PREDICTION OF HETEROTIC GROUPS AND HYBRID PERFORMANCE IN SOUTH AFRICAN SUNFLOWER (*Helianthus annuus* L.) GERMPLASM USING SSR ANALYSIS

By

Tobias Christiaan Lochner

Submitted in fulfilment of the requirements for the degree

Magister Scientiae Agriculturae

Faculty of Natural and Agricultural Sciences

Department of Plant Sciences: Plant Breeding

University of the Free State

Bloemfontein, South Africa

November 2011

Supervisor: Prof. L. Herselman

Declaration

I hereby declare that this dissertation submitted by me for the degree *Magister Scientiae Agriculturae* in Plant Breeding at the University of the Free State, is my own original work and has not been submitted by me previously to any other university/faculty. All sources of materials and financial assistance used for the study have been duly acknowledged. I also agree that the University of the Free State has the sole right to publish this dissertation.

_____ _____

Tobias Christiaan Lochner Date

Dedication

To my wife, soulmate and lifelong friend, Marina

Acknowledgements

I would like to thank the following people, organisations and institutions for their contribution towards the success of this dissertation:

- My Lord God, Who gave me strength, support, encouragement and love. Without Him, nothing is possible.
- My wife, Marina, for always believing.
- Prof. L. Herselman for her supervision, theoretical and practical input, advice, enthusiasm and motivation. Thank you for never giving up.
- Dr. E. Joubert for all her support, encouragement and helping me make sense of the molecular data.
- Pannar Seed (Pty) Ltd, the board and the Research director Mr. R. Drögemöller for the opportunity and financial assistance.
- The sunflower team: Mr. J.J.W. Potgieter, Mr. L. Schoonraad, Mrs. E. Kühn and Mr. A. Pretorius.
- Prof. M.T. Labuschagne and the Plant Breeding division at the University of the Free State.
- My father L.P. and his wife Liezel for their continued support, love and motivation.
- All my friends and family that directly or indirectly have influenced my studies and my life.

Table of Contents

List of tables

List of figures

List of abbreviations

% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage
%	Percentage
°C	Degrees Celsius
µg	Microgram
µl	Microlitre
µM	Micromole
A-line	Cytoplasmic sterile female line
ABI	Applied Biosystems Incorporated
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effects and multiplicative interaction analysis
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
AP-PCR	Arbitrary primed polymerase chain reaction
B-line	Maintainer female line
bp	Base pair
CA	California
CMS	Cytoplasmic male sterility
CV	Coefficient of variation
df	Degrees of freedom
D_m	Nei minimum distance
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
D_R	Rogers distance

E	Environment
EDTA	Ethylenediaminetetraacetic acid
F	Index of genetic similarity
F_1	First inbreeding generation
F_2	Second inbreeding generation
F_{IS}	Fixation index (subpopulation)
F_{IT}	Fixation index (individuals)
Flow days	Days counted until flowering
F_{ST}	Fixation index (total population)
G	Genotype
GCA	General combining ability
GMS	Genetic male sterility
GRAN H2O	Grain moisture expressed as a percentage
h	Hour
h^2	Heritability
ha	Hectare
HO	High oleic acid
I	Identity matrix
IFLP	Intron fragment length polymorphism
INTA	El Instituto Nacional de Tecnología Agropecuaria
L	Likelihood function
LD	Linkage disequilibrium
LG	Linkage group
LL	Log likelihood
LSD	Least significant difference
m	Metre
M	Molar
MANOVA	Multivariate analysis of variance

MAS	Marker-assisted selection
mg	Milligram
$MgCl_2$	Magnesium chloride
min	Minutes
ML	Maximum likelihood
ml	Millilitre
mM	Millimolar
Mn sed	Mean standard error of difference
ms	Recessive male sterility (no male sterility)
Ms	Dominant male sterility
n	Number of observations
n_A	Number of alleles
ng	Nanogram
NJ	Neighbour-Joining method
nm	Nanometer
NMR	Nuclear magnetic resonance
NMS	Nuclear male sterility
o/a	Overall
OD	Optical density
Oil cont	Oil content expressed as a percentage
Oil t/ha	Oil yield expressed in ton per hectare
P	Probability
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
pH	Acidity
PIC	Polymorphic information content
R-line	Fertility restorer line
r^2	Cophenetic correlation coefficient
RAPD	Random amplified polymorphic DNA

REML	Restricted maximum likelihood
Resid	Residual value
rf	Recessive male sterility gene
RFLP	Restriction fragment length polymorphism
RLL	Residual likelihood log
RM	Downy mildew
Rnk	Rank
s	Second
SCA	Specific combining ability
Sed	Standard error of difference
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
STND	Final adult plant stand expressed as a percentage
STRIPED	Striped nature of the seed
t	Ton
TAE	Tris-acetate-EDTA
Taq	*Thermus aquaticus*
TE	Tris-EDTA buffer
TOTL YLD	Total grain yield in ton per hectare
TRAP	Target region amplification polymorphism
Tris-Cl	Tris(hydroxymethyl)aminomethane
U	Unit
UPGMA	Unweighted pair-group method using arithmetic averages
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
V	Volt
V_D	Dominance of loci
V_I	Interaction of loci

Vno	Variety number
v/v	Volume per volume
w/v	Weight per volume
Yld %	Yield expressed as a percentage
Yld %Mn	Yield as a percentage of the mean
Yld t/ha	Yield in ton per hectare

Chapter 1
Introduction

Commercial sunflower is produced worldwide. The largest traditional producer is Russia. A number of other significant sunflower producers include Argentina, the European Union, USA, China, India, Turkey and South Africa. The major sunflower producing states in the USA are North Dakota, South Dakota, Minnesota, Kansas, Colorado, Nebraska, Texas and California (Basra, 1999).

During the 2010 growing season in South Africa, 525 600 tons of sunflower have been delivered up to the 11th of July 2011, which is 41% more than during the same time in 2009. The total projected forecast for the 2010-2011 season is 780 000 tons planted on 643 000 ha. Considering the production, 300 tons of sunflower had to be imported from 1 January 2011 up to 31 May 2011. Should sunflower be imported from the European Union, the price delivered to Randfontein would be R5 474.02 per ton. The sunflower future prices in South Africa are expected to stay stable up to May 2012 at the R4 200 per ton (SAGIS, 2011).

Cultivated sunflower belongs to the genus *Helianthus*. The *Helianthus* genus represents 82 species of which two are utilised as a food source (Heiser, 1978). The most important species for consumption is *H. annuus* L. This species is mainly produced for its oil, but also for bird feed, as a meal supplement for animal feed and for human consumption as confectionary kernels. The other species utilised as a food source is *H. tuberosus* L. (Jerusalem artichoke) of which the tubers are consumed (Dorrel, 1978; Lofgren, 1978).

Sunflower is one of the most important crops produced in the world due to the fact that it is an excellent source of edible vegetable oil. Sunflower oil is used in soft margarines and similar foods and is also a good dietary oil. Sunflower meal is a high quality protein source for stock feed. The high fibre content of the hull however reduces its value to compounders (Weiss, 2000). One of the main focus areas in sunflower breeding is to upgrade the total oil yield per unit area. Yield components such as rows per head, number of flowers per row, the proportion of fertile flowers and seed size constitute equally important objectives in sunflower breeding. An important way to improve seed yield is to select for full fertility in the central area of the head. Seed oil content together with husk thickness and kernel oil

content represent main objectives in sunflower breeding. This selection pressure has assisted in husk percentage being reduced significantly. The kernel oil content has been increased to as much as between 65% and 68% in the best commercial hybrids. Theoretically the biological limit for oil content in sunflower is considered to be 75%. Oil content is however influenced by environmental and agrotechnical conditions (Vrânceanu, 1998).

Sunflower is a versatile crop. This fact and its increasing contribution to oilseed production necessitate increased efforts to develop hybrids with increased productivity and yield (Basra, 1999). Plant breeders are able to follow two possible strategies to increase yield in sunflower. One option would be the development of hybrids that are disease and insect resistant. This type of strategy is called defect elimination or defensive breeding. However, this strategy does not always lead to an increase in yield. The most used option is simply to select for hybrids with increased yield. The genetic constitution of inbreds involved in hybrids depends to a large degree on the way loci segregate during the successive generations of inbreeding and there is virtually nothing breeders can do to change this. It is however possible to calculate the genetic difference between inbred lines through the study of genetic distance among inbreds (Falconer and Mackay, 1996).

Determination of germplasm variety in sunflower backgrounds is time consuming when no prior knowledge is available. DNA marker systems are useful tools for assessing genetic diversity within germplasm. In breeding programmes, information on genetic relationships within species is used for organising germplasm collections, identification of heterotic groups and selection of breeding material (Lee, 1995; Karp *et al*., 1996; Evgenidis *et al*., 2011).

If breeders could predict the potential of crosses for line development and performance prior to the production and testing of lines derived from crosses in field trials, it could potentially increase the efficiency of breeding programmes by focussing breeding efforts on the most promising crosses (Bohn *et al*., 1999).

It will be greatly beneficial to the breeder if a correlation could exist between the genetic distances of inbred lines and the yield obtained from such a cross or hybrid. It could enable the breeder to evaluate a large number of inbreds for genetic distances annually, possibly shorten the testing structure of the breeding programme through initial accurate selection of optimal combinations and possibly reduce the cost of trial evaluation and combination testing

due to the fact that optimal crosses would be made up and tested, therefore reducing numbers tested initially.

This study was therefore based on the following:

1. The establishment of a dendrogram for commercial sunflower lines (R-lines as well as A-lines) to determine the heterotic group layout in the Pannar Seed (Pty) Ltd germplasm (which represents a large variety of germplasm);
2. To determine whether the dendrogram can be of value to be used as a predictor for the best performing combinations between A- and R-lines in the context of South African germplasm as well as to determine whether correlations exist between oil content and dry yield and genetic distance and oil content.

References

Basra, A.S., 1999. Heterosis and hybrid seed production in sunflower. In: Heterosis and hybrid seed production in agronomic crops. Eds. Virupakshappa, K. and Ranganatha, A.R.G. Food Products Press, An imprint of the Haworth Press Inc., New York, USA. pp. 185-215.

Bohn, M., Utz, H.F. and Melchinger, A.E., 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, SSRs and their use for predicting progeny variances. Crop Science 39: 228-237.

Dorrel, D.G., 1978. Processing and utilisation of oilseed sunflower. In: Sunflower science and technology. Ed. Carter, J.F. ASA-CSSA-SSSA, Publishers Madison, WI., USA. pp. 407-440.

Evgenidis, G., Traka-Mavrona, E. and Koutsika-Sotiriou, M., 2011. Principal component analysis as a tool in the assessment of tomato hybrids and cultivars. International Journal of Agronomy, in press. DOI:10.1155/2011/697879.

Falconer, D.S. and Mackay, T.F.C., 1996. Introduction to quantitative genetics. Longman Group Limited, England. pp. 480.

Heiser, C.B., 1978. Taxonomy of *Helianthus* and origin of domesticated sunflower. In: Sunflower science and technology. Ed. Carter, J.F. ASA-CSSA-SSSA, Publishers Madison, WI., USA. pp. 31-53.

Karp, A., Seberg, O. and Buiatti, M., 1996. Molecular techniques in the assessment of botanical diversity. Annals of Botany 78: 143-149.

Lee, M., 1995. DNA markers and plant breeding programmes. Advances in Agronomy 55: 265-344.

Lofgren, J.R., 1978. Sunflower for confectionary food, birdfood and petfood. In: Sunflower science and technology. Ed. Carter, J.F. ASA-CSSA-SSSA, Publishers Madison, WI., USA. pp. 441-456.

SAGIS, 2011. Sunflower, soybean and canola 4 monthly bulletin, 2011/07/11. http://www.sagis.org.za/. Accessed 2011/07/13.

Vrânceanu, A.V., 1998. Sunflower. In: Hybrid cultivar development. Eds. Banga, S.S. and Banga, S.K. Narosa Publishing House, New Delhi, India. pp. 381-382.

Weiss, E.A., 2000. Oilseed crops. Blackwell Science Ltd., USA. pp. 205-244.

Chapter 2
Literature review

2.1 Economic importance of sunflower

Native Americans were among the first to use sunflower (*H. annuus*) in the Southwestern USA (Heiser, 1955) after which the crop spread to northern America. Heiser (1955) also reported that native Americans used sunflower as food and may have cultivated the crop even before they had acquired maize as a food crop. Zukovsky (1950) reported that the initial contacts between North America and Europe have been through Spain. He stated that the earliest records of sunflower seed being introduced to Spain from New Mexico were in 1510 where it was sown in a botanical garden in Spain. He reported that Peter the Great introduced sunflower into Russia during the 18th century. It is important to note that sunflower was reintroduced to North America in its cultivated form after Russia produced it commercially. Seed was imported by American and Canadian farmers from Russia as early as 1880 (Semelczi-Kovacs, 1975; Lentz *et al*., 2001; Lentz *et al*., 2008).

There is limited information available on the development of sunflower in Africa as well as South Africa. Sunflower production in South Africa in 1946 was recorded to be only done on 32 000 ha. There was some production observed in central Africa as well (FAO, 1947-1975). The projected sunflower seed production in South Africa for 2010-2011 was 650 000 ton (Agricommodities, 2011).

Heiser (1955) stated that sunflower consists of 67 species which are all native to the Americas. Most of these species are found in the USA. These species include rare types, some which show elements of natural vegetation and a number of them are weedy types. All the species do however fill niches within the natural ecosystems in the Americas. There are mainly two types which are cultivated as food source. *H. annuus* is largely cultivated for its oil properties and *H. tuberosus* (also known as the Jerusalem artichoke) is cultivated for its tubers.

One of the more important changes in the improvement of sunflower was the development and introduction of dwarf and semi-dwarf types (1-1.5 m) which have small heads. These types were developed because smaller types could be produced that can be harvested easier

than their taller counterparts using mechanical means. Sunflower has also been used as a source of oil in Russia as early as 1779 and the subsequent selection of higher oil content was initiated circa 1860. The oil content of sunflower has since then been increased from 28% to as high as 50% (Zukovsky, 1950; Moghaddasi, 2011). Bâgiu (2007) investigated the development of high oil sunflower hybrids and increased the oil content to a maximum of 53%.

Sunflower oil can be broken down into a number of fatty acids. Palmitic, stearic, oleic and linoleic acids are the primary types. Oleic and linoleic acids make up approximately 90% of the total fatty acid content (Kinman and Earle, 1964; Cummins *et al*., 1967). An inverse relationship seems to exist between oleic and linoleic acid which is influenced specifically by temperature during the growing season (Kinman and Earle, 1964; Canvin, 1965; Jasso de Rodriguez *et al*., 2002; Pacureanu-Joita *et al*., 2005; Chowdhury *et al*., 2007).

2.2 Sunflower morphology

Helianthus annuus is unique in comparison with other cultivated plants due to the fact that it has a single stem and a conspicuous, large inflorescence. It varies greatly with respect to quantitative characteristics which include height, head size, achene size and time to maturity. A number of characteristics can be affected by the environment and measurements of plants should therefore be done under optimum field conditions. The stem of cultivated sunflower is normally unbranched, but branched types do appear in commercial fields and could be used as male parents for the production of hybrid cultivars by opposition companies. The dimensions of the stem as well as the development (including branching) thereof are influenced by the environment. Once the seedling emerges from the soil, the cotyledons unfold and the first pair of true leaves is visible at the tip of the shoot axis. Leaves are then produced in opposite but alternate pairs. After the fifth opposite pair a whorled form of alternate phyllotaxy develops. Leaves on the single stemmed plants can vary in number between eight and 70. Plants with a larger number of leaves tend to be later maturing. Leaves also vary in size, shape of the leaf in general, shape of the tip and base, shape of the margin, shape of the surface, hairiness and petiolar characteristics (Palmer and Phillips, 1963; Lam and Leopold, 1966).

The inflorescence is also important to the plant breeder due to the fact that seed yield is largely determined by the size of the inflorescence as well as the percentage of fertile flowers.

Sunflower is one of the most photogenic crops at flowering stage due to its large inflorescence with yellow-orange ray flowers. The achene (fruit) of the sunflower consists of a seed (also known as the kernel) and an adhering pericarp (also called the hull). When achenes mature over the head, all parts of flowers above the ovary drop away. The achenes tend to grade in size from the largest at the periphery of the head to the smallest at the centre. Achenes also develop a hull whether a seed develops or not. Empty achenes tend to have a pinched appearance. Disk flowers in the center of the head fail to produce seed in some plants and the mature achenes can appear chaff-like. Both the genotype and environment seems to be involved (Fick, 1976; Roth, 1977; Knowles, 1978; Khaleghizadeh, 2011).

2.3 Domestication and genetic development of sunflower for hybrid breeding

2.3.1 Domestication of sunflower

Inbreeding is the natural step to produce pure breeding lines in sunflower, although the risk of narrowing the germplasm base exists. Selection between pure breeding lines will then be done for the best expression of heterosis when crossed. Hybrids are seen as the first generation offspring when two parents of different genotypes are crossed (Fick, 1978; Weiss, 2000). F_1 hybrids are therefore created through inbreeding followed by crossing of dissimilar inbred lines to produce heterozygous though homogeneous hybrids. This procedure ensures uniformity in the seed which is then propagated. Open-pollinated populations on the other hand consist of a variety of genotypes. Genetic homogeneity, which is then combined with high vigour, is achieved through selection within and between inbred lines (Janick, 1999; Škorić *et al*., 2007).

Single cross hybrids, according to Rao and Singh (1978), tend to have significant advantages over three-way hybrids, open-pollinated or synthetic cultivars. This is largely due to higher levels of uniformity for agronomic, disease and seed oil attributes. Fick (1978) also found uniformity in flowering to be useful, largely due to the fact that fewer applications of insecticides are required to control insects such as sunflower moths (*Homoeosoma electellum* Hulst.). The harvesting process could also be made easier through uniformity in maturity, plant height as well as head diameter.

Three-way hybrids are another popular method used in breeding. Two parental lines are used on the female side, which may be related or not. A male line will then be crossed to this single cross as pollinating parent to complete the three-way hybrid. It is possible that slight

segregation might occur in these hybrids resulting in varying flowering periods. Three-way hybrids are an efficient way to reduce seed costs in production systems largely due to the fact that the single cross females have a much higher seed yield than a female inbred line on its own (Van Wijk, 1994; Kaya and Mutlu, 2001; Kaya, 2002).

One of the first major problems initially associated with evaluation of sunflower inbred lines in hybrid combinations was the low hybridisation percentage of crosses. Crossing blocks involving two or more lines were found to exhibit hybridisation percentages which ranged from 21-96%. Current methods employed to produce better hybridisation results are genetic male sterility (GMS) or cytoplasmic male sterility (CMS) to ensure male sterility. Gibberellic acid is another manual method used to ensure male sterility in sunflower breeding material. The gibberellic acid method enables better tester schemes to be used (Fick, 1978; Duca *et al.*, 2008).

Rapid conversion of lines to CMS through the use of glasshouses and winter nurseries can be realised in breeding programmes. Hybrid seed production in isolated crossing blocks (or hand pollinated crossing blocks) using open-pollinated cultivars, synthetics, composites or inbred lines as testers can also be realised using CMS (Fick, 1978).

According to Miller (1999) four distinct heterotic groups are being utilised worldwide in sunflower breeding. Female maintainer inbred lines are being derived from the open-pollinated varieties from Russia. A restorer group, which was acquired from crossing of wild annual sunflower species with domesticated sunflower lines, is uniquely from the USA. These lines tend to be good sources for disease resistance and fertility restorer genes. Romanian and South African female lines (which include CMS lines) are used throughout the industry. The Argentinian INTA (El Instituto Nacional de Tecnología Agropecuaria) group makes up the fourth group which also gives rise to female lines.

2.3.2 Domestication of sunflower through male sterility

An important advance in sunflower domestication and establishing it as a commercially viable crop, was the development of CMS sunflowers. This development made the possibility of hybrid production with much higher yields a viable option (Leclerq, 1969; Leclerq, 1971). Fertility restoration genes were initially identified by Kinman (1970).

Nuclear male sterility (NMS) is another type of sterility which can generally be found in diploid individuals. This type of sterility originates from a spontaneous mutation. This sterility is normally controlled by a single recessive gene. Through the use of a backcross *msms* x *Msms* [*ms* is recessive male sterility (no male sterility) and *Ms* dominant male fertility], the highest level of male steriles obtainable is 50% on average (Poehlman, 1987; Bosemark, 1993). A variety of uses are known for NMS in sunflower. Hand emasculation procedures in self-pollinated crops are no longer necessary, which is a laborious and time consuming exercise. It stands to reason that if a male sterile plant can be used as a female parent, emasculation becomes unnecessary. Natural cross-pollination can also be encouraged in self-pollinated crops. This system can also assist in production of hybrid seed, where a system of pollination control is needed. The problem with NMS is that it does not allow for the production of a uniformly male sterile population and therefore limits the use in hybrid seed production (Poehlman, 1987; Bosemark, 1993). This system was one of the first to replace the use of open-pollinated varieties. It has been replaced commercially by the CMS and fertility restoration method to produce hybrid sunflower. The value of NMS now seems to be an alternative method of hybrid seed production to the CMS system should a problem arise such as was the case in maize. The system is also of value to testcross B-lines prior to conversion to CMS lines (Khan *et al*., 2008).

CMS can generally be divided into two groups, namely alloplasmic and autoplasmic CMS. Alloplasmic CMS is found where CMS was obtained from intergeneric, interspecific or occasional intraspecific crosses and in cases where male sterility could be interpreted as being attributed to inadequate co-operation between the nuclear genome of one species and the organeller genome of another. This would also include CMS in products of interspecific protoplast fusion. Autoplasmic CMS on the other hand is found where CMS has been established within a species due to a result of spontaneous mutational changes in the cytoplasm, which is in all probability in the mitochondrial genome (Bosemark, 1993; Eckardt, 2006).

CMS is an important part of sunflower hybrid production utilised for seed production. This is a much more stable, efficient and economical method than NMS. Inheritance of CMS is under extranuclear genetic control. The combination of so called “sterile” cytoplasms as well as homozygosity for the recessive gene *rf*, sterile (S) *rfrf*, produces male sterility. A genotype which contains normal (N) *rfrf* is normally designated as the maintainer, seeing that a male

sterile plant produces uniform, male sterile progeny only in the case where it is pollinated by plants or isogenic fertile lines of this genotype. Genotypes are also found which inhibit the expression of the CMS characteristic. When such a genotype is used as a pollinating parent on a CMS female and restores the pollen fertility of the progeny, it will be considered to be a restorer. Full restoration often requires the presence of other nuclear genes and could even be accompanied by changes in the mitochondrial genome. CMS is also primarily carried via the female plant. CMS systems vary widely between different crops (Mackenzie and Chase, 1990; Reddy *et al.*, 2008).

CMS systems are widely used in sunflower. The trait is generally incorporated into sunflower female lines through backcrossing. Lines are selected and bred in over seasons. Conversion to CMS only then starts to be implemented by crossing the line in question with a plant containing CMS. The inbred line to be converted will be used as the recurring parent in the backcross and progeny will each time be monitored for the presence of the CMS trait. Ultimately, the final product should ideally be genetically similar to the recurrent parent with the exception that it will be male sterile. The inbred line could have been tested previously for combining ability through the use of a NMS line. Another option will be to test the line with a CMS tester and the resultant cross is then evaluated for combining ability. Conversion of an inbred line to a CMS line can be a long and tedious process, but it is possible to shorten this period through the use of winter nurseries and glasshouses. These methods can assist the breeder in realising as many as three generations per year. A great success in the use of the CMS and accompanying restorer system is that not a lot of problems have been encountered. One of the greatest positive attributes is that the cytoplasm controlling sterility does not influence the agronomic or oil characteristics in any significant way once it is incorporated into inbred lines (Fick, 1978; Reddy *et al.*, 2008).

Unfortunately, there are some negative points involved in using CMS in breeding programmes which include cost and difficulty of use. Maintenance and restoration tend to be dependant on environmental conditions, specifically temperature. Genetic background also tends to have an influence on maintenance and restoration. CMS is associated with some negative traits. Some of these include flower malfunctions as well as chlorophyll deficiencies at lower temperatures. The corresponding genes may need to be introduced into contrasting populations before selection and line development can take place. In certain cases it has been found that hybrid seed production turned out to be impractical as well as uneconomical due to

problems caused by flower morphology and limited pollen dispersal. These problems have however mainly been experienced in self-pollinating crops such as wheat, beans and soybeans. Initially, it is a time consuming and expensive exercise to introduce a CMS-based breeding programme. Once such a system has been established, it can be effective and reliable. Sunflower and sorghum have been found to apply this system effectively (Bosemark, 1993). Fortunately, no mentionable difficulties exist through the use of the CMS and fertility restorer system for the production of hybrid sunflower seed. The cytoplasm controlling sterility does not have any negative effects on the agronomic and oil seed attributes once incorporated into inbred lines. The male sterility source as was discovered by Leclerq (1969) as well as the fertility resoring genes tracing back to Kinman's T66006-2 source have been stable over a number of environments (Velkov and Stoyanova, 1974). It is however important that novel sources of male sterile cytoplasm as well as fertility restoring genes should be found to reduce the potential genetic vulnerability to diseases or other pests (Ardila *et al*., 2010).

2.4 Diseases of sunflower

There are a number of diseases which are known to be antagonistic to sunflower. Most diseases are transferred by soil or windborne fungi (Zimmer and Fick, 1974). There are four major diseases which are of significance worldwide. They include rust (*Puccinia helianthi* Schwein), downy mildew (*Plasmopara halstedii* Farlow), *Verticillium dahliae* (wilt) and *Sclerotinia* stalk and head rot [*S. sclerotiorum* (Lib.) de Bary]. There are certain diseases which can cause damage in specific years should certain climatic conditions be met. These include diseases such as *Phoma* black stem (*Phoma macdonaldii* Boerema), *Alternaria* leaf and stem spot (*Alternaria helianthi* Hansf.), *Septoria* leaf spot (*Septoria helianthi* Ell. At Kell.), *Rhizopus* head rot [*Rhizopus stolonifer* (Ehrenb.) Vuill.], charcoal stem rot (*Sclerotinia bataticola* Taub.) as well as powdery mildew (*Erysiphe cichoracearum* DC) (Sackston, 1981).

There are three major diseases of importance in South Africa. The most significant disease is *Sclerotinia sclerotiorum* which is designated by wilt soon after flowering. A light tan band can be found around the stem at soil level. Grey-black sclerotia form in the rotted heads and stems. The seeds are discoloured and will normally not germinate. *Phoma macdonaldii* is found in South Africa and is recognised through large chocolate coloured blotches on the stems at maturity. *Puccinia helianthi* or rust is found in the western parts of South Africa.

This disease forms rust coloured pustules on leaves, with black specks on the stems. (Bert *et al*., 2004; Zazzerini *et al*., 2005; Vear *et al*., 2007).

2.5 Diversity of sunflower

Helianthus has a basic chromosome number of n=17 and contains diploid (2n=34), tetraploid (2n=68) as well as hexaploid (2n=102) species (Heiser, 1949; Heiser, 1954). Interspecific hybridisation of sunflower has been done for a number of years. Breeders from Russia attempted to use this type of crosses to acquire new sources of resistance to pests. Leclerq (1969) successfully established CMS in the backcross of the cross between *H. petiolaris* with *H. annuus*. It stands to reason that all sunflower hybrids are produced using the CMS-fertility restorer system.

A reduction in genetic diversity occurs mainly due to a population bottleneck. Self-fertilisation which is necessary to achieve pure-breeding lines also adds to this reduction in diversity. Breeders also tend to select for specific agronomically important traits and tend to 'breed out' unwanted genetic material. Liu and Burke (2006) have attempted the first detailed description of patterns of nucleotide polymorphism in wild as well as cultivated sunflower. They found linkage disequilibrium (LD) to be decaying quickly in self-incompatible wild sunflower. Domesticated sunflower (cultivars) showed higher levels of LD. It is therefore important to not only use phenotypic data for description and ultimately registration of cultivars, but also genetic (molecular marker) tools such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). These tools can assist in the detailed description of lines and varieties (Burke *et al*., 2005; Liu and Burke, 2006).

A number of studies on sunflower were done to determine whether genetic diversity losses occurred during the process of inbred line development or whether inbred lines were as diverse as an open-pollinated population. Genetic diversity in sunflower have been shown in a few studies as done by Lawson *et al*. (1994), Jie *et al*. (2003) and Yue *et al*. (2009) among others. There are also studies which found a reduction in genetic diversity in cultivated sunflower when compared with wild sunflower (Gentzbittel *et al*., 1994; Zhang *et al*., 1996). More specifically, Liu and Burke (2006) found that cultivated sunflower is 50-60% less diverse than wild sunflower. Interspecific hybridisation was therefore suggested as a method to create greater diversity in cultivated sunflower. A number of interspecific hybridisation

studies have been established as is the case with Jovanka (2004), Tavoljanskiy *et al*. (2004), Rauf (2008) and Siniša *et al*. (2008).

2.5.1 Genetic distance

Genetic distance as a measurement of diversity is an essential tool to ensure the protection as well as description of plant varieties, more so in commercial crops. Molecular markers have been used extensively to determine genetic distance (Camlin, 2000; Cooke and Reeves, 2003).

Carrera *et al*. (1996) found the difference in gene frequency between sunflower parental genotypes to be important due to the fact that the higher the difference in gene frequency, the higher the level of heterosis should be. Genetic distances among progeny then confirm their origin and the genetic relationship between them and their parents. Smith *et al*. (2009) stated that the breeder can use genetic distance information to make informed decisions regarding the choice of genotypes to cross for the development of populations. It also assists in the identification of diverse parents to cross in hybrid combinations in order to maximise the expression of heterosis.

Genetic distance can be described as the genetic divergence between species or populations within a species. It takes into account a number of parameters used to measure genetic distance. The smaller a genetic distance is, the closer the genetic relationship tends to be and larger genetic distances confer a more distant genetic relationship. Genetic distance can also be used to compare genetic similarity between different species. Genetic distance measurement can be used within species to measure divergence between various subspecies. There are a number of ways to measure genetic distance. Three of the most commonly used distance measures is Nei's genetic distance (Nei, 1972; Nei, 1978), the Cavalli-Sforza chord measure (Cavalli-Sforza and Edwards, 1967) and Reynolds, Weir and Cockerhams genetic distance (Reynolds *et al*., 1983). Nei's standard genetic distance assumes that genetic differences are brought about due to the influence of mutations and genetic drift. The Cavalli-Sforza chord measure and the Reynolds, Weir and Cockerhams genetic distance assume that differences arise due to genetic drift only. In population genetics, the fixation index varies between 0 and 1. The closer the value is to 0, the more identical two populations tend to be and the closer the value is to 1 the more likely is the tendency that two populations belong to

different species. Exceptions do however occur. (Cavalli-Sforza and Edwards, 1967; Nei, 1972; Nei, 1978; Reynolds *et al*., 1983; Takezaki and Nei, 1996).

Studies have been done to determine genetic diversity within the sunflower crop and it is generally based on the following:

2.5.1.1 Morphology

Genetic analysis of sunflower is necessary due to the fact that its germplasm has wide variation in characters such as yield, seed count, plant height, earliness and susceptibility to biotic and abiotic stresses (Thormann *et al*., 1994; Paniego *et al*., 1999). It is important to have a diverse germplasm collection in a successful crop improvement programme. The assessment of genetic diversity within a genetic pool of new breeding germplasm could make crop improvement efficient through the directed accumulation of desired alleles. This process could accelerate the breeding process and reduce the amount of plant material which needs to be screened in experiments. Normally, sunflower cultivar and line identification is based on morphological traits. However, these traits are limited, unstable and not always distinguishable between closely related accessions (Konarev, 2000; Ribeiro *et al*., 2010).

Studies have been done to determine diversity in sunflower through the use of phenotypic and genetic distance determinations. Sujatha *et al*. (2008) used a set of 250 distinct and uniform backcross-derived inbred lines which were developed in sunflower through the use of five interspecific cross combinations. This involved wild diploid annual species namely *H. argophyllus* Torr. & Gray, *H. petiolaris*, *H. debilis* Nutt. and included *H. annuus*. Forty morphologically diverse inbred lines which also included two controls were measured phenotypically and through genetic distance estimation. This included 188 SSR markers of known map location. Their results indicated that the sunflower gene pool could benefit from the introduction of new alleles from the latent genetic diversity present in wild species. The value of morphological traits for evaluation varies according to the intended use of the material. It is important that the level of genetic diversity in pre-breeding material be known so that selection of parental materials can be more effectively done. Melchinger (1999) found that quantitative characters such as yield and heterotic response is expected to increase with parental genetic distance.

Dong *et al.* (2007) studied genetic diversity in sunflower based on eight amplified fragment length polymorphism (AFLP) primers and 17 morphological descriptors. Euclidian distance was used for AFLP (0.32 to 1.56) and morphological data (0.30 to 1.48). The clustering pattern for both AFLP and morphological data indicated unique germplasm which were in general underrepresented in their collection. The morphological based clusters showed a degree of locality separation by germplasm origin, but in general origin did not correspond closely with the clustering pattern.

2.5.1.2 Isozymes

Isozymes were first described by Hunter and Merkert (1957). It is defined as different variants of the same enzyme having identical functions and present in the same individual. By definition this includes enzyme variants that are the product of different genes and therefore represent different loci (isozymes) and enzymes that are the product of different alleles of the same gene (allozymes). Isozymes are a robust and reproducible method. It is seen as a co-dominant marker system and is suited best for the estimation of population genetics parameters as well as genetic mapping. One of the major limitations of isozyme analysis is the low number of markers it provides due to the fact that the number of biochemical assays available to detect them is small. The result of this is that the percentage of genome coverage is not complete enough to allow a thorough enough study of genetic diversity. An additional disadvantage of isozyme analysis lies in the fact that markers are based on phenotype. The problem is that the phenotype may be influenced by environmental factors, with differences in expression making the interpretation of the results more difficult. Due to the fact that differential expression of the genes may occur at different developmental stages or even in different tissues, the same type of material must be used in experiments (Tanksley and Orton, 1983; Hamrick and Godt, 1989; Murphy *et al*., 1996; Rieseberg *et al*., 2007). Studies were conducted in attempting to use isozymes and isozyme systems, with real successes only coming when combined with other marker systems.

Carrera *et al*. (2002) attempted to map sunflower isozymes and used eight isozyme systems. Polymorphisms of the enzyme systems were studied in 25 elite inbred lines. They identified 19 loci, but found only eight to be polymorphic in the germplasm tested. The polymorphic index for the eight informative markers ranged between 0.08 and 0.57 with a mean of 0.36. It was found that several of the isozyme systems used revealed duplicate loci in the sunflower genome, but none of the duplications could be mapped in the F_2 to F_3 populations.

Yordanov *et al.* (2005) used a variety of markers [random amplified polymorphic DNA (RAPDs), arbitrary primed polymerase chain reactions (AP-PCRs), intron fragment length polymorphisms (IFLPs), SSRs and isozymes] to characterise high as well as low regenerative backcross lines and their parents (*H. eggertii* Small. and *H. annuus*). Thirty eight markers specific to *H. eggertii* were developed. Data from the DNA and isozyme analysis were used to determine relationships through the use of a dendrogram. Results exhibited a possibility that the dendrogram could be used as a method for early estimation of advantageous genotypes in plant selection for high regeneration potential.

Problems with the previous marker systems led to the more efficient use of DNA marker systems. This included issues with morphological markers in that they tend to be limited, unstable and not always distinguishable between closely related relatives. Isozyme systems also depend on the phenotype and do not produce sufficient usable markers as was the case in the study done by Carrera *et al.* (2002). DNA-based markers have been proposed by Jaikishen *et al.* (2004) for a more precise and dependable description and differentiation of and among genotypes.

2.6 DNA molecular markers in sunflower as predictor of genetic diversity

By definition, a genetic marker is a gene or DNA sequence with a known location on a chromosome which can be used to identify individuals or even species. It can also be described as a variation which can be observed due to factors such as mutations or alterations in the genomic loci. A genetic marker could consist of a short DNA sequence, such as a sequence around a single base pair change (SNP) or a longer one such as in SSRs. A number of DNA fingerprinting techniques have been developed to provide genetic markers which are capable of detecting differences among DNA samples across a wide range of scales ranging from individual or clone discrimination up to species differences (Vos *et al*., 1995; Blears *et al*., 1998). Some of the available techniques available include: RFLPs [restriction fragment length polymorphisms (Powell *et al*., 1996)], RAPDs (Williams *et al*., 1990), AFLPs (Zabeau and Vos, 1993; Vos *et al*., 1995; Blears *et al*., 1998), SSRs (Tautz, 1989) and SNPs (Brookes, 1999).

A number of factors need to be taken into account before deciding which fingerprinting technique can be used. These include:

1. The application of the technique (DNA genotyping, genetic mapping or population genetics);
2. The organism in question (prokaryotes, plants, animals, humans);
3. Resources available.

Normally, not one DNA-based fingerprinting technique tend to be ideal for all applications (Blears *et al*., 1998).

Some of the techniques are discussed and the various advantages and disadvantages looked at together with some examples of their uses.

