THE USE OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) AND MORPHOLOGICAL DATA TO DETERMINE HETEROTIC GROUPS IN SUNFLOWER (*Helianthus annuus*)

By

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Dedicated to

my brother and lifelong friend, Stephen

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CHAPTER 1
INTRODUCTION

Sunflower is an important edible oilseed crop grown in the world over an area of 21 million hectares with a production of 25 million tons (FAO, 1996).

The largest traditional producer is the former Soviet Union. Other significant producers are Argentina, the combined European Union, China, India, Turkey and South Africa. The Dakotas, Minnesota, Kansas, Colorado, Nebraska, Texas and California are the major producing states in the USA (Basra, 1999).

The world's major oil importers in the three fiscal years 1991, 1992 and 1993 were China, India and Pakistan.

The world's demand for oilseed meals since 1970 has been relatively greater than for vegetable oils. This is due to a rise in demand by the intensive livestock production sector especially for pigs, poultry and aquaculture. The major producers of sunflower meal are the European Union, Russia and Argentina and the major buyer is the European Union (Weiss, 2000).

South Africa's main production areas are the Free State and Northwest province, which are responsible for 80% of the sunflower production. A total of 521 450 hectares was planted during 2000/2001 (Anonymous, 2002).

Sunflower seed (sunseed) provides an important protein source in less-developed societies, and roasted, salted, hulled or whole seed is a popular snack worldwide. Sunseed is also included in many petfoods, especially for birds.

It is however, cultivated mainly for its oil. As a source of edible vegetable oil, it is one of the most important oilseed crops in the world. It is most suitable for use in soft margarines and similar foods and it is also excellent dietary oil. Sunflower meal (sunmeal) is a high quality protein source for stock feed, but the high fiber content of the hull reduces its value to compounders (Weiss, 2000).

The versatile nature of sunflower and its increasing contribution to oilseed production calls for concerted efforts to evolve hybrids with higher productivity (Basra, 1999)

Plant breeders can follow two strategies to enhance yield in sunflower. One option will be to develop hybrids that are disease and insect free. This strategy is called defect elimination. This shall not necessarily give rise to higher yields. The other option is simply to select for hybrids with higher yields. This can only be done by improving the amount of hybrid vigour produced by inbred lines. Hybrid vigour or heterosis is a function of the degree of dominance as well as the difference in gene frequency between the inbred parents. In other words:

$$H = dy^2$$

Where: d = degree of dominance

y^2 = genetic difference between the inbred lines

The only way plant breeders can enhance the yield of hybrids is to manipulate one or both these components. The degree of dominance is a function of the genetic constitution of the inbred parents. The genetic constitution of the inbreds depends largely on the way *loci* segregates during the successive generation of inbreeding and there is almost nothing that breeders can do about it. On the other hand, it is possible to calculate the genetic difference among inbred lines by means of studying the genetic distance among the inbreds (Falconer and Mackay, 1996).

DNA marker systems are useful tools for assessing genetic diversity between germplasm. In breeding programs, information on genetic relationships within species is used for organizing germplasm collections, identifying heterotic groups and for selecting breeding material (Lee, 1995; Karp *et al*, 1996).

If breeders could predict the potential 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 (Bohn *et al*, 1999).

If a correlation exist between the genetic distances of inbreds and the amount of heterosis obtained by such a hybrid, it will be very advantageous to the plant breeder.

a) It will enable the hybrid sunflower breeder to screen thousands of inbreds for genetic distances each year.
b) It will shorten the breeding program with at least one year since it will not be necessary to test hundreds of inbreds for combining ability.
c) It will reduce the farm price of hybrid seeds since the number of crosses, trials and amount of labor will be reduced.

The aim of this study was therefore to determine the genetic distances between 12 sunflower inbreds with the use of the AFLP technique and to correlate these results with the amount of heterosis obtained in the F1-hybrids.

CHAPTER 2
LITERATURE REVIEW

2.1 History and origin

The sunflower originated from Mexico and Peru. It was already present 3 000 years before Christ in Northern America. The Indians used it as food and for decorations of the body. Cultivated sunflower, *Helianthus annuus*, originated from mutations and crosses between wild sunflower types. During the 15th century, the Spaniards brought it to Europe from where it was distributed mainly as an ornamental plant.

It was the Russians who saw the value of this plant as an oil source. A lot of breeding work was done. Selection increased the oil content significantly. The first sunflowers were open pollinated cultivars that were pollinated by insects, mainly bees.

In South Africa, the first sunflower was produced in the beginning of the century, mainly as poultry food. Sunflower was first produced on a larger scale after the Second World War. Because of shortages of open pollinated cultivars, it was less economical to produce sunflower, than other crops like maize and wheat (Greyling, 1990).

Sunflower has made a significant impact in a number of tropical and temperate countries, crossing climatic and geographical boundaries because of its desirable features. The wide adaptability enables the cultivation of the crop in different agroclimatic regions and soil types. It is a short growing season crop and can fit into various multiple cropping systems. It is also an ideal crop for contingency cropping plans (Basra, 1999).

2.2 Genetics

The cultivated sunflower (*Helianthus annuus* L.) is one of the 67 species in the *Helianthus* genus, which includes annual and perennial species.

The basic chromosome number for *H. annuus* is 17(n = 17), which makes it possible to cross with other related species. The common sunflower has been used to produce interspecific and intergeneric hybrids (Pustovoit, 1966). Diploid, tetraploid and hexaploid species are known, although the cultivated sunflower is diploid (Fehr, 1987; Berglund, 1994).

The majority of *Helianthus spp.* have the somatic number 2n = 34, 68, 102, with the exception of 2n=14 and 28 or 2n=32 (Prokopenko, 1975; Weiss, 2000).

The establishment of sunflower gene pools is becoming increasingly urgent and it is important to ensure the availability of the widest possible range of material. In wild sunflower germplasm, considerable variability is available for disease and insect pest resistance and for tolerance to abiotic stresses like drought and salinity. In addition, fatty acid composition and protein quality can be modified, by including wild species in the breeding program. The introgression of traits from wild species can therefore be used to broaden the narrow genetic base of sunflower (Korell *et al*, 1996).

2.3 The development of hybrid breeding

Hybrid breeding in sunflower normally involves the development of pure lines through inbreeding, followed by selection among these lines for the maximum expressions of heterosis when crossed. Hybrids are the first generation offspring of a cross between parents with contrasting genotypes (Allard, 1960; Fick, 1978; Weiss, 2000).

F1 hybrids are created by inbreeding followed by intercrossing divergent inbred lines to create heterozygous but homogeneous hybrids. This breeding system insures uniformity in the seed propagated and allogamous species,

where open-pollinated populations consist of a mixture of genotypes. Genetic homogeneity combined with high vigour is achieved by selecting within and among inbred lines (Janick, 1999).

Single cross hybrids have advantages over three-way hybrids and open pollinated or synthetic cultivars because of the greater uniformity for agronomic, disease and seed oil characters (Rao and Singh, 1978). Uniformity in flowering has been especially useful because fewer applications of insecticides are necessary to control insects such as the sunflower moth (*Homoeosoma electellum Hulst.*). Uniformity in maturity, height, and head diameter has also facilitated harvesting procedures (Fick, 1978). The best single cross will always be higher yielding than any double cross, because of the greater genetic variation among single crosses than among double crosses (Cockerham, 1959; Miller, 1999).

Three-way hybrids are based on the use of two non-associated parental lines on the female side. This gives segregation in planting dates. Therefore, segregation occurs in the hybrids as two different plant heights that result in two different flowering periods. Three-way hybrids are produced primarily to reduce seed costs (Van Rooyen, 1999).

An early problem associated with evaluating inbred lines in hybrid combinations was the low hybridization percentage that often occurred in crossing. In crossing plots involving two or more lines, hybridization percentages ranged from 21 to 96% in seed production studies.

Current methods involving genetic or cytoplasmic male sterility, or induction of male sterility by gibberellic acid, allow complete hybridization of lines and hence greater precision in estimating combining ability. Various tester parents and tester schemes are being used (Fick, 1978).

The finding of cytoplasmic male sterility (CMS) in crosses between *H. petiolaris* and *H. annuus* (Leclerq, 1969), combined with fertility restorer genes (Kinman, 1970) allowed breeders to produce hybrid sunflowers (Weiss,

2000). The first hybrids produced this way were available for commercial production in the United States in 1972, and by 1976, it was estimated that these hybrids accounted for over 80% of the sunflower production in the USA (Fick, 1978; Wan *et al*, 1978; Miller, 1999).

Recent testing by breeders in the United States has included the rapid conversion of lines to cytoplasmic male sterility by using greenhouses and winter nurseries, and subsequent hybrid seed production in isolated crossing blocks using open pollinated cultivars, synthetics, composites, or inbred lines as testers (Fick, 1978).

Breeders throughout the world are now utilizing four distinct heterotic groups within sunflower. The open-pollinated varieties developed in Russia are used in deriving female maintainer inbred lines. The USA restorer group, derived by crossing wild annual species of sunflower with cultivated lines, is a distinct source of disease resistance and fertility restorer genes. Romanian female lines, along with their South African derivatives, are used throughout the industry. Also used are the Argentinean INTA open-pollinated cultivars to develop female lines (Miller, 1999).

2.4 The uses of male sterility (MS), cytoplasmic male sterility (CMS) and restorer genes

Male sterility (MS) in plants implies an inability to produce or to release functional pollen, and is the result of the failure to develop functional stamens, microspores or gametes. These flowers cannot self-pollinate, but can be cross-pollinated. Male sterile genes have been identified in barley, corn, cotton, potatoes, rice, sorghum, soya, sunflower, tobacco, wheat and other crops (Poehlman, 1987; Bosemark, 1993).

Male sterility plays an important role in plant breeding, firstly in the production of hybrid seed, and secondly as a plant breeding tool facilitating population improvement, backcrossing, interspecific hybridization and other intermediate breeding procedures. To utilize hybrid vigour effectively it must be possible to

produce hybrid seed in such quantities that the F1 can be grown directly by the farmer.

Based on its inheritance or origin, male sterility can be divided into:

1) Nuclear male sterility (NMS), also called 'genic', 'genetic' or Mendelian, where the male sterility is governed solely by one or more nuclear genes;
2) Cytoplasmic male sterility (CMS) where male sterility comes about as a result of the combined action of nuclear genes and genic or structural changes in the cytoplasmic organellar genomes resulting in what is often referred to as 'sterile cytoplasm' (S) as opposed to normal 'fertile cytoplasm' (N);
3) Non-genetic, chemically induced male sterility that results from the application of specific chemicals referred to as gametocides or chemical hybridizing agents (CHA) (Bosemark, 1993).

2.4.1 Nuclear male sterility (NMS)

NMS can be found in diploid species. It originates through spontaneous mutation. A single recessive gene usually controls spontaneous NMS. The highest proportion of male steriles that can be realized is 50%, which is obtained in the backcross msms x MSms (Poehlman, 1987; Bosemark, 1993).

Genetic male sterility can be used in the following ways:

1) To eliminate emasculation procedures in self-pollinated crops.
   Emasculation is laborious and time consuming. If a male sterile plant can be used as a female parent, emasculation is unnecessary.
2) To increase natural cross-pollination in self-pollinated crops.
   Male-sterile genes provide a mechanism for increasing cross-pollination in normally self-pollinated crops.
3) To facilitate commercial hybrid seed production.
   In the production of hybrid seed, a mechanism for pollination control is required.

NMS does not however, permit the production of a uniformly male sterile population and this seriously limits its use in hybrid seed production.

2.4.2 Cytoplasmic male sterility (CMS)

CMS can be divided into autoplasmic and alloplasmic CMS. Autoplasmic CMS refers to those cases where CMS has arisen within species as a result of spontaneous mutational changes in the cytoplasm, most likely in the mitochondrial genome. Alloplasmic CMS, on the other hand, would comprise such cases where CMS has arisen from inter-generic, interspecific or occasionally intraspecific crosses and where male sterility can be interpreted as being due to incompatibility or poor co-operation between the nuclear genome of one specie and the organeller genome of another. This category also includes CMS in products of interspecific protoplast fusion (Bosemark, 1993).

Researchers in France reported the discovery of cytoplasmic male sterility (CMS) from an interspecific cross involving *H. petiolaris* Nutt. and *H. annuus* L. This source of CMS was shown to be very stable and it is now the source used almost exclusively in breeding programs around the world (Fick, 1978).

CMS is the most important system used in hybrid seed production and so far, with few exceptions, the only one by which hybrid seed can be produced both effectively and economically. The inheritance of CMS is where the nuclear control is exercised by only one recessive gene. As may be seen in Figure 2.1, it is only the combination of "sterile" cytoplasm and homozygosity for the recessive gene rf, (S) rfrf, that results in male sterility. A genotype of the constitution (N) rfrf is called a maintainer since a male sterile plant will produce a uniformly male sterile offspring only when pollinated by plants of this genotype. A genotype that masks the expression of the CMS trait and which, when used as a pollinator on a CMS female, restores the pollen fertility of the progeny is called a restorer. Full restoration frequently requires the involvement of other nuclear genes and may even be accompanied by changes in the mitochondrial genome (Mackenzie and Chase, 1990). Since the cytoplasm is transferred through the egg, CMS is transmitted only through the female plant.

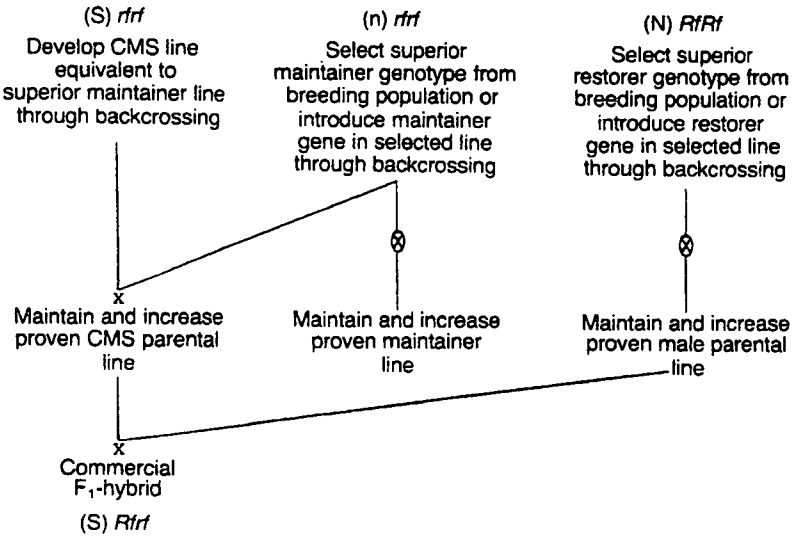

Figure 2.1 Steps in the development of a hybrid seed production system based on cytoplasmic male sterility. Note that for ease of tracing the Rf-gene in backcrosses, restorer lines are usually developed in S-cytoplasm, not in N-cytoplasm as shown here (Bosemark, 1993)

Hybrid breeding and seed production, as illustrated in Figure 2.1, thus requires the following materials and procedures.

2.4.2.1 Maintainer genotypes (N) rfrf

If one wants to use CMS in hybrid breeding, one must first find or introduce maintainer genotypes in one's own breeding material and then through crossing and backcrossing transfer their nuclear genotypes into sterile cytoplasm. Maintainer genotypes, often called B-lines, look precisely the same as any normal fertile genotype with N-cytoplasm. To identify maintainer genotypes, it is thus necessary to testcross fertile plants individually to CMS-plants and to classify the progenies for male sterility. If a particular testcross progeny consists of only 100% male sterile plants, the pollinator plant used in that testcross was of the maintainer genotype. The genotype of the maintainer plant is normally preserved *via* self-pollination, which is also part of the maintainer line development whenever the objective is to develop an inbred CMS line (Bosemark, 1993).

2.4.2.2 Equivalent CMS lines (S) rfrf

Once a new maintainer line has been evaluated and its breeding value proven, its nuclear genotype has to be transferred to the sterile cytoplasm through crossing and repeated backcrossing. The resultant CMS line (A-line) will be largely isogenic to the maintainer line except for the organellar genomes. It can easily be propagated, by further crossing to its maintainer counterpart (B-line) (Bosemark, 1993).

2.4.2.3 Restorer genotypes

For crops like sorghum and sunflower, where the harvested material is the seed, the pollinator parent used on the CMS female parent must be homozygous for the required restorer genes to ensure pollen fertility and seed set in the resultant hybrid. As with maintainers, restorers have to be identified through testcrossing with CMS plants and subsequent classification of the progenies for pollen fertility. Restorers are usually developed in sterile cytoplasm, inbred and then they are selected for agronomic characteristics and combining ability in the same way as the maintainers (Bosemark, 1993).

2.4.3 Chemical hybridizing agents (CHA)

When using CMS for hybrid seed production and the development of maintainers, equivalent CMS lines and restorers will have to precede evaluation for hybrid performance. With CHA a large number of testcrosses for combining ability can be made, by treating one of the parents of a potential hybrid with the CHA. If the performance of the resultant F1 hybrid is good enough, commercial production is possible. CHA are interesting both as a breeding tool in search for good combiners and as a means for large-scale hybrid production.

These chemicals can however, also damage female fertility and it has other weaknesses. While such products may still be useful for intermediate breeding purposes, where only limited seed quantities are required, they do not yet permit the economic production of large quantities of hybrid seed with high and reliable germination (Bosemark, 1993).

2.4.4 Intermediate breeding procedures

Male sterility is very useful in plant breeding programs through facilitating backcrosses, testcrossing for combining ability, and interspecific, and intergeneric hybridization. Although it is generally accepted that a wide gene pool is a prerequisite for successful breeding work, breeders usually use only a very limited amount of the available genetic variation.

In cross-pollinated species, new genetic variability can easily be introduced into breeding populations and these are continuously improved through various methods of recurrent selection. In self-pollinated crops, the use of such methods has been restricted due to the large number of crosses among selected genotypes that are required in each cycle of selection (Bosemark, 1993).

Recurrent selection appears to be one of the most promising methods to increase the frequency of desirable genotypes in a source population, and thus enhance the chances of success in isolating superior inbred lines (Hallauer, 1999). NMS can also be used to make recurrent selection available to breeders of self-pollinated crops. This process is called 'Male Sterile Facilitated Recurrent Selection' (MSFRS) and involves: (a) selecting plants, both male fertile and male sterile, from a population segregating for the desired characters and male sterility; (b) inter-crossing the selected plants; (c) bulking the crossed seed, and growing and harvesting the F1 generation. The resulting F2 generation provides the population from which the next cycle of selection is made. New sources of germplasm may be introduced into the population in any cycle by crossing them to selected male sterile plants. This has been applied in breeding sunflower.

Where good and proven systems of CMS exist, these will most likely remain in use since the immediate benefit of introducing a new system would be relatively small compared to the cost (Bosemark, 1993).

2.4.5 Development of CMS lines

CMS lines are developed through backcrossing. Desirable lines that have undergone inbreeding and selection for several generations are crossed initially to a plant with CMS. Thereafter the inbred line to be converted, is used as the recurrent parent in the backcrossing procedure. The final progeny should be genetically similar to the recurrent parent except that it will be male sterile.

If the inbred line has not been tested previously for combining ability, crosses with a CMS tester and subsequent evaluation of the sterile F1 hybrid can provide valuable information on combining ability. Conversion of an inbred line to cytoplasmic male sterility can be accomplished in a relatively short time, especially by using winter nurseries and greenhouses which allow for as many as three or four generations per year.

No significant problems have been encountered using the cytoplasmic male sterile and fertility restorer system for the production of hybrid seed. The cytoplasm controlling sterility has no apparent adverse effects on agronomic or seedoil characters when it is incorporated into inbred lines (Fick, 1978).

Hybrid breeding based on CMS is frequently tedious and costly and sometimes impractical for one or several of the following reasons:

1) Maintainer and/or restorer genotypes are too scarce in the breeding populations to permit direct isolation, and the corresponding genes may thus have to be introduced into contrasting populations prior to selection and line development.
2) Maintenance as well as restoration are dependant on environmental conditions, especially temperature and genetic background.
3) CMS is sometimes associated with negative traits, e.g. chlorophyll deficiency at low temperatures and flower malformations.
4) There are cases where hybrid seed production has turned out to be impractical and uneconomical because of problems in seed production caused by flower morphology and restricted pollen dispersal. However,

these problems have been experienced mainly in strictly self-pollinating species like wheat, barley, faba, beans and soybeans.

Although it is time consuming and costly to initiate a CMS-based hybrid breeding program, once established it can also be very efficient and reliable, as was found in sorghum, sunflower and sugarbeet (Bosemark, 1993).

2.5 Genetic variance

Breeders are mainly interested in variance, because it assures genetic progress.

The genetic variability of the F1 hybrid is a function of the homozygosity of the parents. Genetic homogeneity in single crosses is a function of the degree of homozygosity of the parents and F1 homogeneity can be increased by increasing the homozygosity of the inbred parents through inbreeding (Janick, 1999).

Genetic variance consists of three major components, namely additive genetic variance, dominance variance and non-allelic interaction. The additive component of genetic variance is the variance, which contributes to genes with a linear effect. The resemblance between parents and offspring is largely due to the additive genetic affects, which is also responsible for the response to selection. The dominance component represents the deviation of the heterozygote from the average of the parents. The interaction deviation is the result of epistatic effects (Wricke and Weber, 1986)

Non-significant mean squares for all traits studied indicated that epistasis was a minor factor in the overall genetic variation in sunflower lines (Miller *et al*, 1980). It is usually necessary to assume that there is no epistasis when giving a genetic interpretation to diallel statistics. Epistasis affects estimates of general and specific combining ability mean squares, variances, and other effects in an unpredictable manner (Baker, 1978).

Quantitative characters (like yield and quality) were measured in terms of variation (V or σ^2). The phenotypic variance of a population is therefore a function of the genotypic and environmental variance.

$$V_P = V_G + V_E$$

Where: V_P = phenotypic variance
V_G = genetic variance
V_E = environmental variance

The genetic variance (V_G) is a function of:

$$V_G = V_A + V_D + V_I + V_E$$

Where: V_A = additive variance
V_D = dominance variance
V_I = interaction variance
V_E = environmental variance

Genetic markers represent genetic variation, which makes it possible to determine the relationship between the different genotypes and to forecast which parings can produce new and superior gene combinations. Genetic markers for specific genes of concern are also useful to screen for recombination between these genes and for accurate selection for genetic superior individuals.

Genetic progress is determined by the identification of genetic variation or diversity, the making of crosses on the establishment of recombination and accurate selection. It is not only the phenotypic composition of a plant that is important but also its breeding value (V_A). The general combining ability of a plant actually measures its breeding value (V_A).

The breeding value of a line is a function of the additive gene action. The additive genes are directly transported from the parents to the offspring. The

additive gene action is therefore responsible for the resemblance between relatives. It can be used to calculate the inheritance of a character.

$$h^2 = V_A/(V_A + V_I + V_D + V_E)$$

Where: h^2 = heritability

V_A = additive variance

V_I = interaction variance

V_D =dominance variance

V_E =environmental variance

(Griffing, 1956).

2.6 Genetic distances

The difference in gene frequency between the parent genotypes is important because the higher the difference in gene frequency, the higher the amount of heterosis. Genetic distances among progeny confirm their origin and the genetic relationships between them and their parents (Carrera *et al*, 1996).

The breeder can use genetic distance information to make informed decisions regarding the choice of genotypes to cross for the development of populations, or to facilitate in the identification of diverse parents to cross in hybrid combinations in order to maximize the expression of heterosis (Smith *et al*, 1990).

The relatively short genetic distances between *H. annuus*, *H. laetiflorus*, *H. salicifolius*, *H. bolanderi*, *H. petiolaris* and *H. tuberosus* suggest that these wild relatives should prove useful donors of valuable genes to *H. annuus*. PCR analysis effectively classified and identified species most related to *H. annuus,* which could be used for the improvement of cultivated sunflower (Baldini *et al*, 1994; Cristov and Vassilevska Ivanova, 1999).

The correlations between genetic distance, heterosis, and hybrid performance for seed yield in sunflower were estimated. Genetic distances were significantly correlated with hybrid seed yield when it was estimated from AFLP fingerprints, but not from co-ancestries (Cheres *et al*, 2000).

