0.52 | 0.39 | 0.44 | 0.46 | 0.48 |
| 5 | 0.63 | 0.61 | 0.55 | 0.56 | | 0.48 | 0.46 | 0.47 | 0.48 | 0.37 | 0.52 | 0.52 | 0.50 | 0.50 | 0.55 | 0.50 | 0.54 | 0.52 | 0.50 | 0.48 | 0.48 | 0.50 | 0.50 | 0.50 | 0.50 | 0.48 | 0.50 | 0.50 | 0.57 | 0.54 | 0.55 | 0.57 | 0.54 | 0.54 | 0.52 | 0.39 | 0.42 | 0.44 | 0.31 |
| 6 | 0.57 | 0.61 | 0.48 | 0.59 | 0.53 | | 0.44 | 0.55 | 0.42 | 0.42 | 0.48 | 0.50 | 0.44 | 0.54 | 0.54 | 0.52 | 0.46 | 0.46 | 0.48 | 0.44 | 0.50 | 0.48 | 0.42 | 0.39 | 0.48 | 0.39 | 0.39 | 0.42 | 0.48 | 0.50 | 0.46 | 0.52 | 0.48 | 0.52 | 0.50 | 0.44 | 0.44 | 0.44 | 0.46 |
| 7 | 0.67 | 0.63 | 0.64 | 0.62 | 0.64 | 0.67 | | 0.53 | 0.46 | 0.48 | 0.46 | 0.48 | 0.50 | 0.52 | 0.55 | 0.52 | 0.52 | 0.50 | 0.50 | 0.52 | 0.50 | 0.52 | 0.46 | 0.42 | 0.46 | 0.48 | 0.50 | 0.39 | 0.52 | 0.54 | 0.46 | 0.52 | 0.46 | 0.50 | 0.46 | 0.42 | 0.46 | 0.37 | 0.52 |
| 8 | 0.60 | 0.65 | 0.59 | 0.61 | 0.61 | 0.54 | 0.69 | | 0.49 | 0.53 | 0.56 | 0.58 | 0.51 | 0.45 | 0.53 | 0.53 | 0.53 | 0.51 | 0.47 | 0.43 | 0.53 | 0.51 | 0.51 | 0.51 | 0.49 | 0.53 | 0.53 | 0.56 | 0.56 | 0.58 | 0.55 | 0.56 | 0.56 | 0.58 | 0.58 | 0.51 | 0.51 | 0.51 | 0.45 |
| 9 | 0.52 | 0.62 | 0.57 | 0.59 | 0.58 | 0.51 | 0.69 | 0.50 | | 0.42 | 0.54 | 0.52 | 0.50 | 0.52 | 0.55 | 0.52 | 0.54 | 0.52 | 0.46 | 0.52 | 0.46 | 0.48 | 0.37 | 0.42 | 0.42 | 0.37 | 0.46 | 0.50 | 0.54 | 0.46 | 0.52 | 0.54 | 0.52 | 0.50 | 0.55 | 0.48 | 0.44 | 0.42 | 0.50 |
| 10 | 0.62 | 0.60 | 0.58 | 0.56 | 0.56 | 0.59 | 0.65 | 0.62 | 0.61 | | 0.54 | 0.52 | 0.44 | 0.50 | 0.54 | 0.46 | 0.50 | 0.48 | 0.44 | 0.50 | 0.46 | 0.42 | 0.44 | 0.48 | 0.46 | 0.37 | 0.48 | 0.52 | 0.52 | 0.46 | 0.52 | 0.52 | 0.52 | 0.48 | 0.54 | 0.34 | 0.37 | 0.44 | 0.46 |
| 11 | 0.62 | 0.60 | 0.56 | 0.58 | 0.57 | 0.59 | 0.60 | 0.59 | 0.58 | 0.58 | | 0.37 | 0.54 | 0.55 | 0.54 | 0.50 | 0.52 | 0.57 | 0.55 | 0.57 | 0.48 | 0.55 | 0.52 | 0.52 | 0.54 | 0.50 | 0.50 | 0.39 | 0.46 | 0.46 | 0.44 | 0.44 | 0.42 | 0.42 | 0.39 | 0.54 | 0.57 | 0.52 | 0.52 |
| 12 | 0.59 | 0.58 | 0.57 | 0.56 | 0.56 | 0.58 | 0.59 | 0.57 | 0.59 | 0.53 | 0.49 | | 0.54 | 0.55 | 0.57 | 0.52 | 0.54 | 0.54 | 0.54 | 0.54 | 0.48 | 0.54 | 0.50 | 0.46 | 0.54 | 0.46 | 0.50 | 0.39 | 0.44 | 0.44 | 0.44 | 0.42 | 0.42 | 0.46 | 0.39 | 0.50 | 0.54 | 0.54 | 0.54 |
| 13 | 0.64 | 0.64 | 0.58 | 0.59 | 0.61 | 0.57 | 0.65 | 0.54 | 0.57 | 0.56 | 0.56 | 0.54 | | 0.42 | 0.54 | 0.39 | 0.34 | 0.39 | 0.39 | 0.50 | 0.46 | 0.28 | 0.42 | 0.44 | 0.42 | 0.42 | 0.46 | 0.52 | 0.50 | 0.52 | 0.50 | 0.54 | 0.48 | 0.54 | 0.55 | 0.39 | 0.37 | 0.46 | 0.48 |
| 14 | 0.55 | 0.66 | 0.63 | 0.59 | 0.63 | 0.54 | 0.68 | 0.53 | 0.49 | 0.58 | 0.65 | 0.61 | 0.61 | | 0.48 | 0.39 | 0.44 | 0.39 | 0.48 | 0.44 | 0.42 | 0.46 | 0.50 | 0.48 | 0.50 | 0.54 | 0.54 | 0.55 | 0.55 | 0.54 | 0.54 | 0.55 | 0.54 | 0.55 | 0.57 | 0.48 | 0.46 | 0.50 | 0.50 |
| 15 | 0.67 | 0.65 | 0.62 | 0.71 | 0.65 | 0.64 | 0.65 | 0.68 | 0.69 | 0.64 | 0.68 | 0.65 | 0.68 | 0.69 | | 0.50 | 0.52 | 0.54 | 0.52 | 0.50 | 0.54 | 0.52 | 0.52 | 0.55 | 0.55 | 0.52 | 0.55 | 0.57 | 0.52 | 0.52 | 0.55 | 0.55 | 0.54 | 0.52 | 0.54 | 0.57 | 0.55 | 0.57 | 0.55 |
| 16 | 0.64 | 0.66 | 0.64 | 0.63 | 0.61 | 0.67 | 0.60 | 0.66 | 0.68 | 0.61 | 0.66 | 0.64 | 0.68 | 0.63 | 0.68 | | 0.37 | 0.37 | 0.39 | 0.54 | 0.39 | 0.44 | 0.50 | 0.44 | 0.52 | 0.50 | 0.50 | 0.55 | 0.52 | 0.50 | 0.54 | 0.52 | 0.54 | 0.52 | 0.50 | 0.46 | 0.46 | 0.52 | 0.52 |
| 17 | 0.54 | 0.61 | 0.54 | 0.59 | 0.60 | 0.49 | 0.67 | 0.52 | 0.44 | 0.63 | 0.58 | 0.59 | 0.57 | 0.53 | 0.68 | 0.66 | | 0.31 | 0.44 | 0.50 | 0.48 | 0.34 | 0.46 | 0.46 | 0.44 | 0.48 | 0.48 | 0.55 | 0.48 | 0.50 | 0.50 | 0.50 | 0.52 | 0.52 | 0.50 | 0.46 | 0.42 | 0.50 | 0.52 |
| 18 | 0.58 | 0.62 | 0.62 | 0.58 | 0.60 | 0.60 | 0.64 | 0.61 | 0.60 | 0.55 | 0.60 | 0.58 | 0.64 | 0.57 | 0.69 | 0.62 | 0.61 | | 0.39 | 0.48 | 0.42 | 0.39 | 0.46 | 0.42 | 0.46 | 0.48 | 0.52 | 0.54 | 0.52 | 0.54 | 0.50 | 0.54 | 0.52 | 0.55 | 0.54 | 0.44 | 0.42 | 0.48 | 0.52 |
| 19 | 0.65 | 0.58 | 0.61 | 0.60 | 0.57 | 0.64 | 0.54 | 0.64 | 0.65 | 0.57 | 0.59 | 0.51 | 0.61 | 0.66 | 0.57 | 0.63 | 0.64 | 0.62 | | 0.50 | 0.42 | 0.34 | 0.44 | 0.42 | 0.44 | 0.46 | 0.52 | 0.52 | 0.50 | 0.52 | 0.54 | 0.57 | 0.52 | 0.55 | 0.54 | 0.44 | 0.46 | 0.50 | 0.52 |
| 20 | 0.63 | 0.54 | 0.53 | 0.58 | 0.52 | 0.59 | 0.59 | 0.57 | 0.59 | 0.59 | 0.54 | 0.50 | 0.54 | 0.64 | 0.62 | 0.61 | 0.61 | 0.59 | 0.55 | | 0.52 | 0.48 | 0.50 | 0.52 | 0.52 | 0.48 | 0.52 | 0.54 | 0.52 | 0.52 | 0.50 | 0.54 | 0.52 | 0.54 | 0.54 | 0.48 | 0.46 | 0.50 | 0.46 |
| 21 | 0.62 | 0.61 | 0.58 | 0.49 | 0.64 | 0.61 | 0.61 | 0.57 | 0.64 | 0.59 | 0.61 | 0.57 | 0.57 | 0.63 | 0.67 | 0.61 | 0.64 | 0.61 | 0.60 | 0.56 | | 0.46 | 0.42 | 0.48 | 0.50 | 0.44 | 0.52 | 0.46 | 0.48 | 0.46 | 0.48 | 0.50 | 0.44 | 0.50 | 0.52 | 0.50 | 0.46 | 0.50 | 0.50 |
| 22 | 0.69 | 0.66 | 0.65 | 0.62 | 0.65 | 0.67 | 0.61 | 0.70 | 0.71 | 0.63 | 0.65 | 0.62 | 0.65 | 0.69 | 0.67 | 0.61 | 0.72 | 0.65 | 0.56 | 0.63 | 0.65 | | 0.44 | 0.46 | 0.42 | 0.42 | 0.50 | 0.54 | 0.46 | 0.48 | 0.50 | 0.54 | 0.50 | 0.54 | 0.55 | 0.42 | 0.39 | 0.50 | 0.48 |
| 23 | 0.59 | 0.55 | 0.52 | 0.57 | 0.54 | 0.57 | 0.62 | 0.62 | 0.59 | 0.57 | 0.54 | 0.56 | 0.60 | 0.63 | 0.67 | 0.64 | 0.56 | 0.58 | 0.61 | 0.56 | 0.59 | 0.65 | | 0.39 | 0.31 | 0.24 | 0.44 | 0.48 | 0.48 | 0.48 | 0.48 | 0.52 | 0.44 | 0.52 | 0.54 | 0.48 | 0.42 | 0.39 | 0.48 |
| 24 | 0.60 | 0.56 | 0.55 | 0.57 | 0.60 | 0.60 | 0.61 | 0.58 | 0.62 | 0.57 | 0.57 | 0.51 | 0.57 | 0.61 | 0.66 | 0.60 | 0.60 | 0.60 | 0.57 | 0.52 | 0.57 | 0.64 | 0.55 | | 0.44 | 0.44 | 0.54 | 0.46 | 0.52 | 0.50 | 0.50 | 0.54 | 0.48 | 0.52 | 0.50 | 0.44 | 0.48 | 0.44 | 0.52 |
| 25 | 0.55 | 0.60 | 0.56 | 0.61 | 0.61 | 0.57 | 0.65 | 0.55 | 0.54 | 0.59 | 0.57 | 0.57 | 0.55 | 0.60 | 0.61 | 0.67 | 0.57 | 0.61 | 0.60 | 0.56 | 0.60 | 0.67 | 0.60 | 0.58 | | 0.34 | 0.46 | 0.52 | 0.52 | 0.54 | 0.50 | 0.54 | 0.52 | 0.55 | 0.52 | 0.46 | 0.44 | 0.37 | 0.46 |
| 26 | 0.57 | 0.62 | 0.57 | 0.60 | 0.58 | 0.58 | 0.65 | 0.53 | 0.56 | 0.57 | 0.59 | 0.51 | 0.52 | 0.57 | 0.64 | 0.62 | 0.54 | 0.61 | 0.58 | 0.55 | 0.60 | 0.67 | 0.58 | 0.52 | 0.52 | | 0.44 | 0.48 | 0.46 | 0.44 | 0.46 | 0.50 | 0.44 | 0.50 | 0.54 | 0.44 | 0.42 | 0.42 | 0.46 |
| 27 | 0.56 | 0.61 | 0.58 | 0.57 | 0.60 | 0.63 | 0.67 | 0.61 | 0.61 | 0.57 | 0.63 | 0.60 | 0.63 | 0.58 | 0.69 | 0.61 | 0.61 | 0.55 | 0.64 | 0.61 | 0.56 | 0.65 | 0.55 | 0.59 | 0.62 | 0.57 | | 0.54 | 0.50 | 0.52 | 0.52 | 0.50 | 0.52 | 0.52 | 0.55 | 0.46 | 0.39 | 0.42 | 0.46 |
| 28 | 0.67 | 0.60 | 0.63 | 0.61 | 0.57 | 0.63 | 0.58 | 0.61 | 0.64 | 0.60 | 0.58 | 0.55 | 0.61 | 0.67 | 0.64 | 0.61 | 0.62 | 0.64 | 0.49 | 0.52 | 0.61 | 0.60 | 0.62 | 0.57 | 0.63 | 0.57 | 0.66 | | 0.48 | 0.50 | 0.34 | 0.42 | 0.31 | 0.46 | 0.42 | 0.50 | 0.52 | 0.46 | 0.54 |
| 29 | 0.63 | 0.63 | 0.58 | 0.54 | 0.59 | 0.63 | 0.57 | 0.59 | 0.63 | 0.60 | 0.57 | 0.53 | 0.54 | 0.66 | 0.67 | 0.62 | 0.62 | 0.62 | 0.56 | 0.52 | 0.50 | 0.63 | 0.58 | 0.56 | 0.58 | 0.55 | 0.61 | 0.57 | | 0.39 | 0.39 | 0.39 | 0.44 | 0.44 | 0.50 | 0.50 | 0.50 | 0.54 | 0.54 |
| 30 | 0.64 | 0.61 | 0.63 | 0.60 | 0.59 | 0.66 | 0.64 | 0.64 | 0.64 | 0.55 | 0.60 | 0.60 | 0.62 | 0.64 | 0.67 | 0.62 | 0.64 | 0.61 | 0.62 | 0.55 | 0.59 | 0.64 | 0.56 | 0.57 | 0.63 | 0.59 | 0.55 | 0.62 | 0.58 | | 0.46 | 0.42 | 0.46 | 0.31 | 0.48 | 0.51 | 0.52 | 0.57 | 0.52 |
| 31 | 0.62 | 0.61 | 0.57 | 0.57 | 0.59 | 0.56 | 0.59 | 0.60 | 0.61 | 0.55 | 0.56 | 0.51 | 0.57 | 0.63 | 0.66 | 0.64 | 0.63 | 0.62 | 0.59 | 0.54 | 0.55 | 0.61 | 0.56 | 0.52 | 0.57 | 0.58 | 0.60 | 0.60 | 0.56 | 0.55 | | 0.24 | 0.31 | 0.42 | 0.48 | 0.50 | 0.50 | 0.48 | 0.52 |
| 32 | 0.64 | 0.60 | 0.59 | 0.60 | 0.59 | 0.61 | 0.59 | 0.61 | 0.64 | 0.59 | 0.58 | 0.53 | 0.59 | 0.66 | 0.63 | 0.63 | 0.64 | 0.59 | 0.53 | 0.52 | 0.59 | 0.62 | 0.60 | 0.52 | 0.56 | 0.56 | 0.61 | 0.52 | 0.54 | 0.57 | 0.55 | | 0.34 | 0.39 | 0.46 | 0.54 | 0.54 | 0.54 | 0.54 |
| 33 | 0.56 | 0.63 | 0.56 | 0.58 | 0.58 | 0.52 | 0.66 | 0.50 | 0.52 | 0.59 | 0.60 | 0.54 | 0.60 | 0.52 | 0.68 | 0.62 | 0.50 | 0.58 | 0.60 | 0.59 | 0.60 | 0.68 | 0.60 | 0.57 | 0.57 | 0.49 | 0.57 | 0.61 | 0.62 | 0.63 | 0.59 | 0.64 | | 0.46 | 0.42 | 0.54 | 0.52 | 0.52 | 0.54 |
| 34 | 0.62 | 0.61 | 0.63 | 0.62 | 0.58 | 0.64 | 0.55 | 0.68 | 0.65 | 0.62 | 0.59 | 0.58 | 0.68 | 0.70 | 0.65 | 0.63 | 0.65 | 0.64 | 0.57 | 0.61 | 0.63 | 0.60 | 0.58 | 0.60 | 0.64 | 0.62 | 0.64 | 0.58 | 0.60 | 0.62 | 0.59 | 0.60 | 0.65 | | 0.46 | 0.55 | 0.54 | 0.52 | 0.54 |
| 35 | 0.59 | 0.59 | 0.60 | 0.51 | 0.55 | 0.61 | 0.63 | 0.62 | 0.62 | 0.56 | 0.59 | 0.57 | 0.61 | 0.63 | 0.71 | 0.62 | 0.63 | 0.57 | 0.56 | 0.55 | 0.51 | 0.62 | 0.56 | 0.57 | 0.64 | 0.60 | 0.54 | 0.55 | 0.51 | 0.56 | 0.57 | 0.57 | 0.61 | 0.60 | | 0.52 | 0.54 | 0.50 | 0.55 |
| 36 | 0.62 | 0.61 | 0.55 | 0.56 | 0.57 | 0.61 | 0.59 | 0.59 | 0.61 | 0.52 | 0.61 | 0.56 | 0.58 | 0.64 | 0.65 | 0.59 | 0.60 | 0.63 | 0.55 | 0.55 | 0.59 | 0.59 | 0.57 | 0.53 | 0.60 | 0.53 | 0.59 | 0.57 | 0.55 | 0.55 | 0.55 | 0.56 | 0.56 | 0.59 | 0.57 | | 0.31 | 0.37 | 0.39 |
| 37 | 0.64 | 0.61 | 0.61 | 0.61 | 0.59 | 0.65 | 0.59 | 0.67 | 0.64 | 0.59 | 0.55 | 0.57 | 0.63 | 0.69 | 0.67 | 0.62 | 0.64 | 0.64 | 0.61 | 0.57 | 0.63 | 0.61 | 0.60 | 0.60 | 0.63 | 0.65 | 0.64 | 0.60 | 0.61 | 0.65 | 0.59 | 0.59 | 0.66 | 0.57 | 0.57 | 0.62 | | 0.31 | 0.44 |
| 38 | 0.61 | 0.63 | 0.58 | 0.59 | 0.56 | 0.61 | 0.67 | 0.61 | 0.58 | 0.59 | 0.55 | 0.55 | 0.59 | 0.65 | 0.65 | 0.64 | 0.59 | 0.62 | 0.62 | 0.59 | 0.65 | 0.65 | 0.56 | 0.59 | 0.62 | 0.58 | 0.61 | 0.64 | 0.59 | 0.65 | 0.61 | 0.63 | 0.62 | 0.59 | 0.60 | 0.60 | 0.54 | | 0.44 |
| 39 | 0.58 | 0.60 | 0.57 | 0.57 | 0.53 | 0.53 | 0.67 | 0.55 | 0.52 | 0.56 | 0.59 | 0.56 | 0.56 | 0.54 | 0.66 | 0.64 | 0.54 | 0.53 | 0.62 | 0.58 | 0.59 | 0.66 | 0.55 | 0.55 | 0.56 | 0.52 | 0.54 | 0.62 | 0.56 | 0.58 | 0.59 | 0.59 | 0.51 | 0.63 | 0.58 | 0.59 | 0.64 | 0.60 | |

* The name of the accessions is given in Table 1.

The dendrogram based on the cluster analysis of agronomical and morphological data show a clear break between different groups of pepper (Fig. 3.2). The estimated cophenetic correlation value ($r_{cop}$ = 0. 829) was high, indicating a good fit with distance values. The dendrogram shows three main clusters. The largest cluster contained genotypes from all varietal groups except from the bell peppers. This cluster further subdivided into two smaller sub-clusters mainly on the basis of fruit size. The first sub-cluster included nine small elongated-fruit types. The second sub-cluster contained 13 large elongated-fruit and two cherry type cultivars. The two cherry cultivars had similar fruit width and pericarp thickness with the large elongated-fruit genotypes and thus grouped together with them. The second cluster contained, as expected, all the bell pepper genotypes. The third cluster contained three cultivars, two small elongated-fruit and one small-fruited cherry type.

The ornamental pepper genotype, C05809, had unique characters such as variegated leaves, purple corolla and dark purple tiny elongated-fruits and was probably separated from the other clusters because of these features. This investigation in general, shows that fruit related traits such as FL, FD, FWT, PCT and FN played a major role in distinguishing cultivars from each other. Zewdie and Zeven (1997) also found fruit number per plant, fruit weight and fruit width were among the morphological characters that played a major role in grouping pepper genotypes into different clusters.

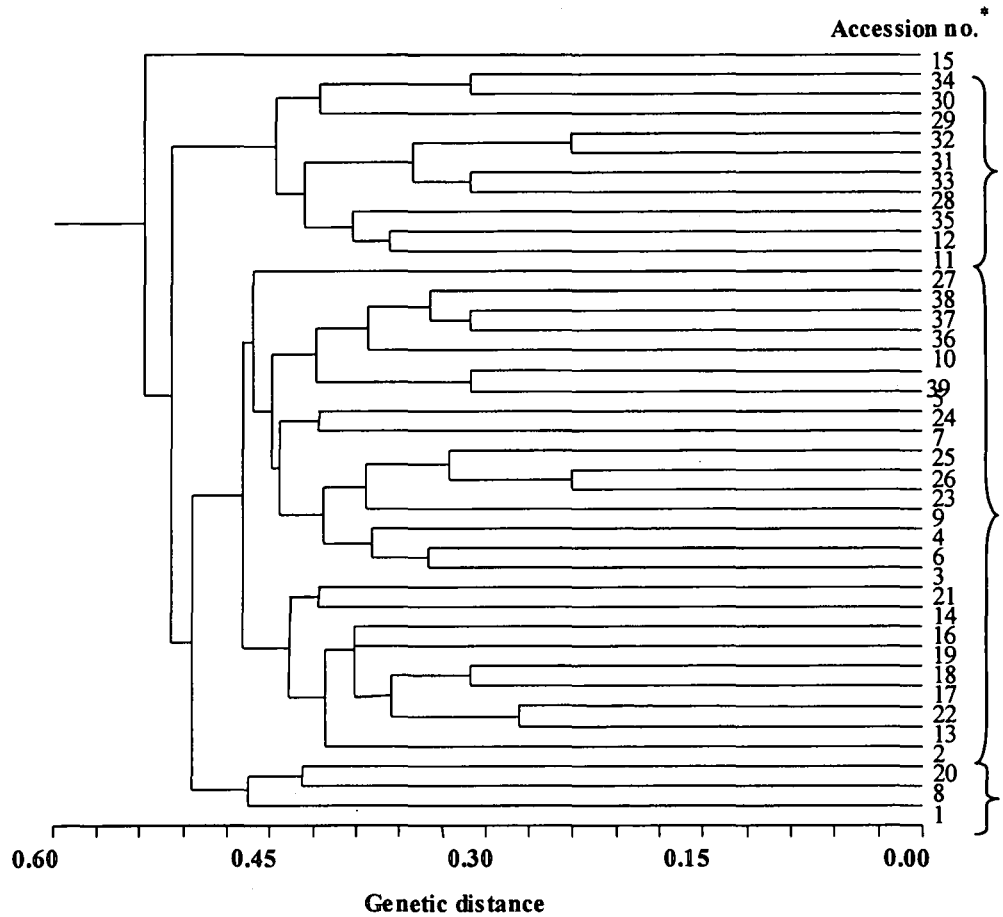


Fig. 3.2. Dendrogram of 39 pepper genotypes obtained from different geographical regions revealed by UPGMA cluster analysis based on morphological traits. * 1 = C00916, 2 = Bakko Local, 3 = Mareko Shote, 4 = Mareko Dubbe, 5 = Kalocsai "A" Cseresznye, 6 = Mareko Fana, 7 = Szegedi 20, 8 = PBC 142A, 9 = C01994, 10 = C03018, 11 = Pepper 1972, 12 = Pepper 1976, 13 = Joyang, 14 = Fire Bomb, 15 = C05809, 16 = PBC 375, 17 = C05692, 18 = PBC 602, 19 = PBC 223, 20 = PBC 612, 21 = 9852-90, 22 = 9852-91, 23 = Quick Set, 24 = Brin III, 25 = Papri King, 26 = Papri Queen, 27 = Caloras PS, 28 = Grande Rico 66, 29 = Florid RG, 30 = C01613, 31 = C03796, 32 = C03804, 33 = C03810, 34 = C01132, 35 = Pepper 1038, 36 = Kalocsai V-2, 37 = Szegedi 178, 38 = Kalocsai 801, 39 = Kalocsai "M" Cseresznye.

## Amplified fragment length polymorphism

Analysis of 39 genotypes using six AFLP primer pairs yielded a total of 352 polymorphic markers. Fragment size ranged from 40 to 400 base pairs. An example of the pattern of amplification products obtained with one AFLP primer pair is presented in Fig. 3.3. Between 50 and 63 bands were identified per primer combination among the studied genotypes, an average being 59 bands per primer combination. Primer combinations M-CAA/E-AAC and M-CTG/E-AAC identified the highest number of polymorphic bands among all the genotypes. Eleven fragments were unique among six genotypes. Genotype Bakko Local had five unique fragments and genotype C05809 had two. In this study a large number of polymorphic bands were observed. This is probably due to the high level of genetic diversity of the studied population. According to Bohn *et al.* (1999), a reliable assessment of genetic similarity in breeding materials requires that molecular markers are (i) highly polymorphic, (ii) abundant in numbers, and (iii) well distributed over the entire genome.

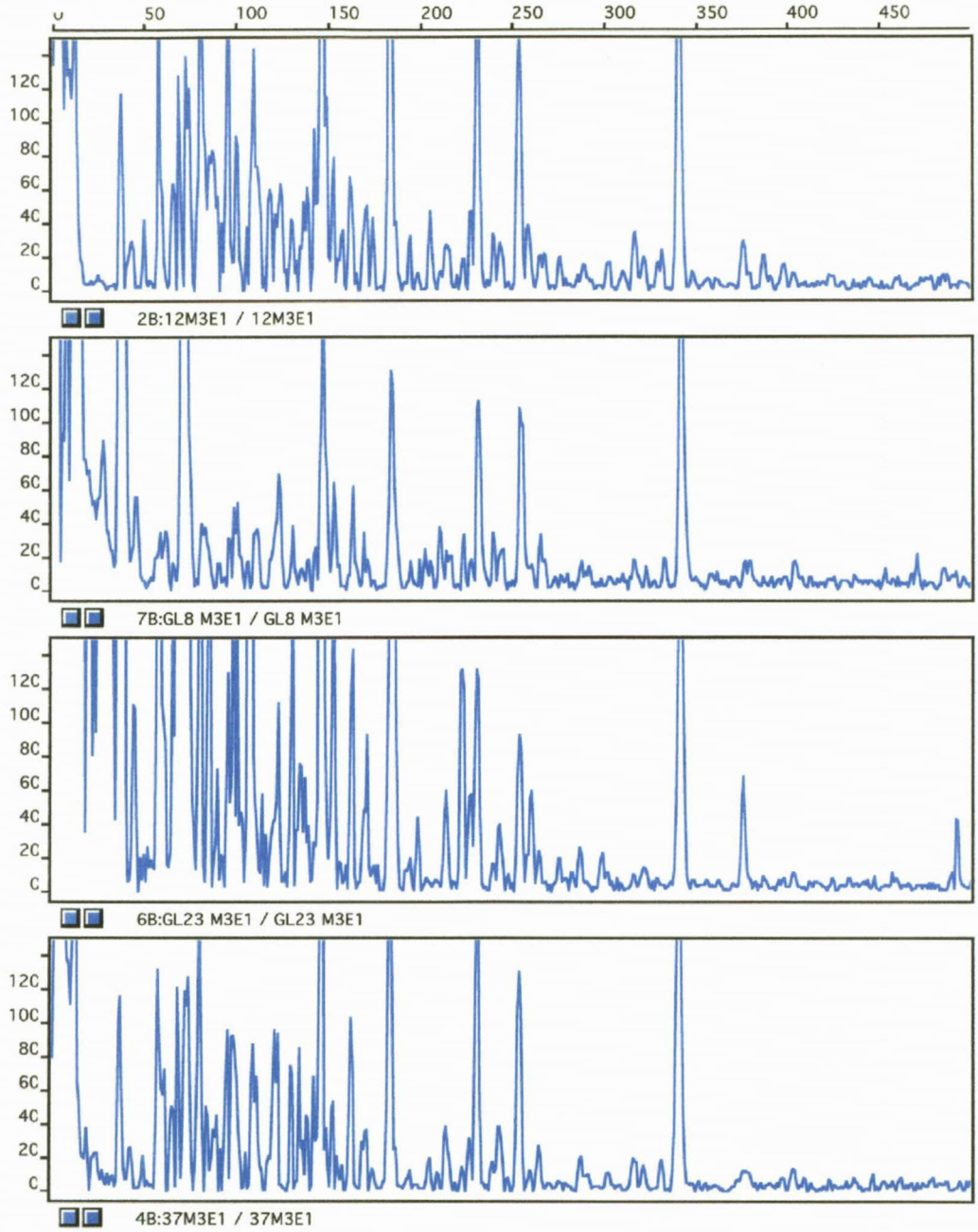


Fig. 3.3. Amplification patterns from the four varietal groups using primer M-CTG/E-ACA. After running the samples in a Perkin Elmer 310 Automated capillary sequence, the peaks were defined by GeneScan software and displayed using the GenoTyper software. The top bar indicates molecular weight in nucleotides. The number to the left of the electropherogram indicates the amplitude of the peaks.

### *Genetic distance and cluster analysis*

Genetic distance estimates for the 741 pairs of cultivars ranged from 0.44 (C01994 vs. C05692) to 0.72 (C05692 vs. C05692) with an average of 0.60 (Table 3.8). The lowest and the highest genetic dissimilarity coefficients were recorded within the pungent elongated-fruit genotypes indicating this group contained both closely and distantly related genotypes. The highest mean genetic dissimilarity coefficient was demonstrated in genotype C05809 (0.66) followed by PBC375 (0.62). As indicated above, C05809 is an ornamental pepper with unique characteristics of plant type, flower and fruit character.

Genetic distances among and between the four varietal groups were compared (Table 3.9). Pungent elongated-fruit types were the most divergent, with a mean GD of 0.61. Genetic dissimilarities between the groups were also found high, the highest being between pungent elongated-fruit and the other groups (0.60). An average GD of 0.57 was recorded between the Ethiopian and the exotic cultivars and was greater than that among the Ethiopian genotypes (0.53). Within the Ethiopian genotypes the distance between Bakko Local and the Mareko types (Mareko Shote, Mareko Dubbe and Mareko Fana) was higher (0.59) than that among the Mareko types (0.54). The GD between Mareko Shote and Mareko Fana was the lowest (0.48), when the four genotypes were compared.

Table 3.9. Mean, minimum, maximum, range and standard deviation (SD) values of genetic distances within and between pepper varietal groups.

| Genotype and group | Number of combination | Morphological trait | | | | | AFLP | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Min. | Max. | Range | SD | Mean | Min. | Max. | Range | SD |
| Total genotype | 741 | 0.48 | 0.24 | 0.58 | 0.34 | 0.06 | 0.60 | 0.44 | 0.72 | 0.28 | 0.04 |
| Cherry | 3 | 0.40 | 0.31 | 0.44 | 0.13 | 0.09 | 0.57 | 0.53 | 0.59 | 0.06 | 0.04 |
| Pungent elongated-fruit (PEF) | 210 | 0.46 | 0.28 | 0.57 | 0.29 | 0.06 | 0.61 | 0.44 | 0.72 | 0.28 | 0.05 |
| Bell | 45 | 0.42 | 0.24 | 0.50 | 0.26 | 0.06 | 0.58 | 0.49 | 0.65 | 0.16 | 0.04 |
| Paprika | 10 | 0.40 | 0.24 | 0.54 | 0.30 | 0.09 | 0.57 | 0.52 | 0.62 | 0.10 | 0.04 |
| Cherry vs. PEF | 63 | 0.49 | 0.37 | 0.55 | 0.37 | 0.04 | 0.60 | 0.52 | 0.69 | 0.17 | 0.04 |
| Cherry vs. Bell | 30 | 0.53 | 0.50 | 0.57 | 0.07 | 0.02 | 0.59 | 0.51 | 0.67 | 0.16 | 0.03 |
| Cherry vs. Paprika | 15 | 0.50 | 0.46 | 0.53 | 0.07 | 0.03 | 0.57 | 0.52 | 0.61 | 0.09 | 0.03 |
| PEF vs. Bell | 210 | 0.52 | 0.39 | 0.58 | 0.19 | 0.03 | 0.60 | 0.49 | 0.71 | 0.22 | 0.04 |
| PEF vs. Paprika | 105 | 0.46 | 0.34 | 0.55 | 0.21 | 0.05 | 0.60 | 0.52 | 0.69 | 0.17 | 0.04 |
| Bell vs. Paprika | 50 | 0.50 | 0.44 | 0.55 | 0.11 | 0.03 | 0.58 | 0.49 | 0.66 | 0.17 | 0.04 |

The dendrogram obtained from UPGMA cluster analysis of 39 genotypes on the basis of AFLP marker-based GDs resulted in distinct separation of the cultivars and shows four major clusters (Fig. 3.4). The largest cluster involved 16 genotypes that included eight bell peppers and eight others, most of them were large elongated-fruit cultivars. Fourteen genotypes were grouped in the second cluster. The third cluster contained one medium elongated-fruit and one bell pepper genotypes. The fourth cluster also involved two genotypes, both from Hungary and had elongate fruits. Five genotypes failed to group with other clusters. Generally, AFLP marker-based diversity analysis showed a tendency of separating genotypes by fruit size. Paran *et al.* (1998) also found that RAPD and AFLP markers separated large-fruited sweet peppers from the small-fruited pungent peppers, and sweet peppers showed less divergent than the small-fruited pungent types. Lefebvre *et al.* (1993) also reported that large-fruited accessions were clustered together and the genetic distances between the small-fruited cultivars were larger than within the bell pepper group.

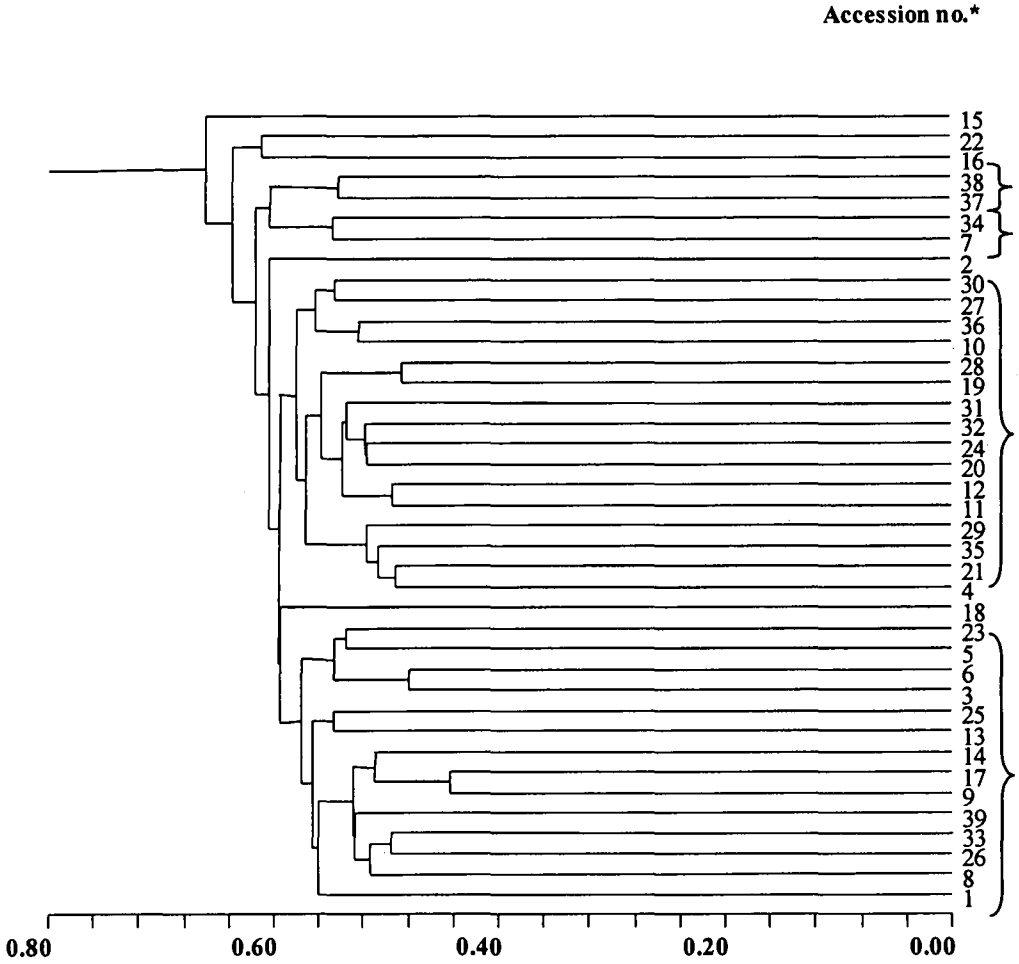


Fig. 3.4. Dendrogram of 39 pepper genotypes obtained from different geographical regions revealed by UPGMA cluster analysis based on AFLP markers. * 1 = C00916, 2 = Bakko Local, 3 = Mareko Shote, 4 = Mareko Dubbe, 5 = Kalocsai "A" Cseresznye, 6 = Mareko Fana, 7 = Szegedi 20, 8 = PBC 142A, 9 = C01994, 10 = C03018, 11 = Pepper 1972, 12 = Pepper 1976, 13 = Joyang, 14 = Fire Bomb, 15 = C05809, 16 = PBC 375, 17 = C05692, 18 = PBC 602, 19 = PBC 223, 20 = PBC 612, 21 = 9852-90, 22 = 9852-91, 23 = Quick Set, 24 = Brin III, 25 = Papri King, 26 = Papri Queen, 27 = Caloras PS, 28 = Grande Rico 66, 29 = Florid RG, 30 = C01613, 31 = C03796, 32 = C03804, 33 = C03810, 34 = C01132, 35 = Pepper 1038, 36 = Kalocsai V-2, 37 = Szegedi 178, 38 = Kalocsai 801, 39 = Kalocsai "M" Cseresznye.