2.6.1 Restriction fragment length polymorphism

RFLPs were the first DNA-based markers to be used and identified for diversity studies and the development of genetic maps. RFLPs detect differences in the genomic DNA due to altered lengths of fragments derived through digestion with restriction enzymes (Powell *et al.,* 1996). The resulting length polymorphism between a certain pair of sites is detected through hybridisation to a labelled DNA probe. The use of the RFLP technique has been known to lack detection of polymorphisms in certain crops such as wheat, holding back the successful construction of linkage maps (Joshi and Nguyen, 1993; Powell *et al.*, 1996). Advantages of RFLP markers over other types of markers include their co-dominant nature as well as the ease with which map information could be transferred to a different mapping population (Beckman and Soller, 1986; Helentjaris, 1987). One of the disadvantages of RFLP analysis is that the technique requires relatively large quantities of high quality DNA. Another drawback of RFLP markers is the fact that RFLP probes are limited in availability (Yu *et al.*, 2003) and RFLP analysis is labour intensive (Mohan *et al.*, 1997).

A number of studies have been published where RFLPs have been used. Jan *et al.* (1998) published sunflower maps through the use of RFLPs. Linkage maps in sunflower have been published by a number of scientists, including Gentzbittel *et al.* (1999). Some of these maps have been used to determine quality traits such as high oleic content (Perez-Vich *et al.*, 2002) as well as seed oil content (Leon *et al.*, 2003).

RFLPs have also been used in diversity studies. Gentzbittel *et al*. (1994) used RFLPs to study the genetic relationships between inbred sunflower lines to determine unique restorer and

maintainer germplasm pools. They used 180 nuclear DNA probes to examine RFLPs in inbred lines of sunflower and calculated genetic distances between inbreds. Estimation of the gene diversity indicated that the available genetic variability in cultivated sunflower (based on allelic frequencies) was lower than that of other crops.

2.6.2 Random amplified polymorphic DNA

RAPD analysis (Welsh and McClelland, 1990; Williams *et al.*, 1990), which is a polymerase chain reaction (PCR)-based technique (Mohan *et al.*, 1997) has overcome a significant number of problems that were encountered by RFLPs (Powell *et al.*, 1996). RAPD analysis is based on the amplification of genomic DNA directed by a single short [10 base pairs (bp)] primer consisting of randomly chosen sequences (Williams *et al*., 1990). Numerous DNA fragments are amplified and separated on standard agarose gels. Advantages of the RAPD technique include the fact that it is cost effective and no prior sequence information of template DNA is required. The DNA template required need not be of high purity or quantity. The technique is fast and easy to use and is able to produce markers in regions which contain repetitive sequences. A disadvantage of this PCR-based technique is that it only allows amplification of a relatively small size range of DNA template so that the priming sites need to be relatively close to each other to ensure amplification. Furthermore it has a low accuracy for linkage analysis due to the dominant nature of the technique. Another negative point of this technique is the high sensitivity it shows to PCR conditions (Monna *et al.*, 1994) making it unrepeatable between laboratories. RAPDs were utilised for mapping purposes, but due to the random nature of their generation, as well as their short primer length, they are challenging to transfer between species (Jones *et al.*, 1997).

Genetic diversity studies in sunflower have been done using RAPDs by among others Lawson *et al.* (1994), Arias and Rieseberg (1995), Rieseberg (1996), Faure *et al.* (1999), Popov *et al.* (2002), Liu *et al.* (2003) and Iqbal *et al.* (2008). Nandini and Chikkadevaiah (2005) did fingerprinting and established phylogenetic relationships between parental lines and open-pollinated varieties of sunflower hybrids. Some of the most popular uses of RAPDs in sunflower have been to identify disease resistance loci (or the tagging of phenotypic loci), such as rust (*Puccinia helianthi*), described by Lawson *et al.* (1998), downy mildew (*Plasmopara halstedii*) as shown by Brahm *et al.* (2000), leaf spot disease (*Alternaria helianthi*) as investigated by Murthy *et al.* (2005) and even broomrape (*Orobanche cumana* Wallr.) which is more common in Europe (Lu *et al.*, 2000).

2.6.3 Amplified fragment length polymorphism

AFLP analysis is based on the amplification of genomic restriction fragment subsets through the use of the PCR technique (Zabeau and Vos, 1993; Vos *et al*., 1995). The AFLP technique mainly consists of the following steps. Firstly, restriction fragments of genomic DNA are produced through the use of two different restriction enzymes. One of these is a frequent cutter (for example a four-base restriction enzyme such as *Mse*I) and also a rare cutter (for example the six-base restriction enzyme such as *Eco*RI). The second step is the ligation of oligonucleotide adapters. Double stranded adapters consist of a core sequence and an enzyme specific sequence. These adapters will be specific for either the *Eco*RI or *Mse*I site. Thirdly, pre-selective amplification takes place. Pre-selective primers are complementary to the core sequence of the adapter as well as the enzyme specific sequence plus one additional selective nucleotide. The fourth step is selective amplification using labelled primers. Selective primers are either radio-actively labelled or fluorescently labelled. Silver staining could also be done which negates the need for labelled primers. Selective primers consist of an identical sequence to that of the pre-selective primers with an additional two selective nucleotides at the 3'-end. The last step is the gel based analysis of the amplified fragments. Labelled fragments can be resolved through gel electrophoresis on among others a Perkin-Elmer Applied Biosystems Inc. automated sequencer. Genescan software can analyse four different fluorescent labels which are visualised as blue, green, yellow and red. It is possible to load multiple samples (amplified with separate primer sets, each labelled with a different fluorescent dye) into a single gel lane along with an internal size standard (Vos *et al*., 1995; Blears *et al*., 1998).

The choice of the number and sequence of the selective nucleotides consequently control the number of DNA fragments obtained (Lin and Kuo, 1995; Mohan *et al*., 1997). AFLP analysis differs from the RFLP technique in the sense that it will display the presence or absence of rectricton fragments rather than length polymorphisms (Vos *et al*., 1995). AFLPs are therefore able to discriminate between closely related organisms, which include near-isogenic lines. A large number of restriction fragments are created which facilitates the detection of polymorphisms. The usefulness of this technique is further accentuated since it requires no previous sequence characterisation of the target genome and can therefore be used for DNA of any origin or complexity (Vos *et al*., 1995). It is also easy to standardise this technique and it can be automated for high throughput applications. High reproducibility, rapid generation

and high frequency of identifiable polymorphisms ensure AFLP analysis to be an attractive technique for identification of polymorphisms and determination of linkages through analysis of individuals from a segregating population before modern techniques were developed (Vos *et al*., 1995; Jones *et al*., 1997).

Brugmans *et al*. (2003) reported that in single locus assays, AFLP markers tend to be less suitable. This includes allele frequency studies, marker-assisted selection (MAS) and map-based cloning. They also found that even though AFLP markers could be used in these applications, many markers tend to be redundant and therefore too expensive and laborious for large-scale single locus screening. One of the major problems is the number of steps involved. The technique can also be expensive due to the cost of the enzymes used. Mba and Tohme (2005) found that simultaneous occurrence of dominant and co-dominant banding patterns are another important factor which limits the application of AFLPs. This can lead to misinterpretation of AFLP data.

This technique has been used as a capable marker system, seeing that it has various applications, for instance genetic mapping, DNA fingerprinting and ultimately diversity analysis (Kusterer *et al.*, 2004). AFLPs have been found to be an effective tool in the DNA fingerprinting of sunflower, as shown by Cheres and Knapp (1998). They attempted to link all current available public lines in the USA to ancestral origins and found that the B-lines (maintainer lines) had a wider genetic diversity base than the R-lines (restorer) and confectionary R-lines. A nearly complete pedigree framework was drawn up, although some lines could not be linked due to incomplete pedigree data. Genetic maps have been successfully drawn up through the use of AFLPs in crops such as rice, maize and sunflower (Rachid Al-Chaarani *et al.*, 2002).

Quagliaro *et al*. (2001) used AFLPs to ascertain the various levels of diversity within and also between populations of *H. argophyllus* which were collected in Mozambique. The data were used for both taxonomic and breeding purposes. They found three primer combinations to exhibit the best results with 92 polymorphic fragments. They were able to discriminate between the wild endemic populations from *H. annuus* and from those of the interspecific hybrids. The majority of the variation was observed within the population. The dendrogram was based on shared fragments and this aimed to divide the *H. argophyllus* into distinct groups resembling various populations. The hybrid genotypes formed unique subgroups with

cultivated sunflower genotypes, which confirmed the applicability of this technique for taxonomic as well as phylogenetic studies. The 12 populations of *H. argophyllus* presented a potentially new and valuable genetic resource, but it was found that only two of them exhibited the majority of the variation observed. This suggested that these two populations could be the most promising material for crossing with cultivated sunflower.

Rönicke *et al*. (2008) studied progenies of partial hybrids between *H. maximilliani* Schrad. (a wild species which have been shown to be resistant to *S. sclerotorium*) and *H. annuus*. Hybrids were characterised by AFLP analysis to determine whether there were introgressions from *H. maximilliani* into cultivated sunflower at molecular level. They found wild species specific fragments as well as fragments not belonging to either parent. The progenies were studied and evaluated for their reaction to *S. sclerotorium* through the use of artificial inoculation on the sunflower heads. Some of the progeny showed a higher level of resistance in comparison with resistant inbred lines. They identified two AFLP fragments which seemed to be linked to *S. sclerotorium* resistance.

Vischi *et al*. (2002) used AFLP data to distinguish between wild material and cultivated sunflower to ascertain the potential value of the material in breeding programmes. Populations were used as well as the wild types of sunflower, *H. argophyllus* and *H. debilis*. They found *H. argophyllus* alleles to be dominant with respect to *H. debilis* alleles and AFLPs as dominant markers did not reveal the heterozygous genotypes. It was found that the wild material appeared quite different at molecular level in comparison with *H. annuus* individuals which suggested potential for its use in breeding programmes.

2.6.4 Simple sequence repeats

SSRs, also known as microsatellites, are commonly used as molecular markers. SSRs are highly mutable loci which could be present at various sites in a genome (Tautz, 1989; Morgante and Olivieri, 1993). Flanking sequences at these sites could possibly be unique and conserved. If SSR loci are cloned and sequenced, it is possible to design primers for these flanking sequences. The sequence tagged microsatellite which is then obtained normally identifies a single locus which is often found to be multi-allelic. The reason for this is that SSRs tend to have a high mutation rate (Jeffreys *et al.*, 1994; Tang *et al.*, 2002). Alleles which then differ in a number of base pairs in length can be resolved on agarose gels. SSRs are however more often visualised on ABI sequencing gels where it is possible to resolve

single repeat differences. All possible alleles are therefore detected. Due to their co-dominant nature (unlike RAPD and AFLP markers), SSRs provide highly informative and polymorphic markers (Tautz, 1989; Morgante and Olivieri, 1993; Koreth *et al.*, 1996). Essential knowledge of SSR markers can be electronically shared and distributed among different laboratories. Duplication of results is quite easily done between facilities. Most SSR markers are monolocus and exhibit Mendelian inheritance. SSR markers are also informative and a large number of public SSRs are available. Genotyping using SSR markers can be easily and rapidly done through the use of a variety of platforms for DNA fragment analysis and quite a number are semi-automated (Cregan *et al.*, 1999; Korzun, 2003). One of the problems associated with SSRs is that the cost involved for primer development is high (Korzun, 2003).

SSRs have been successfully used in the determination of genetic distance in sunflower. Gvozdenović *et al.* (2009) used SSRs to determine the correlation between SSR based genetic distance and heterosis for six agronomic traits. Results obtained were not as expected and it was found that a low correlation existed between genetic distance and heterosis. They came to the conclusion that better results could be obtained should hybrid combinations for each tester and each specific trait were to be analysed separately.

Smith *et al*. (2009) investigated genetic diversity in USA sunflower inbreds as well as hybrids through the use of SSRs. They found associations of inbreds to be consistent with known pedigrees. The male and female parents also tended to group together. Hybrids tested grouped together according to the source company. The SSR profiles could be used to ensure evaluation of distinctness which are necessary to acquire plant variety protection.

Yu *et al.* (2002) used SSRs as a high throughput method for genetic fingerprinting of cultivated sunflower (*H. annuus*) and found that results obtained through this method were similar to previously described methods. They developed 74 SSR markers which were polymorphic when screened for length polymorphisms between 16 elite inbred lines. When cluster analysis was done, they found genetic diversity similar to patterns produced by RFLP fingerprinting. SSRs were found to be slightly more polymorphic than RFLPs. Certain individual SSRs were much more polymorphic than RFLP markers with polymorphic information content (PIC) scores between 0.70 and 0.93. They also found that

polymorphisms observed in cultivated sunflower seemed to be much more reduced than in other crops.

Yordanov *et al.* (2005) used a variety of markers, including SSRs, to establish the high and low regenerative backcross lines and their parents from crosses between *H. annuus* and *H. eggertii*. It was possible to use the resultant dendrogram as a method to estimate the best genotypes when plant selections needed to be done for the best success rate.

Zhang *et al.* (2005) contributed successfully towards the establishment of a set of SSR markers to assist in fingerprinting and variety identification of sunflower lines. They detected low levels of variation within the restorer and maintainer groups. Breeding bottlenecks can be expected to have contributed to this result. A significant difference could however be found between the two groups. The selected set of SSRs was successful in determining sunflower fingerprints and genetic diversity.

Kusterer *et al.* (2004) furthermore reported on the construction of a genetic map in sunflower, as well as the localisation of some of the major traits. A segregating mapping population was developed and the major traits looked at was male fertility vs. sterility, downy mildew resistance vs. susceptibility as well as oleic vs. linoleic acid content. Both AFLP and SSR markers were used. Successful mapping of the mentioned trait genes was done, but consensus was found that more saturated mapping needed to be done, especially on genes coding for oleic vs. linoleic acid content. Kusterer *et al.* (2005) have also carried out finer mapping of the restorer locus. Table 2.1 is a concise table comparing the various molecular marker systems.

2.6.5 Single nucleotide polymorphism

The basic definition of SNPs is the difference in single base pair positions in genomic DNA (Brookes, 1999). Single base insertion/deletion variants or indels would not strictly be considered to be SNPs according to Brookes (1999). It is however true that a number of properties of SNPs also applies to small insertions and deletions. This specific definition is highly dependent on the population used and the non-polymorphic sequence should be seen in this light (Brookes, 1999). SNPs are known to contain the highest level of molecular markers in the genome.

Table 2.1 Comparison of the most commonly used marker systems (Korzun, 2003)

Feature	**RFLP**	**RAPD**	**AFLP**	**SSR**	**SNP**
DNA required (μg)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	High	Moderate	High	Moderate	High
PCR-based	No	Yes	Yes	Yes	Yes
Number of polymorphic loci analysed	1-3	1-50	20-100	1-3	1
Ease of use	Not easy	Easy	Not easy	Easy	Easy
Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Low	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low

RFLP Restriction fragment length polymorphism
RAPD Random amplified polymorphic DNA
AFLP Amplified fragment length polymorphism
SSR Simple sequence repeat
SNP Single nucleotide polymorphism

Plants contain a high density SNPs across the genome, with the number of SNPs in sunflower varying between 1 SNP per 90 bp in coding regions to 1 SNP per 48 bp in non-coding regions. This suggested that the coding regions are more conserved than non-coding regions, most likely due to purifying selection (Bhattramakki *et al.*, 2000; Fusari *et al.*, 2008; Fusari *et al*., 2010). SNPs have been found to be highly abundant in the genome and are useful for creating high-density genetic maps. Furthermore, SNPs have the potential to provide a basis of a superior and informative genotyping platform. SNPs in coding regions could have functional significance if the resulting amino acid change causes changes in the phenotype (Jehan and Lakhanpaul, 2006).

Four possible nucleotides are theoretically involved in SNP variations but in reality only two of the four possibilities have been observed at specific sites in a population. SNPs are therefore bi-allelic in nature. This attribute makes them less informative per locus examined than multi-allelic markers such as RFLPs and SSRs, but the fact that more SNPs can be detected alleviates this problem to some extent (Xiong and Jin, 1999). Fusari *et al.* (2008) confirmed that this major disadvantage can be overcome by the higher level and stability of SNP loci as opposed to SSR loci. SNPs are known to exhibit high information content and produce high levels of polymorphisms. The initial cost involved are however quite high for this technique. SNPs are easily automated and can therefore be more cost effective in the long run (Jehan and Lakhanpaul, 2006).

SNPs have been successfully applied by Kolkman *et al.* (2007) to establish LD in sunflower. It was found that LD decayed more slowly in inbred lines than in wild populations. This was largely attributed to bottlenecks caused by commercial breeding as well as domestication of sunflower. Fusari *et al.* (2008) used SNPs to determine genetic diversity in sunflower and found that there was lower diversity among inbred lines than wild populations.

Freeman *et al.* (2003) identified SNPs in allele sequences of eight to twelve sunflower genotypes which were originally mapped using RFLP markers. High throughput SNP markers were developed for the RFLP loci for amongst other, linkage mapping and diversity analysis.

2.7 Improvement of efficiency of SSR reactions

2.7.1 Multiplex PCR

The effectivity of SSR analysis can be improved through a number of methods. Multiplex PCR is one of these methods. Multiplex PCR is normally a two-amplicon system or it can amplify 13 or more separate regions of DNA (Edwards and Gibbs, 1994). There are a number of advantages to using multiplex PCR. Firstly, it is possible to design internal control amplicons for verification of the presence of target templates. This overcomes possible false negatives which can be found in PCR due to contamination problems (Bej *et al*., 1990). Secondly, it is possible that the quality of the template could be determined much more effectively in multiplex PCR than in regular PCR (Chamberlain *et al*., 1992). Thirdly, it is possible that the amplification as well as the internal standards of multiplex PCR could be used to determine the quantity of a specific template in a sample (Ferre, 1992). Lastly, the effect of cost also plays a role. The expense of reagents and preparation time is also reduced in multiplex PCR compared to normal PCR systems where numerous reaction tubes are used. To maximise efficiency, reactions could be prepared in bulk, then tested for quality and frozen without enzyme or template until it is to be used (Chamberlain *et al*., 1988; Zhang *et al*., 2010).

There are a number of difficulties in the use of multiplex PCR. It is necessary to ensure that the technique is properly optimised. This could be difficult as well as time consuming. There has been limited use of multiplex PCR with SSRs. This includes the fact that the number of polymorphic SSR marker loci for molecular breeding is higher than the number used in multiplex PCR reactions (Hayden *et al*., 2008; Zhang *et al*., 2010).

Baldini *et al*. (2004) used a set of primer combinations selected on the basis of amplicon length to facilitate multiplexing. SSR markers were screened for polymorphism through the use of three colour multiplexes. Markers were used to assist in the evaluation of genetic variability for *S. sclerotorium* resistance in a F_2 population from a cross between susceptible and resistant (derived from *H. argophyllus*) sunflower. They found that the ideal marker for multiplexing should be single locus and not produce non-target bands. It should also be co-dominant and not produce null alleles. Not all of the markers they used were unilocular and co-dominant.

Tang *et al*. (2003) developed PCR-multiplexes to establish a near genome wide framework of SSR marker loci in *H. annuus*. They identified the most outstanding single locus SSR markers from the public collection and screened them for testing in multiplex PCR. They found that the multiplexed PCR markers, when coupled with 17 complimentary SSR marker loci, created a standard genotyping set. This was ideal for first pass scans of the genome, which are necessary for screening bulked segregant DNA samples or mapping phenotypic trait loci. They found that PCR multiplexes increased genotyping throughput, reduced reagent costs and are ideal for repetitive genotyping applications where common sets of SSR marker loci are required or advantageous.

2.7.2 M13 tailed PCR technique

Labelling of PCR products with fluorescently labelled primers has a number of advantages over radio-active labelling or silver staining. This includes the potential for high throughput operations (Oda *et al*., 1997). It is however true that SSR typing through the use of fluorescently labelled primers could be expensive and this will entail that laboratories with limited budgets could be prevented or limited in typing large numbers of SSR markers. Radioactive elements are also not ideal for use in laboratoriums anymore.

An alternative to individually labelled primers is the possibility to use a third primer which is labelled with a fluorescent dye (Oetting *et al*., 1995). One of the two PCR primers then contains a so-called tail (a unique sequence such as the M13 universal primer sequence) in addition to a specific sequence matching a conserved sequence at one side of the microsatellite repeat. PCR products from this amplification will then contain the tail after the initial amplification cycle. A third primer with the tail sequence (the M13 universal primer

sequence) is 5` labelled with a fluorescent dye and also included in the reaction. This will lead to the incorporation of fluorescent labels into the PCR products. It is necessary to mention that this method does not always work well for all SSRs. This entails that special cycling conditions are recommended for different markers (Oetting *et al*., 1995). There have been some studies which have conflictingly reported on the use of M13 tailed primers as well as individually labelled primer methods. Boutin-Ganache *et al*. (2001) compared the tailed primers and individually labelled primers and concluded that the M13 tailed technique improved specificity. Zhou *et al*. (2002) found that the M13 tailed method tended to show inconsistent performance in amplifying the plant genome of *Pinus taeda* L.

There are two stages in a single amplification reaction for the M13 tailed PCR technique:

1. Amplicon 1 is produced through using the tailed forward and the 3' reverse primer. The extension of the forward primer therefore yields a product which contains the tail sequence. When this template therefore anneals with the reverse primer and extends, a product which contains the complement of the mentioned tail sequence will be produced (known as amplicon 2).
2. The last step will then be the production of amplicon 3 through the use of the labelled M13 primer and amplicon 2 as template. The fluorescent reporter is then incorporated into the product during polymerisation and a fluorescent signal is produced. The DNA sequencer will then only detect the labelled amplicon 3.

This process is better visualised through Figure 2.1 as was shown by Zhang *et al*. (2003).

Manual systems are systematically being replaced by semi-automated methods of SSR genotyping in plant breeding and genetics research. These methods are facilitating the effective application of SSR markers for among other applications pedigree analysis (Lexer *et al.*, 1999; Cipriani *et al.*, 2008; Mittal and Dubey, 2009; Singh *et al.*, 2010) as well as assaying of genetic diversity (Macaulay *et al.*, 2001; Zhang *et al.*, 2005; Pervaiz *et al.*, 2010).

2.8 Statistical analysis and sunflower heterotic groups

Sunflower research is normally based on two main structures. The first is the breeding programme and the second multi-location yield trials. The focus of the breeding programme is the development of new inbred lines. The female lines will include isogenic CMS and

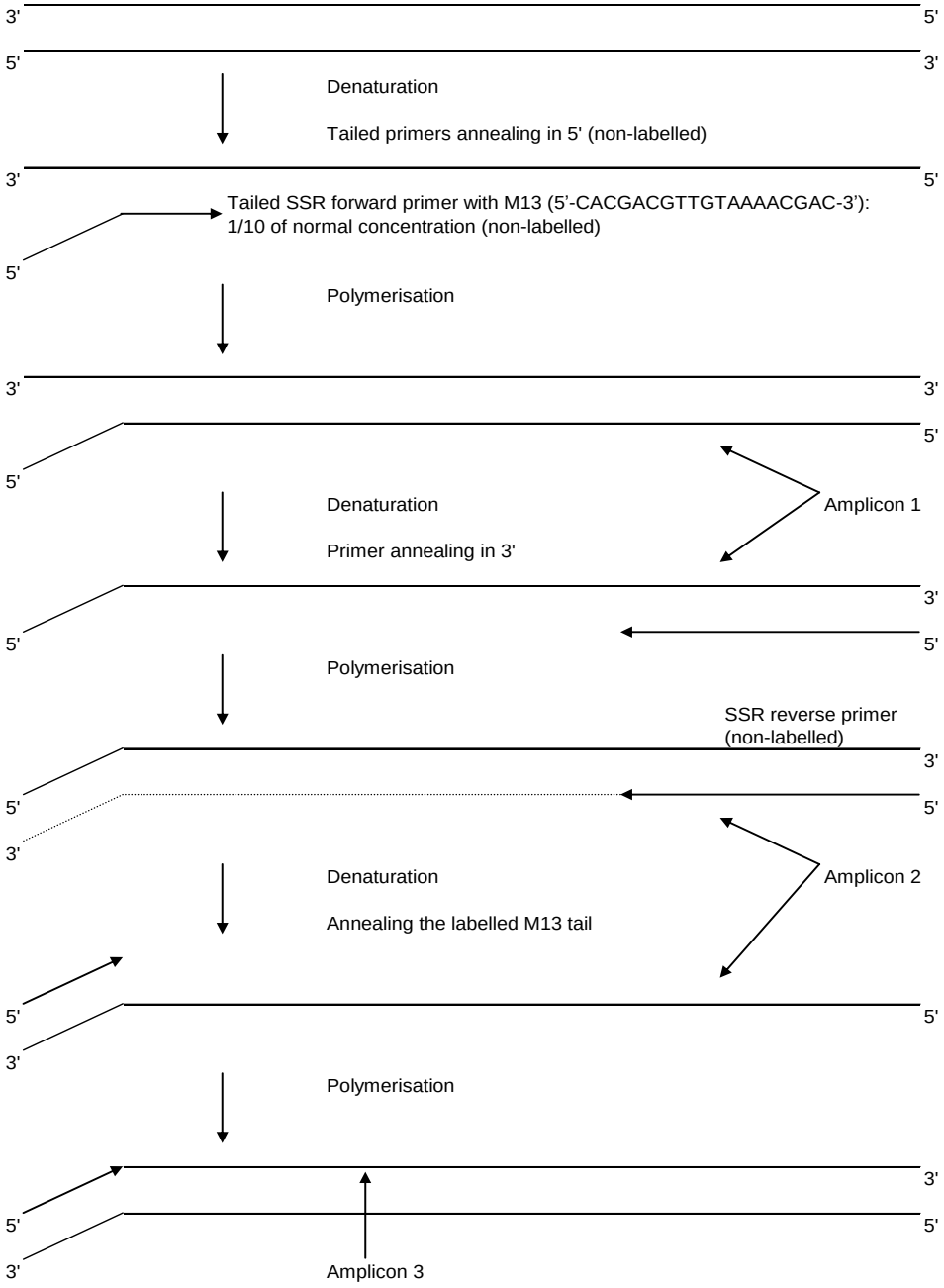

Figure 2.1 The M13 tailed PCR technique (Zhang *et al.*, 2003).

SSR Simple sequence repeat

maintainer lines and on the male side, restorer lines. The CMS and restorer lines are crossed in various combinations to produce hybrids for evaluation in multi-location yield trials. The yield trials serve three basic functions. The first is to accurately estimate and predict the yield based on limited experimental data. The second is to determine yield stability and patterns of response of genotypes across environments. The third is to provide a reliable platform for the selection of genotypes for breeding in the following seasons (Crossa, 1990). To progress significantly in breeding, it is therefore necessary to obtain reliable data from the yield trials. Heterosis also plays a major role in the development of successful hybrids.

Heterosis can be described as the inverse of inbreeding depression and is defined as the difference between the crossbred and inbred means. This translates to the difference between the hybrid and the mean of the parents involved (Falconer and Mackay, 1996; Khan *et al*., 2008). According to Lamkey and Edwards (1999), this definition can also be described as mid-parent heterosis. They stated that high-parent heterosis can be calculated from the mean values of the F_1 cross and the high parent. This is preferred more in self-pollinated crops where the goal is to find a better hybrid than either of the parents.

It is important to determine the relationships between different heterotic groups and ascertain if a line could be used as an elite line in different testing structures. According to Chaudhary (1982) combining ability can be defined as the ability of a parent to produce either inferior or superior combinations in a single or a series of crosses. The general combining ability (GCA) value of a genotype determines the crossing value of the genotype that is (a line or a tester) the optimum combiner in a breeding programme (Falconer and Mackay, 1996). Specific combining ability (SCA) on the other hand shows the minimum as well as the maximum genetic gain of hybrids from specific lines with specific testers. SCA is central in hybrid breeding. The SCA of a cross gives an indication of the proportion of loci which shows dominance (V_D) as well as interaction (V_I). Dominance and interactions are the result of specific gene combinations. Genes involved in dominance and interaction are transferred from parents to offspring (Falconer and Mackay, 1996; Karasu *et al*., 2010). Sprague and Tatum (1942) described SCA as an estimate of the effects of non-additive gene actions. Wricke and Weber (1986) found that combining ability analysis exhibited significant differences between the restorer and CMS lines in their GCA, but no differences were found in the SCA for the relevant yield related traits (Karasu *et al*., 2010).

Cheres *et al.* (1999) investigated the possibility that genetic distance could be a possible predictor of heterosis and resultant hybrid performance within and also between heterotic groups in sunflower. They used 42 female x male (A-line x R-line) and 81 female x female (A-line x B-line) heterotic group crosses. They found heterosis to be significant especially for seed yield. Hybrid seed yield and genetic distances were significantly correlated with each other through the use of AFLP fingerprints. They came to the conclusion that the heterotic groups in sunflower were of significant use, but was not as distinct as is the case in maize.

Turkec and Goksoy (2006) used a more conventional approach to establish general and specific combining ability in sunflower for seed yield and various yield components. Five female lines (A-lines) and five male lines (R-lines) were crossed to produce 25 hybrids. The superior F_1 sunflower hybrids were then evaluated through the use of line x tester analysis. They found a variety of lines to be good general combiners, as well as a variety of crosses to be good selections for SCA for yield as well as good yield components.

2.8.1 REML analysis

Statistical trials can be analysed with a number of methods and programmes. Holland (2006) investigated the use of the restricted maximum likelihood (REML) method as opposed to the multivariate analysis of variance (MANOVA) method. He stated the drawbacks of the MANOVA method as being the possibility of obtaining estimates outside of parameter bounds, reduction in estimation efficiency as well as ignorance of the estimator's distributional properties in cases where data might be missing. The last mentioned problem is also one of the biggest obstacles encountered by plant breeders. REML on the other hand relies on the assumption of normally distributed random effects as well as large sample sizes. REML also requires more computing power, but due to advances in the processing speeds of computers it was made feasible to run REML on personal computers.

There are several advantages of REML estimation in comparison with the MANOVA method. One of the advantages is that REML estimates of the variance and covariance components have known asymptotic distributional properties and then efficiently use information from all experimental units when data are unbalanced. One of the biggest drawbacks of the REML based approaches is that sampling distributions of correlation estimates are usually not available in closed form and tend to be abnormal, according to Liu *et al.* (1997). The REML and analysis of variance (ANOVA) methods were compared using

real data sets as well as simulated data sets. They found that the two methods delivered similar results when the data were balanced or when only 5% of the data were missing. They proved that when more data (15% or 25%) were missing, the REML method in general delivered better results. When one is faced with severely unbalanced data, REML has some important advantages over ANOVA. REML point estimates of parameters are more efficient than those of ANOVA, which means it has a lower variance. Hypothesis tests based on REML tend to have a stronger theoretical justification than approximate *F*-tests. Another advantage is that REML provides a way to test the null hypotheses V_g=0 and V_t=0 (Saxton *et al.*, 2004). Bonate (2006) stated that the parameter estimates in REML are consistent, meaning that as the sample size increases the estimates tend to converge towards the true population values. The standard errors also then decrease simultaneously. Another advantage is that the estimates are also asymptotically normally distributed. The variance of REML estimators is also smaller than the estimators obtained through the use of any other methods (Burch, 2011).

A number of other statistical techniques also exists which have been used in genotype x environment interactions. Crossa (1990) investigated a number of these techniques. Some of these techniques include ANOVA, joint linear regression, additive main effects as well as additive main effects and multiplicative interaction analysis (AMMI). Different multivariate analysis methods have also been used, such as principal component analysis (PCA), principal coordinate analysis, factor analysis as well as cluster analysis. He found that linear regression analysis was mathematically simple and results obtained were biologically interpretable. There are some disadvantages, which includes the following:

1. It is uninformative when linearity fails. One of the assumptions of linear regression is that a linear relationship exists between interaction and environmental means. Results could however be misleading should this linearity not exist. The analysis requires that a large proportion of the genotype by environment effects be attributable to linear regression.
2. It is dependent on the set of genotypes as well as the environments in question. In the regression model, the genotype mean is not independent of the marginal means of the environments. The problem is that once one set of variables is regressed on another that is not independent it violates one of the assumptions of regression analysis. The interdependence could be an issue when a smaller number of genotypes are involved, but not in the case of larger numbers. Should the standard set for stable yield be based on

few genotypes (i.e. 10), each estimated stability coefficient involves the regression of one genotype on an average to which it contributes one tenth (10%). Therefore the smaller the number of genotypes, the smaller the discrepancy is. It also tends to oversimplify the different response patterns through the explanation of the interaction variation in a single dimension (regression coefficient), whilst it might in truth be very complex (Crossa, 1990).

Multivariate methods overcome a number of problems experienced with linear regression. Crossa (1990) describes multivariate methods to have three purposes, namely to eliminate noise from data patterns, summarise data and reveal a structure in the data. Multivariate analyses are well suited for analysis of two-way matrices, genotypes (G) and environments (E), with the aim being to be able to evaluate the response of any genotype in any environment. This response can therefore be conceived as a pattern in E-dimensional space, with the coordinate of an individual axis being the yield or other metric of the genotype in one environment (Kiiveri, 2011).

Another tool available for the analysis of statistical trials is GenStat. Payne (2009) described GenStat to be one of the earliest statistical systems as well as one of the few to be developed outside of North America. One of the greatest features of GenStat is that it was developed by a Statistics Department which also gave rise to popular tools used in applied statistics. Some of these tools include ANOVA, maximum likelihood, general balance, generalised linear models, canonical variates analysis as well as REML analysis of mixed models. GenStat allows for algorithms to be selected as general and comprehensive as possible. One of the best examples of this is the REML algorithm for the analysis of linear mixed models and its extension to the general modelling of covariance structures. This tool not only handles unbalanced designs with several error terms, but it also allows for appropriate analysis of repeated measurements. This is done by fitting autoregressive and ante-dependence models to describe the within subject correlation structures and to model the spatial variation in field trials. This is therefore one of the preferred methods used in the determination of statistical sunflower trials. De la Vega and Chapman (2010) successfully used REML to investigate the influence of environment on the development of genetic progress of especially seed yield in sunflower.

It is clear from literature that limited work has been done on the relationship between genetic distance and yield parameters as well as other agronomic characteristics in sunflower. Determination of genetic distance to determine the relationship between the lines involved would be the first step in attempting to achieve the desired result.

2.9 References

Ardila, F., Echeverria, M.M., Rios, R. and Rodriguez, R.H., 2010. Structural features of a cytoplasmic male sterility source from *Helianthus resinosus*, CMS RES1. Journal of Plant Breeding and Crop Science 2: 168-172.

Agricommodities, 2011. http://www.agricommodityprices.com/futures_prices. Accessed 2011/06/15.

Arias, D.M. and Rieseberg, L.H., 1995. Genetic relationship among domesticated and wild sunflower (*Helianthus annuus*, Asteraceae). Economical Botany 49: 239-248.

Bâgiu, C., 2007. Developing high oil sunflower hybrids. Helia 30: 69-78.

Baldini, M., Vischi, M., Turi, M., Di Bernardo, N., Raranciuc, S., Echeverria, M., Castaño, F., Vannozzi, G.P. and Olivieri, A.M., 2004. Evaluation of genetic variability for *Sclerotina sclerotorium* Lib. De Bary resistance in a F_2 population from a cross between susceptible and resistant sunflower. Helia 27: 159-170.

Beckman, J.S. and Soller, M., 1986. Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35: 111-124.

Bej, A.K., Mahbubani, M.H., Miller, R., DiCesare, D.L., Haft, L. and Atlas, R.M., 1990. Multiplex PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. Molecular and Cellular Probes 4: 353-365.

Bert, P-F., Deschamp-Guillaume, G., Serre, F., Jouan, I., Tourvieille de Labrouhe, D., Nicolas, P. and Vear, F., 2004. Characterisation of QTL involved in resistance to *Sclerotinia sclerotiorum* and *Phoma macdonaldii*. Theoretical and Applied Genetics 109: 865-874.

Bhattramakki, D., Ching, A., Morgante, M., Dolan, M. and Register, J., 2000. Conserved single nucleotide polymorphism (SNP) haplotypes in maize. Plant and Animal Genome VIII Conference (San Diego, CA), January 9-12, 2000.

Blears, M.J., De Grandis, S.A., Lee, H. and Trevors, J.T., 1998. Amplified fragment length polymorphism (AFLP): a review of the procedure and its applications. Journal of Industrial Microbiology and Microbiology 21: 99-114.

Bonate, P., 2006. Pharmacokinetic-Pharmacodynamic modelling and simulation. Springer Science-Business Media, New York. pp. 188-189.

Bosemark, N.O., 1993. Male sterility. In: Plant Breeding: Principles and prospects. Eds. Hayward, M.D., Bosemark, N.O. and Romagosa, I. Chapman and Hall, London. pp. 213-218.

Boutin-Ganache, I., Raposo, M., Raymond, M. and Deschepper, C.F., 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31: 24-28.

Brahm, L., Röcher, T. and Friedt, W., 2000. PCR-based markers facilitating marker assisted selection in sunflower for resistance to downy mildew. Crop Science 40: 676-682.

Brookes, A.J., 1999. The essence of SNPs. Gene 234: 177-186.