2.7 Combining ability

Combining ability is the ability of a parent to produce inferior or superior combinations in one or a series of crosses (Chaudhary, 1982). It is especially useful to test procedures for studying and comparing the performances of lines in hybrid combinations (Griffing, 1956).

The general combining ability (GCA) value of a genotype determines its crossing value that is, whether a line or tester is the best combiner in a breeding program (Falconer and Mackay, 1996). It is recognized as primarily a measure of additive gene action (Sprague and Tatum, 1942).

Specific combining ability (SCA) shows the minimum and maximum genetic gain of hybrids from certain lines by certain testers. SCA is very important in hybrid breeding. The SCA of a cross gives an indication of the proportion of *loci* that shows dominance (V_D) and interaction (V_I). Dominance and interactions are the result of specific gene combinations. These gene combinations split during meioses. When the genes are transferred from the parents to the offspring, different genes are grouped together and they form new combinations in the offspring. Therefore although genes involved in dominance and interaction are transferred from the parents to the offspring, the phenotypic effect of the genes are not directly transferred to the offspring. Thus, *loci* that show dominance or interaction are not contributing to the additive genetic variance or the inheritance of a character (Falconer and Mackay, 1996). SCA is regarded as an estimate of the effects of non-additive gene actions (Sprague and Tatum, 1942).

Most analysis should be limited to estimating GCA and SCA mean squares and effects. Such information is useful in measuring hybrid performance or in assessing the potential of a hybrid breeding program (Baker, 1978).

2.7.1 General and specific combining ability in sunflower

General combining ability values were usually more important than specific combining ability values, indicating the importance of additive genetic controls for organogenesis parameters in sunflower (Becker, 1985).

Combining ability analysis showed significant differences between the restorer and the CMS lines in their GCA, but no differences were found in SCA for the yield-related traits studied (Wricke and Weber, 1986).

Mean squares for GCA and SCA were significant (P=0.01) for all characters. The estimated components for GCA were greater than SCA for days to maturity, weight per bushel, and percent oil in the seed. The components for GCA were higher than SCA for height and yield of seed and essentially the same for days to flower, head diameter and weight per 1000 seeds (Putt, 1966).

The component for GCA was greater than for SCA, which suggests that additive gene action is more important than non-additive gene action in the control of oil content (Putt, 1966; Hussain *et al*, 1998).

The interaction between males and females were also significant. These results indicate that additive genetic effects predominantly influence the expression of kernel cadmium accumulation in hybrids (Li *et al*, 1995).

The environmental conditions influenced to a great extent the evaluations of SCA, while GCA was found to be more stable. The non-additive gene effects for the seed weight/plant and oil content were unstable in variable environments in comparison with additive gene effects (Petakov, 1996).

According to Sprague (1983), additive and dominance gene effects are generally much greater than other types of gene effects. Additive effects are those that respond to selection. Both overdominance and epistasis exist, but neither has been shown to be important at the population level. Additive and dominance effects provide a satisfactory model for heterosis and for the rather remarkable progress achieved through breeding (Crow, 1999). The results on types of gene action in sunflower indicate that additive variance is the most important type of gene action. Dominance variance appeared to be important only for yield, while epistatic effects were minor.

Additive variance was significant for oil percentage, but dominance variance was not (Miller *et al*, 1980). However, Ali *et al* (1992) found that analysis of the data for oil percentage showed that additive gene action with protein percentage and seed yield/plant showed an overdominance type of gene action.

Additive gene effects were important in the inheritance of seed composition and seed oil content, while non-additive effects controlled seed yield (Merinkovic, 1993).

2.8 Genetic correlation

The relationship between two metric characters can be positive or negative. Falconer and Mackay (1996) found that correlated characters are of interest for three reasons namely genetic causes of correlation through the pleiotropic action of genes, in connection to changes brought about by selection and in connection with natural selection.

In plant breeding studies, there are two types of correlations, namely phenotypic and genetic correlations. The genetic correlation is the correlation of breeding values, which is a function of additive gene action. Phenotypic correlation is the association between two characters that can be directly observed and can be determined from measurements of the two characters in a number of individuals of the population. The genetic correlation expresses

the extent to which two measurements reflect what is genetically the same character. A high value of genetic correlation indicates a high genetic association between the characteristics tested. Both genetic and phenotypic correlations are important to indicate the correlated response that may occur during selection of a single trait (Falconer and Mackay, 1996).

Morphological characteristics include plant height, flowering date, days to maturity, stem girth, head diameter, 1000-seed weight and oil percentage.

The head diameter followed by 1000-seed weight, plant height and stem girth showed a significant positive correlation with seed yield. The highest correlation was found with head diameter, which was also revealed in the path coefficient analysis to have the highest positive direct effect. Flowering date showed a direct negative correlation with yield. The positive effect of plant height was correlated with head diameter (Doddamani *et al*, 1997).

2.9 Heterosis and agronomic characteristics

Falconer and Mackay (1996) refer to heterosis as the converse of inbreeding depression and they defined it as the difference between the crossbred and the inbred means i.e. the difference between the hybrid and the mean of the two parents. This definition is usually called mid-parent heterosis (Lamkey and Edwards, 1999).

Most textbooks of genetics and plant breeding describe heterosis as the manifestation of greater vigour in height, leaf area, growth, dry matter accumulation, and higher yield of the F1 hybrid in comparison with its inbred parents (Allard, 1960; Brewbaker, 1964). All these characters are considered to be quantitative and they are usually the end product of a series of reactions (Allard, 1960; Brewbaker, 1964; Lamkey and Edwards, 1999). Heterosis is mainly due to *loci* that are dominant or partially so (Lamkey and Edwards, 1999).

Heterosis in plants has usually been identified with hybrid vigour as a major component (Hayes, 1952; Shull, 1952; Allard, 1960). Shull (1952) defined hybrid vigour as the manifestation of heterosis. Therefore, hybrid vigour is the phenotypic expression of heterosis, which is a genetic phenomenon. In other words, heterosis and hybrid vigour have a relationship that exists between the mechanism and its product. Consequently, the factors that influence genetic expression should affect hybrid vigour. This included inbreeding depression, hybrid stability or homeostasis, general and specific combining ability, and hybrid vigour in its broadest sense as the components of heterosis (Williams, 1959).

The amount of heterosis following a cross between two specific lines or populations depends on the square of the difference of gene frequency (y) between the populations. If the populations that were crossed do not differ in gene frequency, there will be no heterosis. The amount of heterosis will be the greatest when one allele is fixed in one population and the other allele in the other population. If the effect of all *loci* at which the two parent populations differ, is considered, the amount of heterosis produced by the joint effects of all *loci* may be represented as the sum of their separate contributions (as long as the genotypic values attributable to the separate *loci* combine additively). Thus the heterosis in the F1 is:

$$H_{F1} = \Sigma dy^2$$

Where: d = the deviation of the heterozygote from the homozygote midparent
y = gene frequency

Three conclusions can be drawn from the above equation:

1) The occurrence of heterosis after crossing is dependent on directional dominance (like inbreeding depression) and the absence of heterosis is not sufficient to conclude that the individual *loci* show no dominance.

2) The amount of heterosis is specific to each particular cross, because the genes by which two specific lines differ will not be the same for all pairs of lines.
3) If the lines crossed are highly inbred, and thus completely homozygous, the difference in gene frequency between them can only be 0 or 1. The heterosis as shown by the above equation is then the sum of the dominance deviations d of these *loci* that have different alleles in the two lines (Falconer and Mackay, 1996).

Heterosis can also be determined by the following equations:

$$\text{Heterosis} = \frac{F1}{(P1 + P2)/2} \times 100$$

and

$$\text{Heterosis} = \frac{F1 - (P1 + P2)/2}{(P1 + P2)/2} \times 100$$

Where: P = parental lines

When the F1 is better than the parents (P), the occurrence of hybrid vigour and heterosis is obvious (Falconer and Mackay, 1996; Lamkey and Edwards, 1999).

Heterosis in sunflower has been observed for seed yield, time to bloom, plant height, head diameter, seed weight and oil percentage. Therefore, heterosis is of great importance in sunflower breeding. The effect of hybrid vigour in plants is observed in many ways, for example higher yield, improved vigour, plant height, oil percentage and seed weight (Putt, 1966; Fick and Zimmer, 1974; Putt and Dorrell, 1975).

Heterosis is significant for seed yield and is one of the driving forces behind the hybrid seed industry in cultivated sunflower (Cheres *et al*, 2000). Yield is considered a quantitative character and thus is treated as a single character

(Allard, 1960). It appears that if the yield components could be analyzed in depth, they seem to be inherited as Mendelian characters. The F1 hybrids show either dominance or partial dominance. The components then result in higher yield because of the multiplicative effect among them (Cheres *et al*, 2000).

A synthetic variety was evaluated for heterosis of yield and its components. Hybrid vigour under irrigated conditions was 92.62% for oil yield, 77.90% for seed yield, 48.24% for diameter of the seedless center of the head, 8.87% for 1000-seed weight, 7.57% for husk percentage, 5.51% for oil percentage and 4.90% for stalk yield. There was no heterosis for plant height and head diameter.

Under non-irrigated conditions, heterosis was 67.95% for oil yield, 54.03% for seed yield, 11.89% for plant height, 11.49% for head diameter, 7.79% for oil percentage, 6.16% for diameter of the seedless center of the head, 4.92% for stalk yield and 4.80% for husk percentage. There was no heterosis for 1000-seed weight (Yenice and Arslan, 1997).

Heterosis was evident for the important economic characters like yield and oil percentage, as well as for all the other characters. The differences between the mean of all parents and the mean of all crosses were significant at P=0.01 for time to flower, height, head diameter, and seed yield. They were significant at P=0.05 for 1000-seed weight and oil percentage in the seed. The heterosis demonstrated in the study of Putt (1966), emphasized the need for utilizing hybrid vigour in sunflowers. Heterosis is desirable for all characters examined, except possibly height. Short plants are usually considered more desirable for mechanical harvesting. Particularly encouraging is the marked heterosis exhibited for yield and also the heterosis for oil content, the two most important economic characters.

Kovacik (1959, 1960), in a study of intervarietal crosses, observed a superior response with an increase in yield to the extent of one to 20% over the

parents. Only a few lines exceeded the parents in oil percentage (Popov and Lazarov, 1963).

Schuster (1964) observed heterosis for yield where the hybrids were up to 70% better than the parents. Half the hybrids showed heterosis for plant height (47% better). Heterosis for head diameter was 60%. Only 18% of the hybrids showed heterosis for oil percentage.

Shuravina (1972) found that 16 of the 24 hybrids showed heterosis over the tester parents to an extent of 39 and 20% for 1000-seed weight and yield, respectively. In another study, 14 of the 18 hybrids studied showed heterosis of up to 90% for 1000-seed weight and 40% for yield. Only three of the 18 hybrids showed heterosis of 4.8% for oil percentage. Seetharam and colleagues (1977) observed a significant positive heterosis for flowering date, plant height, head diameter, oil percentage and yield.

It is known that diverse genotypes provide the best specific combiners for obtaining heterosis. This is because they bring together several contrasting, but complementary traits or components.

Heterosis is an important component in plant improvement, and efforts will be continued in many plant species in which hybrids are either not currently used or not widely used. It has been used successfully even though its genetic basis has not been determined for the most part (Hallauer, 1999).

2.10 Biotechnology and amplified fragment length polymorphism (AFLP)

Over the past ten years a number of DNA fingerprinting techniques have been developed to provide genetic markers capable of detecting differences among DNA samples across a wide range of scales ranging from individual or clone discrimination up to species level differences (Vos *et al*, 1995; Blears *et al*, 1998).

Currently available techniques include: RFLPs [restriction fragment length polymorphism's (Liu and Furnier, 1993)], DAF [DNA amplification fingerprinting (Caetano-Anolles and Gresshoff, 1994)], AP-PCR (arbitrarily primed PCR, RAPDs [randomly amplified polymorphic DNAs (Williams *et al*, 1990)], microsatellites (Tautz, 1989), and most recently AFLPs [amplified fragment length polymorphism's (Zabeau and Vos, 1993; Vos *et al*, 1995; Blears *et al*, 1998)].

RFLP analysis requires relatively large amounts of very pure DNA. Prior sequence information is necessary if PCR products are to be analyzed. Although this technique is labor intensive and expensive, it is highly repeatable and produces many polymorphic bands. It has been used for the development of detailed genetic maps, screening of resistant genes and cultivar identification in sunflower.

DAF, AP-PCR and RAPD are PCR-based and require much less tissue to produce many polymorphic bands. The DNA fragment patterns generated by these techniques depend on the sequence of the primers and the nature of the template DNA. No prior sequence characterization of the target genome is needed and PCR is performed at low annealing temperatures to allow the primers to hybridize to multiple *loci*. Due to their sensitivity to template and reaction conditions, extraordinary care must be taken to ensure repeatability across multiple reactions. The need to repeat each PCR reaction multiple times and the inability to obtain identical banding patterns in different laboratories have limited the use of these techniques (Blears *et al*, 1998). No studies were done on sunflower with DAF and AP-PCR techniques.

RAPD markers were used to determine genetic diversity in sunflower lines (Moesges and Friedt, 1992; Lawson *et al*, 1994; Teulat *et al*, 1994; Arias and Rieseberg, 1995; Rieseberg *et al*, 1995; Rieseberg, 1996; Roeckel Drevet *et al*, 1997; Faure *et al*, 1999), to identify disease resistance genes (Lawson *et al*, 1996) and to asses the phenetic and phylogenetic relationships in sunflower (Sossey-Alaoui *et al*, 1999).

Microsatellite markers offer many advantages, but the high cost and time that are generally required for the development of primers specific for any given application have limited their use in many laboraties (Whitton *et al*, 1997; Blears *et al*, 1998). It has however, been used for analyzing genetic relationships in cultivated sunflower (Dehmer and Friedt, 1998).

The choice of which fingerprinting technique to use depends on:

1) the application (e.g. DNA genotyping, genetic mapping, population genetics)
2) the organism under investigation (e.g., procaryotes, plants, animals, humans)
3) the resources (time and money) available. In most cases, not one fingerprinting technique is ideal for all applications (Blears *et al*, 1998).

The AFLP technique is one of the number of DNA fingerprinting procedures that takes advantage of PCR to amplify a limited set of DNA fragments from a specific DNA sample (Vos *et al*, 1995; Blears *et al*, 1998).

The technique represents a combination of RFLP and PCR, resulting in highly informative fingerprints. The resemblance with the RFLP technique was the basis to choose the name AFLP. In contrast to the RFLP technique, AFLPs will display the presence or absence of restriction fragments rather than length polymorphisms. The technique is robust and reliable, because stringent reaction conditions are used for primer annealing. The reliability of the RFLP technique is combined with the power of the PCR technique (Vos *et al*, 1995).

AFLP fingerprints can be used to distinguish between even very closely related organisms, including near isogenic lines (Vos *et al*, 1995). The differences in fragment lengths, generated by this technique, can be traced to base changes in the restriction/adapter site, or to insertions or deletions in the body of the DNA fragment. Dependence on sequence knowledge of the target genome is eliminated by the use of adapters of known sequence that

are ligated to the restriction fragments. The PCR primers are specific for the known sequences of the adapters and restriction sites (Blears *et al*, 1998).

Since this technique provides simultaneous coverage of many *loci* in a single assay and it can be tuned to generate DNA fingerprints of complexity by altering the number of selective bases employed. It is proving to be an invaluable tool for studies of diversity, particularly in species where other new-generations markers, such as microsatellites, are not available (Donini *et al*, 1997).

AFLP offers the fastest, most reproducible and most cost effective way to high-density genetic maps for marker assisted selection of desirable traits. It requires relatively small amounts of genomic DNA and unlike microsatellites no taxon-specific primer sets are required. The AFLP technique provides 10 to 100 times more markers than the other techniques. AFLP markers also tend to be more informative than RFLPs or RAPDs, providing data that are 10 to 50 times more informative per rand spent (Blears *et al*, 1998).

A remarkable characteristic of the AFLP reaction is that generally, the labeled primer is completely consumed (the unlabeled primer is in excess), and therefore, the amplification reaction stops when the labeled primer is exhausted. It is also found that further thermo cycling does not affect the band patterns once the labeled primer is consumed.

The AFLP technique is not only a fingerprinting technique. It is also an enabling technology in genome research, because it can bridge the gap between genetic and physical maps.

1) AFLP is a very effective tool to reveal restriction fragment polymorphisms.
2) AFLP markers can be used to detect corresponding genomic clones, e.g. yeast artificial chromosomes (YACs).
3) It can be used for fingerprinting cloned DNA segments like cosmids, P1 clones, bacterial artificial chromosomes (BACs) or YACs.

AFLPs are quickly becoming the tool of choice for many applications and organisms. Potential applications include screening DNA markers linked to genetic traits, percentage analysis, forensic genotyping, diagnostic markers for pathogen borne diseases, and population genetics.

Since the AFLP technique can be applied to a wide variety of organisms (and viral sources) with no prior sequence information, this technique has the potential to become a universal DNA fingerprinting tool (Blears *et al*, 1998).

2.10.1 Primers and adapters

AFLP adapters consist of a core sequence and an enzyme-specific sequence (Table 2.2).

Table 2.2 The structures of the *EcoR1*- and *Mse1*-adapters (Vos *et al*, 1995)

EcoR1-adapter	*Mse1*-adapter
5 – CTCGTAGACTGCGTACC CATCTGACGCATGGTTAA - 5	5 – GACGATGAGTCCTGAG TACTCAGGACTCAT - 5

Adapters for other "rare cutter" enzymes were identical to the *EcoR1*-adapter with the exception that cohesive ends were used, which are compatible with these other enzymes. The *Taq1*-adapter was identical to the *Mse1*-adapter with the exception that a cohesive end was used compatible with *Taq1*.

AFLP primers consist of three parts, namely a core sequence, an enzyme specific sequence (ENZ) and a selective extension (EXT). This is illustrated below (Table 2.3) for *EcoR1*- and *Mse1*-primers with three selective nucleotides (selective nucleotides shown as NNN).

Table 2.3 *EcoR1*- and *Mse1*-primers with a core, an enzyme specific (ENZ) and a selective extension (EXT) sequence (Vos *et al*, 1995)

	CORE	ENZ	EXT
EcoR1	5-GACTGCGTACC	AATTC	NNN-3
Mse1	5-GATGAGTCCTGAG	TAA	NNN-3

AFLP-primers for other "rare cutter" enzymes were similar to the *EcoR1*-primers, and *Taq1*-primers were similar to the *Mse1*-primers, but it had enzyme-specific parts corresponding to the respective enzymes.

Mse1 is therefore preferred for AFLP fingerprinting because it cuts very frequently in most eukaryotic genomes, yielding fragments that are in the optimal size range for both PCR amplification and separation on denaturing polyacrylamide gels. However, *EcoR1* is preferred because it is a reliable (low cost) six-cutter enzyme, which limits problems associated with partial restriction in AFLP fingerprinting.

Careful primer design is crucial for successful PCR amplification. AFLP primers consist of three parts: the 5' part corresponding to the adapter, the restriction site sequence and the 3' selective nucleotides. Therefore, the design of AFLP primers is mainly determined by the design of the adapters, which are ligated to the restriction fragments (Vos *et al*, 1995).

2.10.2 Basic steps of AFLP fingerprinting

AFLP for complex genomes involves five steps:

2.10.2.1 Restriction of the genomic DNA

Restriction fragments of the genomic DNA are produced, by using two different restriction enzymes: a frequent cutter (the four-base restriction enzyme *Mse1*) and a rare cutter (the six-base restriction enzyme *EcoR1*).

Three types of restriction fragments are generated: types with *EcoR1* cuts at both ends, types with *EcoR1* cut at one end and *Mse1* cut at the other end, and types with *Mse1* cuts at both ends.

The frequent cutter will generate small DNA fragments, which will amplify well and are in the optimal size range for separation on denaturing sequencing gels. The number of amplified fragments will be reduced using a rare cutter, since only the rare cutter/frequent cutter fragments are amplified. This limits the number of selective nucleotides needed for the AFLP reaction. The use of two restriction enzymes makes it possible to label only one strand of the double stranded PCR products, which prevents the occurrence of "doublets" on the gels due to unequal mobility of the two strands of the amplified fragments. By using two different restriction enzymes the greatest flexibility in "tuning" the amount of fragments to be amplified is found.

Incomplete restriction of the DNA will cause problems in AFLP fingerprinting, because partial fragments will be generated, which will be detected by the AFLP procedure. When various DNA samples are compared with AFLP fingerprinting, incomplete restriction will result in the deletion of differences in band patterns, which do not reflect true DNA polymorphisms, i.e. when one sample is partially restricted and the others are not.

In complex genomes, the number of restriction fragments that may be detected by the AFLP technique is virtually unlimited. A single enzyme combination (combination of a specific six-base and four-base restriction enzyme) will already permit the amplification of 100 000 of unique AFLP fragments, of which generally 50 to 100 will be selected for each AFLP reaction (Vos *et al*, 1995).

2.10.2.2 Ligation of oligonucleotide adapters

Double stranded adapters consist of a core sequence and an enzyme-specific sequence. They are specific for either the *EcoR1* site or the *Mse1* site. Restriction and ligation take place in a single reaction.

The adapters are designed in such a way that the restriction sites are not restored after ligation. During the ligation reaction, the restriction enzymes are still active. In this way fragment-to-fragment ligation is prevented, since fragment concatamers are restricted. Adapter-to-adapter ligation is not possible because the adapters are not phosphorylated.

Because primers with three selective bases tolerate a low level of mismatch amplification, a two-step amplification strategy was developed for AFLP fingerprinting of complex DNAs. With the preamplification reaction, the genomic DNAs were amplified with AFLP primers both having a single selective nucleotide. The PCR products of the preamplification reaction were then diluted and used as a template for the second AFLP reaction using primers both having three selective nucleotides.

The two-step amplification resulted in two important differences compared with the direct AFLP amplification:

1) background "smears" in the fingerprint patterns were reduced and
2) fingerprints with particular primer combinations lacked one or more bands compared with fingerprints generated without pre-amplification.

An additional advantage to the low level of mismatch, of the two-step amplification strategy is that it provides a virtually unlimited amount of template DNA for AFLP reactions (Vos *et al*, 1995).

2.10.2.3 Preselective amplification

Primers used in this step consist of a core sequence, an enzyme specific sequence and a selective single-base extension at the 3'-end. The sequences of the adapters and restriction sites serve as primer binding sites for the "preselective PCR amplification". Each preselective primer has a "selective" nucleotide that will recognize the subset of restriction fragments having the matching nucleotide downstream from the restriction site. The primary products of the preselective PCR are those fragments having one *Mse1* cut and one *EcoR1* cut, and also having the matching internal

nucleotide. This results in a 16-fold decrease in the complexity of the restriction-ligation products (Blears *et al*, 1998).

2.10.2.4. Selective amplification with labeled primers

Selective primers are either radio-labeled or fluorescently labeled. They consist of an identical sequence to the preselection primers plus two additional selective nucleotides at the 3'-end (i.e. a total of three selective nucleotides). These two additional nucleotides can be any of the 16 possible combinations of the four nucleotides. From the huge number of fragments generated by the two restriction enzymes, only that subset of fragments having matching nucleotides at all three positions will be amplified at this stage (50 to 200 fragments). This step reduces the complexity of the PCR product mixture by 256 fold.

Different primer combinations will generate different sets of fragments. Preliminary screening is used to choose primer pairs that generate suitable levels of variation for the *taxa* being studied. Only one of the two DNA strands of each amplified sequence will be labeled.

2.10.2.5. Gel-based analysis of the amplified fragments

Labeled fragments are resolved by gel electrophoresis on a Perkin-Elmer/Applied Biosystems Inc. automated sequencer. Only the *EcoR1* primer is labeled and therefore only the *EcoR1*-site containing strands will be labeled and then detected. This ensures unambiguous detection of the single strand amplified fragments in denaturing gels by eliminating doublets.