## Comparison of morphological and AFLP genetic variability analyses

Burstin and Charcosset (1997) demonstrated that the magnitude of the correlation coefficient between phenotypic and molecular distances depends on the association between marker loci and quantitative trait loci; the correlation necessarily decreases as the number of loci involved in the variation of quantitative trait increases. Lefebvre *et al.* (2001) suggested that the relationship between molecular distances and phenotypic distances shows that inbred lines with different phenotypes also differ with respect to markers. Thus, a genotype can easily be discriminated with the use of phenotypic distance only. However, molecular markers play an important role while different gene combinations originated from unrelated genetic resources govern the same phenotype.

Distributions of the 741 pairwise morphological and AFLP GD values generated among 39 pepper genotypes were distinctly different (Fig. 3.5). Mean GD estimates for AFLP was higher (0.60) than that for morphological data (0.48), indicating that AFLP had higher discriminating power compared with morphology. When comparisons were made within and between varietal groups, mean morphological and AFLP GD estimates were generally lower within than between varietal groups. Both morphological and AFLP GD estimates were higher for pairwise comparisons within pungent elongated-fruit (mean=0.46 and 0.61, respectively), indicating there was better agreement between the two genetic distance estimates. Values were intermediate (0.42 and 0.58) among bell peppers and lower (0.40 and 0.57) among cherry and paprika groups. Between varietal groups, the mean morphological genetic distance estimates ranged from 0.46 (pungent elongated-fruit vs. paprika) to 0.53 (cherry vs. bell pepper), whereas mean AFLP-measured GD ranged from 0.57 (cherry vs. paprika) to 0.60 (pungent elongated-fruit vs. other groups).

Both methods of GD estimations showed lower distance among the Ethiopian cultivars as compared to that between the Ethiopian and exotic genotypes. These methods also showed that the genetic dissimilarity between Bakko Local and the Mareko types was

higher than that among the Mareko types. Within Mareko types, the lowest genetic distance was detected between Mareko Shote and Mareko Fana by the two methods.

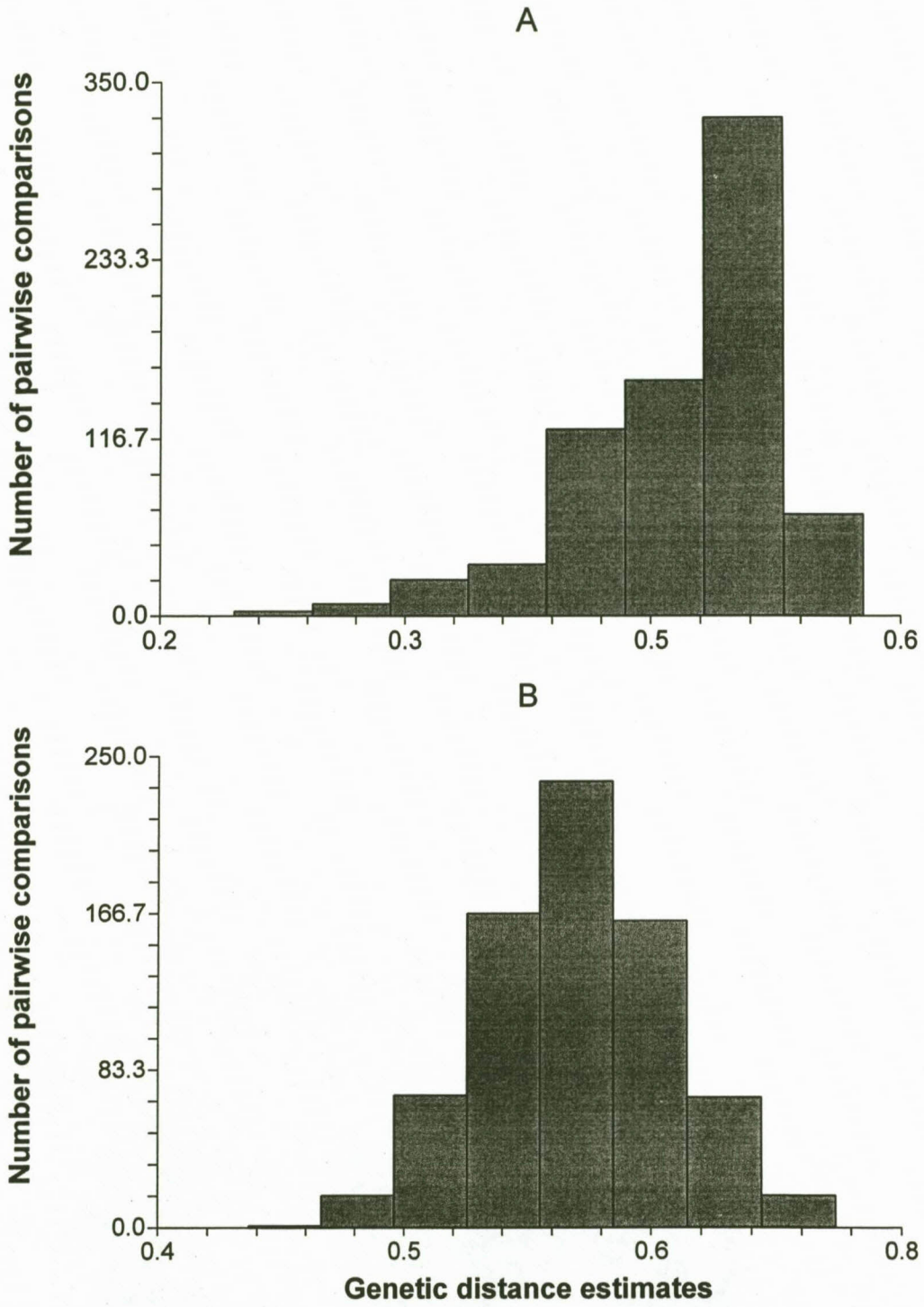


Fig. 3.5. Frequency distribution of genetic diversity estimates based on morphological (A) and AFLP (B) data.

The relationship between distances based on morphological traits and AFLP markers is shown in Fig. 3.6. Although the value was small, a significant, positive ($r$ = 0.101, $P$ < 0.01) correlation was detected between the morphological data and AFLP marker-based matrices, indicating AFLP distance tended to reflect morphological distance. However, variable results were obtained when separate correlations between the matrices of the two methods were calculated for pair-wise comparisons within and between the four varietal groups. The associations were significant and positive between cherry and pungent elongated-fruit ($r$ = 0.320, $P$ < 0.01), and pungent elongated-fruit and paprika ($r$ = 0.193, $P$ < 0.05) cultivars. On the other hand, no correlations were observed between pungent elongated-fruit and bell pepper ($r$ = 0.035), cherry and bell pepper ($r$ = -0.087), cherry and paprika ($r$ = 0.363), and bell and paprika ($r$ = 0.160) groups. Similarly, the correlations between the two genetic distance matrices were low for cultivars of similar fruit characters such as pungent elongated-fruit ($r$ = 0.126), bell pepper ($r$ = -0.062) and paprika ($r$ = 0.093). In general, the low correlation between morphological and AFLP genetic distance estimates was probably due to the high genetic diversity in the population. This was evidenced by the results of other studies in which diverse pedigree comparisons are often excluded to increase the associations between pedigree and DNA markers-based genetic diversity estimates. Using this approach an increase in the correlation between RAPD and pedigree diversity estimates in spring barley (Tinker *et al.*, 1993), and RFLP and coefficient of parentage diversity estimates in soft winter wheat (Kim and Ward, 1997) were reported. Barrett *et al.* (1998) noted that this increased correlation may be attributed to an increase in the proportion of relatedness that accounts for pedigree information as the germplasm gene pool shifts away from its original configuration through cycles of selection.

Morphological data grouped the studied genotypes into different clusters mainly based on fruit characters such as weight, diameter, length, pericarp thickness and number per plant. Similarly, AFLP marker-based genetic distances clustered the genotypes on the basis of these traits. Both methods of genetic dissimilarity measures also separated an ornamental genotype C05809 from other clusters and it was the most divergent genotype.

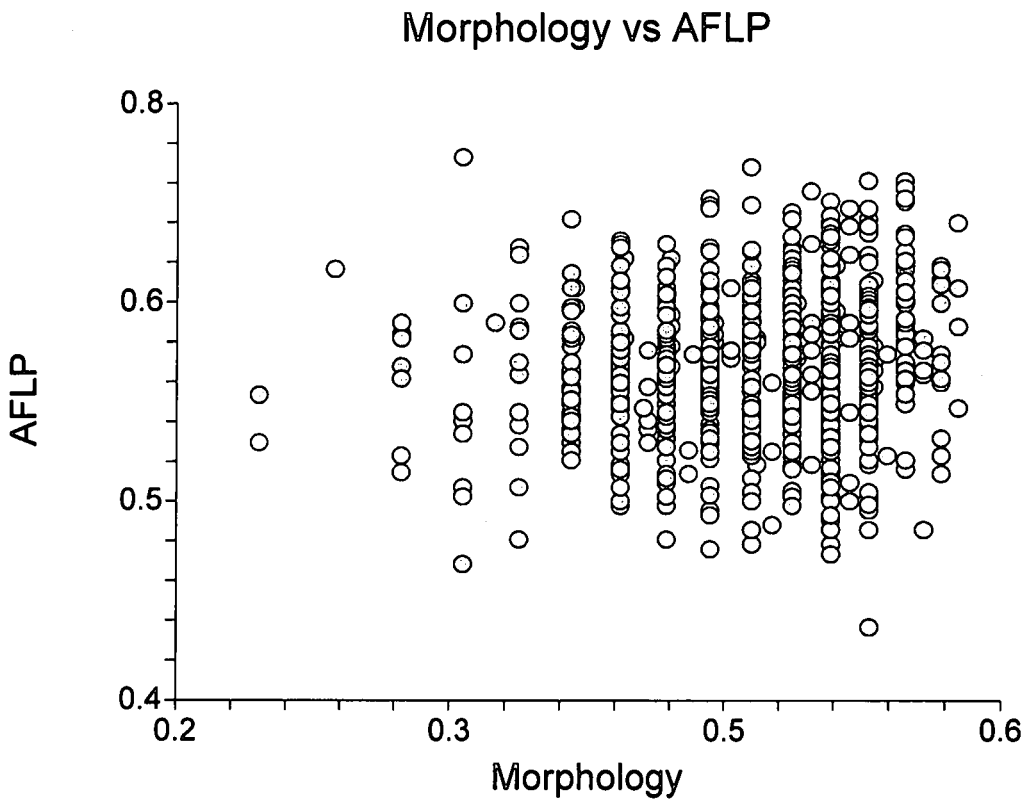


Fig. 3.6. Relationship between distances based on morphological data and amplified fragment length polymorphism markers of all pair-wise comparisons of 39 pepper genotypes.

## CONCLUSION

From a breeding perspective, genetic distance is an important parameter in choosing parents for crossing. In the present investigation, morphological and AFLP-based GD determinations revealed moderately high genetic distances among the genotypes studied. High GDs were also observed among and between the varietal groups. However, when the comparisons were made within and between the Ethiopian cultivars, lower average GD was found among the Ethiopian cultivars compared with that between the Ethiopian and exotic cultivars. Morphological data clearly separated large-fruited genotypes from small-fruited ones. AFLP markers also showed a similar tendency of separating large-fruited from small-fruited genotypes. The significant positive correlation between the two genetic distance estimates indicates AFLP distance tended to reflect morphological distance. On the basis of this study it can be recommended that the combination of morphology and AFLP can provide useful measures of genetic distances. The narrow genetic basis in the Ethiopian cultivars suggests that the pepper breeding program of Ethiopia should focus on enriching its germplasm through local collections and introductions from other parts of the world. In general, the information obtained from the present study will be of practical use for pepper breeding programs in Ethiopia.

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# CHAPTER 4

## Diallel analysis for fruit related traits and other agronomic characters


### ABSTRACT

Estimates of relative importance of additive and non-additive gene actions within breeding populations are important to determine the type of breeding method that effectively improves the performance of the traits of interest. A seven-parent diallel cross (Griffing's Method 2, Model 1) was evaluated in the field and greenhouse at the University of the Free State, South Africa during 2001/02. The objectives of this study were to estimate the combining abilities and genetic effects determining the heritabilities of various characters. The mean squares for general (GCA) and specific (SCA) combining abilities were significant for all measured characters with the exception of SCA for FY in the field and GFTSS in the greenhouse, indicating the presence of both additive and non-additive gene actions for these characters. However, additive gene action was more important compared to the non-additive gene action, as GCA estimates were much higher than that of SCA. The GCA effects revealed that parents behaved genetically as expected with the elongated-fruit, large size and thick pericarp parents exhibiting large positive GCA, and round, small and thin pericarp parents exhibiting high negative GCA effects for these characters, respectively. A number of crosses with significant SCA effects to desired directions and high mean performances were also identified to grow as hybrids. The estimates of predictability ratios for FL, FD, FWT, PCT and FN were close to unity, suggesting the possibility of predicting progeny performance based on parental GCA alone. Theses characters also showed high heritability both in broad- and narrow-senses revealing that their inheritance is less influenced by the environment. Thus, they can be improved using recurrent selection methods, which exploit additive genetic effects.

# INTRODUCTION

Peppers are known to be a versatile crop. They have a wide variety of uses such as flavoring in food manufacturing, adding pungency (organoleptic sensation of heat) and color to foods, coloring for cosmetics, imparting heat to medicines and used as ornamental plants. Peppers are also a good source of income. They are grown in most countries of the world. By volume, red pepper products, pungent and non-pungent, represent one of the important spice commodities in the world (Bosland and Votava, 2000). The report by the FAO (2000) indicates that the production of pepper for use as spice and vegetable has increased by more than 33% between 1991 and 2000. According to this report, the world production of pepper in 2000 was 18 501 000 metric tons, Asia being the largest producer.

Fruit characters determine the products of peppers. In capsicums and chiles, medium-sized fruits with a moderately thin pericarp are required. Medium-sized fruits are preferred to long pods, owing to the fact that in storage they remain intact better than longer pods, which tend to break at the distal ends. A fairly thin pericarp is necessary, as its moisture is less than that of a thick pericarp, and drying is easier. On drying, fruits with thick pericarps show a wrinkled surface and dull appearance. Similarly, medium sized but fleshy fruits are required for peppers grown for vegetable and paprika production. In sweet peppers, plants are selected for large, glossy, firm, thick-fleshed fruits that will withstand shipping and that are resistant to blossom end rot, and uniform in shape and size. However, extra large fruit is undesirable because it is usually associated with lower productivity, irregular shape and poor quality.

Besides yield and fruit related traits, earliness is another important agronomic character. Earliness is characterized by the number of days from sowing/transplanting until 50% of plants have at least one open flower, or by the number of days from sowing/transplanting until 50% of the plants bear mature fruits (IPGRI *et al.*, 1995). The period between flowering and fruit maturity can also be used as a variable for determining earliness, as peppers bear fruits continuously during their life cycle. Cultivars with a shorter life cycle

could reach maturity before the occurrence of severe moisture deficits. Such cultivars also have the advantage of the early market.

Estimates of the relative importance of additive and non-additive gene action within a breeding population are important to determine which breeding procedure will efficiently improve the performance of the traits of interest (Dudley and Moll, 1969). If additive gene action is predominant, selection during the early generations of selfing would be successful. On the other hand if no additive gene action is present, then the selection would be at late generations when these effects are fixed in the homozygous line.

In pepper, several traits are important, thus different breeding methods may be necessary for improvement of a trait under consideration. A number of studies on combining ability for yield and agronomic traits were reported (Ahmed *et al.*, 1997; Bhagyalakshmi *et al.*, 1991; Kordus, 1991; Mishira *et al.*, 1991; Pandian and Shanmugavelu, 1992; Patel *et al.*, 1998; Legesse, 2000; Stevanovic *et al.*, 1997; Szwadiak and Kordus, 1991). These findings have helped the breeders greatly. However, most of the previous studies that were conducted elsewhere were undertaken within sweet pepper or chile varietal groups. Although each type of pepper must conform to its own unique set of characteristics in order to be commercially acceptable, in some cases, required characters may not be found in the genotypes of the same group but can be found in different groups.

Greenleaf (1986) reported that in certain crosses of small oblate or round-fruited with large elongate-fruited cultivars, the $F_1$ was small and oblate but in other studies crosses between oblate and elongate produced intermediate $F_1$ hybrids. Lack of adequate knowledge about the inheritance of fruit size and shape could be one of the factors that hinder the breeding of pepper for required characters. The nature of gene action and magnitude of genetic effects for different traits of interest in a breeding program often determine the selection strategy used to improve that trait. Thus, the objectives of this study were to investigate the combining ability and genetic effects determining the inheritance of various characters and to identify parental lines with good general combining ability for fruit related traits and earliness.

# MATERIALS AND METHODS

## Experimental materials and $F_1$ seed production

Seven parents were selected based on their diverse genetic backgrounds for fruit characters and earliness from the 39 genotypes evaluated for genetic variability (Chapter 3). Description of the parental lines is presented in Table 4.1. The ready-to-open flower buds were hand emasculated and pollinated to produce all possible combinations of $F_1$ hybrids without the reciprocals. Flowers were emasculated early in the morning and late in the afternoon. Pollen for crossing was obtained from freshly dehisced anthers. At this stage pollen is most abundant and viable (Berke, 2000). The crossing was undertaken in the greenhouse. The $F_1$ fruits were harvested at full physiological maturity and cured for two to three days before seed extraction. The extracted seeds were dried in the shade to avoid the cracking of the seed coats.

Table 4.1. Description of parental lines used in the diallel cross.

| Name | Origin | Characteristic | | |
|---|---|---|---|---|
| | | Earliness | Fruit type | Fruit load per plant |
| Kalocsai "M" Cseresznye | Hungary | Early | Small round | Intermediate |
| Szegedi 178 | Hungary | Early | Intermediate elongate | Intermediate |
| Bakko Local | Ethiopia | Late | Intermediate elongate | Intermediate |
| Mareko Shote | Ethiopia | Intermediate | Large elongate | Intermediate |
| C00916 | Hungary | Early | Small round | Many |
| PBC 142A | AVRDC | Late | Small elongate | Many |
| Pepper 1976 | Israel | Intermediate | Large blocky | Few |

## Experiments

The seeds of 21 $F_1$ hybrids and the seven parents, totaling 28 genotypes were sown in the greenhouse on 15 October 2001. The seedlings were grown in seedling trays with 200 cone-shaped cavities filled with growing medium. The plants were fertilized with

hydroponic nutrient powder at the recommended rate and were regularly watered as deemed essential.

The experiments were conducted in the greenhouse and the field at the University of the Free State. For the two sets of experiments six-week old seedlings were transplanted. The experiment was planted in a randomized complete block design with three replications. In the greenhouse, two seedlings were transplanted into 20 cm polythene pots filled with pseudo duplex soil type. The pots had 19 and 24 cm base and top diameters, respectively. Each pot was considered as a plot. The greenhouse temperatures were maintained at 18°C minimum and 28°C maximum. Under the field conditions, each plot contained two plants and the border rows were planted with gourd plants. Nitrogen and phosphorous fertilizers were applied as recommended for pepper production. Weeding and cultivation were done manually as required. Fourteen quantitative characters, such as days to flowering, days to maturity, fruit maturation period, fruit length, fruit width, mean fruit weight, pericarp thickness, plant height, fruit number, fruit yield, total soluble solids (°Brix) at green and red mature stages, and ascorbic acid (mg/100 g) at green and red mature stages. The description of the characters except for total soluble solids and ascorbic acid content is given in the materials and methods of Chapter 3.

The total soluble solids of fruits at both maturity stages were recorded with a hand refractometer calibrated in 0 Brix and values were adjusted at room temperature.

The ascorbic acid or vitamin C content was determined by the 2,6-dichlorophenol indophenol method (AOAC, 1970). An aliquot of 10 ml pepper juice extract was diluted to 50 ml with 3% metaphosphoric acid in a 50 ml volumetric flask. Afterwards, the aliquot was titrated with the standard dye to a pink end-point (persisting for 15 sec). The ascorbic acid content (mg/100 g) was calculated from the titration volume, dye factor, dilution and volume of the sample.

## Statistical analyses

### *Analysis of combing ability*

Analyses of general (GCA) and specific (SCA) combining ability for individual experiments were done following Griffing's Method II Model I (fixed effect) diallel analysis (Griffing, 1956) using AGROBASE 2000 software (Agrobase, 2000). To determine the relative magnitudes of mean squares of GCA and SCA, GCA:SCA ratios were determined for all characters from their respective GCA and SCA mean squares. The GCA and SCA effects were estimated for all measured characters in both experiments using AGROBASE 2000 software (Agrobase, 2000). The significance of the GCA and SCA effects were determined by t-test using $g_i$ and $s_{ij}$ variances, respectively (Griffing, 1956; Singh and Chaudhary, 1979).

### *Estimates of genetic parameters*

The relative contributions of genetic components were determined to obtain estimates of GCA variance ($\sigma^2_{gca}$) and SCA variance ($\sigma^2_{sca}$) for each character. Additive ($V_a$) and dominance ($V_d$) variances were estimated as $V_a = 2(\sigma^2_{gca})$ and $V_d = (\sigma^2_{sca})$. Genotypic variance ($V_g$) and phenotypic variance ($V_p$) were also calculated as $V_g = V_a + V_d$, and $V_p = V_g + V_e$. Broad ($h^2b$) and narrow ($h^2n$) sense heritabilities were calculated from the estimated components of variances as: $h^2b = \frac{V_g}{V_p}$ and $h^2n = \frac{V_a}{V_P}$, respectively.

The relative sizes of variances due to GCA and SCA on progeny performance were estimated following Baker's predictability ratio (PR) (Baker, 1978) as:

$$PR = 2\sigma^2_{gca}/(2(\sigma^2_{gca} + (\sigma^2_{sca})$$

The average degree of dominance was estimated as $\sqrt{H/D} = \sqrt{(\sigma^2_{sca}/\sigma^2_{gca})}$ (Singh and Chaudhary, 1979).

# RESULTS AND DISCUSSION

## Estimates of combining ability

Estimates of relative importance of additive and non-additive gene actions within breeding populations are important to determine the type of breeding method that effectively will improve the performance of the traits of interest (Dudley and Moll, 1969). In this study, the mean squares of GCA were significant for all studied characters and that of SCA were also significant for all the characters except for FY in the field and GFTSS in the greenhouse (Table 4.2). The significance of GCA and SCA for these characters clearly indicates the importance of both additive and non-additive genetic variances in the inheritance of these characters. This finding is in agreement with previous studies (Ahmed *et al.*, 1997; 1999; Bhagyalakshmi *et al.*, 1991; Kordus, 1991; Stevanovic *et al.*, 1997). The high ratios of GCA:SCA mean squares for most of these characters indicate that GCA is more important than SCA (Table 4.2). Ahmed *et al.* (1997) also found greater additive genetic variances for fruit length, fruit diameter, flesh thickness, fruit number and average fruit weight. The preponderance of GCA effects implied that these characters will respond favorably to direct selection.

Table 4.2. Mean squares for GCA and SCA, and GCA:SCA ratio for various agronomic characters, 2001/2002.

| Character[+] | Environment[++] | GCA df = 6 | SCA df = 21 | GCA:SCA - |
|---|---|---|---|---|
| Fruit yield | GH | 13552.5** | 9732.0** | 1.4 |
| | FLD | 90748.3** | 20494.2 | 4.4 |
| Days to flowering | GH | 199.1** | 77.5** | 2.6 |
| | FLD | 98.1** | 24.8** | 4.0 |
| Days to maturity | GH | 264.5** | 119.5** | 2.2 |
| | FLD | 283.6** | 67.6** | 4.2 |
| Fruit maturation period | GH | 65.9** | 17.8* | 3.7 |
| | FLD | 76.8** | 32.0** | 2.4 |
| Plant height | GH | 108.8* | 182.8** | 0.6 |
| | FLD | 250.6** | 267.7** | 0.9 |
| Fruit length | GH | 43.5** | 1.1** | 38.4 |
| | FLD | 70.7** | 4.0** | 17.8 |
| Fruit diameter | GH | 8.5** | 0.3** | 28.6 |
| | FLD | 10.1** | 0.5** | 19.2 |
| Fruit weight | GH | 3151.5** | 277.5** | 11.4 |
| | FLD | 4457.3** | 189.9** | 23.5 |
| Pericarp thickness | GH | 12.0** | 0.7** | 16.1 |
| | FLD | 11.3** | 0.6** | 17.7 |
| Fruit number | GH | 2152.5** | 244.0** | 8.8 |
| | FLD | 6441.8** | 455.9** | 14.1 |
| GFTSS | GH | 0.9** | 0.2 | 4.0 |
| RFTSS | GH | 5.6** | 3.3** | 1.7 |
| GFAA | GH | 2083.4** | 642.2** | 3.2 |
| RFAA | GH | 4503.4** | 697.4** | 6.5 |

[+] GFTSS = green fruit total soluble solid, RFTSS = red fruit total soluble solid, GFAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid, [++] GH = greenhouse, FLD = field, * $P < 0.05$, ** $P < 0.01$.

### *GCA and SCA effects*

The selection of parents for the breeding program was one of the aims of this study. The estimate of GCA of a parent in the diallel is an important indicator of its potential for generating superior breeding populations. A low positive or negative GCA indicates that the mean of a parent in crossing with the other does not differ greatly from the general mean of the crosses. On the other hand, a high GCA estimate indicates that the parental mean is superior or inferior to the general mean. This represents a strong evidence of favorable gene flow from parents to offspring at high frequency and gives information about the concentration of predominantly additive genes (Cruz and Regazzi, 1994). Franco *et al.* (2001) also suggested that crosses involving genotypes with greater estimates of GCA should be potentially superior for the selection of lines in the advanced generations.

The estimates of GCA effects for measured characters are given in Table 4.3. The GCA effects revealed that parents behaved genetically as expected with the elongated-fruit, large size and thick pericarp parents exhibiting large positive GCA, and round, small and thin pericarp parents exhibiting high negative GCA effects for these characters, respectively. Both negative and positive GCA effects for each fruit character are important since they may allow the development of different types of genotypes with characters of interest. Parental genotypes PBC 142A and Pepper 1976, the smallest and the biggest-fruited parents, generally had the highest negative and positive GCA effects for all fruit related characters, indicating that these genotypes could be used to develop $F_1$ hybrids of decreased or increased fruit related traits, respectively. PBC 142A contributed to increased FN in its crosses and had significant positive GCA effects. Significantly high positive GCA effects for FY were observed in Pepper 1976 and Mareko Shote. Mareko Shote also had significantly high positive GCA effects for AA contents both at green and red mature stages and for GFTSS. The highest AA contents at red and green mature fruit stages were recorded in this cultivar (Table 4.3). Of the seven parental genotypes, Mareko Shote had unique characteristics of dark green fruit color at green mature stage and brown fruit color at physiological fruit maturity. The high concentration of AA at the

two fruit development stages was probably due to these characteristics. The investigation indicates that these quality characteristics with yield could be improved by using this parent in hybrid breeding programs for the accumulation of favorable genes.

Table 4.3. Estimates of general combining ability (GCA) effects and mean performance for 14 characters of diallel experiments, 2001/02.

| Character[+] | Enviro nment | Parent[♣] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $P_1$ | | $P_2$ | | $P_3$ | | $P_4$ | | $P_5$ | | $P_6$ | | $P_7$ | | $LSD_{0.05}$ |
| | | GCA | Mean | GCA | Mean | GCA | Mean | GCA | Mean | GCA | Mean | GCA | Mean | GCA | Mean | |
| FY | GH | -5.13 | 98.5 | 11.56 | 142.4 | 12.29 | 192.0 | 39.72$^{*}$ | 245.6 | -37.72$^{*}$ | 199.1 | -62.84$^{**}$ | 78.3 | 42.13$^{**}$ | 339.7 | 81.8 |
| | FLD | -51.55 | 120.0 | -107.42$^{*}$ | 131.7 | 131.43$^{**}$ | 538.0 | 12.75$^{**}$ | 124.7 | -87.49$^{*}$ | 88.3 | -38.25 | 296.0 | 140.53$^{**}$ | 507.7 | 249.0 |
| DF | GH | -3.92$^{**}$ | 83 | -4.80$^{**}$ | 84 | 5.83$^{**}$ | 113 | 1.34 | 97 | -5.58$^{**}$ | 89 | 6.60$^{**}$ | 113 | -1.47 | 92 | 10 |
| | FLD | -3.71$^{**}$ | 77 | -4.27$^{**}$ | 75 | 3.10$^{**}$ | 89 | 1.80$^{*}$ | 85 | -2.08$^{*}$ | 84 | 3.77$^{**}$ | 97 | 1.40 | 88 | 7 |
| DM | GH | -6.94$^{**}$ | 138 | -4.71$^{**}$ | 146 | 7.88$^{**}$ | 181 | 4.58$^{**}$ | 156 | -3.97$^{**}$ | 148 | 2.62$^{*}$ | 167 | 0.55 | 158 | 7 |
| | FLD | -5.74$^{**}$ | 131 | -3.97$^{**}$ | 136 | 7.37$^{**}$ | 162 | 3.66$^{*}$ | 147 | -6.56$^{**}$ | 128 | 5.44$^{**}$ | 166 | -0.19 | 149 | 11 |
| FMP | GH | -3.02$^{*}$ | 55 | 0.09 | 62 | 2.05 | 68 | 3.24$^{*}$ | 59 | -0.39 | 59 | -3.98$^{**}$ | 54 | 2.02 | 66 | 9 |
| | FLD | -2.03 | 54 | 0.30 | 61 | 4.26$^{**}$ | 73 | 1.86 | 63 | -4.48$^{**}$ | 44 | 1.67 | 69 | -1.59 | 61 | 9 |
| PH | GH | -0.94 | 71.3 | -4.13) | 86.0 | 2.24 | 95.7 | 5.24$^{*}$ | 106.3 | 0.02 | 94.7 | 1.87 | 88.3 | -4.31 | 86.0 | 15.6 |
| | FLD | 1.93 | 61.7 | -8.41$^{**}$ | 53.3 | 5.22$^{*}$ | 71.7 | 6.96$^{**}$ | 76.7 | -3.89 | 45.0 | -0.63 | 68.3 | -1.19 | 68.3 | 15.1 |
| FL | GH | -2.14$^{**}$ | 3.1 | 1.00$^{**}$ | 9.0 | 2.23$^{**}$ | 12.2 | 2.13$^{**}$ | 12.5 | -3.32$^{**}$ | 2.6 | -1.14$^{**}$ | 4.5 | 1.23$^{*}$ | 10.5 | 0.7 |
| | FLD | -2.54$^{**}$ | 2.8 | 0.30 | 6.3 | 3.39$^{**}$ | 14.2 | 2.33$^{**}$ | 11.7 | -4.20$^{**}$ | 2.0 | -1.34$^{**}$ | 3.8 | 2.05$^{**}$ | 12.0 | 1.7 |
| FD | GH | 0.22$^{**}$ | 2.9 | -0.18$^{*}$ | 1.9 | -0.42$^{**}$ | 2.1 | -0.05 | 2.9 | -0.08 | 2.6 | -1.37$^{**}$ | 0.8 | 1.88$^{**}$ | 7.8 | 0.4 |
| | FLD | 0.19 | 2.8 | -0.11 | 2.1 | -0.49$^{**}$ | 2.0 | -0.33$^{*}$ | 2.4 | -0.26 | 2.0 | -1.19$^{**}$ | 0.8 | 2.20$^{**}$ | 8.3 | 0.3 |
| FWT | GH | -3.97$^{*}$ | 11.7 | -3.45$^{*}$ | 13.7 | -4.24$^{*}$ | 15.3 | -2.02 | 22.5 | -11.38$^{**}$ | 4.9 | -15.82$^{**}$ | 1.3 | 40.88$^{**}$ | 159.1 | 17.4 |
| | FLD | -3.86$^{**}$ | 8.7 | -3.12$^{*}$ | 11.8 | -4.53$^{**}$ | 14.7 | -4.12$^{**}$ | 15.7 | -13.60$^{**}$ | 3.7 | -19.27$^{**}$ | 2.0 | 48.51$^{**}$ | 154.7 | 7.5 |
| PCT | GH | 0.65$^{**}$ | 3.6 | -0.17 | 2.5 | -0.72$^{**}$ | 2.5 | -0.21 | 3.3 | 0.04 | 3.3 | -1.64$^{**}$ | 0.8 | 2.06$^{**}$ | 9.4 | 0.7 |
| | FLD | 0.66$^{**}$ | 3.0 | -0.11 | 2.3 | -0.55$^{**}$ | 2.0 | -0.39$^{*}$ | 2.1 | -0.18 | 2.2 | -1.51$^{**}$ | 0.8 | 2.07$^{**}$ | 8.5 | 0.8 |
| FN | GH | 2.90 | 12 | -7.06$^{*}$ | 13 | -7.03$^{*}$ | 17 | -7.95$^{*}$ | 13 | 11.60$^{**}$ | 67 | 27.23$^{**}$ | 85 | -19.69 | 3 | 20 |
| | FLD | -11.18$^{*}$ | 15 | -16.03$^{**}$ | 13 | 3.41 | 49 | -10.92$^{*}$ | 9 | -0.81 | 27 | 57.45$^{**}$ | 220 | -21.92$^{**}$ | 5 | 48 |
| GFTSS | GH | -0.16 | 5.5 | 0.16 | 6.0 | 0.03 | 5.5 | 0.29$^{*}$ | 6.0 | -0.53$^{**}$ | 4.3 | 0.40$^{*}$ | 6.7 | -0.19 | 5.1 | 0.9 |
| RFTSS | GH | -0.62 | 9.5 | -0.42 | 9.4 | -0.92$^{*}$ | 9.8 | 0.44 | 12.5 | 0.10 | 12.1 | 1.46$^{**}$ | 18.5 | -0.05 | 10.3 | 3.4 |
| GFAA | GH | -22.01$^{**}$ | 108.1 | -4.89 | 213.3 | -0.29 | 172.9 | 23.31$^{**}$ | 208.6 | -13.57$^{*}$ | 180.3 | 9.37 | 200.5 | 8.08 | 198.0 | 23.3 |
| RFAA | GH | -28.64$^{**}$ | 125.1 | 1.83 | 191.6 | -4.26 | 246.5 | 37.58$^{**}$ | 315.3 | -23.56$^{**}$ | 196.3 | 13.41$^{*}$ | 264.1 | 3.64 | 212.4 | 12.9 |

[+] FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, FL = fruit length, FD = fruit diameter, FWT = fruit weight, PCT = pericarp thickness, FN = fruit number, GFTSS = green fruit total soluble solid, RFTSS = red fruit total soluble solid, GRAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid, [♣] $P_1$ = Kalocsai "M" Cseresznye, $P_2$ = Szegedi 178, $P_3$ = Bakko Local, $P_4$ = Mareko Shote, $P_5$ = C00916, $P_6$ = PBC 142A, $P_7$ = Pepper 1976, GH = greenhouse, FLD = field, $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$.