Brugmans, B., van der Hulst, R.G.M., Visser, R.G.F., Lindhout, P. and van Eck, H.J., 2003. A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. Nucleic Acids Research 31: e55.

Burch, B.D., 2011. Assessing the performance of normal-based and REML-based confidence intervals for the intraclass correlation coefficient. Computational Statistics and Data Analysis 55: 1018-1028.

Burke, J.M., Knapp, S.J. and Rieseberg, L.H., 2005. Genetic consequences of selection during the evolution of cultivated sunflower. Genetics 171: 1933-1940.

Camlin, M.S., 2000. Possible future roles for molecular techniques in the identification and registration of new plant cultivars. ISHS Acta Horticulturae 546: International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture.

Canvin, D.T., 1965. The effect of temperature on the oil content and fatty acid composition of the oils from several different seed crops. Canadian Journal of Botany 43: 63-69.

Carrera, A.D., Pizarro, G., Poverene, M., Feingold, S., León, A.J. and Berry, S.T., 2002. Variability among inbred lines and RFLP mapping of sunflower isozymes. Genetics and Molecular Biology 25: 65-72.

Carrera, A.D, Poverene, M. and Rodriguez, R.H., 1996. Isozyme variability in *Helianthus argophyllus*. Its application in crosses with cultivated sunflower. Helia 19: 19-28.

Cavalli-Sforza, L.L. and Edwards, A.W.F., 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32: 550-570.

Chamberlain, J.S. and 33 co-authors, 1992. Diagnostic of Duchenne and Becker muscular dystrophies by polymerase chain reaction: A multicentre study. The Journal of the American Medical Association 267: 2609-2615.

Chamberlain, J.S., Gibss, R.A., Ranier, J.E., Nguyen, P.N. and Caskey, C.T., 1988. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Research 16: 11141-11156.

Chaudhary, R.C., 1982. Introduction to Plant Breeding. Oxford and IBH Publishing Co. PVT. LD. New Delhi-Bombay. pp. 267.

Cheres, M.T. and Knapp, S.J., 1998. Ancestral origins and genetic diversity of cultivated sunflower: co-ancestry analysis of public germplasm. Crop Science 38: 1476-1482.

Cheres, M.T., Miller, J.F., Crane, J.M. and Knapp, S.J., 1999. Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. Theoretical an Applied Genetics 100: 889-894.

Chowdhury, K., Banu, L.A., Khan, S. and Latif, A., 2007. Studies on the fatty acid composition of edible oil. Bangladesh Journal of Scientific and Industrial Research 42: 311-316.

Cipriani, G., Marrazzo, M.T., Di Caspero, G., Pfeiffer, A., Morgante, M. and Testolin, R., 2008. A set of microsatellite markers with long core repeat optimized for grape (*Vitis* spp.) genotyping. BMC Plant Biology 8: 127.

Cooke, R.J. and Reeves, J.C., 2003. Plant genetic resources and molecular markers: variety registration in a new era. Plant Genetic Resources: Characterization and Utilization 1: 81-87.

Cregan, P.B., Jarvik, T., Bush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.I., Kaya, N., Van Toai, T.T., Lohnes, D.G., Chung, J. and Specht, J.E., 1999. An integrated genetic linkage map of the soybean genome. Crop Science 39: 1464-1490.

Crossa. J., 1990. Statistical analyses of multilocational trials. Advances in Agronomy 44: 55-85.

Cummins, D.G., Marion, J.E., Craigmiles, J.P. and Burns, R.E., 1967. Oil content, fatty acid composition and other agronomic characteristics of sunflower introductions. Journal of the American Oil Chemists Society 44: 581-582.

De la Vega, A.J. and Chapman, S.C., 2010. Mega-environment differences affecting genetic progress for yield and relative value of component traits. Crop Science 50: 574-583.

Dong, G.J., Liu, G.S. and Li, K.F., 2007. Studying genetic diversity in the core germplasm of confectionary sunflower (*Helianthus annuus* L.) in China based on AFLP and morphological analysis. Russian Journal of Genetics 43: 627-635.

Duca, M., Port, A., Orozco-Cardenas, M. and Lovatt, C., 2008. Gibberellin-induced gene expression associated with cytoplasmic male sterility in sunflower. Biotechnology and Biotechnological Equipment 22: 691-698.

Eckardt, N.A., 2006. Cytoplasmic male sterility and fertility restoration. Plant Cell 18: 515-517.

Edwards, M.C. and Gibbs, R.A., 1994. Multiplex PCR: advantages, development and applications. Genome Research 3: S65-S75.

Falconer, D.S. and Mackay, T.F.C., 1996. Introduction to quantitative genetics. Longman Group Limited, England. pp. 480.

FAO, 1947-1975. Yearbook of food and agricultural statistics. Vol. 1-29. Food and Agricultural Organization of the United Nations, Rome, Italy.

Faure, N., Quillet, M.C., Serieys, H., Tersac, M., Berville, A., Kaan, F., Berville, A. and Tesac, M., 1999. Wild *Helianthus annuus* for sunflower improvement. Proceedings of the Fourth European Conference on Sunflower Biotechnology, Montpellier, France, 20-23 October 1998. Helia 22: 37-48.

Ferre, F., 1992. Quantitative or semi-quantitative PCR: Reality vs. myth. PCR Methods and Applications 2: 1-9.

Fick, G.N., 1976. Genetics of floral color and morphology in sunflowers. The Journal of Heredity 67: 227-230.

Fick, G.N., 1978. Breeding and genetics. In: Sunflower science and technology. Ed. Carter, J.F. Madison, WI. The American Society of Agronomy, pp. 279-338.

Freeman, C.A., Kolkman, J.M., Slabough, M.B., Berry, S., Olungu, C., Maes, N., Leon, A.J. and Knapp, S.J., 2003. SNP marker development and diversity in domesticated and wild sunflowers. Plant and Animals Genomes XI Conference (San Diego, CA). (http://www.intl-pag.org/pag/11/abstracts/).

Fusari, C.M., Lia, V.V., Hopp, H.E., Heinz, R.A. and Paniego, N.B., 2008. Identification of single nucleotide polymorphisms and analysis of linkage disequilibrium in sunflower elite inbred lines using the candidate gene approach. BMC Plant Biology 8: 7.

Fusari, C.M., Lia, V.V., Nishinakamasu, V., Zubrzycki, J.E., Puebla, A.F., Maligne, A.E., Hopp, H.E., Heinz, R.A. and Paniego, N.B., 2010. Single nucleotide polymorphism genotyping by heteroduplex analysis in sunflower (*Helianthus annuus* L.). Molecular Breeding 28: 73-89.

Gentzbittel, L., Mestries, E., Mouzeyar, S., Mazeyrat, F., Badaoul, S., Vear, F., Tourvieill de Labrouhe, D. and Nicolas, P., 1999. A composite map of expressed sequences and phenotypic traits of the sunflower (*Helianthus annuus* L.) genome. Theoretical and Applied Genetics 99: 218-234.

Gentzbittel, L., Zhang, Y.X., Vear, F., Griveau, B. and Nicolas, P., 1994. RFLP studies of genetic relationships among inbred lines of the cultivated sunflower, *Helianthus annuus* L.: evidence for distinct restorer and maintainer germplasm pools. Theoretical and Applied Genetics 89: 419-425.

Gvozdenović, S., Saftić-Panković, D., Jocić, S. and Radić, V., 2009. Correlation between heterosis and genetic distance based on SSR markers in sunflower (*Helianthus annuus* L.). Journal of Agricultural Sciences 54: 1-10.

Hamrick, J.L. and Godt, M.J., 1989. Allozyme diversity in plant species. Plant population genetics, breeding, and genetic resources. Sinauer Associates, Sunderland, MA. pp. 43-63.

Hayden, M.J., Nguyen, T.M., Waterman, A. and Chalmers, K.J., 2008. Multiplex-ready PCR: A new method for multiplexed SSR and SNP genotyping. BMC Genomics 9: 80.

Heiser, C.B., 1949. Study in the evolution of the sunflower species *Helianthus annuus* and *H. bolanderi*. University of California Publications in Botany 23: 157-196.

Heiser, C.B., 1954. Variation and subspeciation in the common sunflower *Helianthus annuus*. The American Midland Naturalist 51: 287-305.

Heiser, C.B., 1955. Origin and development of the cultivated sunflower. American Biology Teacher 17: 161-167.

Helentjaris, T., 1987. A genetic linkage map for maize based on RFLPs. Trends in Genetics 3: 217-221.

Holland, J.B., 2006. Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. Crop Science 46: 642-654.

Hunter, R.L. and Merkert, C.L., 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125: 1294-1295.

Iqbal, M.A., Sadaqat, H.A. and Khan, I.A., 2008. Estimation of genetic diversity among sunflower genotypes through random amplified polymorphic DNA analysis. Genetics and Molecular Research 7: 1408-1413.

Jaikishen, I., Ramesha, M.S., Rajendrakumar, P., Rao, K.S., Neeraja, C.N., Balachandran, S.M., Viraktamath, B.C., Sujatha, K. and Sundaram, R.M., 2004. Characterization of genetic diversity in hybrid rice parental lines using EST-derived and non-EST SSR markers. Rice Genetics Newsletter 23: 24-28.

Jan, C.C., Vick, B.A., Miller, J.F., Kahler, A.L. and Butler III, E.T., 1998. Construction of an RFLP linkage map for cultivated sunflower. Theoretical and Applied Genetics 96: 15-22.

Janick, J., 1999. Exploitation of heterosis: Uniformity and stability. In: The genetics and exploitation of heterosis in crops. Eds. Coors, J.G. and Pandey, S. ASA, CSSA and SSSA. Madison, WI. USA. pp. 319-333.

Jasso de Rodriguez, D., Phillips, B.S., Rodriguez-García, R. and Angulo-Sánchez, J.L., 2002. Grain yield and fatty acid composition of sunflower seed for cultivars developed under dry land conditions. Eds. Janick, J. and Whipkey, A. Trends in New Crops and New Uses. ASHS Press, Alexandria, VA, USA. pp. 139-142.

Jeffreys, A.J., Tamaki, K., MacLeod, A., Monckton, D.G., Neil, D.L. and Armour, J.A.L., 1994. Complex gene conversion events in germline mutations at human minisatellites. Nature Genetics 6: 136-145.

Jehan, T. and Lakhanpaul, S., 2006. Single nucleotide polymorphism (SNP) - Methods and applications in plant genetics: A review. Indian Journal of Biotechnology 5: 435-459.

Jie, L., Gong-She, L. and Jan, C.C., 2003. Comparison of genetic diversity of the germplasm resources of confectionary sunflower (*Helianthus annuus* L.) based on RAPDs and AFLPs. Acta Botanica Sinica 45: 352-358.

Jones, N., Ougham, H. and Thomas, H., 1997. Markers and mapping: We are all geneticists now. New Phytologist 137: 165-177.

Joshi, C.P. and Nguyen, H.T., 1993. RAPD (random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats. Plant Science 93: 95-103.

Jovanka, A., 2004. Roles of interspecific hybridisation and cytogenetic studies in sunflower breeding. Helia 27: 1-24.

Karasu, A., Oz, M., Sincik, M., Goksoy, A.T. and Turan, Z.M., 2010. Combining ability and heterosis for yield and yield components in sunflower. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38: 260-264.

Kaya, Y. 2002. The problems of hybrid sunflower seed production in Turkey. Proceedings of the 1st Turkish seed congress, Izmir. September 11-13. pp. 259-266.

Kaya, Y. and Mutlu, H., 2001. The use of three-way hybrids in sunflower production in Turkey. Proceedings of the 4th Turkish field crops congress, Tekirdag. September 17-21. pp. 1-6.

Khaleghizadeh, A., 2011. Effect of morphological traits of plant, head and seed of sunflower hybrids on house sparrow damage rate. Crop Protection 30: 360-367.

Khan, H., Rahman, H.-U., Ahmad, H., Ali, H., Amullah, I. and Alam, M., 2008. Magnitude of heterosis and heritability in sunflower over environments. Pakistan Journal of Botany 40: 301-308.

Kiiveri, H.T., 2011. Multivariate analysis of microarray data: differential expression and differential connection. BMC Bioinformatics 12: 42.

Kinman, M.L., 1970. New development in USDA and State Experimental Station sunflower breeding programmes. Proceedings of the 4th International Sunflower Conference, Memphis, Tennessee, pp: 181-183.

Kinman, M.L. and Earle, F.R., 1964. Agronomic performance and chemical composition of the seed of sunflower hybrids and introduced varieties. Crop Science 4: 417-420.

Knowles, P., 1978. Sunflower science and technology. Agronomy 19: 55-87.

Kolkman, J.M., Berry, S.T., Leon, A.J., Slabaugh, M.B., Tang, S., Gao, W., Shintani, D.K., Burke, J.M. and Knapp, S.J., 2007. Single nucleotide polymorphisms and linkage disequilibrium in sunflower. Genetics 177: 457-468.

Konarev, V.G., 2000. Cultivar identification and gene pool registration by seed proteins in cultivated plants. St. Petersburg: Institute of Rastenievod, Russia. pp. 186.

Koreth, J., O'Leary, J.J. and McGee, J.O., 1996. Microsatellites and PCR genome analyses. Journal of Pathology 178: 239-248.

Korzun, V., 2003. Molecular markers and their applications in cereals breeding. A paper presented during the FAO international workshop on "Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding?". 17-18 October 2003, Turin, Italy.

Kusterer, B., Horn, R. and Friedt, W., 2005. Molecular mapping of the fertility restoration locus *Rf1* in sunflower and development of diagnostic markers for the restorer gene. Euphytica 143: 35-42.

Kusterer, B., Rozynek, B., Brahm, L., Prufe, M., Tzigos, S., Horn, R. and Friedt, W., 2004. Construction of a genetic map and localization of major traits in sunflower (*Helianthus annuus* L.). Helia 27: 15-24.

Lam, S.L. and Leopold, A.C., 1966. Role of leaves in phototropism. Plant Physiology 41: 847-851.

Lamkey, K.R. and Edwards, J.W., 1999. Quantitative genetics of heterosis. In: The genetics and exploitation of heterosis in crops. Eds. Coors, J.G. and Pandey, S. The American Society of Agronomy and The Crop Science Society of America USA. pp. 31-48.

Lawson, W.R., Goulter, R.J., Henry, R.J., Kong, G.A. and Kochman, J.K., 1998. Marker-assisted selection for two rust resistance genes in sunflower. Molecular Breeding 4: 227-234.

Lawson, W.R., Henry, R.J., Kochman, J.K. and Kong, G.A., 1994. Genetic diversity in sunflower (*Helianthus annuus* L.) as revealed by random amplified polymorphic DNA analysis. Australian Journal of Agricultural Research 45: 1319-1327.

Leclerq, P., 1969. Une sterilite male cytoplasmique chez le tournesol. Annual Amelior Plantes 19: 99-106.

Leclerq, P., 1971. La sterilite male cytoplasmique der Tournesol. Annual Amelior Plantes 21: 45-54.

Lentz, D.L., Pohl, M.D., Alvarado, J.L., Tarighat, S. and Bye, R., 2008. Sunflower (*Helianthus annuus* L.) as a pre-Columbian domesticate in Mexico. Proceedings of the National Academy of Sciences of the United States of America 105: 6232-6237.

Lentz, D.L., Pohl, M.D., Pope, K. and Wyatt, A., 2001. Prehistoric sunflower (*Helianthus annuus* L.) domestication in Mexico. Economic Botany 55: 370-376.

Leon, A.J., Andrade, F.H. and Lee, M., 2003. Genetic analysis of seed oil concentration across generations and environments in sunflower (*Helianthus annuus* L.). Crop Science 43: 135-140.

Lexer, C., Heinze, B., Steinkellner, H., Kampfer, S., Ziegenhagen, B. and Glössl, J., 1999. Microsatellite analysis of maternal half-sib families of *Quercus robur*, pedunculate oak: Detection of seed contaminations and inference of the seed parents from the offspring. Theoretical and Applied Genetics 99: 185-191.

Lin, J. and Kuo, J., 1995. AFLP™: A novel PCR-based assay for plant and bacterial DNA fingerprinting. Focus 17: 2.

Liu, A. and Burke, J.M., 2006. Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics 173: 321-330.

Liu, B.H., Knapp, S.J. and Birkes, D., 1997. Sampling distributions, biases, variances and confidence intervals for genetic correlations. Theoretical and Applied Genetics 94: 8-19.

Liu, J., Liu, G.-S. and Jan, C.C., 2003. Comparison of genetic diversity of the germplasm resources of confectionary sunflower (*Helianthus annuus*) in China based on RAPDs and AFLPs. Acta Botanica Sinica 45: 352-358.

Lu, Y.H., Melero-Vara, J.M., Garcia-Tejada, J.A. and Blanchard, A., 2000. Development of SCAR markers linked to the gene *Or5* conferring rust resistance to broomrape (*Orobanche cumana* Wallr.) in sunflower. Theoretical and Applied Genetics 100: 625-632.

Macaulay, M., Ramsay, L., Powell, W. and Waugh, R., 2001. A representative highly informative genotyping set of barley SSRs. Theoretical and Applied Genetics 102: 801-809.

Mackenzie, A.S. and Chase, C.D., 1990. Fertility restoration is associated with loss of a portion of the mitochondrial genome in cytoplasmic male-sterile common bean. Plant Cell 2: 905-912.

Mba, C. and Tohme, J., 2005. Use of AFLP markers in surveys of plant diversity. Methods in Enzymology 395: 177-201.

Melchinger, A.E., 1999. Genetic diversity and heterosis. In: The genetics and exploitation of heterosis in crops. Eds. Coors, J.G. and Pandey, S. ASA, CSSA and SSSA. Madison, Wisconsin. pp. 99-118.

Miller, J.F. 1999. Oilseeds and heterosis. In: The genetics and exploitation of heterosis in crops. Eds. Coors J.G. and Pandey. S. ASA, CSSA, and SSSA. Madison, WI. pp. 399-404.

Mittal, N. and Dubey, A.K., 2009. Microsatellite markers - A new practice of DNA based markers in molecular genetics. Pharmacognosy Reviews 3: 235-246.

Moghaddasi, M.S., 2011. Sunflower tissue culture. Advances in Environmental Biology 5: 746-755.

Mohan, M., Nair, S., Bhagwai, A., Krishma, T.G., Yano, M., Bhaita, C.R. and Sasaki, T., 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 3: 87-103.

Monna, L., Miyao, A., Inoue, T., Fukuoka, S., Yamazaki, M., Zhong, H.S., Sasaki, T. and Minobe, Y., 1994. Determination of RAPD markers in rice and their conversion into sequence tagged sites (STSs) and STS-specific primers. DNA Research 1: 139-148.

Morgante, M. and Olivieri, A.M., 1993. PCR-amplified microsatellites as markers in plant genetics. Plant Journal 3: 175-182.

Murphy, R.W., Sites, J.W., Buth, D.G. and Haufler, C.H., 1996. Proteins: isozyme electrophoresis. Molecular Systematics (2nd ed.). Sinauer Associates, Sunderland, MA. pp. 20-51.

Murthy, U.K., Lyngdoh, I.E., Gopalakrishna, T., Shivanna, M.B. and Theerta Prasad, D., 2005. Assessment of heritability of *Alternaria helianthi* resistance trait in sunflower using molecular markers. Helia 28: 33-42.

Nandini, R. and Chikkadevaiah, S.H.L., 2005. DNA fingerprinting of sunflower genotypes (*Helianthus annuus* L.). Helia 28: 9-18.

Nei, M., 1972. Genetic distance between populations. American Naturalist 106: 283-292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 76: 379-390.

Oda, S., Oki, E., Maehara, Y. and Sugimachi, K., 1997. Precise assessment of microsatellite instability using high resolution fluorescent microsatellite analysis. Nucleic Acids Research 25: 3415-3420.

Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, G.L., Sellers, T.A. and King, R.A., 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infra-red fluorescence and M13 tailed primers. Genomics 30: 450-458.

Pacureanu-Joita, M., Stanciu, D., Petcu, E., Raranciuc, S. and Sorega, I., 2005. Sunflower genotypes with high oleic acid content. Romanian Agricultural Research 22: 23-26.

Palmer, J.H. and Phillips, I.D.J., 1963. The effect of the terminal bud, indoleacetic acid and nitrogen supply on the growth and orientation of the petiole of *Helianthus annuus*. Physiologia Plantarum 16: 572-584.

Paniego, N., Muñoz, M., Echaide, M., Fernandez, L., Faccio, P., Zandomeni, R., Suarez, E. and Hopp, E., 1999. Microsatellite development for sunflower. Plant and Animal Genome VII Conference conference: 17-21 January 1999, San Diego, CA. pp. 464.

Payne, R.W., 2009. GenStat. Wiley Interdisciplinary Reviews: Computational Statistics 1: 255-258.

Perez-Vich, B., Fernandez-Martinez, J.M., Grondona, M., Knapp, S.J. and Berry, S.T., 2002. Stearoyl-ACP and oleoyl-PC desaturase genes co-segregate with quantitative trait loci underlying high stearic and high oleic acid mutant phenotypes in sunflower. Theoretical and Applied Genetics 104: 338-349.

Pervaiz, Z.H., Rabbani, M.A., Khaliq, I., Pearce, S.R. and Malik, S.A., 2010. Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. Electronic Journal of Biotechnology 13 http://www.ejbiotechnology.cl/content/vol13/issue3/full/5/index.html. Accessed 2010/06/14.

Poehlman, J.M., 1987. Breeding field crops. AVI Publishing Company Inc, Westport. pp. 104-110.

Popov, V.N., Urbanovich, O.Y. and Kirichenko, V.V., 2002. Studying genetic diversity in inbred sunflower lines by RAPD and isozyme analyses. Russian Journal of Genetics 38: 785-790.

Powell, W., Morganante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225-238.

Quagliaro, G., Vischi, M., Tyrka, M. and Olivieri, A.M., 2001. Identification of wild and cultivated sunflower for breeding purposes by AFLP markers. The American Genetic Association 98: 38-42.

Rachid Al-Chaarani, G., Roustaee, A., Gentzbittel, L., Modrani, L., Barrault, G., Dechamp-Guillaume, G. and Sarrafi, A., 2002. A QTL analysis of sunflower partial resistance to downy mildew (*Plasmopara halstedii*) and black stem (*Phoma macdonaldii*) by the

use of recombinant inbred lines (RILs). Theoretical and Applied Genetics 104: 490-496.

Rao, M.N. and Singh, B., 1978. Inheritance of some quantitative characters in sunflower (*Helianthus annuus* L.). Pantnagar Journal of Research 4: 144-146.

Rauf, S., 2008. Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. Communications in Biometry and Crop Science 3: 29-44.

Reddy, C.V.C.M., Sinha, B., Reddy, A.V.V. and Reddy, Y.R., 2008. Maintenance of male sterility and fertility restoration in different CMS sources of sunflower (*Helianthus annuus* L.). Asian Journal of Plant Sciences 7: 762-766.

Reynolds, J.B., Weir, B.S. and Cockerham, C.C., 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105: 767-779.

Ribeiro, A., Gouveia, A., Bessa, A., Ferreira, A., Magumisse, A.T., Manjate, M. and Faria, T., 2010. Population structure and genetic diversity of wil *Helianthus* species from Mozambique. Genetika 46: 1086-1094.

Rieseberg, L.H., 1996. Homology among RAPD fragments in interspecific comparisons. Molecular Ecology 5: 99-105.

Rieseberg, L.H., Kim, S.-C., Randell, R.A., Whitney, K.D., Gross, B.L., Lexer, C. and Clay, K., 2007. Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129: 149-165.

Rönicke, S., Hahn, V., Horn, R., Grone, I., Brahm, L., Schnabl, H. and Friedt, W., 2008. Interspecific hybrids of sunflower as a source of *Sclerotinia* resistance. Plant Breeding 123: 152-157.

Roth, I., 1977. Fruits of angiosperms. Encyclopaedia of Plant Anatomy 10: 258-290.

Sackston, W.E., 1981. The sunflower crop and disease: progress, problems and prospects. Plant Disease 65: 643-648.

Saxton, A.M., Balzarini, M.G. and Cappio-Borlino, A., 2004. Genetic analysis of complex traits using SAS. SAS Publishing, University of Tennessee. pp. 15-16.

Semelczi-Kovacs, A., 1975. Acclimatization and dissemination of the sunflower in Europe. Acta Ethnographica Academiae Scientarum Hungaricae 24: 47-88.

Singh, R.K., Mishra, S.K., Singh, S.P., Mishra, N. and Sharma, M.L., 2010. Evaluation of microsatellite markers for genetic diversity analysis among sugarcane species and commercial hybrids. Australian Journal of Crop Science 4: 116-125.

Siniša, J., Zvonimir, S., Nada, L. and Dragan, S., 2008. Genetic possibilities for altering sunflower oil quality to obtain novel oils. Canadian Journal of Physiology and Pharmacology 86: 215-221.

Škorić, D., Jocić, S., Hladni, N. and Vannozzi, G.P., 2007. An analysis of heterotic potential for agronomically important traits in sunflower (*Helianthus annuus* L.). Helia 30: 55-74.

Smith, J.S.C., Hoeft, E., Cole, G., Lu, H., Jones, E.S., Wall, S.J. and Berry, D.A., 2009. Genetic diversity among sunflower inbreds and hybrids: assessing probability of ancestry and potential for use in plant variety protection. Crop Science 49: 1295-1303.

Sprague, G.F. and Tatum, L.A., 1942. General versus specific combining ability in a single cross of corn. American Journal of the Society of Agronomists 34: 923-932.

Sujatha, M., Prabakaran, A.J., Dwivedi, S.L. and Chandra, S., 2008. Cytomorphological and molecular diversity in backcross-derived inbred lines of sunflower (*Helianthus annuus* L.). Genome 51: 282-293.

Takezaki, N. and Nei, M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389-399.

Tang, S., Kishore, V.K. and Knapp, S.J., 2003. PCR-multiplexes for a genome-wide framework of simple sequence repeat marker loci in cultivated sunflower. Theoretical and Applied Genetics 107: 6-19.

Tang, S., Yu, J.K., Slabaugh, M.B., Shintani, D.K. and Knapp, S.J., 2002. Simple sequence repeat map of the sunflower genome. Theoretical and Applied Genetics 105: 1124-1136.

Tanksley, S.D. and Orton, T.J., 1983. Isozymes in plant genetics and breeding, parts A and B. Elsevier Science, Amsterdam, Netherlands. pp. 55-77.

Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA. Nucleic Acids Research 17: 6463-6471.

Tavoljanskiy, N.P., Tavoljanskiy, A.N., Chiryaev, P.V. and Tikhomirov, V.T., 2004. Interspecific hybridisation in sunflower breeding for economically valuable characteristics. Helia 27: 143-148.

Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tivang, J.G. and Osborn, T.C., 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theoretical and Applied Genetics 88: 973-980.

Turkec, A. and Goksoy, A.T., 2006. Identification of inbred lines with superior combining ability for hybrid sunflower (*Helianthus annuus*) production in Turkey. New Zealand Journal of Crop and Horticultural Science 34: 7-10.

Van Wijk, J., 1994. Hybrids, bred for superior yields or for control? Biotechnology and Development Monitor 19: 3-5.

Vear, F., Serre, F., Roche, S., Walser, P. and Tourvieille de Labrouhe, D., 2007. Improvement of *Sclerotinia sclerotiorum* head rot resistance in sunflower by recurrent selection of a restorer population. Helia 30: 1-12.

Velkov, V. and Stoyanova, Y., 1974. Biological peculiarities of cytoplasmic male sterility and schemes of its use. Proceedings of the 6th international sunflower conference (Bucharest, Romania), July 22-24, 1974. pp. 361-365.

Vischi, M., Nonino, F. and Olivieri, A.M., 2002. AFLP markers for the study of introgression between wild sunflower species (*H. argophyllus* and *H. debilis* spp. *cucumerifolius*) and in relation with *H. annuus*. Helia 25: 47-58.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 18: 7213-7218.

Weiss, E.A., 2000. Oilseed crops. Blackwell Science Ltd, USA. pp. 205-244.

Welsh, J. and McClelland, M., 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingy, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Wricke, E.G. and Weber, W.E., 1986. Quantitative genetics and selection in plant breeding. Walter de Gruyter and Co. Berlin. pp. 263-264.

Xiong, M. and Jin, L., 1999. Comparison of the power and accuracy of biallelic and microsatellite markers in population based gene-mapping methods. American Journal of Human Genetics 64: 629-640.

Yordanov, Y., Hristov, E., Yordanova, E., Atanassov, I. and Georgiev, S., 2005. Using DNA and isozyme markers to study genetic relationship among high regenerative

interspecific hybrids of *Helianthus eggertii* Small x *Helianthus annuus* L. General and Applied Genetics 19: 27-32.

Yu, J., Mangor, J., Thompson, L., Edwards, K.J., Slabaugh, M.B. and Knapp, S.J., 2002. Allelic diversity of simple sequence repeats among elite inbred lines of cultivated sunflower. Genome 45: 652-660.

Yu, J., Tang, S., Slabaugh, M.B., Heesacker, A., Cole, G., Herring, M., Soper, J., Han, F., Chu, W., Webb, D.M., Thompson, L., Edwards, K.J., Berry, S., Leon, A.J., Grondona, M., Olungu, C., Maes, N. and Knapp, S.J., 2003. Towards a saturated molecular genetic linkage map for cultivated sunflower. Crop Science 43: 367-387.

Yue, B., Cai, X., Vick, B.A. and Hu, J., 2009. Genetic diversity and relationships among 177 public sunflower inbred lines assessed by TRAP markers. Crop Science 49: 1242-1249.

Zabeau, M. and Vos, P., 1993. Selective restriction fragment amplification : A general method for DNA fingerprinting. European Patent Application 92402697.

Zazzerini, A., Tosi, L. and Mondjana, A.M., 2005. First report of *Puccinia helianthi* (Race 314) on sunflower in Mozambique. Journal of Phytopathology 153: 733-735.

Zhang, L.S., Bacquet, V., Li, S.H. and Zhang, D., 2003. Optimization of multiplex PCR and multiplex gel electrophoresis in sunflower SSR analysis using infrared fluorescence and tailed primers. Acta Botanica Sinica 45: 1312-1318.

Zhang, L.S., Le Clerq, V., Li, S. and Zhang, D., 2005. Establishment of an effective set of simple sequence repeat markers for sunflower variety identification and diversity assessment. Canadian Journal of Botany 83: 66-72.

Zhang, T.P., Zhao, S.Y., Gao, J.Y. and Guan, J.P., 1996. Esterase isozyme research for varieties of sunflower from south mountain district in Guizhou. China Agricultural Science and Technology Press, Beijing, pp. 571-575.

Zhang, Y.Y., Xu, Y.F., Hussain, K., Liu, Y.Z. and Lin, F., 2010. Establishment of a multiplex-PCR system for identification of genetically modified maize events. Middle-East Journal of Scientific Research 6: 563-568.

Zhou, Y., Bui, T., Auckland, L.D. and Williams, C.G., 2002. Direct fluorescent primers are superior to M13-tailed primers for *Pinus taeda* microsatellites. Biotechniques 32: 46-52.

Zimmer, D.E. and Fick, G.N., 1974. Some diseases of sunflowers in the United States - their occurrence, biology and control. In Proceedings of the 6th International Sunflower Conference, Bucharest, Romania. pp. 673-680.

Zukovsky, P.M., 1950. Cultivated plants and their wild relatives. Commonwealth Agricultural Bureaux, Farnham Royal, England. pp. 595.

Chapter 3

Genetic diversity of inbred sunflower lines as defined by SSR markers

3.1 Summary

The gene pool of commercial sunflower (*H. annuus*) is the result of a variety of breeding and domestication bottlenecks (Yu *et al.*, 2002; Yu *et al.*, 2003; Zhang *et al.*, 2005). A singular point of domestication has been hypothetically determined by early genetic studies (Harter *et al.*, 2004). It was therefore important in this study to assess the level of genetic diversity in elite female (A-lines) and male restorer sunflower lines (R-lines) from Pannar Seed (Pty) Ltd's breeding programme and compare germplasm on grounds of estimates of genetic similarities derived through the means of SSR (microsatellite) marker analysis.

A set of 55 SSR markers with documented map positions were used to ascertain the genetic similarity in sunflower lines consisting of female (A) and male (R) inbred lines. An average PIC value of 0.510 and an average major allele frequency of 0.534 were observed. The PowerMarker programme was used to determine genetic distances and data were evaluated genotypically and not haplotypically in order to allow for heterozygotes. Rogers as well as Nei distance matrixes showed an excellent correlation with the breeding background as well as information gathered from breeders on the inbred lines involved. A distinct differentiation between A- and R-lines was observed through cluster analysis, which indicated the presence of heterotic groups within the inbred lines.

3.2 Introduction

There is limited information available regarding African as well as South African sunflower breeding material. There is furthermore limited breeding work being done by government departments not only in South Africa, but in Africa in general. The fact that private seed companies do not share data on sunflower germplasm and genetic backgrounds also limits the availability of data to sunflower breeders. Traditional plant breeding methods have been effective for generation of conventional lines, but with the introduction of more competitive hybrids as well as traits, it has become more important to make use of molecular marker techniques. Mitchell *et al.* (1997) stated that integration of technologies which enable acquisition of significant quantities of genetic information for improved genotype identification will largely assist in conservation of plant genetic resources. This can also be

extrapolated to sunflower and the preservation of genetic material, especially since the current traits in sunflower is of a non-genetically modified nature.

The availability of genetic diversity within breeding germplasm is important for maintaining a competitive edge in the commercial market. A number of studies have been conducted which indicated that once a crop is domesticated, a reduction in genetic diversity occurs. This includes studies by among others Liu and Burke (2006) which found that LD decays rapidly in self-incompatible sunflower. Higher levels of LD were found in domesticated sunflower. The process of self-fertilisation to obtain inbred lines also assists in the reduction of genetic diversity. This is where genetic or molecular marker tools such as SSRs can assist in the description of sunflower cultivars (Burke *et al*., 2005).

It was important to search for alternate sources of genetic diversity outside of domesticated sunflower to attempt to broaden the genetic base. Studies have been conducted to look at genetic diversity between species as well as between lines of various breeding programmes. Some of these studies investigated the levels of diversity between wild and domesticated sunflower. Arif *et al*. (2010) investigated a number of techniques used for assessment of plant diversity. They reported that sequencing based molecular techniques provided better resolution at intra-genus level and above. RAPDs, AFLPs and SSRs provide a way to classify individuals into genotypic categories and are ideally suited to describe intra-species variation. Dong *et al*. (2007) used AFLPs to assess collection diversity among 70 germplasm accessions in confectionary sunflower. They found that morphologically based clusters showed a degree of locality separation by germplasm origin. The general finding, however, was that origin did not correspond closely with the clustering pattern. Quagliaro *et al*. (2001) used AFLPs to determine the level of diversity within and between populations of *H. argophyllus* which were collected in Mozambique. They confirmed the use of this technique for both phylogenetic and taxonomic studies. Ellis *et al*. (2006) used both nuclear and chloroplast SSRs to assist in the investigation of the population genetics of *H. verticillatus* Small. and compared the data to that of *H. angustifolius* L. They concluded that *H. verticillatus* was not of hybrid origin seeing that it did not exhibit a mixture of parental alleles at nuclear loci. They found that it did not share a chloroplast DNA haplotype with either of its putative parents.

SSRs have been successfully used in the determination of genetic distance in sunflower. Gvozdenović *et al.* (2009) used SSRs to determine the correlation between SSR based genetic distance and heterosis for six agronomic traits. They found no significant positive correlation between genetic distance and mid- and high-parent heterosis, SCA and mean value in any of the examined traits for the 60 hybrids tested. A highly significant negative correlation was found between genetic distance and mean oil percentage. Kolkman *et al*. (2007) investigated SNP frequencies, nucleotide diversity and LD in sunflower cultivars. They found LD to decay more slowly in inbred lines than in wild populations. Fusari *et al*. (2008) investigated a set of 28 genes in 19 sunflower inbred lines and identified two gene pools. They detected a high frequency of SNPs and concluded that high resolution mapping in sunflower could be achieved with marker densities lower than those reported in literature until 2008.

Another area which has become paramount lately is ways to ensure plant variety protection. One of the studies conducted by Smith *et al.* (2009) looked at the potential use of SSRs to discriminate between sunflower inbred lines and hybrids in the USA. They found that inbreds with similar pedigrees associated with each other and groupings within these inbreds could be determined as male or female. Pedigree analysis of known hybrids was used to establish the parental inbreds. SSR profiles were successfully used to facilitate the identification of uniqueness of the lines.

An important study on the possibility that genetic distance could be a possible predictor of heterosis and resultant hybrid performance within and also between heterotic groups in sunflower was done by Cheres *et al.* (2000). Their results indicated that heterotic groups in sunflower were of significant use.

The aim of this study was to determine the applicability of SSR marker systems for analysis of diversity in sunflower, as well as to evaluate the diversity found in a set of inbred sunflower lines.