The GeneScan software analyzes four different fluorescent labels that are visualized as blue, green, yellow and red. Multiple samples (amplified with separate primer sets, each labeled with a different fluorescent dye) can be loaded in a single gel lane along with an internal DNA size standard (also labeled). Such "multiplexing" reduces the cost of the analysis.

The GeneScan results are displayed as a reconstructed gel image, electropherograms, or tabular data. GeneScan results can be imported into

the Genotyper program for subsequent data analysis. This software identifies and measures bands ranging in size from 50 to 500 base pairs. The bands (alleles) are scored as present/absent, and a binary matrix is constructed. The matrix is then analyzed using phenetic methods such as UPGMA and cluster analysis.

This technique provides numerous informative bands and can be accurately sized using fluorochrome-labeled primers and an automated sequencing gel scanner for electrophoresis and data analysis.

2.10.3 AFLPs and heterosis

Heterosis was significant for seed yield and plant height, but not for seed oil concentration and flowering date. Genetic distances were significantly correlated with hybrid seed yield when estimated from AFLP fingerprints. Substantial genetic diversity seems to be present within and between heterotic groups of sunflower (Cheres *et al*, 2000).

Genetic similarities were lower overall for maintainer (B) x restorer (R) crosses than for B x B or R x R crosses. Principle-coordinate and cluster analysis separated lines into two groups, one for B-lines and another for R-lines. These groupings illustrate the breeding history and basic heterotic pattern (B x R) of sunflower and the widespread practice of using B x B and R x R crosses to develop new lines. There were, nevertheless, distinct subgroups within these groups. These subgroups may represent unique heterotic groups and create a basis for formally describing heterotic patterns in sunflower (Hongtrakul *et al*, 1997).

CHAPTER 3
GENETIC DIVERSITY OF INBRED LINES

3.1 Introduction

The difference in gene frequency between parent genotypes is very important to the plant breeder. If there is a high difference in gene frequency, the breeder can expect a high amount of heterosis. Furthermore, the genetic distance among progeny confirms their origin and the genetic relationship between them and their parents (Vranceanu *et al*, 1994).

The breeder can use genetic distance information to make informed decisions regarding the choice of genotypes to cross for the development of populations, or to facilitate in the identification of diverse parents to cross in hybrid combinations in order to maximize the expression of heterosis (Smith *et al*, 1990).

If breeders could predict the potential of crosses for line development before producing and testing lines in field trials, this would increase the efficiency of breeding programs by concentrating the efforts on the most promising crosses (Bohn *et al*, 1999).

DNA marker systems are useful tools for assessing genetic diversity between germplasm. In breeding programs, information on genetic relationships within species is used to organize germplasm collections, to identify heterotic groups and to select breeding material. AFLP analysis is a rapid and efficient method for producing DNA fingerprints and to determine genetic diversity (Lee, 1995; Karp *et al*, 1996).

The correlations between genetic distance, heterosis, and hybrid performance for seed yield in sunflower were estimated. Genetic distances were

significantly correlated with hybrid seed yield when estimated from AFLP fingerprints (Cheres *et al*, 2000).

The objective of this study was therefore to determine the genetic diversity of 12 sunflower inbred lines with the use of the AFLP technique and different combinations of primers. These results will then be used to identify heterotic groups in a hybrid breeding program.

3.2 Materials and methods

3.2.1 Plant material

A total of 12 inbred lines (Table 3.1) were used in this study, consisting of six female inbred lines (lines) and six male inbred lines (testers). The female lines were cytoplasmic male sterile, while the male lines had the restorer gene. The material was obtained from the genebank of a private seed company.

Table 3.1 The female and male inbred lines used as parents in this study

Female (CMS)	Male (Restorer)
1A	11R
2A	12R
3A	13R
4A	14R
5A	15R
6A	16R

3.2.2 Methods

3.2.2.1 Growing conditions

Twelve plants (three per pot) of each inbred line were grown in the glasshouse at the University of the Free State (UFS), in Bloemfontein, South Africa. Curaterr (10 GR) and N:P:K fertilizer of 3:2:1(25)+0,5 Zn were mixed

with the soil to enhance the growth development of the plants. The plants were watered every second day. A constant temperature of 27°C (day/night) was maintained throughout the experiment. The leaves were collected after each plant formed five to seven leaves.

3.2.2.2 DNA-extraction

DNA was extracted from young fresh leaves using a monocot extraction procedure (Edwards *et al*, 1991). The fresh leaves were collected on ice and sealed in plastic bags. In the laboratory the plant material was homogenised using a mortar and pestle. The leaf material was frozen in liquid nitrogen and ground to a fine powder. The ground powder was transferred to a clean 50ml polypropylene tube containing 10ml extraction buffer (5M NaCl, 0.5M Tris-HCl, 0.25M EDTA and 20% SDS at pH8). The homogenate was vortexed and incubated at 65°C for 30min.

Clean-up buffer was added (1M Tris-HCl, 0.25M EDTA and 5g of CTAB) and the extract was incubated for 1h at 65°C with periodic shaking every 10min. Thereafter, 10ml chloroform-isoamylalcohol (24:1 v/v) was added and it was mixed gently. Centrifugation of the extraction was performed at 10 000 rpm for 15min at 2°C. The aqueous layer was transferred to a clean tube and the DNA was precipitated with 100% cold ethanol (1:1 v/v). The DNA was spooled and washed twice in 70% ethanol. The DNA pellet was resuspended in 1ml of sterile water (Sabax, Non Pyrogenic). The DNA concentration was determined with a spectrophotometer. The DNA samples were diluted with Sabax water to a final concentration of 250ng/µl. The concentrations were measured at 260nm and 280nm. At 260nm the DNA concentration was determined, while the protein concentration was determined at 280nm. The samples were aliquoted for storage at -20°C.

3.2.2.3 Primers

DNA was digested with *Mse1* (frequent 4-base cutter) and *EcoR1* (rare 6-base cutter) as described by Vos *et al* (1995). The *EcoR1*-primer was

fluorescently labelled. Oligonucleotide sequences used for adapters and primers are listed in Table 3.2.

Table 3.2 A list of adapter and primer sequences used in AFLP reactions

Mse-adapter 5'-GACGATGAGTCCTGAG-3' *Mse*-primers (5'-GATGAGTCCTGAGTAA-3')	*Eco*-adapter 5'-CTCGTAGACTGCGTACC-3' *Eco*-primers (5'-GATGCGTACCAATTC-3')
Mse + CTT	*Eco* + ACA (FAM)
Mse + CAG	*Eco* + AAC (NED)
Mse + CTC	

3.2.2.4 Amplified fragment length polymorphism (AFLP) reactions

AFLP analysis was performed using bulk segregant analysis (BSA). The DNA from the 12 inbred lines was bulked. The AFLP reactions were done according to the manufacturer's instructions (Gibco BRL).

Restriction endonuclease digestion of genomic DNA

Genomic DNA (250ng) was digested with *Mse1* and *EcoR1* to determine if both enzymes digest entirely.

The DNA sample (250ng/µl), 5 x restriction ligation buffer and *EcoR1/Mse1* (0.5µl) were mixed and diluted with AFLP grade water to a final volume of 25µl. The contents were collected after a brief centrifugation and incubated for 2h at 37°C.

Thereafter the mixture was incubated for 15min at 70°C to inactivate the restriction enzymes. The tubes were placed on ice and the contents were collected after brief centrifugation.

Ligation of adaptors

The digested fragments were then ligated with *EcoR1* and *Mse1* adapters (Table 3.2).

T4 DNA ligase (1μl) and adaptor ligation solution (24μl) were added to the 25μl double digested DNA. It was gently mixed at room temperature, centrifuged to collect the contents and incubated at 20°C for 2 hours.

A 1:10 dilution of the ligation mixture was performed by adding 90μl of TE to a 10μl reaction mixture, mixing it thoroughly.

Pre-selective AFLP amplification

A 51μl pre-selective PCR reaction was performed with 5μl diluted ligation product, pre-amp primer mix, 10 x PCR buffer for AFLP and 1U of *Taq* DNA polymerase (Gibco BRL). A touchdown Hybaid thermal cycler (Vos *et al*, 1995) was used to perform the amplification reaction for 20 cycles with the following profile: a 30s denaturing step at 94°C, a 60s annealing step at 56°C and a 60s extension step at 72°C. A 1:50 dilution of the preselective PCR products was performed by adding 147μl TE at 3μl reaction after running 15μl on 1% agarose gels at 90V.

The diluted PCR products of the pre-amplification reaction were used as templates for the second AFLP reaction, using primers that have three selective nucleotides.

Selective AFLP amplification

Selective amplification was carried out using various primer combinations of primer *Mse1* and primer *EcoR1* (Table 3.2)(Zabeau and Vos, 1993; Vos *et al*, 1995).

Selective PCR-reactions were performed in a 20μl PCR reaction containing 5μl of the diluted pre-selective reaction, 4.5μl of the Mse+3 primer (Table 3.2), 1μl *EcoR1*+3 primer (fluorescently labelled), 2μl of 10 x PCR buffer and 5U of

Taq DNA polymerase. Reactions were performed for 30 cycles with the following cycle profile: a 30s denaturing step at 94°C, a 30s annealing step at 65°C and a 2min extension step at 72°C. PCR was started at a very high annealing temperature to obtain optimal primer selectivity. Then the annealing temperature was reduced by 0.7°C for 12 consecutive cycles and then continued at 56°C, where efficient primer binding occurred. A total of six primer combinations were tested.

After amplification, 5µl of each of the selective reactions were added to a new tube containing 24µl of formamide and 1µl of Rox standard size marker, denatured at 94°C for 10min and run on a Perkin Elmer ABI Prism 310 Automated capillary sequencer (PE Biosystems). The *EcoR1* primers (PE Biosystems) were labelled with NED and FAM respectively (Table 3.2).

3.2.2.5 Gel analysis

Amplification products through the AFLP process were resolved by electrophoresis on 1% agarose gels consisting of 0.5g agarose (0.5M) and 3µl ethidium bromide. DNA (3µl) and 3µl loading buffer were loaded on the gel. The loading buffer contained 2ml glycerol (20%), 7ml Sabax water, 1ml Tris-HCl (1M) and 0.025g bromophenol blue. Electrophoresis was performed at a constant power of 90V for 2h. Ethidium bromide (0.5 µg/ml) made it possible to detect the amplification products under UV light after staining (Vos *et al*, 1995). The AFLP profiles were resolved using an ABI377 automatic sequencer.

3.2.2.6 Data collection and analysis

AFLP fragment data for three primer pairs was coded using a binary system and it was summarised in a data matrix. The statistical program NCSS 2001 was used to determine the genetic distances and to group the inbred lines into clusters. The pairwise genetic distance was calculated directly from an input data matrix containing absence (0) and presence (1) values of all AFLP markers. Generally the fraction of bands shared between any two individuals

is used to calculate a similarity coefficient (S), which is converted to a genetic distance (D) value using either:

$$D = 1-S$$

or

$$D = -\ln(S)$$

This calculation is possible for all the possible pairwise groupings of individuals and the pairwise distance values grouped in a table of pairwise distance matrix (Barrett and Kidwell, 1998).

3.2 Results and discussion

3.3.1 Genetic distances

Primers *Mse*-CTT + *Eco*-ACA

The genetic distances for the female lines showed that 1A and 4A (0.30), and 2A and 4A (0.30) were closely related when primers *Mse*-CTT + *Eco*-ACA were used (Table 3.3). The highest amount of dissimilarity was found between 1A and 6A (0.47). Of the male lines 11R and 13R (0.28) were closely related, but 13R and 16R (0.47) showed the highest amount of dissimilarity. The F1 progeny showed that 4A and 16R (0.30) were closely related. The highest amount of dissimilarity was found between 6A and 12R (0.53). According to the genetic distances, 12R should be crossed with 6A to obtain maximum heterosis. The least amount of heterosis could be expected if 4A and 16R were crossed.

Table 3.3 The genetic distances of female, male and F1 hybrid lines when the *Mse*-CTT + *Eco*-ACA primer combination was used

	1A	2A	3A	4A	5A	6A	11R	12R	13R	14R	15R	16R
1A		0.46	0.35	0.30	0.42	0.47	0.41	0.46	0.32	0.39	0.41	0.34
2A			0.42	0.30	0.35	0.44	0.44	0.43	0.47	0.32	0.36	0.46
3A				0.41	0.31	0.41	0.32	0.37	0.41	0.35	0.37	0.35
4A					0.36	0.41	0.39	0.32	0.34	0.36	0.35	0.30
5A						0.46	0.44	0.37	0.36	0.43	0.35	0.35
6A							0.37	0.53	0.46	0.41	0.37	0.44
11R								0.36	0.28	0.39	0.41	0.34
12R									0.42	0.39	0.41	0.41
13R										0.34	0.35	0.47
14R											0.39	0.42
15R												0.41
16R												

Primers *Mse*-CAG + *Eco*-ACA

It was found that the genetic distances for female lines, 1A and 4A (0.33) were closely related when primers *Mse*-CAG + *Eco*-ACA were used (Table 3.4). The highest amount of dissimilarity was found between 1A and 6A (0.54). Male lines 12R and 16R (0.35) were closely related, but 11R and 13R (0.50) showed the highest amount of dissimilarity. The F1 progeny showed that 4A and 11R (0.30) were closely related, as well as 4A and 15R (0.30). The highest amount of dissimilarity was found between 6A and 15R (0.50). According to the genetic distances 15R should be crossed with 6A to obtain maximum heterosis.

Table 3.4 The genetic distances of female, male and F1 hybrid lines when the *Mse*-CAG + *Eco*-ACA primer combination was used

	1A	*2A*	*3A*	*4A*	*5A*	*6A*	*11R*	*12R*	*13R*	*14R*	*15R*	*16R*
1A		0.40	0.44	0.33	0.35	0.54	0.41	0.49	0.36	0.32	0.48	0.31
2A			0.40	0.51	0.49	0.36	0.39	0.35	0.35	0.32	0.35	0.31
3A				0.42	0.42	0.51	0.41	0.49	0.42	0.35	0.37	0.31
4A					0.49	0.36	0.30	0.33	0.28	0.32	0.30	0.31
5A						0.38	0.37	0.38	0.31	0.39	0.35	0.40
6A							0.45	0.49	0.38	0.39	0.50	0.33
11R								0.41	0.50	0.44	0.49	0.43
12R									0.42	0.39	0.45	0.35
13R										0.45	0.45	0.40
14R											0.42	0.41
15R												0.43
16R												

Primers *Mse*-CTC + *Eco*-ACA

The genetic distances for primers *Mse*-CTC + *Eco*-ACA (Table 3.5) for the female lines showed that 2A and 5A (0.23) were closely related. The highest amount of dissimilarity was found between 1A and 4A (0.49). The male lines, 12R and 14R (0.34) as well as 12R and 15R (0.34) were closely related, but 11R and 16R (0.53) showed the highest amount of dissimilarity. At the F1 progeny, 1A and 14R (0.28), and 2A and 15R (0.28) were closely related. The highest amount of dissimilarity was found between 4A and 16R (0.54). According to the genetic distances 16R should be crossed with 4A to obtain maximum heterosis.

Table 3.5 The genetic distances of female, male and F1 hybrid lines when the *Mse*-CTC + *Eco*-ACA primer combination was used

	1A	*2A*	*3A*	*4A*	*5A*	*6A*	*11R*	*12R*	*13R*	*14R*	*15R*	*16R*
1A		0.43	0.47	0.49	0.36	0.39	0.40	0.36	0.32	0.28	0.30	0.46
2A			0.38	0.38	0.23	0.36	0.41	0.31	0.35	0.33	0.28	0.39
3A				0.42	0.41	0.36	0.37	0.31	0.35	0.35	0.33	0.43
4A					0.43	0.46	0.43	0.45	0.41	0.35	0.41	0.54
5A						0.39	0.36	0.32	0.32	0.40	0.32	0.36
6A							0.49	0.49	0.41	0.35	0.35	0.45
11R								0.40	0.44	0.40	0.40	0.53
12R									0.40	0.34	0.34	0.42
13R										0.48	0.42	0.48
14R											0.46	0.48
15R												0.46
16R												

Primers *Mse* + *Eco*-ACA

When all the data of the *Eco*-ACA and three *Mse*-primers were combined (Table 3.6), it was found that the female lines 2A and 5A (0.35) were closely related. The highest amount of dissimilarity was found between 1A and 6A (0.46). The male lines 12R and 14R (0.37) were closely related, but 13R and 16R (0.45) showed the highest amount of dissimilarity. At the F1 progeny, 2A and 14R (0.32), as well as 3A and 14R (0.32) were closely related. The highest amount of dissimilarity was found between 6A and 12R (0.50). According to the genetic distances 12R should therefore be crossed with 6A to obtain maximum heterosis. The least amount of heterosis would be obtained if 2A or 3A were crossed with 14R.

Table 3.6 The genetic distances of the female, male and F1 hybrid lines when all the *Eco*-ACA combinations and the three *Mse*-primers were combined

	1A	2A	3A	4A	5A	6A	11R	12R	13R	14R	15R	16R
1A		0.43	0.42	0.37	0.37	0.46	0.40	0.43	0.34	0.33	0.39	0.37
2A			0.40	0.40	0.35	0.38	0.38	0.36	0.38	0.32	0.33	0.38
3A				0.42	0.38	0.42	0.37	0.39	0.39	0.32	0.36	0.36
4A					0.43	0.41	0.37	0.37	0.34	0.34	0.35	0.38
5A						0.40	0.39	0.39	0.36	0.40	0.37	0.40
6A							0.44	0.50	0.41	0.38	0.40	0.40
11R								0.39	0.41	0.41	0.43	0.43
12R									0.41	0.37	0.40	0.39
13R										0.43	0.41	0.45
14R											0.43	0.44
15R												0.43
16R												

Primers *Mse*-CTT + *Eco*-AAC

The genetic distances for the female lines showed that 2A and 5A (0.40), as well as 5A and 6A (0.40) were closely related when primers *Mse*-CTT + *Eco*-AAC were used (Table 3.7). The highest amount of dissimilarity was found between 1A and 2A (0.59). Of the male lines 11R and 12R (0.32) were closely related, but 15R and 16R (0.52) showed the highest amount of dissimilarity. The F1 progeny showed that 2A and 11R (0.27) were closely related. The highest amount of dissimilarity was found between 1A and 13R (0.52). According to the genetic distances 13R should therefore be crossed with 1A to obtain maximum heterosis.

Table 3.7 The genetic distances of female, male and F1 hybrid lines when the *Mse*-CTT + *Eco*-AAC primer combination was used

	1A	2A	3A	4A	5A	6A	11R	12R	13R	14R	15R	16R
1A		0.59	0.49	0.47	0.46	0.42	0.30	0.49	0.52	0.43	0.37	0.37
2A			0.47	0.43	0.40	0.42	0.27	0.49	0.46	0.36	0.37	0.35
3A				0.52	0.49	0.47	0.29	0.48	0.49	0.39	0.33	0.43
4A					0.43	0.49	0.31	0.48	0.47	0.44	0.36	0.43
5A						0.40	0.30	0.40	0.40	0.35	0.29	0.35
6A							0.41	0.49	0.42	0.41	0.46	0.42
11R								0.32	0.40	0.37	0.51	0.40
12R									0.47	0.48	0.40	0.47
13R										0.51	0.46	0.44
14R											0.51	0.51
15R												0.52
16R												

Primers *Mse*-CAG + *Eco*-AAC

The genetic distances for primers *Mse*-CAG + *Eco*-AAC showed that the female lines 4A and 6A (0.30) were closely related (Table 3.8). The highest amount of dissimilarity was found between 1A and 6A (0.62). The male lines 15R and 16R (0.32) were closely related, but 11R and 16R (0.50) showed the highest amount of dissimilarity. The F1 progeny showed that 4A and 11R (0.30) were closely related. The highest amount of dissimilarity was found between 6A and 11R (0.48). According to the genetic distances for this primer combination 11R should therefore be crossed with 6A to obtain maximum heterosis.

Table 3.8 The genetic distances of female, male and F1 hybrid lines when the *Mse*-CAG + *Eco*-AAC primer combination was used

	1A	*2A*	*3A*	*4A*	*5A*	*6A*	*11R*	*12R*	*13R*	*14R*	*15R*	*16R*
1A		0.42	0.38	0.31	0.35	0.62	0.43	0.36	0.42	0.37	0.33	0.41
2A			0.36	0.42	0.43	0.39	0.37	0.34	0.40	0.47	0.38	0.35
3A				0.40	0.39	0.43	0.39	0.38	0.31	0.43	0.36	0.39
4A					0.49	0.30	0.30	0.34	0.31	0.41	0.34	0.37
5A						0.36	0.34	0.39	0.35	0.42	0.43	0.44
6A							0.48	0.37	0.45	0.42	0.35	0.44
11R								0.35	0.43	0.44	0.35	0.50
12R									0.40	0.41	0.38	0.37
13R										0.43	0.40	0.37
14R											0.49	0.44
15R												0.32
16R												

Primers *Mse*-CTC + *Eco*-AAC

Genetic distances showed that the female lines 1A and 4A (0.29) were closely related when primers *Mse*-CTC + *Eco*-AAC were used (Table 3.9). The highest amount of dissimilarity was found between 4A and 5A (0.54). Male lines 11R and 15R (0.34), as well as 12R and 15R (0.34) were closely related, but 11R and 12R (0.48) showed the highest amount of dissimilarity. At the F1 progeny, 2A and 15R (0.30) were closely related. The highest amount of dissimilarity was found between 6A and 11R (0.50). According to the genetic distances 11R should therefore be crossed with 6A to obtain maximum heterosis. It is interesting to note that with this primer combination, 14R and 16R were identical (0.00). It was however, not found in any of the other primer combinations tested.

Table 3.9 The genetic distances of female, male and F1 hybrid lines when the *Mse*-CTC + *Eco*-AAC primer combination was used

	1A	2A	3A	4A	5A	6A	11R	12R	13R	14R	15R	16R
1A		0.41	0.33	0.29	0.35	0.41	0.31	0.29	0.31	0.34	0.34	0.34
2A			0.42	0.37	0.34	0.43	0.43	0.43	0.35	0.35	0.30	0.35
3A				0.53	0.46	0.47	0.41	0.40	0.37	0.32	0.34	0.32
4A					0.54	0.47	0.44	0.35	0.38	0.34	0.38	0.34
5A						0.48	0.42	0.38	0.39	0.39	0.40	0.39
6A							0.50	0.40	0.43	0.40	0.41	0.40
11R								0.48	0.46	0.38	0.34	0.38
12R									0.38	0.35	0.34	0.35
13R										0.43	0.39	0.43
14R											0.44	0.00
15R												0.44
16R												

Primers Mse + Eco-AAC

When all the data of the *Eco*-AAC combinations and *Mse*-primers were combined (Table 3.10), it showed that the female lines 1A and 4A (0.31) were closely related. The highest amount of dissimilarity was found between 4A and 5A (0.48). Male lines 12R and 15R (0.32) were closely related, but 14R and 16R (0.54) showed the highest amount of dissimilarity. At the F1 progeny 3A and 15R (0.28) were closely related. The highest amount of dissimilarity was found between 6A and 11R (0.43). According to the genetic distances 11R should be crossed with 6A to obtain maximum heterosis.

Table 3.10 The genetic distances of the female, male and F1 hybrid lines when the *Eco*-AAC combinations and the three *Mse*-primers were combined

	1A	*2A*	*3A*	*4A*	*5A*	*6A*	*11R*	*12R*	*13R*	*14R*	*15R*	*16R*
1A		0.45	0.35	0.31	0.36	0.44	0.30	0.34	0.38	0.34	0.30	0.33
2A			0.36	0.36	0.35	0.36	0.30	0.38	0.35	0.33	0.29	0.29
3A				0.45	0.41	0.40	0.31	0.37	0.33	0.31	0.28	0.31
4A					0.48	0.36	0.30	0.35	0.34	0.34	0.31	0.32
5A						0.37	0.32	0.35	0.34	0.34	0.33	0.35
6A							0.43	0.37	0.38	0.36	0.35	0.35
11R								0.35	0.39	0.34	0.35	0.38
12R									0.38	0.36	0.32	0.34
13R										0.41	0.37	0.36
14R											0.44	0.54
15R												0.37
16R												

Primers *Mse* + *Eco*

When all the data of Eco-ACA and Eco-AAC with the respective different *Mse*-primer combinations were combined (Table 3.11), it was found that the female lines 1A and 4A (0.29) were closely related. The highest amount of dissimilarity was found between 1A and 6A (0.44). The male lines 12R and 15R (0.30) were closely related, but 14R and 15R (0.45) showed the highest amount of dissimilarity. The F1 progeny showed that 4A and 15R (0.22) were closely related. The highest amount of dissimilarity was found between 6A and 11R (0.43). According to the genetic distances 11R should therefore be crossed with 6A to obtain maximum heterosis.