Table 4.3 also shows that parental genotypes such as Kalocsai "M" Cseresznye, Szegedi 178, and C00916 had significant GCA effects for early flowering and early maturity, and contributed significantly to earliness in their crosses. They also showed significantly fewer mean numbers of days from sowing to flowering and to fruit maturity. When genetic effects are mainly additive, breeding programs that produce pure lines are logical choices for autogamous crop species (Cockerham, 1961; Dudley and Moll, 1969).

Statistically significant positive or negative SCA effects were observed in some cross combinations (Table 4.4). Since SCA was defined by Sprague and Tatum (1942) as "those cases in which certain combinations do relatively better or worse than would be expected on average performance of the lines involved," the high SCA effects for these crosses suggest that they performed better or poorer than would be expected from the GCA effects of their respective parents. The mean squares for SCA were non-significant only for FY in the field and for GFTSS in the greenhouse indicating the lack of non-additive gene effects for these characters. Vasal *et al.* (1993) also observed lack of non-additive gene actions for grain yield and endosperm hardness in maize.

Hybrid combinations, which had high means, favorable SCA estimates and involved the parents with high GCA, would tend to increase concentration of favorable alleles (Cruz and Regazzi, 1994). In the present study, some hybrids that involved high GCA parents for certain traits showed high tendency towards increased mean performance and high SCA effects (Table 4.4). These hybrids include Kalocsai "M" Cseresznye/Pepper 1976 for FD and PCT, and Szegedi 178/Bakko Local and Szegedi 178/Mareko Shote for FL. Some other crosses that involved high and low GCA parents demonstrated significant mean performance and SCA effects. Kalocsai "M" Cseresznye/PBC 142A and C00916/Pepper 1976 had the lowest number of DM and highly significant negative SCA effects for the trait in both environments but involved contrasting parents regarding the GCA, where Kalocsai "M" Cseresznye had a highly significant negative GCA effect but PBC 142A had a significant positive GCA for this trait. The negative SCA effect of the cross from these parents may be due to additive × dominance type of gene action. Although C00916/Pepper 1976 for DF, and Kalocsai "M" Cseresznye/PBC 142A and

C00916/Pepper 1976 for DM had large negative SCA effects, GCA effects were generally more important than the SCA effects as shown by high GCA:SCA mean square ratios (Table 4.2). This suggests that earliness is influenced to a greater extent by additive than non-additive gene effects among the genotypes studied.

Five crosses (Mareko Shote × PBC 142A, PBC 142A × Pepper 1976, Kalocsai "M" Cseresznye × Szegedi 178, Szegedi 178 × Bakko Local and Szegedi 178 × Marko Shote) showed positive and significant SCA effects for FL (Table 4.4). Of the parents involved in these crosses, all except Kalocsai "M" Cseresznye and PBC 142A had positive significant GCA effects for this character. The large positive SCA effects of hybrids Kalocsai "M" Cseresznye/Szegedi 178, Mareko Shote/PBC 142A and PBC 142A/Pepper 1976 may be due to the unexpected interaction between these genotypes. The high GCA:SCA ratios of 38.4 and 17.8 (Table 4.2) under greenhouse and field conditions, respectively, also suggest that FL is more influenced by additive than non-additive gene effects. $F_1$ hybrids between small round and elongate parents generally had intermediate FL but with the tendency towards the round-fruited parent. This was evidenced by hybrids obtained from Kalocsai "M" Cseresznye × Mareko Shote, Mareko Shote × C00916, and Bakko Local × C00916.

High negative SCA effects were observed in crosses derived from high negative × high positive GCA parents. Hybrid PBC 142A/Pepper 1976 exhibited significant negative SCA effect for FD, revealing high negative GCA parent, PBC 142A, contributed more towards decreased FD than high positive GCA parent, Pepper 1976, could have done towards increased FD.

Certain parental genotypes that had similar geographical origin and were good general combiners for a particular character showed SCA effects to undesired directions in their progenies. Bakko Local × Mareko Shote, both of which were obtained from Ethiopia and were good general combiners for FL, had low negative SCA effects for this character. Similarly, Kalocsai "M" Cseresznye × C00916, in which both parents were obtained from Hungary and had high GCA for DF and DM, gave low positive SCA effects for these

traits. This may suggest that these parents that originated from the same countries probably have a high concentration of the same favorable genes for these characters and have a low degree of gene complementations. Thus, the present study also emphasizes the need to exploit the full advantage of crosses from parental genotypes of different geographical origins.

Table 4. 4. Mean performance, SCA and GCA effects of 21 $F_1$ hybrids for various characters evaluated in the greenhouse (GH) and field (FLD), 2001/02.

| Character* | Env | Cross | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $P_1 \times P_2$ | | | $P_1 \times P_3$ | | | $P_1 \times P_4$ | | | $P_1 \times P_5$ | | | $P_1 \times P_6$ | | | $P_1 \times P_7$ | | | $P_2 \times P_3$ | | |
| | | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect |
| FY | GH | 236 | -31.6 | L×L | 279 | 11.1 | L×L | 415 | 119.7** | L×L | 335 | 116.7* | L×L | 339 | 146.5** | L×L | 240 | -58.2 | L×H | 423 | 138.3** | L×L |
| | FLD | 299 | 53.6 | L×L | 485 | 1.6 | L×H | 416 | 51.3 | L×H | 437 | 172.0 | L×L | 258 | -56.2 | L×L | 633 | 139.8 | L×H | 464 | 36.1 | L×H |
| DF | GH | 73 | -1.7 | H×H | 78 | -7.6** | H×L | 81 | -0.2 | H×L | 77 | 1.1 | H×H | 82 | -4.4 | H×L | 78 | -0.7 | H×L | 77 | -7.4** | H×L |
| | FLD | 76 | 3.0 | H×H | 79 | -1.7 | H×L | 82 | 2.6 | H×L | 78 | 2.8 | H×H | 68** | -13.1** | H×L | 79 | 0.3 | H×L | 81 | 1.2 | H×L |
| DM | GH | 129 | -1.2 | H×H | 140 | -2.8 | H×L | 138 | -2.2 | H×L | 131 | 0.1 | H×H | 130 | -8.2** | H×L | 131 | -5.1 | H×L | 134 | -11.7** | H×L |
| | FLD | 129 | 1.3 | H×H | 136 | -3.0 | H×L | 139 | 4.0 | H×L | 129 | 3.9 | H×H | 119 | -17.4** | H×L | 131 | 0.2 | H×L | 135 | -5.1 | H×L |
| FMP | GH | 56 | 0.5 | H×L | 62 | 4.9 | H×L | 57 | -2.0 | H×L | 54 | -1.0 | H×L | 48 | -3.8 | H×H | 53 | -4.4 | H×L | 56 | -4.3 | L×L |
| | FLD | 52 | -1.6 | L×L | 57 | -1.3 | L×L | 57 | 1.5 | L×L | 50 | 1.1 | L×H | 51 | -4.3 | L×L | 52 | -0.1 | L×L | 54 | -6.3 | L×L |
| PH | GH | 112 | 15.2** | L×L | 112 | 8.5 | L×L | 108 | 1.8 | L×H | 115 | 14.0** | L×L | 126 | 22.8** | L×L | 92 | -5.0 | L×L | 112 | 11.7 | L×L |
| | FLD | 90 | 12.8* | L×L | 103 | 11.9* | L×H | 101 | 8.1 | L×H | 107 | 25.0** | L×L | 77 | -8.3 | L×L | 87 | 2.3 | L×L | 87 | 6.9 | L×H |
| FL | GH | 6.2 | -0.4 | L×H | 8.7 | 0.8* | L×H | 6.5 | -1.2** | L×H | 3.0 | 0.7 | L×L | 4.5 | 0.0 | L×L | 7.7 | 0.8* | L×H | 11.9 | 1.0* | H×H |
| | FLD | 7.9 | 0.7 | L×L | 12.0 | 1.7** | L×H | 8.4 | -0.8 | L×H | 4.3 | 1.6** | L×L | 4.9 | -0.6 | L×L | 9.6 | 0.6 | L×H | 16.3 | 3.2** | L×H |
| FD | GH | 3.4 | 0.4* | H×L | 3.0 | 0.2 | H×L | 3.2 | 0.0 | H×L | 2.9 | -0.2 | H×L | 1.9 | 0.0 | H×L | 5.7 | 0.6** | H×H | 2.6 | 0.2 | L×L |
| | FLD | 3.5 | 0.3 | L×L | 2.9 | 0.0 | L×L | 3.4 | 0.3 | L×L | 3.0 | -0.2 | L×L | 2.2 | -0.0 | L×L | 6.7 | 1.1** | L×H | 2.7 | 0.1 | L×L |
| FWT | GH | 18.7 | 2.6 | L×L | 18.3 | 3.1 | L×L | 21.2 | 3.8 | L×L | 6.4 | -1.7 | L×L | 5.8 | 2.2 | L×L | 58.0 | -2.4 | L×H | 20.9 | 5.1 | L×L |
| | FLD | 28.0 | 6.1* | L×L | 19.7 | -0.9 | L×L | 27.3 | 6.4* | L×L | 12.3 | 0.9 | L×L | 7.0 | 1.2 | L×L | 85.0 | 11.4** | L×H | 24.3 | 3.1 | L×L |
| PCT | GH | 6.2 | 2.2** | H×L | 2.9 | -0.6* | H×L | 4.5 | 0.6* | H×L | 4.5 | 0.3 | H×L | 2.3 | -0.2 | H×L | 6.4 | 0.2 | H×H | 2.8 | 0.2 | L×L |
| | FLD | 4.6 | 0.8* | H×L | 3.2 | -0.1 | H×L | 4.4 | 0.9** | H×L | 4.5 | 0.8* | H×L | 2.0 | -0.4 | H×L | 7.2 | 1.2** | H×H | 3.1 | 0.5 | L×L |
| FN | GH | 19 | -7.4 | L×L | 24 | -2.8 | L×L | 30 | 4.8 | L×L | 79 | 33.6** | L×H | 90 | 29.6** | L×H | 5 | -9.1 | L×L | 26 | 9.2 | L×L |
| | FLD | 15 | 3.8 | L×L | 29 | -1.9 | L×L | 22 | 5.4 | L×L | 54 | 27.0* | L×L | 50 | -34.6** | L×H | 9 | 3.7 | L×L | 22 | -3.8 | L×L |
| GFTSS | GH | 5.0 | -0.15 | L×L | 4.9 | -0.1 | L×L | 4.5 | -0.8 | L×H | 4.3 | -0.1 | L×L | 5.2 | -0.2 | L×H | 4.6 | -0.2 | L×L | 4.8 | -0.5 | L×L |
| RFTSS | GH | 9.0 | -0.6 | L×L | 11.8 | 2.7* | L×L | 9.9 | -0.5 | L×L | 9.7 | -0.4 | L×L | 9.7 | -1.8 | L×H | 10.3 | 0.3 | L×L | 9.2 | -0.1 | L×L |
| GFAA | GH | 146 | -2.4 | L×L | 216 | 62.5 | L×L | 191 | 14.4 | L×H | 124 | -16.2 | L×L | 158 | -4.4 | L×L | 154 | -7.5 | L×L | 142 | -28.5 | L×L |
| RFAA | GH | 216 | 27.6* | L×L | 245 | 62.7** | L×L | 220 | -3.6 | L×H | 140 | -22.3 | L×L | 186 | -13.4 | L×H | 204 | 13.8 | L×L | 207 | -5.6 | L×L |

Table 4. 4. Continued...

| Character | Environment | Cross | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $P_2 \times P_4$ | | | $P_2 \times P_5$ | | | $P_2 \times P_6$ | | | $P_2 \times P_7$ | | | $P_3 \times P_4$ | | | $P_3 \times P_5$ | | | $P_3 \times P_6$ | |
| | | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect |
| FY | GH | 380 | 67.8 | L×H | 134 | -100.3* | L×L | 262 | 52.7 | L×L | 471 | 156.3** | L×H | 423 | 110.0* | L×H | 209 | -26.3 | L×L | 161 | -49.1 | L×L |
| | FLD | 385 | 75.7 | L×H | 112 | -96.6 | L×L | 367 | 108.8 | L×L | 374 | -63.0 | H×H | 585 | 36.8 | H×H | 603 | 154.7 | H×L | 442 | -55.4 | H×L |
| DF | GH | 77 | -3.6 | L×L | 71 | -4.0 | H×H | 79 | -6.5** | H×L | 80 | 2.9 | L×L | 85 | -5.6** | L×L | 81 | -4.6** | L×H | 93 | -2.8 | L×L |
| | FLD | 79 | 0.1 | L×L | 65** | -10.0** | H×H | 79 | -1.5 | H×L | 82 | 3.5 | L×L | 87 | 0.8 | L×L | 81 | -1.0 | L×H | 87 | -1.2 | L×L |
| DM | GH | 137 | -5.1 | H×L | 132 | -1.5 | H×H | 132 | -7.8** | H×L | 139 | 0.7 | L×L | 150 | -4.3 | L×L | 139 | -6.8** | L×H | 147 | -6.0** | L×L |
| | FLD | 136 | -0.7 | H×L | 125 | -1.2 | H×H | 132 | -6.5 | H×L | 131 | -1.6 | L×L | 143 | -5.4 | L×L | 138 | 0.5 | L×H | 146 | -4.2 | L×L |
| FMP | GH | 60 | -1.4 | L×L | 61 | 2.5 | L×L | 53 | -1.2 | L×H | 58 | -2.2 | L×L | 65 | 1.3 | L×L | 58 | -2.1 | L×L | 53 | -3.2 | L×H |
| | FLD | 57 | -0.9 | L×L | 60 | 8.8 | L×H | 53 | -5.0 | L×L | 49 | -5.1 | L×L | 57 | -6.2 | L×L | 57 | 1.5 | L×H | 59 | -3.0 | L×L |
| PH | GH | 111 | 7.7 | L×H | 83 | -14.5** | L×L | 110 | 10.4 | L×L | 78 | -15.1** | L×L | 98 | -11.4 | L×H | 108 | 3.5 | L×L | 105 | -1.0 | L×L |
| | FLD | 87 | 4.4 | L×H | 64 | -7.0 | L×L | 91 | 16.4** | L×L | 68 | -6.4 | H×L | 97 | 1.5 | H×H | 97 | 12.3* | H×L | 94 | 6.1 | H×L |
| FL | GH | 12.7 | 1.8** | H×H | 4.2 | -1.3** | H×L | 7.7 | 0.1 | H×L | 10.3 | 0.4 | H×H | 11.8 | -0.3 | H×H | 5.7 | -0.9* | H×L | 7.9 | -0.9* | H×L |
| | FLD | 14.3 | 2.8** | L×H | 4.1 | -1.4* | L×L | 9.9 | 1.5* | L×L | 12.5 | 0.7 | H×H | 15.0 | -0.2 | H×H | 8.1 | -0.6 | H×L | 11.5 | 0.0 | H×L |
| FD | GH | 3.6 | 0.9** | L×L | 3.0 | 0.3 | L×L | 1.4 | -0.0 | L×L | 4.4 | -0.2 | L×H | 2.3 | -0.2 | L×L | 2.7 | 0.2 | L×L | 1.4 | 0.2 | L×L |
| | FLD | 2.9 | 0.2 | L×L | 2.7 | -0.1 | L×L | 3.5 | 1.6** | L×L | 5.0 | -0.3 | L×H | 2.2 | -0.1 | L×L | 2.8 | 0.4 | L×L | 1.9 | 0.4 | L×L |
| FWT | GH | 20.5 | 2.5 | L×L | 9.7 | 1.0 | L×L | 7.0 | 2.9 | L×L | 52.4 | -8.5* | L×H | 19.9 | 2.7 | L×L | 11.3 | 3.5 | L×L | 5.5 | 2.1 | L×L |
| | FLD | 28.7 | 7.0* | L×L | 9.7 | -2.5 | L×L | 8.0 | 1.5 | L×L | 81.3 | 7.0* | L×H | 21.7 | 1.4 | L×L | 16.0 | 5.2 | L×L | 8.0 | 2.9 | L×L |
| PCT | GH | 2.9 | -0.3 | L×L | 2.8 | -0.5 | L×L | 2.1 | 0.4 | L×L | 4.6 | -0.8** | L×H | 2.5 | -0.1 | L×L | 3.2 | 0.4 | L×L | 1.0 | -0.1 | L×L |
| | FLD | 3.3 | 0.6 | L×L | 2.7 | -0.3 | L×L | 2.0 | 0.4 | L×L | 4.7 | -0.5 | L×H | 2.4 | 0.1 | L×L | 2.9 | 0.4 | L×L | 1.3 | 0.2 | L×L |
| FN | GH | 23 | 7.1 | L×L | 15 | -20.1** | L×H | 63 | 12.3 | L×H | 9 | 5.2 | L×L | 29 | 13.1 | L×L | 23 | -12.5 | L×H | 39 | -12.1 | L×H |
| | FLD | 16 | 4.3 | L×L | 15 | -6.5 | L×L | 63 | -17.5 | L×H | 7 | 6.3 | L×L | 31 | 0.1 | L×L | 56 | 15.0 | L×L | 88 | -11.6 | L×H |
| GFTSS | GH | 5.9 | 0.3 | L×H | 4.3 | -0.5 | L×L | 56 | -0.0 | L×H | 4.9 | -0.2 | L×L | 5.2 | -0.3 | L×H | 4.5 | -0.2 | L×L | 5.7 | 0.2 | L×H |
| RFTSS | GH | 11.7 | 1.0 | L×L | 10.8 | 0.5 | L×L | 12.1 | 0.4 | L×H | 9.8 | -0.4 | L×L | 9.7 | -0.4 | L×L | 9.1 | -0.7 | L×L | 7.0 | -4.2** | L×H |
| GFAA | GH | 183 | -10.9 | L×H | 144 | -13.2 | L×L | 158 | -21.4 | L×L | 160 | -19.1 | L×L | 201 | 2.5 | L×H | 137 | -24.5* | L×L | 176 | -8.9 | L×L |
| RFAA | GH | 227 | -27.1* | L×H | 172 | -21.0 | L×L | 219 | -11.0 | L×H | 201 | -19.0 | L×L | 264 | 15.9 | L×H | 176 | -11.1 | L×L | 202 | -22.4 | L×H |

Table 4.4. Continued...

| Character[+] | Environment | Cross[♣] | | | | | | | | | | | | | | | | | | | | LSD$_{0.05}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $P_3 \times P_7$ | | | $P_4 \times P_5$ | | | $P_4 \times P_6$ | | | $P_4 \times P_7$ | | | $P_5 \times P_6$ | | | $P_5 \times P_7$ | | | $P_6 \times P_7$ | | |
| | | Mean | SCA | GCA effect[♦] | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | Mean | SCA | GCA effect | |
| FY | GH | 318 | 3.0 | L×H | 274 | 11.1 | H×L | 172 | -65.2 | H×L | 289 | -54.0 | H×H | 179 | 18.2 | L×L | 219 | -46.8 | L×H | 251 | 10.6 | L×H | 120.2 |
| | FLD | 759 | 83.5 | H×H | 532 | 202.6 | H×L | 408 | 29.8 | H×L | 770 | 213.2 | H×H | 230 | -48.4 | L×L | 354 | -103.2 | L×H | 590 | 84.0 | L×H | 263.9 |
| DF | GH | 81 | -7.1 | L×L | 78 | -3.5 | L×H | 86 | -6.0** | L×L | 81 | -2.3 | L×L | 82 | -4.4 | H×L | 70 | -8.7** | H×L | 82 | -7.2** | L×L | 4 |
| | FLD | 85 | -0.2 | L×L | 79 | -1.7 | L×H | 88 | 1.1 | L×L | 82 | -2.2 | L×L | 85 | 1.6 | H×L | 75 | -6.0** | H×L | 83 | -3.2 | L×L | 4 |
| DM | GH | 137 | -13.9** | L×L | 140 | -2.8 | L×H | 146 | -3.4 | L×L | 155 | 7.7** | L×L | 136 | -4.8 | H×L | 128 | -11.1** | H×L | 137 | -8.3** | L×L | 7 |
| | FLD | 142 | -2.6 | L×L | 136 | 1.5 | L×H | 142 | -4.5 | L×L | 140 | -0.9 | L×L | 138 | 1.7 | H×L | 116 | -14.6** | H×L | 137 | -5.5 | L×L | 7 |
| FMP | GH | 56 | -6.8** | L×L | 62 | 0.7 | L×L | 60 | 2.6 | L×H | 74 | 10.0** | L×L | 54 | -0.4 | L×H | 58 | -2.4 | L×L | 55 | -1.1 | L×L | 7 |
| | FLD | 56 | -2.4 | L×L | 56 | 3.3 | L×H | 54 | -5.6 | L×L | 57 | 1.4 | L×L | 53 | 0.1 | H×L | 41 | -8.6 | H×L | 54 | -2.1 | L×L | 7 |
| PH | GH | 110 | 10.1 | L×L | 110 | 2.8 | H×L | 103 | -6.0 | H×L | 120 | 17.2** | H×L | 112 | 7.9 | L×L | 98 | 0.7 | L×L | 100 | 0.6 | L×L | 16.2 |
| | FLD | 94 | 6.3 | H×L | 104 | 17.4** | H×L | 85 | -5.3 | H×L | 105 | 15.6** | H×L | 92 | 12.5 | L×L | 80 | 1.4 | L×L | 89 | 6.8 | L×L | 14.8 |
| FL | GH | 11.7 | 0.5 | H×H | 5.8 | -0.7 | H×L | 9.7 | 1.0* | H×L | 9.6 | -1.5** | H×H | 4.2 | 0.9* | L×L | 4.0 | -1.6** | L×H | 8.7 | 0.9* | L×H | 1.1 |
| | FLD | 14.8 | -0.1 | H×H | 7.3 | -0.3 | H×L | 12.9 | 2.5** | H×L | 14.8 | 1.0 | H×H | 4.2 | 0.3 | L×L | 5.8 | -1.5* | L×H | 12.4 | 2.3** | L×H | 1.4 |
| FD | GH | 3.9 | -0.5** | L×H | 3.0 | 0.1 | L×L | 1.5 | -0.1 | L×L | 4.2 | -0.7** | L×H | 1.6 | 0.1 | L×L | 4.8 | 0.0 | L×H | 2.2 | -1.3** | L×H | 0.4 |
| | FLD | 4.6 | -0.3 | L×H | 3.2 | 0.6 | L×L | 1.4 | -0.3 | L×L | 4.6 | -0.5 | L×H | 1.9 | 0.2 | L×L | 5.5 | 0.4 | L×H | 2.4 | -1.8** | L×H | 1.4 |
| FWT | GH | 43.0 | -17.1** | L×H | 14.7 | 4.6 | L×L | 7.1 | 1.5 | L×L | 40.9 | -21.4** | L×H | 3.6 | 7.4 | L×L | 29.7 | -23.3** | L×H | 13.5 | -35.0** | L×H | 5.9 |
| | FLD | 71.7 | -1.2 | L×H | 17.7 | 6.5* | L×L | 7.3 | 1.8 | L×L | 60.3 | -13.0** | L×H | 4.7 | 8.6** | L×L | 41.3 | -22.5** | L×H | 19.0 | -39.2** | L×H | 7.3 |
| PCT | GH | 4.1 | -0.7* | L×H | 3.6 | 0.3 | L×L | 1.3 | -0.3 | L×L | 4.7 | -0.7* | L×H | 2.1 | 0.2 | L×L | 5.5 | -0.1 | L×H | 2.6 | -1.3** | L×H | 0.7 |
| | FLD | 4.1 | -0.7** | L×H | 3.2 | 0.5 | L×L | 1.2 | -0.2 | L×L | 3.8 | -1.1** | L×H | 1.6 | 0.0 | L×L | 5.2 | 0.0 | L×H | 2.7 | -1.1** | L×H | 0.9 |
| FN | GH | 9 | 4.8 | L×L | 25 | -9.6 | L×H | 34 | -16.2* | L×H | 8 | 4.7 | L×L | 68 | -1.1 | H×H | 6 | -16.8* | H×L | 26 | -12.4 | H×L | 19 |
| | FLD | 15 | -5.5 | L×L | 38 | 10.7 | L×L | 69 | -16.2 | L×H | 17 | 10.8 | L×L | 74 | -21.7 | L×H | 12 | -3.6 | L×L | 42 | -31.9** | H×L | 23 |
| GFTSS | GH | 5.1 | 0.1 | L×L | 5.3 | 0.4 | H×L | 5.1 | -0.7 | H×H | 5.8 | 0.6 | H×L | 5.1 | 0.1 | L×H | 4.2 | -0.2 | L×L | 4.5 | -0.8 | L×L | 0.9 |
| RFTSS | GH | 10.0 | 0.7 | L×L | 10.3 | -0.9 | L×L | 10.3 | -2.3* | L×H | 12.1 | 1.1 | L×L | 11.3 | -0.9 | L×H | 10.5 | -0.2 | L×L | 10.9 | -1.2 | H×L | 2.3 |
| GFAA | GH | 184 | 0.8 | L×L | 184 | -1.2 | H×L | 200 | -8.4 | H×L | 237 | 30.4* | H×L | 191 | 19.4 | L×L | 141 | -28.4* | L×L | 204 | 10.9 | L×L | 32.8 |
| RFAA | GH | 204 | -10.2 | L×L | 205 | -23.8 | H×L | 236 | -29.9* | H×H | 274 | 17.8 | H×L | 223 | 17.8 | L×H | 198 | 3.0 | L×L | 246 | 13.8 | H×L | 35.9 |

[+] FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, FL = fruit length, FD = fruit diameter, FWT = mean fruit weight, PCT = pericarp thickness, FN = fruit number, GFTSS = green fruit total soluble solid, RFTSS = red fruit total soluble solid, GRAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid, [♣] GH = greenhouse, FLD = field,, [♣] $P_1$ = Kalocsai "M" Cseresznye, $P_2$ = Szegedi 178, $P_3$ = Bakko Local, $P_4$ = Mareko Shote, $P_5$ = C00916, $P_6$ = PBC 142A, $P_7$ = Pepper 1976, [♦] H = high parent, L = Low parent, * $P < 0.05$, ** $P < 0.01$.

**Estimates of genetic parameters**

General combining ability variances ($\sigma^2_{gca}$) were much higher than SCA variances ($\sigma^2_{sca}$) for FL, FD, FWT, PCT and FN in both environments (Table 4.5), indicating additive variability was of greater importance in the inheritance of the characters and that they should respond favorably to direct selection. However, since the parents were not randomly selected but rather selected for extreme values of fruit size, shape and number, the relative amount of GCA variances may have been overestimated. In these characters, however, significant SCA effects were observed, which, as suggested by Stuber (1970) were probably the result of additive × additive epistatic effects. Stuber (1970) also proposed that additive × additive epistatic variation is more important in determining the inheritance of quantitative traits in self-pollinating crops. In general, high heritability estimates in both the broad- and narrow-sense were also recorded for these characters indicating again the greater importance of additive variability in their inheritance. The low heritability that was observed in other characters indicates that environmental factors have more pronounced effects relative to the genetic effects.

The average degree of dominance for DF, DM, FMP, FY, GFTSS, RFTSS, GFAA and RFAA were greater than 1 (Table 4.5), indicating the presence of over dominance. Similarly, Zecevic and Stevanovic (1997) reported over dominance for earliness and yield per plant. Bhatt *et al.* (2001) found an average degree of dominance of more than unity for total soluble solid and vitamin C in tomato. On the other hand, the degrees of dominances for FL, FD, FWT, PCT and FN ranged between 0 and 1, suggesting partial dominance.

Baker (1978) indicated that when SCA mean squares are not significant, the hypothesis that the performance of a single-cross progeny can be adequately predicted on the basis of a GCA would be accepted. If, on the other hand, the SCA mean square is significant the relative importance of GCA and SCA should be assessed by estimating components of variance in determining progeny performance. The closer the ratio to unity, the greater the predictability based on GCA alone. The predictability ratios (PR) for FL, FD, FWT,

PCT and FN were very high (Table 4.5), therefore it can be inferred that the possibility of determining progeny performance for these fruit characters from parental GCA alone is high.

Table 4.5. Estimates of genetic parameters for various characters evaluated under greenhouse (GH) and field (FLD) conditions, 2001/02.

| Character[+] | Environment | Genetic parameter | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $\sigma^2$gca | $\sigma^2$sca | $\sigma^2$A | $\sigma^2$D | $\sigma^2$e | $h^2$b (%) | $h^2$n (%) | PR | $\sqrt{D/H}$ |
| FY | GH | 424.5 | 7600.3 | 849.0 | 7600.3 | 2131.7 | 79.9 | 8.0 | 0.10 | 4.23 |
| | FLD | 7806.0 | 8556.3 | 15612.0 | 8556.3 | 11937.9 | 66.9 | 43.2 | 0.65 | 1.05 |
| DF | GH | 21.3 | 71.8 | 42.6 | 71.8 | 5.7 | 95.3 | 35.5 | 0.37 | 1.84 |
| | FLD | 8.1 | 20.5 | 16.3 | 20.5 | 4.3 | 89.5 | 39.7 | 0.44 | 1.59 |
| DM | GH | 16.1 | 111.4 | 32.2 | 111.4 | 8.1 | 94.7 | 21.2 | 0.22 | 2.63 |
| | FLD | 24.0 | 54.4 | 48.0 | 54.4 | 13.2 | 88.6 | 41.5 | 0.47 | 1.51 |
| FMP | GH | 5.3 | 8.9 | 10.6 | 8.9 | 9.0 | 68.4 | 37.2 | 0.54 | 1.30 |
| | FLD | 4.98 | 21.35 | 9.97 | 21.35 | 10.61 | 74.7 | 15.3 | 0.32 | 2.07 |
| PH | GH | -8.2 | 138.1 | -16.4 | 138.1 | 44.7 | 73.1 | -9.9 | -0.13 | - |
| | FLD | -1.9 | 230.7 | -3.8 | 230.7 | 37.0 | 86.0 | -1.4 | -0.02 | - |
| FL | GH | 4.7 | 1.0 | 9.4 | 1.0 | 0.2 | 98.1 | 88.7 | 0.90 | 0.46 |
| | FLD | 7.4 | 3.6 | 14.8 | 3.6 | 0.4 | 97.9 | 78.7 | 0.80 | 0.70 |
| FD | GH | 0.9 | 0.3 | 1.8 | 0.3 | 0.03 | 98.6 | 84.5 | 0.86 | 0.58 |
| | FLD | 1.1 | 0.3 | 2.2 | 0.3 | 0.2 | 92.6 | 81.1 | 0.88 | 0.52 |
| FWT | GH | 319.3 | 261.3 | 638.6 | 261.3 | 16.2 | 98.2 | 69.7 | 0.71 | 0.90 |
| | FLD | 474.2 | 180.8 | 948.3 | 180.8 | 9.1 | 99.2 | 83.3 | 0.84 | 0.62 |
| PCT | GH | 1.2 | 0.7 | 2.4 | 0.7 | 0.1 | 96.9 | 75.0 | 0.77 | 0.76 |
| | FLD | 1.2 | 0.5 | 2.4 | 0.5 | 0.1 | 96.7 | 80.0 | 0.83 | 0.62 |
| FN | GH | 212.0 | 178.2 | 424.0 | 178.2 | 65.8 | 90.1 | 63.5 | 0.70 | 0.92 |
| | FLD | 665.1 | 303.2 | 1330.2 | 303.2 | 152.8 | 91.4 | 74.5 | 0.81 | 0.68 |
| GFTSS | GH | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 75.0 | 50.0 | 0.67 | 1.00 |
| RFTSS | GH | 0.3 | 2.2 | 0.6 | 2.2 | 1.2 | 70.0 | 15.0 | 0.21 | 2.71 |
| GFAA | GH | 160.1 | 481.3 | 320.1 | 481.3 | 161.3 | 83.2 | 33.3 | 0.40 | 1.73 |
| RFAA | GH | 422.9 | 515.3 | 845.8 | 515.3 | 182.1 | 88.2 | 54.8 | 0.62 | 1.10 |

[+] FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, FL = fruit length, FD = fruit diameter, FWT = fruit weight, PCT = pericarp thickness, FN = fruit number, GFTSS = green fruit total soluble solid, RFTSS = red fruit total soluble solid, GRAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid, PR = predictability ratio, D = SCA variance, H = GCA variance.

## CONCLUSION

Information on diallel analysis of fruit related traits and agronomic characters in available Ethiopian and exotic pepper germplasm are meager. The present study considered the combining ability of seven diverse pepper parents for fruit related traits, earliness, ascorbic acid content, total soluble solids, plant height and fruit yield per plant. Since parents were not selected at random, inferences must be limited to the respective populations of the seven parent diallel experiments.

This investigation indicated that GCA and SCA were significant sources of variation for all measured characters. However, the higher magnitude of GCA as compared to SCA showed that additive genetic effects are more important for the inheritance of these characters. Thus, high genetic effects could be achieved per breeding cycle. Since GCA estimates were much larger than SCA estimates for all measured characters with the exception of PH, the correlation between *per se* value and GCA will give an indication about the possibility to use means of the two parents to predict the value of the $F_1$ hybrid. The significant estimates of GCA effects that were observed among the parents for measured characters show that individual parents contributed differently to the specific character. Parents such as Kalocsai "M" Cseresznye and Szegedi 178 for earliness; Bakko Local and Mareko Shote for FL; Mareko Shote for FY and AA; PBC 142A for FN; and Pepper 1976 for FL, FN, FWT, PCT and FY, are good combiners and an ideal choice as parents for the pepper breeding program in Ethiopia. In general, the breeding materials used in this study were found to be useful sources for genetic variability for the development of new genotypes of desired fruit size and shape.

Some crosses demonstrated significantly high SCA effects to the desired direction indicating that they can be grown as hybrids. Certain cross combinations that involved parental lines that were good general combiners for certain characters revealed favorable SCA effects and higher means would tend to increase favorable alleles. On the other hand, significant mean performance and SCA effects were also observed in some crosses that involved contrasting parents regarding GCA.

Of the studied characters, FL, FD, FWT, PCT and FN showed very high heritability both in narrow- and broad-sense indicating that the environment had less effect on their inheritances. The high predictability ratios of these characters also showed that the prediction of progeny performance only from the GCA effects of parental lines could be possible. Partial dominance, dominance and over dominance of the studied characters were observed.

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# CHAPTER 5

## Hybrid performance and heterosis for yield and other agronomic characters


### ABSTRACT

Seven genetically diverse pepper inbred lines were crossed in a half diallel fashion to evaluate the performance of hybrids and determine heterosis over mid-parent, high-parent and standard check for various characters. The study was undertaken in the field and greenhouse using a randomized complete block design with three replications during 2001/02. Hybrids generally showed good overall performance for most of the characters compared to the inbred lines. Two of the 21 hybrids significantly out yielded the standard check (Bakko Local). In addition, five inbred lines and all the hybrids were earlier to flower and mature compared to the check. Substantial mid-parent (MPH), high-parent (HPH) and standard (SH) heterosis were observed for the majority of studied characters. Mean MPH and SH were high and positive for fruit yield, plant height, fruit diameter, fruit weight, pericarp thickness and fruit number per plant. High positive HPH was observed in fruit yield per plant and plant height. For days to flowering, days to maturity and fruit maturation period, the overall mean MPH, HPH and SH were negative values. Thus, it can be concluded that with the proper choice of parents, pepper hybrids of higher yield potential, good fruit characteristics and early types can be developed.

# INTRODUCTION

Pepper is an important spice and vegetable crop in Ethiopia. Various types and forms of peppers are grown in the country. The two major types are capsicums or "berbere" and chiles or "mitmita". *Berbere* is used to add color and pungency to the local dishes and *mitmita* is utilized entirely for its high pungency in certain foods. The former is more important in terms of consumption and area of production. However, yield per unit area is very low mainly due to the local cultivars being low yielding, late maturing and susceptible to biotic and abiotic stresses.

Crop plants are crossed to produce superior $F_1$ individuals. The lines that are crossed are from different populations. If the two populations differ in gene frequencies, a cross between them will show heterosis (Falconer and Mackay, 1996). Yield heterosis is a variable trait and depends not only on the parent combinations but also on environmental conditions. Young and Virmani (1990) and Virmani *et al.* (1982) reported variable yield heterosis in rice. Generally, heterosis is environment-dependent, but the nature of interactions depends on the species and the trait under consideration (Knight, 1973).

Heterosis is a genetic phenomenon resulting from heterozygosity (Shigeru *et al.*, 1998), usually described as superiority of $F_1$ hybrid performance, i.e. hybrid vigor. Hybrid vigor or heterosis usually refers to the increase in size or rate of growth of offspring over parents (Duvick, 1999). Falconer and Mackay (1996) described heterosis as the difference between the hybrid and the mean of the two parents and this is often expressed as a percentage of the mid-parent. The other type of heterosis is high parent heterosis, which is the difference between the hybrid and the high parent. Lamkey and Edward (1999) suggested that high parent heterosis is preferred in some circumstances, particularly in self-pollinated crops, for which the goal is to find a better hybrid than either of the parents. The third measure of heterosis, standard heterosis, is the difference between the hybrid and the standard variety. From the plant breeding viewpoint standard heterosis is of practical significance (Young and Virmani, 1990).