3.3 Materials and methods

3.3.1 Plant material and DNA extraction

A total of 93 inbred sunflower lines were used for DNA analysis using SSR markers. The inbred lines included 49 R- and 40 A-lines. Four additional B-lines were added [B4, B41(HO), B42(HO) and B43(HO)] to determine the relationship between the A- and B-lines. All lines used were obtained from Pannar Seed (Pty) Ltd and were selected based largely on the fact that they have been thoroughly tested through an advanced testing programme and were among the highest yielding lines in the trials. They were furthermore selected based on good oil content as well as disease resistance and standability. Due to confidentiality reasons, the 93 lines were coded and the coded system will be used throughout the study. There were two cases where some of the lines used in this study were converted to obtain specific traits. The first line was the rust resistant line R41 which has been converted to a high oleic line and the line was designated R42(HO). This rust resistant line has also been converted to contain downy mildew resistance and the designation was R44(RM). Furthermore the R-line R46 has been converted to contain downy mildew resistance and was designated R47(RM). R46 is also related to R41 in having R41 as part of its pedigree.

The 93 inbred lines were planted in a glasshouse at Greytown, KwaZulu-Natal (South Africa). Ten to fifteen seeds per line were planted per line in seedling trays. The temperature was kept between 18°C and 25°C. After germination, seedlings were allowed to grow until day seven to allow for sufficient leaf material to be available for DNA extraction. Total genomic DNA was isolated from approximately 100 mg of fresh leaf material using the GenElute^{TM} Plant Genomic DNA mini prep kit (Sigma-Aldrich Biotechnology LP, Saint Louis, Missouri, USA and/or Sigma-Aldrich Co., Saint Louis, Missouri, USA) according to manufacturer's specifications. DNA concentrations and purity were determined spectrophotometrically by measuring absorbances at 260 nm and 280 nm. DNA concentrations were calculated using the formula: DNA concentration = optical density (OD_{260}) x dilution factor x constant (50 µg/ml). DNA samples were diluted to a working concentration of 10 ng/µl in TE-buffer [10 mM tris(hydroxymethyl)aminomethane (Tris-Cl), pH = 8 and 1 mM ethylenediaminetetraacetic acid (EDTA)] and stored at 4°C. The integrity of the DNA was confirmed by 0.7% (w/v) agarose gel electrophoresis in Tris-acetate-EDTA (TAE) buffer for 1 h at 80 V with visualisation under ultraviolet (UV) light after staining with ethidium bromide.

3.3.2 SSR markers

Fifty-five SSR markers were used to establish SSR profiles for the 93 inbreds. The selected markers were spread throughout the sunflower genome. Tang *et al.* (2002) found that 459 SSR markers coalesced into 17 linkage groups which they presumed corresponded to the 17 chromosomes in the haploid sunflower (x=17) genome. The distribution of SSR markers used in the study through the sunflower genome is given in Table 3.1 and were selected based on the linkage map published by Tang *et al.* (2002) and Yu *et al.* (2002).

SSR analyses were performed in 12 µl reaction mixtures containing 20 ng of template DNA, 1x Kapa *Taq* Polymerase buffer (buffer information proprietary), 2.5 mM $MgCl_2$, 100 µM of each deoxyribonucleotide triphosphate (dNTP) and 1.5 U of *Taq* DNA polymerase (KapaBiosystems, Cape Town, South Africa). Primers were synthesised using a tailed primer strategy and labelled using a fluorescent dye (Zhang *et al.*, 2005). With the tailing strategy a M13 (5'-CACGACGTTGTAAAACGAC-3') tail was added to the forward primer during primer synthesis. Primers were synthesised by Inqaba Biotechnical Industries (Pty) Ltd (Hatfield, South Africa). Amplification then required three primers: one tailed forward primer (1 µM), one normal reverse primer (3 µM) and 10 µM of the fluorescently-labelled tailed primer (synthesised by Applied Biosystems™ by Life Technologies™, Carlsbad, CA, USA). Primer sequences were obtained from the Sunflower Genome Database (www.css.orst.edu/knapp-lab/sunflower - Accessed 2010/08/04) and are given in Appendix 1.

A common problem with PCR is that spontaneous amplification occurs. This phenomenon can be reduced through the use of a Touchdown PCR cycling programme. Cycle parameters included an initial denaturation step of 5 min at 94°C, followed by seven cycles of 30 s at 94°C, 30 s at 63°C and 45 s at 72°C. The annealing temperature was decreased by 1°C with each cycle. This was followed by 30 cycles of 30 s at 94°C, 30 s at 57°C and 45 s at 72°C, with a final extension step at 72°C for 10 min.

PCR of different loci were pooled based on size of amplicon or fluorophore used. One µl of the resulting mixture of the PCR pool was combined with 9 µl of a 1x HiDi loading buffer (containing formamide) containing 5.5% (v/v) LIZ-250 internal size standard (Applied Biosystems™, Foster City, CA, USA). Samples were denatured for 5 min at 95°C, quickly cooled on ice and genotyped on an ABI 3130xl Sequencer (Applied Biosystems™), using

GeneScan® and Genotyper® software (Applied Biosystems). Fragment scoring was done manually.

Table 3.1 Fifty-five sunflower simple sequence repeat (SSR) markers exhibiting linkage group, number of markers per linkage group and marker names

Linkage group number	Number of markers	Marker names
1	4	ORS 371, ORS 543, ORS716, ORS 837
2	3	ORS 342, ORS 925, ORS 1065
3	4	ORS 665, ORS 1036, ORS 1114, ORS 1222
4	3	ORS 366, ORS 674, ORS 785
5	2	ORS 505, ORS 1024
6	3	ORS 381, ORS 483, ORS 650
7	2	ORS 331, ORS 1041
8	3	ORS 456, ORS 894, ORS 1161
9	3	ORS 428, ORS 887, ORS 938
10	2	ORS 437, ORS 878
11	5	ORS 457, ORS 621, ORS 630, ORS 733, ORS 1227
12	4	ORS 502, ORS 761, ORS 778, ORS 1085
13	2	ORS 316, ORS 1179
14	3	ORS 307, ORS 1079, ORS 1248
15	4	ORS 420, ORS 687, ORS 857, ORS 1141
16	4	ORS 407, ORS 656, ORS 750, ORS 885
17	4	ORS 297, ORS 561, ORS 735, ORS 1245

3.3.3 Data collection and analysis

Fragments with the same mobility were considered identical and coded as such. The presence of one allele (single mobility) was considered as a homozygous state, assuming the absence of null alleles. Different mobilities were recoded alphabetically with the fastest or smallest fragments as "A". A variety x marker matrix was created, recording genotypic SSR data as A (AA) or AB/AC/AD etc.

The evaluation of results needs to be processed through software programmes. There are a number of software programmes which can apply the various coefficients on the available data. PowerMarker (version 3.0) consists of a data-driven, integrated analysis environment for genetic data and has a powerful graphical interface. PowerMarker can handle a variety of marker data, including SSR data. It computes a number of statistics for each marker locus which includes allele number, missing proportion, heterozygosity, gene diversity, PIC and stepwise patterns for SSR data. PowerMarker further incorporates all common methods for testing Hardy-Weinberg and linkage equilibrium, which includes χ^2 tests, likelihood ratio tests and exact tests. The most commonly used measures of LD are also calculated which includes D^1 and R^2 (Liu and Muse, 2005). The variety x marker matrix was used to estimate the genetic similarity between genotypes using Rogers (1972) and Nei (1973) similarity coefficients. The PowerMarker programme (Liu and Muse, 2005) was used to calculate pairwise similarities. MEGA5 (Kumar *et al.*, 2008) was used as treeviewer. Both the Rogers distance matrix as well as the Nei distance matrix were calculated. Only the Rogers distance matrix is shown and was used for construction of the dendrogram. This is due to the fact that the Rogers distance is metric and produces the maximum statistical validity for any PCA analysis (unlike Nei or percentage fixed differences) (Kitchener *et al*., 1994). The unweighted pair-group method using arithmetic averages (UPGMA) [as well as Neighbour-Joining (NJ)] was used to construct dendrograms, depicting the relationships among accessions. UPGMA assumes equal rates of evolution, so that branch tips come out equal. The NJ algorithm allows for unequal rates of evolution, so that the branch lengths are proportional to amount of change. Should rates on the different branches not be markedly unequal, the branching orders produced by the two methods will therefore not be different.

The cophenetic correlation coefficient (r^2) was calculated. The cophenetic correlation is a measure of how faithfully a dendrogram preserves the pairwise distances between the original unmodeled data points. The calculation of the cophenetic correlation can be described in the following manner: The original data (X_i) can be modelled through the use of a cluster method to produce a dendrogram (T_i). This can be translated as a simplified model in which data that are closely grouped observed to be grouped in a hierarchial tree. The following definitions then apply where $x(i.j) = |X_i - X_j|$, which is the ordinary Euclidian distance between the i^{th} and j^{th} observations. The dendrogram distance between model points T_i and T_j is defined as $t(i,j)$. This distance is the height of the node at which these two points are first joined

together. Therefore, by letting x be the average of the $x(i,j)$ and letting t be the average of the $t(i,j)$, the cophenetic correlation coefficient r^2 is given by

$$r^2 = [\sum_{i<j}(x(i,j) - x)(t(i,j) - t)]/\sqrt{[\sum_{i<j}(x(i,j) - x)^2][\sum_{i<j}(t(i,j) - t)^2]}$$

(Sokal and Rohlf, 1962).

Genetic distance is calculated for the various methods using the assumptions that

X_u = uth allele frequency from the first population and

Y_u = uth allele frequency from the second population.

The Rogers distance D_R (Rogers, 1972) is calculated as

$$D_R = \sqrt{[\sum_u (X_u - Y_u)^2]}/2$$

and the Nei minimum distance D_m (Nei, 1973) is calculated as

$$D_m = (J_X + J_Y)/2 - J_{XY}$$

With J_{XY} being the probability

PCA can be utilised to obtain a two- or three dimensional scatter plot of individuals. This can be done in such a manner that the geometrical distances between individuals in the plot reflect the genetic distances between them with limited distortion. Aggregations of individuals in this type of plot will reveal sets of genetically similar individuals (Melchinger, 1993; Warburton and Crossa, 2000). Wiley (1981) defined PCA as a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables. This can allow for the visualisation of differences between individuals and target possible groups. The reduction achieved is through linear transformation of the original variables into a novel set of uncorrelated variables described as principal components (PCs). The PCA was developed to determine genetically similar individuals and for comparison with the dendrogram. The first step involves calculation of eigen values. Eigen values define the

amount of total variation that is displayed on the PC axis. The first PC summarises most of the variability present in the original data relative to all remaining PCs. The second PC explains most of the variability not explained by the first PC and uncorrelated with the first. The process continues in this manner (Jolliffe, 1986). The genetic distances were determined through the PowerMarker programme. The information was then transferred to NTSYS-pc (version 2.20) to determine the PCA (Rohlf, 2005).

One method for the measurement of estimation of population differentiation is through the use of the analysis of molecular variance (AMOVA). AMOVA is a method used for the estimation of population differentiation directly from molecular data and the testing of hypotheses about such differentiation. Molecular data such as molecular marker data, direct sequence data or phylogenetic trees based on molecular data could be analysed through the use of this method. AMOVA treats any kind of raw molecular data as a Boolean vector p_i which represents a 1 x n matrix of 1s and 0s, with 1 representing the presence of an allele and 0 its absence. A marker could be defined as being a nucleotide base, a base sequence, a restriction fragment or a mutational event (Excoffier *et al*., 1992). The sums of squares can be analysed in a nested ANOVA framework. A nested ANOVA differs from a simple ANOVA in that data are arranged hierarchically and mean squares are computed for groupings at all levels of the hierarchy. This allows for hypothesis tests of between group and within group differences at several hierarchical levels (Excoffier *et al*., 1992; Excoffier, 2001). The AMOVA analysis was performed through the use of the software programme Arlequin version 3.5 (Excoffier and Lischer, 2010).

The AMOVA analysis was done through the calculation of Euclidian distances between pairs of vectors through the subtraction of the Boolean vector of one haplotype from another. This was done through the formula $(p_j - p_k)$. Should p_j and p_k be visualised as points in *n*-dimensional space indicated by the intersections of the values in each vector with *n* being equal to the length of the vector, then the Euclidian distance is a scalar that is equal to the shortest distance between the two points. The squared Euclidian distance could then be calculated through the use of the equation

$$\delta^2_{jk} = (p_j - p_k)\,{}^{/}W(p_j - p_k)$$

W is a weighting matrix and can be described by default to be an identity matrix. W can be a matrix with a number of values depending on how one measures molecular change at different locations on a sequence or phylogenetic tree (Excoffier *et al*., 1992).

Squared Euclidian distances were calculated for all pairwise arrangements of Boolean vectors, which were then arranged into a matrix. The distances were then partitioned into submatrices corresponding to subdivisions within the population (Excoffier *et al*., 1992):

$$D^2 = \begin{bmatrix} \begin{bmatrix} \delta^2_{11} & \delta^2_{12} \\ \delta^2_{21} & \delta^2_{22} \end{bmatrix} & \cdots & \delta^2_{1k} \\ \cdots & \cdots & \cdots \\ \delta^2_{j1} & \cdots & \delta^2_{jk} \end{bmatrix}$$

The squared Euclidian distances are arranged in such a manner that the submatrices on the diagonal of the larger matrix are pairs of individuals in the same population while those on the off-diagonal represent pairs of individuals from different populations. The sums of the diagonals in the matrix and submatrices yield sums of squares for the various hierarchial levels of the population.

3.4 Results

A total of 93 inbred lines were genotyped through the use of 55 mapped SSR markers dispersed throughout the sunflower genome. The selected SSR markers each amplified a single locus across the 93 inbred lines. The SSR markers were screened for polymorphisms among a few elite inbred lines to estimate allele length ranges, assess genotyping qualities and also identify SSR markers for the purpose of testing in PCR multiplexes. Information generated for the 55 markers used in this study are shown in Table 3.2.

Table 3.2 Fifty-five sunflower simple sequence repeat (SSR) markers exhibiting gene diversity, heterozygosity, number of alleles, linkage groups and polymorphic information content

Marker name	Gene diversity	Heterosygosity	n_A	LG	PIC
ORS 371	0.56	0.0000	3	1	0.47
ORS 543	0.68	0.0430	5	1	0.63
ORS 716	0.63	0.0645	7	1	0.55
ORS 837	0.57	0.0323	3	1	0.51
ORS 342	0.25	0.0222	3	2	0.22
ORS 925	0.78	0.0753	7	2	0.75
ORS 1065	0.65	0.0215	4	2	0.60
ORS 665	0.63	0.0430	5	3	0.56
ORS 1036	0.50	0.0215	2	3	0.37
ORS 1114	0.63	0.0645	3	3	0.56
ORS 1222	0.63	0.0220	3	3	0.55
ORS 366	0.70	0.0111	6	4	0.63
ORS 674	0.65	0.0538	5	4	0.61
ORS 785	0.77	0.1667	6	4	0.74
ORS 505	0.75	0.0645	5	5	0.70
ORS 1024	0.72	0.0538	6	5	0.68
ORS 381	0.52	0.4432	3	6	0.47
ORS 483	0.55	0.0109	2	6	0.45
ORS 650	0.06	0.0118	3	6	0.06
ORS 331	0.70	0.0000	4	7	0.65
ORS 1041	0.67	0.0645	5	7	0.61
ORS 456	0.55	0.0323	3	8	0.46
ORS 894	0.50	0.0317	4	8	0.40
ORS 1161	0.49	0.0440	5	8	0.46
ORS 428	0.70	0.0538	4	9	0.64
ORS 887	0.29	0.0109	3	9	0.27
ORS 938	0.51	0.0222	5	9	0.40
ORS 437	0.42	0.0899	4	10	0.38
ORS 878	0.64	0.0000	5	10	0.59
ORS 457	0.70	0.0337	4	11	0.65
ORS 621	0.72	0.0769	5	11	0.66
ORS 630	0.68	0.2258	4	11	0.61
ORS 733	0.55	0.0215	4	11	0.45
ORS 1227	0.72	0.0111	4	11	0.67
ORS 502	0.33	0.0753	4	12	0.28
ORS 761	0.55	0.0000	4	12	0.45
ORS 778	0.30	0.0222	2	12	0.27
ORS 1085	0.45	0.0108	2	12	0.35
ORS 316	0.57	0.0430	4	13	0.54
ORS 1179	0.46	0.0000	3	13	0.36
ORS 307	0.52	0.0323	3	14	0.40
ORS 1079	0.46	0.0000	6	14	0.43
ORS 1248	0.64	0.0220	3	14	0.56
ORS 420	0.59	0.0952	4	15	0.52
ORS 687	0.56	0.0323	3	15	0.46
ORS 857	0.22	0.0000	3	15	0.21
ORS 1141	0.76	0.0538	3	15	0.71
ORS 407	0.62	0.0215	4	16	0.57
ORS 656	0.69	0.0909	6	16	0.64
ORS 750	0.61	0.0109	3	16	0.53
ORS 885	0.60	0.0222	3	16	0.53
ORS 297	0.75	0.0215	5	17	0.70
ORS 561	0.35	0.0227	4	17	0.30
ORS 735	0.72	0.0753	5	17	0.68
ORS 1245	0.61	0.0430	7	17	0.56
Average	0.57	0.0480	4.1	9.27	0.51

n_A Number of alleles
LG Linkage group
PIC Polymorphic information content

The number of alleles per SSR locus varied between two and seven with an average of 4.1. The PIC value (expected heterozygosity) per locus ranged between 0.06 (ORS 650) and 0.75 (ORS 925) with an average of 0.51. The gene diversity ranged from 0.06 (ORS 650) to 0.78 (ORS 925) with an average of 0.57. The average heterosygosity was found to be 0.048 with a number of markers exhibiting a heterosygosity of 0 (ORS 331, ORS 371, ORS 761, ORS 857, ORS 878, ORS 1079 and ORS 1179). The highest level of heterosygosity was found to be 0.4432 (ORS 381).

UPGMA dendrograms were constructed from distance matrices of both Nei and Rogers. Seeing that the Nei matrix and Rogers matrix produced UPGMA dendrograms with similar topologies, only the Rogers dendrogram is presented (Figure 3.1). In addition, a NJ tree was constructed. Since they had identical topologies, only a UPGMA dendrogram is shown.

The Rogers distance matrix showed significant differences between the various lines involved in the determination of genetic distance. The smallest genetic distance was found to be 0.0000 between R14 and R41 which are two closely related lines. The second smallest genetic distance was 0.0189 [between R42(HO) and R43(HO) - Appendix 2]. These two lines are known to be closely related with R43(HO) being another high oleic selection of the same line. The largest genetic distance was found to be 0.8721, which was between A9 and A40 (Appendix 2). The average Rogers distance was 0.5739.

The Nei distance matrix was also calculated between the lines. The smallest genetic distance was found between R14 and R41 (0.0000) and the second smallest genetic distance was found to be 0.0094 [between R42(HO) and R43(HO)] which was similar to results obtained using the Rogers distance matrix. The largest genetic distance was found to be 0.8673 which was found between A40 and A9. This was once again similar to the results obtained using the Rogers distance. The average Nei distance for the entire data set was 0.5570 which was slightly lower than the average obtained using the Rogers distance, but similar nonetheless.

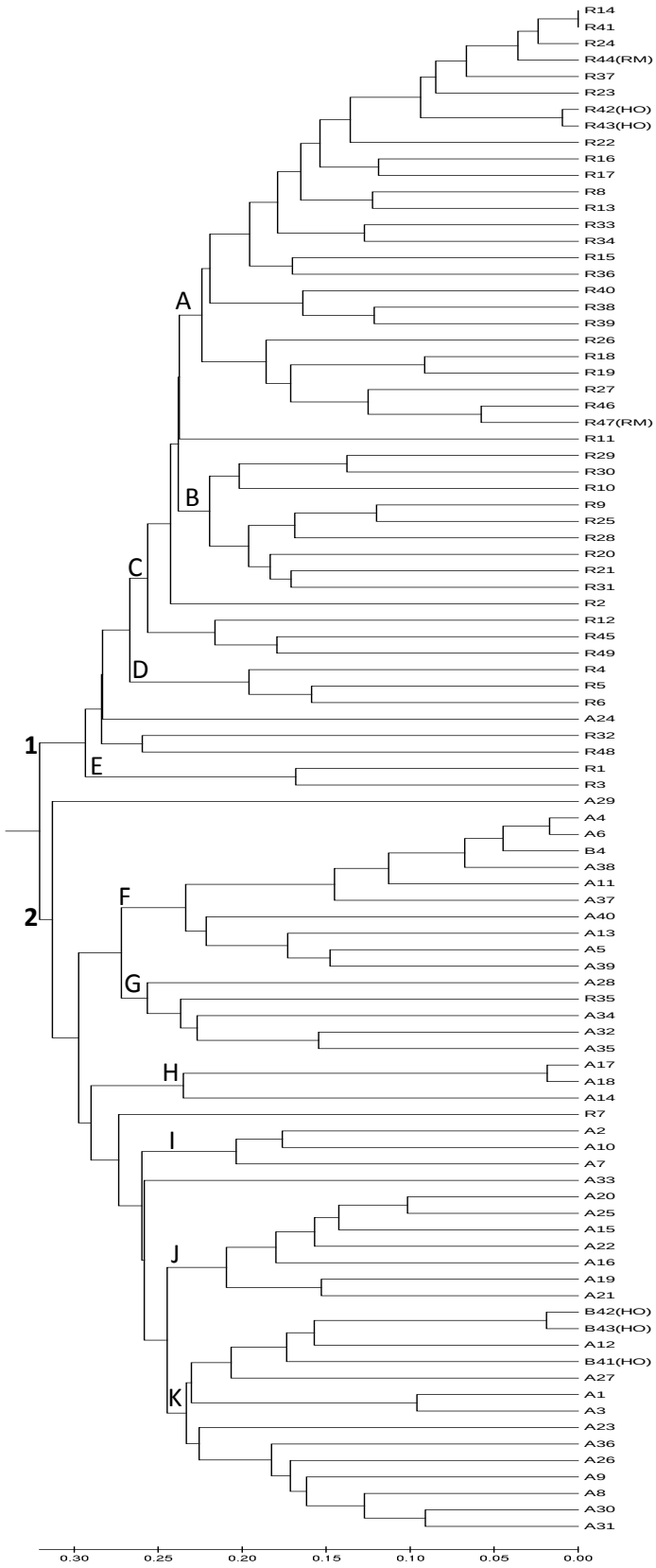

Figure 3.1 Evolutionary relationships of 93 inbred sunflower lines based on SSR marker data and determined using Rogers genetic distance and the unweighted pair-group method using arithmetic averages.

The seven smallest genetic distances in the Rogers distance matrix were found between the following line combinations: 0.0000 between R14 and R41 (Nei – 0.0080), 0.0189 between R42(HO) and R43(HO) (Nei – 0.0094), 0.0341 between A6 and A4 (Nei – 0.0284), 0.0370 between A17 and A18 (Nei – 0.0370), 0.0377 between B42(HO) and B43(HO) (Nei – 0.0283), 0.0472 between R41 and R24 (Nei – 0.0425) and 0.0481 between R24 and R14 (Nei – 0.0433). The comparative distance in the Nei distance matrix for the same lines is shown in brackets. The Rogers and Nei distances were similar and in one case exactly the same.

The six largest genetic distances in the Rogers distance matrix were: 0.8721 between A9 and A40 (Nei – 0.8673), 0.8415 between A23 and R8 (Nei – 0.8389), 0.8218 between A8 and R47(RM) (Nei – 0.8077), 0.8192 between A37 and R6 (Nei – 0.8029), 0.8122 between A16 and R46 (0.8029) and 0.8038 between A23 and R47(RM) (Nei – 0.7841).

It is important to note that there was a distinct tendency for groups to develop within the dendrogram. Firstly a distinct group of R-lines were found as designated by 1 in Figure 3.1. This group normally contains the male, branched parents in sunflower hybrid breeding. This group did not consist of R-lines exclusively, since the female line A24 was found in group 1. This line was selected from European germplasm and might possibly have been selected closer to the wild sunflower background which is generally where male germplasm originated from. A second distinct group of lines was found, namely the A-lines, and included, as expected, the B-lines. This group is designated by 2 in Figure 3.1. This group of lines is generally used as female lines and is single headed and unbranched in nature. This group did not consist of only A- and B-lines but also contained the R-lines R35 and R7. Both of these lines have been partially bred from populations and this could explain why they grouped within group 2. The average genetic distance within the R-line group was 0.5129 and in the A-line group 0.5992. The cophenetic correlation coefficient (r^2) was calculated (Sokal and Rohlf, 1962) to be 0.89. A relatively high correlation was therefore found between the accuracy of the dendrogram's representation of the pairwise distances as determined by the distance matrix.

Within the R-line group (cluster 1), five groupings could be identified, designated A to E in cluster 1, Figure 3.1. In the A- and B-line group (cluster 2), six groupings were identified designated F to K in cluster 2 of Figure 3.1. Lines that formed singletons (grouped separately

from all other lines) were not considered as a group. Cluster A contained a number of rust resistant (*P. helianthi*) R-lines. This included R42(HO), R44(RM), R43(HO) and R47(RM). These lines differ only in the type of trait attached to them which includes high oleic (HO) and downy mildew (RM) resistance. These lines clustered in the same group as the original R41 line. This group also contained two lines, R33 and R34, which are two different selections from the same genetic background. The two lines R23 and R24 also share a common parent in their pedigree (R41), and also clustered closely together and to their common parent R41. The pedigree of R14 in cluster A contains R41 and is closely related to it. The clustering with R41 suggests that selection for this line was strongly in favour of the R41 background. R46 and R47(RM) was found in this cluster and is related to the R41 background.

Cluster B contained R-lines which included lines whose pedigree contains South African germplasm combined with foreign germplasm. R11 was one of the exceptions in this case which was expected to group in cluster A. A number of the lines in cluster B have R41 background in the pedigree such as R9 and R10. R20 and R21 share a similar pedigree and clustered together.

Cluster C contained three lines which included R12 and R45. R12 is well suited to South America. R45 is related to R41 but most of the pedigree is related to foreign germplasm. R49 is a foreign R-line which grouped in this cluster.

Cluster D contained the three R-lines R4, R5 and R6. R5 and R6 share a common pedigree and clustered closely together. R4 share a similar pedigree to the other two lines in this cluster.

Cluster E contained a number of R-lines such as R32 and R48. R32 has exceptional oil content. The R-line R48 was found within this cluster and represents foreign germplasm. R1 and R3 clustered closely together and share a similar pedigree and grouped into their own sub-cluster. Both the lines share a pedigree of USA origin. The exception was the female A-line A24 which clustered with this set of R-lines.

Cluster F is the first cluster which mainly represented the A and B female lines. This cluster contained a number of lines which have a foreign pedigree. Of particular interest is one of the

A and B combinations in the dendrogram. As expected, A4 and B4 closely grouped together. The only difference between them should be the presence of the CMS trait in the A-line. A4 and A6 clustered closely together. This is due to the fact that it is the same line, but different breeding sources of it. It was planted from different nurseries, A4 obtained from the South African nursery and A6 from the United States Department of Agriculture (USDA). The lines A5 and A40 which are of foreign origin clustered together in cluster F. Both A37 and A38, found in this cluster, are related to A4. A39 found in this cluster is related to A5. A13 and A39 are also related to A5.

Cluster G contained the A-lines A28, A34, A32 and A35. The pedigrees of these A-lines consisted of lines bred from populations. The two R-lines in the A-line cluster R35 and R7 consists of lines bred from populations which could explain why this line was found in this cluster. Selection for the line was likely done more in favour of the genes derived from the population in its pedigree.

Cluster H contained three female lines, A17, A18 and A14. A17 and A18 are more foreign in nature and are well suited to South America. A14 has a pedigree which consists of lines made up from populations. The three lines from cluster I are from USA origin.

Cluster J contained a number of lines which are well suited to South African, African and South American environments. This group contained an A-line A20, which is well adapted to South America. A19, A15, A25 and A21 are lines which have proven to be high yielding and stable. The A-line A16 is well adapted to Africa as well as South America and has a high oil content.

Cluster K consisted of a collection of A-lines with foreign germplasm. This included three high oleic lines B41(HO), B42(HO) and B43(HO). A24 that clustered between cluster D and E is part of the germplasm of A23. A23 does contain a selection of other lines in its pedigree. A30 and A31 share a similar pedigree and clustered together. A12 is more related in its pedigree to B42(HO) and B43(HO) than the two high oleic lines are to B41(HO).

A two-dimensional PCA was drawn up and is shown in Figure 3.2. The eigen values used to construct the PCA are given in Appendix 3.

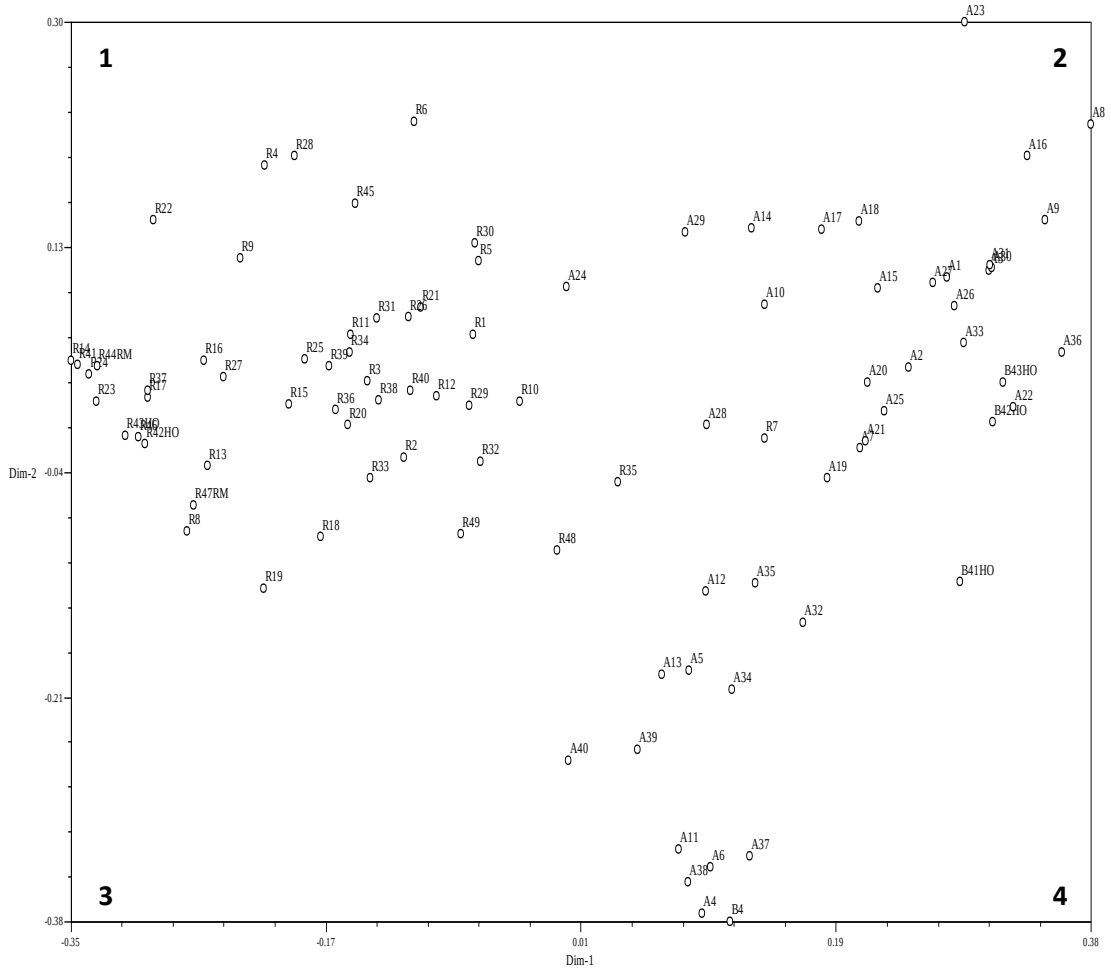

Figure 3.2 Evolutionary relationships based on SSR analysis of 93 inbred sunflower lines as determined using two-dimensional principal component analysis.

The PCA was drawn up for comparison to the dendrogram. The two-dimensional PCA (Figure 3.2) can be divided into four quadrants, as indicated in Figure 3.2. There is a strong tendency for the PCA to show the same trends with clustering of lines as in the dendrogram in Figure 3.1. The following similarities were seen between the dendrogram and the PCA: Most of the R-line males could be found in the first quadrant with some in the third quadrant. The majority of the A- and B-line females could be found in the second and fourth quadrants. It is important to note that the A4 and B4 lines grouped closely together in the fourth quadrant. A6 was found in the fourth quadrant close to A4 and B4 similar to clustering in Figure 3.2. B42(HO) and B43(HO) grouped closely together in the second quadrant. A17 and A18 grouped together in the second quadrant. R41 and its various trait versions grouped closely together in quadrant one.

Figure 3.3 includes the PCA but with vectors included. Here it is clear that B42(HO) and B43(HO) grouped closely together. The relationship between A4, B4 and A6 were more distinct and grouped more closely together. A17 and A18 still grouped close together. The grouping between A17, A18 and A16 was clearer in Figure 3.3. A dense grouping of lines was found to the left of the figure, which represents the R41, R46 and R33/R34 lines and lines with similar genetic backgrounds. The resolution is not clear due to the similarities in the group of R-line males. The R-line males again grouped together to the left of Figure 3.3 and the female A-and B-lines two the right.

Three distinct groups were formed. The first group (group A) contained most of the R-line males. Group B and group C contained the majority of the A- and B-line females. Group B contained a number of lines which are more related to the European backgrounds. A13 is related in its pedigree to A5. The lines A37, A38 and A11 were more related to the A4 and B-lines in their pedigree. This was shown in Figure 3.3 where they grouped in B. The groupings in the PCA corresponded with the groupings in the dendrogram. Group A from the PCA was consistent with cluster 1 in the dendrogram. Group B in the PCA corresponded with clusters H to K in the dendrogram. Group C in the PCA corresponded with clusters F and G in the dendrogram. The clusters F to K represents cluster 2 in the dendrogram.

Group A included a diverse group of A-line females, which represented germplasm from the USA as well. This was represented by the lines A8, A9, A10 and A2. A number of the most important A-line females was found here and included A25, A19, A16 and A23.

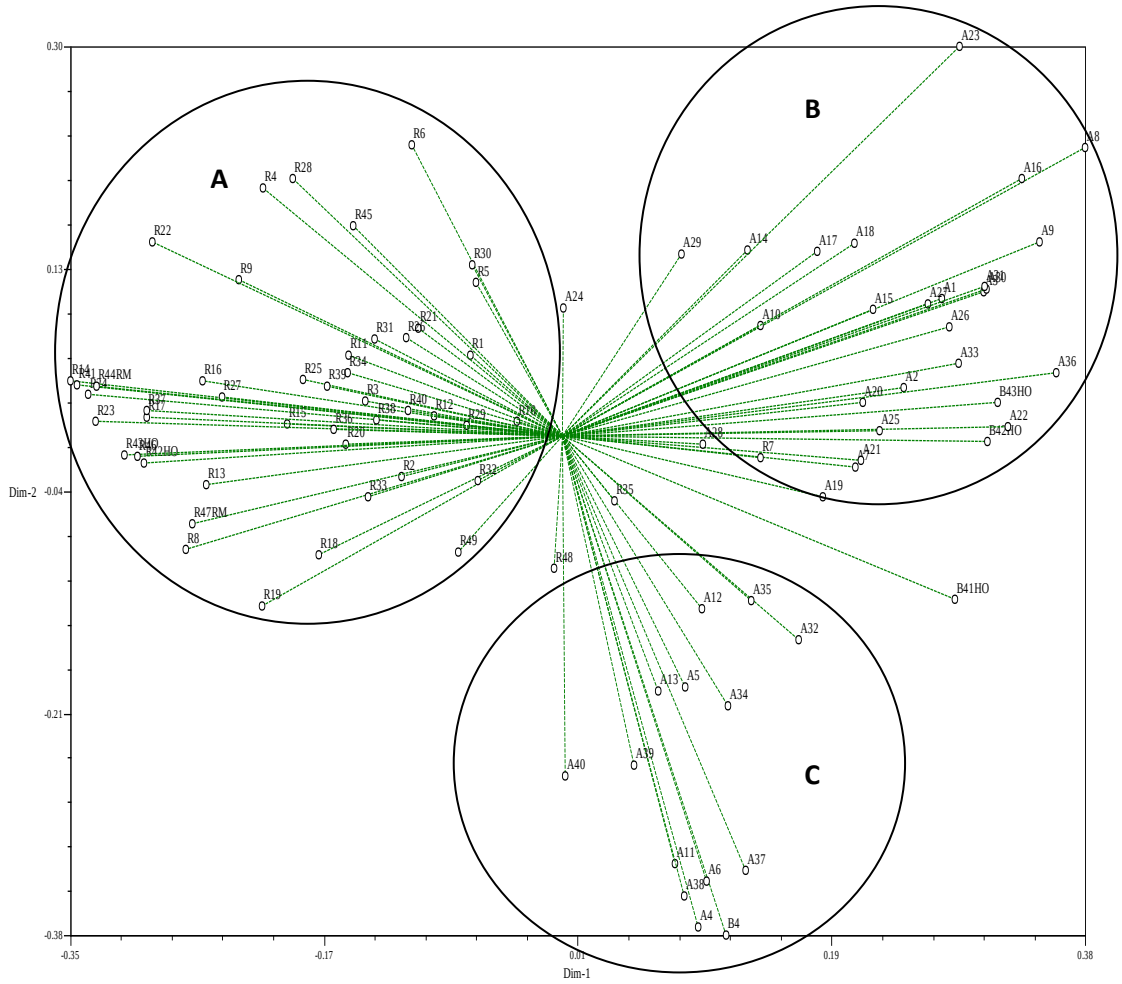

Figure 3.3 Evolutionary relationships of 93 inbred sunflower lines determined using principal component analysis, with vectors.