Table 3.11 The combined genetic distances of the six primer combinations for the female, male and F1 hybrid lines

	1A	2A	3A	4A	5A	6A	11R	12R	13R	14R	15R	16R
1A		0.41	0.37	0.29	0.33	0.44	0.37	0.37	0.35	0.31	0.28	0.32
2A			0.34	0.32	0.31	0.34	0.32	0.34	0.34	0.29	0.23	0.28
3A				0.39	0.39	0.42	0.33	0.37	0.35	0.27	0.23	0.32
4A					0.40	0.31	0.26	0.30	0.26	0.27	0.22	0.28
5A						0.33	0.28	0.33	0.29	0.33	0.27	0.32
6A							0.43	0.42	0.38	0.33	0.30	0.36
11R								0.36	0.38	0.34	0.33	0.36
12R									0.35	0.33	0.30	0.32
13R										0.37	0.36	0.36
14R											0.45	0.44
15R												0.35
16R												

Discussion

If one compare the genetic distances of all the *Mse* + *Eco* primer combinations (Table 3.3 to 3.11) the following is observed:

1. *Mse*-CTT (Table 3.3) and *Mse*-CAG (Table 3.4) with *Eco*-ACA showed that 1A and 4A were closely related and 1A and 6A had the highest amount of dissimilarity of the female lines. However, *Mse*-CTC + *Eco*-ACA (Table 3.5) showed that 1A and 4A had the highest amount of dissimilarity that is in direct contrast with the first two primer combinations. When the combined data of *Eco*-ACA with three *Mse*-primers (Table 3.6) was analyzed, it showed that 2A and 5A were very similar as was found with *Mse*-CTC + *Eco*-ACA. The highest amount of dissimilarity in this combination was also 1A and 6A. It is thus possible to use *Mse*-CTT + *Eco*-ACA or *Mse*-CAG + *Eco*-ACA or the combined data of *Eco*-ACA with the three *Mse*-primers to determine dissimilarity between female lines.
2. When the different primer combinations were used to compare the genetic distances of the male lines, *Mse*-CTT + *Eco*-ACA showed that 11R and 13R were very similar, but it was in contrast with *Mse*-CAG + *Eco*-ACA that showed that these two lines had the highest amount of dissimilarity. The combined data of *Eco*-ACA with the three *Mse*-primers and *Mse*-CTC + *Eco*-ACA showed that 12R and 14R were closely related, while the combined data of *Eco*-ACA and the three *Mse*-primers and *Mse*-CTT + *Eco*-ACA showed that 13R and 16R had the highest amount of dissimilarity.
3. Different results were found when the *Mse-Eco* primer combinations were compared in the F1, and it was not possible to determine which lines would give the least amount of heterosis. The combined data of *Eco*-ACA with the three *Mse*-primers and *Mse*-CTT + *Eco*-ACA however, showed that when 6A and 12R were crossed, the highest amount of heterosis could be expected.
4. *Mse*-CTC + *Eco*-AAC and the combined data of *Eco*-AAC with the three *Mse*-primers gave the same results, except for the least amount of heterosis in the F1 hybrid. These combinations showed that 1A and 4A were very similar, while 4A and 5A had the highest amount of dissimilarity of the female lines. The male lines 12R and 15R were found to be

similar. However, the combined data of *Eco*-AAC with the three *Mse*-primers showed that 14R and 16R had the highest amount of dissimilarity, while *Mse*-CTC + *Eco*-AAC found these lines to be identical. Both these two combinations and *Mse*-CAC + *Eco*-AAC showed that if one cross 6A with 11R one would obtain the highest amount of heterosis.

5. The combined analysis of genetic distances of all the *Eco* and *Mse* primers found that 1A and 4A were very similar as was found in *Mse*-CTT + *Eco*-ACA, *Mse*-CAG + *Eco*-ACA and *Mse*-CTC + *Eco*-AAC. It was thus also in direct contrast with the results of *Mse*-CTC + *Eco*-ACA that found these two lines highly dissimilar. The combined analysis also showed that 1A and 6A had the highest amount of dissimilarity as was found with *Mse*-CTT + *Eco*-ACA, *Mse*-CAG + *Eco*-ACA and *Mse*-CAG + *Eco*-AAC. The combined analysis showed that the male lines 12R and 15R were very similar as was found in *Mse*-CTC + *Eco*-ACA and *Mse*-CTC + *Eco*-AAC. No primer combination gave the same results as the combined analysis to the highest amount of dissimilarity between the male lines. The combined analysis showed that a cross between 6A and 11R would give the highest amount of heterosis as was found in *Mse*-CAC + *Eco*-AAC and *Mse*-CTC + *Eco*-AAC. The least amount of heterosis would be found when 4A was crossed with 15R as was found in *Mse*-CAG + *Eco*-ACA.

It can therefore be concluded, that:

1. *Mse*-CTT + *Eco*-ACA, *Mse*-CAG + *Eco*-ACA and *Mse*-CTC + *Eco*-AAC could be used to identify similarity in female lines, while *Mse*-CTT + *Eco*-ACA, *Mse*-CAG + *Eco*-ACA and *Mse*-CAG + *Eco*-AAC would identify, the same lines with the highest amount of dissimilarity.
2. *Mse*-CTC + *Eco*-ACA and *Mse*-CTC + *Eco*-AAC could be used to identify similarity in male lines, while *Mse*-CTC + *Eco*-ACA and *Mse*-CAG + *Eco*-AAC would identify the male lines with the highest amount of dissimilarity.
3. *Mse*-CAG with the two *Eco*-primers or *Mse*-CTC with the two *Eco*-primers will identify similarity in F1 hybrids.
4. *Mse*-CAG + *Eco*-AAC and *Mse*-CTC + *Eco*-AAC will identify the same lines that will give maximum heterosis.

3.3.2 Dendrograms

Primers *Mse*-CTT + *Eco*-ACA

According to the dendrogram for *Mse*-CTT + *Eco*-ACA (Figure 3.1) the lines were grouped into two main clusters. Cluster 1 consisted of lines 4A and 3A. Cluster 2 was divided into two groups. Group A consisted of 15R, 14R, 11R and 5A. Group B was further divided into two subgroups. Subgroup a consisted of 16R, 13R and 2A, while subgroup b consisted of 12R, 6A and 1A.

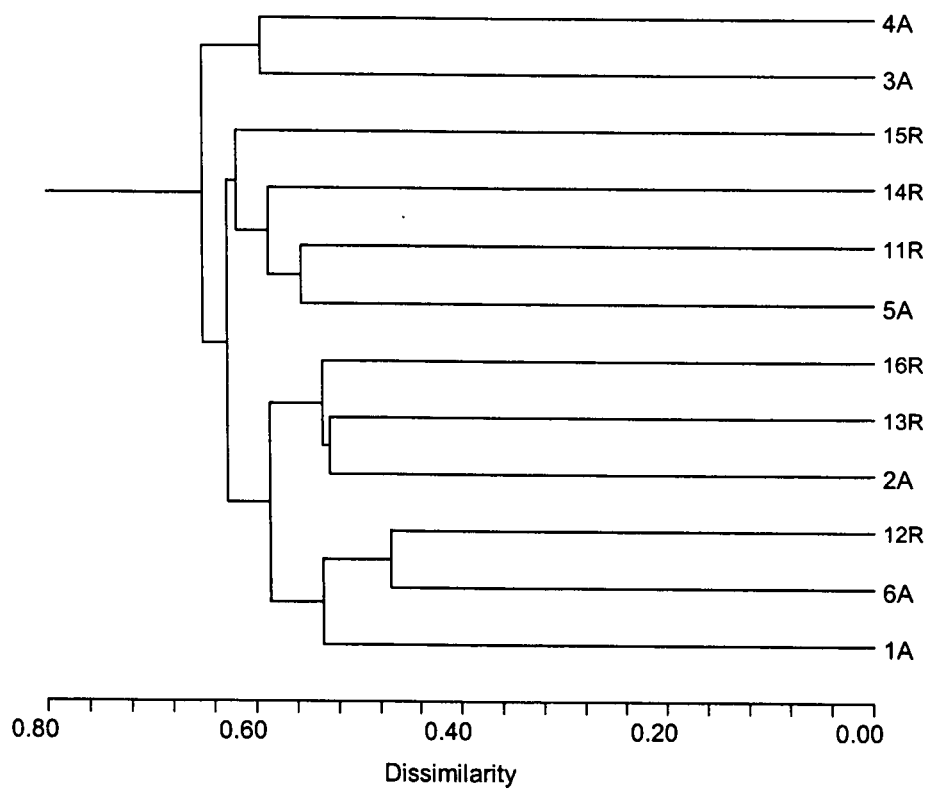

Figure 3.1 Dendrogram of primer combination *Mse*-CTT + *Eco*-ACA for male and female lines

Primers *Mse*-CAG + *Eco*-ACA

Figure 3.2 showed that the lines were grouped into two clusters when *Mse*-CAG + *Eco*-ACA primers were used. Cluster 1 consisted of 5A, 4A and 2A. Cluster 2 was divided into two groups. Group A consisted of 16R, 14R, 13R and 11R. Group B was again divided into two subgroups. Subgroup a consisted of 12R and 3A, and subgroup b consisted of 15R, 6A and 1A.

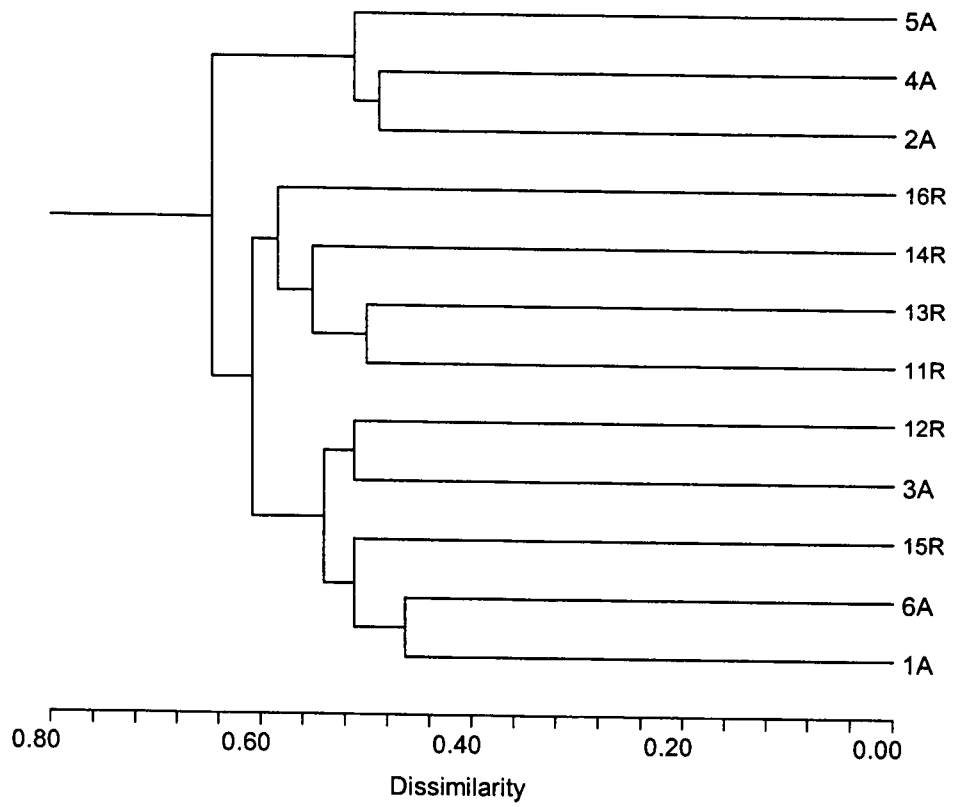

Figure 3.2 Dendrogram of primer combination *Mse*-CAG + *Eco*-ACA for male and female lines

Primers *Mse*-CTC + *Eco*-ACA

According to *Mse*-CTC + *Eco*-ACA (Figure 3.3) all the lines were grouped into two clusters. Cluster 1 consisted of only 2A, while cluster 2 was divided into three groups. Group A consisted of 15R, 14R and 13R. Group B consisted of 12R, 11R and 6A, while group C consisted of 5A, 3A, 16R, 4A and 1A. Group C could further be divided into three subgroups, where subgroup a consisted of 5A, subgroup b consisted of 3A and subgroup c consisted of 16R, 4A and 1A.

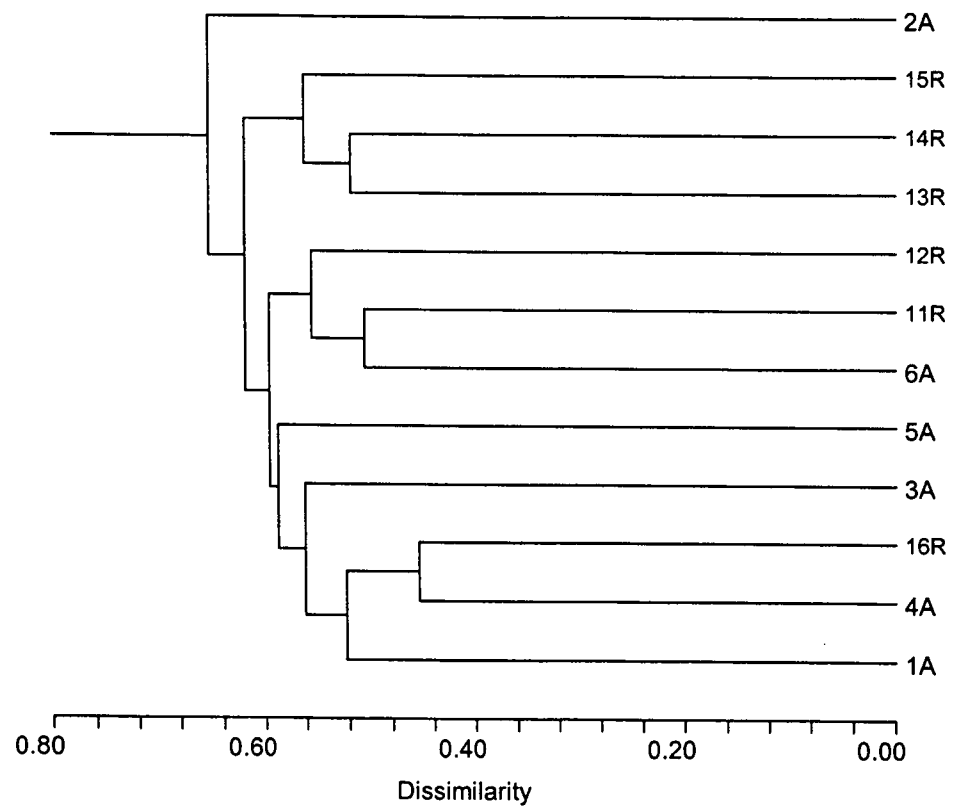

Figure 3.3 Dendrogram of primer combination *Mse*-CTC + *Eco*-ACA for male and female lines

Primers *Mse* + *Eco*-ACA

The dendrogram for the combined *Eco*-ACA combinations with the three *Mse*-primers (Figure 3.4) showed that the lines were grouped into two clusters. Cluster 1 was divided into two groups. Group A consisted of 14R, 16R and 13R. Group B consisted of 15R and 11R. Cluster 2 was also divided into two groups. Group C consisted of 5A and 4A. Group D was divided into three subgroups. Subgroup a consisted of 2A, subgroup b of 3A and subgroup c of 12R, 6A and 1A.

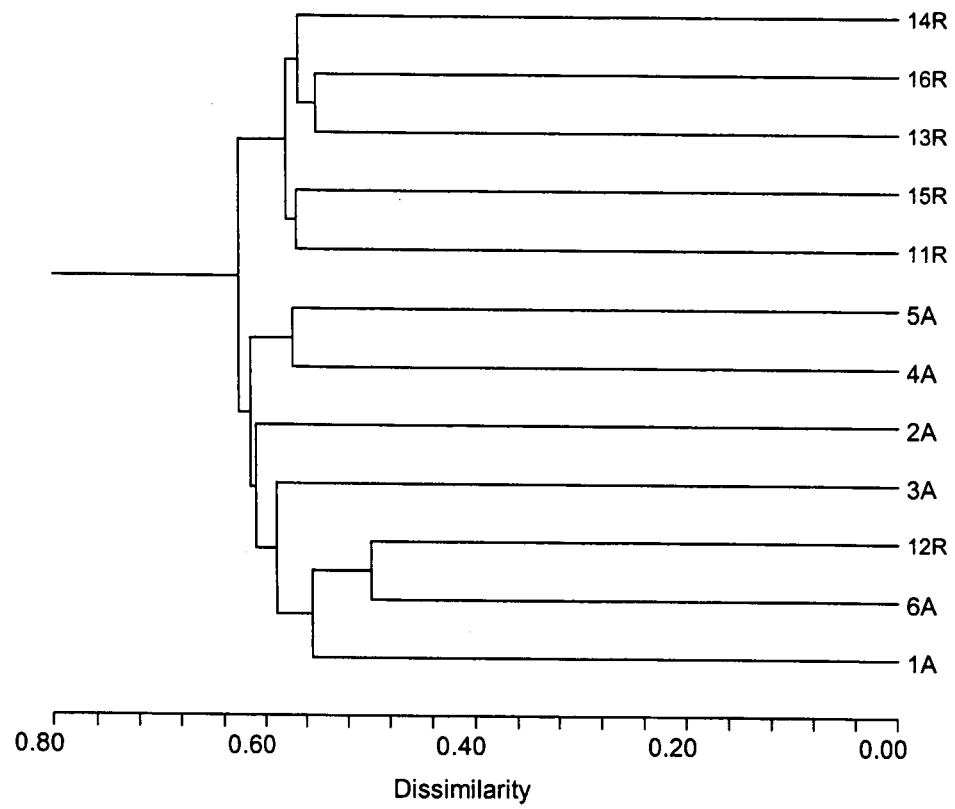

Figure 3.4 Dendrogram of the combined *Eco*-ACA combinations with the three *Mse*-primers for the male and female lines

Primers *Mse*-CTT + *Eco*-AAC

Figure 3.5 showed that the lines were grouped into two main clusters with *Mse*-CTT + *Eco*-AAC. Cluster 1 was divided into two groups. Group A consisted of 16R, 15R, 14R, 13R and 11R and could again be divided into two subgroups. Subgroup a consisted of 16R and 15R and subgroup b consisted of 14R and 13R. Group B consisted of 11R. Cluster 2 was also divided into two groups. Group C consisted only of 5A. Group D consisted of 6A, 4A, 3A, 12R, 2A and 1A, and was again divided into two subgroups. Subgroup c consisted of 6A, 4A and 3A, while subgroup d consisted of 12R, 2A and 1A.

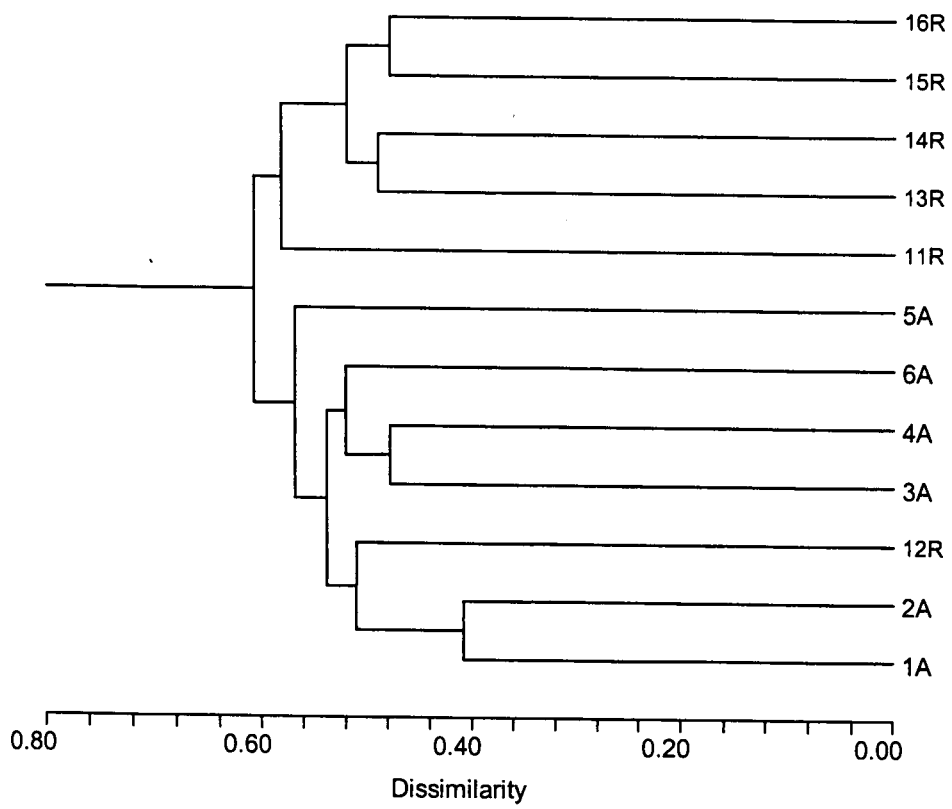

Figure 3.5 Dendrogram of primer combination *Mse*-CTT + *Eco*-AAC for male and female lines

Primers *Mse*-CAG + *Eco*-AAC

According to the dendrogram for *Mse*-CAG + *Eco*-AAC (Figure 3.6) the lines were grouped into two main clusters. Cluster 1 was divided into three groups. Group A consisted of 12R, group B consisted of 3A and group C was divided into two subgroups. Subgroup a consisted of 5A and 4A, and subgroup b consisted of 15R, 14R and 2A. Cluster 2 was divided into two groups. Group D consisted of 13R and group E could be divided into two subgroups. Subgroup c consisted of 16R and 11R, and subgroup d consisted of 6A and 1A.

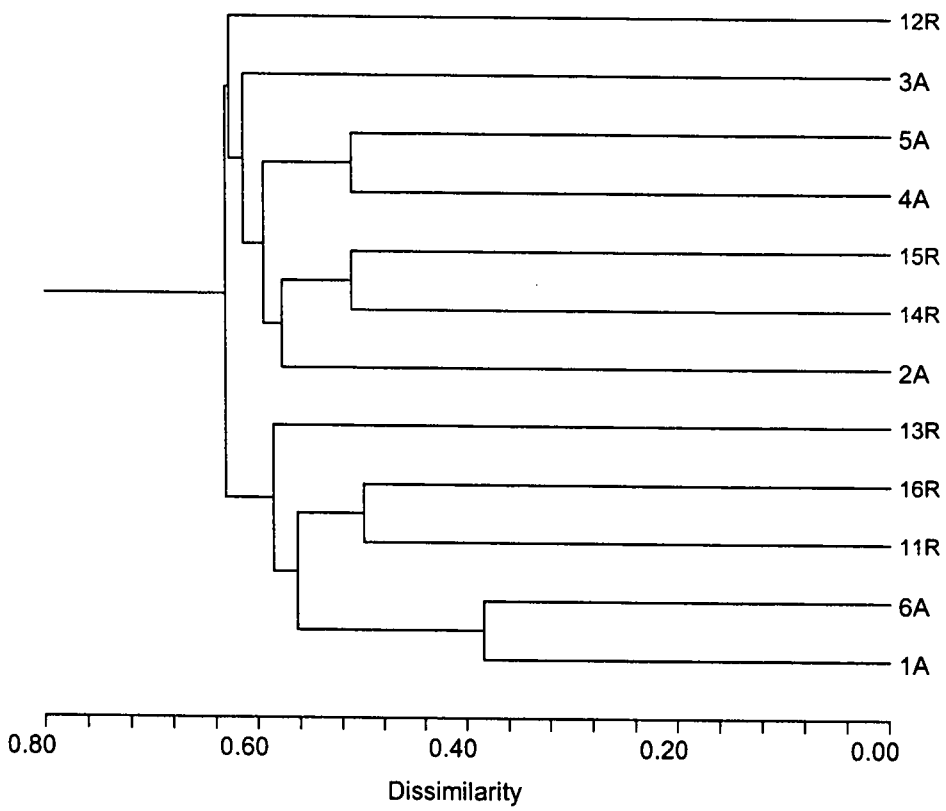

Figure 3.6 Dendrogram of primer combination *Mse*-CAG + *Eco*-AAC for male and female lines

Primers *Mse*-CTC + Eco-*ACA*]

According to Figure 3.7 the lines were grouped into two clusters for *Mse*-CTC + *Eco*-ACA. Cluster 1 was divided into two groups. Group A consisted of 15R, 16R, 14R and 13R and was further divided into two subgroups. Subgroup a consisted of 15R, 16R and 14R, and subgroup b consisted of 13R. It is again important to notice that 16R and 14R were identical. Group B was divided into 3 subgroups. Subgroup a consisted of 11R and 6A, subgroup b consisted of 5A, 4A and 3A, and subgroup c of 12R and 2A. Cluster 2 consisted only of 1A.