Heterosis for yield and other agronomic characters were reported in peppers (Ahmed *et al.*, 1999; Bhagyalakshmi *et al.*, 1991; Kaul and Sharma, 1988; Kordus, 1991; Zecevic and Stevanovic, 1997). However, information on heterotic patterns between the available Ethiopian and exotic cultivars is meager. According to Berke (2000) pepper hybrids are gaining increasing popularity among farmers throughout the world. Bosland and Votava (2000) also indicated that peppers grown from hybrid seeds are highly uniform and usually higher yielding. The importance of growing peppers from hybrid seeds can also be judged from the high price of pepper hybrid seeds. Berke (2000) noted that the price can range from \$300 to \$25000 $kg^{-1}$ depending on the company and where it is sold.

Pepper production in many areas of Ethiopia is generally characterized by water deficit, where plants are entirely dependent on stored soil moisture for growth and maturity. Furthermore, they are generally grown under poor management by subsistence farmers. The supply of limited and erratic water and nutrients can further affect the productivity of the available unimproved local cultivars. Studies have shown that heterosis is greater under stress environments than under favorable conditions. Axtell *et al.* (1999) reported that in sorghum and pearl millet hybrids yield heterosis increased by 58% over the best parent under dry land conditions. The objectives of this study were to evaluate the performance of hybrids, and estimate mid-parent, high-parent and standard heterosis in hybrids obtained from crosses between parental genotypes of diverse genetic backgrounds.

# MATERIALS AND METHODS

## Experimental materials and $F_1$ seed production

These are presented in the materials and methods of Chapter 4.

## Experiments

All the detail is given in the materials and methods part of Chapter 4. Measurements are also similar to that of Chapter 4.

## Statistical analyses

Analyses of variances for various characters evaluated at two environments were done using AGROBASE 2000 Software (Agrobase, 2000). The mean squares were compared with F-values to assess the significances of the differences among the genotypes.

### *Estimates of heterosis*

$$\text{Mid-parent heterosis (MPH)}(\%) = \frac{F_1 - MP}{MP} \times 100$$

$$\text{High-parent heterosis (HPH)}(\%) = \frac{F_1 - HP}{HP} \times 100$$

$$\text{Standard heterosis (SH)}(\%) = \frac{F_1 - SV}{SV} \times 100$$

where, $F_1$ = $F_1$ hybrid performance, MP = $(P_1 + P_2)/2$ in which $P_1$ and $P_2$ are the performances of inbred parents, respectively; HP = high parent value; SV = standard variety value.

Statistical significance of mid-parent, high-parent and standard heterosis values was tested by comparing these values with the LSD values.

## RESULTS AND DISCUSSION

Analyses of variance for various characters evaluated in two environments showed that the mean squares of genotypes, parental lines and hybrids were all significant with the exception of a non-significant parental mean square for FMP in the greenhouse (Table 5.1). Significant mean squares of parental lines revealed the presence of wide genetic differences among the parental genotypes. As it is shown in the table, parents vs. hybrids mean squares, which is the measure of average heterosis, was significant for DF, PH and GFTSS in both environments indicating the importance of dominant genetic effects in the inheritance of these characters. Similarly, Zecevic and Stevanovic (1997) reported that non-additive gene action plays a more important role than additive gene action in the inheritance of earliness. On the contrary, the non-significance of average heterosis for FMP, FN, RFTSS, GFAA and RFAA suggest the absence of dominant genetic effects for these characters. The significance of average heterosis for DM, FL, FD, FWT, PCT, and FY was variable across the two environments.

Table 5.1. Mean squares of diallel crosses among seven parents evaluated for 14 agronomic characters in the greenhouse (GH) and field (FLD), 2001/2002.

| Character[+] | Env. | Source of variation | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Rep | Genotype | Parent (P) | Hybrid ($F_1$) | P vs. $F_1$ | Error | CV, % |
| | | df = 2 | df = 27 | df= 6 | df = 20 | df = 1 | df = 54 | - |
| FY | GH | 3177.9 | 31743.0$^{**}$ | 24254.4$^{**}$ | 27537.3$^{**}$ | 15313.0$^{**}$ | 6395.1 | 30.7 |
| | FLD | 179942.2$^{**}$ | 108318.6$^{**}$ | 111919.1$^{*}$ | 82875.2$^{**}$ | 56722.2 | 35813.8 | 46.9 |
| DF | GH | 3.6 | 313.6$^{**}$ | 474.3$^{**}$ | 77.1$^{**}$ | 388.6$^{**}$ | 17.2 | 5.0 |
| | FLD | 8.0 | 123.2$^{**}$ | 168.9$^{**}$ | 97.9$^{**}$ | 33.8$^{*}$ | 13.0 | 4.4 |
| DM | GH | 5.1 | 455.2$^{**}$ | 610.4$^{**}$ | 154.6$^{**}$ | 527.1$^{**}$ | 24.4 | 3.5 |
| | FLD | 20.6 | 346.8$^{**}$ | 652.0$^{**}$ | 170.8$^{**}$ | 193.7 | 39.6 | 4.5 |
| FMP | GH | 13.4 | 85.6$^{**}$ | 77.7 | 86.6$^{**}$ | 10.5 | 27.0 | 8.9 |
| | FLD | 42.9 | 125.8$^{**}$ | 279.8$^{**}$ | 51.3$^{*}$ | 65.7 | 31.8 | 10.1 |
| PH | GH | 619.8$^{**}$ | 499.1$^{**}$ | 344.3$^{*}$ | 378.8$^{**}$ | 365.2$^{*}$ | 134.2 | 11.4 |
| | FLD | 140.9 | 791.7$^{*}$ | 367.9$^{*}$ | 393.1$^{**}$ | 1076.9$^{**}$ | 111.0 | 12.4 |
| FL | GH | 1.1 | 31.6$^{**}$ | 55.3$^{**}$ | 26.1$^{**}$ | 0.0 | 0.6 | 9.6 |
| | FLD | 0.1 | 56.4$^{**}$ | 74.8$^{**}$ | 48.6$^{**}$ | 9.7$^{*}$ | 1.2 | 11.5 |
| FD | GH | 0.1 | 6.4$^{**}$ | 15.0$^{**}$ | 4.1$^{**}$ | 0.0 | 0.1 | 9.8 |
| | FLD | 0.4 | 7.9$^{**}$ | 17.9$^{**}$ | 5.2$^{**}$ | 0.2$^{*}$ | 0.5 | 22.6 |
| FWT | GH | 98.8 | 2748.5$^{**}$ | 9470.9$^{**}$ | 750.6$^{**}$ | 225.7$^{*}$ | 48.5 | 29.7 |
| | FLD | 16.8 | 3414.7$^{**}$ | 9125.4$^{**}$ | 1870.1$^{**}$ | 3.9 | 27.3 | 18.1 |
| PCT | GH | 0.04 | 9.7$^{**}$ | 21.7$^{**}$ | 6.6$^{**}$ | 0.0 | 0.3 | 14.8 |
| | FLD | 0.1 | 9.0$^{**}$ | 19.0$^{**}$ | 6.3$^{**}$ | 0.2$^{*}$ | 0.4 | 18.4 |
| FN | GH | 128.3 | 2004.4$^{**}$ | 3101.5$^{**}$ | 1750.0$^{**}$ | 1.0 | 197.5 | 45.9 |
| | FLD | 1429.8$^{*}$ | 5358.4$^{**}$ | 17905.8$^{**}$ | 1730.9$^{**}$ | 249.8 | 458.3 | 55.3 |
| GFTSS | GH | 0.3 | 1.2$^{**}$ | 1.7$^{**}$ | 0.8 | 0.6$^{*}$ | 22.4 | 12.5 |
| RFTSS | GH | 1.3 | 11.5$^{**}$ | 31.6$^{**}$ | 4.3 | 3.3 | 3.5 | 17.5 |
| GFAA | GH | 401.8 | 2888.0$^{**}$ | 3914.3$^{**}$ | 2640.4$^{**}$ | 160.1 | 484.0 | 12.6 |
| RFAA | GH | 470.4 | 4629.6$^{**}$ | 11069.6$^{**}$ | 2864.4$^{**}$ | 123.4 | 546.4 | 10.9 |

[+] FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, FL = fruit length, FD = fruit diameter, FWT = fruit weight, PCT = pericarp thickness, FN = fruit number, GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GFAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid, $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$.

## Performance of genotypes and heterosis

Mean performance of all measured characteristics for the two environments are summarized in Table 5.2. Genotypes flowered and matured earlier in the field than in the greenhouse probably due to the higher day temperature (about 31°C) in the field. Berke (2000) indicated that in peppers, flowering is a function primarily of the genotypes although some interaction with temperature does occur. Significant differences were also observed for PH, FL and FWT. On the other hand, FY, FMP, FD, PCT and FN were not significantly affected by the environment.

Table 5.2. Means of greenhouse and field trials evaluated for various characters.

| Environment | Character[+] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | FY | DF | DM | FMP | PH | FL | FD | FWT | PCT | FN |
| Greenhouse | 260.9 | 83.7 | 142.2 | 58.4 | 101.9 | 7.7 | 3.0 | 23.5 | 3.5 | 31 |
| Field | 403.9 | 81.4 | 137.1 | 55.7 | 83.7 | 9.4 | 3.2 | 28.9 | 3.2 | 39 |
| $LSD_{0.05}$ | ns | 1.4 | 2.1 | ns | 11.6 | 0.3 | ns | 4.5 | ns | ns |

[+] FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, PH = plant height, FL = fruit length, FD = fruit diameter, FWT = fruit weight, PCT = pericarp thickness, FN = fruit number.

The commercial exploitation of the phenomenon of heterosis is one of the most important contributions to plant breeding. The extent of heterotic response of the $F_1$ hybrid largely depends on the breeding value and genetic diversity of the parents included in crosses, and on the environmental conditions under which hybrids are grown (Bhatt, 1971; Knobel *et al.*, 1997; Jordaan, 1999; Young and Virmani, 1990). Cultivars are known to differ in their ability to combine with others when they are crossed. Identification of those specific combinations of parents is therefore essential in the exploitation of heterosis in agricultural crops (Bhatt, 1971; Jordaan *et al.*, 1999).

A large number of hybrids showed superiority over their parents for various characters revealing substantial heterosis in the hybrids (Tables 5.3 and 5.4). Although no hybrid showed increase for all the characters, there were significant differences between means of the hybrids for all measured characters. FY among crosses ranged from 123.4 to 538.8 g with an average yield of 369.5 g. As expected, crosses with the bell pepper parent in general gave bigger fruit size and higher yield per plant followed by crosses between intermediate fruit-sized parents. Some of the best yielding crosses were Bakko Local × Pepper 1976, Mareko Shote × Pepper 1976, Bakko Local × Mareko Shote, Szegedi 178 × Bakko Local, Kalocsai "M" Cseresznye × Pepper 1976, Szegedi 178 × Pepper 1976, PBC 142A × Pepper 1976, Kalocsai "M" Cseresznye × Mareko Shote, Bakko Local × C00916 and Mareko Shote × C00916.

Significantly high mid-parent, high-parent and standard heterosis were recorded for FY (Tables 5.3 and 5.4). The best cross that showed the highest mid-parent yield heterosis was Mareko Shote × C00916. Many of the $F_1$ hybrids out yielded the high parent, thus displaying "true" heterosis. The highest percent of HPH of 175.7 and 118.3% for FY were observed between Mareko Shote × C00916 and Szegedi 178 × Mareko Shote, respectively. Kaul and Sharma (1988) reported high parent heterosis of 34.0% in bell pepper. Twelve of the 21 crosses showed SH ranging from 28.0 to 68.8%. Szegedi 178/Pepper 1976, Bakko Local/Pepper 1976 and Bakko Local/Mareko Shote were among the best hybrids that showed the highest SH for fruit yield per plant.

Of the total hybrids, five crosses showed significantly high heterosis over mid- and high-parent each, and four showed significantly high heterosis over the standard variety for FL (Table 5.3). Szegedi 178/Bakko Local, Szegedi 178/Mareko Shote, Szegedi 178/Pepper 1976, and Bakko Local/Pepper 1976 were some of the hybrids that showed longer fruits. The crosses between Bakko Local × Mareko Shote, Bakko Local × Pepper 1976 and Mareko Shote × Pepper 1976, all of which had high GCA and *per se* performance for FL, in general showed low heterosis for this character. Morgan *et al.* (1989) found that heterosis for wheat grain yield was less where the parents were higher yielding because

the parental lines already had many of the genes beneficial for yield in the homozygous state and so, were unable to show much heterosis.

The mean FWT of the crosses ranged between 4.2 and 71.5 g, the highest being between Kalocsai "M" Cseresznye × Pepper 1976 and Szegedi 178 × Pepper 1976. In Ethiopia, because bell (sweet) pepper is not pungent, it is less preferred by the majority of the local consumers. The cross between Kalocsai "M" Cseresznye × Pepper 1976 (pungent cherry × sweet pepper) had the highest mean fruit weight, bell pepper fruit shape and mild pungency as determined by taste. This hybrid pungent bell pepper can be grown in Ethiopia to meet the demand of the local consumers. More over, it was observed that hybrids obtained from the crosses between the pungent and the sweet pepper parents showed lower pungency as compared to the pungent parent. Zewdie *et al.* (2001) reported parents with negative GCA effects for capsaicinoid (alkaloid compound) content contributed most to its reduction.

Hybrid PBC 142A/Pepper 1976 (small-fruited chile × bell) was found to be a typical capsicum (berbere) type in terms of fruit related traits and can be considered promising. Generally, the higher or lower magnitudes of fruit related traits demonstrated by certain crosses indicate that the parental genotypes involved in the current study had high genetic variability to genetically improve these characters based on the breeding objectives. The SH for FWT ranged from –72.4 for hybrid C00916/PBC 142A to 381.6% for hybrid Kalocsai "M" Cseresznye/Pepper 1976 with mean performance ranging from 4.2 to 71.5 g per fruit. As expected, a higher percentage SH was observed in hybrids with Pepper 1976 (bell) as one of their parents. Many crosses also showed high MPH, HPH and SH for FD. Regarding the SH, the highest percentage heterosis for this trait was recorded in the hybrid Kalocsai "M" Cseresznye/Pepper 1976. Similarly, percent standard heterosis for PCT ranged from low to high. One, five, and three of the 21 crosses showed statistically significant percentages MPH, HPH and SH, respectively for FN.

Table 5.3. Mean performance and percentage mid-parent (MPH), high-parent (HPH) and standard (SH) heterosis of seven parents and 21 crosses for various characters at two environments.

| Genotype | Fruit yield per plant | | | | Days to flowering | | | | Days to maturity | | | | Fruit maturation period | | | | Plant height | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH |
| | | | | | | | | | | | Parent | | | | | | | | | |
| $P_1$ | 129.6 | - | - | - | 79.8 | - | - | - | 134.5 | - | - | - | 54.7 | - | - | - | 54.7 | - | - | - |
| $P_2$ | 137.1 | - | - | - | 79.5 | - | - | - | 141.0 | - | - | - | 61.5 | - | - | - | 61.5 | - | - | - |
| $P_3$ | 365.0 | - | - | - | 100.8 | - | - | - | 171.2 | - | - | - | 70.3 | - | - | - | 70.3 | - | - | - |
| $P_4$ | 185.2 | - | - | - | 90.8 | - | - | - | 151.8 | - | - | - | 61.0 | - | - | - | 61.0 | - | - | - |
| $P_5$ | 143.7 | - | - | - | 86.5 | - | - | - | 137.8 | - | - | - | 51.3 | - | - | - | 51.3 | - | - | - |
| $P_6$ | 187.2 | - | - | - | 104.8 | - | - | - | 166.3 | - | - | - | 61.5 | - | - | - | 61.5 | - | - | - |
| $P_7$ | 423.7 | - | - | - | 90.1 | - | - | - | 153.7 | - | - | - | 63.5 | - | - | - | 63.5 | - | - | - |
| Mean | 221.5 | - | - | - | 90 | - | - | - | 151 | - | - | - | 61 | - | - | - | 76.9 | - | - | - |
| $LSD_{0.05}$ | 125.8 | - | - | - | 6.0 | - | - | - | 6.4 | - | - | - | 6.1 | - | - | - | 10.4 | - | - | - |
| | | | | | | | | | | | Cross | | | | | | | | | |
| $P_1 \times P_2$ | 267.1 | 82.3 | 51.2 | -7.6 | 75 | -4.9 | -3.7 | -24.3 | 129 | -6.2 | -3.9 | -26.3 | 54 | -6.5 | 0.2 | -22.5 | 101.0 | 49.8 | 35.7 | 22.8 |
| $P_1 \times P_3$ | 382.2 | 71.4 | 28.7 | 28.7 | 79 | -12.3 | -1.3 | -20.7 | 138 | -9.6 | 2.8 | -19.2 | 60 | -4.8 | 9.0 | -14.7 | 107.2 | 47.1 | 32.4 | 32.4 |
| $P_1 \times P_4$ | 415.7 | 152.8 | 90.8 | 57.4 | 82 | -4.5 | 2.5 | -17.7 | 138 | -3.3 | 3.1 | -18.8 | 57 | -1.7 | 4.8 | -18.9 | 104.3 | 34.9 | 17.1 | 29.6 |
| $P_1 \times P_5$ | 385.8 | 177.7 | 87.0 | 34.3 | 78 | -6.2 | -1.3 | -21.4 | 130 | -3.7 | -1.9 | -23.9 | 52 | -1.1 | 7.2 | -25.2 | 110.8 | 73.8 | 47.2 | 38.4 |
| $P_1 \times P_6$ | 398.6 | 105.3 | 72.6 | 17.9 | 75 | -18.7 | -6.0 | -25.0 | 125 | -13.8 | -7.3 | -27.2 | 49 | -14.3 | -5.7 | -29.9 | 101.2 | 39.1 | 27.4 | 23.0 |
| $P_1 \times P_7$ | 436.2 | 37.6 | -2.0 | 39.3 | 79 | -6.7 | -1.2 | -20.6 | 131 | -8.8 | -2.4 | -23.2 | 52 | -10.3 | -1.6 | -24.9 | 89.2 | 24.4 | 15.9 | 9.4 |
| $P_2 \times P_3$ | 443.5 | 104.9 | 56.1 | 59.8 | 79 | -10.8 | 0.5 | -19.7 | 134 | -13.5 | -4.3 | -21.1 | 55 | -16.0 | -9.3 | -21.1 | 99.5 | 31.7 | 17.8 | 21.2 |
| $P_2 \times P_4$ | 382.4 | 151.8 | 118.3 | 41.5 | 78 | -8.4 | -1.5 | -21.4 | 137 | -6.6 | -3.0 | -20.0 | 59 | -3.7 | -0.1 | -16.1 | 98.7 | 24.1 | 8.7 | 19.3 |
| $P_2 \times P_5$ | 123.4 | 8.9 | -21.8 | -52.5 | 68 | -17.8 | -13.7 | -31.6 | 129 | -6.1 | -5.3 | -24.7 | 61 | 8.5 | 22.1 | -13.6 | 73.8 | 12.4 | 0.0 | -9.7 |
| $P_2 \times P_6$ | 314.6 | 86.5 | 56.8 | 8.4 | 79 | -13.7 | 0.1 | -20.1 | 132 | -10.4 | -5.3 | -22.5 | 53 | -13.1 | -5.6 | -24.3 | 100.5 | 37.5 | 27.0 | 22.8 |
| $P_2 \times P_7$ | 422.4 | 61.9 | 8.6 | 68.8 | 81 | -3.2 | 3.1 | -17.9 | 135 | -8.1 | -4.1 | -20.9 | 53 | -13.1 | -9.6 | -22.6 | 73.0 | 1.3 | -6.0 | -9.9 |
| $P_3 \times P_4$ | 503.9 | 86.8 | 41.3 | 63.5 | 86 | -8.6 | -5.2 | -13.2 | 147 | -7.0 | -3.5 | -14.3 | 60 | -7.6 | -0.6 | -13.1 | 97.7 | 15.0 | 9.6 | 21.5 |
| $P_3 \times P_5$ | 405.8 | 63.7 | 9.2 | 13.1 | 81 | 1.3 | -4.8 | -18.0 | 139 | -3.5 | 1.2 | -18.6 | 58 | -5.0 | 15.6 | -17.8 | 102.5 | 42.7 | 20.0 | 26.2 |
| $P_3 \times P_6$ | 301.4 | -0.3 | -21.6 | -7.9 | 90 | -11.7 | -6.7 | -9.4 | 146 | -8.2 | -4.2 | -14.3 | 56 | -14.5 | -6.7 | -20.2 | 99.7 | 25.0 | 16.2 | 23.8 |
| $P_3 \times P_7$ | 538.8 | 33.9 | 6.5 | 67.9 | 83 | -9.9 | -5.2 | -15.7 | 139 | -11.2 | -8.0 | -18.3 | 59 | -15.9 | -8.0 | -20.4 | 102.0 | 27.1 | 19.2 | 24.4 |
| $P_4 \times P_5$ | 402.8 | 327.7 | 175.7 | 31.8 | 79 | -10.0 | -7.5 | -20.6 | 138 | -0.9 | 0.4 | -19.3 | 59 | 5.6 | 18.8 | -15.5 | 107.2 | 44.2 | 20.9 | 32.7 |
| $P_4 \times P_6$ | 290.3 | 38.0 | 8.2 | -17.9 | 87 | -10.5 | -3.9 | -12.2 | 144 | -5.7 | -1.8 | 108.5 | 57 | -5.6 | 0.3 | -18.5 | 93.8 | 11.2 | 1.2 | 14.9 |
| $P_4 \times P_7$ | 529.5 | 87.2 | 35.9 | 55.2 | 82 | -8.2 | -6.5 | -17.4 | 147 | -2.6 | -0.8 | -13.8 | 66 | 5.9 | 13.8 | -5.8 | 112.5 | 34.0 | 22.2 | 39.3 |
| $P_5 \times P_6$ | 204.1 | 5.3 | -27.6 | -27.2 | 84 | -12.3 | -2.9 | -15.6 | 137 | -6.3 | 1.4 | -19.8 | 53 | -5.2 | 11.7 | -23.7 | 101.7 | 43.7 | 27.4 | 23.0 |
| $P_5 \times P_7$ | 286.1 | 5.4 | -25.0 | -5.9 | 72 | -17.0 | -14.6 | -26.7 | 122 | -16.3 | -11.6 | -28.9 | 49 | -14.0 | -2.2 | -29.1 | 89.2 | 23.7 | 10.8 | 9.1 |
| $P_6 \times P_7$ | 420.5 | 35.9 | 3.0 | 28.0 | 83 | -11.9 | -7.6 | -16.7 | 137 | -10.0 | -7.0 | -19.7 | 55 | -12.0 | -1.3 | -22.1 | 94.3 | 22.1 | 20.1 | 15.8 |
| Mean | 369.3 | 81.8 | 35.8 | 23.7 | 83 | -9.8 | -4.2 | -19.3 | 136 | -7.7 | -3.1 | -14.6 | 56 | -6.9 | 2.5 | -20.0 | 98.1 | 31.7 | 18.6 | 20.5 |
| $LSD_{0.05}$ | 143.3 | 99.5 | 69.8 | 43.8 | 2.9 | 7.3 | 5.2 | 2.9 | 5.0 | 4.8 | 4.7 | 4.8 | 4.8 | 9.8 | 12.6 | 6.8 | 10.8 | 20.1 | 16.1 | 13.9 |
| | | | | | | | | | | | Overall | | | | | | | | | |
| Mean | 332.4 | - | - | - | 83 | - | - | - | 140 | - | - | - | 57 | - | - | - | 92.8 | - | - | - |
| $LSD_{0.05}$ | 139.2 | - | - | - | 3.7 | - | - | - | 5.4 | - | - | - | 5.2 | - | - | - | 10.6 | - | - | - |

Table5.3. Continued...

| Genotype[♣] | Fruit length | | | | Fruit diameter | | | | Mean fruit weight | | | | Pericarp thickness | | | | Fruit number per plant | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH |
| | | | | | | | | | | | Parent | | | | | | | | | |
| $P_1$ | 2.9 | - | - | - | 2.9 | - | - | - | 10.2 | - | - | | 3.3 | - | - | - | 15 | - | - | - |
| $P_2$ | 7.7 | - | - | - | 2.0 | - | - | - | 12.7 | - | - | | 2.4 | - | - | - | 13 | - | - | - |
| $P_3$ | 13.2 | - | - | - | 2.1 | - | - | - | 15.0 | - | - | | 2.3 | - | - | - | 33 | - | - | - |
| $P_4$ | 12.1 | - | - | - | 2.7 | - | - | - | 19.1 | - | - | | 2.7 | - | - | - | 11 | - | - | - |
| $P_5$ | 2.3 | - | - | - | 2.3 | - | - | - | 4.3 | - | - | | 2.7 | - | - | - | 47 | - | - | - |
| $P_6$ | 4.2 | - | - | - | 0.8 | - | - | - | 1.7 | - | - | | 0.8 | - | - | - | 153 | - | - | - |
| $P_7$ | 11.3 | - | - | - | 8.1 | - | - | - | 156.9 | - | - | | 8.9 | - | - | - | 4 | - | | - |
| Mean | 7.6 | - | - | - | 3.0 | - | - | - | 31.4 | - | - | - | 3.3 | - | - | - | 36 | - | - | - |
| $LSD_{0.05}$ | 0.9 | - | - | - | 0.2 | - | - | - | 9.1 | - | - | - | 0.5 | - | - | - | 25 | | | |
| | | | | | | | | | | | Cross | | | | | | | | | |
| $P_1 \times P_2$ | 7.0 | 41.8 | 1.5 | -46.7 | 3.3 | 43.2 | 7.6 | 68.8 | 23.3 | 116.1 | 92.3 | 58.1 | 5.4 | 90.2 | 66.1 | 140.8 | 17 | 25.5 | 4.1 | -26.6 |
| $P_1 \times P_3$ | 10.3 | 28.2 | -21.5 | -21.5 | 3.0 | 20.8 | 4.4 | 43.8 | 19.0 | 52.4 | 28.3 | 28.3 | 3.1 | 14.4 | -3.5 | 42.3 | 26 | 25.8 | -0.4 | -0.4 |
| $P_1 \times P_4$ | 7.5 | 0.2 | -37.5 | -43.4 | 3.3 | 21.3 | 12.6 | 59.2 | 24.3 | 74.6 | 36.4 | 64.5 | 4.5 | 54.3 | 39.8 | 101.9 | 26 | 108.1 | 79.2 | 13.2 |
| $P_1 \times P_5$ | 3.7 | 44.1 | 27.4 | -72.3 | 3.0 | 18.1 | 2.4 | 43.0 | 9.4 | 39.3 | 0.9 | -36.4 | 4.5 | 52.9 | 38.6 | 105.0 | 66 | 182.3 | 86.2 | 194.4 |
| $P_1 \times P_6$ | 4.7 | 37.9 | 14.5 | -64.2 | 2.0 | 10.4 | -29.0 | -1.9 | 6.4 | 0.8 | -38.6 | -57.0 | 2.2 | -11.0 | -32.8 | -1.4 | 70 | 27.1 | -28.5 | 231.7 |
| $P_1 \times P_7$ | 8.6 | 21.5 | -23.3 | -34.5 | 6.2 | 13.4 | -23.2 | 200.5 | 71.5 | -12.1 | -53.3 | 381.6 | 6.8 | 12.8 | -22.8 | 213.0 | 7 | -26.1 | -50.3 | -74.6 |
| $P_2 \times P_3$ | 14.1 | 38.9 | 6.8 | 6.8 | 2.6 | 28.3 | 24.0 | 26.7 | 22.6 | 66.1 | 50.0 | 52.2 | 3.0 | 27.1 | 15.7 | 34.7 | 24 | 27.3 | -2.2 | 1.2 |
| $P_2 \times P_4$ | 13.8 | 46.0 | 16.0 | 4.7 | 3.3 | 41.2 | 24.8 | 59.3 | 24.6 | 63.4 | 40.1 | 66.1 | 3.1 | 25.2 | 15.5 | 42.8 | 19 | 62.4 | 42.6 | -13.9 |
| $P_2 \times P_5$ | 4.1 | -11.9 | -41.5 | -68.3 | 2.9 | 38.5 | 26.0 | 38.6 | 9.7 | 16.3 | -21.8 | -35.0 | 2.8 | 7.9 | -7.4 | 24.2 | 15 | -29.9 | -48.0 | -37.4 |
| $P_2 \times P_6$ | 8.8 | 60.0 | 26.7 | -33.6 | 2.5 | 65.2 | 15.6 | 20.4 | 7.5 | -2.3 | -42.0 | -48.9 | 2.1 | 9.1 | -15.0 | -7.1 | 63 | -8.9 | -48.2 | 156.4 |
| $P_2 \times P_7$ | 11.4 | 23.6 | 1.5 | -13.2 | 4.7 | -6.2 | -41.4 | 129.2 | 66.9 | -19.2 | -56.4 | 349.6 | 4.6 | -17.8 | -47.6 | 111.3 | 8 | 5.5 | -29.7 | -67.8 |
| $P_3 \times P_4$ | 13.4 | 6.5 | -0.4 | 1.8 | 2.3 | -3.0 | -12.8 | 10.0 | 20.8 | 26.1 | 14.1 | 40.1 | 2.5 | 3.5 | -2.8 | 11.9 | 30 | 53.6 | 34.7 | 18.1 |
| $P_3 \times P_5$ | 6.9 | -11.1 | -47.9 | -47.9 | 2.7 | 30.7 | 20.7 | 32.6 | 13.7 | 43.9 | -7.9 | -7.9 | 3.0 | 25.6 | 11.0 | 36.9 | 40 | 13.4 | -27.5 | 25.2 |
| $P_3 \times P_6$ | 9.7 | 12.0 | -26.7 | -26.7 | 1.7 | 14.0 | -19.7 | -19.7 | 6.8 | -26.9 | -56.1 | -54.4 | 1.2 | -36.2 | -50.3 | -46.1 | 63 | -22.9 | -55.4 | 111.4 |
| $P_3 \times P_7$ | 13.3 | 8.9 | 0.8 | 0.8 | 4.3 | -15.7 | -47.1 | 107.4 | 57.3 | -32.7 | -63.1 | 288.1 | 4.1 | -26.3 | -54.0 | 87.8 | 12 | -27.5 | -57.8 | -57.8 |
| $P_4 \times P_5$ | 6.6 | -7.2 | -44.8 | -50.2 | 3.2 | 32.2 | 19.9 | 51.2 | 16.2 | 47.2 | -8.8 | 8.6 | 3.4 | 29.8 | 21.6 | 53.5 | 31 | 111.5 | 76.1 | 15.2 |
| $P_4 \times P_6$ | 11.3 | 41.3 | -5.6 | -14.5 | 1.4 | -17.9 | -45.9 | -31.5 | 7.2 | -33.4 | -60.2 | -51.7 | 1.3 | -37.8 | -52.8 | -41.9 | 51 | -34.6 | -63.7 | 67.4 |
| $P_4 \times P_7$ | 12.2 | 4.4 | -2.9 | -8.3 | 4.4 | -18.0 | -45.8 | 112.7 | 50.6 | -41.6 | -67.3 | 239.4 | 4.3 | -26.6 | -52.3 | 89.4 | 12 | 72.8 | 21.8 | -59.0 |
| $P_5 \times P_6$ | 4.2 | 39.2 | 16.4 | -68.2 | 1.8 | 20.3 | -15.2 | -14.2 | 4.2 | 12.5 | -24.0 | -72.4 | 1.9 | -3.8 | -25.0 | -19.1 | 71 | -18.4 | -39.6 | 189.1 |
| $P_5 \times P_7$ | 4.9 | -27.7 | -56.6 | -62.9 | 5.2 | 1.1 | -35.9 | 150.8 | 35.5 | -55.6 | -77.2 | 139.3 | 5.3 | -6.8 | -39.7 | 143.6 | 9 | -25.5 | -47.8 | -59.9 |
| $P_6 \times P_7$ | 10.6 | 36.8 | -6.5 | -20.2 | 2.3 | -48.0 | -71.4 | 11.7 | 16.2 | -79.4 | -69.4 | 9.6 | 2.6 | -49.1 | -70.3 | 19.5 | 34 | -50.1 | -74.5 | 22.9 |
| Mean | 8.9 | 20.6 | -9.7 | -32.5 | 3.1 | 13.8 | -10.9 | 52.3 | 24.4 | 12.2 | -18.3 | 64.8 | 3.4 | 6.5 | -12.8 | 54.6 | 33 | 22.4 | -10.9 | 31.0 |
| $LSD_{0.05}$ | 0.9 | 19.6 | 20.2 | 6.9 | 0.6 | 34.8 | 26.3 | 29.8 | 4.6 | 26.1 | 23.3 | 30.9 | 0.6 | 26.6 | 21.6 | 27.6 | 14.6 | 69.8 | 55.4 | 65.2 |
| | | | | | | | | | | | Overall | | | | | | | | | |
| Mean | 8.6 | - | - | - | 3.1 | - | - | - | 26.2 | - | - | - | 3.4 | - | - | - | 35 | - | - | - |
| $LSD_{0.05}$ | 0.9 | - | - | - | 0.5 | - | - | - | 5.9 | - | - | - | 0.5 | - | 21.6 | - | 17.3 | - | - | - |

[♣] $P_1$ = Kalocsai "M" Cseresznye, $P_2$ = Szegedi 178, $P_3$ = Bakko Local, $P_4$ = Mareko Shote, $P_5$ = C00916, $P_6$ = PBC 142A, $P_7$ = Pepper 1976.

Early maturity genotypes are probably very important under Ethiopian conditions, where the growing period is limited by the short rainy season. In the present study, most of the crosses involving at least one early parent flowered and matured up to 8 to 22 and 8 to 25 days earlier than the other crosses, respectively. In general, most of the crosses were earlier to flower and mature than the late flowering and late maturing parents. Hybrid Bakko Local/PBC 143A was the latest to flower and involved late flowering parents, flowered 15 days earlier than the latest parent, Bakko Local. Similarly, the late maturing hybrid, Mareko Shote/Pepper 1976, matured 24 days earlier than the late maturing parent, Bakko Local. Interestingly, the yield potential and fruit characteristics of certain early type crosses were higher than that of the standard variety, Bakko Local. The crosses between Bakko Local × Pepper 1976, Mareko Shote × Pepper 1976, Bakko Local × Mareko Shote and Szegedi 178 × Bakko Local gave yield advantages of 47.6, 45.1, 38.1 and 21.5% compared to the standard variety. The majority of the hybrids were earlier than their parents for flowering, maturity and fruit maturation period. In addition, the SH for the three measures of earliness were much higher than the MPH and HPH (Table 5.3).