An AMOVA analysis was done (Table 3.3) as well as an AMOVA with calculations done on individual or line level (Table 3.4) on sunflower lines which were grouped into A- and B-lines and R-lines, which then represented populations. The A- and B-lines represented one population and the R-lines another.

A total of 81.44% of the total variation was attributed to within population variation and the remaining 18.56% to between population variation by the AMOVA (Table 3.3). As was expected with cross pollinated species, genetic diversity within the populations was high and accounted for most of the total variation while between population variation was moderate.

Table 3.3 Analysis of molecular variance of sunflower populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	P
Among populations	2	366.06	2.78 Va	18.56	0.1857	0.00
Within populations	195	2379.13	12.20 Vb	81.44		0.00
Total	197	2745.18	14.98			

d.f. Degrees of freedom
F_{ST} Fixation index (total population)
P Probability

The fixation index (F_{ST}) was calculated. The fixation index is the measure of the diversity of randomly chosen alleles within the same subpopulation relative to that found in the entire population. It can be expressed as the proportion of genetic diversity due to allele frequency differences among populations (Holsinger and Weir, 2009). The comparison of genetic variability within and between populations is used in the population genetics field. Values range from zero to one. A value of zero implies complete panmixis where two populations are interbreeding freely. A value of one implies that the two populations are separate. In Table 3.3 the fixation index was found to be 0.1857 which is low and suggests that the lines included in this study are interbreeding freely. Breeding records might prove this to be incorrect.

Table 3.4 Analysis of molecular variance of sunflower populations calculated on individual level

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	F_{IS}	F_{IT}	P
Among populations	2	366.06	2.59 Va	17.31	0.173	0.926	0.939	0.00
Among individuals within populations	96	2288.63	11.46 Vb	76.59				0.00
Within individuals	99	90.50	0.91 Vc	6.11				0.00
Total	197	2745.18	14.98					

d.f. Degrees of freedom
F_{ST} Fixation index (total population)
F_{IS} Fixation index (subpopulation)
F_{IT} Fixation index (individuals)
P Probability

In Table 3.4 a total of 17.31% of the total variation was attributed to among population variation, 76.59% to among individuals within populations and the remaining 6.11% to within individuals variation by the AMOVA calculated on individual level. Variation among individuals within populations was the greatest and represents diversity in the sunflower lines tested. The fixation indices (F_{ST}, F_{IS} and F_{IT}) were calculated. The fixation index (F_{ST}) for the

total population in Table 3.4 was calculated to be 0.173 and indicates that the populations are relatively freely interbreeding. The fixation index for the sub-population (F_{IS}) was calculated to be 0.926 and the fixation index for the individuals (F_{IT}) was calculated to be 0.939. These values indicate that both the subpopulations and individuals were separate. This indicate that the A- and B-line groups were separate from the R-line group. It further suggested that the lines were separate.

3.5 Discussion

A relatively large number of lines were evaluated in the current study through the use of SSR markers. In a previous study Paniego *et al*. (2002) developed SSR markers for sunflower. They sequenced 503 SSR clones and designed 271 PCR primer sequences. Sixteen sunflower accessions were analysed and 170 of the primers tested were shown to be polymorphic for the selected lines. The SSRs produced an average of 3.5 alleles per locus and the PIC value was 0.55. The SSRs tested in the current study produced 4.1 alleles per locus and the PIC value was slightly lower at 0.51. This compared well to the findings of Yu *et al*. (2002) who developed 130 unique SSRs through the design and testing of primers for 171 unique SSRs. They found the average number of alleles per locus to be 3.7 for dinucleotide, 3.6 for trinucleotide and 9.5 for tetranucleotide repeats. The PIC value was found to be 0.53 for dinucleotide, 0.53 for trinucleotide and 0.83 for tetranucleotide repeats.

Zhang *et al.* (2005) also investigated the establishment of a set of SSR markers for sunflower fingerprinting. A total of 78 SSR markers were selected and subsequently used to assess genetic variability between a set of 124 sunflower inbred lines. This included 67 female lines and 57 male restorer lines. They found an average of 3.5 alleles per SSR locus and a PIC per locus which ranged from 0.06 to 0.81 with an average of 0.51. They also used Rogers distances to determine relationships between inbred lines. The majority of distances were found to be between 0.4 and 0.6. Some of the pairs did however exhibit distances lower than 0.1. They found the genetic diversity value within each subset of male and female lines to be relatively low.

The current dendrogram was constructed using 55 selected SSR markers. This resulted in grouping of the 93 inbred lines into two major clusters. The first of the groups (1) included most of the R-lines which represents the restorer or male lines. The genetic means observed among the 49 R-lines was 0.513. The second group (2) consisted mainly of A-lines and a few

isogenic B-lines (as is the case with A4 and B4). This group represented the female group which included sterile A-lines as well as maintaining B-lines. The genetic means observed between the 44 female lines were 0.599. It is expected that the lowest genetic mean is to be found between specific pairs of lines within either the female group or the male group. The second smallest genetic distance was found to be 0.0189 between R42(HO) and R43(HO). It is important to note that certain lines in the R-line group have been converted from a conventional inbred line to lines containing traits such as high oleic content and downy mildew resistance. This is the case with both R41 and R46. R46 also has some resemblance to R41 due to the fact that R41 was one of the lines included in the breeding and selection of R46.

Four versions of the R41 lines were included in the study. The genetic distance between the isogenic lines were as follows: 0.0741 between R41 and R44(RM), 0.1604 between R41 and R42(HO), 0.1759 between R41 and R43(HO), 0.0189 between R42(HO) and R43(HO), 0.1484 between R42(HO) and R44(RM) and 0.1642 between R44(RM) and R43(HO). Two versions of the R46 line were included in the study. The genetic distance between the two isogenic lines, R46 and R47(RM), was 0.1154.

There are slight differences between the various versions of the abovementioned lines. The genetic differences between R41 and R42(HO) and R41 and R43(HO) were larger than the difference between R41 and R44(RM). The difference between the various versions of the line could be attributed to the trait which was incorporated into the original line. Several generations of conventional backcrossing could also contribute to the slight genetic deviation from the original line. The difference between the conventional line R41 and the downy mildew resistant line R44(RM) was smaller than that of R41 and R42(HO). The difference between R46 and R47(RM) was also relatively similar to that of R41 and R44(RM). The larger difference between the conventional line and the high oleic version could be attributed to the fact that the high oleic trait is an induced mutation which included modifier genes (Soldatov, 1976) and more genetic material therefore gets transferred.

It was found that the results showed a good similarity to the expected data obtained from pedigree information. Within the dendrogram, R42(HO), R44(RM) and R43(HO) fell in the same cluster. As was expected, R33 and R34 also fell into the same cluster. This was due to the fact that these lines share a common pedigree. Furthermore, R15 clustered together with

R41, correlating with known pedigree data. R46 and R47(RM), also closely related to each other and to R41, did cluster closely and all three lines grouped together in cluster A. The small difference between R46 and R47(RM) could possibly be due to the trait selection in R47(RM). Looking at their pedigree data, all of these lines exhibit a strong tendency towards the R41 germplasm in their pedigrees. This also implies that selection was strongly favoured in the direction of the R41 genetic background.

Comparing the PCA to the UPGMA dendrogram there were a number of similarities. A4 and B4 grouped closely together both in the dendrogram and in the PCA. The A-line A6 which is closely related to A4 and B4 grouped close to the mentioned lines in the PCA. Furthermore, lines B42(HO) and B43(HO) grouped closely together both in the dendrogram and the PCA. Another similarity was that lines A17 and A18 grouped closely together in quadrant two of the PCA and in the dendrogram. R9 and R33 in quadrant one grouped further from each other in the dendrogram (group A) as it did in the PCA. The PCA with vectors confirmed the groupings found in the two-dimensional PCA. The tendencies seem much the same in the PCA as in the dendrogram. The three groups found in the PCA with vectors indicated three major groups between the lines. As expected, group A contained the majority of the male R-lines. The A- and B- line females were divided into two groups. Group B contained A- and B-lines representing European germplasm. Group C represented USA and South African germplasm. There were similarities between the dendrogram and the PCA. The dendrogram confirmed with cluster 1 the R-lines found in group A of the PCA. Cluster 2 from the dendrogram corresponded to group B and C in the PCA. This makes it possible to select lines for specific countries and assist in the selection of backgrounds when deciding on new crosses for new breeding lines.

It is therefore clear that lines or populations previously used in the development of the current lines included in the dendrograms, were definitive in the grouping of the developed lines. The lines used in the study are used as reference lines for varietal verification. It does happen that there might be slight changes to the lines as they continue to be improved and slight selection differences might occur in the maintenance of the lines. There is however low levels of phenotypic variation within the lines used in the study. This is largely due to the fact that the lines are at a high inbred level.

According to the AMOVA data most of the diversity was attributed to variation within the populations (81.44%) and then among individuals within populations (76.59%). This translated to the fact that the lines used in this study were very diverse. Diversity thus exists within the groups and it represents variation in the sunflower germplasm. With this variation present in Pannar Seed (Pty) Ltd sunflower germplasm successful variety crosses can be made between the various lines as have been proven with crosses between the A- and B-line groups. An important point to be made is that this translates to the fact that genetic narrowing has not occurred in the Pannar Seed (Pty) Ltd sunflower germplasm and that variety does exist between the lines. Care should be taken to ensure that the same lines such as the successful lines A19, A16, A21, A25, A24, R41, R34 and R29 are not used repeatedly for line development. New introductions should continually be made into the germplasm backgrounds to broaden the genetic variety in the sunflower programme.

A well distributed set of SSRs were used in this study to determine the genetic diversity among the 93 inbred lines involved. There was a distinct split between the R- and A-lines in the dendrogram. The genetic distance between various lines varied from as small as 0.0000 between R41 and R14 to as big as 0.8721 between A9 and A40. There are a number of options available for the use of this data. It is possible to use the data to attempt to predict better combinations for use in possible line breeding combinations. It could also be possible to use the data to attempt to determine optimal crosses and attempt to predict the best hybrid combinations. It would be advisable to include more lines to obtain a more complete picture. The inclusion of linked markers (for instance qualitative or quantitative linked markers) into the set of markers can enable the screening of future lines.

3.6 References

Arif, I.A., Bakir, M.A., Khan, H.A., Al Farhan, A.H., Al Homaidan, A.A., Bahkali, A.H., Al Sadoon, M. and Shobrak, M., 2010. A brief review of molecular techniques to assess plant diversity. International Journal of Molecular Sciences 11: 2079-2096.

Burke, J.M., Knapp, S.J. and Rieseberg, L.H., 2005. Genetic consequences of selection during the evolution of cultivated sunflower. Genetics 171: 1933-1940.

Cheres, M.T., Miller, J.F., Crane, J.M. and Knapp, S.J., 2000. Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. Theoretical an Applied Genetics 100: 889-894.

Dong, G.J., Liu, G.S. and Li, K.F., 2007. Studying genetic diversity in the core germplasm of confectionary sunflower (*Helianthus annuus* L.) in China based on AFLP and morphological analysis. Russian Journal of Genetics 43: 762-770.

Ellis, J.R., Pashley, C.H., Burke, J.M. and McCauley, D.E., 2006. High genetic diversity in a rare and endangered sunflower as compared to a common congener. Molecular Ecology 15: 2345-2355.

Excoffier, L., 2001. Analysis of population subdivision. In: Handbook of statistical genetics. Eds. Balding, D.J., Bishop, M. and Cannings, C. John Wiley & Sons, Chichester, UK. pp. 271-307.

Excoffier, L. and Lischer, H.E.L., 2010. Arlequin suite version 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564-567.

Excoffier, L., Smouse, P.E. and Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Fusari, C.M., Lia, V.V., Hopp, H.E., Heinz, R.A. and Paniego, N.B., 2008. Identification of single nucleotide polymorphisms and analysis of linkage disequilibrium in sunflower elite inbred lines using the candidate gene approach. Plant Biology 8: 1-14.

Gvozdenović, S., Saftić-Panković, D., Jocić, S. and Radić, V., 2009. Correlation between heterosis and genetic distance based on SSR markers in sunflower (*Helianthus annuus* L.). Journal of Agricultural Sciences 54: 1-10.

Harter, A.V., Gardner, K.A., Falush, D., Lentz, D.L., Bye, R.A. and Rieseberg, L.H., 2004. Origin of extant domesticated sunflowers in eastern North America. Nature 430: 201-205.

Holsinger, K.E. and Weir, B.S., 2009. Genetics in geographically structured populations: defining, estimating and interpreting FST. Nature Reviews Genetics 10: 639-650.

Jolliffe, I.T., 1986. Principal Component Analysis. Springer, NY. pp. 28.

Kitchener, D.J., Adams, M. and Boeadi, K., 1994. Morphological and genetic relationships among populations of *Scotorepens saborni* (Chiroptera: Vespertilionidae) from Papua New Guinea, Australia and Indonesia. Australian Mammalogy 17: 31-42.

Kolkman, J.M., Berry, S.T., Leon, A.J., Slabaugh, M.B., Tang, S., Gao, W., Shintani, D.K., Burke, J.M. and Knapp, S.J., 2007. Single nucleotide polymorphisms and linkage disequilibrium in sunflower. Genetics 177: 457-468.

Kumar, S., Nei, M., Dudley, J. and Tamura, K., 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics 9: 299-306.

Liu, A. and Burke, J.M., 2006. Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics 173: 321-330.

Liu, K. and Muse, S.V., 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128-2129.

Melchinger, A.E., 1993. Use of RFLP markers for analyses of genetic relationships among breeding materials and prediction of hybrid performance. Proceedings of the International Crop Science Congress, July 1992. pp. 621-628.

Mitchell, S.E., Kresovich, S., Jester, C.A., Hernandez, C.J. and Szewe-McFadden, A.K., 1997. Application of multiplex PCR and fluorescence based, semi-automated allele sizing technology for genotyping plant genetic resources. Crop Science 37: 617-624.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America 70: 3321-3323.

Paniego, N., Echaide, M., Muñoz, M., Fernãndez, L., Torales, S., Faccio, P., Fuxan, I., Carrera, M., Zandomeni, R., Suãrez, E.Y. and Hopp, H.E., 2002. Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.). Genome 4: 34-43.

Quagliaro, G., Vischi, M., Tyrka, M. and Olivieri, A.M., 2001. Identification of wild and cultivated sunflower for breeding purposes by AFLP markers. The American Genetic Association 92: 38-42.

Rogers, J.S., 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII, University of Texas Publication 7213: 145-153.

Rohlf, F.J., 2005. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.2. Exeter Software: Setauket, NY.

Smith, J.S.C., Hoeft, E., Cole, G., Lu, H., Jones, E.S., Wall, S.J. and Berry, D.A., 2009. Genetic diversity among U.S. sunflower inbreds and hybrids: assessing probability of ancestry and potential for use in plant variety protection. Crop Science 49: 1295-1303.

Sokal, R.R. and Rohlf, F.J., 1962. The comparison of dendrograms by objective methods. Taxon 11: 30-40.

Soldatov, K.I., 1976. Chemical mutagenesis in sunflower breeding. Proceedings of the 7th international sunflower conference, Krasnodar, Russia, 27 June - 3 July 1976. pp. 352-357.

Tang, S., Yu, J.K., Slabaugh, M.B., Shintani, D.K. and Knapp, S.J., 2002. Simple sequence repeat map of the sunflower genome. Theoretical and Applied Genetics 105: 1124-1136.

Warburton, M. and Crossa, J., 2000. Data analysis in the CIMMYT Applied Biotechnology Center for Fingerprinting and Genetic Diversity Studies. CIMMYT, Mexico. pp. 20-21.

Wiley, E.O., 1981. Phylogenetics: The theory and practice of phylogenetics and systematics. John Wiley, New York. pp. 439.

Yu, J., Mangor, J., Thompson, L., Edwards, K.J., Slabaugh, M.B. and Knapp, S.J., 2002. Allelic diversity of simple sequence repeats among elite inbred lines of cultivated sunflower. Genome 45: 652-660.

Yu, J., Tang, S., Slabaugh, M.B., Heesacker, A., Cole, G., Herring, M., Soper, J., Han, F., Chu, W., Webb, D.M., Thompson, L., Edwards, K.J., Berry, S., Leon, A.J., Grondona,

M., Olungu, C., Maes, N. and Knapp, S.J., 2003. Towards a saturated molecular genetic linkage map for cultivated sunflower. Crop Science 43: 367-387.

Zhang, L.S., Le Clerq, V., Li, S. and Zhang, D., 2005. Establishment of an effective set of simple sequence repeat markers for sunflower variety identification and diversity assessment. Canadian Journal of Botany 83: 66-72.

Chapter 4

Correlation between SSR genetic data and yield data

4.1 Summary

Little is known about the application of genetic distances for the prediction of sunflower hybrid combinations. It is important to determine heterotic groups in order to predict the best possible hybrid combinations. Optimal crosses can then be made between parental lines with maximum potential to optimise heterosis. The aims of this study were to analyse sunflower yield trials using GenStat (REML) and to evaluate and compare data obtained from yield trials and the dendrogram obtained through the use of SSR markers. A total of 133 yield trials were evaluated over six locations. Crosses were made between 11 R-lines (testers) and 33 A-lines (male sterile female lines). Combinations of these crosses were compared with a commercial hybrid to ascertain a pattern between yield and combining ability. Differences were found within crosses between R- and A-lines. No significant trend was found between genetic distance and yield.

4.2 Introduction

Determination of genetic distance through the use of molecular markers is increasingly becoming important for international seed companies to assist plant registrations and Plant Breeders Rights' protection, as well as in the investigation of heterotic groups (Camlin, 2000; Cooke and Reeves, 2003). Yu *et al.* (2002; 2003) developed 1 089 SSR markers specific for cultivated sunflower to help eliminate long standing bottlenecks brought about by the scarcity of single copy DNA markers available in the public domain. The first genetic linkage map of sunflower constructed using SSR markers was developed by Tang *et al.* (2002). This could lead the way for germplasm security.

Plant variety protection is an important issue for plant breeding companies in general. The use of marker based techniques has become increasingly important in plant variety protection. Smith *et al.* (2009) investigated the use of SSRs to discriminate between sunflower inbred lines and hybrids in the USA. Inbreds with similar pedigrees clustered together and groupings within these inbreds could be easily classified as being male and female. Pedigree analysis of known hybrids was used to establish the parental inbreds as well. SSR profiles were successfully used to facilitate the identification of uniqueness of lines.

Zhang *et al.* (2005) contributed successfully to the establishment of a set of SSR markers to assist in fingerprinting and variety identification of sunflower lines. They detected low values of variation within the restorer and maintainer groups. The selected set of SSRs was successful in determining sunflower fingerprints and genetic diversity.

It is important to understand the genetic diversity of parental lines in order to guarantee the success of plant breeding programmes. This is especially important when the main goal is hybrid seed production. Genetic diversity information assists the breeder to determine heterotic groups and therefore the optimal crosses to perform on parental lines with maximum potential to optimise heterosis (Betrán *et al.*, 2003). Knowledge obtained from genetic diversity data of parental lines can generally be used in all commercially bred crops to obtain maximum combinability.

Cheres *et al.* (2000) investigated the possibility that genetic distance could be a possible predictor of heterosis and resultant hybrid performance within and also between heterotic groups in sunflower. They used heterotic group crosses and then compared hybrid seed yield and genetic distances obtained through the use of AFLPs. They found that genetic distances were significantly correlated with hybrid seed yield when estimated from AFLP fingerprints but not from coancestries. They came to the conclusion that the heterotic groups in sunflower were of significant use, but was not as distinct as is the case in maize. Gvozdenović *et al.* (2009) used SSRs to determine the correlation between SSR based genetic distance and heterosis for six agronomic traits in sunflower. Results obtained were not as expected and it was found that a low correlation existed between genetic distance and heterosis.

Turkec and Goksoy (2006) used a more conventional approach to establish general and specific combining ability in sunflower for seed yield and various yield components. They crossed female and male lines to produce hybrids and then evaluated the F_1 hybrids through line x tester analysis. They found a variety of lines to be good general combiners, as well as a variety of crosses to be good selections for specific combining ability as well as good yield.

According to Chaudhary (1982), combining ability can be defined as the ability of a parent to produce either inferior or superior combinations in a single or a series of crosses. The GCA of a genotype determines the crossing value of the genotype (a line or a tester) that is the optimum combiner in a breeding programme (Falconer and Mackay, 1996). This can be

described best as being primarily the measure of additive gene action (Sprague and Tatum, 1942).

SCA on the other hand shows the minimum as well as the maximum genetic gain of hybrids from specific lines with specific testers. SCA is central in hybrid breeding. The SCA of a cross gives an indication of the proportion of loci which shows dominance (V_D) as well as interaction (V_I). Dominance and interaction are the result of specific gene combinations. Genes involved in dominance and interaction are transferred from parents to offspring. The phenotypic effect of the genes is not transferred directly to the offspring due to the fact that the different genes are grouped together and form new combinations in the offspring. Loci that show dominance or interaction are therefore not contributing to the additive genetic variance or the inheritance of a character (Falconer and Mackay, 1996). Sprague and Tatum (1942) described SCA as an estimate of the effects of non-additive gene actions.

Sprague (1983) reported that additive and dominance gene effects are as a rule much greater than other types of gene effects. Additive effects are those effects that respond to selection. Overdominance as well as epistasis exists, but neither one of these have been found to be important at population level. Additive and dominance effects provide a satisfactory model for heterosis and for progress attained through breeding (Crow, 1999). Additive variance was found to be the most important type of gene action in sunflower. Dominance variance appeared to be important for yield only, while epistatic effects were minor (Miller *et al*., 1980).

A number of statistical methods have been developed for the analysis of data and to determine various parameters such as GCA and SCA. One of the most commonly used group of statistical models is the ANOVA. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal and subsequently generalises the *t*-test to more than two groups (Anscombe, 1948). Other statistical models have been developed after the advent of ANOVA.

REML has been used in this study to analyse the single data from each trial. McCulloch (1997) described and defined REML in the following manner: The main advantage of REML is that it can handle missing data. Seeing that maximum likelihood estimates are used in REML, it is important to describe the maximum likelihood estimates method and how it is

adapted to obtain REML estimates. The likelihood function (L) is used to measure the likelihood of data when model parameters are given. L is best defined through the use of the density function. In mixed models, the observations tend to be independent and the likelihood has to be based on a multivariate density function. Seeing that the expected value of the random effects vector is 0, the mean of the distribution of Y is Xα which in turn leads to the likelihood function,

$$L = (1/((2\pi)^{(1/2)n}|V|^{1/2}))exp[-1/2(y - X\alpha)^{/}V^{-1}(y - X\alpha)]$$

where n is the number of observations.

The maximum likelihood (ML) estimates need to be derived, which is done by maximising the likelihood function with respect to the parameter estimated. In a normal distribution, the extreme value will always be a maximum. The maximum point of a function will also coincide with the maximum point of the logarithm of the same function. Seeing that this will be easier to handle, the logarithm of the likelihood will be used and maximised instead. The log likelihood will be designated LL and in a multivariate normal distribution the equation will be,

$$LL = C - 1/2[\log|V| + (y - X\alpha)^{/}V^{-1}(y - X\alpha)]$$

where C is a constant which can be ignored in the maximisation process. The ML estimates of α is obtained by taking the derivative of the above formula and setting the derivative equal to 0,

$$X^{/}V^{-1}(y - X\alpha) = 0$$

which will then give the estimate of α,

$$\hat{\alpha} = (X^{/}V^{-1}X)^{-1} X^{/}V^{-1}y$$

The variance of the estimate $\hat{\alpha}$ is,

$$var(\hat{\alpha}) = (X^{/}V^{-1}X)^{-1} X^{/}V^{-1}var(y)V^{-1}X(X^{/}V^{-1}X)^{-1}$$
$$= (X^{/}V^{-1}X)^{-1} X^{/}V^{-1}VV^{-1}X(X^{/}V^{-1}X)^{-1}$$

$= (X^{/}V^{-1}X)^{-1}$

It is important to note that by taking the derivative of the variance of the estimate of $\hat{\alpha}$ does not lead to a linear equation and a solution cannot be specified in a single equation. Iterative processes therefore have to be used to obtain the parameters in V.

The estimate of V largely depends on α, but seeing that α is unknown the expression of the estimated $\hat{\alpha}$ which will lead to downward biased estimates of the components in V will have to be used. A classical example of this is the univariate normal distribution whereby the ML estimate of the variance is

$var(y) = 1/n\sum(x_i - \bar{x})$,

which is different from the unbiased estimate
$var(y) = 1/(n-1)\sum(x_i - \bar{x})$

Looking at the REML equation, the likelihood function is based on a linear transformation of y. For all practical reasons, it will be designated y^+, so that y^+ does not contain any fixed effects. This is necessary so that a transformation can be found where $E(y^+) = 0$. It is possible to write the transformation as

$y^+ = Ay = AX\alpha + AZu + Ae$,

and should A be chosen as

$A = I - X(X^{/}X)^{-1}X^{/}$, where I is the identity matrix, $AX = 0$ will be obtained and the likelihood function will then be based on the residual terms $y - X\hat{\alpha}$. The residual likelihood log will then be

$RLL = ½[\log|V| + |(X^{/}V^{-1}X| + (y - X\hat{\alpha})^{/}V^{-1}((y - X\hat{\alpha})]$

When looking at fixed effetcs, ML estimates and REML estimates will give the same results. If variance estimates are taken into account, there will be a difference in the two methods (Diggle, 1992; Paterson and Lello, 2003; Kelly *et al.*, 2009).

Yield trials are the primary experiment in plant breeding. It is crucial to ensure effective use of the available entries (hybrids or varieties). It is also an expensive exercise to increase the number of entries and locations to test these entries. Should an existing database with fingerprinted sunflower lines be available to evaluate possible hybrid predictions, a much more efficient testing system could be established.

The main aims of the study were therefore:

1. Analysis of sunflower yield trials using GenStat (REML).
2. Evaluation and comparison of yield trials and the dendrogram obtained through the use of SSR data.

4.3 Materials and methods

4.3.1 R-line testers as male parents

Trials were planted containing 11 R-lines which were crossed (or used as testers) with 33 A-lines and the resultant hybrids were used in the trials. The R-lines were used as testers or the pollinating parent in an isolation block that included the A-line females. The R-lines used were chosen out of the total group of R-lines (Figure 3.1, Chapter 3) due to specific characteristics stated in the next paragraphs and due to their previous performance in yield trials and being selected as elite lines. All female A-lines were included in the isolation blocks seeing that they were initially identified as B-lines and crossed with NMS lines. Once they are selected from these trials they are converted to CMS and are subsequently treated as elite lines.

The first R-line that was selected was R44(RM). R44(RM) is a line which also has a conventional form in R41 as well as a high oleic version R42(HO). R44(RM) exists in commercial hybrids and combines well with A-lines. This line exhibits resistance to brown rust as well as downy mildew. The R34 R-line was selected for high yielding abilitiy and *Puccinia* resistance. R9, R13 and R15 were selected for their yielding characteristics. R11 was selected for its yielding properties and because it performed well in Argentina. R47(RM) was selected for its yielding properties and has a conventional version R46. R47(RM) has resistance against downy mildew. R32 was not only selected for its yielding attributes, but also high oil content. R29 is a newer line which has been found to be good in combination with the A-line females. This line is also resistant to rust. R10 is a line which has proven to

be high yielding in combinations and R48 is a line which has proven to be an elite line in combinations.

4.3.2 Trial design and locations used

All trials were lattice designs with three replications. Different randomisations were used at different sites to maximise randomisation effects between the different single trials. Trials were planted as two row plots, with 0.91 m spacing between rows, spacing between plants set at 0.30 m and a row length of 6 m. An alley of 1.5 m was allowed between ranges. The plant population was approximately 36 000 plants per ha, which was reached through overplanting. Overplanting is done because in sunflower not all seedlings survive to the adult stage due to factors such as pests.

Trials were planted on six sites over the country as shown in Table 4.1. Trials with the following males as parents were planted during the planting seasons as indicated: R9, R11, R44(RM) and R48: 2002-2003; R12 and R13: 2003-2004; R34, R32 and R44(RM): 2004-2005; R15 and R47(RM): 2005-2006; R10: 2006-2007 and R29: 2007-2008. The different R-line tester groups were not crossed to the A-lines in the same season due to logistical difficulties and therefore all trials were not planted in the same season due to the sheer number of entries and trials involved. All trials were dry-land trials and no irrigation was used. Nutrient deficiencies were prevented with fertilisation where necessary. Weeds and insect pests were controlled chemically. Plot data of grain yield were determined by machine harvesting the two row plots with the use of Wintersteiger dual plot harvesters. Grain oil concentration was determined on a sample from each plot and analysed on a Spinlock NMR (nuclear magnetic resonance) machine. Oil yield was calculated as the product of grain yield and grain oil concentration. Analyses of trials were done with GenStat employing the REML method (Payne, 2009).

Table 4.1 R-line male elite testers used as parents in crosses with the A-lines planted at six localities in South Africa

Location	Province	R-line testers
Kroonstad	Free State	R44(RM); R34; R9; R13; R15; R11; R47(RM); R32; R29; R10; R48
Villiers	Free State	R44(RM); R9; R11; R48
Delmas	Mpumalanga	R44(RM); R9; R15; R11; R47(RM); R29; R10; R48
Standerton	Mpumalanga	R34; R13; R32
Lichtenburg	North-West	R34; R15; R47(RM); R32; R29; R10
Klerksdorp	North-West	R11; R48

Leeuwner (2005) investigated the different environment and genotype interactions in sunflower in South Africa. He found that the long-season cultivars were better adapted to the northern and western environments. The medium-season cultivars were better suited to the eastern environments. The eastern environments included Balfour, Bethal, Delmas, Kinross, Leandra, Senekal, Standerton and Tweespruit, the western locations included Bloemfontein, Klerksdorp, Kroonstad, Lichtenburg, Makokskraal and Rysmierbult and the northern locations were Dwaalboom and Settlers. Selection of trial sites in this study was based on his description of the best localities suited for sunflower hybrids.

The 33 A-lines (female lines) (Figure 3.1, Chapter 3) involved were crossed to the R-line testers (Table 4.1) and the resultant hybrids were evaluated for yield. However, some of the crosses did not yield sufficient seed to be included in the trials. Trials were split into maturity groups. The same commercial check (PAN 7351) was included in all trials to ensure a method to measure the performance of the crosses against.

Other commercial checks that were included were PAN 7033, PAN 7049 and PAN 7355. Lines were also classified into trials on the basis of the flowering dates of the A-lines as collected when they were planted in the crossing blocks. This was to ensure that the same maturing lines grouped together as much as possible, although the differences in flowering dates were not very large. It is important to note that the trend in South Africa is for later maturing hybrids due to the fact that these types of hybrids tend to be higher yielding. Flowering dates were only collected at Delmas due to logistical reasons.

4.3.3 Single analysis

Each trial used in the combined analysis was first analysed before inclusion in the combined analysis through the use of REML as shown in Table 4.2. In these analyses varieties were ranked according to oil yield in t/ha. A comparative randomised block design analysis is shown next to the REML analysis to show the amount of loss or gain obtained through using REML. The coefficient of variation (CV) was relatively high (18.80% in the case of Table 4.2) due to the fact that oil was brought into calculation when determining the oil yield in t/ha. Oil readings vary between cultivars and bring an additional variable to the calculation of oil yield. It is important to then look at the normal distribution of the trial and not only at the CV percentage to determine the accuracy of the trial.

4.3.4 Combined analyses

The combined analysis as shown in the results was used instead of the single analysis due to the fact that hybrid crosses occurred at more than one locality. This enables the breeder to better evaluate summarised data. The combined analysis is a product of an in-house development as was done under the leadership of Dr. Heinz Kaiser from Pannar Seed (Pty) Ltd. The specific software is Excel based. Calculation of the data is specifically done by taking the means of each variety's percentage of the mean of the trial. By doing this, the effects of higher and lower yielding trials affecting the rank of a particular variety are reduced.