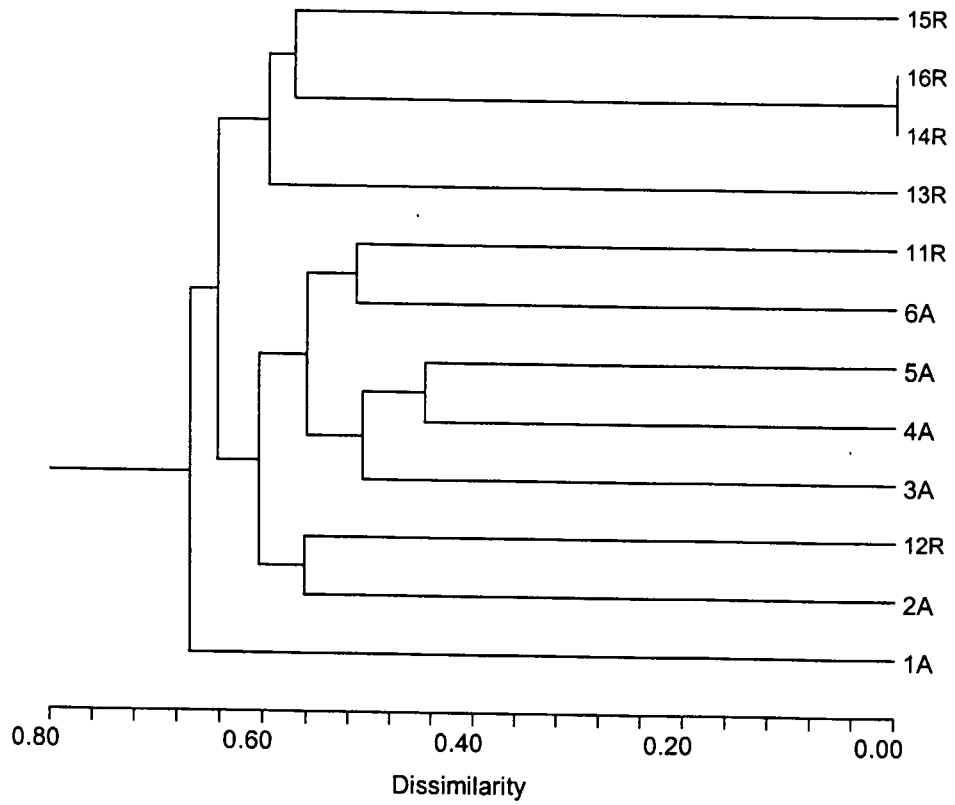

Figure 3.7 Dendrogram of primer combination *Mse*-CTC + *Eco*-AAC for male and female lines

Primers *Mse* + *Eco*-AAC

The dendrogram for all the *Eco*-AAC combinations with the 3 *Mse*-primers (Figure 3.8) showed that the lines were grouped into two clusters. Cluster 1 consisted of 15R, 16R and 14R. Cluster 2 was divided into two groups. Group A consisted of 5A, 4A and 3A. Group B was further divided into two subgroups. Subgroup a consisted of 12R, 13R, 11R and 6A, and subgroup b consisted of 2A and 1A.

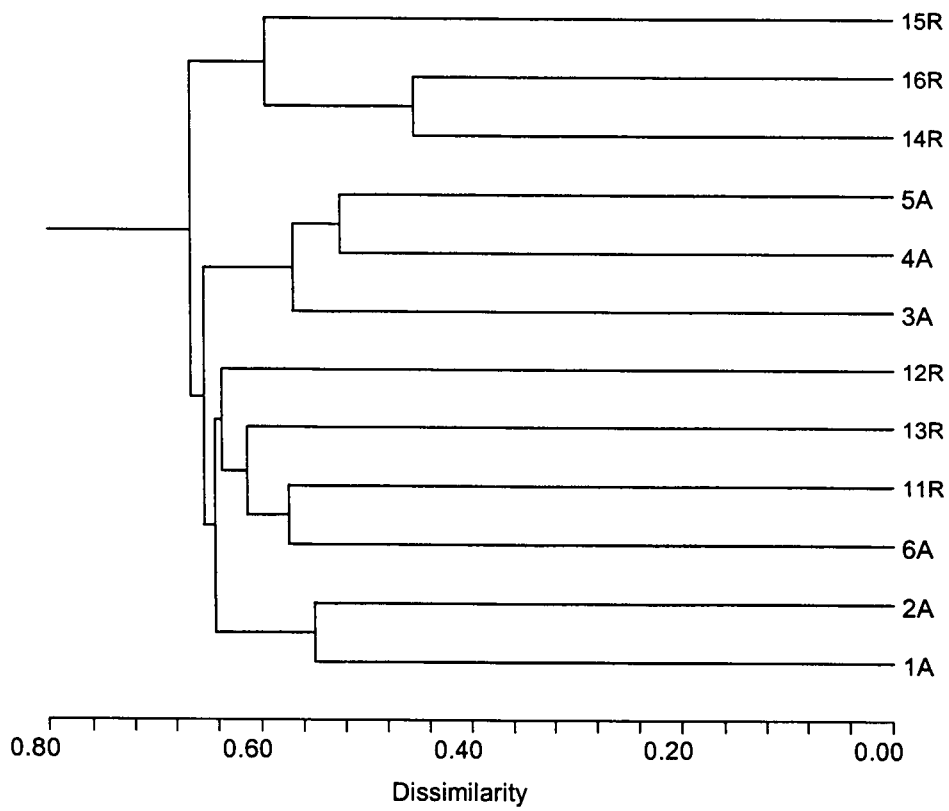

Figure 3.8 Dendrogram of all the *Eco*-AAC combinations with the three *Mse*-primers for the male and female lines.

Primers *Mse* + *Eco*

One combined dendrogram for the six different primer combinations (Figure 3.9) showed that the lines were grouped into two clusters. Cluster 1 consisted of 16R, 15R and 14R. Cluster 2 was divided into two groups. Group A was divided into two subgroups. Subgroup a consisted of 5A and 4A, and subgroup b consisted of 3A. Group B was divided into five subgroups. Subgroup c consisted of 2A, subgroup d consisted of 13R, subgroup e of 12R, subgroup f consisted of 11R and subgroup g of 6A and 1A.

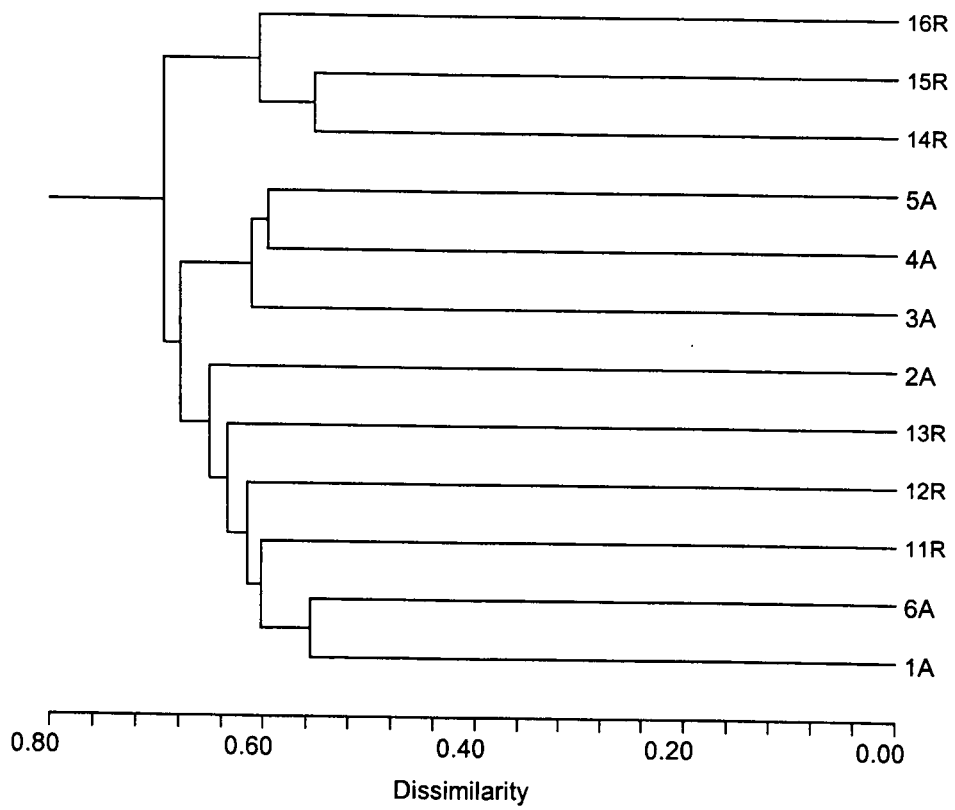

Figure 3.9 Combined dendrogram of the six primer combinations for the male and female lines

Discussion

The male and female lines were clustered into separate groups and subgroups. Hongtrakul *et al* (1997) found that B-lines and R-lines were also separated into two groups. They concluded that these groupings illustrated the breeding history and basic heterotic pattern of sunflower. Subgroups within these groups may however, represent unique heterotic groups and create a basis for formally describing heterotic patterns in sunflower.

The following is observed if one compares the dendrograms of the *Mse* + *Eco*-primer combinations (Figure 3.1 to 3.9):

1. The combined dendrogram of *Eco*-ACA with the three *Mse*-primers (Figure 3.4) separated the male and female lines. All the male lines were grouped together as one cluster. The female lines were grouped as a different cluster. The only exception was 12R that was found between the female lines. Dendrograms that were similar to the combined dendrogram of *Eco*-ACA were *Mse*-CTC + *Eco*-ACA (Figure 3.3), *Mse*-CTT + *Eco*-AAC (Figure 3.5) and *Mse*-CTC + *Eco*-AAC (Figure 3.7).
2. The combined dendrogram of *Eco*-AAC with the three *Mse*-primers (Figure 3.8) also separated the male and female lines from each other. The male and female lines were grouped into four groups, in the order male, female, male, female. No other combination of *Mse* and *Eco*-primers gave similar results than this combined dendrogram.
3. The combined dendrogram of all the *Mse* + *Eco*-primers (Figure 3.9) also separated the male and female lines into four groups. The only dendrogram that gave the same results as the combined *Mse* + *Eco*-primer dendrogram, is the combined *Eco*-AAC dendrogram (Figure 3.8).

According to the combined dendrogram of all the *Mse* + *Eco* primers (Figure 3.9) and the combined dendrogram of *Eco*-AAC with the three *Mse*-primers (Figure 3.8), it can be concluded that if 14R, 15R or 16R are crossed with 1A or 6A, one can expect maximum heterosis.

CHAPTER 4
HYBRID BREEDING IN SUNFLOWER

4.1 Introduction

Plant breeders enhance yield in sunflower simply by selecting for hybrids with higher yields. This can only be done by improving the amount of hybrid vigour produced by inbred lines.

Every year breeders produce a multitude of potentially useful crosses and evaluate their varietal ability by testing either the selfed progenies or doubled haploid lines in field experiments. The availability of adapted elite varieties of different genetic backgrounds allows the farmer to hedge the risk of reduced performance by any one variety under unpredictable environmental stress conditions (Bohn *et al*, 1999).

Hybrid breeding in sunflower normally involves the development of pure lines through inbreeding, followed by selection among these lines for the maximum expression of heterosis when crossed. Hybrids are the first generation offspring of a cross between parents with contrasting genotypes. When the F1 hybrids are better than the parents the occurrence of hybrid vigour and heterosis is obvious (Allard, 1960; Fick, 1978, Weiss, 2000).

Heterosis in sunflower has been observed for seed yield, time to bloom, plant height, head diameter, seed weight and oil percentage. Therefore, heterosis is one of the driving forces behind the hybrid seed industry in cultivated sunflower. The effect of hybrid vigour in plants are observed in many ways, for example higher yield, improved vigour, plant height, oil percentage and 1000-seed weight (Putt, 1966; Fick and Zimmer, 1974; Putt and Dorrell, 1975).

If breeders could predict the potential of crosses for line development before producing and testing lines derived from them in field trials, it would increase

the efficiency of breeding programs by concentrating the efforts on the most promising crosses. One strategy used in breeding programs to predict the prospects of crosses for line development, is based on the assumption that the specific combining ability (SCA) expressed by a hybrid is related to the genetic distance between its parental lines (Bohn *et al*, 1999).

The aim of this study was:

1) To determine the combining ability of the inbred lines,
2) To determine if there was a correlation between the different characteristics measured,
3) To determine the expression of heterosis for the different characteristics.

4.2 Materials and Methods

4.2.1 Inbred lines

A total of six female sunflower inbred lines (lines) and six male sunflower inbred lines (testers), developed in a hybrid breeding program of a private seed company, were used as parental inbreds in this study. No detail regarding the source or pedigree of the genotypes is known. These materials were chosen due to their unique characteristics, heterotic grouping and their maturity.

The agronomic characteristics of the six lines and six testers shown in Table 4.1.

Table 4.1 The agronomic characteristics of the six lines and six testers

	Agronomic characteristics
Lines	
1A	Is a single headed line with a medium sized, slightly convex head that is hollow at the back of the head. Plants are tall (approximately 1.67m) and it flowered last of the six lines. A disk floret abnormality occurred in 4 of the 28 plants.
2A	Is a single headed line with a medium sized, concave head. The back of head is flat and the plants are tall (approximately 1.43m).
3A	Is a single headed line with a medium sized, convex head where the back of the head forms a deep hollow. This line is short (approximately 1.30m) and it flowered first of the six lines.
4A	Is a branching line with more than one head. The heads are small, concave, and slightly hollowed at the back of the head. This is a short line (approximately 1.28m).
5A	Is a single headed line with a medium sized, concave head. The back of the head is flat and this line is very short (approximately 1.09m).
6A	Is a single headed line with a large, slightly convex head. The back of the head is very hollow and the plant is tall (approximately 1.48m).
Testers	All the testers are branching types.
11R	Small heads, 1.30m tall and it flowered second of the testers.
12R	Small heads, 1.09m tall and it flowered fourth of the testers.
13R	Small heads, very tall (2.15m) and it flowered last of the six testers
14R	Small heads, 1.28m tall and it flowered first of the testers
15R	Small heads, 1.39m tall and it flowered third of the testers.
16R	Small heads, 1.40m tall and it flowered fifth of the six testers. The heads provided little pollen.

4.3 Crossing block

The seed of the parental inbred lines were germinated in petri dishes. After germination, it was planted in 3l pots in a greenhouse at the University of the Free State, in Bloemfontein in October 2000. To synchronize pollination, the planting of the male plants was replicated twice with a 10-day interval. A total of 28 pots of each female line and 14 pots of each male line (seven pots per planting) were planted. Three seeds were planted in each pot and it was later thinned out to one plant per pot.

At planting, Curaterr (10 GR) and N:P:K fertilizer [3:2:1(25)+0.5 Zn] were mixed with the soil to enhance the growth development of the plants. Every second week Chemicult (Hydroponic nutrient powder) was given to the plants. At 1m plant height the fertilizer was changed to KAN. The temperature in the glasshouse was maintained at 27°C during the day and night.

Plants were watered regularly. Aphids and red spider mite infestation were controlled by spraying the plants every two weeks, alternatively with Talstar TAB 5,0% or Wonder Red Spiderspray. The heads were covered with transparent plastic bags to prevent pollen contamination.

Pollination was done, by transferring the pollen from the male plants to the female plants. All six lines (1A, 2A, 3A...) were individually crossed with each tester (11R, 12R, 13R...) to produce F1 hybrid seed (Table 4.2). The pollinated heads were then covered with the same bag to prevent unnecessary pollination from other plants. After maturity the F1 hybrid seeds of each individual cross were harvested and threshed out separately by hand. Thirty six crosses were made and sufficient seed was generated to plant three trials.

Table 4.2 Crossing block of the six lines by six testers used to develop F1 hybrids

Entry No.	Lines	Testers	F1 Hybrids
1	1A	11R	1Ax11R
2	1A	12R	1Ax12R
3	1A	13R	1Ax13R
4	1A	14R	1Ax14R
5	1A	15R	1Ax15R
6	1A	16R	1Ax16R
7	2A	11R	2Ax11R
8	2A	12R	2Ax12R
9	2A	13R	2Ax13R
10	2A	14R	2Ax14R
11	2A	15R	2Ax15R
12	2A	16R	2Ax16R
13	3A	11R	3Ax11R
14	3A	12R	3Ax12R
15	3A	13R	3Ax13R
16	3A	14R	3Ax14R
17	3A	15R	3Ax15R
18	3A	16R	3Ax16R
19	4A	11R	4Ax11R
20	4A	12R	4Ax12R
21	4A	13R	4Ax13R
22	4A	14R	4Ax14R
23	4A	15R	4Ax15R
24	4A	16R	4Ax16R
25	5A	11R	5Ax11R
26	5A	12R	5Ax12R
27	5A	13R	5Ax13R
28	5A	14R	5Ax14R
29	5A	15R	5Ax15R

Table 4.2 (Continued)

Entry No.	Lines	Testers	F1 Hybrids
30	5A	16R	5Ax16R
31	6A	11R	6Ax11R
32	6A	12R	6Ax12R
33	6A	13R	6Ax13R
34	6A	14R	6Ax14R
35	6A	15R	6Ax15R
36	6A	16R	6Ax16R

The cross between 4A and 16R gave a sterile hybrid (4Ax16R) with no seed. Therefore, 4Ax16R was not used due to a lack of seed, but it was replaced with a standard. The standard was HV3037 and it was planted under the hybrid name 4Ax16R. Therefore, the results given under 4Ax16R are those of HV3037.

4.4 Trials

The F1 hybrid material was planted according to a randomized complete block design with three replications. Three trials were planted on the farm Doornpan, in Bainsvlei, Bloemfontein, on a Bainsvlei soil type. One trial was planted on 20 December 2001 and the other two trials on 28 December 2001. There were no other crops planted previously on this plot.

The plots were ploughed with a tandem disc plough 50 days before planting. Two days before planting N:P:K fertilizer [3:2:1(25)+0.5 Zn] was worked in.

Each plot consisted of two 5m rows of each line and tester, with 90cm intra row spacing and 20cm inter row spacing. Plots were planted with 25 seeds per row. This produced an average plant density of approximately 20 000 plants per ha.

Two guard rows were planted around the trials, but not between the plots. To ensure population density and even distribution of plants in the plot, double sowing and thinning out at the three to five leaf stage were done. Thinning out to two plants per hole occurred 14 days after planting. One week later (21 days), the weaker of the two plants was also removed.

No irrigation was necessary due to the high rainfall during the growing season. The plants were hand weeded 21 and 50 days after sowing.

Weather conditions were obtained from the nearest weather station. The average minimum and maximum temperature and average rainfall data per month received at Doornpan for the 2001/2002 growing season are presented in Table 4.3.

Table 4.3 Climatological data for the average minimum and maximum temperatures and rainfall (mm) at Doornpan in the 2001/2002 growing season

	Oct 2001	Nov 2001	Dec 2001	Jan 2002	Feb 2002	Mar 2002	April 2002
Av. Min. Temp. (°C)	12.0	13.2	14.1	14.7	15.3	14.1	10.4
Av. Max. Temp. (°C)	26.6	24.9	26.8	28.3	29.0	28.2	27.1
Av. Rainfall (mm)	46.2	166.3	102.0	158.1	36.6	26.8	25.9

After maturity, each plant was harvested and threshed out separately by hand. Kernel samples were taken from each plant and it was analyzed for various characteristics.

4.5 Characteristics measured

The following characteristics were measured as described by Miller *et al* (1980):

Plant height: The distance in centimeters between the soil surface and stem attachment to the capitulum. The mean of 10 measurements/plot was calculated.

Flowering date: Number of days from planting to the date when 50% of the heads/plot had started anthesis.

Head diameter: Was measured with a measuring tape and the mean of 10 measurements/plot was calculated in centimeters.

1000-Seed weight: A random sample of seeds, expressed as g/1000 seeds.

Yield: The mean yield of 10 heads/plot was calculated and expressed in t/ha.

Oil percentage: Determined by nuclear magnetic resonance (NMR) on a random sample of approximately 10g of seed. It was expressed as a percentage (10% moisture basis).

4.6 Statistical analysis

A range of statistical analyses using the statistical software computer program, Microsoft EXCELL and AGROBASE 20 were calculated.

4.6.1 Analysis of variance (ANOVA)

Analysis of variance is an arithmetric technique by which total variation presented in a set of data is partitioned into different components. The ordinary factorial analysis of variance for single data was analyzed separately for each trial using the AGROBASE 20 Computer program. Differences among significant treatment means were separated using least significant differences (LSD) at $P \leq 0.05$. These means were summarized in the histogram charts form. The charts were drawn using the Microsoft EXCELL computer program.

4.6.2 Genetic analysis

Genetic parameters were calculated using the Line x Tester analysis. Line x Tester analysis is a type of diallel analysis, that is used in hybrid breeding programs, where new lines are tested against known testers, to see how well they will perform in hybrid combinations. This is a type of diallel analysis where general- and specific combining ability and other genetic parameters are calculated for lines in a hybrid breeding program. Specific testers are tested against specific lines.

The components of variance of the ANOVA were interpreted genetically by translating them into covariance of relatives based on a factorial model (Singh and Chaudhary, 1979; Wricke and Weber, 1986). The statistical model for the ANOVA was:

$$Y_{hijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + R_h + \varepsilon_{hijk}$$

Where: Y_{hijk} = the observation of the k-th full sib progeny in a plot in h-th replication of the i-th paternal plant and the j-th maternal plant.

$M\mu$ (μ) = common to all observations,

α_i = the effect of the i-th paternal plant,

β_j = the effect of the j-th maternal plant,

$(\alpha\beta)_{ij}$ = the interaction of the paternal and maternal plants,

R_h = the effect of the h-th replication, and

ε_{hijk} = the environmental effect and reminder of the genetic effect between full sibs on the sample plot.

All effects are random (except for replication), normal independent with exceptions equal to zero (Becker, 1985).

The analysis of variance consisted of two variance components, which estimate the covariance between half sibs, one from the sample of lines, and

one from the samples of testers. These estimates might differ due to maternal effects (Wricke and Weber, 1986).

4.6.2.1 Combining ability effects

Combining ability is the ability of a parent to produce inferior or superior combinations in one or a series of crosses (Chaudhary, 1982). A line with good or high combining ability values for traits of economic importance can be selected to improve these traits.

GCA effects

Poehlman (1987) defined general combining ability (GCA) as the average performance of a line in a hybrid combination, and as such, GCA is recognized as primarily a measure of additive gene action. The GCA value of a genotype determines its crossing value. Falconer and Mackay (1996) defined GCA as the mean performance of the line in all crosses.