Table 5.4. Mean performance and percentage mid-parent (MPH), high-parent (HPH) and standard parent (SH) heterosis (%) for total soluble solids and ascorbic acid (mg/g) of seven parents and 21 crosses tested in the greenhouse.

| Genotype[♣] | Green fruit total soluble solids | | | | Red fruit total soluble solids | | | | Green fruit ascorbic acid | | | | Red fruit ascorbic acid | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH | Mean | MPH | HPH | SH |
| | | | | | | | | | Parent | | | | | | | |
| $P_1$ | 5.5 | - | - | - | 9.5 | - | - | - | 108.1 | - | - | - | 125.1 | - | - | - |
| $P_2$ | 6.0 | - | - | - | 9.4 | - | - | - | 213.3 | - | - | - | 246.5 | - | - | - |
| $P_3$ | 5.5 | - | - | - | 9.8 | - | - | - | 172.9 | - | - | - | 191.6 | - | - | - |
| $P_4$ | 6.0 | - | - | - | 12.5 | - | - | - | 208.6 | - | - | - | 315.3 | - | - | - |
| $P_5$ | 4.3 | - | - | - | 12.1 | - | - | - | 180.5 | - | - | - | 196.3 | - | - | - |
| $P_6$ | 6.7 | - | - | - | 18.5 | - | - | - | 200.5 | - | - | - | 264.1 | - | - | - |
| $P_7$ | 5.1 | - | - | - | 10.3 | - | - | - | 198.0 | - | - | - | 212.4 | - | - | - |
| Mean | 5.6 | - | - | - | 11.7 | - | - | - | 183.1 | - | - | - | 221.6 | - | - | - |
| $LSD_{0.05}$ | 0.9 | - | - | - | 3.4 | - | - | - | 23.3 | - | - | - | 12.9 | - | - | - |
| | | | | | | | | | Cross | | | | | | | |
| $P_1 \times P_2$ | 5.0 | 15.8 | -16.8 | -9.8 | 9.0 | -4.6 | -13.3 | -8.0 | 146 | -8.8 | -31.0 | -15.6 | 216 | 16.1 | -12.4 | 12.6 |
| $P_1 \times P_3$ | 4.9 | 12.9 | -13.7 | -9.8 | 11.8 | 22.2 | 17.4 | 20.6 | 216 | 53.1 | 24.4 | 24.4 | 245 | 54.8 | 28.0 | 28.0 |
| $P_1 \times P_4$ | 4.5 | 27.8 | -27.8 | -18.0 | 9.9 | -8.5 | -19.7 | 1.7 | 191 | 21.6 | -6.5 | 10.4 | 220 | 0.1 | -30.2 | 15.2 |
| $P_1 \times P_5$ | 4.3 | 20.5 | -21.3 | -21.4 | 9.7 | -9.0 | -21.4 | 0.4 | 124 | -14.0 | -31.2 | -28.5 | 140 | -12.7 | -28.5 | -26.8 |
| $P_1 \times P_6$ | 5.2 | 17.9 | -22.2 | -5.7 | 9.7 | -30.4 | -46.8 | -2.1 | 158 | 2.2 | -21.4 | -8.5 | 186 | -3.9 | -29.2 | -2.4 |
| $P_1 \times P_7$ | 4.6 | 15.2 | -17.4 | -16.3 | 10.3 | 4.7 | -3.6 | 5.7 | 154 | 0.6 | -22.3 | -10.9 | 204 | 20.3 | 14.3 | 6.9 |
| $P_2 \times P_3$ | 4.8 | 19.9 | -20.0 | -13.0 | 9.2 | -4.2 | -11.4 | -6.7 | 142 | -26.6 | -33.4 | -18.1 | 207 | -5.4 | -15.9 | 8.2 |
| $P_2 \times P_4$ | 5.9 | 8.9 | -4.4 | 6.1 | 11.7 | 7.8 | -6.5 | 20.1 | 183 | -13.9 | -19.9 | 6.0 | 227 | -19.1 | -28.0 | 18.4 |
| $P_2 \times P_5$ | 4.3 | 25.1 | -28.5 | -22.0 | 10.8 | 1.3 | -5.6 | 9.3 | 144 | -27.0 | -32.3 | -16.9 | 172 | -22.2 | -30.1 | -10.1 |
| $P_2 \times P_6$ | 5.6 | 12.5 | -15.1 | 2.7 | 12.1 | -10.6 | -31.2 | 24.9 | 158 | -23.3 | -28.0 | -8.2 | 219 | -14.2 | -17.0 | 14.4 |
| $P_2 \times P_7$ | 4.9 | 14.9 | -17. | -11.0 | 9.8 | -0.2 | -6.2 | 0.5 | 160 | -22.5 | -25.2 | -7.6 | 201 | -12.2 | -18.5 | 5.3 |
| $P_3 \times P_4$ | 5.2 | 11.2 | -12.5 | -6.1 | 9.7 | -11.5 | -20.6 | 0.1 | 201 | 5.6 | -2.9 | 16.2 | 264 | 4.6 | -16.0 | 38.5 |
| $P_3 \times P_5$ | 4.5 | 14.1 | -21.7 | -19.2 | 9.1 | -15.0 | -22.4 | -6.2 | 137 | -22.3 | -23.8 | -20.8 | 174 | -9.3 | -11.2 | -7.9 |
| $P_3 \times P_6$ | 5.7 | 7.7 | -12.7 | 3.6 | 7.0 | -14.7 | -61.1 | -28.4 | 176 | -6.0 | -12.3 | 1.4 | 202 | -11.6 | -23.7 | 5.1 |
| $P_3 \times P_7$ | 5.1 | 7.6 | -8.8 | -7.3 | 10.0 | 3.5 | -0.4 | 6.4 | 184 | -0.9 | -7.1 | 6.3 | 204 | 0.9 | -4.3 | 7.0 |
| $P_4 \times P_5$ | 5.3 | 1.1 | -13.1 | -3.7 | 10.3 | -14.9 | -21.4 | 5.7 | 184 | -4.9 | -12.3 | 6.4 | 205 | -19.8 | -34.9 | 7.1 |
| $P_4 \times P_6$ | 5.1 | 25.3 | -23.9 | -7.5 | 10.3 | -31.1 | -41.7 | 6.4 | 200 | -2.2 | -6.8 | 15.3 | 236 | -18.6 | -25.2 | 23.1 |
| $P_4 \times P_7$ | 5.8 | -3.8 | -3.2 | 5.0 | 12.1 | 7.8 | -1.8 | 24.4 | 237 | 21.6 | 10.7 | 37.2 | 274 | 4.0 | -13.1 | 43.1 |
| $P_5 \times P_6$ | 5.1 | 11.4 | -23.6 | -7.6 | 11.3 | -25.0 | -37.0 | 16.0 | 191 | -0.1 | -5.0 | 10.1 | 223 | -3.3 | -15.7 | 16.2 |
| $P_5 \times P_7$ | 4.2 | 21.8 | -17.6 | -24.1 | 10.5 | -5.4 | -11.0 | 8.4 | 141 | -25.1 | -28.7 | -18.1 | 198 | -3.4 | -7.0 | 3.8 |
| $P_6 \times P_7$ | 4.5 | 31.1 | -31.9 | -18.3 | 10.9 | -22.8 | -38.5 | 11.8 | 204 | 2.3 | -0.6 | 17.6 | 246 | -23.2 | -6.9 | 28.4 |
| Mean | 5.0 | 15.2 | -17.8 | -9.7 | 10.3 | -9.3 | -19.2 | 5.3 | 172.8 | -4.5 | -15.0 | -0.1 | 212.5 | -3.7 | -15.5 | 11.1 |
| $LSD_{0.05}$ | 0.9 | 24.7 | 20.0 | 16.1 | 2.3 | 27.1 | 26.9 | 23.3 | 32.8 | 17.8 | 17.0 | 18.9 | 35.9 | 20.0 | 12.4 | 18.8 |
| | | | | | | | | | Overall | | | | | | | |
| Mean | 5.1 | - | - | - | 10.6 | - | - | - | 175.4 | - | - | - | 214.8 | - | - | - |
| $LSD_{0.05}$ | 0.9 | - | - | - | 2.5 | - | - | - | 30.1 | - | - | - | 31.9 | - | - | - |

[♣] $P_1$ = Kalocsai "M" Cseresznye, $P_2$ = Szegedi 178, $P_3$ = Bakko Local, $P_4$ = Mareko Shote, $P_5$ = C00916, $P_6$ = PBC 142A, $P_7$ = Pepper 1976.

Table 5.4 shows mean performance and percent heterosis for fruit TSS and ascorbic acid at green and red mature stages. There were highly significant differences for these traits among the genotypes. Mareko Shote/Pepper 1976 and Kalocsai "M" Cseresznye/Bakko Local were among the hybrids that showed the highest heterosis of 37.2 and 24.4%, respectively. Six hybrids (Szegedi 178/Mareko Shote, Bakko Local/Mareko Shote, Mareko Shote/PBC 142A, Mareko Shote/Pepper 1976, PBC 142A/Pepper 1976 and Kalocsai "M" Cseresznye/Bakko Local) gave significantly high ascorbic acid at red mature stage compared to the standard variety. Most of these crosses were derived from parental line Mareko Shote that had high mean ascorbic acid. For TSS, only PBC 142A at green mature stage, Szegedi 178/PBC 142A and Mareko Shote/Pepper 1976 at red mature stage surpassed the standard variety. Significantly high positive heterosis for TSS and ascorbic acid as compared to mid-parent, high-parent and standard check were also observed. High positive mean MPH for TSS at green mature stage, and positive mean SH for TSS and ascorbic acid at red mature stages were observed. Bhatt *et al.* (2001) also observed high positive significant heterosis for ascorbic acid and TSS over the top, the better and commercial control in tomato (*Lycopersicon esculentum*).

## CONCLUSION

The significance of the heterotic performance was highly affected by the genetic backgrounds of the parental genotypes. Superiority of a number of hybrid combinations over the standard variety was observed for FY, DF, FM, FMP, FWT, PCT, PH and FN. The high heterosis among these germplasm for most of the characters studied indicates that considerable potential exists in these materials for developing hybrids. The present studies provided useful information on the performance of some new hybrid combinations thus to eliminate inferior hybrids to Bakko Local (standard variety) and to promote for further testing those superior to the standard variety. Routine testing over locations and years helps to accumulate and collect information on the new $F_1$ hybrids compared to the standard variety.

From this study it was observed that $F_1$ pepper hybrids did not only have high yield potential and overall plant performance but they also increase daily productivity on the account of their earlier maturity. Therefore, hybrid breeding can be used effectively to improve yield, yield components, fruit quality, and overall plant performance in peppers. The results of this study also show that spice and vegetable type hybrid varieties that can satisfy the local demand can be developed. Pepper breeding programs in Ethiopia should aim at superiority of new $F_1$ hybrids over the available local cultivars.

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# CHAPTER 6

## Relationship between heterosis and hybrid performance and parental genetic distance


### ABSTRACT

Genetic diversity is considered as one of the criteria of selection of parents for hybrid breeding. The present study was carried out to evaluate genetic divergence among seven pepper cultivars and to assess the relationship between heterosis and parental genetic distance. Twenty-one $F_1$ hybrids and seven parents were evaluated for 15 morphological and agronomic characters in the greenhouse and field. The seven parents were also examined for DNA polymorphisms using six AFLP primer combinations. Both methods of genetic distance measurements showed moderately high genetic distances among the parental lines. Cluster analysis using the two genetic distance measures generally grouped the seven parents differently. Moderate to high mid-parent and high-parent heterosis were observed for most of measured characters. Most of the hybrids performed better than most of the parental lines for fruit yield, traits of earliness and plant height. Both methods of distance measurements allocated pepper genotypes into heterotic groups. The correlations of morphological distances with mid-parent heterosis were negative and significant for days to flowering and days to maturity suggesting earliness in pepper can be predicted from morphological distances of parental lines. However, the correlations of AFLP measured genetic distances with mid- and high-parent heterosis were non-significant for all characters with the exception of fruit diameter and proved to be of no predictive value.

## INTRODUCTION

Parental selection is the first step in any plant breeding program. This is because the development of improved varieties of crop plants in breeding and selection programs depends on the existence of genetic diversity on which selection can act. Identification of combinations with strong yield heterosis is the most important step in developing hybrids. Parents with a higher general combining ability and a large genetic distance between them, in general, can produce a hybrid with better yield performance (Boppenmaier *et al.*, 1993; Cox and Murphy, 1990; Diers *et al.*, 1996). Transgressive segregants are also obtained when parents are genetically divergent. Busch *et al.* (1974) indicated that the probability of recovering a superior progeny genotype is greater if both parents are similar in performance as opposed to one parent being inferior for one or more traits. In addition to this, Moll *et al.* (1962) demonstrated that there is an optimum genetic distance between germplasm pools and exceeding it may actually decrease hybrid performance.

The identification of parental combinations that produce hybrids of superior yield is one of the most costly and time consuming steps in any hybrid breeding program. This is because it is necessary to cross the available inbred lines and evaluate the hybrids in extensive yield trials. Moreover, in some cases it is even difficult to evaluate all possible single-cross hybrid combinations between the available inbred lines because the number of possible hybrids is often prohibitive. Thus, because of space limitations only a limited number of hybrids generated from a relatively small number of inbred parents can be evaluated (Bernardo, 1992; Smith, 1986). In addition, trait expression is often highly influenced by environmental factors. In some studies it was also reported that *per se* performance does not predict the performance of hybrids (Hallauer and Miranda, 1988). Recent advances in genome research have generated considerable interest in predicting hybrid performance using molecular markers in crop breeding programs.

Identification of methods that efficiently detect desirable combinations could be one of the important steps in a hybrid breeding. Morphological and molecular marker genetic

diversity in relation to hybrid performance has been studied in several crops. However, these studies indicate variable results. Positive associations between morphological genetic distance and heterosis were reported among crosses in wheat (Cox and Murphy, 1990). Riday *et al.* (2003) in their study on alfalfa also indicated that a morphological distance matrix based on agronomic and forage-quality traits was significantly correlated with heterosis but genetic distance did not correlate with yield specific combining ability (SCA) and mid-parent heterosis.

Investigations in maize, rice and oilseed rape have shown that the molecular genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted by using molecular markers (Bernardo, 1994; Betran *et al.*, 2003; Boppenmaier *et al.*, 1993; Diers *et al.*, 1996; Riaz *et al.*, 2001; Zhang *et al.*, 1994). On the contrary, other studies in maize (Benchimol *et al.*, 2000), pearl millet (Chowdari *et al.*, 1998), rice (Kwon *et al.*, 2002), wheat (Liu *et al.*, 1999, Fabrizius *et al.*, 1998), alfalfa (Riday *et al.*, 2003) and chickpea (Sant *et al.*, 1999) showed low correlation between genetic distance and hybrid performance/heterosis. Xu *et al.* (2002) and Zhao *et al.* (1999) also reported that the association between the two is complex in rice. Cowen and Frey (1987a, b) were also unable to predict yield heterosis in oat (*Avena sativa* L.) from diversity measures based on coefficient of parentage, Euclidean distance based on quantitative traits, or two distance measures based on information from diallel matings. In addition to the identification of potentially high yielding hybrids, genetic distance measurements help to assign new pure lines to heterotic groups.

Heterosis has been documented in hot and sweet peppers, and hybrids are gaining popularity among farmers throughout the world (Berke, 2000). Bosland and Votava (2000) reported that peppers grown from hybrid seeds are highly uniform and usually higher yielding. This has spurred interest in developing hybrids in peppers. In pepper it has been observed that crosses between parents of different origins generally have greater heterosis than crosses between parents of similar origins. However, the use of molecular markers to measure allelic differences between parents for the estimation of hybrid yield and heterosis has not been reported on pepper. Thus, the aims of this study were to

measure morphological traits and AFLP marker-based genetic diversity among the seven parental lines and assess the relationship between genetic distance and the heterosis/performance of hybrids derived from them.

## MATERIALS AND METHODS

### Plant materials

The detail is given in materials and methods part of Chapter 4.

*Measurements*: Fruit shape, days to flowering, days to maturity, fruit maturation period, plant height, fruit length, fruit diameter, fruit weight, pericarp thickness, fruit number per plant, fruit yield per plant, fruit total soluble solids and fruit ascorbic acid contents at green and red maturity stages were used for the morphological characterization of the seven parental lines and their 21 non-reciprocal crosses. Six AFLP primer combinations were also used to measure the DNA polymorphisms of the seven parental lines. The details for AFLP analysis and agronomic evaluations are given in the materials and methods part of Chapter 3.

### Statistical analysis

#### *Estimation of heterosis*

The formulae for estimating mid- and high-parent heterosis are presented in the materials and methods part of Chapter 5.

#### *Analysis of combining ability*

GCA and SCA effects were analyzed as described in the materials and methods part of Chapter 4.

### *Distance measurements and cluster analysis*

*Morphology*: As indicated in the materials and methods of Chapter 3, in developing the binary data, the method of Gerdes and Tracy (1994) was applied. The means and least significant differences ($LSD_{0.05}$) of the combined analysis of variance over the two environments were determined for each quantitative character using AGROBASE 2000 software (Agrobase, 2000). The genotypes were then compared pair-wise for each trait, and if two inbred lines were not significantly different from each other, they were given a score of 1 for that trait. Likewise, if the two inbred lines were significantly different they were given a score of 0. For fruit shape, scores of 1 or 0 were given to the genotypes depending on the presence or absence of this trait, respectively. Genetic distances and dendrogram were determined via the unweighted pair group mean (UPGMA) method using Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998).

*AFLP*: The details of data collection and analyses are given in the materials and methods part of Chapter 3. Band profiles generated by AFLP markers were designated for each parent as 1 or 0 indicating the presence or absence of a specific band, respectively. Genetic distances among all 21 pairs of the seven parents were estimated using 217 polymorphic bands according to Nei (1987) as: $GD = 1\text{-}2N_{ij}/(N_i + N_j)$, where $N_{ij}$ is the number of bands common to lines i and j, $N_i$ and $N_j$ are the number of fragments in lines i and j, respectively. The inbred lines were grouped using UPGMA method with dissimilarity matrix using Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998).

*Correlation:* Values of genetic distances as measured by morphological traits and AFLP markers were correlated with mean values of hybrids (hybrid performance), mid- and high-parent heterosis, and SCA effects to estimate their relationships.

# RESULTS AND DISCUSSION

## Genetic distance

Potential applications of molecular markers in plant breeding are fingerprinting of genotypes for plant variety identification and protection as well as assessing the genetic similarity among parents for prediction of quantitative-genetic parameters such as heterosis or progeny variance (Bohn *et al.*, 1999). Among other techniques like speed of assay and amount of DNA required from the plant of interest, a well-suited marker system for fingerprinting should reveal a high degree of polymorphism.

In this study, analysis of six AFLP primer pairs yielded a total of 236 bands among the seven parental genotypes of which 217 were polymorphic. Between 34 and 47 bands were revealed per primer combination from the studied genotypes, a mean of 39.3 bands per primer combination. M - CAA/E - AAC primer pair gave the maximum number of polymorphic bands. Twenty bands were unique among six of the parental genotypes. Parental line Bakko Local had eight unique bands and genotype Szegedi 178 had six. The high percent of polymorphic bands observed in this study was probably due to the diverse background of the parental lines used for the diallel cross.

AFLP genetic distances (GDs) calculated for all 21 combinations of the seven parents using 217 polymorphic bands are presented in Table 6.1. The highest morphological and AFLP genetic distances were observed between Kalocsai "M" Cseresznye vs. PBC 142A and Szegedi 178 vs. PBC 142A, respectively. Kalocsai "M" Cseresznye and Szegedi 178 were obtained from Hungary but PBC 142A was from India. Link *et al.* (1996) indicated that geographical distance between the regions of origin can be a major cause of genetic distance because it usually implies an independent evolution of germplasm pools. However, it is often associated with a lack of agronomic adaptation in one group in the region of cultivation of the other group.

Table 6.1. Estimates of genetic distances based on morphological (upper diagonal) and AFLP (lower diagonal) data for all pairwise combinations of seven parental genotypes.

| Parental line[+] | KCM | S178 | BL | MS | C00916 | PBC 142A | P1976 |
|---|---|---|---|---|---|---|---|
| KCM | | 0.614 | 0.673 | 0.644 | 0.583 | 0.727 | 0.673 |
| S178 | 0.737 | | 0.644 | 0.476 | 0.644 | 0.583 | 0.614 |
| BL | 0.667 | 0.625 | | 0.614 | 0.583 | 0.673 | 0.673 |
| MS | 0.658 | 0.700 | 0.629 | | 0.644 | 0.614 | 0.549 |
| C00916 | 0.676 | 0.704 | 0.710 | 0.707 | | 0.673 | 0.673 |
| PBC 142A | 0.655 | 0.799 | 0.730 | 0.700 | 0.710 | | 0.673 |
| P1976 | 0.710 | 0.647 | 0.662 | 0.658 | 0.704 | 0.669 | |

[+] KMC = Kalocsai "M" Cseresznye, S178 = Szegedi 178, BL = Bakko Local, MS = Mareko Shote, C00916 = C00916, PBC 142A = PBC 142A, P1976 = Pepper 1976

PBC 142A had the highest mean morphological distance (0.664) and was the most divergent; on the other hand AFLP measured GD showed C00916 the most divergent genotype with a mean genetic dissimilarity coefficient of 0.704. Generally, the mean AFLP distance was higher (0.689) than that of morphological distance (0.635), indicating AFLP markers had high discriminating power as compared to morphological traits.

Cluster analyses based on morphological and AFLP measured distances provided fairly good divisions of the parental genotypes into their heterotic groups (Fig. 6.1A, B). However, the two measures of distance grouped the seven parents differently but in both cases the parental lines that had similar fruit characters were clustered together. Although the morphological dendrogram placed the two Ethiopian cultivars separately, Bakko Local and Mareko Fana, the AFLP dendrogram placed them together, indicating the two cultivars are genetically closely related compared with other parental lines. Small and cherry type parents were grouped closely compared with large and elongated type parents, and vice versa, indicating the evidence of a larger-fruited vs. smaller-fruited heterotic pattern. Lefebvre *et al.* (1993) also reported that cultivars of bell pepper (all of European and North American origin) were much more similar to one another than were

small-fruited accessions of European, Mexican, Indian and Ethiopian accessions. In this study the correlation between the two methods of diversity measures were low and non-significant suggesting they probably have reflected the diversity in different parts of the pepper genome.

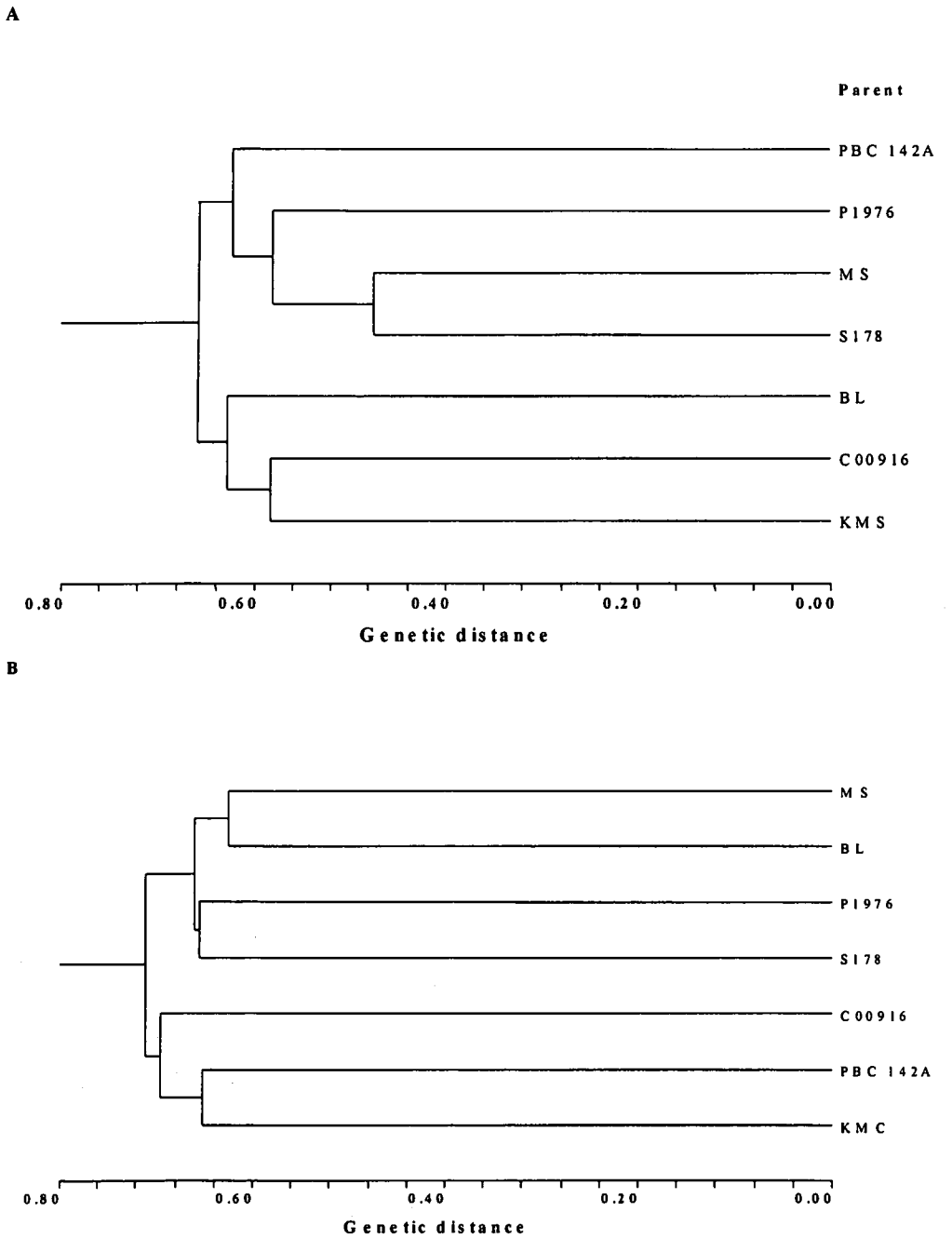


Fig. 6.1. Dendrogram of the seven parental lines clustered on the basis of morphological data (A) and AFLP marker (B) based genetic distance estimates. KMC = Kalocsai "M" Cseresznye, S178 = Szegedi 178, BL = Bakko Local, MS = Mareko Shote, C00916 = C00916, PBC 142A = PBC 142A, P1976 = Pepper 1976.

**Performance of parental lines and hybrids**

Measurements of 14 quantitative characters for the 21 hybrids and seven parents averaged over two environments are presented in Table 6.2. Nine of 21 hybrids gave significantly higher FY than six of the parental lines. Similarly, significantly shorter periods for DF, DM and FMP were recorded among the hybrids. Conversely, although the majority of the crosses performed better than their respective parents, none of the hybrids surpassed Pepper 1976 (the largest-fruited parent) for FD, FWT and PCT. The highest RFTSS and RFAA were also observed in the parental lines, PBC 142A and Mareko Shote, respectively. Some pictures of $F_1$ hybrids developed from crosses between parents of different fruit types revealing fruit and general plant performances are shown in Fig. 6.2.

Table 6.2. Measurements of 14 quantitative characters for 21 hybrids and seven parents averaged over two environments, 2001/02.

| Entry[+] | DF | DM | FMP | PH | FL | FD | FWT | PCT | FN | FY | GFTSS | RFTSS | GFAA | RGAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $P_1 \times P_2$ | 75 | 129 | 54 | 101.0 | 7.0 | 3.5 | 23.3 | 5.4 | 17 | 267.1 | 5.0 | 9.0 | 146.1 | 215.6 |
| $P_1 \times P_3$ | 79 | 138 | 60 | 107.3 | 10.3 | 3.0 | 19.0 | 3.1 | 26 | 382.2 | 4.9 | 11.8 | 215.8 | 244.6 |
| $P_1 \times P_4$ | 82 | 138 | 57 | 104.3 | 7.5 | 3.3 | 24.3 | 4.5 | 26 | 415.7 | 4.5 | 9.9 | 191.1 | 220.2 |
| $P_1 \times P_5$ | 78 | 130 | 52 | 110.8 | 3.8 | 3.0 | 9.4 | 4.5 | 66 | 385.8 | 4.3 | 9.7 | 123.6 | 140.3 |
| $P_1 \times P_6$ | 75 | 125 | 49 | 101.2 | 4.7 | 2.0 | 6.4 | 2.2 | 70 | 298.6 | 5.2 | 9.7 | 158.4 | 186.2 |
| $P_1 \times P_7$ | 79 | 131 | 53 | 89.2 | 8.6 | 6.2 | 7.5 | 6.8 | 7 | 436.2 | 4.6 | 10.3 | 153.9 | 203.6 |
| $P_2 \times P_3$ | 79 | 135 | 55 | 99.5 | 14.1 | 2.6 | 22.6 | 3.0 | 24 | 443.5 | 4.8 | 9.2 | 141.6 | 206.8 |
| $P_2 \times P_4$ | 78 | 137 | 59 | 98.7 | 13.8 | 3.3 | 24.6 | 3.1 | 19 | 382.4 | 5.9 | 11.7 | 182.9 | 227.1 |
| $P_2 \times P_5$ | 68 | 129 | 60 | 73.8 | 4.1 | 2.9 | 9.7 | 2.8 | 15 | 123.4 | 4.3 | 10.8 | 143.7 | 172.1 |
| $P_2 \times P_6$ | 79 | 132 | 53 | 100.5 | 8.8 | 2.5 | 7.5 | 2.1 | 63 | 314.6 | 5.6 | 12.1 | 158.4 | 219.3 |
| $P_2 \times P_7$ | 81 | 135 | 54 | 73.0 | 11.4 | 4.7 | 66.9 | 4.6 | 8 | 422.4 | 4.9 | 9.8 | 159.5 | 201.3 |
| $P_3 \times P_4$ | 68 | 147 | 60 | 97.7 | 13.4 | 2.3 | 20.8 | 2.4 | 30 | 503.8 | 5.2 | 9.7 | 200.8 | 264.1 |
| $P_3 \times P_5$ | 81 | 139 | 58 | 102.5 | 6.9 | 2.7 | 13.7 | 3.0 | 40 | 405.8 | 4.5 | 9.1 | 137.0 | 175.9 |
| $P_3 \times P_6$ | 90 | 146 | 56 | 99.7 | 9.7 | 1.7 | 6.8 | 1.2 | 63 | 301.4 | 5.7 | 7.0 | 175.5 | 201.6 |
| $P_3 \times P_7$ | 83 | 140 | 56 | 102.0 | 13.3 | 4.3 | 57.3 | 4.1 | 12 | 538.8 | 5.1 | 10.4 | 183.9 | 204.0 |
| $P_4 \times P_5$ | 80 | 138 | 59 | 107.2 | 6.6 | 3.1 | 16.2 | 3.4 | 32 | 402.8 | 5.3 | 10.3 | 183.9 | 205.1 |
| $P_4 \times P_6$ | 87 | 144 | 57 | 93.8 | 11.3 | 1.4 | 7.2 | 1.3 | 51 | 290.3 | 5.1 | 10.3 | 199.6 | 235.9 |
| $P_4 \times P_7$ | 82 | 147 | 66 | 112.5 | 12.2 | 4.4 | 50.6 | 4.3 | 12 | 529.5 | 5.8 | 12.1 | 237.1 | 273.8 |
| $P_5 \times P_6$ | 84 | 137 | 53 | 101.7 | 4.2 | 1.8 | 4.2 | 1.9 | 71 | 204.1 | 5.1 | 11.3 | 190.5 | 222.5 |
| $P_5 \times P_7$ | 72 | 122 | 49 | 89.2 | 4.9 | 5.2 | 35.5 | 5.3 | 11 | 286.1 | 4.2 | 10.5 | 141.4 | 197.9 |
| $P_6 \times P_7$ | 83 | 137 | 55 | 94.3 | 10.6 | 2.3 | 16.2 | 2.6 | 34 | 420.5 | 4.5 | 10.9 | 203.7 | 245.7 |
| $P_1$ | 80 | 135 | 55 | 66.5 | 2.9 | 2.9 | 10.2 | 3.3 | 15 | 129.6 | 5.5 | 9.5 | 108.1 | 125.1 |
| $P_2$ | 79 | 141 | 62 | 69.7 | 7.7 | 2.0 | 12.7 | 2.4 | 13 | 137.1 | 6.0 | 9.4 | 213.3 | 264.5 |
| $P_3$ | 101 | 171 | 70 | 83.7 | 13.2 | 2.1 | 14.0 | 2.3 | 33 | 365.0 | 5.5 | 9.8 | 172.9 | 191.6 |
| $P_4$ | 91 | 152 | 61 | 91.5 | 12.1 | 2.7 | 19.1 | 2.7 | 11 | 185.1 | 6.0 | 12.5 | 208.6 | 315.3 |
| $P_5$ | 87 | 138 | 51 | 69.8 | 2.3 | 2.6 | 4.3 | 2.7 | 47 | 143.7 | 4.3 | 12.1 | 180.3 | 196.3 |
| $P_6$ | 105 | 166 | 62 | 78.3 | 4.2 | 0.8 | 1.7 | 0.8 | 153 | 187.2 | 6.7 | 18.5 | 200.5 | 264.1 |
| $P_7$ | 90 | 154 | 64 | 78.7 | 11.3 | 8.1 | 156.9 | 8.9 | 4 | 423.7 | 5.1 | 10.3 | 198.0 | 212.4 |
| Mean | 83 | 140 | 57 | 92.8 | 8.6 | 3.1 | 26.2 | 3.4 | 35 | 333.1 | 5.2 | 10.6 | 175.4 | 214.8 |
| CV, 100 | 4.7 | 4.1 | 9.5 | 11.9 | 10.9 | 17.8 | 23.5 | 16.6 | 52.1 | 43.7 | 12.5 | 17.5 | 12.6 | 10.9 |
| $LSD_{0.05}$ | 3.7 | 5.4 | 5.2 | 10.6 | 0.9 | 0.5 | 5.9 | 0.5 | 17 | 139.4 | 0.9 | 2.5 | 30.1 | 31.9 |

[+] $P_1$ = Kalocsai "M" Cseresznye, $P_2$ = Szegedi 178, $P_3$ = Bakko Local, $P_4$ = Mareko Shote, $P_5$ = C00916, $P_6$ = PBC142A, $P_7$ = Pepper 1976; DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, FWT = fruit weight, FL = fruit length, FD = fruit diameter, PCT = pericarp thickness, PH = plant height, FN = fruit number. FY = fruit yield, GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GFAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid.

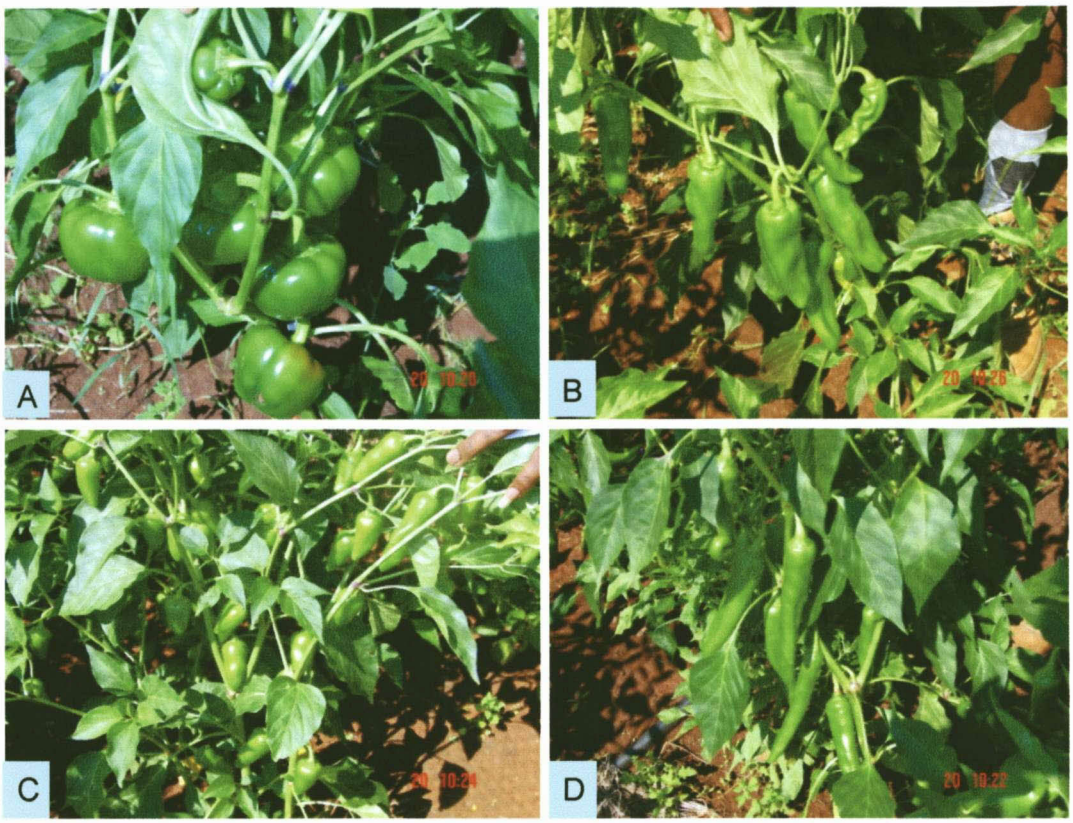


Fig. 6.2. Performance of $F_1$ pepper hybrids for fruit related traits. A = Kalocsai "M" Cseresznye × Pepper 1976, B = Mareko Shote × Pepper 1976, C = Kalocsai "M" Cseresznye × PBC 142A and D = Bakko Local × Mareko Shote. Fruit types of parental genotypes: Kalocsai "M" Cseresznye is small round, Pepper 1976 is blocky, Bakko Local and Mareko Shote are large elongate, and PBC 142A is small-elongate chile.

Correlation coefficients between parental means and general combining ability (GCA) effects were positive and significant for DF, DM, FL, FD, FWT, PCT, FN, GFTSS, RFTSS and RFAA content (Table 6.3). The high associations of parental means with GCA effects indicate the predominance of additive gene actions for these characters. This further indicates the performance of these traits can be predicted using the parental GCA. Since parental means and GCA effects were strongly correlated, the use of mid-parent means could predict cross performance for the above-mentioned characters. The close agreement between GCA and *per se* performance also indicates the presence of high genetic diversity in the parental genotypes (Pandian and Shanmugavelu, 1992).

Table 6.3. Correlation coefficients between parental means and GCA effects for 14 characters.

| Environment | FY | DF | DM | FMP | FL | FD | FWT | PCT | PH | FN | GDTSS | RFTSS | GFAA | RFAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Greenhouse | 0.723 | 0.965** | 0.922** | 0.760 | 0.982** | 0.966** | 0.987** | 0.938* | 0.648 | 0.924* | 0.969** | 0.944* | 0.735 | 0.890* |
| Field | 0.896* | 0.923* | 0.950** | 0.945* | 0.967** | 0.982** | 0.980** | 0.937* | 0.799 | 0.985** | - | - | - | - |

FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, FWT = fruit weight, FL = fruit length, FD = fruit diameter, PCT = pericarp thickness, PH = plant height, FN = fruit number. GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GFAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid, * $P < 0.05$, ** $P < 0.01$.