Some of the trials at certain localities which were not usable are not shown. An additional column is shown where the relevant cross yield is expressed as a percentage of dry yield of the standard, PAN 7351. The yield of PAN 7351 is shown in each specific trial and calculated for that trial. This method was used as all trials could not be combined into one coherent trial. The relative yield percentage was calculated using the overall yield (o/a yield) which represents the dry yield or in other words the yield without factoring in the oil content. The formula used was

% relative yield = (variety o/a yield/PAN 7351 o/a yield) x 100

Table 4.2 Example of a single analysis on a single trial (60 entries) on one locality with R29 as the male tester using REML

PANNAR (Pty) Ltd.
2009/05/29 08:29
08H 737 A KROONSTAD LATE(2) A-LINES X R29
REML - Spatial Model - rows and col(AR). Vers. 2008.02.03

			REML		Randomised Blocks			TOTL	OIL	GRAN		STRI	Rep 1			Rep 2			Rep 3		
Rnk	Vno	Code Pedigree	Yld t/ha	Yld %Mn	Yld t/ha	Yld %	Rnk	YLD	CONT	H2O	STND	PED.	Rnk	Yld	Resid.	Rnk	Yld	Resid.	Rnk	Yld	Resid.
1	6	PAN 7031	1.37	144.73	1.35	142.64	1	3.04	44.38	5.89	66.67	2.67	9	1.24	-0.081	1	1.47	0.064	3	1.34	0.011
2	4	PAN 7050	1.33	140.76	1.31	138.96	2	2.95	44.51	6.35	79.17	4.00	4	1.36	0.079	13	1.07	-0.219	1	1.52	0.130
3	43	A86	1.27	134.35	1.24	130.67	5	3.03	40.86	6.02	77.50	4.67	1	1.41	0.092	7	1.15	-0.058	11	1.15	-0.108
4	10	A63	1.27	134.21	1.30	137.15	3	3.09	42.01	6.08	71.67	5.00	5	1.32	0.006	3	1.32	0.006	5	1.26	-0.026
5	47	A90	1.23	130.47	1.27	134.57	4	2.78	45.72	6.21	61.67	4.00	24	1.06	-0.210	4	1.29	0.071	2	1.47	0.179
6	40	A83	1.22	128.42	1.21	127.54	6	2.91	41.49	5.99	77.50	5.00	10	1.23	-0.050	2	1.32	0.088	16	1.07	-0.107
7	41	A84	1.18	124.69	1.17	123.77	8	2.78	42.21	6.00	68.33	5.00	6	1.31	0.106	18	1.03	-0.193	9	1.17	0.032
8	37	A80	1.15	121.32	1.10	115.72	16	2.64	41.48	6.21	59.17	4.33	41	0.92	-0.173	11	1.11	0.024	8	1.26	0.091
9	46	A89	1.14	120.20	1.10	116.03	15	2.68	40.97	6.08	74.17	5.00	35	0.98	-0.108	16	1.05	-0.024	4	1.26	0.110
10	17	A19	1.13	119.40	1.12	118.02	13	2.78	40.13	6.03	59.17	4.00	13	1.20	0.005	31	0.89	-0.175	6	1.26	0.192
11	56	A58(HO)	1.12	117.97	1.14	120.89	11	2.72	42.00	5.97	70.83	4.67	8	1.26	0.113	6	1.16	0.052	19	1.02	-0.115
12	34	A77	1.12	117.84	1.15	121.12	9	2.87	39.88	6.12	69.17	4.00	15	1.15	-0.002	8	1.12	-0.032	10	1.17	0.037
13	59	A61(HO)	1.10	116.62	1.15	121.09	10	2.73	41.93	6.07	66.67	4.33	18	1.13	-0.010	5	1.22	0.101	14	1.09	-0.065
14	55	A57(HO)	1.10	116.42	1.12	118.30	12	2.61	42.85	6.01	68.33	5.00	2	1.39	0.198	21	1.00	-0.040	26	0.97	-0.128
15	1	PAN 7033	1.10	115.88	1.18	125.20	7	2.93	40.50	5.90	69.17	4.67	12	1.21	0.023	12	1.09	-0.051	7	1.26	0.109
16	38	A81	1.09	115.02	1.10	116.55	14	2.73	40.34	5.98	54.17	5.00	3	1.37	0.246	29	0.94	-0.140	22	1.00	-0.022
17	44	A87	1.08	114.29	1.07	113.37	17	2.74	39.14	6.14	59.17	5.00	19	1.13	0.010	27	0.96	-0.061	12	1.14	0.093
18	2	PAN 7049	1.07	113.17	1.06	112.40	19	2.62	40.62	5.92	58.33	4.00	7	1.30	0.166	34	0.87	-0.137	21	1.01	-0.018
19	21	A69	1.05	111.25	1.03	109.29	21	2.80	36.87	5.83	80.83	5.33	14	1.15	0.053	26	0.96	-0.108	23	1.00	0.006
20	5	PAN 7351	1.05	110.83	0.98	103.79	27	2.45	40.15	5.92	62.50	5.00	22	1.09	0.089	35	0.87	-0.136	24	0.99	0.005
21	39	A82	1.04	109.36	1.05	110.74	20	2.69	38.99	5.90	76.67	5.00	29	1.03	-0.032	17	1.04	-0.010	15	1.07	0.048
22	58	A60(HO)	1.02	108.20	1.07	112.63	18	2.55	41.73	5.98	75.83	4.67	17	1.13	0.013	10	1.11	0.077	27	0.95	-0.007
23	35	A78	1.01	107.23	1.02	107.33	22	2.41	42.18	6.02	70.83	4.33	26	1.05	-0.007	9	1.12	0.115	32	0.88	-0.097
24	16	A67	1.00	106.07	0.98	103.07	29	2.41	40.45	6.02	65.83	4.00	21	1.10	0.050	20	1.02	0.078	40	0.81	-0.158
25	19	A73	0.99	104.16	0.98	103.97	26	2.74	35.83	5.87	80.00	6.00	30	1.03	-0.026	15	1.07	0.092	34	0.86	-0.119
26	18	A68	0.98	103.25	0.99	104.67	25	2.35	42.24	6.21	75.00	4.33	27	1.05	0.026	43	0.81	-0.154	13	1.11	0.177
27	45	A88	0.97	102.91	1.00	105.26	24	2.42	41.14	6.05	73.33	4.00	28	1.03	-0.033	30	0.91	-0.019	17	1.04	0.078
28	22	A70	0.97	102.80	0.98	103.40	28	2.69	36.38	5.96	75.83	5.00	31	1.02	0.011	24	0.97	0.040	28	0.95	-0.043
29	57	A59(HO)	0.97	102.27	0.93	98.77	31	2.28	41.02	5.94	57.50	4.33	25	1.05	0.053	28	0.94	0.018	41	0.81	-0.122
30	30	A75	0.96	100.99	1.00	105.53	23	2.69	37.09	5.83	65.00	4.67	33	1.00	-0.048	22	0.98	0.067	20	1.02	0.045
31	32	A51	0.94	99.79	0.95	100.43	30	2.50	37.95	5.95	83.33	4.00	23	1.07	0.098	25	0.96	0.006	38	0.82	-0.088
32	54	A43(HO)	0.94	99.51	0.90	94.68	36	2.21	40.52	6.42	63.33	6.00	37	0.97	-0.003	23	0.97	0.074	46	0.75	-0.131
33	7	PAN 7034	0.94	98.95	0.88	92.96	37	2.05	42.86	6.18	55.00	2.00	46	0.85	-0.038	46	0.76	-0.114	18	1.03	0.137
34	11	A64	0.93	98.46	0.90	95.53	34	2.43	37.17	5.77	72.50	5.33	44	0.88	0.000	37	0.86	-0.058	25	0.97	0.047
35	3	PAN 7048	0.92	96.73	0.87	92.14	38	2.13	40.96	6.04	63.33	4.67	11	1.21	0.257	54	0.63	-0.221	44	0.77	-0.086
36	15	A66	0.90	95.26	0.93	98.16	32	2.42	38.45	5.87	62.50	4.00	38	0.96	-0.007	33	0.88	-0.008	29	0.94	0.049
37	36	A79	0.89	94.32	0.91	96.04	33	2.19	41.51	6.11	81.67	5.33	16	1.13	0.173	19	1.03	0.094	58	0.57	-0.261
38	53	A42(HO)	0.88	92.51	0.87	92.03	39	2.15	40.48	6.28	74.17	6.00	34	0.98	0.040	47	0.72	-0.111	31	0.91	0.016
39	33	A76	0.87	92.29	0.90	94.77	35	2.30	39.04	5.73	72.50	5.00	32	1.00	0.040	41	0.83	-0.032	35	0.86	0.020
40	13	A18	0.86	90.90	0.86	91.37	40	2.34	36.88	6.05	71.67	5.67	42	0.92	0.030	40	0.84	0.037	37	0.84	-0.014
41	14	A65	0.84	88.29	0.76	80.00	47	2.04	37.11	5.52	64.17	4.00	50	0.77	-0.010	42	0.83	0.054	52	0.67	-0.129
42	27	A53	0.83	88.03	0.83	87.45	42	1.97	42.10	6.09	50.83	5.00	55	0.66	-0.203	14	1.07	0.195	45	0.76	-0.012
43	20	A74	0.83	87.78	0.83	87.76	41	2.13	39.05	6.00	75.00	5.00	20	1.10	0.203	53	0.67	-0.100	47	0.72	-0.066
44	52	A41(HO)	0.81	85.19	0.81	85.53	43	2.22	36.45	6.11	58.33	6.67	48	0.78	-0.051	50	0.71	-0.040	30	0.94	0.116
45	60	A62(HO)	0.80	84.02	0.80	84.95	45	2.11	38.01	5.97	38.33	4.00	56	0.66	-0.181	32	0.89	0.160	33	0.86	0.017
46	24	A72	0.78	82.71	0.76	80.60	46	1.93	39.51	5.92	60.00	5.00	39	0.93	0.054	48	0.72	0.000	54	0.64	-0.101
47	12	A17	0.76	80.51	0.74	77.82	51	2.02	36.43	6.06	74.17	6.00	49	0.78	0.071	49	0.71	-0.069	48	0.72	0.000
48	28	A54	0.75	78.79	0.81	85.41	44	2.04	39.68	5.88	54.17	5.00	51	0.77	-0.042	36	0.87	0.081	42	0.79	-0.010
49	25	A56	0.74	78.53	0.73	77.11	53	1.74	41.85	5.98	62.50	5.00	45	0.86	0.070	55	0.63	-0.070	49	0.70	-0.003
50	23	A71	0.74	78.38	0.74	78.53	49	2.01	37.00	5.65	42.50	4.00	53	0.70	-0.069	38	0.85	0.151	51	0.68	-0.116
51	8	AGS 5671	0.74	77.85	0.73	77.43	52	1.73	42.29	6.78	65.83	2.00	40	0.93	0.156	45	0.76	0.013	60	0.51	-0.190
52	9	AGS 8251	0.73	77.12	0.74	77.96	50	1.90	38.73	6.07	46.67	6.00	60	0.59	-0.083	44	0.77	0.021	36	0.85	0.072
53	49	A92(RM)	0.72	75.66	0.68	72.34	58	1.85	37.03	5.72	71.67	4.00	43	0.89	0.144	58	0.58	-0.069	55	0.58	-0.069
54	50	A93(RM)	0.70	73.65	0.75	79.74	48	2.01	37.55	5.76	80.83	4.00	59	0.61	-0.127	39	0.84	0.105	39	0.81	0.060
55	29	A55	0.69	73.41	0.70	74.16	55	1.97	35.62	6.14	45.83	4.00	58	0.63	-0.096	52	0.69	-0.044	43	0.78	0.097
56	42	A85	0.68	72.15	0.70	73.95	56	1.84	38.06	5.87	58.33	5.33	52	0.71	-0.040	51	0.71	0.082	50	0.69	-0.050
57	31	A50	0.66	69.23	0.69	73.27	57	1.94	35.82	5.73	77.50	9.00	47	0.79	0.045	56	0.63	-0.018	53	0.66	0.046
58	26	A52	0.65	68.71	0.71	74.85	54	1.87	37.90	6.00	79.17	5.33	36	0.97	0.232	57	0.62	-0.045	59	0.53	-0.134
59	51	A94(RM)	0.62	65.68	0.59	62.12	59	1.51	38.82	5.96	65.83	4.00	57	0.64	-0.011	59	0.54	-0.019	56	0.58	-0.032
60	48	A91(RM)	0.57	60.52	0.55	58.50	60	1.42	38.98	5.85	51.67	4.67	54	0.67	0.007	60	0.41	-0.099	57	0.58	0.043
		Means of all treatments	0.95	100.00	0.95	100.00		2.37	39.82	6.00	66.56	4.72									
		Means of standards																			

Plant Date	2008/12/02	%cv	18.7949	%cv	20.333
Harvest Date	2009/04/20	Mn sed	0.10268	sed	0.1111
Population	36000	% gain	8.18441		
Land					
Comments					

Rnk	Rank
Vno	Variety number
Yld t/ha	Yield in ton per hectare
Yld %Mn	Yield as a percentage of the mean
Yld %	Yield expressed as a percentage
TOTL YLD	Total grain yield in ton per hectare
OIL CONT	Oil content expressed as a percentage
GRAN H2O	Grain moisture expressed as a percentage
STND	Final adult plant stand expressed as a percentage
STRIPED	Striped nature of the seed expressed as a rating from one to nine (one being black seed and nine being almost white)
Resid	Residual value
%cv	Coefficient of variance
Mn sed	Mean standard error of difference
Sed	Standard error of difference

The ranking in each instance was calculated based on the overall yield. Not all entries in each of the trials are shown due to other entries being irrelevant for this study. In specific cases such as Table 4.3, only data for the Kroonstad locality were available and therefore results in the combined analysis will be identical to that of the single analysis.

4.3.5 Comparison with genetic distance

Comparisons were done to determine if genetic distance can be a predictor of yield or more specifically determine the best combinations to be made between the A-lines and the R-lines. The relative yield (expressed as a percentage of PAN 7351) was plotted against genetic distance as obtained from Appendix 2. A simple linear regression was done with each of the A-line x tester combinations to determine whether there was any meaningful correlation between genetic distance and yield as proposed by Cheres *et al*. (2000). A simple linear regression was done with each of the A-line x tester combinations to determine if there was a correlation between genetic distance and yield, genetic distance and oil content and yield and oil content. Agrobase 20 (Agrobase, 2000) was used to determine the linear regression. For all hybrid combinations, a graph was plotted for each R-line tester crossed to the relevant females to attempt to determine whether the specific female x male combination tended to be relatively higher yielding with a larger genetic distance as measured on the Rogers distance matrix (Appendix 2). The A-lines were plotted against each individual R-line with the A-line with the smallest genetic distance from the relevant R-line to the left of the graph and the largest genetic distance between the A-line and the relevant R-line to the right.

4.3.6 Combining ability and heritability

4.3.6.1 GCA and SCA effects

The GCA of lines and testers was calculated from a line x tester analysis through the use of the Agrobase 20 programme (Agrobase, 2000). The GCA estimates for the lines and testers for the relative yield characteristic were calculated to select the best line and tester for the use of hybrid breeding.

The SCA estimates for the crosses were calculated. This explains the minimum and maximum genetic gain of hybrids from certain lines by certain testers.

4.3.6.2 GCA:SCA ratio

The GCA:SCA mean square ratio was determined to evaluate the performance of the effects and to assess the relative importance of additive gene or non-additive gene effects. This ratio indicates whether a character is controlled by either additive or non-additive gene action (Singh and Chaudhary, 1979). The GCA:SCA ratio was determined 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. A high ratio suggests additive gene action while a low ratio suggests specific gene action such as non-additive gene action.

4.3.6.3 Heritability

Heritability (h^2) can be defined as the ratio of the genotypic variance to the phenotypic variance (Fehr, 1987). The genotypic variance is the variance by genetic differences between individuals. Heritability can be described in a broad-sense or a narrow-sense. Broad-sense heritability expresses the extent to which an individual's phenotypes are determined through its genotype. Broad-sense heritability can be estimated from the ratio of the total genetic variance to the phenotypic variance. Broad-sense heritability expresses the extent to which phenotypes are determined by the genes carried over from the parents. Narrow-sense heritabilities are estimated from the ratio of the additive portion of the genetic variance to the phenotypic variance. The heritabilities were determined from genetic components of the line x tester analysis through use of the Agrobase 20 programme (Agrobase, 2000).

4.4 Results

4.4.1 Combined analysis

In the following combined analysis trials, not all trials at the various localities were successful because some of the trials failed. This will be described for each locality. Varieties were grouped according to maturity or flowering date. The grouping on flowering date is to distinguish between earlier maturing lines in crosses which could be sent to the USA or Europe for further testing and later maturing lines in crosses which are more suited to Africa and South America. Hybrids maturing at the same time have the benefit that birds will not pick out the earliest material and influence the early entries in the trial negatively. Crosses should mature at the same time and damage (if any) should be spread evenly over trials. The number of entries differed for each combined group of trials. Trials were combined based firstly on the R-line parent used and then on maturity. The different localities were then combined into one summary. The trial planted at each locality had three replications. Trials

were not planted in the same year due to logistical problems with the number of trials needed to be done. Commercial hybrids were included in each set of trials and differed between the different combined analyses. The commercial hybrids were chosen based on their commercial relevance. Not all commercial hybrids are shown in the combined analyses. The yield value of the commercial standard PAN 7351 differed between the various combined analyses due to the fact that the combined trial analysis did not include certain localities. Yields differed between locations. Trials tended to differ from each other depending on where they were planted as well as different planting dates, seasonal influences, disease and pest pressures and flowering dates. This was one of the main reasons why commercial standards were always included to ensure that new varieties could be compared relative to the commercial standards. The CV was given under each locality name in each of the combined analyses.

4.4.1.1 R44(RM) male as tester on A-lines

Table 4.3 shows the very early group and Table 4.4 the early A-lines crossed with R44(RM). The very early trial was planted at Kroonstad, Villiers and Delmas localities. Only data from the Kroonstad locality were used due to problems with the other localities. No flowering data were available for this specific combined analysis. The early trial was planted at Kroonstad, Villiers and Delmas. Data from both Kroonstad and Delmas were available but not from Villiers. Flowering data were available for this trial. For all trials, the CV for each location is given directly under the locality name as part of the column heading, e.g. 19.00 for Kroonstad in Table 4.3. The rest of the values in the column are the yield for the specific location indicated in t/ha.

Table 4.3 Very early A-line trials using the R44(RM) tester

	No. of Trials	1		1	1	1	0	
		O/a	%	Kroonstad	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	19.00	t/ha	cont	days	PAN 7351
	Treatments mean	2.24	100.00	2.24	0.89	39.82		
1	PAN 7355	3.05	136.39	3.05	1.23	40.10		120.55
3	A10	2.84	126.75	2.84	1.34	47.10		112.25
9	PAN 7351	2.53	113.21	2.53	1.02	40.30		

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.5 shows the early-medium, Table 4.6 the medium and Table 4.7 the medium-late trials. The early-medium, medium and medium-late trials were planted at Kroonstad, Villiers and Delmas. In the early-medium trials the Villiers trial was unusable. In the medium trials all three localities were usable, while with the medium-late trials the Villiers trial was unusable. Flowering data were collected in both the early-medium and medium-late trials.

Table 4.4 Early A-line trials using the R44(RM) tester

	No. of Trials	2		1	1	2	2	1	
		O/a	%	Kroonstad	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	19.09	39.89	t/ha	cont	days	PAN 7351
	Treatments mean	2.09	100.00	2.25	1.93	0.83	39.60	65.38	
1	PAN 7355	3.04	145.74	3.04	3.01	1.22	40.25	69.00	106.67
5	PAN 7351	2.85	136.62	2.76	2.90	1.18	41.80	67.00	
8	A38	2.42	116.07	2.63	2.22	0.90	37.00	68.00	84.91
10	A34	2.38	113.99	2.74	2.05	0.99	41.20	67.00	83.51
15	A37	2.28	109.06	2.39	2.15	0.90	39.75	66.00	80.00
30	A32	2.10	100.39	2.20	1.98	0.82	39.25	69.00	73.68
34	A5	1.97	94.40	2.66	1.36	0.77	38.80	66.00	69.12
44	A36	1.70	81.69	2.14	1.31	0.73	42.25	62.00	59.65

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.5 Early-medium A-line trials using the R44(RM) tester

	No. of Trials	2		1	1	2	2	1	
		O/a	%	Kroonstad	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	19.39	34.21	t/ha	cont	days	PAN 7351
	Treatments mean	2.22	100.00	2.20	2.23	0.88	39.51	66.57	
3	A19	2.97	133.94	3.04	2.90	1.23	41.35	68.00	100.68
4	PAN 7351	2.95	133.33	2.78	3.13	1.18	39.80	68.00	
5	PAN 7355	2.89	130.59	2.79	3.00	1.16	40.20	69.00	97.97
6	A16	2.84	128.26	2.91	2.77	1.24	43.80	69.00	96.27
23	A31	2.27	102.28	2.26	2.28	0.84	37.20	68.00	76.95
31	A28	2.17	97.96	2.16	2.19	0.82	37.85	67.00	73.56
36	A6	2.06	93.11	2.01	2.12	0.81	39.15	68.00	69.83
38	A7	2.01	90.71	2.26	1.76	0.82	40.50	57.00	68.14
48	A29	1.81	81.81	1.86	1.77	0.73	40.25	67.00	61.36

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.6 Medium A-line trials using the R44(RM) tester

	No. of Trials	3		1	1	1	3	3	0	
		O/a	%	Kroonstad	Villiers	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	20.69	29.24	37.74	t/ha	cont	days	PAN 7351
	Treatments mean	1.86	100.00	2.19	1.22	2.17	0.72	38.52		
3	PAN 7355	2.39	128.50	2.87	1.84	2.24	0.89	38.17		105.75
4	A15	2.32	124.72	2.51	1.71	2.59	0.94	41.30		102.65
6	PAN 7351	2.26	121.35	2.67	1.74	2.16	0.86	38.83		
34	A30	1.63	87.46	2.12	1.03	1.77	0.63	38.13		72.12
36	A8	1.56	83.92	1.79	1.15	1.64	0.62	40.20		69.03

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.7 Medium-late A-line trials using the R44(RM) tester

	No. of Trials	2		1	1	2	2	1	
		O/a	%	Kroonstad	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	18.32	32.04	t/ha	cont	days	PAN 7351
	Treatments mean	2.26	100.00	2.28	2.24	0.91	40.42	67.54	
3	PAN 7355	3.04	134.81	2.89	3.19	1.26	41.35	68.00	116.03
4	A25	2.76	122.12	2.55	2.95	1.21	43.80	68.00	105.34
5	PAN 7351	2.62	115.99	2.54	2.69	1.10	42.00	67.00	
7	A22	2.56	113.31	2.32	2.79	1.07	41.75	69.00	97.71
16	A26	2.40	106.46	2.26	2.54	1.02	42.60	64.00	91.60

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.8 includes the late and Table 4.9 the very late trials. The late and the very late trials were planted at Kroonstad, Villiers and Delmas. In the late trial the Kroonstad and Delmas trials were usable. In the very late trial only the Kroonstad trial was usable. Flowering data were collected at the late trial only.

Table 4.8 Late A-line trials using the R44(RM) tester

	No. of Trials	2		1	1	2	2	1	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 12.37	Delmas 21.01	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.10	100.00	2.09	2.11	0.79	37.64	68.41	
2	PAN 7355	2.71	129.19	2.75	2.67	1.01	37.10	70.00	113.39
9	PAN 7351	2.39	113.67	2.50	2.28	0.93	39.15	68.00	
37	A9	1.92	91.40	1.92	1.92	0.70	36.65	68.00	80.33

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.9 Very late A-line trials using the R44(RM) tester

	No. of Trials	1		1	1	1	0	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 17.00	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.05	100.00	2.05	0.83	40.19		
2	PAN 7355	2.82	137.58	2.82	1.13	40.00		116.05
7	PAN 7351	2.43	118.69	2.43	0.96	39.30		
12	A23	2.26	110.38	2.26	0.84	37.10		93.00
15	A24	2.18	106.51	2.18	0.84	38.30		89.71
16	A17	2.16	105.53	2.16	0.83	38.40		88.89
40	A18	1.84	89.80	1.84	0.74	40.50		75.72

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Combinations made with the R-line tester R44(RM) and with the A-lines A36, A29, A7, A8, A5, A6, A30, A28, A32, A18, A31, A37, A9, A34, A38, A17, A24, A26, A23, A16 and A22 performed poorer than the commercial check PAN 7351 when looking at overall yield. The A-lines A19, A15, A25 and A10 performed better than the commercial check PAN 7351 when looking at overall yield. Flowering dates were captured at the Delmas locality and ranged from 57 days for the USA line A7 in combination with R44(RM) to 70 days for PAN 7355. Most of the South African A-line germplasm combinations with R44(RM) expressed a flowering date of 67-69 days. The Pannar Seed (Pty) Ltd germplasm is selected to be longer maturing to ensure maximum use of the growing season. The oil content of the A-line combinations with R44(RM) ranged from 36.65% for A9 in combination with R44(RM) to 47.10% for A10 in combination with R44(RM). The average oil content for all combinations

was 40.17%. The genetic distance varied from 0.552 between A12 and R44(RM) to 0.777 between A9 and R44(RM) (Table 4.10).

Table 4.10 Genetic distance and relative yield for each A-line combination with R44(RM)

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.5515	No data	No data
A25	0.5551	105.34	43.80
A28	0.5599	73.56	37.85
A24	0.5691	89.71	38.30
A26	0.5691	91.60	42.60
A32	0.5784	73.68	39.25
A29	0.5958	61.36	40.25
A34	0.5987	83.51	41.20
A6	0.6071	69.83	39.15
A35	0.6154	No data	No data
A38	0.6265	84.91	37.00
A13	0.6407	No data	No data
A5	0.6408	69.12	38.80
A4	0.6466	No data	No data
A39	0.6484	No data	No data
A7	0.6691	68.14	40.50
A37	0.6712	80.00	39.75
A19	0.6777	102.11	41.35
A10	0.6798	112.25	47.10
A42(HO)	0.6836	No data	No data
A30	0.6895	72.12	38.13
A17	0.6931	88.89	38.40
A18	0.6931	75.72	40.50
A43(HO)	0.6968	No data	No data
A15	0.6980	102.66	41.30
A31	0.6987	76.95	37.20
A41(HO)	0.7025	No data	No data
A22	0.7055	97.71	41.75
A36	0.7173	59.65	42.25
A16	0.7333	96.27	43.80
A8	0.7543	69.03	40.20
A23	0.7630	93.00	37.10
A9	0.7771	80.33	36.65

As shown in Figure 4.1, relative yield from hybrids obtained from crosses made between R44(RM) and 25 A-line females were plotted against the relevant genetic distances. Certain crosses were not realised and zero values were found. The A-lines were plotted with the A-line found to have the smallest genetic distance from R44(RM) to the left of the graph and the A-line with the largest genetic distance to the right. The relative yield (compared with PAN 7351) is found on the Y-axis as was obtained from the combined analysis. The A-lines in order from smallest to largest genetic distance from R44(RM) are shown in Table 4.10. A simple linear regression was done to determine whether genetic distance was significantly correlated with yield. The R^2 value for this set of data was found to be 0.0013 and the p-value (non-directional) 0.8652. A simple linear regression was done to determine whether genetic distance was correlated with oil content. The R^2 value for this set of data was found to be 0.0055 and the p-value 0.7256. A simple linear regression was done to determine if there was a correlation between yield and oil content. The R^2 value for this set of data was found to be 0.2323 and the p-value 0.0147. This translates to the fact that the fit of the line was not perfect and translates that no fit was possible at all. Only in the case of yield versus oil content for the group of A-lines crossed to R44(RM) was $p<0.05$ and a meaningful correlation found. The significance of the correlation continues to be the most important factor as the correlation itself can change depending on the size of the dataset.

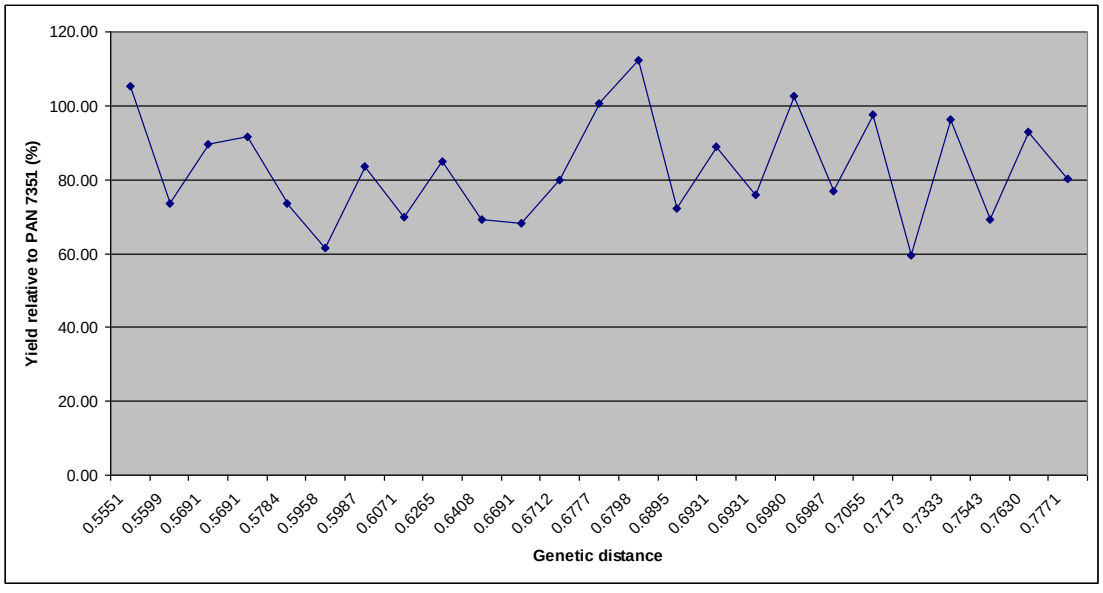

Figure 4.1 Relative yield versus genetic distance of hybrids obtained from crosses of R44(RM) as a male tester with 25 female A-lines.

4.4.1.2 R34 male as tester on A-lines

Table 4.11 shows the set of early A-lines used with R34 as tester at Standerton, Kroonstad and Lichtenburg. Only the Standerton and Lichtenburg trials were used and no flowering data could be recorded.

Table 4.11 Early A-line trials using the R34 tester

	No. of Trials	2		1	1	2	2	0	
Rnk	Hybrid	O/a Yield	% Yield	Standerton 18.82	Lichtenburg 32.45	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.25	100	2.51	1.99	0.90	37.80		
1	A38	3.00	137.48	2.51	3.49	1.10	38.10		145.63
3	PAN 7033	2.73	120.63	3.15	2.30	1.02	37.90		132.52
6	A19	2.69	118.26	3.21	2.17	1.08	38.30		130.58
21	A37	2.43	108.69	2.61	2.25	0.89	37.70		117.96
22	A34	2.42	107.67	2.69	2.16	0.91	37.20		117.48
39	A10	2.28	101.38	2.56	2.00	0.89	37.80		110.68
57	A5	2.15	93.18	2.89	1.42	0.93	38.50		104.37
61	PAN 7351	2.06	92.43	2.11	2.00	0.79	38.20		
70	A35	2.04	89.34	2.53	1.55	0.92	38.80		99.03

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.12 includes the set of medium A-lines crossed with R34 as tester as used at Standerton, Kroonstad and Lichtenburg. Trials at the Standerton and Kroonstad localities were used. No flowering data were obtained.

Table 4.13 includes the late A-lines and Table 4.14 the high oleic A-lines. The late A-line trials included all trials planted at Standerton, Kroonstad and Lichtenburg. The high oleic trials were successfully planted on Standerton, Kroonstad and Lichtenburg. No flowering data were recorded.

Table 4.12 Medium A-line trials using the R34 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Standerton 22.33	1 Kroonstad 20.19	2 Oil t/ha	2 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.53	100	2.61	2.45	0.90	37.20		
1	A25	3.25	128.12	3.72	2.78	1.07	38.20		163.32
2	PAN 7033	3.13	124.10	3.15	3.12	0.99	36.10		157.29
4	A6	3.08	121.59	3.48	2.69	0.97	36.90		154.77
32	A13	2.65	105.10	2.65	2.66	0.85	36.50		133.17
51	A23	2.47	97.49	2.73	2.22	0.84	37.50		124.12
87	PAN 7351	1.99	79.26	1.72	2.27	0.67	34.40		
88	A22	1.96	77.65	2.03	1.90	0.71	35.70		98.49

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.13 Late A-line trials using the R34 tester

Rnk	Hybrid	3 O/a Yield	% Yield	1 Standerton 19.73	1 Kroonstad 23.54	1 Lichtenburg 29.24	3 Oil t/ha	3 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.34	100	2.86	2.24	1.92	0.90	38.80		
4	A24	2.77	118.27	3.33	2.83	2.15	0.99	35.60		130.05
9	PAN 7033	2.67	112.59	3.55	2.49	1.96	1.05	39.00		125.35
20	A39	2.44	105.98	2.51	2.80	2.01	0.89	36.40		114.55
32	A15	2.35	98.94	3.22	2.06	1.76	0.97	40.40		110.33
46	A18	2.26	95.40	3.08	2.05	1.66	0.83	36.40		106.10
49	A16	2.19	94.13	2.50	2.42	1.66	0.94	42.50		102.82
51	PAN 7351	2.13	91.23	2.62	2.07	1.72	0.83	38.40		

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.14 High oleic A-line trials using the R34 tester

Rnk	Hybrid	3 O/a Yield	% Yield	1 Standerton 14.57	1 Kroonstad 12.46	1 Lichtenburg 25.77	3 Oil t/ha	3 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.41	100	2.86	2.44	1.94	0.90	36.80		
3	PAN 7033	2.79	118.13	3.02	2.50	2.83	1.06	38.10		117.72
12	A12	2.47	102.90	2.71	2.63	2.06	0.90	36.30		104.22
19	PAN 7351	2.37	98.23	2.69	2.50	1.90	0.88	36.90		

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The combinations made with the R-line tester R34 which included the A-lines A25, A6, A38, A13, A19, A24, A23, A37, A34, A39, A10, A15, A18, A5, A12 and A16 performed better than the commercial check PAN 7351 for overall yield. A35 and A22 in combination with R34 performed worse than the commercial check PAN 7351 for overall yield. Unfortunately the other 15 A-line crosses with R34 did not realise and yield data could not be determined. The oil content of the A-line combinations with R34 ranged from 35.60% for A24 to 42.50% for A16. The average oil content for all combinations was 37.71%. Combinations of A-lines with the male R-line R34 tend not to have high oil content due to the fact that the R-line lowered the oil content of combinations. The genetic distance varied from 0.482 between A12 and R34 to 0.701 between A23 and R34 (Table 4.15).

As shown in Figure 4.2, relative yield from hybrids obtained from crosses made between R34 and 18 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R34 are shown in Table 4.15. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0014; p=0.8822) and oil content (R^2=0.0971; p=0.2070) as well as between yield and oil content (R^2=0.0214; p=0.5628). The R^2 values observed in all three cases translated to the fact that no line could be fit. In none of the cases for the group of A-lines crossed to R34 was $p<0.05$ and no resulting meaningful correlations were found.

4.4.1.3 R9 male as tester on A-lines

Table 4.16 includes the early and Table 4.17 the early-medium A-lines crossed with R9 as tester. In the early group, the trials were planted at Kroonstad, Villiers and Delmas but only the data from Kroonstad could be used. In the early medium group, the trials were planted at Kroonstad, Villiers and Delmas. Only the Kroonstad locality was used. No flowering data could be collected.

Table 4.15 Genetic distance and relative yield for each A-line combination with R34

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.4815	104.22	36.30
A28	0.4860	No data	No data
A10	0.5197	110.68	37.80
A42(HO)	0.5370	No data	No data
A26	0.5612	No data	No data
A41(HO)	0.5741	No data	No data
A6	0.5743	154.77	36.90
A43(HO)	0.5755	No data	No data
A24	0.5794	130.05	35.60
A19	0.5860	130.58	38.30
A30	0.5885	No data	No data
A32	0.5885	No data	No data
A5	0.5901	104.37	38.50
A25	0.5936	163.32	38.20
A8	0.5976	No data	No data
A22	0.6067	98.49	35.70
A38	0.6067	145.63	38.10
A39	0.6082	114.55	36.40
A34	0.6086	117.48	37.20
A29	0.6166	No data	No data
A15	0.6173	110.33	40.40
A9	0.6200	No data	No data
A7	0.6247	No data	No data
A31	0.6248	No data	No data
A17	0.6271	No data	No data
A18	0.6271	106.10	36.40
A4	0.6304	No data	No data
A36	0.6339	No data	No data
A37	0.6538	117.96	37.70
A35	0.6612	99.03	38.80
A13	0.6679	133.17	36.50
A16	0.6679	102.82	42.50
A23	0.7007	124.12	37.50

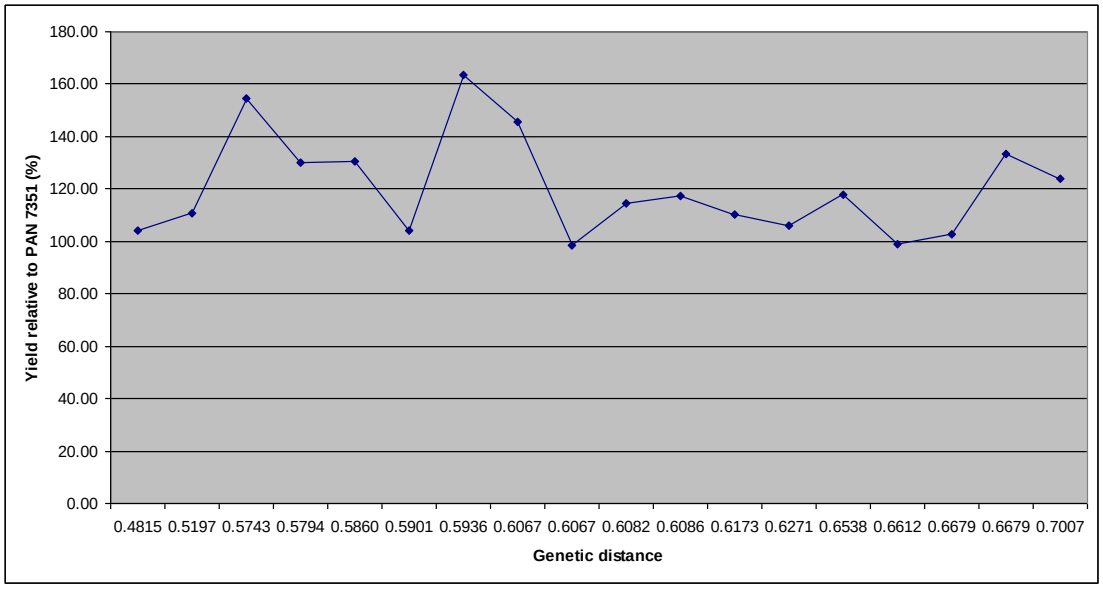

Figure 4.2 Relative yield versus genetic distance of hybrids obtained from R34 as a male tester with 18 female A-lines.

Table 4.16 Early A-line trials using the R9 tester

Rnk	No. of Trials / Hybrid	1 O/a Yield	% Yield	1 Kroonstad 20.52	1 Oil t/ha	1 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.96	100.00	1.96	0.75	38.18		
1	A34	3.09	157.72	3.09	1.19	38.30		137.33
3	A5	2.64	134.44	2.64	1.03	39.10		117.33
7	PAN 7355	2.43	123.73	2.43	0.93	38.10		108.00
12	PAN 7351	2.25	114.62	2.25	0.79	35.30		
14	A37	2.21	112.80	2.21	0.89	40.40		98.22
16	A4	2.13	108.78	2.13	0.78	36.50		94.67
19	A19	2.06	104.80	2.06	0.81	39.30		91.56
30	A32	1.85	94.07	1.85	0.69	37.50		82.22
49	A38	0.95	48.27	0.95	0.33	35.20		42.22

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.17 Early-medium A-line trials using the R9 tester

	No. of Trials	1		1	1	1	0	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 21.31	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.94	100.00	1.94	0.75	37.83		
2	PAN 7355	2.56	132.15	2.56	1.01	39.50		100.39
3	PAN 7351	2.55	131.61	2.55	0.97	38.10		
17	A16	2.12	109.54	2.12	0.96	45.00		83.14
26	A28	2.02	104.41	2.02	0.78	38.30		79.22
41	A36	1.82	93.83	1.82	0.68	37.40		71.37
50	A31	1.54	79.50	1.54	0.57	37.10		60.39
53	A29	1.42	73.35	1.42	0.56	39.10		55.69

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.18 shows the medium and Table 4.19 the medium-late A-lines. The localities used for both the trials were Kroonstad, Villiers and Delmas. Only the data from the Delmas location had to be discarded. No flowering data could be collected.

Table 4.18 Medium A-line trials using the R9 tester

	No. of Trials	2		1	1	2	2	0	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 22.57	Villiers 20.76	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.84	100.00	2.02	1.65	0.70	37.95		
2	A15	2.56	139.54	2.76	2.36	1.03	40.40		109.87
3	PAN 7355	2.55	138.97	2.73	2.36	0.95	37.10		109.44
6	PAN 7351	2.33	127.18	2.78	1.93	0.86	36.25		
7	A13	2.28	124.27	2.32	2.21	0.89	39.10		97.85
18	A8	2.01	109.64	2.25	1.79	0.78	38.35		86.27

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.19 Medium-late A-line trials using the R9 tester

	No. of Trials	2		1	1	2	2	0	
		O/a	%	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	23.81	22.10	t/ha	cont	days	PAN 7351
	Treatments mean	1.84	100.00	1.99	1.70	0.70	38.06		
3	PAN 7355	2.44	132.17	2.47	2.38	0.89	36.65		109.42
4	A25	2.40	129.96	2.67	2.14	1.00	41.75		107.62
7	A22	2.27	123.06	2.02	2.46	0.88	39.40		101.79
9	PAN 7351	2.23	120.99	2.10	2.32	0.82	37.10		
15	A26	2.03	110.15	1.88	2.14	0.79	39.15		91.03
34	A39	1.75	94.88	1.81	1.68	0.63	36.05		78.48

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.20 includes the late and Table 4.21 the very late A-line trials. The late A-line trial was evaluated at Kroonstad, Villiers and Delmas although the trial at Delmas had to be discarded. The very late trial was planted at the Kroonstad, Villiers and Delmas localities, but the Villiers locality had to be discarded. Flowering data could be collected at the very late A-line trial.