The GCA of the lines and testers was computed from a Line x Tester analysis using the AGROBASE 20 computer program. The GCA estimates for lines and testers for all characters were calculated to select the best line and tester to use for hybrid breeding. The GCA estimates for the lines (g_i) and testers (g_t) was calculated as follow:

a) Lines (g_i)

$$g_i = x_i.../tr - x../ltr$$

Where: l = number of lines
t = number of testers
r = number of replications

Standard error (SE) for g_i effects

$$SE \text{ (GCA for lines)} = (Me/r \times t)^{1/2}$$

Where: Me = error mean square

b) Testers (g_t)

$$g_t = x.j./lr - x.../ltr$$

Standard error (SE) for g_t effects

$$S.E.(GCA\ for\ tester) = (Me/r \times l)½$$

The LSD between GCA was calculated as:

$$LSD = q\alpha;\ t,f.\ S^2\sqrt{E/r} \qquad (t = 0.5)$$

Where: $q\alpha;t,f$ = α value at t treatment's degree of freedom and error's degree of freedom

SCA effects

Poehlman (1987) defined specific combining ability (SCA) as the performance of specific combinations of genetic strains in crosses in relation to the average performance of all combinations and as an estimate of the effects of non-additive gene action. Falconer and Mackay (1996) described SCA as the deviation to a greater or lesser extent from the expected value of any particular cross, which is the sum of the general combining abilities of its two parental lines.

The SCA estimates for crosses were also calculated. This shows the minimum and maximum genetic gain of hybrids from certain lines by certain testers. The SCA effects estimation (S_{ij}) for crosses was calculated as follow:

SCA effects (S_{ij}):

$$S_{ij} = x_{ij}/r - xj../tr - x.j./lr + x.../ltr$$

Standard error (SE) for S_{ij} effects

$$SE\ (SCA\ effects) = (Me/r)½$$

The LSD between SCA effects was calculated as:

$$LSD = q\alpha;t,f.\ S^2\sqrt{E/r} \qquad (t = 0.5)$$

Where: $q\alpha;t,f =$ α value at t treatment's degree of freedom and error's degree of freedom.

4.6.2.2 General combining ability (GCA): Specific combining ability (SCA) ratio

The GCA:SCA ratio was calculated to study the performance of the effects and to assess the relative importance of additive gene or non-additive gene effects. The ratio indicates whether a character is mainly controlled by additive or non-additive gene action (Singh and Chaudhary, 1979). The GCA:SCA ratio was calculated from the estimates of genetic components of the Line x Tester analysis of variance, as the ratio of sum of additive genetic variances to the dominance genetic variance ($\sigma^2_A : \sigma^2_D$). A high ratio indicates additive gene action, while a low ratio indicates specific gene action i.e. non-additive gene action.

4.6.3 Correlation coefficient

4.6.3.1 Genetic correlation

Genetic correlations (r_A) can be obtained according to the formula of Falconer and Mackay (1996):

$$r_A = COV_{xy}/\sqrt{(Var_x\ Var_y)}$$

Where: COV_{xy} = covariance of the characteristic x and y
Var_x = variance of character x
Var_y = variance of character y

Simple genetic correlation between characteristics was calculated from GCA effects estimates of crosses, using AGROBASE 20 sub-menu statistic command Corr. The analysis provided both positive and negative correlation coefficient estimates together with their probabilities, such that a probability

near zero indicates a significant correlation, and near 1.00 indicates no correlation (AGROBASE, 2000).

4.6.4 Heritability

Heritability (h^2) is defined as the ratio of the genotypic variance (σ^2_g) to the phenotypic variance (σ^2_p)(Wricke and Weber, 1986; Fehr, 1987). The genotypic variance is thus the variation by genetic differences among individuals. Heritability can be expressed in a broad-sense or a narrow-sense. Broad-sense heritability expresses the extent to which an individual's phenotypes are determined by their genotypes. Therefore, broad-sense heritability is estimated from the ratio of the total genetic variance to the phenotypic variance. Narrow-sense heritability expresses the extent to which phenotypes are determined by the genes transmitted from the parents. Narrow-sense heritabilities are estimated from the ratio of the additive portion of the genetic variance to the phenotypic variance. Heritabilities were calculated from genetic components of the Line x Tester analysis, using the AGROBASE 20 computer program.

Broad-sense heritability was calculated from the formula:

$$h^2 = \sigma^2_A + \sigma^2_D /(\sigma^2_A + \sigma^2_D + MSE_{GCA})$$

Narrow-sense heritability was calculated from the formula:

$$h^2 = \sigma^2_A /(\sigma^2_A + \sigma^2_D + MSE_{GCA})$$

Where: σ^2_A = additive genetic variance

σ^2_D = dominance genetic variance

MSE_{GCA} = mean square error

4.6.5 Heterosis

Heterosis is a function of the degree of dominance and the difference in gene frequency between the parent lines. The level of heterosis was determined

for all the characteristics measured. Two types of heterosis were calculated based on the mean values of the genotypes.

Mid-parent heterosis

This is measured as the deviation of the offspring from the mid-parent value, often expressed as a percentage of mid-parent value. Mid-parent heterosis can be calculated from the formula:

$$H_{F1} = \frac{(F1 - MP)}{MP} \times 100\%$$

Where: H_{F1} = heterosis for F1 cross
F1 = mean value of F1 cross
MP = mean mid-parent value

High parent heterosis

This was calculated from the mean values of the F1 cross and high parent, using the formula:

$$H_{F1} = \frac{(F1 - HP)}{HP} \times 100\%$$

Where: H_{F1} = heterosis for F1 cross
F1 = mean value of F1 cross
HP = mean value of high parent

4.7 Results and discussion

4.7.1 Analysis of variance

The results of the analysis of variance for the agronomic characteristics are given in Table 4.4. The mean squares for the agronomic characteristics of all the parents were significantly different. Significant differences were found between the crosses as well as all the lines for all the characteristics

measured. The testers that were used were significantly different for all the characteristics, except for head diameter. Significant differences were also found for the Line x Testers.

Table 4.4 Analysis of variance for agronomic characteristics

Source	Df	Plant height	Flowering date	Head diameter	1000-Seed weight	Yield	Oil %
Replications	2	0.06	19.68	12.10**	77.63**	0.054	17.96*
Treatments	47	17.13**	2381.31	3431.89**	11236.36**	86.65**	774.04**
Parents	11	2.65**	625.64**	240.97**	3506.76**	6.99**	112.73**
Crosses	35	5.51**	1478.55**	118.92**	2486.49**	13.49**	597.53**
Par.vs crosses	1	8.93**	277.12**	3072.00**	5243.11**	66.18**	63.79**
Lines	5	1.66**	253.38**	37.42**	1112.77**	0.87*	211.99**
Testers	5	1.39**	659.16**	12.08	435.69**	5.36**	213.02**
Line x Testers	25	2.45**	566.01**	69.42**	938.04**	7.25**	172.53**
Residual	94	0.04	224.99	105.24	932.47	5.79	228.78
Total	143	17.17	2625.97	3549.22	12246.45	92.50	1020.78

** significant at level 0.01 and * significant at level 0.05.

4.7.1.1. Plant height

The plant height of the parental lines and their F1 hybrids are illustrated in Figure 4.1. The highest ranking parent was 13R, followed by 1A and 6A. Parent 13R differed significantly from the other 11 parental lines. The parents, 12R and 5A were the lowest and second lowest ranking parents and they differed significantly from the other 10 parents. There were five F1 hybrids (6Ax14R, 1Ax13R, 4Ax13R, 6Ax15R and 6Ax13R) that were taller than 13R. Hybrid 6Ax14R was the highest ranking hybrid of all the entries and it was significantly higher than the other 35 F1 hybrids. The hybrid 3Ax12R had the lowest height and it differed significantly from the other 35 F1

hybrids. The standard (4Ax16R) ranked ninth of the F1 hybrids and 13R was the only parent that was taller than the standard.

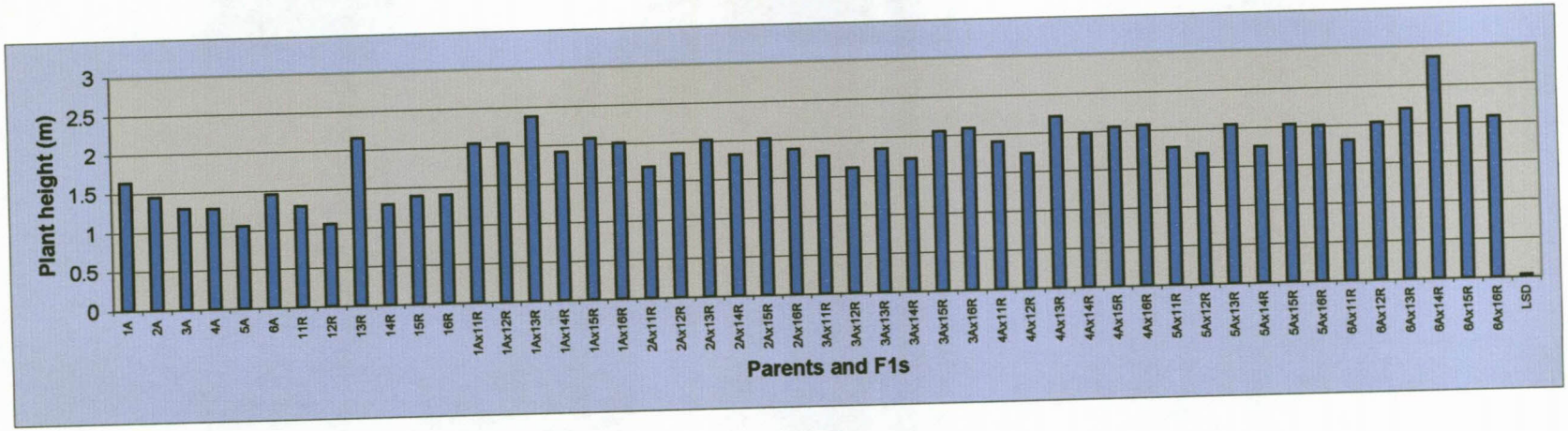

Figure 4.1 Plant height for the parents and their F1 hybrids

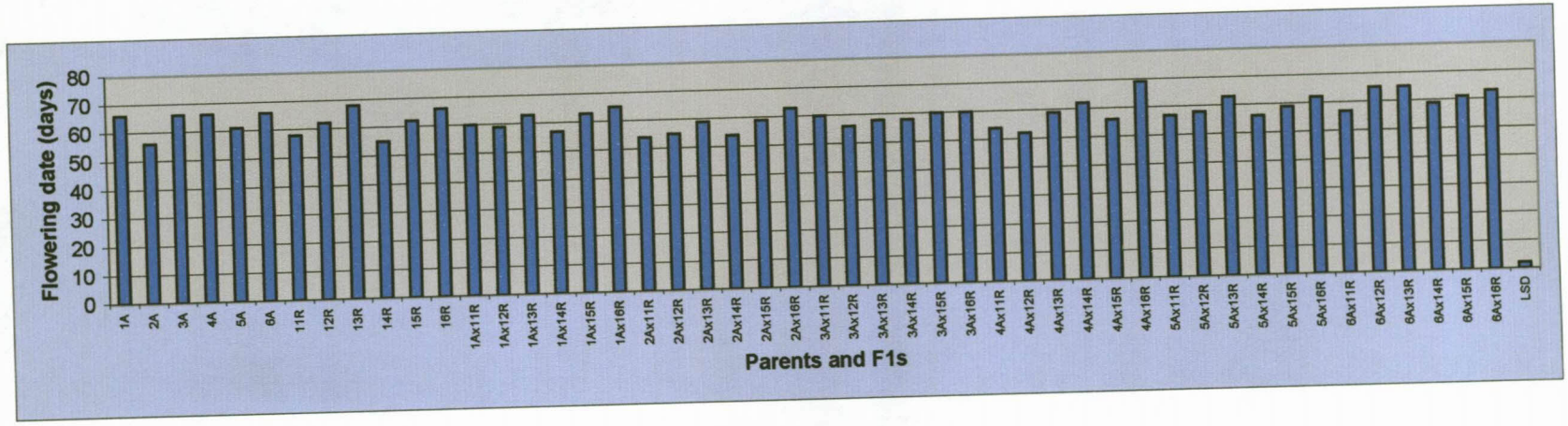

Figure 4.2 Flowering date for the parents and their F1 hybrids

4.7.1.2 Flowering date

The flowering date of the parents and their F1 hybrids are illustrated in Figure 4.2. The highest ranking parent was 13R and it differed significantly from the other parents, except from 16R, 4A, 3A, 1A and 6A. The parent 14R was the lowest ranking parent and it differed significantly from the other parents, except from 2A. Only one hybrid ranked higher than 13R and it was 4Ax16R (standard). The standard 4Ax16R differed significantly from all the F1 hybrids. The second highest ranking F1 hybrid was 1Ax16R, but it did not perform better than six of the parents. The flowering date of this hybrid (1Ax16R) was significantly later than all the F1 hybrids, except 6Ax12R, 6Ax13R, 1Ax15R, 1Ax13R and 6Ax16R. The shortest flowering date was found in 4Ax12R. It differed significantly from the other F1 hybrids, except from 2Ax11R and 2Ax14R.

4.7.1.3 Head diameter

The head diameter for the parents and their F1 hybrids are illustrated in Figure 4.3. The highest ranking parent was 6A, followed by 2A, 5A and 1A. This parent (6A) differed significantly from the other parental lines. The parent with the smallest head diameter was 15R and it differed significantly from all the female parents. All the hybrids ranked higher than the parents. The highest ranking F1 hybrids with the same head diameter were 1Ax11R, 1Ax12R and 3Ax15R. They were significantly bigger than most of F1 hybrids, except from 6Ax15R, 3Ax13R, 5Ax14R, 6Ax12R, 3Ax12R, 3Ax14R, 5Ax13R, 5Ax12R, 3Ax11R, 6Ax11R, 6Ax16R, 4Ax11R, 2Ax11R, 4Ax14R, 4Ax12R, 5Ax11R and 4Ax16R. The standard, 4Ax16R, had the smallest head diameter and it differed significantly from all the F1 hybrids, except from 5Ax11R. The second lowest ranking F1 hybrid was 5Ax11R and it was significantly different from the other F1 hybrids, except from 4Ax12R, 4Ax14R, 2Ax11R and 4Ax11R.

4.7.1.4 1000-Seed weight

The parents and their F1 hybrids for 1000-seed weight are illustrated in Figure 4.4. The parent with the highest 1000-seed weight was 5A and it differed

significantly from the other parents. The parent 12R was the lowest ranking parent and it differed significantly from the other parents, except from 15R. Nine hybrids ranked higher than 5A. The highest ranking F1 hybrid was 4Ax16R (standard) and it differed significantly from all the F1 hybrids, except from 5Ax11R, 4Ax14R, 1Ax16R, 1Ax13R and 5Ax16R. The second highest ranking F1 hybrid was 5Ax11R and it was significantly different from all the F1 hybrids, except 4Ax14R, 1Ax16R, 1Ax13R, 5Ax16R, 6Ax13R, 6Ax11R and 3Ax11R. The hybrid 2Ax15R had the lowest 1000-seed weight and it differed significantly from the other F1 hybrids, except from 2Ax12R, 6Ax15R, 2Ax13R and 2Ax11R.

4.7.1.5 Yield

The yield of the parental lines and their F1 hybrids are illustrated in Figure 4.5. The highest ranking parent was 5A, followed by 4A, 3A and 6A. The parent 5A differed significantly from all the parental lines, except from 4A and 3A. The lowest ranking parent was 12R and it differed significantly from all the female parents, but not from the male parents (testers). All the F1 hybrids out yielded the parents. The highest yield of all the entries was found for hybrid 6Ax13R. It was significantly higher than the other F1 hybrids, except 1Ax13R, 4Ax13R, 3Ax13R and 3Ax15R. The lowest yield was found in 3Ax14R and it differed significantly from the other F1 hybrids, except from 6Ax12R, 6Ax16R, 5Ax12R, 1Ax14R and 4Ax11R. The standard (4Ax16R) ranked tenth of the F1 hybrids, thus nine of the F1 hybrids out-yielded the standard.

4.7.1.6 Oil percentage

The oil percentage of the parents and their F1 hybrids are illustrated in Figure 4.6. The parent with the highest oil percentage was 6A and it differed significantly from the other parents except from 4A and 1A. The lowest ranking parent was 11R and it differed significantly from all the female parents. No F1 hybrid ranked higher than 6A. The F1 hybrid with the highest oil content was 1Ax12R, but it did not have a higher oil percentage than any of the parents. This hybrid 1Ax12R was significantly higher than all the F1 hybrids, except 1Ax13R, 5Ax12R, 1Ax11R, 3Ax14R and 1Ax14R. The

lowest oil percentage was found in 4Ax11R. It differed significantly from the other F1 hybrids, except from 3Ax11R, 2Ax11R and 6Ax15R. The female lines had a higher oil percentage than the male lines.

Discussion

The parental lines and the F1 hybrids showed significant differences for all the characteristics measured. The female parent (line) that overall had the longest flowering date, the highest head diameter and highest oil percentage was 6A. The female 5A had the shortest plant height, but the highest 1000-seedweight and yield. Line 2A had the shortest flowering date and lowest 1000-seedweight and yield. The male parent (tester) that had the longest flowering date and the highest yield was 13R. Tester 14R had the highest oil percentage. The male parent that had the shortest plant height, the lowest 1000-seedweight and lowest yield was 12R. The F1 hybrid that had the highest head diameter and highest oil percentage was 1Ax12R. Hybrid, 6Ax13R had the longest flowering date and the highest yield. For the latest flowering date, the highest ranking parent produced the highest ranking crosses, thus indicating the transfer of the genes to the offspring. Some crosses performed equally or better than their best parents indicating the presence of heterosis effects. If yield is the most important selection criteria, the hybrid 6Ax13R will perform the best in a breeding program as it had the highest yield.

4.7.2 Combining ability

4.7.2.1 General combining ability (GCA)

Estimates for GCA effects of the parents are given in Table 4.5. The GCA of the parental line, 6A was the best for plant height and it was significantly better than all the other parents. It was followed by 13R that had the second highest GCA for plant height. The significant GCA effect of 6A for plant height was associated with positive and high GCA effects for flowering date.

The GCA of 16R was the highest for flowering date and it was significantly better than all the other lines. The line 6A had the second highest GCA for flowering date. The line 16R was associated with positive GCA effects

for plant height, head diameter and 1000-seed weight.

The parental line 1A had the highest GCA for head diameter and it differed significantly from the other parents, except from 2A, 3A, 13R and 15R. The line 15R had the second highest head diameter. The line 1A was associated with positive GCA effects for all the characteristics measured. The line 15R was associated with positive GCA effects for plant height and flowering date.

For 1000-seed weight, the GCA of the parental line 16R was the highest and it was significantly better than all the parents except 1A and 4A. Line 1A had the second highest 1000-seed weight and it was associated with high positive GCA effects for all the characteristics measured. The line 4A had the third highest 1000-seed weight and it was associated with high positive GCA effects for plant height and yield.

Parental line 13R had the highest GCA for yield and it was significantly better than all the parents. It was also associated with positive GCA effects for all the characteristics measured.

The GCA of 1A was the best for oil percentage and it was significantly better than the other parents except 12R. The line 12R was not associated with any other positive GCA effects.

Discussion

Significant GCA effects were found between the parents for the different characteristics measured. These results indicate that the parental line 6A was the best general combiner for plant height and it can be used to improve this characteristic. The best general combiner for flowering date and 1000-seedweight was 16R. The best general combiner for head diameter was 1A. The best general combiner for yield was 13R and for oil percentage, 12R was the best combiner. However 1A could be used to improve head diameter, 1000-seed weight, yield and oil percentage, as it had the highest or second highest GCA effects for these characteristics. To increase plant height and flowering date, one could use 6A. The line 13R could also be used to