Quantitative genetic theory states that heterosis is the function of increasing genetic diversity among parents (Falconer, 1989). In the present investigation, the means of the hybrids were significantly greater than the parental means for FY, DF, DM, FMP, FD, FWT, PH, and GFTSS (Table 6.4), indicating that heterosis was present for these traits.

The means and ranges of performance and heterosis of the hybrids are given in Table 6.5. Although the amount of heterosis varied from trait to trait, the average mid-parent heterosis for measured traits was in the desired direction except for RFTSS, GFAA and RFAA. In addition, FY, DF, DM and PH demonstrated average HPH in the desired direction indicating the presence of true-heterosis or heterobeltiosis. The advantage for the $F_1$ hybrids showing HPH for these characters might be explained by desirable genetic complementation between the inbred genotypes (Dubreuil, *et al.*, 1996). On the other hand, there was no evidence of positive heterosis for RFTSS, GFAA and RFAA as the means of the hybrids were lower than the means of the parental lines.

Table 6.4. Means for fruit yield, yield components and other agronomic characters for parents and $F_1$ hybrids from a seven-parent half-diallel mating set in pepper, 2001/02.

| Entry | FY | DF | DM | FMP | FL | FD | FWT | FW | PH | FN | GFTSS | RFTSS | GRAA | RFAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean of parents | 221.5 | 90.4 | 150.9 | 60.5 | 7.6 | 3.0 | 31.4 | 3.3 | 76.9 | 39 | 5.6 | 11.7 | 183.1 | 221.6 |
| Mean of $F_1$ hybrids | 369.3 | 79.9 | 135.8 | 55.9 | 8.9 | 3.2 | 24.4 | 3.4 | 98.1 | 33 | 5.0 | 10.3 | 172.3 | 212.5 |
| Parents vs. hybrids | ** | ** | ** | * | ** | * | ** | ns | ** | ns | * | ns | ns | ns |

FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, FWT = fruit weight, FL = fruit length, FD = fruit diameter, PCT = pericarp thickness, PH = plant height, FN = fruit number. GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GRAA = green fruit ascorbic acid, RFAA =red fruit ascorbic acid, ns = non-significant, $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$.

Table 6.5. Means and ranges of heterosis (%) for 14 quantitative characters of the 21 $F_1$ hybrids.

| Heterosis | | FY | DF | DM | FMP | FL | FD | FWT | FW | PH | FN | GFTSS | RFTSS | GFAA | RFAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPH | Mean | 82.7 | -9.8 | -7.7 | -6.7 | 20.6 | 13.8 | 12.2 | 6.5 | 31.7 | 22.4 | 15.2 | -7.6 | -4.3 | -3.7 |
| | Range | -0.3-327.7 | -18.7-1.3 | -16.3- -0.9 | -16.0-8.5 | -27.7-60.0 | -48.0-65.2 | -79.4-116.1 | -49.1-90.2 | 1.3-73.8 | -50.1-182.3 | -3.8-31.1 | -31.1-22.2 | -27.0-53.1 | -23.2-54.8 |
| HPH | Mean | 35.8 | -4.2 | -3.1 | 2.5 | -9.7 | -10.9 | -18.3 | -12.8 | 18.6 | -10.9 | -17.8 | -19.2 | -15.0 | -15.5 |
| | Range | -27.6-175.7 | -14.6-3.1 | -11.6-3.1 | -9.6-22.1 | -56.6-27.4 | -71.4-26.0 | -77.2-92.3 | -70.3-66.1 | -6.0-47.2 | -74.5-86.2 | -31.9- -3.2 | -61.1-17.4 | -33.4-24.4 | -34.9-28.0 |

FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, FWT = fruit weight, FL = fruit length, FD = fruit diameter, PCT = pericarp thickness, PH = plant height, FN = fruit number. GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GFAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid.

Crosses between widely divergent materials may be potentially useful in hybrid breeding because poor adaptation of one parent may be counterbalanced by increased heterotic response (Moll *et al.*, 1962; Paterniani and Lonnquist, 1963). As indicated above, the two distance measurements generally separated the parental lines into two major heterotic groups: smaller- and larger-fruited (Fig. 6.1). The $F_1P$ and MPH for the intra- and inter-group hybrids, as clustered by AFLP, for measured quantitative characters varied from trait to trait (Table 6.6). Average $F_1$ performances for FY, FWT and FN for intra-group hybrids were greater than for inter-group ones, suggesting intermediate divergence of parental lines could improve the performance of hybrids for these traits. Melchinger (1999) indicated that the efficiency of predicting of hybrid performance from GD is greater with crosses between inbred lines from the same heterotic groups than in crosses between inbred lines from different heterotic groups.

Inter-group crosses showed on average higher superiority only for PCT over intra-group crosses (Table 6.6). Link *et al.* (1996) reported the inter-pool crosses showed on average a minor but non-significant yield superiority over intra-pool crosses in faba beans (*Vicia faba* L.). The $F_1$ performances for inter- and intra-group hybrids for other characters were generally similar.

Heterotic relationship between germplasm groups is a function of their genetic distance (Falconer, 1981). However, according to Moll *et al.* (1965), with divergence of parents, heterosis increases only within a restricted range but it decreases once a certain optimum genetic distance is surpassed. In this investigation, the highest estimates of heterosis for FY, FN, FL and FWT were observed in intra-group hybrids indicating that higher heterosis for these traits can be achieved through selecting parents form intermediate divergence groups. Contrarily, higher heterosis for FD, GFTSS and PCT were recorded in inter-group crosses revealing the performance of these traits can be increased when parents are selected from different clusters.

Table 6.6. Mid-parent heterosis (MPH) and mean $F_1$ performance ($F_1P$) of 14 quantitative characters for inter- and intra-group hybrids on the basis of AFLP measurements.

| | No. of F1 | FY | DF | DM | FMP | PH | FL | FD | FWT | PCT | FN | GFTSS | RFTSS | GFAA | RFAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPH (%) | | | | | | | | | | | | | | | |
| Inter-group | | | | | | | | | | | | | | | |
| I-II | 12 | 75.8 ± 94.2 | -10.0 ± 5.7 | -7.4 ± 4.3 | -6.1 ± 7.8 | 31.3 ± 13.9 | 15.3 ± 28.3 | 17.9 ± 30.9 | 11.7 ± 59.7 | 9.5 ± 41.1 | 7.2 ± 55.9 | 17.5 ± 9.2 | -8.0 ± 14.2 | -3.5 ± 23.7 | -2.6 ± 24.0 |
| Intra-group | | | | | | | | | | | | | | | |
| I | 6 | 87.8 ± 43.7 | -8.2 ± 2.9 | -8.2 ± 4.2 | -8.4 ± 9.3 | 22.2 ± 13.4 | 21.4 ± 19.5 | 4.4 ± 26.8 | 10.4 ± 52.7 | -2.5 ± 27.1 | 32.4 ± 41.9 | 9.8 ± 8.8 | 0.5 ± 8.2 | -6.1 ± 20.1 | -4.5 ± 10.5 |
| II | 3 | 96.1 ± 106.0 | -12.4 ± 7.7 | -7.9 ± 6.4 | -6.9 ± 8.3 | 52.2 ± 23.1 | 40.4 ± 4.0 | 16.3 ± 6.4 | 17.5 ± 24.2 | 12.7 ± 42.9 | 63.7 ± 128.8 | 16.6 ± 5.7 | -27.7 ± 5.4 | -4.0 ± 10.7 | -6.6 ± 6.4 |
| Average | | 92.0 ± 8.3 | -10.3 ± 4.2 | -8.1 ± 0.3 | -7.7 ± 1.5 | 37.2 ± 30.0 | 30.9 ± 19.0 | 10.4 ± 11.9 | 14.0 ± 7.1 | 5.1 ± 15.2 | 48.1 ± 31.3 | 13.2 ± 6.8 | -13.6 ± 28.2 | -5.1 ± 2.1 | -5.6 ± 2.1 |
| $F_1P$ | | | | | | | | | | | | | | | |
| Inter-group | | | | | | | | | | | | | | | |
| I-II | 12 | 337.2 ± 95.1 | 79.6 ± 6.3 | 135.3 ± 7.1 | 55.9 ± 3.4 | 96.9 ± 10.0 | 8.0 ± 2.4 | 3.2 ± 1.4 | 15.6 ± 9.2 | 3.5 ± 1.8 | 32.1 ± 19.9 | 4.9 ± 0.5 | 10.1 ± 1.4 | 170.8 ± 28.7 | 211.5 ± 24.8 |
| Intra-group | | | | | | | | | | | | | | | |
| I | 6 | 470.1 ± 69.4 | 78.5 ± 6.0 | 140.2 ± 6.1 | 58.3 ± 4.8 | 97.2 ± 14.3 | 13.0 ± 1.1 | 3.6 ± 1.1 | 40.5 ± 22.1 | 3.6 ± 1.0 | 17.5 ± 9.0 | 5.3 ± 0.5 | 10.5 ± 1.3 | 184.3 ± 36.3 | 229.5 ± 35.1 |
| II | 3 | 296.2 ± 111.3 | 79.0 ± 5.6 | 130.7 ± 7.4 | 51.3 ± 2.6 | 104.6 ± 6.6 | 4.2 ± 0.6 | 2.3 ± 0.8 | 6.7 ± 3.2 | 2.9 ± 1.7 | 69.0 ± 3.2 | 4.9 ± 0.6 | 10.2 ± 1.1 | 157.5 41.0 | 183.0 ± 50.5 |
| Average | | 383.2 ± 173.9 | 78.8 ± 0.5 | 135.5 ± 9.5 | 54.8 ± 7.0 | 100.9 ± 7.4 | 8.6 ± 8.8 | 3.0 ± 1.3 | 23.6 ± 33.8 | 2.3 ± 0.7 | 43.3 ± 51.5 | 5.1 ± 0.4 | 10.4 ±0.3 | 170.9 ± 26.5 | 206.3 ± 46.5 |

FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, FWT = fruit weight, FL = fruit length, FD = fruit diameter, PCT = pericarp thickness, PH = plant height, FN = fruit number, GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GFAA = green fruit ascorbic acid, RFAA = red fruit ascorbic acid content [+] average mid-parent heterosis

Of the studied characters, FY had the highest mean MPH (82.7%) with a range of –0.3 to 327.7% (Table 6.5). As can be also seen in Fig. 5.3A, all hybrids performed better than the mid-parent values for FY except for the cross-combination Szegedi 178 × C00916. The highest mid-parent positive heterosis deviation for this trait was observed in the crosses between Mareko Shote and C00916; Kalocsai "M" Cseresznye and Mareko Shote; and Szegedi 178 and Mareko Shote, suggesting it is advisable to select parents from inter-cluster rather than from intra-cluster (Fig. 6.1A, B) in order to obtain promising hybrids. A cross between Kalocsai "M" Cseresznye and C00916, both from intra-cluster, on the other hand revealed very high heterosis deviation for FY. Hybrids produced from some very closely related parents such as C00916 and PBC 142A and from other distantly related parents such as C00916 and Pepper 1976 had low heterosis deviation. Crosses produced from parents of intermediate divergence classes tended to show higher heterosis for FY however, although not consistent. Hybrids obtained from very closely or distantly related parents generally showed low heterosis. Liu *et al.* (1999) also reported similar results in wheat where, when GD between parents was over 0.7 or below 0.3 the $F_1$ generation generally displayed a lower mid-parent heterosis.

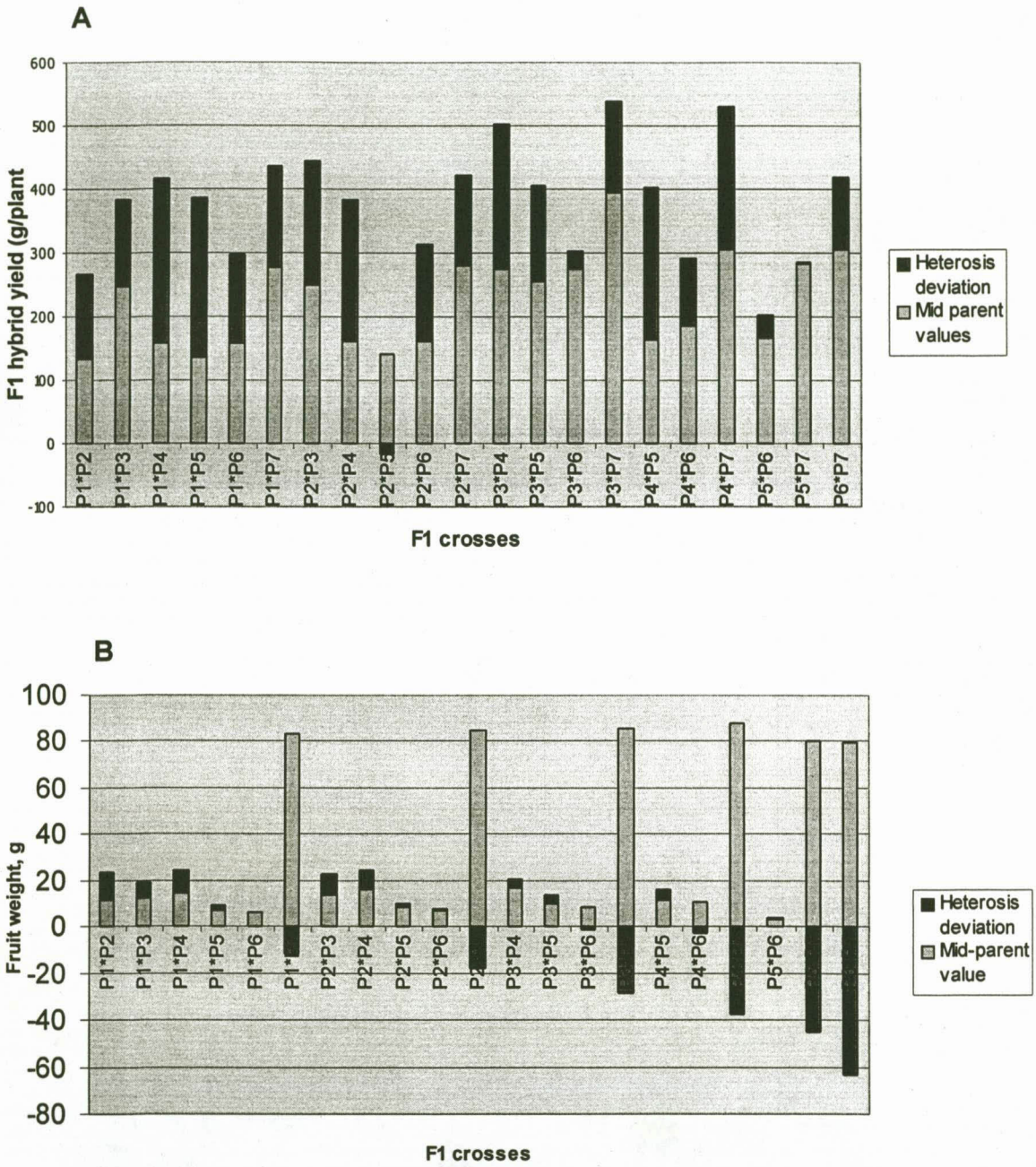


Fig. 6.3. Figure depicting heterosis deviation of mean fruit yield per plant (A) and mean fruit weight (B) over the mid-parent values for 21 $F_1$ hybrids. $P_1$ = Kalocsai "M" Cseresznye, $P_2$ = Szegedi 178, $P_3$ = Bakko Local, $P_4$ = Mareko Shote, $P_5$ = C00916, $P_6$ = PBC 142A, $P_7$ = Pepper 1976.

For FWT, the majority of the progenies derived from parents of intermediate divergence showed positive heterosis deviations from their respective mid-parent values than those derived from distantly related ones (Fig. 6.3B). All crosses that involved Pepper 1976 (the largest-fruited inbred line) as one of the parents performed below the mid-parent value for fruit weight indicating extreme diversity for this trait is not desirable to develop larger-fruited hybrid genotypes. The cross between PBC 142A and Pepper 1976 was among the hybrids that involved extremely divergent parents for FWT and showed very high negative heterosis deviation from the mid-parent value. This result also supports a previous report of Moll *et al.* (1965) which states that heterosis decreases beyond a certain level of genetic diversity because of incompatible gene combinations when two highly divergent parental populations are crossed. Busch *et al.* (1974) also indicated that the probability of recovering a superior progeny genotype is greater if both parents are similar in performance as opposed to one parent being inferior for one or more of the traits.

In this study, the complex and low heritability traits such as yield tended to show a greater level of heterosis and the parental performance (additive gene action) contributed less to $F_1$ performance. On the other hand, traits such as FL, FD, FWT, PCT and earliness in general demonstrated low heterosis and parental performance contributed more for these characters.

**Comparison of heterosis and genetic distance**

Table 6.7 illustrates the correlation coefficients of morphological and AFLP genetic distances with the MPH, HPH, $F_1P$ and SCA effects. Morphological distance was inversely related with the heterosis of DF, DM and FMP. The correlation values of GD measured by AFLP markers with MPH and HPH of all measured characters were non-significant with the exception of MPH for FD.

The correlations of GD generated by the two methods with $F_1P$ for all characters were non-significant with the exception of negative significant correlation of AFLP distance

with FY. The significant correlation of AFLP marker-based GD with this character as compared to other characters indicates the GD can account only for variation in performance due to the dominance effects (Bernardo, 1992). As mentioned above, FY had much greater heterosis and likely dominance effects than other characters.

Correlations between AFLP-distance and SCA effects of FY and FD were significantly positive. It is assumed that the SCA effect expressed by a hybrid is related to the genetic distance between its parental lines (Lee *et al.*, 1989). The correlation coefficients between morphological and AFLP distances with SCA effects of FY and FN were significant and greater than for $F_1P$. This is because the inbreeding between related lines is characterized by negative SCA values (Burstin *et al.*, 1995). Although poor correlations between morphological and AFLP distances with the heterosis of most of the measured characters, were observed, the majority of the progenies expressed appreciable levels of heterosis in the desired directions (Table 6.5). Thus, heterosis probably also exists due to different allelic combinations at particular loci in each parent, that, when brought together in hybrid combination, complement each other, resulting in heterosis expression (Bingham *et al.*, 1994). Riday *et al.* (2003) also indicated that such loci may not directly be related to observable morphological differences but could have an effect on the physiology of the plant. Molecular marker heterozygosity would be predictive of hybrid performance when dominance effects are strong, allele frequencies are negatively correlated between parents, heritabilities are high, and there is linkage between most markers and quantitative trait loci (Bernardo, 1992).

The relationship between morphological and AFLP distances with the MPH of FY and FWT were presented in Fig. 6.4A, B, C, D. As it is seen in Fig. 6.4A, B, when GDs between parents were lower or higher, their $F_1$ generations displayed lower MPH for FY. However, this trend was not consistent for all hybrid combinations for both distance measurements indicating that GD is not the only factor determining heterosis in pepper and that, heterosis cannot be predicted by GD alone. A similar trend was also observed for FWT in both genetic distance measurements (Fig. 6.4C, D).

Table 6.7. Correlation coefficients of genetic distance (GD) estimates with hybrid heterosis (MPH = mid-parent heterosis, HPH = high parent heterosis), mean performance ($F_1P$) and SCA effects averaged over two environments, 2001/02.

| Trait | Morphology | | | | | AFLP | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r(MPH, GD) | r(HPH, GD) | r($F_1P$, GD) | r(SCA, GD) | | r(MPH, GD) | r(HPH, GD) | r($F_1P$, GD) | r(SCA, GD) | |
| | | | | GH | FLD | | | | GH | FLD |
| FY | -0.294 | -0.356 | -0.112 | -0.040 | -0.469* | -0.136 | -0.127 | -0.610** | -0.509** | -0.005 |
| DF | -0.482* | -0.260 | -0.029 | -0.256 | -0.413 | -0.111 | -0.164 | -0.169 | -0.030 | -0.024 |
| DM | -0.492* | -0.246 | -0.286 | -0.427 | -0.423 | 0.091 | -0.051 | -0.253 | -0.052 | 0.110 |
| FMP | -0.387 | -0.176 | -0.423 | -0.351 | -0.140 | 0.061 | 0.156 | -0.235 | -0.040 | 0.171 |
| PH | -0.037 | 0.083 | -0.143 | -0.022 | -0.317 | 0.142 | 0.120 | -0.109 | -0.036 | 0.231 |
| FL | -0.178 | -0.263 | -0.335 | -0.116 | -0.401 | 0.138 | -0.090 | -0.423 | -0.020 | -0.099 |
| FD | -0.273 | -0.342 | -0.049 | -0.199 | -0.083 | 0.545** | 0.271 | -0.081 | 0.349 | 0.562** |
| FWT | -0.281 | -0.284 | -0.039 | -0.099 | -0.167 | 0.017 | -0.080 | -0.298 | 0.196 | 0.120 |
| PCT | -0.274 | -0.318 | -0.046 | -0.111 | -0.183 | 0.151 | 0.102 | -0.092 | 0.006 | 0.321 |
| FN | -0.397 | -0.419 | 0.157 | -0.138 | -0.548* | -0.299 | -0.198 | 0.263 | -0.403 | -0.111 |
| GFTSS | 0.290 | -0.446 | -0.366 | -0.311 | - | -0.028 | -0.062 | 0.104 | 0.173 | - |
| RFTSS | -0.289 | -0.243 | -0.269 | -0.112 | - | -0.132 | -0.265 | 0.087 | -0.164 | - |
| GFAA | 0.214 | 0.116 | -0.0345 | 0.170 | - | -0.358 | -0.335 | -0.304 | -0.256 | - |
| RFAA | 0.253 | 0.258 | -0.146 | 0.246 | - | -0.195 | -0.054 | -0.225 | -0.143 | - |

FY = fruit yield, DF = days to flowering, DM = days to maturity, FMP = fruit maturation period, FWT = fruit weight, FL = fruit length, FD = fruit diameter, PCT = pericarp thickness, PH = plant height, FN = fruit number. GFTSS = green fruit total soluble solids, RFTSS = red fruit total soluble solids, GFAA = greed fruit ascorbic acid, RFAA = red fruit ascorbic, * $P < 0.05$, ** $P < 0.01$.

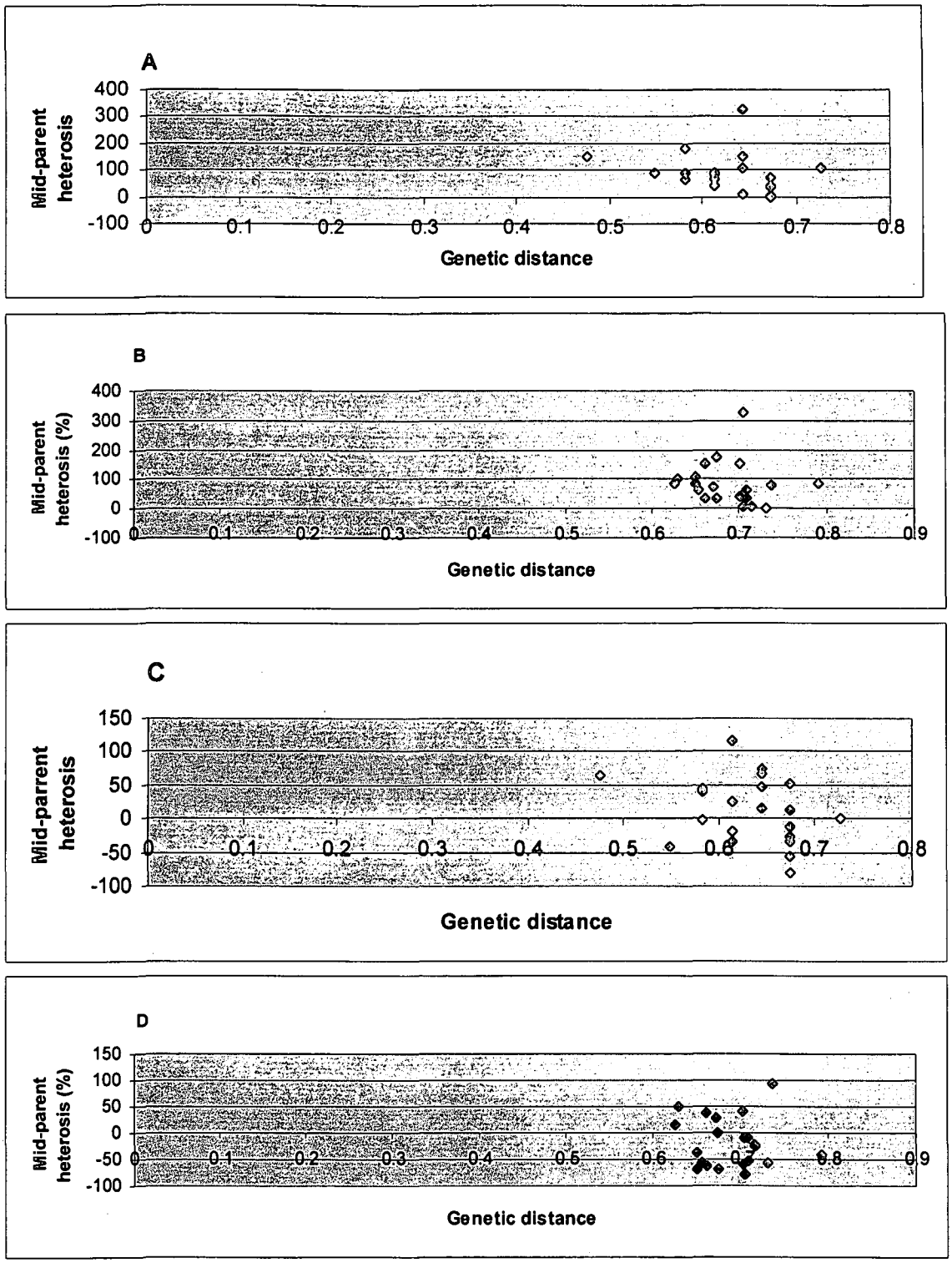


Fig. 6.4. Relationships between mid-parent heterosis of fruit yield vs. morphological data (A), mid-parent heterosis of fruit yield vs. AFLP marker (B), mid-parent heterosis of fruit weight vs. morphological data (C) and heterosis of fruit weight vs. AFLP marker (D) based estimate of genetic distances.

In the present study, GD measured by morphological traits and AFLP markers showed low correlations with heterosis and hybrid performance. Previous studies in various crop species such as maize (Ajmone-Marsal *et al.*, 1998; Benchimol *et al.*, 2000), rice (Kwon *et al.*, 2002), wheat (Bohn *et al.*, 1999; Martin *et al.*, 1995), alfalfa (Riday *et al.*, 2003) and chickpea (Sant *et al.*, 1999) also showed low correlations of GD with heterosis/hybrid performance. Liu *et al.* (1999) also reported that it is possible to differentiate wheat lines into heterotic groups from which superior hybrids can be developed, but it is impossible to predict hybrid performance by GD itself. Similarly, Xu *et al.* (2002) reported that random sets of SSR markers and pedigree based genetic diversity measures had no significant correlations with mid-parent heterosis for grain yield and biomass in rice. Several suggestions have been given concerning the low correlation of GD with hybrid performance/heterosis. Kwon *et al.* (2002) suggested that this could be firstly as the result of a lack of linkage between genes controlling the traits measured and the markers used to estimate distance. Secondly, chromosomal regions can differ in their contribution to $F_1$ performance and heterosis. Saghai Maroof *et al.* (1997) concluded that the level of correlations between marker distance and hybrid performance is dependent on the germplasm used. Riday *et al.* (2003) suggested that progeny heterosis can be accounted for by the interaction of genes controlling morphologically divergent traits between the parents. They further indicated progeny heterosis could also be due to the divergence between the parents at particular genetic loci that do not control field-level phenotypic differences.

## CONCLUSION

Very high heterosis for fruit yield, yield components and other agronomic characters in pepper has created interest in the development of hybrids. Hybrids offer some advantages over pure-line cultivars in that complementary traits from parental lines can be combined in a single $F_1$ genotype. In the present study also from low to high heterosis was observed for the studied characters. The investigation of genetic divergence among the seven parents with morphological traits indicated that extreme divergence for characters like

fruit related traits is not desirable for hybrid breeding in peppers. However, if the objective of a breeding program is, for example, to develop a hybrid of medium sized-fruit, smaller- and larger-fruited parents can be used in the crossing programs. As observed in the present study all fruit related traits showed partial dominance.

In this study, AFLP markers showed a higher tendency of differentiating pepper into heterotic groups from which superior hybrids can be derived than morphology. The significant correlations between morphological distance and mid-parent heterosis of days to maturity and fruit maturation period indicate the predictive value of this method for the heterosis of these traits. Although the values were non-significant, the correlations of genetic distances measured by the two methods with fruit related traits were negative, suggesting crosses between distantly related parents produce smaller fruits than the mid-parent values. The parental genotypes used in this study had diverse morphological backgrounds and were from different market types. Since each type of pepper must conform to its own unique set of characteristics, in order to be commercially acceptable, pepper hybrid breeding should deal with parental lines of similar varietal groups (market types).

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# CHAPTER 7

## Comparative performance and heterosis in single, three-way and double cross hybrids


### ABSTRACT

Hybrids are superior to pure line varieties or open-pollinated land-race cultivars. Depending on the structure of the parental groups hybrids can be of several types. It is hypothesized that yield stability is high in three-way and double-cross hybrids owing to higher genetic heterogeneity as compared to single-cross hybrids. Twenty-six genotypes including six inbred lines, eight single cross, six three-way cross and six double cross hybrids were evaluated for heterosis and performance of fruit yield (FY) and other agronomic characters in two environments using a randomized complete block design with three replications, during 2002 and 2003. The genotypes performed differently across the environments and showed high variation for the majority of characters studied. Higher performance for all the studied characters was observed in the field than in the greenhouse. The level of genotype-environment interaction for FY was higher in single-cross than in the three-way and double cross hybrids indicating that the latter two were more stable than the single cross hybrids. Higher mean FY per plant was obtained from the three-way cross followed by double cross hybrids. In general, three-way cross hybrids showed promising performance for the studied traits, indicating that it can be used in pepper hybrid breeding programs. The highest estimates of mid-parent heterosis and high-parent heterosis were also observed in the three-way and double cross hybrids.

## INTRODUCTION

Hybrid varieties are superior to pure line varieties or open-pollinated land-race cultivars. They can be single, three-way or double crosses. Considering all possible hybrids from a given sample of inbred lines, the expected genetic variance and predicted yield potential decline from single to three-way, to double and to top crosses (Cockerham, 1961). Yield stability is high in three-way and double-cross hybrids owing to higher genetic heterogeneity among populations within a cultivar from three-way and double cross hybrids as compared to single-cross hybrids. Patanothai and Atkins (1974) also indicated that three-way hybrids may be more stable than single crosses in their performance over a range of environmental conditions and stability of performance in different environments can be a result of heterogeneity within the population, or it can be a characteristic of specific genotype. Eberhart *et al.* (1964) found higher genotype-year interactions in single crosses than in three-way crosses.

The three types of hybrids have their own merits and demerits. Although three-way and double cross hybrids are probably higher yielding, they are heterogeneous as compared to single cross hybrids. Crop uniformity is considered a desirable character in modern agriculture because product uniformity is essential in marketing; uniformity in maturity permits crop scheduling; and uniformity in plant structure and maturation permits effective mechanical harvest (Janick, 1999). It is also an essential feature of crop quality especially in horticultural commodity.

Production of $F_1$ hybrids is a successful breeding technique because it exploits, and promotes homogeneity and is a way for commercial breeders to control their product. The uniformity of the hybrids has been considered one of their special benefits. Janick (1999) indicated that there are two dimensions of uniformity of hybrids: genetic homogeneity and genetic stability. Genetic homogeneity refers to the presence of identical genotypes while genetic stability refers to phenotypic uniformity (homeostasis) in different environments. On the other hand, crop diversity is considered desirable in some

environments and situations; because it is assumed to produce population buffering under stress, as diversity spreads risk.

Studies have been undertaken on different crops to determine which hybrid type the breeder should use in order to optimize chances of combining high yield performance with satisfactory yield stability and reported different results. In sorghum (*Sorghum bicolor* L.) the utilization of heterosis was made possible initially through three-way crosses (Stephens *et al.*, 1952). Performance evaluation trials conducted by Weatherspoon (1970) on single, double and three-way crosses indicated that single crosses produced the highest grain yields in maize, followed by the three-way crosses, while the double crosses gave the lowest yield. Saleh *et al.* (2002) reported high estimates of heterosis for grain yield and yield components but no obvious differences in performance among single, three-way and double cross hybrids in maize. According to Becker *et al.* (1982) double cross or an equivalent form with a two-line synthetic as pollinator parent is probably the most favorable hybrid type for rye (*Secale cereale* L.).

In the past, only double cross seed could be produced in adequate quantity at reasonable price in maize, since in this case both parents are single crosses and are vigorous. However, most new maize varieties in the United States are three-way and single crosses (Wricke and Weber, 1986). Although single cross hybrids are less stable in some situations owing to high uniformity, different methods of pest control and production techniques have allowed the increase of its yield stability. Thus, in crops such as maize, the double cross hybrid has been replaced by three-way and single-cross hybrids. However, in pepper, although single cross hybrids are widely used particularly in the developed countries, there is no report on the merit of producing and growing three-way and double cross hybrids.

As pepper pure lines are in general weaker, susceptible to diseases and pests, it is more difficult and expensive to produce enough single cross hybrid seeds using pure lines as seed parents. In Ethiopia, peppers are mainly grown by subsistence farmers and hybrid seeds, particularly single crosses, could be expensive for the farmers. Since single cross

hybrids are highly uniform, there could also be the risk of crop failure due to severe stress. Thus, the production of hybrid seeds on strong and competitive single-cross hybrid could make it possible to produce three-way or double cross hybrid seeds in higher quantity and affordable price. The objectives of this study were thus, to compare the three types of hybrids for yield and yield components, to estimate heterosis and broad-sense heritabilities for these characters.

## MATERIALS AND METHODS

### Plant materials and production of hybrid seeds

Based on high general combining ability (GCA) and good general plant performance, six inbred parental genotypes and eight $F_1$ single cross hybrids were selected from the studies of diallel crosses conducted in 2001 and 2002. The values of the three-way and double crosses were predicted from the single-crosses as suggested by Wricke and Weber (1986) and six three-way and six double cross hybrids were selected for the experimental investigations. The prediction of the values of the three-way and double crosses were made from the single crosses not directly involved in the three-way or double crosses using the formulae given by Wricke and Weber (1986) assuming the absence of epistasis:

$$T_{ij.l} = \frac{1}{2}(S_{il} + S_{jl})$$

$$D_{ij.lm} = \frac{1}{4}(S_{il} + S_{im} + S_{jl} + S_{jm})$$

where $S_{il}$, $S_{jl}$, $S_{im}$, $S_{jm}$ = the values of the single crosses between parents $i$, $j$, $l$, and $m$; $T_{ij\cdot l}$ = the predicted value of the three-way cross $(i \times j) \times l$; and $D_{ij\cdot lm}$ = the predicted value of the double cross $(i \times j) \times (l \times m)$.

The description of the inbred lines, and predicted yield and yield components for the six three-way and six double crosses on which the selection was based are presented in Tables 7.1 and 7.2, respectively. The seeds of the six inbred lines and the eight single cross hybrids were raised and planted in the greenhouse in 2002. The eight single crosses were used to form six double cross hybrids. In addition, six three-way crosses were developed from the single crosses and the parental inbred genotypes. Inbred lines were used as pollen parents and single crosses as seed parents. Emasculation and pollination were done by hand. Crosses were performed in one way, without reciprocals, following the procedure outlined by Wricke and Weber (1986). Crosses and inbred combinations for single, three-way and double cross hybrids are shown in Table 7.3.