Table 4.20 Late A-line trials using the R9 tester

	No. of Trials	2		1	1	2	2	0	
		O/a	%	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	21.22	20.49	t/ha	cont	days	PAN 7351
	Treatments mean	1.77	100.00	1.90	1.65	0.67	37.64		
1	PAN 7355	2.57	144.78	2.65	2.47	0.94	36.65		120.66
8	PAN 7351	2.13	120.00	2.30	1.96	0.81	37.70		
13	A17	1.98	111.80	2.25	1.73	0.84	42.30		92.96
45	A9	1.50	84.88	1.76	1.27	0.56	36.95		70.42

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.21 Very late A-line trials using the R9 tester

	No. of Trials	2		1	1	2	2	1	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 24.06	Delmas 20.88	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.07	100.00	1.99	2.15	0.85	40.97	70.17	
1	PAN 7355	2.91	140.72	2.99	2.82	1.16	40.15	69.00	119.26
6	PAN 7351	2.44	117.86	2.16	2.73	1.02	41.25	67.00	
11	A23	2.32	111.95	2.16	2.48	0.97	41.80	66.00	95.08
30	A24	1.90	91.73	1.75	2.05	0.77	40.40	69.00	77.87
38	A18	1.66	80.43	1.51	1.82	0.68	40.50	70.00	68.03

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The A-lines A38, A29, A31, A18, A9, A36, A24, A39, A28, A32, A16, A8, A26, A19, A17, A4, A23, A13 and A37 in combination with the tester R9 performed worse than the commercial check PAN 7351 when looking at overall yield. The A-lines A22, A25, A15, A5 and A34 in combination with the R-line R9 performed better than the commercial check PAN 7351 on overall yield. The oil content of the A-line combinations with R9 ranged from 35.20% for A38 to 45.00% for A16. The average oil content for all combinations was 39.14%. The genetic distance varied from 0.514 between A12 and R9 to 0.736 between A9 and R9 (Table 4.22).

As shown in Figure 4.3, relative yield from hybrids obtained from crosses made between R9 and 24 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R9 are shown in Table 4.22. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0109; p=0.1689) and oil content (R^2=0.005; p=0.9174) as well as between yield and oil content (R^2=0.1122; p=0.1096). The R^2 values observed in all three cases translated to the fact that no line could be fit. In none of the cases for the group of A-lines crossed to R9 was $p<0.05$ and no resulting meaningful correlations were found.

Table 4.22 Genetic distance and relative yield for each A-line combination with R9

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.5138	No data	No data
A24	0.5228	77.87	40.40
A5	0.5396	117.33	39.10
A13	0.5691	97.85	39.10
A28	0.5944	79.22	38.30
A10	0.5953	No data	No data
A39	0.6006	78.48	36.05
A32	0.6247	82.22	37.50
A15	0.6248	109.87	40.40
A31	0.6271	60.39	37.10
A26	0.6314	91.03	39.15
A16	0.6339	83.14	45.00
A29	0.6347	55.69	39.10
A34	0.6365	137.33	38.30
A41(HO)	0.6390	No data	No data
A35	0.6432	No data	No data
A6	0.6490	No data	No data
A19	0.6500	91.56	39.30
A18	0.6528	68.03	40.50
A17	0.6528	92.96	42.30
A8	0.6549	86.27	38.35
A43(HO)	0.6609	No data	No data
A30	0.6642	No data	No data
A38	0.6660	42.22	35.20
A4	0.6674	94.67	36.50
A22	0.6734	101.79	39.40
A42(HO)	0.6767	No data	No data
A23	0.6829	95.08	41.80
A36	0.6920	71.37	37.40
A25	0.6935	107.62	41.75
A7	0.6956	No data	No data
A37	0.7104	98.22	40.40
A9	0.7358	70.42	36.95

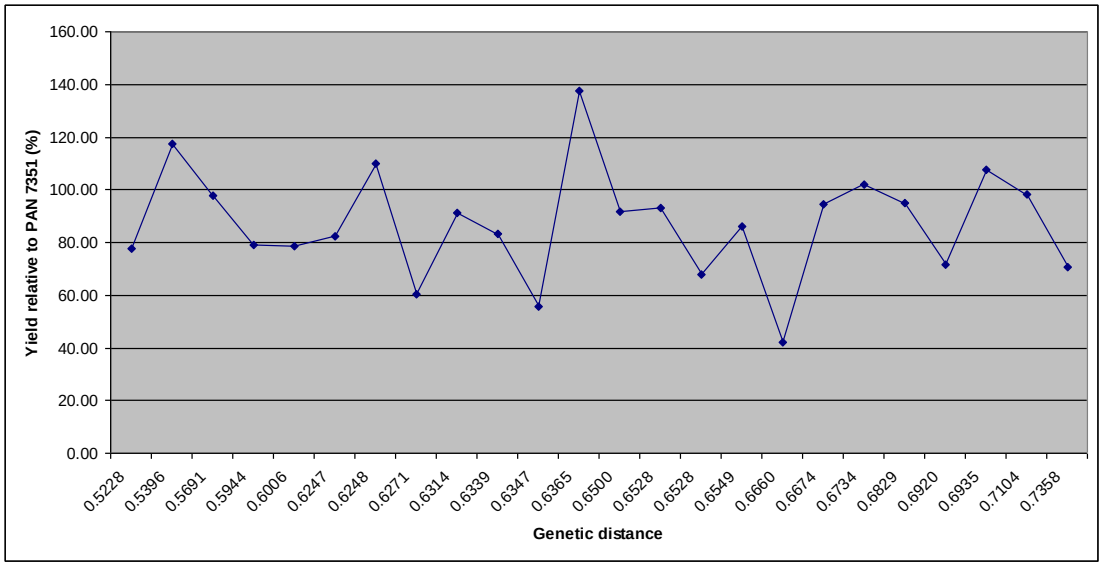

Figure 4.3 Relative yield versus genetic distance of hybrids obtained from crosses with R9 as a male tester crossed to 25 female A-lines.

4.4.1.4 R13 male as tester on A-lines

Table 4.23 includes the early A-lines crossed with R13 as tester planted at Kroonstad and Standerton. Both the localities were successfully harvested. No flowering data were recorded.

Table 4.24 shows the medium A-lines evaluated at Kroonstad as well as Standerton. Trials from these localities were both successful. No flowering data could be obtained. It is interesting to note that A4 and A6 performed very similarly. This is to be expected seeing that these lines are closely related.

Table 4.23 Early A-line trials using the R13 tester

	No. of Trials	1		1	1	2	2	0	
		O/a	%	Kroonstad	Standerton	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	16.69	21.17	t/ha	cont	days	PAN 7351
	Treatments mean	3.14	100	3.48	2.79	1.33	42.57		
2	PAN 7351	3.78	120.28	4.12	3.42	1.56	41.70		
5	PAN 7355	3.62	115.32	4.09	3.16	1.45	40.20		95.77
18	A10	3.16	100.65	3.56	2.77	1.33	42.15		83.60
41	A26	2.50	79.50	2.74	2.24	1.08	43.70		66.14

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.25 includes the late A-lines that were planted at Kroonstad and Standerton. Both of the localities were successful. No flowering data were collected.

Table 4.24 Medium A-line trials using the R13 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 17.66	1 Standerton 23.1	2 Oil t/ha	2 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	3.10	100	3.28	2.92	1.39	44.72		
1	PAN 7351	3.85	124.17	3.92	3.77	1.68	43.55		
3	PAN 7355	3.64	117.34	3.76	3.51	1.57	43.05		94.55
11	A34	3.40	109.55	3.56	3.23	1.50	44.25		88.31
16	A4	3.28	105.71	3.37	3.18	1.49	45.55		85.19
17	A6	3.27	105.35	3.39	3.14	1.50	46.05		84.94
33	A23	3.06	98.62	2.81	3.26	1.37	45.25		79.48
34	A13	3.04	97.84	3.04	3.01	1.32	43.60		78.96
41	A25	2.90	93.51	3.38	2.45	1.37	46.70		75.32
55	A22	2.41	77.61	2.53	2.29	1.13	47.05		62.60

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.25 Late A-line trials using the R13 tester

Rnk	Hybrid	1 O/a Yield	% Yield	1 Kroonstad 12.43	1 Standerton 14.12	2 Oil t/ha	2 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	3.16	100	3.63	2.69	1.39	44.65		
2	PAN 7355	3.93	124.37	4.02	3.71	1.61	41.70		102.08
4	PAN 7351	3.85	121.91	4.20	3.45	1.64	43.05		
11	A15	3.56	112.74	4.00	3.10	1.63	46.20		92.47
15	A17	3.45	109.07	3.38	3.37	1.43	42.50		89.61
22	A16	3.31	104.66	3.67	2.91	1.56	47.85		85.97
23	A39	3.25	102.71	3.64	2.83	1.45	45.20		84.42
31	A18	3.13	99.19	3.78	2.54	1.40	45.30		81.30

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The A-lines A15, A17, A34, A16, A4, A6, A39, A10, A18, A23, A13, A25, A26 and A22 were crossed to the R13 R-line tester and all these combinations performed worse than the commercial check PAN 7351 when looking at overall yield. Nineteen A-lines did not produce usable data. The oil content of the A-line combinations with R13 ranged from 42.15% for A10 to 47.85% for A16. The average oil content for all combinations was 45.10%. The crosses derived from crossing the A-lines to the R13 R-line have high oil content and this R-line seems to enhance oil content in combinations. The genetic distance varied from 0.507 between A13 and R13 to 0.773 between A23 and R13 (Table 4.26).

As shown in Figure 4.4, relative yield from hybrids obtained from crosses made between R13 and 14 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R13 are shown in Table 4.26. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0000; p=0.9832) and oil content (R^2=0.1400; p=0.1876) as well as between yield and oil content (R^2=0.0187; p=0.6414). The R^2 values observed in all three cases translated to the fact that no line could be fit. In none of the cases for the group of A-lines crossed to R13 was $p<0.05$ and no resulting meaningful correlations were found.

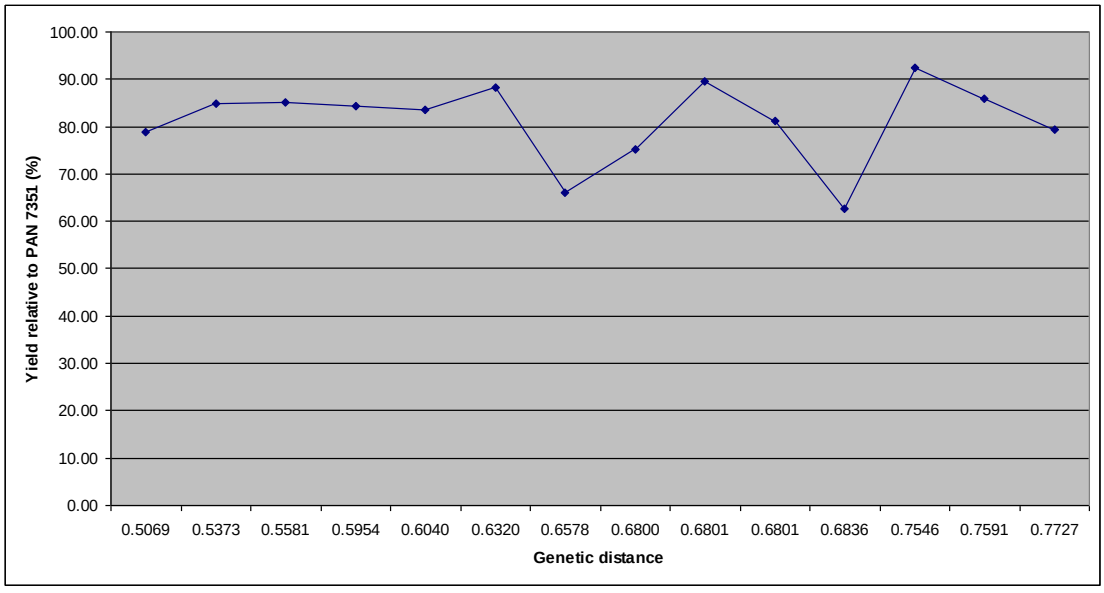

Figure 4.4 Relative yield versus genetic distance of hybrids obtained from crosses with R13 as a male tester crossed to 14 female A-lines.

Table 4.26 Genetic distance and relative yield for each A-line combination with R13

A-line	Genetic distance	% Relative yield	% Oil content
A13	0.5069	78.96	43.60
A12	0.5263	No data	No data
A6	0.5373	84.94	46.05
A4	0.5581	85.19	45.55
A32	0.5635	No data	No data
A41(HO)	0.5673	No data	No data
A24	0.5729	No data	No data
A29	0.5854	No data	No data
A39	0.5954	84.42	45.20
A38	0.5987	No data	No data
A10	0.6040	83.60	42.15
A28	0.6082	No data	No data
A5	0.6173	No data	No data
A37	0.6273	No data	No data
A34	0.6320	88.31	44.25
A7	0.6487	No data	No data
A26	0.6578	66.14	43.70
A35	0.6578	No data	No data
A42(HO)	0.6635	No data	No data
A19	0.6648	No data	No data
A25	0.6800	75.32	46.70
A17	0.6801	89.61	42.50
A18	0.6801	81.30	45.30
A22	0.6836	62.60	47.05
A36	0.6862	No data	No data
A30	0.6956	No data	No data
A43(HO)	0.6961	No data	No data
A31	0.7145	No data	No data
A9	0.7402	No data	No data
A8	0.7428	No data	No data
A15	0.7546	92.47	46.20
A16	0.7591	85.97	47.85
A23	0.7727	79.48	45.25

4.4.1.5 R15 male as tester on A-lines

Table 4.27 includes the early and Table 4.28 the early-medium A-line crosses with R15 as tester. The early trials were planted at Kroonstad, Delmas and Lichtenburg. Both the Kroonstad and Lichtenburg localities yielded successful trials. Flowering data were collected at the early trials. The early-medium trials were planted at Kroonstad, Delmas and Lichtenburg. Trials were successful at Kroonstad and Delmas and the flowering data were collected. All trials at the Lichtenburg locality failed due to drought.

Table 4.27 Early A-line trials using the R15 tester

	No. of Trials	2		1	1	2	2	1	
		O/a	%	Kroonstad	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	24.79	21.29	t/ha	cont	days	PAN 7351
	Treatments mean	1.74	100	2.10	1.38	0.70	38.90	58.10	
2	A37	2.16	123.17	2.67	1.65	0.90	39.30	59.00	111.34
6	A5	2.10	118.79	2.67	1.53	0.88	40.60	58.00	108.25
9	A35	2.02	114.55	2.55	1.49	0.73	37.60	57.00	104.12
12	PAN 7033	1.94	111.92	2.30	1.58	0.83	38.20	67.00	100.00
14	PAN 7351	1.94	110.59	2.40	1.48	0.82	39.30	64.00	
20	A34	1.95	108.93	2.60	1.30	0.77	37.90	63.00	100.52
24	A19	1.78	106.18	1.84	1.73	0.74	41.10	58.00	91.75
35	A38	1.70	96.75	2.10	1.29	0.57	36.30	60.00	87.63
36	A10	1.67	96.48	1.97	1.37	0.63	41.70	55.00	86.08

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.28 Early-medium A-line trials using the R15 tester

		2		1	1	2	2	1	
		O/a	%	Kroonstad	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	23.75	23.29	t/ha	cont	days	PAN 7351
	Treatments mean	1.99	100	2.58	1.40	0.80	38.60	59.40	
3	PAN 7033	2.46	124.81	3.11	1.81	1.03	40.00	67.00	102.07
9	PAN 7351	2.41	117.28	3.35	1.47	0.92	37.90	60.00	
31	A6	1.93	98.88	2.37	1.48	0.67	36.90	60.00	80.08
47	A4	1.83	88.71	2.56	1.10	0.63	36.30	60.00	75.93

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.29 shows the data for the medium trials which were planted at Kroonstad, Lichtenburg and Delmas. Trials at Kroonstad and Delmas were successful. Flowering data were collected. Table 4.30 contains the medium-late A-lines and was planted at Kroonstad, Lichtenburg and Delmas. The trials at Kroonstad and Delmas were successful. Flowering data were collected.

Table 4.29 Medium A-line trials using the R15 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 24.67	1 Delmas 27.19	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.93	100	2.56	1.31	0.70	38.20	60.90	
14	PAN 7351	2.11	107.02	2.93	1.30	0.77	38.20	60.00	
20	A25	2.04	103.41	2.82	1.26	0.77	41.10	60.00	96.68
26	A22	1.94	101.85	2.49	1.39	0.64	37.10	63.00	91.94
48	PAN 7033	1.83	92.23	2.57	1.10	0.75	38.30	67.00	86.73

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.30 Medium-late A-line trials using the R15 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 20.86	1 Delmas 27.89	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.02	100	2.67	1.37	0.80	39.10	61.50	
7	PAN 7351	2.31	114.94	3.03	1.60	0.90	38.70	64.00	
10	A39	2.17	113.13	2.57	1.78	0.77	38.90	63.00	93.94
14	PAN 7033	2.24	109.76	3.01	1.46	0.98	39.20	67.00	96.97
15	A16	2.21	109.71	2.89	1.52	0.84	41.30	63.00	95.67
17	A24	2.21	109.40	2.92	1.50	0.72	37.50	60.00	95.67
26	A23	2.02	103.56	2.47	1.57	0.88	38.80	58.00	87.45
51	A15	1.79	86.89	2.46	1.12	0.73	38.90	63.00	77.49

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.31 indicates the late A-line trial which were planted at Kroonstad, Lichtenburg and Delmas. The trials were successful at Kroonstad and Delmas. Flowering data were collected.

Table 4.31 Late A-line trials using the R15 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 22.80	1 Delmas 25.43	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.07	100	2.54	1.59	0.80	36.10	67.90	
1	PAN 7351	2.60	126.06	3.19	2.01	1.02	37.00	68.00	
23	PAN 7033	2.17	103.76	2.75	1.58	0.82	35.70	70.00	83.46
52	A17	1.80	88.54	2.08	1.51	0.58	33.60	67.00	69.23
53	A18	1.75	86.79	1.98	1.52	0.56	35.10	67.00	67.31

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

In Tables 4.32-4.35, all A-line females which did not have sufficient seed for more than one locality were included. These trials were only planted at Kroonstad. Flowering data were not collected.

Table 4.32 Additional female (Group 1) A-line trials using the R15 tester

Rnk	No. of Trials / Hybrid	1 O/a Yield	% Yield	1 Kroonstad 22.10	1 Oil t/ha	1 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.43	100.00	2.43	0.91	37.32		
5	PAN 7351	2.90	119.26	2.90	1.02	39.10		
8	PAN 7033	2.78	114.05	2.78	1.25	39.40		95.86
11	A28	2.72	111.76	2.72	1.22	39.00		93.79
25	A26	2.46	101.20	2.46	0.83	39.10		84.83
29	A31	2.45	100.58	2.45	0.85	35.20		84.48
39	A29	2.26	92.69	2.26	0.73	35.40		77.93
51	A30	1.93	79.20	1.93	0.67	37.00		66.55

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.33 Additional female (Group 2) A-line trials using the R15 tester

Rnk	No. of Trials / Hybrid	1 O/a Yield	% Yield	1 Kroonstad 21.11	1 Oil t/ha	1 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.58	100.00	2.58	0.92	35.82		
1	PAN 7033	3.37	130.86	3.37	1.85	42.10		102.43
2	PAN 7351	3.29	127.60	3.29	1.24	35.10		
15	A36	2.88	111.96	2.88	0.90	34.10		87.54
22	A32	2.81	108.94	2.81	1.22	38.20		85.41

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.34 Additional female (Group 3) A-line trials using the R15 tester

Rnk	No. of Trials / Hybrid	1 O/a Yield	% Yield	1 Kroonstad 20.82	1 Oil t/ha	1 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.65	100.00	2.65	0.96	35.65		
2	PAN 7033	3.77	142.28	3.77	1.77	40.10		117.08
7	PAN 7351	3.22	121.48	3.22	1.23	38.40		
36	A8	2.49	93.91	2.49	1.02	36.10		77.33

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.35 Additional female (Group 4) A-line trials using the R15 tester

Rnk	No. of Trials / Hybrid	1 O/a Yield	% Yield	1 Kroonstad 19.18	1 Oil t/ha	1 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	2.39	100.00	2.39	0.84	34.84		
10	PAN 7033	2.79	116.91	2.79	1.41	38.10		135.44
20	A12	2.54	106.37	2.54	0.90	34.50		123.30
28	A7	2.36	98.99	2.36	1.03	39.00		114.56
30	A9	2.35	98.43	2.35	1.04	35.50		114.08
44	PAN 7351	2.06	86.25	2.06	1.00	36.50		

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Seven of the A-lines crossed with the R-line tester R15 performed better than the commercial check PAN 7351 and included the lines A12, A7, A9, A37, A5, A35 and A34 when looking at overall yield. Combinations which performed worse than the PAN 7351 commercial check when looking at overall yield included A25, A16, A24, A39, A28, A22, A19, A38, A36, A23, A10, A32, A26, A31, A6, A29, A15, A8, A4, A17, A18 and A30. Four A-line combinations failed to produce results. Flowering dates were captured at the Delmas locality and ranged from 55 days for the USA line A10 in combination with R15 to 70 days for PAN 7033. Most of the South African A-line germplasm combinations with R15 expressed an average flowering date of 62.3 days. The oil content of the A-line combinations with R15 ranged from 33.60% for A17 to 41.70% for A10. The average oil content for all combinations was 37.69%. The R15 R-line did not produce combinations with high oil content in combination with the A-lines. The genetic distance varied from 0.533 between A12 and R15 to 0.789 between A9 and R15 (Table 4.36).

As shown in Figure 4.5, relative yield from hybrids obtained from crosses made between R15 and 29 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R15 are shown in Table 4.36. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0000; p=0.9959) and oil content (R^2=0.0009; p=0.8776) as well as between yield and oil content (R^2=0.0657; p=0.1797). The R^2 values observed in all three cases translated to the fact that the no line could be fit. In none of the cases for the group of A-lines crossed to R15 was $p<0.05$ and no resulting meaningful correlations were found.

Table 4.36 Genetic distance and relative yield for each A-line combination with R15

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.5327	123.30	34.50
A24	0.5506	95.67	37.50
A29	0.5693	77.93	35.40
A19	0.5716	91.75	41.10
A37	0.5936	111.34	39.30
A30	0.5969	66.55	37.00
A22	0.5994	91.94	37.10
A6	0.6150	80.08	36.90
A4	0.6189	75.93	36.30
A38	0.6197	87.63	36.30
A32	0.6222	85.41	38.20
A28	0.6222	93.79	39.00
A31	0.6339	84.48	35.20
A25	0.6346	96.68	41.10
A34	0.6415	100.52	37.90
A10	0.6447	86.08	41.70
A13	0.6457	No data	No data
A15	0.6653	77.49	38.90
A41(HO)	0.6698	No data	No data
A43(HO)	0.6705	No data	No data
A35	0.6710	104.12	37.60
A42(HO)	0.6767	No data	No data
A36	0.6920	87.54	34.10
A16	0.6963	95.67	41.30
A26	0.7055	84.83	39.10
A39	0.7064	93.94	38.90
A8	0.7104	77.33	36.10
A7	0.7119	114.56	39.00
A17	0.7239	69.23	33.60
A18	0.7239	67.31	35.10
A23	0.7471	87.45	38.80
A5	0.7660	108.25	40.60
A9	0.7889	114.08	35.50

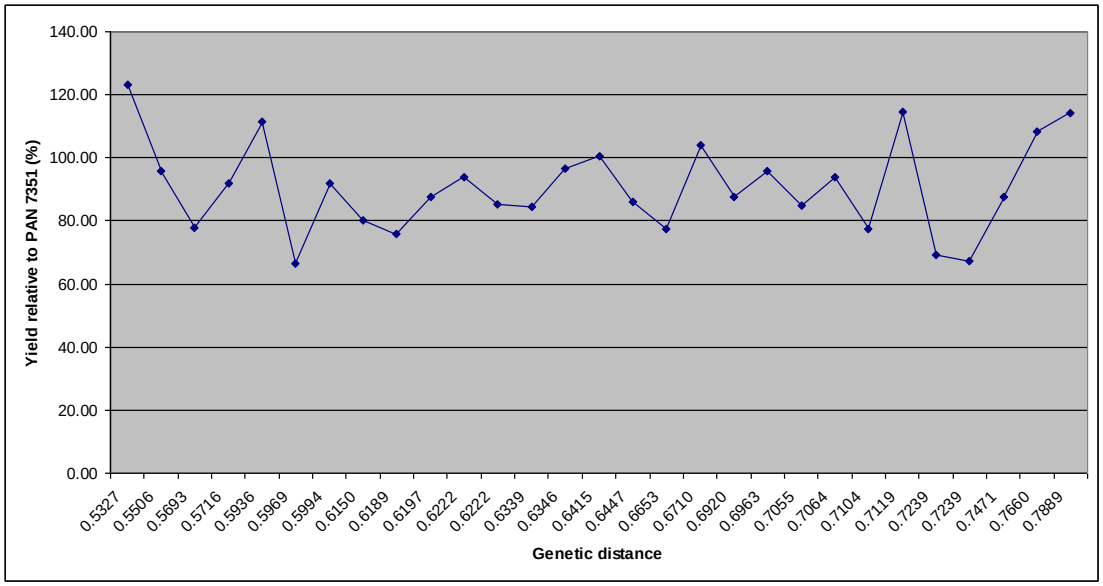

Figure 4.5 Relative yield versus genetic distance of hybrids obtained from crosses with R15 as a male tester crossed to 29 female A-lines.

4.4.1.6 R11 male as tester on A-lines

Table 4.37 represents the very early trials which were planted at Klerksdorp, Kroonstad, Villiers and Delmas. Only the trials at Kroonstad and Villiers were successful. No flowering data were collected. Table 4.38 depicts the early A-lines used with R11 as tester. The early trial was planted at Klerksdorp, Kroonstad and Villiers with the trials at all three locations being successful. No flowering data were collected.

Table 4.37 Very early A-line trials using the R11 tester

	No. of Trials	2		1	1	2	2	0	
		O/a	%	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	15.69	18.22	t/ha	cont	days	PAN 7351
	Treatments mean	1.67	100.00	2.26	1.08	0.71	41.32		
2	PAN 7355	2.67	160.31	2.74	2.15	0.95	38.70		108.54
4	PAN 7351	2.46	147.47	2.73	1.88	0.94	40.30		
13	A10	1.96	117.30	2.37	1.40	0.81	42.25		79.67
36	A7	1.62	97.24	2.31	0.99	0.74	43.20		65.85

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.38 Early A-line trials using the R11 tester

	No. of Trials	3		1	1	1	3	3	0	
		O/a	%	Klerksdorp	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	9.47	15.87	14.23	t/ha	cont	days	PAN 7351
	Treatments mean	2.20	100.00	2.95	2.23	1.43	0.95	42.76		
1	A19	3.18	144.56	4.14	2.60	2.52	1.32	42.97		123.74
3	PAN 7355	2.87	130.36	3.29	2.81	2.19	1.11	39.80		111.67
7	A34	2.79	126.51	3.60	2.97	1.78	1.13	40.67		108.56
8	A37	2.74	124.49	3.74	2.64	1.83	1.23	44.57		106.61
10	PAN 7351	2.57	116.45	3.31	2.34	1.89	1.01	40.00		
16	A4	2.44	110.68	3.05	2.76	1.50	1.08	43.93		94.94
22	A32	2.30	104.23	2.79	2.04	1.81	0.99	44.30		89.49
25	A5	2.26	102.69	2.86	2.20	1.61	0.94	42.90		87.94
42	A38	2.05	93.20	2.61	2.57	1.08	0.86	40.93		79.77

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.39 includes the early-medium, Table 4.40 the medium and Table 4.41 the medium-late trials. All three of these trials were planted at Klerksdorp, Kroonstad, Villiers and Delmas. In the early-medium group, trials were successful at Klerksdorp, Kroonstad and Villiers. The same holds true for the medium and medium-late trials. No flowering data were collected.

Table 4.39 Early-medium A-line trials using the R11 tester

	No. of Trials	3		1	1	1	3	3	0	
		O/a	%	Klerksdorp	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	12.72	14.21	18.18	t/ha	cont	days	PAN 7351
	Treatments mean	2.25	100.00	3.04	2.42	1.30	0.94	41.30		
9	A16	2.52	111.65	3.50	2.40	1.57	1.15	45.97		108.62
13	PAN 7355	2.49	110.54	2.93	2.62	1.66	0.89	36.87		107.33
27	PAN 7351	2.32	103.09	3.68	2.41	1.16	0.94	38.67		
35	A35	2.25	99.97	3.27	2.39	1.22	1.00	42.67		96.98
45	A28	2.07	91.82	2.83	2.20	1.19	0.86	41.07		89.22
46	A6	2.05	91.15	2.68	2.48	1.08	0.87	41.23		88.36
47	A31	2.04	90.68	2.64	2.04	1.31	0.84	41.67		87.93
58	A29	1.87	83.12	2.74	2.10	0.94	0.80	41.53		80.60

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.40 Medium A-line trials using the R11 tester

	No. of Trials	3		1	1	1	3	3	0	
		O/a	%	Klerksdorp	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	10.90	15.27	18.95	t/ha	cont	days	PAN 7351
	Treatments mean	2.26	100.00	2.94	2.59	1.26	0.96	41.76		
1	A15	2.91	128.72	3.71	3.14	1.74	1.27	44.13		118.29
6	PAN 7355	2.74	121.00	3.32	3.34	1.53	1.07	38.77		111.38
16	PAN 7351	2.46	108.67	3.16	2.70	1.44	0.94	38.23		
18	A13	2.44	107.55	3.09	2.63	1.46	1.01	41.63		99.19
22	A8	2.39	105.60	2.53	2.40	1.74	1.01	45.23		97.15
44	A30	2.12	93.75	2.68	2.80	1.03	0.95	42.97		86.18

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.41 Medium-late A-line trials using the R11 tester

	No. of Trials	3		1	1	1	3	3	0	
		O/a	%	Klerksdorp	Kroonstad	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	12.83	14.73	13.53	t/ha	cont	days	PAN 7351
	Treatments mean	2.24	100.00	2.85	2.07	1.80	0.99	43.66		
2	A22	2.66	118.81	3.21	2.32	2.36	1.23	46.73		112.71
3	PAN 7355	2.60	116.04	3.21	2.28	2.25	1.05	40.50		110.17
10	A25	2.53	113.00	3.07	2.35	2.12	1.19	47.53		107.20
22	PAN 7351	2.36	105.41	3.44	1.89	1.87	0.96	39.53		
49	A39	2.05	91.58	2.54	2.23	1.40	0.87	41.67		86.86
54	A26	1.97	88.00	2.65	1.70	1.60	0.85	42.27		83.47

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.42 includes the late and Table 4.43 the very late A-line trials. The late A-line trial was planted at Klerksdorp, Kroonstad, Villiers and Delmas with only the Klerksdorp and Kroonstad trials being successful. No flowering data were collected. The very late trial was planted at Klerksdorp, Kroonstad, Villiers and Delmas and all of the trials were successful. Flowering data were collected

Table 4.42 Late A-line trials using the R11 tester

	No. of Trials	2		1	1	2	2	0	
Rnk	Hybrid	O/a Yield	% Yield	Klerksdorp 18.67	Kroonstad 23.11	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.70	100.00	3.07	2.33	1.13	41.64		
2	PAN 7355	3.22	119.22	3.62	2.81	1.22	37.75		107.33
8	PAN 7351	3.00	110.96	3.27	2.69	1.18	39.40		
37	A9	2.60	96.11	3.14	2.10	1.13	43.20		86.67

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.43 Very late A-line trials using the R11 tester

	No. of Trials	4		1	1	1	1	4	4	1	
Rnk	Hybrid	O/a Yield	% Yield	Klerksdorp 16.21	Kroonstad 12.41	Villiers 14.13	Delmas 22.70	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.10	100.00	2.96	2.24	1.73	1.47	0.54	39.37	70.94	
6	A18	2.37	112.70	3.39	1.95	2.19	1.81	0.60	40.10	72.00	108.22
13	PAN 7355	2.25	107.24	2.92	2.76	2.33	1.08	0.58	36.43	72.00	102.74
18	PAN 7351	2.19	104.23	2.42	2.72	1.64	1.76	0.54	34.90	69.00	
27	A24	2.12	100.81	3.00	2.26	1.71	1.50	0.50	35.93	70.00	96.80
31	A17	2.05	97.60	2.45	2.36	1.71	1.53	0.54	38.33	70.00	93.61
37	A23	1.95	92.77	3.37	1.85	1.80	1.04	0.47	39.90	69.00	89.04

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The A-lines A7, A10, A38, A29, A26, A30, A9, A39, A31, A5, A6, A23, A28, A32, A17, A4, A24, A35, A8 and A13 in combination with the R-line tester R11 performed worse than the commercial check PAN 7351 when looking at overall yield. The A-lines A19, A15, A22, A16, A34, A18, A25 and A37 in combination with the R-line R11 performed better than the commercial check PAN 7351 when considering overall yield. Flowering dates were captured on the one trial at the Delmas locality and ranged from 69 days for the A-line A23 in combination with R11 and 69 days for PAN 7351 to 72 days for HS9032 in combination with R11 and 72 days for PAN 7355. Most of the South African A-line germplasm combinations with R11 expressed an average flowering date of 70.42 days. The oil content of the A-line combinations with R11 ranged from 35.93% for A24 to 47.53% for A25. The average oil

content for all combinations was 42.48%. The R11 R-line produced combinations with good oil content in combination with the A-lines. The genetic distance varied from 0.546 between A12 and R11 to 0.764 between both A17 and A18 and R11 (Table 4.44).

As shown in Figure 4.6, relative yield from hybrids obtained from crosses made between R11 and 28 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R11 are shown in Table 4.44. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0175; p=0.5026) and oil content (R^2=0.0357; p=0.3354) as well as between yield and oil content (R^2=0.0794; p=0.1462). The R^2 values observed in all three cases translated to the fact that no line could be fit. In none of the cases for the group of A-lines crossed to R11 was $p<0.05$ and no resulting meaningful correlations were found.

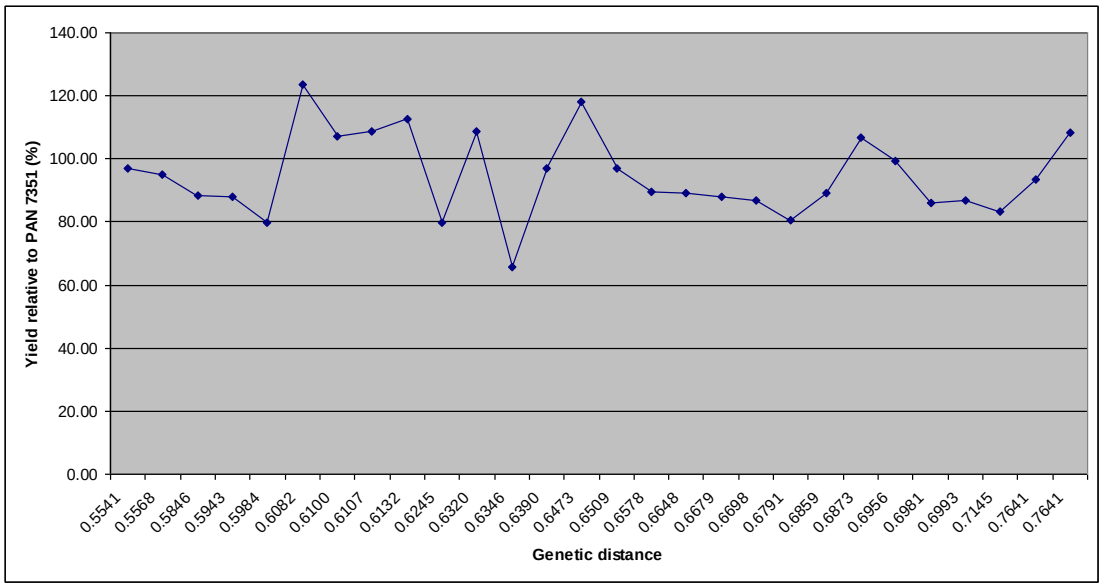

Figure 4.6 Relative yield versus genetic distance of hybrids obtained from crosses with R11 as a male tester crossed to 28 female A-lines.