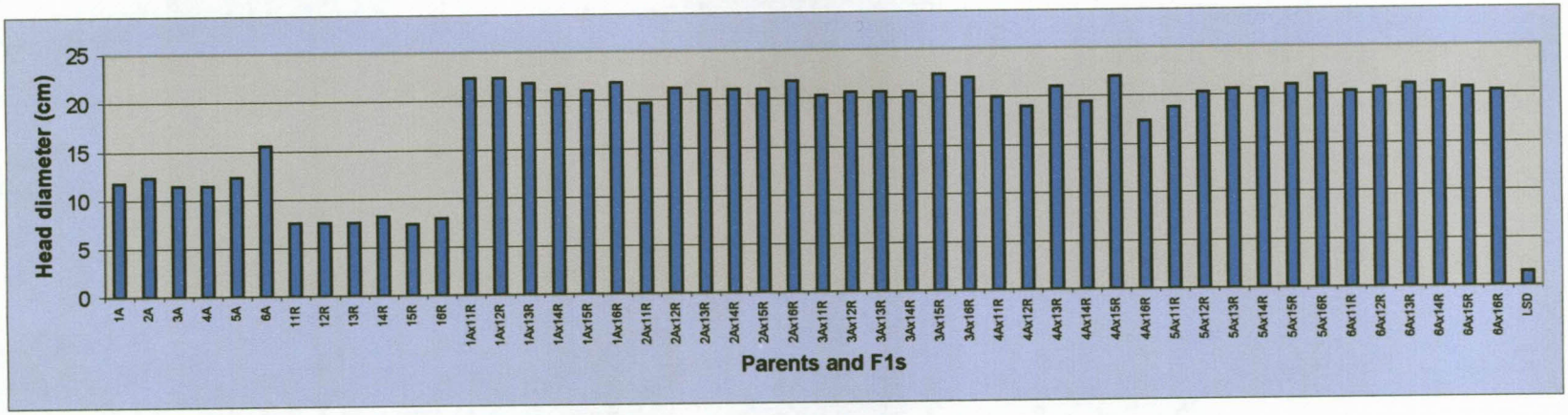

Figure 4.3 Head diameter of the parents and their F1 hybrids

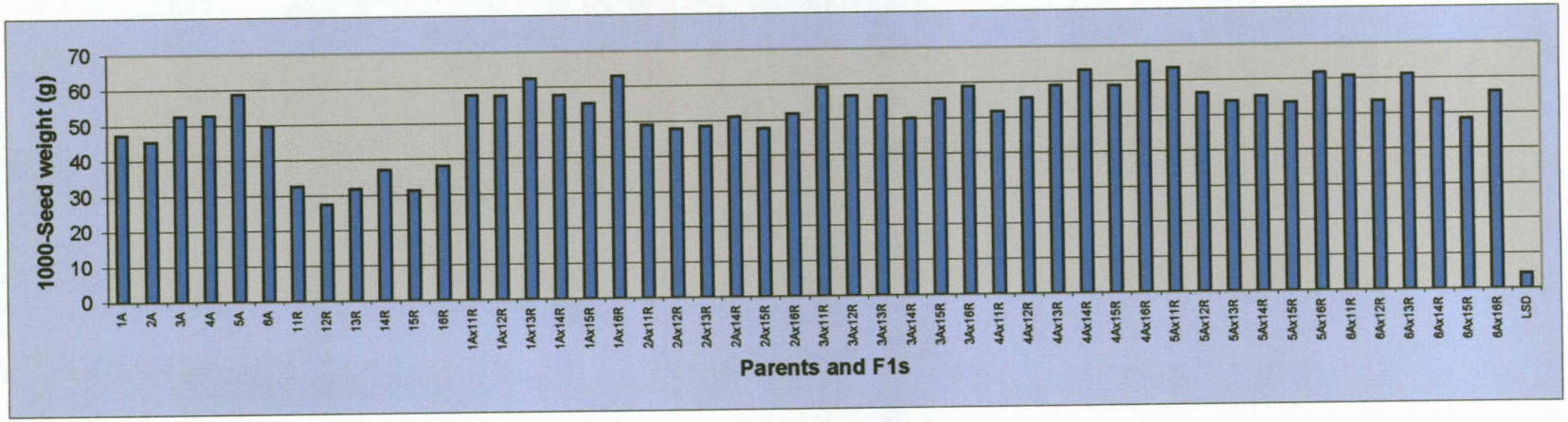

Figure 4.4 1000-Seed weight of the parents and their F1 hybrids

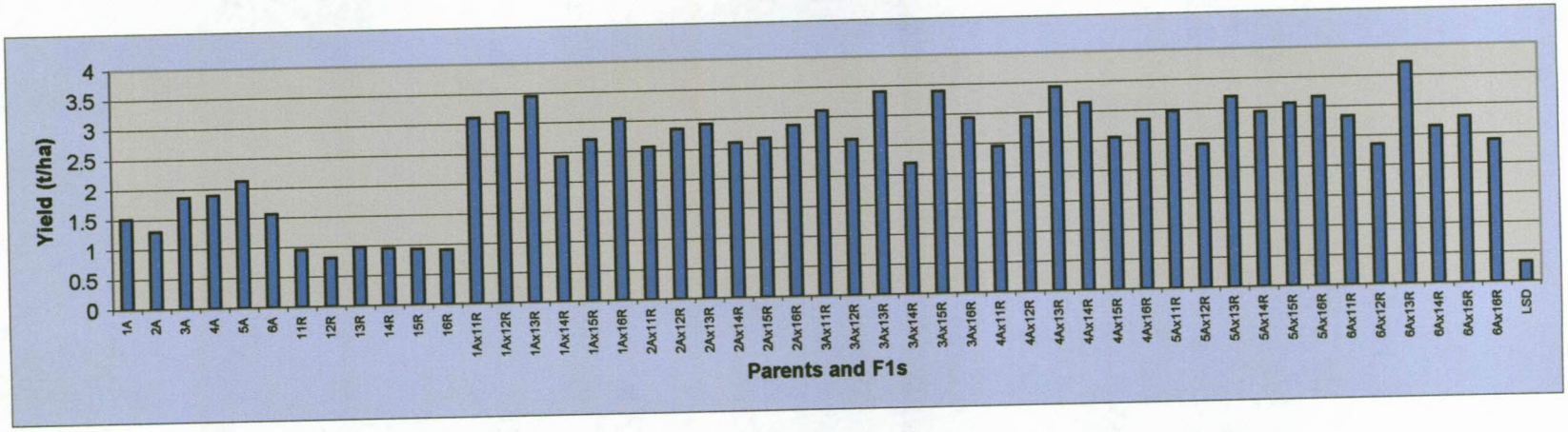

Figure 4.5 Total yields of the parents and their F1 hybrids

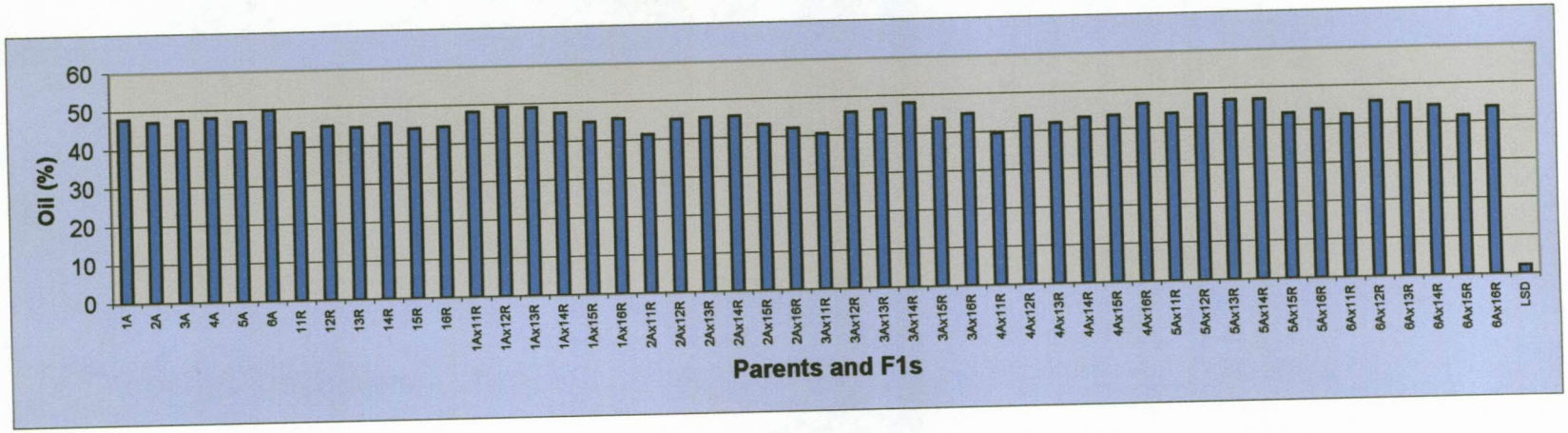

Figure 4.6 Oil percentage of the parents and their F1 hybrids

increase plant height and yield. To increase flowering date and 1000-seed weight, one can use 16R.

Table 4.5 General combining ability effects for the agronomic characteristics measured

Parents	Plant height	Flowering date	Head diameter	1000-Seed weight	Yield	Oil %
1A	0.098	1.732	0.917	3.009	0.104	2.612
2A	-0.099	-2.324	0.139	-6.685	-0.139	-0.916
3A	-0.133	-0.602	0.306	0.176	0.056	0.157
4A	0.014	-0.713	-1.028	2.426	0.006	-1.655
5A	-0.096	-0.269	-0.250	1.426	0.072	0.718
6A	0.216	2.176	-0.083	-0.352	-0.099	-0.916
11R	-0.149	-2.602	-0.583	1.065	-0.047	-2.110
12R	-0.154	-2.046	-0.083	-1.380	-0.162	1.673
13R	0.139	1.843	0.194	0.398	0.467	1.040
14R	0.026	-1.824	-0.083	-0.824	-0.226	1.162
15R	0.096	0.287	0.528	-2.796	-0.021	-1.427
16R	0.043	4.343	0.028	3.537	-0.011	-0.338
LSD	0.034	0.769	0.788	1.358	0.120	1.016

4.7.2.2 Specific combining ability

Estimates of specific combining ability effects for all the agronomic characteristics are given in Table 4.6.

The hybrid combination 6Ax14R ranked first for SCA effect for plant height, thus, it is the best specific combiner for plant height. It was followed by 3Ax16R and 1Ax13R. There were significant differences between these three combinations. The hybrid 6Ax14R also had a significant better SCA effect than the other 33 F1 combinations.

The hybrid 4Ax16R had the highest SCA effect for flowering date. It was however the standard. The hybrid 6Ax12R had the second highest SCA effect for flowering date followed by 4Ax14R and 3Ax11R. There were no significant differences between these three hybrids, but they were however, significantly better than the other 32 hybrid combinations.

Hybrid 4Ax15R expressed the highest SCA effects for head diameter followed by 5Ax16R, 1Ax11R and 4Ax13R. Hybrid 4Ax15R was significantly different from 22 of the F1 hybrids. Hybrids 4Ax12R and 2Ax11R had significantly smaller heads.

The hybrid 4Ax14R expressed the highest SCA effects for 1000-seed weight. It was followed by 6Ax13R and 5Ax11R. Hybrids 5Ax16R, 1Ax16R, 5Ax12R and 1Ax12R had significantly lower SCA effects.

Hybrid 4Ax14R had the highest SCA effect for yield followed by 6Ax13R and 3Ax15R. The SCA effect of 4Ax14R was significantly better than 25 other F1 hybrids.

The standard, 4Ax16R, expressed the highest SCA effects for oil percentage. The second highest ranking hybrid was 1Ax11R, followed by 3Ax14R and 4Ax15R. Hybrid 1Ax11R differed significantly from 28 other hybrids.

Discussion

Significant SCA effects between the crosses were found for the different agronomic characteristics measured. F1 hybrid 3Ax15R was the only hybrid that had positive SCA effects for all the characteristics measured. Hybrid 6Ax14R had the highest SCA effect for plant height and it can be used in a breeding program to enhance plant height. To select for short plants 6Ax11R could be used. The best specific combiner for a late flowering date was 6Ax12R, while 4Ax12R need the shortest time till flowering. For head diameter, 4Ax15R had the highest positive SCA effect. F1 hybrid 4Ax14R was the best specific combiner for 1000-seed weight and yield and could be

Table 4.6 Specific combining ability (SCA) effects for agronomic characteristics

F1 hybrids	Plant height	Flowering date	Head diameter	1000-Seed weight	Yield	Oil %
1Ax11R	0.122	1.102	1.194	-2.232	0.170	2.927
1Ax12R	0.120	-0.120	0.694	0.046	0.366	0.244
1Ax13R	0.160	0.324	-0.250	3.102	-0.003	0.477
1Ax14R	-0.194	-2.676	-0.306	-0.509	-0.334	-1.145
1Ax15R	-0.093	1.880	-1.250	-0.870	-0.262	-1.057
1Ax16R	-0.114	-0.509	-0.083	0.463	0.064	-1.445
2Ax11R	-0.038	-0.843	-0.694	-1.370	-0.130	-0.379
2Ax12R	0.127	-0.398	0.472	-0.093	0.262	-0.295
2Ax13R	0.004	0.380	-0.139	-1.204	-0.297	0.538
2Ax14R	-0.090	-1.620	0.139	2.519	0.092	0.616
2Ax15R	0.047	1.269	-0.472	1.157	-0.059	0.871
2Ax16R	-0.050	1.213	0.694	-1.009	0.131	-1.351
3Ax11R	0.069	3.769	-0.194	2.102	0.212	-2.218
3Ax12R	-0.086	-0.454	-0.361	1.8791	-0.159	-0.601
3Ax13R	-0.133	-2.343	-0.639	-0.065	-0.008	0.532
3Ax14R	-0.163	0.991	-0.361	-5.343	-0.519	1.877
3Ax15R	0.114	0.880	0.694	2.130	0.470	0.166
3Ax16R	0.200	-2.843	0.861	-0.704	0.003	0.244
4Ax11R	0.052	-1.787	0.806	-7.648	-0.395	-1.373
4Ax12R	-0.100	-4.676	-0.694	-1.537	0.197	-0.923
4Ax13R	0.077	-1.232	1.028	-3.148	0.062	-2.190
4Ax14R	-0.033	4.769	-0.361	5.574	0.484	-0.945
4Ax15R	-0.033	-3.009	1.694	3.213	-0.311	1.844
4Ax16R	0.037	5.935	-2.472	3.546	-0.037	3.588
5Ax11R	0.036	0.102	-1.306	5.019	0.056	0.321
5Ax12R	-0.053	0.546	-0.139	0.296	-0.389	1.371
5Ax13R	0.031	1.657	-0.083	-3.815	-0.224	0.305
5Ax14R	-0.147	-1.009	0.194	-1.259	0.198	0.282
5Ax15R	0.047	-0.120	-0.083	-1.120	0.133	-0.962
5Ax16R	0.087	-1.176	1.417	0.880	0.227	-1.318
6Ax11R	-0.240	-2.343	0.194	4.130	0.087	0.721
6Ax12R	-0.008	5.102	0.028	-0.593	-0.277	0.205
6Ax13R	-0.138	1.213	0.083	5.130	0.471	0.338
6Ax14R	0.628	-0.454	0.694	-0.982	0.079	-0.684
6Ax15R	-0.082	-0.898	-0.583	-4.509	0.028	-0.862
6Ax16R	-0.159	-2.620	-0.417	-3.176	-0.388	0.282
LSD	0.028	2.372	1.592	4.685	0.384	2.384

used to improve these characteristics. Hybrid 1Ax11R could be used to improve oil percentage.

4.7.2.3 GCA:SCA ratio

The GCA:SCA ratios were calculated and are given in Table 4.7. The GCA:SCA ratio gives an indication of additive or non-additive gene action. The SCA variance in this study was found higher than the GCA variance for all the characteristics measured. This means that a large part of the total genetic variability associated with the measured traits was the result of non-additive gene action.

The ratios were positive for all the traits. All the ratios were less than one and this indicates that non-additive effects were more important than additive effects for all the characteristics measured. The low GCA:SCA ratio is the result of the high SCA effects.

Table 4.7 The ratios between the mean squares of general combining ability and specific combining ability

Parameters	GCA	SCA	GCA:SCA
Plant height	0.005	0.130	0.04 : 1
Flowering date	1.525	26.996	0.06 : 1
Head diameter	0.048	2.210	0.02 : 1
1000-Seed weight	2.607	36.802	0.07 : 1
Yield	0.007	0.305	0.02 : 1
Oil percentage	0.791	5.956	0.13 : 1

Discussion

Putt (1966) and Hussain *et al* (1998) found that the GCA effect was higher than the SCA effect for plant height and yield, thus indicating that additive gene action was important. The results of this study however, showed that the SCA effect was greater, thus indicating non-additive gene action. This was

found in the studies of Merinkovic (1993) who concluded that non-additive gene effects controlled seed yield. Putt (1966) also found that non-additive gene effects controlled the inheritance of flowering date, head diameter and 1000-seed weight as was found in this study.

4.7.3 Genetic correlation

Genotypic correlations were calculated between all the characteristics measured to determine the influence that the different agronomic characteristics had on each other. A genotypic correlation matrix is given in Table 4.8.

In this study plant height was positively correlated with head diameter (r=0.69), 1000-seed weight (r=0.45) as well as yield (r=0.65). Plant height was negatively correlated with oil percentage (r=-0.18), but it was however non-significant.

Flowering date showed a significantly negative correlation with head diameter (r=-0.29), but had a significantly positive correlation with oil percentage (r=0.27). It also showed a non-significant negative correlation with yield (r=-0.16).

Head diameter was positively correlated with both 1000-seed weight (r=0.72) and yield (r=0.86).

There was a significantly positive correlation between 1000-seed weight and yield (r=0.79).

Yield and oil percentage were negatively correlated (r=-0.14), but it was however non-significant.

Table 4.8 Correlation coefficients between the agronomic characteristics

	Plant height	Flowering date	Head diameter	1000-Seed weight	Yield	Oil %
Plant height	1	0.055	0.694*	0.451*	0.650*	-0.179
Flowering date	0.055	1	-0.294*	-0.031	-0.155	0.265*
Head diameter	0.694*	-0.294*	1	0.716*	0.860*	-0.135
1000-Seed weight	0.451*	-0.031	0.716*	1	0.794*	0.021
Yield	0.650*	-0.155	0.860*	0.794*	1	-0.138
Oil %	-0.179	0.265*	-0.135	0.021	-0.138	1

* significant at level 0.01

Discussion

The genetic correlation is the correlation of breeding values and expresses the extent to which two measurements reflect what is genetically the same characteristic. An increase or decrease in one characteristic is generally associated with an increase or decrease in the other.

The significant positive correlation of plant height with head diameter, 1000-seed weight and yield indicates that an increase in plant height will result in an increase in head diameter, 1000-seed weight and yield. Oil percentage however, will decrease.

Flowering date was positively correlated with oil percentage. This indicates that oil percentage can be improved by selecting for genotypes that require more days to flower. Head diameter was negatively correlated and indicates that an increase in the flowering date will have a decrease in the head diameter.

By increasing the head diameter, the 1000-seed weight and yield would be

improved, due to the positive correlation that exists. The 1000-seed weight was positively correlated with yield and by selecting for higher 1000-seed weight, the yield would thus increase. Yield and oil percentage were not significantly correlated, but it is important for the breeder to know that there exists a negative correlation between them. An increase in yield will lead to a decrease in the oil percentage.

Correlations of interest to the breeder were that an increase in head diameter, lead to an increase in 1000-seed weight and yield. Selecting for 1000-seed weight, would also improve yield. Selecting for higher plant height would increase the head diameter, 1000-seed weight and yield, but would result in a decrease in the oil percentage. Increasing the flowering date would increase the oil percentage, but reduce the head diameter.

Doddamani et al (1997) also found that head diameter, 1000-seed weight and plant height had a significant positive correlation with yield. They also found that flowering date had a negative correlation with yield. The results of this study thus confirm their results.

4.7.4 Heritability

The broad-sense (h^2b) and narrow-sense (h^2n) heritabilities were calculated for all the characteristics and can be seen in Table 4.9.

All the characteristics measured had very high broad-sense heritabilities, which varied from $h^2b = 0.84$ for yield to $h^2b = 0.99$ for flowering date. Flowering date (0.99) had the highest broad-sense heritability, followed by 1000-seed weight (0.98) and plant height (0.96). Yield (0.84) had the lowest broad-sense heritability.

All the traits had very low narrow-sense heritabilities. Oil percentage (0.11) had the highest narrow-sense heritability, followed by 1000-seed weight (0.07) and flowering date (0.05). Yield (0.02) and head diameter (0.02) had the lowest narrow-sense heritability.

Table 4.9 Estimates of heritabilities for the agronomic characteristics

	Plant height	Flowering date	Head diameter	1000-Seed weight	Yield	Oil %
σ^2_A	0.01	1.53	0.05	2.61	0.01	0.79
σ^2_D	0.13	27.00	2.21	36.80	0.31	5.96
σ^2_E	0.01	0.37	0.25	0.74	0.06	0.37
h^2b	0.96	0.99	0.90	0.98	0.84	0.95
h^2n	0.04	0.05	0.02	0.07	0.02	0.11

Discussion

The narrow-sense heritability is of great importance to the plant breeder because it measures the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation of offspring. The narrow-sense heritability was very low for all the characteristics measured with oil percentage (11%) the highest. The low narrow-sense heritibility was caused by low additive effects and high dominant gene actions for all the characteristics measured.

4.7.5 Heterosis

Mid-parent (MP) and high-parent (HP) heterosis were calculated for all the characteristics measured. Estimated values are presented in Table 4.10.

All the F1 hybrids expressed positive MP heterosis for plant height, which ranged from 6.9% to 106.5%. The F1 hybrid 6Ax14R expressed the highest MP heterosis (106.5%) followed by 1Ax16R (93.3%) and 5Ax15R (63.7%). Thirty-three of the 36 hybrids had positive HP heterosis for plant height. The hybrid 6Ax14R (95.2%) had the highest HP heterosis, followed by 5Ax12R (55.6%) and 4Ax14R (54.3%).

Five of the F1 hybrids had positive mid-parent heterosis for flowering date. The standard (4.3%) expressed the highest MP heterosis. The second highest was 2Ax16R (2.2%) followed by 4Ax14R (1.7%) and 6Ax12R (1.1%). Only two F1 hybrids had positive HP heterosis for flowering date, namely 2Ax12R (11.8%) and 4Ax16R (standard) (4.0%).

All the F1 hybrids had positive MP heterosis for head diameter, which ranked from 71.7% to 135.8%. The hybrid 4Ax15R (135.8%) had the highest positive MP heterosis followed by 3Ax15R (135.1%), 1Ax11R (130.9%) and 1Ax12R (130.9%). All the hybrids had positive HP heterosis for head diameter. The highest HP heterosis was found in 4Ax15R (94.2%) followed by 1Ax11R, 1Ax12R and 3Ax15R (all 91.4%).

For 1000-seed weight all 36 F1 hybrids had positive MP heterosis. The hybrid 1Ax13R (58.0%) had the highest MP heterosis, followed by 1Ax12R (54.4%) and 6Ax13R (51.8%). Twenty-nine of the 36 hybrids had positive HP heterosis. The hybrid 1Ax16R (33.2%) had the highest amount of HP heterosis followed by 1Ax13R (32.1%) and 6Ax13R (22.9%).

Positive MP heterosis was found for all the F1 hybrids for yield, which ranked from 56.0% to 190.6%. The hybrid 6Ax13R (190.6%) had the highest MP heterosis followed by 1Ax12R (172.7%) and 1Ax13R (173.8%). All the hybrids had positive HP heterosis. The hybrid 6Ax13R (136.9%) had the highest amount of HP heterosis followed by 1Ax13R (127.0%) and 2Ax13R (124.6%).

Ten of the 36 hybrids expressed positive MP heterosis for oil percentage. The hybrid 1Ax12R (5.7%) ranked the highest followed by 1Ax13R (5.4%) and 5Ax12R (5.3%). Seven F1 hybrids showed positive HP heterosis, where 5Ax12R (3.6%) had the highest amount of HP heterosis followed by 1Ax13R (2.0%) and 1Ax12R (2.8%).

Table 4.10 Heterosis (%) estimates for agronomic characteristics

F1 hybrids	Plant height		Flowering date		Head diameter		1000-Seed weight		Yield		Oil %	
	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP
1Ax11R	39.5	25.0	-3.5	-9.6	130.9	91.4	44.7	22.3	150.8	104.6	5.1	0.5
1Ax12R	50.0	24.4	-7.8	-10.6	130.9	91.4	54.4	21.9	172.7	109.9	5.7	2.8
1Ax13R	25.3	10.2	-5.5	-6.9	120.1	85.7	58.0	32.1	173.8	127.0	5.4	2.0
1Ax14R	29.9	16.5	-6.6	-14.2	113.3	82.8	36.8	21.9	96.0	59.9	1.1	-1.1
1Ax15R	36.8	26.8	-0.8	-4.0	121.1	80.0	40.9	17.0	120.3	78.3	-2.6	-6.3
1Ax16R	93.3	22.6	-1.8	-2.0	120.2	85.7	47.7	33.2	149.2	100.0	-1.3	-4.9
2Ax11R	22.5	16.6	-5.8	-6.9	96.7	59.5	25.5	7.9	127.4	97.7	-8.9	-5.7
2Ax12R	45.7	27.6	-7.6	11.8	113.3	73.0	31.3	5.3	168.7	119.2	-2.1	-4.0
2Ax13R	11.6	-6.5	-4.6	-12.8	110.0	70.3	25.6	6.8	153.9	124.6	-1.2	-3.5
2Ax14R	32.9	25.5	-3.9	-3.0	103.3	70.3	23.7	12.3	131.0	100.8	-1.7	-3.1
2Ax15R	42.3	39.3	0.6	-4.9	113.6	70.3	24.4	4.9	138.4	105.4	-5.2	-8.1
2Ax16R	30.8	29.0	2.2	-5.5	108.2	75.8	24.3	14.1	158.6	120.8	-7.9	-10.5
3Ax11R	36.2	36.2	-2.7	-8.6	110.2	74.2	39.3	12.9	120.6	67.2	-11.0	-14.6
3Ax12R	35.3	23.9	-11.8	-14.2	113.8	77.1	41.7	7.8	95.5	40.9	-1.0	-3.2
3Ax13R	6.9	-14.4	-12.7	-14.2	113.8	77.1	33.9	7.5	137.8	82.8	0.6	-2.2
3Ax14R	31.5	31.5	-4.2	-11.7	106.7	77.1	11.6	-4.9	56.0	18.3	2.8	0.9
3Ax15R	52.6	48.2	-5.8	-8.6	135.1	91.4	32.5	5.6	142.1	82.3	-4.9	-8.1
3Ax16R	54.8	49.3	-8.6	-9.1	123.6	88.5	30.3	12.2	110.8	57.5	-2.6	-5.7
4Ax11R	46.2	46.2	-12.2	-17.7	110.5	76.5	21.4	-1.8	71.3	29.6	-13.6	-17.4
4Ax12R	46.2	34.9	-18.8	-21.2	100.0	67.7	38.4	5.2	117.0	55.0	-6.0	-8.5
4Ax13R	27.8	2.3	-11.5	-12.8	121.1	85.4	31.6	5.5	137.5	81.0	-9.6	-12.5
4Ax14R	54.3	54.3	1.7	-6.6	96.6	70.6	40.7	19.7	120.3	66.7	-7.6	-9.7
4Ax15R	53.7	48.2	-12.3	-15.2	135.8	94.2	40.1	11.5	81.6	35.5	-5.6	-9.2
4Ax16R	53.3	47.9	4.3	4.0	79.2	53.0	44.3	24.1	102.9	50.3	0.3	-3.3
5Ax11R	48.7	36.2	-4.2	-6.6	86.7	64.9	39.0	8.1	91.6	29.1	-35.1	-6.7
5Ax12R	55.6	55.6	-6.0	-7.0	103.3	64.9	30.8	-4.1	64.0	13.1	5.3	3.6
5Ax13R	26.5	-2.8	-2.6	-7.8	106.7	67.6	19.3	-8.1	105.1	50.2	2.1	1.0
5Ax14R	47.9	36.4	-2.9	-7.2	100.1	67.6	15.6	-5.8	89.6	80.0	1.3	0.2
5Ax15R	63.7	46.0	-3.0	-3.8	113.6	70.3	18.9	-8.9	100.7	44.1	-5.4	-8.0
5Ax16R	61.3	42.9	-1.3	-5.5	116.3	78.4	27.8	5.3	108.6	48.8	-4.0	-6.4
6Ax11R	21.5	24.0	-8.4	-14.2	74.2	29.7	47.7	22.2	122.8	80.3	-9.2	-14.8
6Ax12R	59.8	39.0	1.1	-2.0	77.1	31.9	39.1	7.8	97.5	49.7	-3.9	-8.2
6Ax13R	21.6	18.5	-3.5	-4.9	80.0	34.0	51.8	22.9	190.6	136.9	-4.5	-9.2
6Ax14R	106.5	95.2	-2.2	-10.1	77.8	36.1	24.1	8.2	107.9	68.2	-7.3	-11.0
6Ax15R	54.6	51.4	-4.5	-7.6	79.7	31.9	19.3	-2.9	123.2	77.7	-11.7	-16.6
6Ax16R	45.5	42.5	-4.3	-4.5	71.7	29.7	27.6	12.5	92.7	52.2	-7.1	-12.1

MP = mid-parent

HP = high-parent

Discussion

Generally, the most crosses showed a positive mid-parent (MP) and high-parent (HP) heterosis. Yordanov (1983) reported that when suitable pairs with high combining abilities are combined, the respective high heterosis effect could be expected. The heterosis effects are normally the highest in the F1-generation and cannot be predicted exactly beforehand. The hybrid 6Ax14R showed high MP and HP heterosis for plant height. The hybrid 2Ax12R had high HP heterosis for flowering date. For head diameter, 4Ax15R had the highest MP and HP heterosis. The hybrid 1Ax16R had the highest HP heterosis for 1000-seed weight. The hybrid with the highest MP and HP heterosis for yield was 6Ax13R. Hybrid 1Ax12R had the highest MP heterosis and 5Ax12R the highest HP heterosis for oil percentage. The three hybrids that expressed the highest heterosis overall, were 1Ax12R, 1Ax13R and 6Ax13R.