Table 7.1. Inbred parental genotypes used to develop the single, three-way and double cross hybrids.

| Code | Name/pedigree | Fruit type | Origin |
|---|---|---|---|
| $P_1$ | Kalocsai "M" Cseresznye | Medium, round | Hungary |
| $P_2$ | Szegedi 178 | Medium long | Hungary |
| $P_3$ | Bakko Local | Medium long | Ethiopia |
| $P_4$ | Mareko Shote | Long | Ethiopia |
| $P_5$ | PBC 142A | Small long | India |
| $P_6$ | Pepper 1976 | Blocky | Israel |

Table 7.2. Predicted and actual yield and yield components for three-way and double cross hybrids.

| Hybrid | DF | | FL | | FD | | FWT | | FW | | FN | | FY | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRD | ACL | PRD | ACL | PRD | ACL | PRD | ACL | PRD | ACL | PRD | ACL | PRD | ACL |
| Three-way cross | | | | | | | | | | | | | | |
| TWC-1 | 78 | 63.8 | 9.2 | 8.2 | 4.1 | 3.0 | 45.1 | 21.2 | 5.0 | 3.0 | 13 | 12 | 344.8 | 255.8 |
| TWC-2 | 82.0 | 72.7 | 13.6 | 12.2 | 2.8 | 2.4 | 22.7 | 17.9 | 3.1 | 2.5 | 26 | 16 | 443.2 | 247.9 |
| TWC-3 | 81.2 | 70.3 | 11.8 | 10.4 | 4.6 | 4.0 | 62.1 | 53.0 | 4.5 | 4.1 | 10 | 6 | 402.4 | 232.2 |
| TWC-4 | 82.3 | 73.3 | 12.8 | 11.7 | 4.4 | 3.5 | 54.0 | 35.1 | 4.2 | 3.1 | 12 | 9 | 534.2 | 231.0 |
| TWC-5 | 79.5 | 74.3 | 12.6 | 9.8 | 4.0 | 3.5 | 45.8 | 36.6 | 3.9 | 3.2 | 14 | 7 | 402.4 | 176.2 |
| TWC-6 | 84.0 | 71.0 | 11.8 | 11.1 | 2.9 | 2.5 | 28.9 | 18.2 | 2.8 | 2.4 | 32 | 16 | 409.9 | 228.0 |
| Mean | 81.2 | 70.9 | 12.0 | 10.6 | 3.8 | 3.2 | 43.1 | 30.3 | 3.9 | 3.1 | 17.8 | 11 | 422.8 | 228.5 |
| Double cross | | | | | | | | | | | | | | |
| DC-1 | 79.7 | 75.3 | 9.5 | 6.4 | 4.0 | 3.9 | 41.3 | 30.0 | 4.7 | 4.0 | 16 | 9 | 408.7 | 180.8 |
| DC-2 | 79.7 | 71.8 | 12.9 | 12.3 | 3.7 | 3.0 | 42.4 | 26.0 | 3.6 | 2.9 | 17 | 13 | 461.9 | 285.1 |
| DC-3 | 82.0 | 71.8 | 13.1 | 10.9 | 3.5 | 3.0 | 40.2 | 22.6 | 3.6 | 2.9 | 19 | 13 | 474.9 | 203.0 |
| DC-4 | 85.4 | 79.0 | 11.6 | 11.0 | 3.0 | 2.3 | 30.5 | 16.5 | 2.7 | 2.2 | 36 | 19 | 415.0 | 240.0 |
| DC-5 | 82.4 | 73.3 | 10.7 | 8.8 | 4.0 | 3.4 | 43.5 | 26.2 | 4.5 | 3.3 | 19 | 8 | 473.7 | 187.8 |
| DC-6 | 82.1 | 70.8 | 10.9 | 9.9 | 3.3 | 2.7 | 33.0 | 20.9 | 3.1 | 2.8 | 34 | 14 | 389.2 | 212.3 |
| Mean | 81.9 | 73.7 | 11.5 | 9.9 | 3.6 | 3.1 | 38.5 | 23.7 | 3.7 | 3.0 | 23.5 | 13 | 437.2 | 218.2 |

DF = days to flowering, FL = fruit length, FD = fruit diameter, FWT = fruit weight, FN = fruit number, FY = fruit yield, PRD = predicted performance, ACL = actual performance.

Table 7.3. Hybrids and inbred line combinations for single, three-way and double crosses.

| Hybrid | Inbred combination |
|---|---|
| Single cross | |
| SC-1 | Kalocsai "M" Cseresznye × Pepper 1976 |
| SC-2 | Szegedi 178 × Bakko Local |
| SC-3 | Szegedi 178 × Mareko Shote |
| SC-4 | Bakko Local × Mareko Shote |
| SC-5 | Mareko Shote × Pepper 1976 |
| SC-6 | PBC 142A × Pepper 1976 |
| SC-7 | Kalocsai "M" Cseresznye × Bakko Local |
| SC-8 | Bakko Local × Pepper 1976 |
| Three-way cross | |
| TWC-1 | (Kalocsai "M" Cseresznye × Pepper 1976) × Szegedi 178 |
| TWC-2 | (Szegedi 178 × Bakko Local) × Mareko Shote |
| TWC-3 | (Szegedi 178 × Mareko Shote) × Pepper 1976 |
| TWC-4 | (Bakko Local × Mareko Shote) × Pepper 1976 |
| TWC-5 | (Mareko Shote × Pepper 1976) × Szegedi 178 |
| TWC-6 | (PBC 142A × Pepper 1976) × Mareko Shote |
| Double cross | |
| DC-1 | (Kalocsai "M" Cseresznye × Pepper 1976) × (Szegedi 178× Mareko Shote) |
| DC-2 | (Szegedi 178 × Bakko Local) × (Mareko Shote × Pepper 1976) |
| DC-3 | (Szegedi 178 × Mareko Shote) × (Bakko Local × Pepper 1976) |
| DC-4 | (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976) |
| DC-5 | (Mareko Shote × Pepper 1976) × (Kalocsai "M" Cseresznye × Bakko Local) |
| DC-6 | (PBC 142A × Pepper 1976) × (Szegedi 178 × Mareko Shote) |

## Experiments

Seedlings of the 26 genotypes comprising six inbred lines, eight single crosses, six double crosses and six three-way crosses were raised in the greenhouse and transplanted in the field as well as greenhouse during 2002/03. Transplanting was performed at about 45 days after sowing using a spacing of 0.7 m between rows and 0.3 m between plants

within rows in the field. Each replicate comprised one row of four plants. The border rows were planted with gourd plants and as a result all the plants in each replication were used to measure the necessary data. In the greenhouse, two seedlings were established in each pot filled with soil. In both environments, the experiments were carried out in randomized complete block design with three replications. All the necessary cultural practices were applied as per recommended for pepper production.

### *Data collection*

The following data were collected:

*Days to flowering*: number of days from sowing to the first open flower

*Fruit length* (cm): recorded at the second harvest

*Fruit width* (cm): measured at point of maximum width at the second harvest

*Fruit weight* (g): measured at the second harvest

*Pericarp thickness* (mm): recorded at the second harvest

*Fruit number*: total fruit number per plant from first harvest to last harvest.

*Fruit yield* (g): total fresh fruit yield from first harvest to last harvest

## Statistical analyses

### *Analyses of variances*

Combined analyses of variance for all measured characters over the two environments were undertaken using AGROBASE 2000 software (Agrobase, 2000). The mean squares were compared with F-values to assess the significance of the differences among the genotypes.

### *Estimates of heterosis*

Mid- and high-parent heterosis demonstrated by the hybrids for the various measured traits were estimated using the formulae given in the materials and methods part of Chapter 5. The heterosis estimates were tested for their significance using t-tests, taking individual values from each replication as units of observation.

### *Estimates of heritability*

Broad-sense heritability ($h^2b$) estimates for measured characters were estimated following the ANOVA components variance procedure as used by Vogel *et al.* (1981):

$$h^2b = \sigma^2_g/(\sigma^2_g + \sigma^2_e/r)$$

The variance components were calculated as:

$$\sigma^2_g = \frac{MS_g - MS_e}{r}$$

$$\sigma^2_e = \frac{MS_e}{r}$$

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

where $\sigma^2_g$ = genotypic variance, $\sigma^2_e$ = error variance, $\sigma^2_p$ = phenotypic variance, $MS_g$ = mean square of genotype, $MS_e$ = mean square of error, $r$ = number of replications.

## RESULTS AND DISCUSSION

### Analyses of variance and performance of genotypes

Mean squares were statistically significant for the majority of studied traits among the different types of hybrids. Results of combined analyses of variance for single crosses showed highly significant differences ($P < 0.01$) among the genotypes evaluated for characters measured (Table 7.4) indicating the hybrids were genetically variable. Environment also caused significant differences among the genotypes revealing that the genotypes performed differently across the environments. Lower stabilities of FY, FL, FD and FN per plant across the environments were evidenced by significant genotype-environment interaction.

As in the case of single crosses, the three-way crosses performed differently across the environments and showed very high variation for all characters except for FY per plant (Table 7.4). Genotype-environment interaction was highly significant for FY, FWT, FD and FN but the level of significance was lower for FD and FN. The non-significant genotype-environment variations observed in DF, FL and PCT suggest that they were stable across the environments.

In double cross hybrids, variations among the crosses were significant for DF, FL, FD, PCT and FN per plant but non-significant for FY and FWT (Table 7.4). In both three-way and double cross hybrids, the level of genotype-environment interaction on FY was lower than that in single cross hybrids, revealing that the former two were more stable than the latter. Genotype-environment interactions for simply inherited characters such as FL and PCT were non significant both in three-way and double cross hybrids. As indicated by Piepho (1996), stable yields play a major role for risk-minimization especially in developing countries. Stable yields are the key to sustainable food supplies. Patanothai and Atkins (1974) indicated that because single crosses are homogeneous they must depend entirely on individual buffering for stability of performance. On the other hand, a three-way hybrid can have stability resulting from population as well as individual

buffering. The analyses of variance for the six inbred lines were significant for all the traits indicating the presence of high genetic diversity among them for measured traits.

Table 7.4. Mean square from combined analyses of variance for single crosses, three-way crosses, double crosses, inbred lines and total genotypes evaluated for yield and other characters, 2002/03.

| Source | df | FY | DF | FL | FD | FWT | PCT | FN |
|---|---|---|---|---|---|---|---|---|
| Single cross | | | | | | | | |
| Env. (E) | 1 | 236191.2** | 21.3 | 63.4** | 0.1 | 544.1** | 0.1 | 415.7** |
| Genotype (G) | 7 | 12053.6** | 72.6** | 37.2** | 151.7** | 582.8** | 4.3** | 126.2** |
| G×E | 7 | 15235.0** | 22.4 | 1.6** | 0.1* | 23.9 | 0.2 | 39.5** |
| Error | 28 | 2886.7 | 14.2 | 0.7 | 0.1 | 33.6 | 0.4 | 6.9 |
| Three-way cross | | | | | | | | |
| Env. (E) | 1 | 233921.5** | 78.0* | 70.6** | 3.5** | 2943.1** | 4.7** | 183.5** |
| Genotype (G) | 5 | 4656.5 | 85.3** | 12.5** | 2.4** | 1155.5** | 2.3** | 109.0** |
| G×E | 5 | 17057.5* | 17.2 | 3.3 | 0.4* | 24.6** | 0.4 | 48.6* |
| Error | 20 | 6091.5 | 17.1 | 2.5 | 0.1 | 45.1 | 0.3 | 13.3 |
| Double cross | | | | | | | | |
| Env. (E) | 1 | 210902.0** | 434.0** | 63.7** | 1.0 | 697.8** | 0.1 | 245.3** |
| Genotype (G) | 5 | 9048.9 | 55.3* | 25.5** | 1.9** | 135.1 | 2.0** | 86.6** |
| G×E | 5 | 25833.7* | 62.0* | 1.3 | 0.2 | 15.7 | 0.6 | 80.3** |
| Error | 20 | 7909.3 | 17.0 | 1.8 | 0.4 | 63.0 | 0.7 | 16.0 |
| Total hybrids | | | | | | | | |
| Env. (E) | 1 | 67861.4** | 367.5** | 196.1** | 3.2** | 3473.2** | 2.6* | 834.8** |
| Genotype (G) | 19 | 11926.0** | 71.1** | 25.1** | 4.2** | 644.1** | 2.8** | 101.7** |
| G×E | 19 | 17023.4** | 37.8** | 1.9 | 0.3 | 164.1** | 0.5 | 49.0** |
| Error | 76 | 5130.5 | 15.3 | 1.6 | 0.2 | 44.1 | 0.4 | 11.5 |
| Inbred lines | | | | | | | | |
| Env. (E) | 1 | 269840.2** | 6.3 | 27.6** | 0.2 | 2878.3* | 0.6 | 6876.0** |
| Genotype (G) | 5 | 11726.1* | 285.6** | 77.7** | 38.3** | 12886.9** | 18.1** | 6379.8** |
| G×E | 5 | 13455.2* | 9.7 | 1.4 | 0.2 | 1650.4* | 0.1 | 2565.2** |
| Error | 20 | 3550.8 | 16.4 | 0.8 | 0.2 | 541.5 | 0.2 | 27.0 |
| Total genotype | | | | | | | | |
| Env. (E) | 1 | 933925.7** | 219.2** | 219.2** | 3.2** | 5279.2** | 3.2** | 4247.7** |
| Genotype (G) | 25 | 11844.9** | 38.5** | 38.5** | 10.9** | 3610.3** | 5.7** | 1641.4** |
| G×E | 4 | 16159.3** | 1.9 | 1.9 | 0.3 | 315.6** | 0.4 | 688.8** |
| Error | 100 | 4861.4 | 1.5 | 1.5 | 0.2 | 98.8 | 0.4 | 16.4 |

FY = fruit yield, DF = days to flowering, FL = fruit length, FD = fruit diameter, FWT = fruit weight, FN = fruit number, * $P < 0.05$, ** $P < 0.01$.

Many breeding programs consider wide adaptation as a primary objective. Crossa *et al.* (1990) indicated that a vital goal in breeding and agronomic research is to provide reliable guidance for selecting the best genotypes for planting in future years and at new sites, i.e., to predict yield as precisely as possible based on limited experimental data. Farmers are also interested in a constantly superior performance of a cultivar on their own farm. Breeding for sustainability has been defined as a process of fitting cultivars to an environment instead of altering the environment (by adding fertilizer, water, pesticides, etc.) to fit cultivars (Coffman and Smith, 1991).

In Ethiopia, pepper is grown mainly by subsistence farmers in many parts of the country including marginal areas. Basically, heterogeneous pepper landraces or farmers' varieties are the backbone of agricultural systems in Ethiopia, the country where environmental variation is high and unpredictable (MOA, 1998). In such circumstances, the production of modern, genetically uniform varieties bred for favorable environments could be a difficult task at the levels of inputs farmers can afford (Ceccarelli, 1994). The development of heterogeneous high yielding and disease resistant genotypes can perhaps be an alternative solution for subsistence farmers of Ethiopia. Ceccarelli *et al.* (1991) indicated that, in marginal environments, genotypes representing different combinations of traits are probably the best solution for long-term stability.

The comparison between predicted and actual yield and yield components for three-way and double cross hybrids is presented in Table 7.2. The actual performance for complex characters such as FY was highly variable (Table 7.5) and was not generally as predicted from the single crosses. On the contrary, the actual performance for highly heritable characters such as FL, FD, FWT and PCT corresponded with the predicted performances of these characters from their single crosses although the degrees of performances were lower than the predicted values. According to these results, simply heritable characters are more predictable than complex characters.

Table 7.5 also shows the performance of all categories of genotypes for measured characters. Yield performance was highly variable among the 26 genotypes. The highest

fruit yield was recorded in nine of the genotypes (five three-way crosses, two double crosses, one single cross and one inbred line). The two double cross and four of the five three-way cross hybrids involved bell pepper inbred line, Pepper 1976, in the combinations, indicating that larger fruit size and consequently high fruit mass per plant, was contributed by this parent. For instance, the highest fruit yields of 285.1 and 255.8 g per plant were recorded in the double cross (PBC 142A × Pepper 1976) × (Szegedi 178 × Mareko Shote) and the three-way cross (Kalocsai "M" Cseresznye × Pepper 1976) × Szegedi 178, respectively. Both hybrids involved Pepper 1976 in their hybrid combinations. On the other hand, the highest FN per plant was shown by the inbred genotype PBC 142A, and no hybrid surpassed this genotype for this trait. Cross combination (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976) was among the hybrids that showed a relatively larger number of fruits per plant, and contained PBC 142A in the cross combination. Similarly, two hybrids [(Kalocsai "M" Cseresznye × Pepper 1976) × Szegedi 178 and Kalocsai "M" Cseresznye × Pepper 1976)] and an inbred line Szegedi 178 were the earliest types among all genotypes. In these two hybrids, earliness was probably contributed by Kalocsai "M" Cseresznye and Szegedi 178, and Kalocsai "M" Cseresznye, respectively.

Table 7.5. Mean performance for fruit yield and other characters measured on single crosses, three-way crosses, double crosses and inbred lines pepper genotypes

| Genotype+ | FY | | | DF | | | FL | | | FD | | | FWT | | | PCT | | | FN | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE |
| Single cross | | | | | | | | | | | | | | | | | | | | | |
| SC-1 | 199.7 | 209.3 | 204.5 | 63.3 | 69.3 | 66.3 | 4.0 | 5.2 | 4.6 | 5.2 | 5.6 | 5.4 | 39.9 | 43.8 | 41.9 | 5.0 | 4.3 | 4.7 | 6.0 | 4 | 5 |
| SC-2 | 82.1 | 358.6 | 220.4 | 70.0 | 72.7 | 71.3 | 10.1 | 13.1 | 11.6 | 1.8 | 2.4 | 2.1 | 10.8 | 21.3 | 16.1 | 2.8 | 2.9 | 2.9 | 8.0 | 21 | 15 |
| SC-3 | 113.4 | 241.5 | 177.5 | 72.3 | 70.0 | 71.2 | 10.4 | 11.5 | 11.0 | 2.6 | 2.2 | 2.4 | 17.8 | 20.1 | 19.2 | 2.5 | 2.9 | 2.7 | 7.3 | 14 | 11 |
| SC-4 | 77.1 | 201.0 | 139.1 | 78.7 | 77.0 | 77.8 | 10.5 | 14.1 | 12.3 | 1.7 | 1.9 | 1.8 | 8.6 | 17.3 | 13.0 | 2.0 | 2.1 | 2.1 | 9.7 | 16 | 13 |
| SC-5 | 66.9 | 96.7 | 81.8 | 73.0 | 73.7 | 73.3 | 7.6 | 10.1 | 8.9 | 3.6 | 3.4 | 3.5 | 21.6 | 27.6 | 24.6 | 2.9 | 2.8 | 2.9 | 4.3 | 4 | 4 |
| SC-6 | 66.5 | 270.4 | 168.4 | 73.3 | 77.3 | 75.3 | 6.8 | 9.8 | 8.3 | 2.0 | 2.0 | 2.0 | 9.7 | 15.7 | 12.7 | 1.6 | 2.0 | 1.8 | 8.7 | 21 | 15 |
| SC-7 | 148.8 | 233.7 | 191.3 | 67.0 | 72.7 | 69.8 | 7.5 | 8.4 | 8.0 | 2.6 | 2.8 | 2.7 | 14.7 | 17.2 | 15.9 | 2.4 | 3.0 | 2.7 | 13.7 | 18 | 16 |
| SC-8 | 71.5 | 337.1 | 204.3 | 74.3 | 70.0 | 72.2 | 9.2 | 12.2 | 10.7 | 3.2 | 3.4 | 3.3 | 21.0 | 34.7 | 27.8 | 3.1 | 3.0 | 3.1 | 4.3 | 10 | 7 |
| Mean | 103.3 | 243.5 | 173.4 | 71.5 | 72.8 | 72.2 | 8.3 | 10.6 | 9.4 | 2.9 | 3.0 | 2.9 | 18.0 | 24.8 | 21.4 | 2.8 | 2.9 | 2.9 | 7.6 | 14 | 10.8 |
| $LSD_{0.05}$ | 45.2 | 99.5 | 52.8 | 5.9 | ns | 3.7 | 1.1 | 1.3 | 0.8 | 0.3 | 0.4 | 0.2 | 3.7 | 11.2 | 5.7 | 1.0 | 0.8 | 0.6 | 2.1 | 5 | 2.6 |
| Three-way cross | | | | | | | | | | | | | | | | | | | | | |
| TWC-1 | 72.6 | 439.0 | 255.8 | 61.0 | 66.7 | 63.8 | 5.8 | 10.6 | 8.2 | 2.8 | 3.2 | 3.0 | 14.1 | 28.2 | 21.2 | 2.4 | 3.6 | 3.0 | 5.7 | 19 | 12 |
| TWC-2 | 176.1 | 319.6 | 247.9 | 71.0 | 74.3 | 72.7 | 11.4 | 13.0 | 12.2 | 2.4 | 2.4 | 2.4 | 17.8 | 18.1 | 17.9 | 2.4 | 2.5 | 2.5 | 12.0 | 20 | 16 |
| TWC-3 | 167.6 | 296.8 | 232.2 | 70.3 | 70.3 | 70.3 | 8.1 | 12.7 | 10.4 | 3.4 | 4.7 | 4.0 | 29.5 | 76.6 | 53.0 | 3.5 | 4.7 | 4.1 | 7.0 | 6 | 6 |
| TWC-4 | 192.6 | 269.3 | 231.0 | 73.7 | 73.0 | 73.3 | 10.5 | 12.9 | 11.7 | 3.4 | 3.6 | 3.5 | 30.9 | 39.4 | 35.1 | 3.2 | 3.1 | 3.1 | 8.3 | 10 | 9 |
| TWC-5 | 134.9 | 217.4 | 176.2 | 70.3 | 78.3 | 74.3 | 9.1 | 10.6 | 9.8 | 2.9 | 4.1 | 3.5 | 22.7 | 50.4 | 36.6 | 2.7 | 3.7 | 3.2 | 7.3 | 6 | 7 |
| TWC-6 | 143.6 | 312.4 | 228.0 | 70.3 | 71.7 | 71.0 | 10.1 | 12.0 | 11.1 | 2.2 | 2.7 | 2.5 | 12.8 | 23.5 | 18.2 | 2.0 | 2.8 | 2.4 | 12.3 | 19 | 16 |
| Mean | 147.9 | 309.1 | 228.5 | 69.4 | 72.4 | 70.9 | 9.2 | 12.0 | 10.6 | 2.8 | 3.5 | 3.2 | 21.3 | 39.4 | 30.3 | 2.7 | 3.4 | 3.1 | 8.8 | 13 | 11 |
| $LSD_{0.05}$ | ns | ns | ns | 5.6 | ns | 4.1 | 2.2 | ns | 1.6 | 0.5 | 0.6 | 0.4 | 5.3 | 13.0 | 6.7 | 0.6 | 1.1 | 0.6 | 3.7 | 7 | 4 |

Table 7.5. Continued...

| Genotype | FY | | | DF | | | FL | | | FD | | | FWT | | | PCT | | | FN | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE | GH | FLD | APE |
| Double cross | | | | | | | | | | | | | | | | | | | | | |
| DC-1 | 217.3 | 144.3 | 180.8 | 66.3 | 84.3 | 75.3 | 5.4 | 7.5 | 6.4 | 3.6 | 4.1 | 3.9 | 23.8 | 36.3 | 30.0 | 4.1 | 3.8 | 4.0 | 12 | 5 | 9 |
| DC-2 | 143.9 | 426.2 | 285.1 | 68.3 | 75.3 | 71.8 | 11.7 | 13.0 | 12.3 | 2.8 | 3.3 | 3.0 | 21.9 | 30.1 | 26.0 | 2.8 | 2.9 | 2.9 | 7 | 18 | 13 |
| DC-3 | 148.4 | 257.5 | 203.0 | 70.7 | 73.0 | 71.8 | 9.4 | 12.4 | 10.9 | 2.9 | 3.1 | 3.0 | 19.4 | 25.8 | 22.6 | 2.3 | 3.4 | 2.9 | 11 | 14 | 13 |
| DC-4 | 136.8 | 343.1 | 240.0 | 79.3 | 78.7 | 79.0 | 9.8 | 12.1 | 11.0 | 2.2 | 2.3 | 2.3 | 13.0 | 20.0 | 16.5 | 2.2 | 2.2 | 2.2 | 14 | 23 | 19 |
| DC-5 | 124.1 | 251.4 | 187.8 | 70.3 | 76.3 | 73.3 | 7.2 | 10.4 | 8.8 | 3.5 | 3.3 | 3.4 | 23.4 | 29.0 | 26.2 | 3.6 | 2.9 | 3.3 | 7 | 9 | 8 |
| DC-6 | 79.0 | 345.5 | 212.3 | 66.3 | 75.3 | 70.8 | 7.9 | 11.9 | 9.9 | 2.3 | 3.1 | 2.7 | 14.3 | 27.4 | 20.9 | 2.4 | 3.1 | 2.8 | 7 | 20 | 14 |
| Mean | 141.6 | 294.7 | 218.2 | 70.2 | 77.2 | 73.7 | 8.6 | 11.2 | 9.9 | 2.9 | 3.2 | 3.1 | 19.3 | 28.1 | 23.7 | 2.9 | 3.1 | 3.0 | 10 | 15 | 13 |
| $LSD_{0.05}$ | 85.6 | ns | ns | 6.0 | ns | 4.1 | 1.4 | 2.5 | 1.3 | ns | ns | 0.6 | Ns | ns | ns | ns | ns | 0.8 | 4 | 7 | 12 |
| Inbred line | | | | | | | | | | | | | | | | | | | | | |
| $P_1$ | 99.2 | 313.3 | 206.3 | 69.0 | 67.7 | 68.3 | 2.4 | 2.9 | 2.6 | 3.2 | 3.8 | 3.5 | 12.0 | 19.7 | 15.9 | 3.3 | 4.1 | 3.7 | 13 | 26 | 19 |
| $P_2$ | 77.7 | 263.1 | 170.4 | 64.7 | 67.0 | 65.8 | 8.9 | 10.9 | 9.9 | 2.3 | 2.2 | 2.3 | 14.6 | 22.6 | 18.6 | 2.7 | 2.8 | 2.7 | 7 | 13 | 10 |
| $P_3$ | 95.4 | 408.7 | 252.1 | 84.0 | 81.7 | 82.8 | 10.9 | 12.6 | 11.8 | 1.8 | 1.5 | 1.7 | 10.3 | 11.6 | 10.9 | 1.9 | 1.9 | 1.9 | 13 | 48 | 31 |
| $P_4$ | 92.3 | 210.7 | 151.5 | 79.0 | 74.3 | 76.7 | 9.6 | 12.7 | 11.2 | 2.3 | 2.9 | 2.6 | 12.7 | 17.8 | 15.3 | 2.4 | 2.6 | 2.5 | 10 | 14 | 12 |
| $P_5$ | 38.4 | 215.6 | 127.0 | 81.3 | 81.0 | 81.2 | 4.8 | 5.6 | 5.2 | 0.8 | 0.8 | 0.8 | 1.6 | 1.6 | 1.6 | 0.7 | 0.8 | 0.7 | 37 | 145 | 91 |
| $P_6$ | 204.8 | 211.7 | 208.3 | 71.3 | 72.7 | 72.0 | 6.4 | 8.8 | 7.6 | 7.9 | 8.0 | 7.9 | 102.4 | 167.7 | 135.0 | 5.6 | 6.0 | 5.8 | 2 | 2 | 2 |
| Mean | 101.3 | 270.5 | 185.9 | 74.9 | 74.1 | 74.5 | 7.2 | 8.9 | 8.1 | 3.0 | 3.2 | 3.1 | 25.6 | 40.2 | 32.9 | 2.8 | 3.0 | 2.9 | 14 | 41 | 27.5 |
| $LSD_{0.05}$ | 63.8 | 101.1 | 56.9 | 7.1 | 4.6 | 4.0 | 1.2 | 1.4 | 0.8 | 0.6 | 0.8 | 3.1 | 16.8 | 31.1 | 32.9 | 0.5 | 0.9 | 5.2 | 8 | 8 | 0.5 |
| Mean, overall | 122.3 | 276.7 | 199.3 | 71.5 | 74.0 | 72.8 | 8.3 | 10.7 | 9.5 | 2.9 | 3.2 | 3.0 | 20.8 | 32.5 | 26.6 | 2.8 | 3.1 | 2.9 | 9.8 | 20.2 | 15 |
| $LSD_{0.05}$, overall | 59.0 | 121.3 | 66.8 | 5.7 | 5.0 | 3.8 | 1.5 | 1.6 | 1.2 | 0.6 | 0.6 | 0.4 | 9.7 | 16.6 | 9.5 | 0.9 | 0.8 | 0.6 | 4.4 | 6.5 | 4 |
| Mean, all $F_1$s | 128.1 | 278.6 | 203.4 | 70.5 | 74.0 | 72.2 | 8.6 | 10.7 | 9.9 | 2.9 | 3.2 | 3.0 | 19.4 | 32.5 | 24.8 | 2.8 | 3.1 | 3.0 | 9 | 20 | 11 |
| $LSD_{0.05}$, all $F_1$s | 59.3 | 126.2 | 68.9 | 5.5 | 5.3 | 3.8 | 1.6 | 1.9 | 1.2 | 0.6 | 0.5 | 0.4 | 7.2 | 10.7 | 6.4 | 1.0 | 0.9 | 0.6 | 3.0 | 5.9 | 3 |

[+] For cross combination see Table 6.3. FY = fruit yield, DF = days to flowering, FL = fruit length, FD = fruit diameter, FWT = fruit weight, FN = fruit number, GH = greenhouse, FLD = field, APE = average performance over environments, ns = non-significant.

The combined analyses of variance among single, three-way and double cross hybrids showed significant differences for some of the measured traits (Table 7.6). The variations for FY, DF, FWT and FN per plant were significantly high, indicating that the hybrids were highly variable for these characters. The environment also had a significant effect for all traits measured, indicating that the genotypes performed differently across the environments. When comparisons were made between the single, three-way and double cross hybrids for the mean performance (Table 7.7), three-way hybrids showed the highest mean FY per plant (228.5 g) followed by double cross hybrids (218.1 g). Similarly, the highest FD was recorded in these two forms of hybrids. In general, three-way crosses showed promising performance for the studied traits and overall plant performance, indicating that it can effectively be used in the pepper hybrid breeding program of Ethiopia. On the other hand, single crosses performed poorly. However, Weatherspoon (1970) reported more superior yield performance in single crosses than in three-way and double crosses hybrids in maize. However, among the three forms of hybrids, the highest uniformity in maturity and fruit characters were observed in single crosses followed by three-way crosses (Fig. 7.1). Double cross hybrids exhibited the highest heterogeneity, especially when inbred lines of diverse genetic background were used in the hybrid development.

Table 7.6. Mean squares from combined analysis of variance for comparison between single, three-way and double cross hybrids evaluated for yield and other characters at two environments, 2002/03.

| Source | df | FY | DF | FL | FD | FWT | PCT | FN |
|---|---|---|---|---|---|---|---|---|
| Env. (E) | 1 | 103327.4** | 63.0** | 30.1** | 0.6** | 563.2** | 0.4** | 122.0** |
| Genotype (G) | 2 | 5144.4** | 11.6* | 2.0* | 0.1 | 129.5** | 0.1 | 4.5 |
| G × E | 2 | 166.9 | 12.5* | 0.1 | 0.1 | 54.8** | 0.2* | 0.7 |
| Error | 8 | 534.0 | 1.8 | 0.3 | 0.03 | 5.0 | 0.04 | 1.8 |
| CV (%) | | 11.2 | 1.8 | 5.8 | 6.1 | 8.9 | 7.0 | 11.8 |

FY = fruit yield, DF = days to flowering, FL = fruit length, FD = fruit diameter, FWT = fruit weight, FN = fruit number, * $P < 0.05$, ** $P < 0.01$

Table 7.7. Mean values of yield and other characters measured on single, three-way and double cross hybrids, 2001/02.

| Genotype | FY | DF | FL | FD | FWT | PCT | FN |
|---|---|---|---|---|---|---|---|
| Single cross | 173.4 | 72.2 | 9.4 | 2.9 | 21.4 | 2.8 | 10.7 |
| Three-way cross | 228.5 | 70.9 | 10.6 | 3.2 | 30.3 | 3.0 | 11.0 |
| Double cross | 218.1 | 73.7 | 9.9 | 3.0 | 23.7 | 3.0 | 12.3 |
| $LSD_{0.05}$ | 24.9 | 1.4 | 0.6 | 0.2 | 2.4 | 0.2 | 1.4 |

FY = fruit yield, DF = days to flowering, FL = fruit length, FD = fruit diameter, FWT = fruit weight, FN = fruit number.


Fig. 7.1. Figures depicting the performance of single (top), three-way (middle) and double (bottom) cross hybrids for uniformity of fruit length and shape in pepper. Single cross hybrids gave the most uniform fruits followed by the three-way cross hybrids. The double cross hybrids were the least uniform.

## Heterosis

Hybrid vigor, or heterosis, usually refers to the increase in size or rate of growth of offspring over parents; for example, hybrid vigor in crop plants can be observed as increase in yield of grain, or decrease in number of days to flowering (Duvick, 1999). Development of hybrid seeds can enhance crop yield and performance.

Yield heterosis is a variable trait and depends not only on the parent combinations but also on the environmental conditions (Young and Virmani, 1990; Virmani *et al.*, 1982). Stuber (1999) also indicated that environmental variability may affect the relationships of specific physiological components of heterosis. It is also a common hypothesis that heterosis would be more valuable in stressed environments. Generally, heterosis is environment dependent, but the nature of interactions depends on the species and the trait under consideration (Knight, 1973). According to the concept of genetic homeostasis proposed by Lerner (1954), heterozygotes (hybrids) are likely to be better buffered than homozygotes (parents) against environmental variation. Heterosis gives the crop considerable resilience in response to environmental fluctuations. Usually farmers prefer hybrids not only for higher yields but also for stable yields in different locations and in different years. Hybrids, by being able to successfully encounter varying kinds of stresses imposed in different locations and years, exceed homozygous lines in their stability of performance (Tsaftaris *et al.*, 1999).

Variable estimates of heterosis were observed among the different categories of hybrids (Tables 7.8 and 7.9). Two double [(Szegedi 178× Bakko Local) × (Mareko Shote × Pepper 1976) and (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976)], and three three-way [(Szegedi 178× Bakko Local) × Mareko Shote , (Mareko Shote × Pepper 1976) × Szegedi 178 and (PBC 142A × Pepper 1976) × Mareko Shote] crosses of total hybrids had significant MPH and HPH for FY per plant across the two environments. The highest mid-parent and high-parent heterosis of 93.4 and 56.6% were shown by the two double cross hybrids, respectively.

Across environment data further showed that 12 and eight crosses demonstrated positive MPH and HPH for FWT. Five of 12 hybrids that showed positive MPH ranging from 2.8 to 40.0% with an average of 25.3% were double crosses. Similarly, four of the eight crosses that had positive HPH were double crosses and the other four were three-way crosses. No single cross hybrid showed positive HPH for this trait.

Table 7.8. Estimates of mid-parent heterosis (%) for seven characters in single, three-way and double cross hybrids under greenhouse (GH) and field (FLD) conditions, 2002/03.

| Hybrid[+] | Fruit yield | | | Days to flowering | | | Fruit length | | | Fruit diameter | | | Fruit weight | | | Pericarp thickness | | | Fruit number | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GH | FLD | Com[++] | GH | FLD | Com | GH | FLD | Com. | GH | FLD | Com | GH | FLD | Com | GH | FLD | Com | GH | FLD | Com |
| SC-1 | 47.1 | -6.0 | 20.5 | -9.2 | -1.4 | -5.6 | -7.5 | 12.0 | 2.2 | -6.2 | -4.5 | -5.3 | 45.0 | -47.3 | -1.2 | 19.5 | 2.5 | 11.0 | -36.1 | -69.8 | -52.9 |
| SC-2 | -3.8 | 13.6 | 4.9 | -0.1 | -2.2 | -1.1 | 2.6 | 11.9 | 7.2 | -10.4 | 30.8 | 10.1 | -11.4 | 33.2 | 10.9 | 2.6 | 3.5 | 3.1 | 6.4 | 23.4 | 14.9 |
| SC-3 | 41.6 | 1.7 | 21.6 | 0.7 | -0.8 | -0.1 | 11.1 | 4.9 | 8.0 | 11.7 | -10.3 | 0.7 | 17.3 | 4.1 | 10.7 | -2.8 | 1.3 | -0.7 | 2.9 | 5.2 | 4.0 |
| SC-4 | -18.4 | -33.4 | -25.9 | -3.5 | -1.3 | -2.4 | 2.1 | 13.7 | 7.9 | -12.4 | -12.9 | -12.6 | -13.7 | 17.9 | 2.1 | 3.4 | 5.6 | 4.5 | -15.3 | -48.8 | -32.0 |
| SC-5 | -50.4 | -53.8 | -52.1 | -2.8 | 0.2 | -1.3 | -3.3 | -5.7 | -4.5 | -28.5 | -35.5 | -14.0 | -60.1 | -68.8 | -64.4 | 9.6 | 4.3 | 6.9 | -37.8 | -55.6 | -46.7 |
| SC-6 | -40.2 | 34.6 | -2.8 | -3.9 | 0.5 | -1.7 | 23.1 | 36.5 | 29.8 | -24.1 | -55.0 | -39.6 | 103.8 | -81.0 | 11.4 | 41.8 | 41.6 | 41.7 | -61.1 | -75.1 | -68.1 |
| SC-7 | 59.4 | -29.4 | 15.0 | -6.4 | -3.1 | -4.7 | 79.0 | 8.8 | 43.9 | 5.4 | 4.5 | 4.9 | -24.1 | -6.1 | -15.1 | -15.8 | -14.9 | -15.4 | 4.6 | -16.7 | -6.1 |
| SC-8 | -49.2 | -2.2 | -25.7 | -4.3 | -8.4 | -6.4 | 7.8 | 5.9 | 6.8 | -32.6 | -27.7 | -30.1 | -61.2 | -60.0 | -60.6 | 24.1 | 22.8 | 23.4 | -50.1 | -64.9 | -57.5 |
| TWC-1 | -47.0 | 107.6 | 30.3 | -4.6 | -6.6 | -5.6 | -3.2 | 19.4 | 8.1 | -26.9 | -16.3 | -21.6 | -48.1 | -11.1 | -29.6 | -35.3 | -10.1 | -22.7 | -2.2 | 60.9 | 29.4 |
| TWC-2 | 123.4 | 18.8 | 71.1 | -4.5 | 1.2 | -1.7 | 16.9 | 4.8 | 10.8 | 0.4 | -5.3 | -2.5 | 58.7 | -8.7 | 25.0 | -3.1 | -7.9 | -5.5 | 19.6 | -2.7 | 8.4 |
| TWC-3 | 16.3 | 33.6 | 24.9 | -2.2 | -1.4 | -1.8 | -2.6 | 25.7 | 11.5 | -34.4 | -7.7 | -21.0 | -46.2 | -15.3 | -30.7 | 15.8 | 24.5 | 20.2 | -2.2 | -40.6 | -21.4 |
| TWC-4 | 38.0 | 31.8 | 34.9 | -1.6 | -2.4 | -2.0 | 26.0 | 12.8 | 19.4 | 2.9 | -26.1 | -11.6 | -39.2 | -55.1 | -47.1 | 21.8 | 18.6 | 20.2 | -8.1 | -23.8 | -15.9 |
| TWC-5 | 95.0 | 19.1 | 57.2 | 2.3 | 11.6 | 6.9 | 18.9 | 0.8 | 9.8 | -6.1 | 49.4 | 21.7 | 25.5 | 109.0 | 67.3 | -2.6 | 13.1 | 5.3 | 25.3 | 19.8 | 22.6 |
| TWC-6 | 79.9 | 26.9 | 53.4 | -7.6 | -4.5 | -6.1 | 23.7 | 2.7 | 13.2 | 2.7 | 14.1 | 8.4 | 16.3 | 33.5 | 24.9 | 9.1 | 18.0 | 13.6 | 16.4 | -7.1 | 4.6 |
| DC-1 | 34.8 | -32.0 | 1.4 | -2.1 | 21.1 | 9.5 | 0.4 | -8.6 | -4.1 | -7.2 | 5.1 | -1.0 | -21.4 | 27.2 | 2.9 | -12.8 | -6.1 | -9.4 | 33.8 | 8.9 | 21.4 |
| DC-2 | 102.6 | 84.2 | 93.4 | -4.4 | 3.0 | -0.7 | 34.4 | 12.8 | 23.6 | 3.2 | 12.7 | 7.9 | 36.8 | 17.4 | 27.1 | 5.0 | -1.5 | 1.8 | -8.5 | -9.1 | -8.8 |
| DC-3 | 60.6 | -7.2 | 26.7 | -3.6 | 4.3 | 0.4 | -1.7 | 4.9 | 1.6 | 10.9 | 3.9 | 7.4 | -7.5 | -6.4 | -7.0 | -5.2 | 8.7 | 1.8 | 20.3 | -3.3 | 8.5 |
| DC-4 | 100.2 | 49.0 | 74.6 | 4.6 | 2.3 | 3.4 | 6.1 | 2.1 | 4.1 | 15.3 | 20.9 | 18.1 | 43.1 | 20.0 | 31.4 | 3.5 | 2.4 | 2.9 | 18.5 | 16.3 | 17.4 |
| DC-5 | 10.8 | 48.9 | 29.9 | 0.6 | 5.8 | 3.2 | -4.1 | 13.3 | 4.6 | 12.8 | 4.7 | 8.7 | 29.5 | 20.8 | 25.1 | 12.1 | 0.9 | 6.5 | 19.3 | 40.0 | 29.6 |
| DC-6 | -8.7 | 33.3 | 12.3 | -8.9 | 2.3 | -3.4 | -7.3 | 12.6 | 2.6 | -2.1 | 49.8 | 23.8 | 3.0 | 77.1 | 40.0 | 19.4 | 21.7 | 20.5 | -8.3 | -0.2 | -4.4 |
| Mean | 29.6 | 17.0 | 23.3 | -3.1 | 1.0 | -1.1 | 11.1 | 9.6 | 10.3 | -6.3 | -0.3 | -3.3 | 2.3 | 0.0 | 1.2 | 5.5 | 7.4 | 6.5 | -3.1 | -12.2 | -7.6 |
| $LSD_{0.05}$ | 78.0 | 60.3 | 49.6 | ns | 9.1 | 6.9 | ns | ns | ns | ns | 27.5 | 24.4 | ns | 57.9 | 63.5 | 19.8 | 19.7 | 13.8 | 19.8 | 21.4 | 14.4 |

[+] Parental combination is given in Table 6.3, [++] Com = means obtained from combined analysis, ns = non-significant

Table 7.9. Estimates of high-parent heterosis (%) for seven characters in single, three-way and double cross hybrids under greenhouse (GH) and field (FLD) conditions, 2002/03.

| Hybrid[+] | Fruit yield | | | Days to flowering | | | Fruit length | | | Fruit diameter | | | Fruit weight | | | Pericarp thickness | | | Fruit number | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GH | FLD | Com[++] | GH | FLD | Com | GH | FLD | Com | GH | FLD | Com | GH | FLD | Com | GH | FLD | Com | GH | FLD | Com |
| SC-1 | 21.1 | -23.9 | -1.4 | -4.0 | 2.0 | -1.0 | -36.3 | -9.8 | -23.1 | -33.9 | -29.2 | -31.6 | 31.1 | -70.3 | -19.6 | -11.9 | -24.4 | -18.2 | -51.9 | -81.8 | -66.8 |
| SC-2 | -21.0 | -12.9 | -17.0 | 10.9 | 9.0 | 9.9 | -7.1 | 1.4 | -2.9 | -20.9 | 13.9 | -3.5 | -22.4 | 15.1 | -3.7 | 15.4 | 9.8 | 12.6 | -39.0 | -55.7 | -47.3 |
| SC-3 | 29.7 | -13.2 | 8.2 | 12.0 | 3.6 | 8.5 | 3.4 | 2.2 | 2.8 | 0.1 | -21.7 | -10.8 | 6.0 | -7.8 | -0.9 | -5.2 | 4.4 | -0.4 | -21.8 | -11.8 | -16.8 |
| SC-4 | -25.2 | -50.4 | -37.8 | 1.4 | 2.3 | 2.5 | -3.9 | 11.6 | 3.9 | -20.3 | -32.3 | -26.3 | -17.3 | -3.8 | -10.6 | -12.1 | -7.1 | -9.6 | -25.9 | -64.7 | -45.3 |
| SC-5 | -60.1 | 16.7 | -59.6 | 2.4 | 6.3 | 2.3 | -19.7 | -19.9 | -19.8 | -53.9 | -56.1 | -55.0 | -77.5 | -82.6 | -80.0 | -49.2 | -50.9 | -50.0 | -54.6 | -71.4 | -63.0 |
| SC-6 | -63.6 | -33.5 | -23.4 | 2.8 | 6.4 | 4.5 | 8.2 | 11.5 | 9.8 | -52.4 | -75.2 | -63.8 | 96.1 | -90.4 | 2.9 | -71.0 | -66.4 | -68.7 | -75.8 | -85.6 | -80.1 |
| SC-7 | 46.4 | -33.5 | 6.5 | -1.0 | -3.6 | 2.7 | 53.2 | -33.3 | 10.0 | -18.8 | -26.5 | -22.7 | -40.6 | -12.0 | -26.3 | -26.0 | -25.2 | -25.6 | 8.0 | -61.8 | -26.9 |
| SC-8 | -59.3 | -13.2 | -36.2 | 4.2 | 6.4 | 0.3 | -13.7 | -3.4 | -8.6 | -58.6 | -56.7 | -57.6 | -78.4 | -78.5 | -78.4 | -44.5 | -48.0 | -46.2 | -66.7 | -78.0 | -72.4 |
| TWC-1 | -60.4 | 79.0 | 9.3 | -3.0 | 2.0 | -0.5 | -26.3 | 0.4 | -12.9 | -45.8 | -42.6 | -44.2 | -64.6 | -30.3 | -47.4 | -52.0 | -12.6 | -32,3 | -21.3 | 59.2 | 18.9 |
| TWC-2 | 108.8 | -5.8 | 51.5 | 1.6 | 2.3 | 2.0 | 12.4 | -3.3 | -24.0 | -12.6 | -12.3 | -12.5 | 48.2 | -13.5 | 17.3 | -12.6 | -13.5 | -13.0 | 29.2 | -1.7 | 13.7 |
| TWC-3 | -0.2 | 16.5 | 8.2 | 0.5 | 0.5 | 0.5 | -21.5 | 11.3 | 4.5 | -56.4 | -40.7 | -48.6 | -68.2 | -52.1 | -60.1 | -37.6 | -0.1 | -18.9 | -10.8 | -57.2 | -34.0 |
| TWC-4 | 3.6 | 12.4 | 8.0 | 3.3 | 0.5 | 1.9 | 1.2 | -8.3 | -5.1 | -20.7 | -54.0 | -37.4 | -66.8 | -75.0 | -70.9 | -43.2 | -47.7 | -45.4 | -9.7 | -37.2 | -23.5 |
| TWC-5 | 66.0 | -16.4 | 24.8 | 8.9 | 17.9 | 13.4 | 10.4 | -5.1 | -3.6 | -20.3 | 20.1 | -0.1 | 4.8 | 85.6 | 45.2 | -7.7 | 32.9 | 12.6 | 38.9 | -51.9 | -6.5 |
| TWC-6 | 39.5 | 13.2 | 26.3 | -4.1 | -0.9 | -2.5 | 7.3 | -12.1 | 2.6 | -6.9 | -3.2 | -5.1 | -1.5 | 18.7 | 8.6 | -16.7 | 10.5 | -3.1 | 18.5 | -8.7 | 4.9 |
| DC-1 | 6.4 | -42.6 | -18.1 | 4.8 | 22.2 | 13.5 | -14.1 | -33.9 | -2.4 | -29.7 | -26.0 | -27.9 | -39.9 | 9.4 | -15.2 | -15.6 | -6.3 | -11.0 | 63.9 | -64.0 | -0.1 |
| DC-2 | 84.2 | 17.1 | 50.7 | -1.4 | 4.2 | 1.4 | 16.8 | 0.0 | -24.0 | -21.4 | -4.5 | -12.9 | 20.8 | 9.2 | 15.0 | -14.2 | -5.7 | -10 | -15.0 | -13.6 | -14.3 |
| DC-3 | 32.3 | -17.8 | 7.3 | 2.0 | 4.8 | 3.4 | -10.4 | -1.5 | 8.4 | 0.6 | -11.3 | -5.3 | -18.2 | -24.4 | -21.3 | -23.2 | 1.2 | -11.0 | 51.4 | -5.5 | 22.9 |
| DC-4 | 80.7 | 32.5* | 56.6 | 9.2 | 6.6 | 7.9 | 3.2 | -13.3 | -6.0 | 5.4 | 17.1 | 11.3 | 39.3 | 16.1 | 27.7 | 8.3 | -4.0 | 2.1 | 36.5 | 28.6 | 32.5 |
| DC-5 | -20.6 | 6.1 | -7.2 | 5.0 | 7.3 | 6.2 | -18.3 | 4.9 | -6.7 | -2.9 | -6.3 | -4.6 | 29.6 | 4.1 | 16.8 | 23.1 | -5.2 | 9.0 | -49.9 | 18.7 | -15.6 |
| DC-6 | -29.7 | 25.1 | -2.3 | -6.6 | 7.7 | 0.6 | -23.0 | 4.7 | -9.2 | -13.9 | 41.5 | 13.8 | -19.0 | 70.7 | 25.9 | 14.9 | 13.8 | 14.3 | -16.6 | 2.7 | -7.0 |
| Mean | 8.9 | -3.5 | 2.7 | 2.4 | 5.3 | 3.9 | -3.9 | -4.8 | -4.4 | -24.2 | -20.3 | -22.2 | -11.9 | -15.6 | -13.8 | -19.0 | -12.2 | -15.6 | -10.6 | -32.1 | -21.4 |
| $LSD_{0.05}$ | 78.1 | ns | 46.0 | ns | ns | 7.2 | ns | ns | ns | ns | 26.6 | 23.5 | ns | 55.1 | ns | 32.6 | 65.4 | 23.8 | 34.5 | 53.9 | 31.6 |

[+] Parental combination is given in Table 6.3, [++] Com = means obtained from combined analysis, ns = non-significant

For DF, 15 of 20 estimates of MPH were negative and significant but only three estimates of HPH were negative across environments (Tables 7.8 and 7.9). All single cross and five three-way cross hybrids had negative MPH for this trait. In the present study, except for FL, heterosis estimates were in general significant. Tables 7.8 and 7.9 also show, for FL, although the estimates were non-significant, the values ranged from -4.5 to 43.9% for MPH and from -24.0 to 10.0% for HPH. When the three hybrid categories were compared for heterosis estimates of the characters studied, three-way and double crosses showed very high MPH and HPH for FY compared with single cross hybrids. The estimated mean values of heterosis for FY were 45.3 and 39.7% for mid-parent and 21.4 and 14.5% for high-parent heterosis, respectively (Tables 7.8 and 7.9). On the other hand, single and three-way crosses showed higher mean MPH for FL and PCT as compared to double cross hybrids. Contrarily, characters such as FD, FWT and FN had the highest estimates of mid-parent heterosis in double cross hybrids.

The levels of heterosis were also variable across the two testing sites. The overall performance of the stand was better in the field than in the greenhouse. The main limiting factor in the greenhouse was that the plants were grown in pots whereas in the field they were grown under normal conditions. Higher magnitudes of MPH for FY, DF, FL and FWT were observed in the greenhouse than in the field (Table 7.8). Similarly, a higher estimate of mean HPH of 8.9% for FY was recorded in the greenhouse (Table 7.9). However, in a study of the influence of temperature on heterosis for several maize seedling growth traits, Rood *et al.* (1988) found that the level of heterosis for these traits could not be explained simply by the ability of a hybrid to better tolerate cool temperature. They concluded that hybrids derived from a group of four elite inbred lines displayed heterosis similarly under either favorable or cool temperature conditions. In the current investigation, the indication of environmental effect on heterosis for yield and other characters in pepper is interesting and needs further research. This is particularly of great interest under Ethiopian conditions, where environmental factors are highly variable and means to modify them is scarce. The ability of hybrids to perform better under low input conditions will also be another encouraging point for starting a pepper hybrid breeding in Ethiopia.

**Variance components and heritability**

Genotypic and phenotypic variances and broad-sense heritability estimates for yield and other characters among 20 hybrid varieties evaluated in the greenhouse and field are given in Table 7.10. The highest genotypic variance was recorded for FY per plant followed by FWT under both environments. On the other hand, PCT and FD demonstrated the lowest genotypic variances.

Heritability is the measurement of the genetic variation in a population relative to the total phenotypic variation of a trait. It is highly influenced by the methods of determination and the genotypes used. The estimation of heritability is specific to the material used, place and time of evaluation. In this study, broad-sense heritability estimates for complex characters such as FY were lower compared to less complex characters at both environments. Lower heritability estimates for complex characters than for less complex characters were also reported in other studies (Sughroue and Hallauer, 1997). High heritability estimates for FD (93.9 and 89.4%), FWT (90.3 and 85.1%), FL (86.9 and 89.4%) and FN (85.1 and 82.6%) were observed in the greenhouse and field conditions, respectively. Heritability estimates for DF and PCT were moderately high. The high heritability estimates for fruit related traits indicate these traits are less influenced by environment. On the other hand, low heritability estimates for FY under greenhouse (61.9%) and field (73.1%) conditions were recorded, indicating that environment had a more pronounced effect on yield performance. Selection progress to increase this trait will therefore be slow.

Table 7.10. Genotypic variance ($\sigma^2_g$), phenotypic variance ($\sigma^2_p$) and broad-sense heritability estimates ($h^2b$) for yield and yield components in pepper hybrids, greenhouse and field, 2001/02.

| Character | Greenhouse | | Field | | $h^2b$ (%) | |
|---|---|---|---|---|---|---|
| | $\sigma^2_g$ | $\sigma^2_p$ | $\sigma^2_g$ | $\sigma^2_p$ | GH | FLD |
| Fruit yield | 4545.15 | 7346.49 | 1684.29 | 2303.44 | 61.9 | 73.1 |
| Days to flowering | 11.31 | 16.25 | 14.77 | 20.06 | 69.6 | 73.6 |
| Fruit length | 4.10 | 4.72 | 3.84 | 4.30 | 86.9 | 89.4 |
| Fruit diameter | 0.80 | 0.85 | 0.57 | 0.64 | 93.9 | 89.4 |
| Fruit weight | 187.32 | 207.55 | 52.05 | 61.19 | 90.3 | 85.1 |
| Pericarp thickness | 0.33 | 0.46 | 0.45 | 0.61 | 72.0 | 73.5 |
| Fruit number | 35.03 | 41.14 | 7.32 | 8.86 | 85.1 | 82.6 |

The data in Table 7.5 shows that the greenhouse was a low yielding environment. The mean performances of all the measured characters with the exception of DF were higher in the field than in the greenhouse. The most common justification of conducting selection in optimum environments, regardless of the nature of the environment, is the lower heritability found in a low yielding environment. In the present study, higher heritability estimates were shown by FY, FL and PCT in the good yielding environment, field. On the other hand, although FD, FWT and FN per plant were higher in the field, they showed higher heritability estimates in the greenhouse conditions (poor environment). Thus, from this study it is not possible to conclude that the level of heritability is determined by the type of environment. Ceccarelli (1994) also indicated that the conclusion that heritability in low yielding environments is lower than that in high yielding environments is not supported by experimental evidence.

## CONCLUSION

In the present study, the hybrid varieties evaluated, showed varied performance for fruit yield and yield components. Hybrids with good performance generally consisted of high performing inbred lines. All three-way cross hybrids except (Mareko Shote × Pepper 1976) × Szegedi 178 showed superior performance for fruit yield, therefore they can be grown as hybrid varieties. These hybrids were also generally uniform in respect to days to flowering and fruit related traits such as fruit length, fruit diameter and pericarp thickness. Two double cross hybrids [(Szegedi 178 × Bakko Local) × (Mareko Shote × Pepper 1976) and (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976)] also showed superior performance for fruit yield and were moderately uniform in pericarp thickness, fruit diameter and length but of these two hybrids, (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976) was late flowering. For days to flowering, one single cross, Bakko Local × Pepper 1976, and one three-way cross, (PBC 142A × Pepper 1976) × Mareko Shote, showed high negative heterosis, indicating that dominance for earliness is favorable.

Generally, hybrids that involved inbred lines that had similar background for earliness and fruit related characters revealed high uniformity for these characters. Therefore, it can be concluded that to obtain nearly uniform three-way and double cross hybrids, inbred lines with similar backgrounds for fruit characters and earliness should be carefully selected. Three-way cross and double cross hybrids are genetically heterogeneous and, as a result help the population buffer biotic and abiotic stresses. The production of three-way and double cross hybrid seeds was also easier since vigorous and competitive single cross hybrids were used as seed parents. Mean heterosis estimates produced by most of the hybrids for fruit yield were high, indicating the preponderance of dominance and gene dispersion among the parental inbred lines.

From low to high heritability estimates were observed for studied characters. Under both greenhouse and field conditions, fruit related traits showed high heritability estimates revealing that they were less influenced by the environment. The result from this study

also indicated it is not possible to conclude that heritability depends on the type of environment as the level of heritability for good and poor environments were not clearly correlated with the type of environment.

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# CHAPTER 8

## General conclusions and recommendations

In Ethiopia, pepper plays an essential role in the sustainability of livelihood of smallholder farmers and their families, providing food and generating income. Different types of peppers are grown as important spice and vegetable crops almost everywhere in the country both under rain-fed and irrigation conditions.

Pepper is used in different forms based on the fruit characters such as size, pungency level and color. Dry pods of moderately pungent pepper are ground into powder and used in daily preparation of local dishes. Chili powder, made from the dry, small-fruited, highly pungent types, is used to add pungency to certain food types. Pepper is also used as vegetable at green mature stage. It is not only one of the cash-generating crops, particularly for small-scale farmers, but is also used as a raw material for agro-industries that produce paprika and capsicum oleoresins for the export market. Due to its economical importance, proportionally a large area of land is allocated for pepper in major pepper production areas of the country. However, the average national yield is very low, dry fruit production is only about 0.41 tons/ha. This is mainly due to lack of improved high yielding pure lines or hybrid varieties.

Although the demand for domestic consumption and export is increasing, production and productivity of pepper is decreasing. Besides the low productivity of the existing farmer's varieties, the production of the crop is highly affected by farmers' low-land holding which is caused by a high rate of population increase. Thus, to produce more pepper from less land, with less water and fewer pesticides, the replacement of the old with improved varieties is essential.

Genetic diversity is the basis of genetic improvement. Thirty-nine pepper genotypes obtained from different countries were grown in the greenhouse at the University of the Free State, South Africa during 2001 and 2002 for morphological characterizations, using

20 different quantitative and qualitative traits. In addition, six AFLP primer pairs were used to determine pairwise genetic distances among the germplasms. Both methods showed moderately high genetic distances among the genotypes studied. High genetic distances were also observed within and between the varietal groups. However, when the comparisons were made within and between the Ethiopian cultivars, a lower average genetic distance was observed among the Ethiopian cultivars compared with that between the Ethiopian and exotic cultivars.

Morphological data separated large-fruited genotypes from small-fruited ones. Similarly, AFLP markers separated the genotypes generally on the basis of fruit size. Although the value of the correlation coefficient between the two genetic distance estimations was low, it was significant and positive, indicating AFLP distance tended to reflect morphological distance. On the basis of this study, it can be recommended that the combination of morphology and AFLP can provide useful measures of genetic distances. The narrow genetic basis in the Ethiopian cultivars suggests that the pepper breeding program of Ethiopia should focus on enriching its germplasm through local collections and introductions from other parts of the world.

Diallel analysis is the concept defined as making all possible crosses among a group of genotypes. It is used by breeders to obtain information on value of varieties as parents, to assess the gene action involved in various characters, and thereby develop appropriate selection procedures and understand heterotic patterns of the progenies at an early stage of the hybridization program. Information on combining ability and heterosis of fruit related traits and agronomic characters in available Ethiopian and exotic pepper germplasms are meager. The present study considered the combining ability and heterosis of seven diverse pepper parents for days to flowering, days to maturity, fruit maturation period, fruit length, fruit diameter, fruit weight, pericarp thickness, ascorbic acid content, total soluble solids, plant height, fruit number and fruit yield per plant. Since parents were not selected at random, inferences must be limited to the respective populations of the seven parent diallel experiments.

This study showed that GCA and SCA were significant sources of variation for all measured characters. However, the magnitude of GCA was higher than that of SCA for all characters with the exception of plant height, indicating additive genetic effects are more important for the inheritance of these characters. The correlation between *per se* value and GCA will give an indication about the possibility to use means of the two parents to predict the value of the $F_1$ hybrid. The significant estimates of GCA effects for measured characters show that individual parents contributed differently to the specific character. Among others, parents such as Kalocsai "M" Cseresznye and Szegedi 178, and Bakko Local and Mareko Shote contributed towards earliness and increased fruit length in their progenies, respectively. Additionally, Mareko Shote was a good general combiner for fruit yield and ascorbic acid content. Pepper 1976 had high positive GCA for fruit yield, fruit length, fruit weight and pericarp thickness. In general, the breeding materials used in this study were found to be useful sources for genetic variability for the development of new genotypes of desired fruit size and shape.

Of the studied characters, fruit length, fruit diameter, fruit weight, pericarp thickness and fruit number showed very high heritability both in narrow and broad senses, indicating that the environment has less effect on their inheritance. The high predictability ratios of these characters also showed that the prediction of progeny performance only from the GCA effects of parental line could be possible.

Some hybrids demonstrated significantly high SCA effects in the desired direction. Hybrids that involved high GCA parents for certain traits showed high tendency towards increased mean performance and high SCA effects. These hybrids include Kalocsai "M" Cseresznye/Pepper 1976 for fruit diameter and pericarp thickness, and Szegedi 178/Bakko Local and Szegedi 178/Mareko Shote for fruit length. On the other hand, significant mean performance and SCA effects were observed in some other hybrids that involved contrasting parents regarding GCA.

The significance of the heterotic performance was highly affected by the genetic backgrounds of the parental genotypes. Crosses that involved bell pepper as one of their

parents in general gave bigger fruit size and higher yield per plant followed by crosses that involved intermediate fruit-size parents. Among the hybrids, the best yielding crosses were Bakko Local × Pepper 1976, Mareko Shote × Pepper 1976, Bakko Local × Mareko Shote, Szegedi 178 × Bakko Local, Kalocsai "M" Cseresznye × Pepper 1976, Szegedi 178 × Pepper 1976, PBC 142A × Pepper 1976, Kalocsai "M" Cseresznye × Mareko Shote, Bakko Local × C00916 and Mareko Shote × C00916. For yield and other agronomic performance, these hybrids should be further tested across environments. The high heterosis among these germplasm for most of the characters studied indicates that considerable potential exists in these materials for developing hybrids. From this study it was also observed that $F_1$ pepper hybrids did not only have high yield potential and overall plant performance but also they increase daily productivity on the account of their earlier maturity. Therefore, hybrid breeding can be effectively used in Ethiopia to improve yield, yield components, fruit quality and overall plant performance in peppers. The results of this study also clearly show that spice and vegetable type hybrids or pure line varieties that can satisfy the local demand, can be developed.

Prediction of the performance of hybrids was one of the objectives of this study. Because, prediction of the prospects of crosses for line and hybrid development before production and field testing could increase the efficiency of a breeding program by concentrating the efforts on the most promising ones. AFLP markers showed higher tendency of differentiating parental lines into heterotic groups from which superior hybrids can be derived than morphological traits. However, the two methods expressed little or no promise for predicting $F_1$ heterosis or performance for measured characters. However, although the correlations between morphological distance and hybrid performance for fruit length, fruit diameter, fruit weight and pericarp thickness were small negative, crosses that were obtained from extremely divergent parental lines for these traits produced smaller fruits. The parental genotypes used in this study had diverse morphological backgrounds and were from different market types. Since each type of pepper must conform to its own unique set of characteristics, in order to be commercially acceptable, pepper hybrid breeding should deal with parental lines of similar varietal

groups (market types) unless the objective is to develop a hybrid or pure line variety of different fruit character than of the parental lines.

Hybrids are not always single crosses, but three-way and double cross hybrids also exist. The practical difficulties associated with the low productivity of inbred lines are usually overcome by the use of three-way and double cross hybrids, although there is some loss in performance and uniformity in these crosses.

In the present study, single, three-way and double crosses and inbred lines showed variable performance for fruit yield and yield components. Hybrids that involved high performing inbred lines showed good performance for the measured traits. All three-way cross hybrids except (Mareko Shote × Pepper 1976) × Szegedi 178 showed superior performance for fruit yield so that they can be grown as hybrid varieties. These hybrids were also generally uniform in respect to days to flowering and fruit related traits such as fruit length, fruit diameter and pericarp thickness. Two double cross hybrids [(Szegedi 178× Bakko Local) × (Mareko Shote × Pepper 1976) and (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976)] also showed superior performance for fruit yield and were moderately uniform in pericarp thickness, fruit diameter and length but of the two hybrids, (Bakko Local × Mareko Shote) × (PBC 142A × Pepper 1976) was late flowering.

For days to flowering, a single cross, Bakko Local × Pepper 1976, and a three-way cross, (PBC 142A × Pepper 1976) × Mareko Shote, showed high negative heterosis, indicating that dominance for earliness is favorable. Generally, hybrids that involved inbred lines that had similar genetic background for earliness and fruit related characters, revealed high uniformity for these characters. Therefore, it can be recommended that to obtain nearly uniform three-way and double cross hybrids, inbred lines with similar backgrounds for fruit characters and maturity should be carefully selected. The double cross and three-way cross hybrids are genetically heterogeneous and, as a result, help the population buffer biotic and abiotic stresses. Hybrid seed production in three-way and double crosses was also easier, since vigorous and competitive single cross hybrids were

used as seed parents. Mean heterosis estimates produced by most of the hybrids for fruit yield were high, indicating the preponderance of dominance and gene dispersion among the parental inbred lines.

In general, the results of the present studies that involved analyses of genetic diversity, diallel analysis and heterosis, prediction of hybrid combination and comparative performance of different types of hybrids, will have considerable practical importance for pepper breeding programs in Ethiopia.

# CHAPTER 9

## Summary/Opsomming

## Summary

The knowledge of genetic similarity and dissimilarity among crop cultivars is of vital importance for the plant breeder. The genetic variability of 39 pepper (*Capsicum annuum* L.) genotypes of different varietal groups that were obtained from different geographical origins was studied using morphological traits and amplified fragment length polymorphisms (AFLP) markers. Both methods showed moderately high genetic distances among the different genotypes indicating genetic diversity among the total genotypes. However, when a comparison was made between the Ethiopian and the exotic genotypes, the mean genetic distance among Ethiopian genotypes was lower than that between the Ethiopian and the exotic ones. The dendrogram based on morphological data clustered the genotypes on the basis of fruit size and was generally consistent with different varietal groups. Similarly, with AFLP data, genotypes with similar fruit sizes clustered together.

Combining ability and heterosis estimates are important to determine the direction and goals of a breeding program. Seven diverse parental lines were selected from the 39 genotypes and crossed in a half-diallel method. The parental lines and their 21 $F_1$ hybrids were evaluated to estimate the combining abilities and genetic effects determining the heritability of various characters, and to determine heterosis of hybrids over mid-parent, high-parent and standard checks for various characters. Generally, significant general (GCA) and specific (SCA) combining abilities were observed for all measured characters indicating the presence of both additive and non-additive gene actions. However, additive gene action is more important than non-additive gene action, as the magnitude of GCA effects was much higher than SCA effects. The estimates of predictability ratios for fruit length, fruit diameter, fruit weight, pericarp thickness and fruit number were closer to unity, suggesting the possibility of predicting progeny performance based on parental

GCA alone. High heritability both in broad and narrow senses was also recorded for these characters indicating their inheritance is less influenced by the environment.

Substantial heterosis over mid-parent, high-parent and the standard check was observed. Many crosses demonstrated high heterosis for fruit yield, fruit diameter, mean fruit weight and pericarp thickness over the standard check. For the traits of earliness (days to flowering, days to maturity and fruit maturation period), the overall mean mid-parent, high-parent and standard heterosis were negative. Thus, it can be suggested that with the proper choice of parents, pepper hybrids that have higher yield potential, good fruit characteristics and early maturity can be developed to increase pepper productivity in Ethiopia.

Genetic diversity between parents may contribute positively to both heterosis and trangressive segregation. The relationship between genetic diversity of the seven parental lines, and heterosis and hybrid performance was assessed. The genetic diversity was measured using 15 morphological traits and six AFLP primer combinations. Cluster analysis using the two genetic distance measures generally grouped the seven parents differently. Morphological distance was negatively correlated only with mid-parent heterosis (MPH) for days to flowering and days to maturity. The correlations of AFLP measured genetic distances with mid-parent and high-parent heterosis were non-significant for all characters with the exception of fruit diameter and proved to be of no predictive value.

In addition to single crosses, three-way and double crosses can be used to overcome the low productivity of inbred lines. It is hypothesized that yield stability is high in three-way and double-cross hybrids owing to higher genetic heterogeneity as compared to single cross hybrids. Twenty-six genotypes, including six inbred lines, eight single, six three-way and six double crosses were evaluated for yield and other agronomic characters in two environments using a randomized complete block design with three replications. The three categories of hybrids performed differently across the environments and showed high variations for the majority of characters studied. Three-way crosses gave the highest

mean fruit yield per plant followed by double crosses. The two types of hybrids were also more stable than the single crosses. In general, three-way crosses showed promising performance for the studied traits. The highest estimates of mid- and high-parent heterosis were also observed in the three-way and double cross hybrids. Therefore, the low productivity of local pepper cultivars in Ethiopia can be overcome through developing and utilizing three-way and double cross hybrids.

## Opsomming

Die kennis van genetiese ooreenkomste en verskille tussen cultivars is van kritiese belang vir die planteteler. Die genetiese variabiliteit van 39 rissie (*Capsicum annuum* L.) genotipes van verskillende groepe en geografiese oorspronge is bestudeer met die gebruik van morfologiese eienskappe en AFLP (amplified fragment length polymorphism) merkers. Beide metodes het redelike hoë genetiese afstande getoon tussen genotipes, wat die teenwoordigheid van genetiese diversiteit tussen genotipes aantoon. Toe 'n vergelyking getref is tussen Etiopiese en eksotiese genotipes, was die gemiddelde genetiese afstand tussen Etiopiese genotipes laer as tussen Etiopiese en eksotiese cultivars. Die dendrogram wat gebasseer is op morfologiese data, het die genotipes gegroepeer op die basis van vrug grootte en dit was oor die algemeen in ooreenstemming met die verskillende produksie groepe. Net so, met AFLP data het genotipes met dieselfde vrug grootte saam gegroepeer.

Kombineervermoë en heterose bepalings is belangrik om die rigting en die doelstellings van 'n teelprogram te bepaal. Sewe diverse ouer lyne is geselekteer van die 39 genotipes en is gekruis in 'n half-dialleel. Die ouer lyne en hulle 21 F1 basters is geëvalueer om kombineervermoë en genetiese effekte te bereken wat oorerflikheid bepaal van verskillende eienskappe, en om heterose van basters oor die mid-ouer, hoogste ouer en standaarde vir verskillende eienskappe vas te stel. Oor die algemeen was daar betekenisvolle algemene- (GCA) en spesifieke (SCA) kombineervermoë vir alle gemete eienskappe, wat die aanwesigheid van beide additiewe en nie-additiewe geen aksie aantoon. Additiewe geen aksie was egter meer belangrik as nie-additiewe geen aksie, omdat die grootte van GCA effekte baie hoër was as die SCA effekte. Die berekening van voorspelbaarheids verhoudings van vrug lengte, vrug deursnee, vrug gewig, perikarp dikte en aantal vrugte was naby een, wat aangedui het dat die nageslag se eienskappe voorspel kan word vanaf die ouerlike GCA. Hoë oorerflikhede in die breë en nou sin was ook teenwoordig vir hierdie eienskappe wat aangetoon het dat oorerflikheid nie baie deur die omgewing beïnvloed is nie.

'n Hoë vlak van heterose oor die mid-ouer en standaarde is uitgedruk. Heelwat kruisings het hoë vlakke van heterose getoon vir vrug opbrengs, vrug deursnee, gemiddelde vrug gewig en perikarp dikte teenoor die standaard. Vir vroegheids eienskappe(dae tot blom, dae tot rypheid en vrug rypheids tyd) was die algehele mid-ouer, hoogste ouer en standaard heterose negatief. Daar kan dus gesê word dat met 'n goeie keuse van ouers, rissie basters met 'n groter opbrengs potensiaal, goeie vrug eienskappe en vroeë rypheid ontwikkel kan word om rissie produktiwiteit in Etiopië te verhoog.

Genetiese diversiteit tussen ouers kan positief bydra tot beide heterose en transgressiewe segregasie. Die verhouding tussen genetiese diversiteit van die sewe ouer lyne en heterose en baster prestasie is geassesseer. Die genetiese diversiteit is gemeet deur die gebruik van 15 morfologiese eienskappe en ses AFLP kombinasies. Groeperings analise wat twee genetiese afstands metings metodes gebruik, het die sewe ouers verskillend gegroepeer. Morfologiese afstand was negatief gekorreleer net met mid-ouer heterose vir dae tot blom en dae tot rypheid. Die korrelasie van AFLP gemete genetiese afstande met mid-ouer en hoogste ouer heterose was nie betekenisvol nie vir alle eienskappe behalwe vrug deursnee, en was van geen voorspellende waarde nie.

Buiten enkel kruisings, kan drierigting en dubbel kruisings gebruik word om lae produktiwiteit van ingeteelde lyne te oorkom. Daar is 'n hipotese dat opbrengs stabiliteit hoog is in drierigting- en dubbel kruis basters a.g.v. hoër genetiese heterogeniteit in vergelyking met enkelkruis basters. Ses en twintig genotipes, insluitend ses ingeteelde lyne, agt enkel, ses drierigting en ses dubbel krusings is geëvalueer vir opbrengs en ander agronomiese eienskappe in twee omgewings met 'n gerandomiseerde blokontwerp met drie herhalings. Die drie kategorië van basters het verskillend gereageer oor omgewings en het groot variasie getoon vir die meeste eienskappe wat gemeet is. Drierigting kruisings het die meeste vrugte per plant gelewer, gevolg deur dubbel kruisings. Die twee tipes basters was ook meer stabiel as die enkel kruisings. In die algemeen het drierigting kruisings belowende potensiaal getoon vir gemete eienskappe. Die hoogste bepalings van mid-ouer en hoogste ouer heterose is gesien vir drierigting en dubbel kruis basters.

Daarom kan die lae produktiwiteit van plaaslike rissie cultivars in Etiopië oorkom word deur die ontwikkeling en gebruik van drierigting en dubbel kruis basters.