Seetharam *et al* (1977) observed a significant positive heterosis for flowering date, plant height, head diameter, oil percentage and yield. According to Schuster (1964), heterosis for yield for the hybrids was up to 70% better than that of the parents. Half the hybrids showed heterosis for plant height (47% better) and heterosis for head diameter was 60%. Popov and Lazarov (1963) as well as Shuravina (1972) found that only a few hybrids exceeded the parents for oil percentage (heterosis of 4.8%). Above is all similar to the results found in this thesis.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATION

If it is possible to predict the outcome of crosses before field testing, it would increase the efficiency of a breeding program (Bohn *et al*, 1999). This would be possible if one can find a correlation between the genetic distances of inbreds and the amount of heterosis obtained from hybrids. The aim of this study, was therefore to correlate the genetic distances found with the AFLP technique with the amount of heterosis obtained in the F1 hybrids.

Table 5.1 gives the genetic distances of the F1 hybrids for all the primer combinations that was higher than D=0.45. Please note that none of the F1 hybrids had genetic distances higher than 0.45 when the *Mse*-primers and *Eco*-ACA or *Eco*-AAC data were combined or when all the data of the *Mse*-primers and all the data of the *Eco*-primers were combined. It is thus not included in Table 5.1.

Table 5.1: The genetic distances of the F1 hybrids, higher than D=0.45

Mse-CTT + *Eco*-ACA	*Mse*-CAG + *Eco*-ACA	*Mse*-CTC + *Eco*-ACA	*Mse*-CTT + *Eco*-AAC	*Mse*-CAG + *Eco*-AAC	*Mse*-CTC + *Eco*-AAC
1Ax12R (0.46)	1Ax12R (0.49)	1Ax16R (0.46)	1Ax12R (0.49)	2Ax14R (0.47)	6Ax11R (0.5)
2Ax13R (0.47)	1Ax15R (0.48)	4Ax16R (0.54)	1Ax13R (0.52)	6Ax11R (0.48)	
2Ax16R (0.46)	3Ax12R (0.49)	6Ax11R (0.49)	2AX12R (0.49)	6Ax13R (0.45)	
6Ax12R (0.53)	6Ax11R (0.45)	6Ax12R (0.49)	2AX13R (0.46)		
6Ax13R (0.46)	6Ax12R (0.49)	6Ax16R (0.45)	3Ax12R (0.48)		
	6Ax15R (0.50)		3Ax13R (0.49)		
			4Ax12R (0.48)		
			4Ax13R (0.47)		
			6Ax12R (0.49)		
			6Ax15R (0.46)		

Table 5.2 summarizes the three hybrids with the highest amount of mid-parent (MP) and high-parent (HP) heterosis for all the characteristics measured.

Table 5.2: The three hybrids with the highest MP and HP heterosis for the different characteristics measured

Heterosis	Plant height	Flowering date	Head diameter	1000-Seedweight	Yield	Oil %
Mid parent	6Ax14R (106.5) 1Ax16R (93.3) 5Ax15R (63.7)	2Ax16R (2.2) 4Ax14R (1.7) 6Ax12R (1.1)	4Ax15R (135.8) 3Ax15R (135.1) 1Ax11R(130.9) 1Ax12R (130.9)	1Ax13R (58.0) 1Ax12R (54.4) 6Ax13R (51.8)	6Ax13R (190.6) 1Ax12R (172.7) 1Ax13R (173.8)	1Ax12R (5.7) 1Ax13R (5.4) 5Ax12R (5.3)
High parent	6Ax14R (95.2) 5Ax12R (55.6) 4Ax14R (54.3)	2Ax12R (11.8) 4Ax16R (4.0)	4Ax15R (94.2) 1Ax11R (91.4) 1Ax12R (91.4) 3Ax15R (91.4)	1Ax16R (33.2) 1Ax13R (32.1) 6Ax13R (22.9)	6Ax13R (136.9) 1Ax13R (127.0) 2Ax13R (124.6)	5Ax12R (3.6) 1Ax12R (2.8) 1Ax13R (2.0)

Mse-CTT + *Eco*-ACA showed that 1A and 12R was genetically dissimilar and this hybrid also had a high amount of heterosis for head diameter, 1000-seed weight, yield and oil percentage. It also showed that 2Ax16R and 6Ax12R were genetically dissimilar and these two hybrids had a high amount of heterosis for flowering date. Hybrid 6Ax13R had a high amount of heterosis for 1000-seed weight and yield, while 2Ax13R had a high amount of heterosis for yield.

Mse-CAG + *Eco* ACA showed that 1Ax12R was genetically dissimilar and this hybrid had a high amount of heterosis for head diameter, 1000-seed weight, yield and oil percentage. Hybrid 6Ax12R was also dissimilar and it had a high amount of heterosis for flowering date.

Mse-CTC + *Eco*-ACA showed that 1Ax16R was genetically dissimilar and this hybrid had a high amount of heterosis for plant height and 1000-seed weight. It also showed that 4Ax16R and 6Ax12R were genetically dissimilar and both had a high amount of heterosis for flowering date.

Mse-CTT + *Eco*-AAC showed that 1Ax12R was genetically dissimilar and this hybrid had a high amount of heterosis for head diameter, 1000-seed weight, yield and oil percentage. Hybrid 1Ax13R had a high amount of heterosis for 1000-seedweight, yield and oil percentage. It also showed that 2Ax12R and 6Ax12R were genetically dissimilar and these two hybrids had a high amount of heterosis for flowering date. Hybrid 2Ax13R had a high amount of heterosis for yield.

Mse-CAG + *Eco*-AAC found that hybrid 6Ax13R was dissimilar and it had a high amount of heterosis for 1000-seed weight and yield. The results of *Mse*-CTC + *Eco*-AAC was not correlated with any hybrid with a high amount of heterosis.

It can therefore be concluded that it is possible to correlate the genetic distances found with AFLP data with the amount of heterosis that can be expected in F1 hybrids. However, not all primer combinations would give the same results and the best primer combination for each crop need to be identified. In this study *Mse*-CTT + *Eco*-ACA had the highest correlation with the amount of heterosis found in the F1 hybrids and it is recommended that it can be used to identify heterosis in sunflower. It will thus be possible to screen many inbreds and to shorten the breeding program with the help of the AFLP technique.

The disadvantage of this technique however, was that it was not able to detect that inbreds 4A and 16R would produce a sterile hybrid (4Ax16R). *Mse*-CTC + *Eco*-ACA showed that these two lines was highly dissimilar and could therefore be used in a breeding program.

CHAPTER 6
SUMMARY

Breeders would like to predict the outcome of crosses, before producing and testing lines derived from them in field trials. One way to ensure this is by finding a correlation between the genetic distances of inbreds and the amount of heterosis obtained by such a hybrid. The aim of this study was to determine the genetic distances between 12 sunflower inbred lines with the use of the AFLP technique and to correlate these results with the amount of heterosis obtained in F1-hybrids.

Twelve inbred lines, consisting of six females (lines) and six males (testers), was planted in a glasshouse at the University of the Free State (UFS) in Bloemfontein, South Africa. Two experiments were done on these parental lines.

Young leaves were collected from each line. DNA was extracted from the leaves and AFLP analysis was performed on the DNA. Six different primer combinations were used, namely: *Mse*-CTT + *Eco*-ACA; *Mse*-CAG + *Eco*-ACA; *Mse*-CTC + *Eco*-ACA; *Mse*-CTT + *Eco*-AAC; *Mse*-CAG + *Eco*-AAC and *Mse*-CTC + *Eco*-AAC. The objective was to determine the genetic distances of the 12 lines with the use of the AFLP technique and different primer combinations. These results would then be used to identify heterotic groups in a hybrid breeding program.

The genetic similarities were lower overall for CMS (A) x restorer (R) crosses than for AxA or RxR . This was confirmed by Hongtrakul *et al* (1997). *Mse*-CTT + *Eco*-ACA, *Mse*-CAG + *Eco*-ACA and *Mse*-CTC + *Eco*-AAC identified the highest amount of dissimilarity between female lines 1A and 4A. The highest amount of dissimilarity between male lines 14R and 16R were identified by *Mse*-CTC + *Eco*-ACA and *Mse*-CTC + *Eco*-AAC.

According to Hongtrakul *et al* (1997), the cluster analysis separated lines into two groups, one for A-lines (females) and another for R-lines (males). This was also found in this study. These groupings illustrate the breeding history and basic heterotic pattern of sunflower.

In the second experiment, all six testers were individually crossed with each line to produce F1 hybrid seed. Thirty six crosses were made and sufficient seed was generated, except from the cross between parental lines 4A and 16R that resulted in a sterile hybrid with no seed. Therefore, 4Ax16R was replaced with a standard, HV3037. The 36 F1 hybrids were planted according to a randomized complete block design with three replications. The plant height, flowering date, head diameter, 1000-seed weight, yield and oil percentage of each hybrid was determined with the Line x Tester analysis. The aim was to determine the combining ability of the inbred lines, to determine if there are genetic correlations between the different characteristics and to determine the expression of heterosis for the different characteristics.

Hybrid 6Ax13R had the latest flowering date and the highest yield. The F1 hybrid that had the highest head diameter and highest oil percentage was 1Ax12R. Some crosses performed equally or better than their best parents indicating the presence of heterotic effects. If yield is the most important selection criteria, the hybrid 6Ax13R would perform the best in a breeding program as it ranked the highest.

Line 1A could be used to improve head diameter, 1000-seed weight, yield and oil percentage, as it had the highest or second highest GCA effects for these characteristics. The tester 13R could also be used to increase plantheight and yield. To increase flowering date and 1000-seedweight, one can use 16R.

F1 hybrid 3Ax15R was the only hybrid that had positive SCA effects for all the characteristics measured. The hybrid 4Ax14R was the best specific combiner

for 1000-seed weight and yield, while 1Ax11R had the highest positive effects for oil percentage.

According to the GCA:SCA ratio the SCA was greater, indicating non-additive gene action. This was found in the studies of Merinkovic (1993) who concluded that non-additive gene effects controlled yield. Putt (1966) also found that non-additive gene effects controlled the inheritance of flowering date, head diameter and 1000-seed weight.

Correlations of interest were that when selecting for increased plant height one would increase the head diameter, 1000-seed weight and yield, but it would however, result in a decrease in the oil percentage. By increasing the flowering date, one would increase the oil percentage, but reduce the head diameter. Doddamani *et al* (1997) also found that head diameter, 1000-seed weight and plant height had a significant positive correlation with yield. They also found that flowering date had a negative correlation with yield. The results of this study thus confirm their results.

Flowering date had the highest broad-sense heritability, followed by 1000-seed weight and plant height. Oil percentage had the highest narrow-sense heritability, followed by 1000-seed weight and flowering date.

The hybrid with the highest MP and HP heterosis for yield was 6Ax13R. Hybrid 1Ax12R had the highest MP heterosis while 5Ax12R the highest HP heterosis for oil percentage. The three hybrids that expressed the highest heterosis overall, were 1Ax12R, 1Ax13R and 6Ax13R.

Seetharam *et al* (1977) observed a significant positive heterosis for flowering date, plant height, head diameter, oil percentage and yield. According to Schuster (1964), heterosis for yield for the hybrids was up to 70% better than that of the parents. Half the hybrids showed heterosis for plant height (47% better) and heterosis for head diameter was 60%. Popov and Lazarov (1963) as well as Shuravina (1972) found that only a few hybrids exceeded the

parents for oil percentage (heterosis of 4.8%). Above is all similar to the results found in this study.

Correlations between genetic distance, heterosis, and hybrid performance for yield in sunflower were estimated. Genetic distances from AFLP fingerprints were correlated with the amount of heterosis found in F1 hybrids.

Mse-CTT + *Eco*-ACA had the highest correlation with the amount of heterosis in the F1 generation. It can therefore be recommended that this primer combination can be used to identify heterosis for flowering date, head diameter, yield and oil percentage in hybrids.

Therefore, it is possible to correlate the genetic distances found with AFLP data with the amount of heterosis that can be expected in F1 hybrids. This makes it possible to screen thousands of inbred lines and shorten the hybrid breeding program. The number of crosses, trails and amount of labor will decrease and will result in a lower farm price for hybrid seeds.

HOOFSTUK 6
OPSOMMING

Telers verkies om die resultate van kruisings te kan voorspel voordat die toets en produksie van basters op die lande, plaasvind. Een manier om dit te doen is om 'n korrelasie tussen die genetiese afstande van die ingeteelde lyne en die hoeveelheid heterose in 'n baster, te bepaal. Die doel van hierdie studie was om die genetiese afstande te bepaal tussen 12 sonneblom ingeteelde lyne met die gebruik van die AFLP tegniek en om hierdie resultate te korreleer met die hoeveelheid heterose gevind in F1 basters.

Twaalf ingeteelde lyne, bestaande uit ses vroulike (lyne) en ses manlike (toetsers) lyne, is geplant in 'n glashuis by die Universiteit van die Vrystaat in Bloemfontein, Suid-Afrika. Twee eksperimente is gedoen op hierdie ouer lyne.

Jong blare van elke lyn is versamel. Die DNA is ge-ekstraheer vanaf die blare en AFLP analises is uitgevoer op die DNA. Ses verskillende primer kombinasies is gebruik, naamlik: *Mse*-CTT + *Eco*-ACA; *Mse*-CAG + *Eco*-ACA; *Mse*-CTC + *Eco*-ACA; *Mse*-CTT + *Eco*-AAC; *Mse*-CAG + *Eco*-AAC en *Mse*-CTC + *Eco*-AAC. Die doelwit was om die genetiese afstande te bepaal van die 12 lyne met die gebruik van die AFLP tegniek en verskillende primer kombinasies. Hierdie resultate sou dan gebruik word om heterotiese groepe in 'n baster teelprogram, te identifiseer.

Die genetiese ooreenkomste was oor die algemeen laer vir CMS (A) x hersteller (R) kruisings as vir AxA of RxR. Dit is bevestig deur Hongtrakul *et al* (1997). *Mse*-CTT + *Eco*-ACA, *Mse*-CAG + *Eco*-ACA en *Mse*-CTC + *Eco* - AAC het almal dieselfde mees onverwante vroulike lyne (1A en 4A) geïdentifiseer. Dieselfde mees onverwante manlike lyne (14R en 16R) is deur *Mse*-CTC + *Eco*-ACA en *Mse*-CTC + *Eco*-AAC geïdentifiseer.

Volgens Hongtrakul *et al* (1997), verdeel die dendrogram analise die lyne in twee groepe, een vir A-lyne (vroulik) en 'n ander vir R-lyne (manlik). Dit is ook

in die studie gevind. Hierdie groeperings illustreer die teelgeskiedenis en basiese heterotiese patroon van sonneblom.

In die tweede eksperiment, is al ses toetsers individueel gekruis met elke lyn om F1 bastersaad te produseer. Ses en dertig kruisings is gemaak en voldoende saad is gegenereer, behalwe vanaf die kruising tussen ouerlyne 4A en 16R wat 'n steriele baster met geen saad gevorm het. Daarom is 4Ax16R vervang met 'n standaard, HV3037. Die 36 F1 basters is geplant volgens 'n gerandomiseerde volledige blokontwerp met drie replikasies. Die planthoogte, blomdatum, kopdeursnee, 1000-saad massa, opbrengs en olie persentasie van die basters is bepaal met die Lyn x Toetser analise. Die doelwitte was om die kombineervermoë van die ingeteelde lyne te bepaal, om te sien of daar genetiese korrelasies tussen die verskillende eienskappe is en om die uitdrukking van heterose vir die verskillende eienskappe te bepaal.

Baster 6Ax13R het die langste blomdatum en die hoogste opbrengs gegee. Die F1 baster met die grootste kopdeursnee en hoogste olie persentasie was 1Ax12R. Sommige kruisings het dieselfde of beter as hul beste ouers gepresteer, wat dui op die teenwoordigheid van heterotiese effekte. As opbrengs die belangrikste seleksie kriteria is, sal die baster 6Ax13R die beste presteer in 'n teelprogram aangesien dit die hoogste opbrengs gehad het.

Lyn 1A kan gebruik word om kopdeursnee, 1000-saad massa, opbrengs en olie persentasie te verbeter, aangesien dit die hoogste of tweede hoogste GCA effekte gehad het vir hierdie eienskappe. Die toetster 13R kan ook gebruik word om planthoogte en opbrengs te verbeter. Om blomdatum en 1000-saad massa te verbeter, kan 16R gebruik word.

Die F1 baster 3Ax15R was die enigste baster wat positiewe SCA effekte vir al die eienskappe gehad het. Die baster 4Ax14R was die beste spesifieke kombineerder vir 1000-saad massa en opbrengs, terwyl 1Ax11R die hoogste positiewe effekte vir olie persentasie gehad het.

Volgens die GCA:SCA verhouding was SCA groter, wat dui op nie-additiewe geenaksie. Merinkovic (1993) het ook tot die gevolgtrekking gekom dat nie-

additiewe geen effekte die oorerwing van blomdatum, kopdeursnee en 1000-saad massa beheer.

Korrelasies van belang was dat wanneer daar geselekteer word vir hoër planthoogte, die kopdeursnee, 1000-saad massa en opbrengs sal toeneem, alhoewel dit 'n afname in die olie persentasie tot gevolg sal hê. Deur die blomdatum te verhoog, sal die olie persentasie toeneem, maar kopdeursnee sal afneem. Doddamani *et al* (1997) het ook gevind dat die kopdeursnee, 1000-saadmassa en planthoogte 'n betekenisvolle positiewe korrelasie met opbrengs gehad het. Hulle het ook gevind dat die blomdatum 'n negatiewe korrelasie met opbrengs gehad het. Die resultate van hierdie studie bevestig dus hulle gevolgtrekking.

Blomdatum het die hoogste breë sin oorerflikheid gehad, gevolg deur 1000-saad massa en planthoogte. Olie persentasie, gevolg deur 1000-saad massa en blomdatum het die hoogste nou sin oorerflikheid gehad.

Die baster met die hoogste MP en HP heterose vir opbrengs was 6Ax13R. Baster 1Ax12R het die hoogste MP heterose, terwyl 5Ax12R die hoogste HP heterose vir olie persentasie gehad het. Die drie basters wat oor die algemeen die hoogste heterose gehad het, was 1Ax12R, 1Ax13R en 6Ax13R.

Seetharam *et al* (1977) het 'n betekenisvolle positiewe heterose vir blomdatum, planthoogte, kopdeursnee, olie persentasie en opbrengs waargeneem. Volgens Schuster (1964) was heterose vir opbrengs vir die basters tot 70% beter as die van die ouers. Die helfte van die basters het heterose vir planthoogte (47% beter) gehad en heterose vir kopdeursnee was 60%. Popov en Lazarov (1963), so wel as Shuravina (1972) het gevind dat net enkele basters die ouers met olie persentasie oorskry (heterose van 4.8%). Bogenoemde is alles in ooreenstemming met die resultate van hierdie tesis.

Korrelasies tussen genetiese afstand, heterose en baster prestasie vir opbrengs in sonneblom is bepaal. Genetiese afstande vanaf AFLP vinger-

afdrukke was gekorreleer met die hoeveelheid heterose gevind in die F1 basters.

Mse-CTT + *Eco*-ACA het die hoogste korrelasies met die hoeveelheid heterose in die F1 generasie gehad. Dit kan dus aanbeveel word dat hierdie primer kombinasies gebruik kan word om heterose te identifiseer vir blomdatum, kopdeursnee, opbrengs en olie persentasie in basters.

Dit is dus moontlik om die genetiese afstande gevind met die AFLP data, te korreleer met die hoeveelheid heterose wat verwag kan word in F1 basters. Dit maak dit moontlik om duisende ingeteelde lyne te evalueer en kan dus die baster teelprogram verkort. Die aantal kruisings, proewe en hoeveelheid arbeid nodig, sal afneem en dit kan 'n laer prys vir die boere vir bastersaad tot gevolg hê.

LIST OF ABBREVIATIONS

µl	microliter
°C	degrees Celsius
A	cytoplasmic sterile female line
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AP-PCR	Arbitrarily primed PCR
Av.	average
BACs	Bacterial artificial chromosomes
B	maintainer female line
BSA	Bulked segregant analysis
CHA	Chemical hybridizing agents
cm	centimeter
CMS	Cytoplasmic male sterility
CTAB	Cetyltrimethylammonium bromide
D	genetic distance
DAF	DNA amplification fingerprinting
Df	degrees of freedom
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra-acetic acid
e.g.	for example
EMS	Expected mean square
ENZ	Enzyme specific sequence
EXT	selective extension
F	index of genetic similarity
F1	first generation
F2	second generation
FAM	*Eco* + ACA
g	grams
GCA	General combining ability

H	heterosis
h	hour
h^2	heritability
ha	hectare
HP	high parent
i.e.	that is
l	liter
LSD	Least significant difference
m	meter
M	molar
max.	maximum
Me	mean error
min	minutes
min.	minimum
ml	milliliter
mm	millimeter
MP	mid parent
MS	Mean squares
MSE	Mean squares for error
MSFRS	Male sterile facilitated recurrent selection
N	normal cytoplasm
NaCl	sodium chloride
NCSS	Number cruncher statistical system
NED	*Eco* + AAC
ng	nanogram
ng/µl	nanogram per microliter
nm	nanometer
NMS	nuclear male sterility
NPK	Nitrogen phosphorus potassium
PCR	Polymerase chain reaction
pH	acidity
R	fertility restorer line
r	correlation coefficient

RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
rpm	revolutions per minute
s	seconds
S	similarity coefficient
S	sterile cytoplasm
SCA	Specific combining ability
SDS	Sodium dodecyl sulphate
SE	Standard error
spp.	species
t	ton
TE	Tris EDTA buffer
Temp.	temperature
Tris-HCl	Tris (hydroxymethyl) – aminomethane hydrochloride
U	units
UFS	University of the Free State
UPGMA	Unweighted pair-group mean arithmetic
UV	ultraviolet
V	Volt
v/v	volume per volume
YACs	Yeast artificial chromosomes
Zn	zinc

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