Table 4.44 Genetic distance and relative yield for each A-line combination with R11

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.5455	No data	No data
A24	0.5541	96.80	35.93
A4	0.5568	94.94	43.93
A6	0.5846	88.36	41.23
A31	0.5943	87.93	41.67
A10	0.5984	79.67	42.25
A19	0.6082	123.74	42.97
A25	0.6100	107.20	47.53
A16	0.6107	108.62	45.97
A36	0.6132	No data	No data
A22	0.6132	112.71	46.73
A38	0.6245	79.77	40.93
A34	0.6320	108.56	40.67
A7	0.6346	65.85	43.20
A35	0.6390	96.98	42.67
A15	0.6473	118.29	44.13
A8	0.6509	97.15	45.23
A42(HO)	0.6538	No data	No data
A43(HO)	0.6569	No data	No data
A32	0.6578	89.49	44.30
A28	0.6648	89.22	41.07
A5	0.6679	87.94	42.90
A9	0.6698	86.67	43.20
A41(HO)	0.6731	No data	No data
A29	0.6791	80.60	41.53
A23	0.6859	89.04	39.90
A37	0.6873	106.61	44.57
A13	0.6956	99.19	41.63
A30	0.6981	86.18	42.97
A39	0.6993	86.86	41.67
A26	0.7145	83.47	42.27
A17	0.7641	93.61	38.33
A18	0.7641	108.22	40.10

4.4.1.7 R47(RM) male as tester on A-lines

Table 4.45 contains the early and Table 4.46 the early-medium A-lines used in the trials with R47(RM) as tester. The early trial was planted at Kroonstad, Lichtenburg and Delmas. Only the Kroonstad trial was successful, but no flowering data were recorded. The early-medium trial was planted at Kroonstad, Lichtenburg and Delmas. The Kroonstad and Delmas trials were successful. Flowering data were recorded.

Table 4.45 Early A-line trials using the R47(RM) tester

	No. of Trials	1		1	1	1	0	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 28.18	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.62	100.00	1.62	0.61	34.95		
1	PAN 7351	2.79	172.74	2.79	1.25	38.30		
2	PAN 7033	2.31	142.72	2.31	1.18	40.10		82.80
13	A5	1.90	117.36	1.90	0.53	37.10		68.10
15	A10	1.85	114.50	1.85	0.61	38.20		66.31
23	A37	1.68	103.86	1.68	0.53	37.50		60.22
26	A38	1.62	100.42	1.62	0.53	35.40		58.06
30	A19	1.44	89.17	1.44	0.83	42.00		51.61
35	A34	1.18	72.89	1.18	0.64	37.40		42.29

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.46 Early-medium A-line trials using the R47(RM) tester

		2		1	1	2	2	1	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 36.19	Delmas 23	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.1	100	2.73	1.47	0.80	37.10	68.90	
3	PAN 7351	2.34	116.89	2.70	1.98	0.99	38.80	69.00	
4	PAN 7033	2.60	113.05	4.06	1.14	1.20	40.10	71.00	111.11
11	A4	2.08	104.64	2.35	1.81	0.58	38.10	68.00	88.89

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.47 includes the medium, Table 4.48 the medium-late and Table 4.49 the late A-lines used in the trials. The medium trials were planted at Kroonstad, Lichtenburg and Delmas, but

only the Kroonstad trial was successful. No flowering data were collected. The medium-late trial was planted at Kroonstad, Lichtenburg and Delmas and both the Kroonstad and Delmas trials were succesful. Flowering data were collected. The late trial was planted at Kroonstad, Lichtenburg and Delmas. Only the Kroonstad trial was successful. No flowering data were collected.

Table 4.47 Medium A-line trials using the R47(RM) tester

	No. of Trials	1		1	1	1	0	
		O/a	%	Kroonstad	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	20.19	t/ha	cont	days	PAN 7351
	Treatments mean	2.35	100.00	2.35	0.92	35.25		
1	PAN 7351	3.74	159.12	3.74	1.68	41.00		
4	PAN 7033	3.27	139.48	3.27	1.48	40.40		87.43
17	A25	2.61	111.10	2.61	1.09	46.00		69.79
21	A22	2.60	110.92	2.60	0.99	40.10		69.52

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.48 Medium-late A-line trials using the R47(RM) tester

		2		1	1	2	2	1	
		O/a	%	Kroonstad	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	21.79	23.5	t/ha	cont	days	PAN 7351
	Treatments mean	2.33	100	2.80	1.85	0.90	38.90	68.00	
1	PAN 7351	3.20	136.99	3.93	2.47	1.32	38.40	67.00	
6	A39	2.57	112.87	2.85	2.29	0.87	37.30	68.00	80.31
8	PAN 7033	2.46	108.29	2.71	2.22	1.10	38.70	70.00	76.88
11	A23	2.45	106.27	2.85	2.05	1.07	39.20	65.00	76.56
14	A16	2.44	104.08	3.01	1.86	0.98	41.70	69.00	76.25
15	A24	2.37	103.81	2.63	2.10	0.80	35.90	68.00	74.06

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.49 Late A-line trials using the R47(RM) tester

Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 22.31	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	No. of Trials	1		1	1	1	0	
	Treatments mean	2.38	100.00	2.38	0.90	36.87		
1	PAN 7351	3.82	160.21	3.82	1.80	40.00		
3	PAN 7033	3.16	132.79	3.16	1.64	41.10		82.72
26	A17	2.04	85.71	2.04	0.91	35.10		53.40
27	A18	1.93	80.98	1.93	0.81	34.50		50.52

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The combinations made with the R47(RM) R-line included A4, A39, A23, A16, A24, A25, A22, A5, A10, A37, A38, A17, A19, A18 and A34, which performed worse than the commercial check PAN 7351 for overall yield. The remaining 18 A-line crosses did not realise and data could not be collected on them. Flowering dates were captured on the two trials at the Delmas locality and ranged from 65 days for the A-line A23 in combination with R47(RM) to 71 days for PAN 7033. Most of the South African A-line germplasm combinations with R47(RM) expressed an average flowering date of 68.35 days. The oil content of the A-line combinations with R47(RM) ranged from 34.50% for A18 to 46.00% for A25. The average oil content for all combinations was 38.37%. The R47(RM) R-line did not produce combinations with high oil content in combination with the A-lines except for the A-lines A19, A16 and A25. All three of these lines were conducive for high oil content in combinations. The genetic distance varied from 0.605 between A6 and R47(RM) to 0.822 between A8 and R47(RM) (Table 4.50).

As shown in Figure 4.7, relative yield from hybrids obtained from crosses made between R47(RM) and 15 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R47(RM) are shown in Table 4.50. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0002; p=0.9597) and oil content (R^2=0.1766; p=0.1172) as well as between yield and oil content (R^2=0.0596; p=0.3807). The R^2 values observed in all three cases translated to the fact that no line could be fit. In none of the cases

for the group of A-lines crossed to R47(RM) was $p<0.05$ and no resulting meaningful correlations were found.

Table 4.50 Genetic distance and relative yield for each A-line combination with R47(RM)

A-line	Genetic Distance	% Relative yield	% Oil content
A6	0.6046	No data	No data
A39	0.6150	80.31	37.30
A24	0.6154	74.06	35.90
A5	0.6169	68.10	37.10
A38	0.6198	58.06	35.40
A12	0.6320	No data	No data
A13	0.6391	No data	No data
A35	0.6461	No data	No data
A37	0.6503	60.22	37.50
A32	0.6583	No data	No data
A4	0.6674	88.89	38.10
A25	0.6680	69.79	46.00
A34	0.6738	42.29	37.40
A15	0.6755	No data	No data
A10	0.6798	66.31	38.20
A7	0.6820	No data	No data
A26	0.6820	No data	No data
A28	0.6916	No data	No data
A18	0.7006	50.52	34.50
A17	0.7006	53.40	35.10
A29	0.7256	No data	No data
A41(HO)	0.7300	No data	No data
A22	0.7448	69.52	40.10
A19	0.7448	51.61	42.00
A9	0.7493	No data	No data
A42(HO)	0.7496	No data	No data
A43(HO)	0.7693	No data	No data
A31	0.7711	No data	No data
A30	0.7807	No data	No data
A36	0.7833	No data	No data
A16	0.8000	76.25	41.70
A23	0.8038	76.56	39.20
A8	0.8218	No data	No data

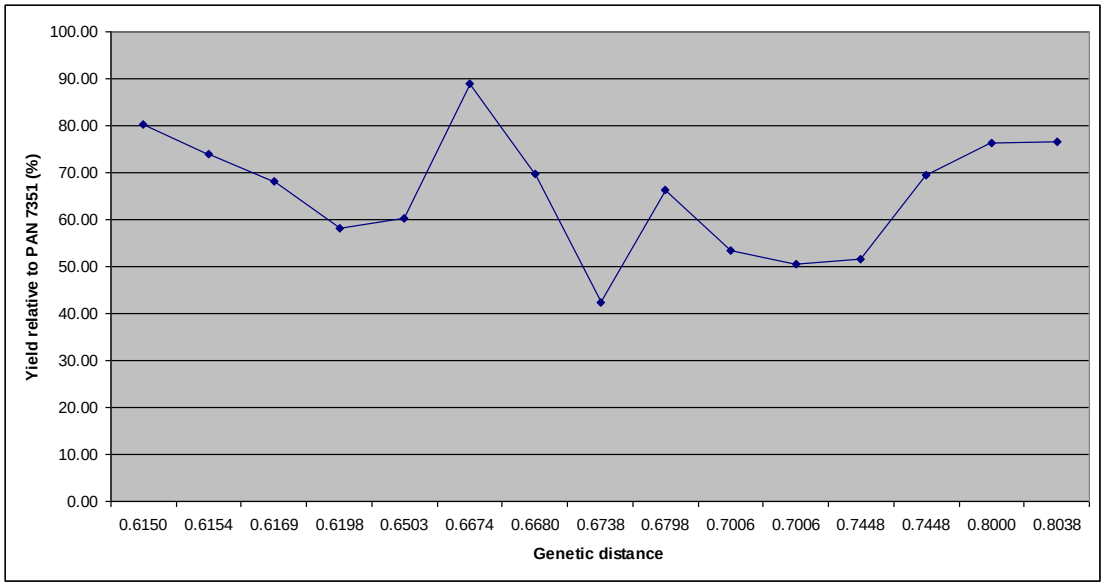

Figure 4.7 Relative yield versus genetic distance of hybrids obtained from crosses with R47(RM) as a male tester crossed to 15 female A-lines.

4.4.1.8 R32 male as tester on A-lines

Table 4.51 included the early A-lines used in the trials with R32 as tester. The trials were planted at Standerton, Lichtenburg and Kroonstad. Only data from Lichtenburg and Kroonstad were used. No flowering data were collected.

Table 4.51 Early A-line trials using the R32 tester

	No. of Trials	2		1	1	2	2	0	
		O/a	%	Lichtenburg	Kroonstad	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	20.81	25.96	t/ha	cont	days	PAN 7351
	Treatments mean	1.73	100	1.83	1.62	0.70	41.90		
2	PAN 7351	2.16	126.00	2.01	2.31	0.85	39.30		
5	PAN 7033	2.07	119.14	2.29	1.84	0.75	36.50		95.83
9	A37	1.99	112.68	2.76	1.22	0.83	41.20		92.13
13	A19	1.85	108.05	1.74	1.97	0.77	41.80		85.65
18	A35	1.76	102.01	1.87	1.65	0.73	41.50		81.48
27	A5	1.64	94.89	1.78	1.51	0.69	42.20		75.93
30	A38	1.58	90.52	1.85	1.30	0.65	41.40		73.15
40	A34	1.42	81.65	1.57	1.26	0.56	39.30		65.74

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The medium A-lines which were used in the trials are shown in Table 4.52. Trials were planted at Standerton, Lichtenburg and Kroonstad. Both Lichtenburg and Kroonstad were used for these trials. No flowering data were collected.

Table 4.52 Medium A-line trials using the R32 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Lichtenburg 20.63	1 Kroonstad 21.61	2 Oil t/ha	2 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.73	100	1.90	1.55	0.70	41.30		
2	PAN 7351	2.11	124.85	1.93	2.30	0.80	38.20		
4	PAN 7033	2.09	121.50	2.26	1.93	0.78	37.20		99.05
11	A22	1.86	108.05	2.01	1.71	0.81	43.30		88.15
25	A26	1.71	98.26	2.07	1.36	0.72	42.10		81.04

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.53 contains the late A-lines for the R32 tester. The trials were planted at Standerton, Kroonstad and Lichtenburg. All three of the localities were successful. No flowering data were collected. Unfortunately, not all crosses were successful for this specific R-line tester.

Table 4.53 Late A-line trials using the R32 tester

Rnk	Hybrid	3 O/a Yield	% Yield	1 Standerton 20.37	1 Kroonstad 19.00	1 Lichtenburg 20.76	3 Oil t/ha	3 Oil cont	0 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.98	100	2.39	1.93	1.62	0.80	42.20		
1	PAN 7033	3.07	153.47	3.98	2.86	2.36	1.21	39.80		131.20
6	PAN 7351	2.34	121.35	2.12	2.67	2.23	0.90	38.40		
10	A15	2.24	111.62	2.93	2.22	1.58	1.02	45.00		95.73
12	A39	2.13	108.15	2.56	1.87	1.96	0.89	41.80		91.03
15	A16	2.10	104.80	2.84	1.74	1.72	0.96	45.60		89.74

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Combinations made with the R32 R-line tester included A-lines A15, A37, A39, A16, A22, A19, A35, A26, A5, A38 and A34 which all performed worse than the commercial check

PAN 7351 when looking at overall yield. Unfortunately 22 of the A-line crosses failed and could not be used in data analysis. The oil content of the A-line combinations with R32 ranged from 39.30% for A34 to 45.60% for A16. The average oil content for all combinations was 42.29%. The R32 R-line produces combinations with good oil content in combination with the A-lines. The genetic distance varied from 0.500 between A39 and R32 to 0.727 between A16 and R32 (Table 4.54).

As shown in Figure 4.8, relative yield from hybrids obtained from crosses made between R32 and 11 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R32 are shown in Table 4.54. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0112; p=0.7557) and oil content (R^2=0.1568; p=0.2249) as well as between yield and oil content (R^2=0.4562; p=0.0226). No line could be fitted. Only in the case of yield versus oil content for the group of A-lines crossed to R32 was $p<0.05$ and a meaningful correlation found.

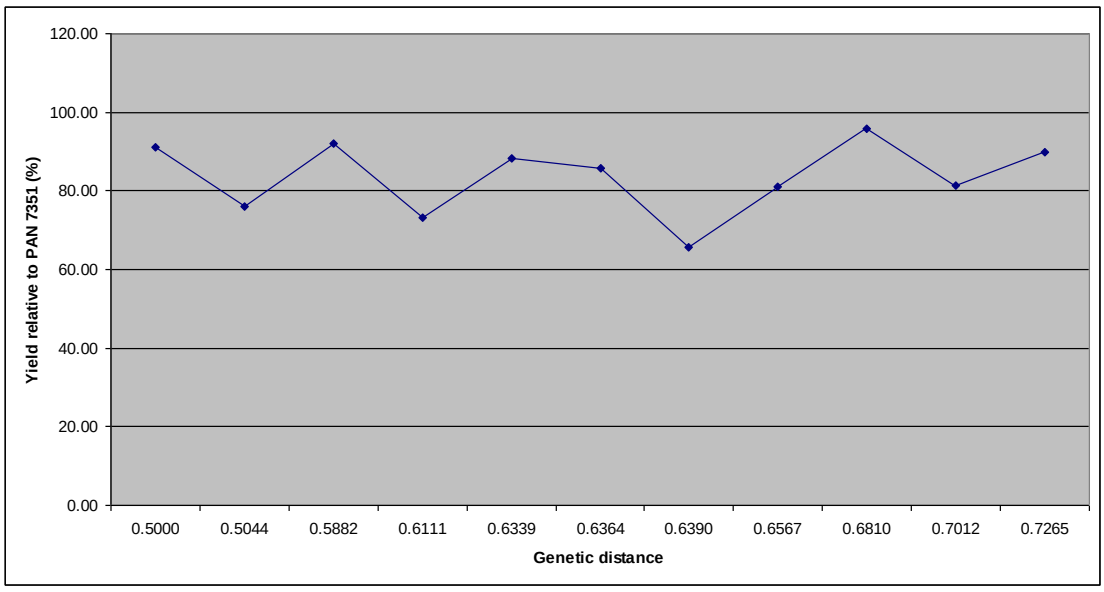

Figure 4.8 Relative yield versus genetic distance of hybrids obtained from crosses with R32 as a male tester crossed to 11 female A-lines.

Table 4.54 Genetic distance and relative yield for each A-line combination with R32

A-line	Genetic distance	% Relative yield	% Oil content
A39	0.5000	91.03	41.80
A5	0.5044	75.93	42.20
A13	0.5278	No data	No data
A12	0.5377	No data	No data
A10	0.5434	No data	No data
A8	0.5438	No data	No data
A4	0.5682	No data	No data
A24	0.5741	No data	No data
A6	0.5856	No data	No data
A37	0.5882	92.13	41.20
A9	0.5969	No data	No data
A31	0.5969	No data	No data
A36	0.5994	No data	No data
A38	0.6111	73.15	41.40
A41(HO)	0.6132	No data	No data
A42(HO)	0.6201	No data	No data
A7	0.6245	No data	No data
A32	0.6271	No data	No data
A23	0.6333	No data	No data
A22	0.6339	88.15	43.30
A19	0.6364	85.65	41.80
A34	0.6390	65.74	39.30
A28	0.6407	No data	No data
A29	0.6421	No data	No data
A30	0.6524	No data	No data
A26	0.6567	81.04	42.10
A43(HO)	0.6705	No data	No data
A25	0.6738	No data	No data
A15	0.6810	95.73	45.00
A35	0.7012	81.48	41.50
A17	0.7264	No data	No data
A18	0.7264	No data	No data
A16	0.7265	89.74	45.60

4.4.1.9 R29 male as tester on A-lines

R29 is one of the more important lines and was successfully crossed with most of the A-lines to produce trials as shown below. Tables 4.55-4.57 include three groups of early A-line testers. Trials were planted at Kroonstad, Delmas and Lichtenburg and all localities produced successful trials. Flowering data were recorded.

Table 4.55 Early A-line trials (Group 1) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 32.25	Lichtenburg 28.42	Delmas 19.01	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.95	100.00	0.79	0.74	0.86	0.80	40.74	60.74	
1	PAN 7049	3.02	154.39	1.23	1.15	1.30	1.23	42.01	67.00	126.23
4	PAN 7033	2.64	138.22	1.12	1.10	1.07	1.10	40.95	68.00	110.44
6	PAN 7351	2.39	128.20	0.88	1.20	0.96	1.01	41.83	67.00	
15	A7	1.96	104.28	0.83	0.75	0.91	0.83	42.72	60.00	81.91
22	A8	1.81	96.20	0.72	0.61	1.00	0.77	44.80	64.00	75.44

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.56 Early A-line trials (Group 2) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 26.57	Lichtenburg 34.38	Delmas 29.67	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.72	100.00	0.76	0.55	0.81	0.71	39.42	60.56	
7	PAN 7033	2.26	132.82	1.10	0.83	0.82	0.92	40.74	70.00	102.26
8	PAN 7049	2.25	137.31	1.22	0.76	0.91	0.96	41.11	69.00	101.85
9	PAN 7351	2.21	131.80	0.87	0.88	0.97	0.91	42.12	66.00	
23	A24	1.95	101.99	0.83	0.45	0.93	0.74	39.33	63.00	87.97
54	A36	1.54	84.02	0.77	0.46	0.54	0.59	42.25	64.00	69.76

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.57 Early A-line trials (Group 3) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	24.72	37.91	33.35	t/ha	cont	days	PAN 7351
	Treatments mean	1.89	100.00	0.87	0.73	0.74	0.78	41.28	64.85	
1	PAN 7049	2.54	126.70	1.22	0.76	1.01	0.99	41.45	72.00	121.63
7	A38	2.40	115.17	1.03	0.74	0.93	0.90	39.47	65.00	115.06
8	PAN 7033	2.39	124.83	0.95	0.93	1.01	0.97	39.59	77.00	114.53
11	A5	2.16	115.89	0.96	0.80	0.93	0.90	41.38	66.00	103.74
13	PAN 7351	2.09	105.08	0.84	0.85	0.75	0.81	40.59	69.00	
19	A10	2.08	117.35	1.15	0.79	0.83	0.92	42.80	63.00	99.90

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Tables 4.58-4.60 include the group of medium A-lines in the trials. This set of trials was planted at Kroonstad, Lichtenburg and Delmas. Trials at all three localities were used. Flowering data were collected.

Table 4.58 Medium A-line trials (Group 1) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	22.10	34.24	32.08	t/ha	cont	days	PAN 7351
	Treatments mean	1.72	100.00	0.81	0.62	0.66	0.70	39.58	65.32	
3	PAN 7049	2.34	130.66	1.00	0.98	0.74	0.91	40.52	76.00	147.04
5	PAN 7033	2.31	127.67	1.02	0.84	0.81	0.89	40.52	75.00	145.35
17	A37	1.84	109.03	0.84	0.64	0.80	0.76	40.76	67.00	115.47
37	A32	1.76	99.97	0.71	0.62	0.75	0.69	39.11	69.00	110.57
43	PAN 7351	1.59	92.50	0.69	0.65	0.59	0.64	40.76	69.00	
52	A9	1.52	81.31	0.79	0.40	0.54	0.58	41.84	65.00	95.41

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.59 Medium A-line trials (Group 2) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	25.40	32.75	30.66	t/ha	cont	days	PAN 7351
	Treatments mean	1.87	100.00	0.86	0.66	0.78	0.76	40.92	68.87	
2	PAN 7049	2.63	137.41	1.08	1.06	0.97	1.04	40.29	74.00	131.76
10	PAN 7033	2.36	118.76	0.83	0.86	1.00	0.90	40.19	74.00	117.99
13	A28	2.13	109.65	1.00	0.82	0.68	0.83	41.60	71.00	106.56
15	PAN 7351	2.00	112.44	0.98	0.78	0.81	0.86	40.51	74.00	
49	A31	1.61	81.15	0.95	0.45	0.50	0.63	39.99	66.00	80.86
51	A23	1.47	84.09	0.81	0.39	0.76	0.66	40.14	69.00	73.60
52	A30	1.45	81.47	0.89	0.35	0.68	0.64	42.48	61.00	72.60
60	A29	1.35	69.70	0.70	0.31	0.63	0.55	41.82	70.00	67.84

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.60 Medium A-line trials (Group 3) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	23.27	37.59	20.74	t/ha	cont	days	PAN 7351
	Treatments mean	1.91	100.00	0.83	0.66	0.84	0.77	40.52	70.35	
1	PAN 7049	2.64	139.64	0.98	1.14	1.06	1.06	40.65	75.00	126.48
3	PAN 7033	2.55	128.27	1.11	0.93	0.92	0.98	39.38	76.00	122.13
14	PAN 7351	2.09	111.98	0.96	0.72	0.94	0.87	40.45	71.00	
26	A35	1.90	99.91	0.88	0.62	0.83	0.78	42.02	71.00	91.00

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Tables 4.61-4.62 include the first late group of A-lines used in the trials. These trials were planted at Kroonstad, Lichtenburg and Delmas. All three localities produced successful trials. Flowering data were collected.

Table 4.61 Late A-line trials (Group 1) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	22.18	38.18	28.74	t/ha	cont	days	PAN 7351
	Treatments mean	1.97	100.00	0.89	0.62	0.87	0.80	40.22	69.73	
1	PAN 7033	2.71	141.28	1.30	0.99	1.03	1.11	40.67	77.00	130.04
2	PAN 7049	2.47	129.32	1.21	0.81	1.06	1.03	41.19	74.00	118.43
24	PAN 7351	2.08	106.59	0.97	0.77	0.76	0.83	40.43	72.00	
43	A13	1.91	95.15	0.86	0.51	0.93	0.77	39.33	71.00	91.60
54	A12	1.63	77.89	0.45	0.57	0.79	0.61	38.70	71.00	78.31

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.62 Late A-line trials (Group 2) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	18.79	35.90	23.23	t/ha	cont	days	PAN 7351
	Treatments mean	1.96	100.00	0.95	0.62	0.84	0.80	41.01	71.07	
3	PAN 7033	2.43	127.47	1.10	0.93	0.98	1.00	41.05	77.00	121.11
4	PAN 7049	2.43	127.50	1.07	1.02	0.88	0.99	40.63	76.00	120.91
11	A6	2.15	104.49	0.95	0.59	0.99	0.84	39.36	73.00	107.22
12	A19	2.12	103.31	1.13	0.60	0.78	0.84	40.84	73.00	105.43
21	PAN 7351	2.01	106.09	1.05	0.73	0.76	0.84	41.07	74.00	
24	A43(HO)	1.87	104.99	0.95	0.71	0.85	0.83	42.21	68.00	93.28
25	A41(HO)	1.84	83.72	0.80	0.45	0.78	0.68	37.91	69.00	91.64
50	A42(HO)	1.68	93.30	0.88	0.54	0.85	0.75	42.17	71.00	83.37
55	A18	1.62	75.27	0.86	0.31	0.71	0.63	37.92	68.00	80.59
59	A17	1.57	79.34	0.77	0.44	0.72	0.64	39.02	70.00	77.90

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Tables 4.63-4.65 show the second group of late A-lines included in the trials. These trials were planted at Kroonstad, Lichtenburg and Delmas. The three localities produced successful trials. Flowering data were collected.

Table 4.63 Late A-line trials (Group 3) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	28.11	38.48	26.21	t/ha	cont	days	PAN 7351
	Treatments mean	1.80	100.00	0.86	0.56	0.81	0.74	40.04	62.95	
1	PAN 7049	2.76	162.64	1.17	1.03	1.36	1.19	41.54	69.00	138.65
7	PAN 7033	2.26	134.15	0.87	0.97	1.04	0.96	40.84	71.00	113.35
12	A4	2.13	116.37	0.70	0.75	1.08	0.84	40.14	66.00	107.13
17	PAN 7351	1.99	105.30	1.07	0.47	0.87	0.80	41.28	66.00	

Rnk Rank
O/a yield Overall yield
Oil t/ha Oil yield expressed in ton per hectare
Oil cont Oil content expressed as a percentage
Flow days Days counted until flowering
% yield rel PAN 7351 The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.64 Late A-line trials (Group 4) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	20.98	39.79	23.84	t/ha	cont	days	PAN 7351
	Treatments mean	1.81	100.00	0.92	0.59	0.71	0.74	39.60	66.06	
1	PAN 7049	2.70	146.26	1.23	0.90	1.08	1.07	41.14	73.00	159.94
11	A16	2.15	127.71	1.09	0.84	0.87	0.93	44.13	69.00	127.60
15	PAN 7033	2.14	121.13	1.04	0.63	1.02	0.89	40.29	79.00	127.12
36	A39	2.12	97.03	0.80	0.52	0.81	0.71	36.71	66.00	125.82
38	A34	1.76	90.35	1.09	0.40	0.60	0.70	40.56	67.00	104.15
39	A26	1.74	93.31	0.89	0.56	0.62	0.69	42.07	65.00	103.44
45	PAN 7351	1.69	90.13	0.99	0.63	0.38	0.67	39.70	69.00	

Rnk Rank
O/a yield Overall yield
Oil t/ha Oil yield expressed in ton per hectare
Oil cont Oil content expressed as a percentage
Flow days Days counted until flowering
% yield rel PAN 7351 The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.65 Late A-line trials (Group 5) using the R29 tester

	No. of Trials	3		1	1	1	3	3	1	
		O/a	%	Kroonstad	Lichtenburg	Delmas	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	22.40	39.75	32.64	t/ha	cont	days	PAN 7351
	Treatments mean	1.81	100.00	0.89	0.58	0.77	0.75	40.16	68.28	
2	PAN 7049	2.30	119.84	1.30	0.59	0.87	0.92	41.61	72.00	113.42
4	PAN 7033	2.18	109.32	1.13	0.52	0.86	0.84	40.93	74.00	107.45
6	A15	2.13	124.17	0.91	0.87	0.93	0.90	42.77	74.00	105.13
8	PAN 7351	2.03	118.23	1.00	0.82	0.78	0.87	43.28	74.00	
10	A22	1.86	107.38	1.05	0.54	0.85	0.82	41.39	66.00	91.76
13	A25	1.84	111.95	1.19	0.64	0.71	0.85	43.77	72.00	90.58

Rnk Rank
O/a yield Overall yield
Oil t/ha Oil yield expressed in ton per hectare
Oil cont Oil content expressed as a percentage
Flow days Days counted until flowering
% yield rel PAN 7351 The performance of each variety relative to PAN 7351 expressed as a percentage

The R29 R-line tester in combination with A29, A36, A30, A23, A8, A17, A12, A18, A31, A7, A42(HO), A24, A25, A35, A13, A41(HO), A22, A43(HO), A9 and A10 performed worse than the commercial check PAN 7351 when looking at overall yield. A26, A5, A34, A15, A19, A28, A4, A6, A32, A38, A37, A39 and A16 performed better than the commercial check PAN 7351 in combination with the R-line tester R29 for overall yield. Flowering dates were captured on the one trial at the Delmas locality and ranged from 60 days for the A-line A7 in combination with R29 to 79 days for PAN 7033. Most of the South African A-line germplasm combinations with R29 expressed an average flowering date of 69.45 days. The oil content of the A-line combinations with R29 ranged from 36.71% for A39 to 44.13% for A16 and 44.80% for A8. The average oil content for all combinations was 40.95%. The R29 R-line produces combinations with good oil content in combination with the A-lines. The genetic distance varied from 0.444 between A12 and R29 to 0.671 between A23 and R29 (Table 4.66).

As shown in Figure 4.9, relative yield from hybrids obtained from crosses made between R29 and 33 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R29 are shown in Table 4.66. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0157; p=0.4867) and oil content (R^2=0.0011; p=0.8559) as well as between yield and oil content (R^2=0.0170; p=0.4697). The R^2 values observed in all three cases translated to the fact that no line could be fitted. In none of the cases for the group of A-lines crossed to R29 was $p<0.05$ and no resulting meaningful correlations were found.

Table 4.66 Genetic distance and relative yield for each A-line combination with R29

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.4444	78.31	38.70
A13	0.4636	91.60	39.33
A43(HO)	0.5000	93.28	42.21
A28	0.5067	106.56	41.60
A10	0.5083	99.90	42.80
A8	0.5091	75.44	44.80
A42(HO)	0.5093	83.37	42.17
A29	0.5193	67.84	41.82
A4	0.5222	107.13	40.14
A6	0.5359	107.22	39.36
A31	0.5364	80.86	39.99
A22	0.5455	91.76	41.39
A32	0.5545	110.57	39.11
A38	0.5636	115.06	39.47
A41(HO)	0.5648	91.64	37.91
A30	0.5727	72.60	42.48
A5	0.5809	103.74	41.38
A34	0.5833	104.15	40.56
A16	0.5976	127.60	44.13
A24	0.6000	87.97	39.33
A36	0.6000	69.76	42.25
A35	0.6091	91.00	42.02
A19	0.6157	105.43	40.84
A37	0.6346	115.47	40.76
A18	0.6364	80.59	37.92
A17	0.6364	77.90	39.02
A39	0.6415	125.82	36.71
A9	0.6430	95.41	41.84
A15	0.6513	105.13	42.77
A25	0.6538	90.58	43.77
A7	0.6574	81.91	42.72
A26	0.6612	103.44	42.07
A23	0.6710	73.60	40.14

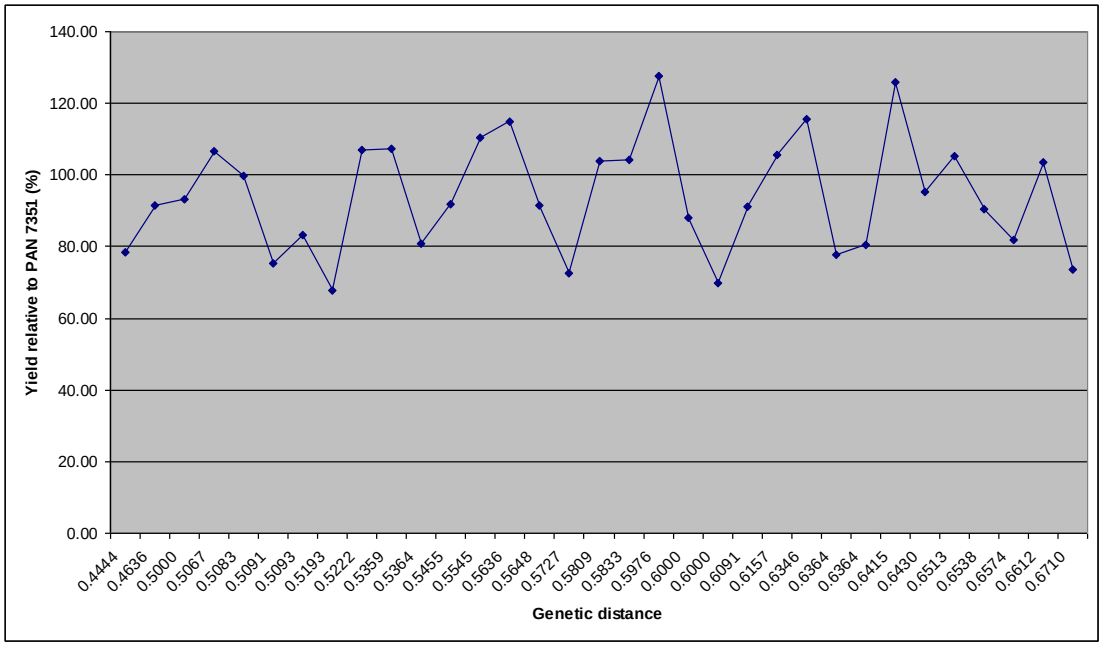

Figure 4.9 Relative yield versus genetic distance of hybrids obtained from crosses with R29 as a male tester crossed to 33 female A-lines.

4.4.1.10 R10 male as tester on A-lines

Tables 4.67-4.69 show the early group of A-lines used in the trials with R10 as a tester. The early group was planted at Kroonstad, Lichtenburg and Delmas. The early trial (1) at Lichtenburg was used and flowering data were collected. The early trial (2) at both Kroonstad and Lichtenburg was used and flowering data collected. The early trial (3) at Kroonstad and Lichtenburg was used and flowering data collected. One of the primary reasons for the lack of data from the Delmas locality was the severe *Sclerotinia* infection. It was possible to capture flowering data, but yield was negatively influenced.

Table 4.67 Early A-line trials (Group 1) using the R10 tester

	No. of Trials	1		1	1	1	1	
Rnk	Hybrid	O/a Yield	% Yield	Lichtenburg 33.42	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.50	100	0.61	0.61	40.10	71.90	
3	PAN 7049	2.18	145.89	0.89	0.89	41.30	80.00	104.81
5	PAN 7033	2.16	134.12	0.82	0.82	39.30	68.00	103.85
6	PAN 7351	2.08	142.64	0.87	0.87	40.70	76.00	
17	A5	1.83	115.51	0.71	0.71	40.70	74.00	87.98
18	A7	1.69	115.45	0.71	0.71	43.60	70.00	81.25
33	A10	1.50	97.69	0.60	0.60	40.60	72.00	72.12

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.68 Early A-line trials (Group 2) using the R10 tester

		2		1	1	2	2	1	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 38.47	Lichtenburg 35.21	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.00	100	0.69	0.75	0.72	38.50	74.90	
3	PAN 7049	2.48	105.37	0.61	0.92	0.77	39.20	81.00	128.50
4	A32	2.48	102.43	0.75	0.72	0.74	38.70	76.00	128.50
16	PAN 7033	2.38	110.96	0.74	0.87	0.80	38.90	79.00	123.32
25	A19	2.28	127.36	0.76	1.09	0.93	41.10	76.00	118.13
37	PAN 7351	1.93	94.71	0.45	0.94	0.69	41.00	79.00	
39	A36	1.74	96.01	0.59	0.81	0.70	40.70	75.00	90.16
48	A38	1.66	86.31	0.50	0.75	0.63	37.70	77.00	86.01

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.69 Early A-line trials (Group 3) using the R10 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 29.68	1 Lichtenburg 31.59	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.80	100	0.69	0.61	0.65	38.40	75.90	
2	PAN 7033	2.28	122.79	0.72	0.85	0.79	38.70	78.00	168.89
7	A34	1.97	102.18	0.58	0.73	0.65	36.80	78.00	145.93
12	PAN 7049	1.96	133.24	0.78	0.92	0.85	40.10	80.00	145.19
24	A35	1.91	117.24	0.79	0.73	0.76	39.20	76.00	141.48
25	A28	1.91	103.04	0.78	0.56	0.67	38.10	76.00	141.48
35	A12	1.79	98.10	0.69	0.58	0.63	37.50	76.00	132.59
48	PAN 7351	1.35	86.40	0.72	0.41	0.57	42.20	77.00	
57	A29	1.21	69.07	0.32	0.55	0.44	39.00	76.00	89.63

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Tables 4.70-4.72 contain three groups of medium A-lines in the trials. All three groups were planted at Kroonstad, Lichtenburg and Delmas. Trials were successful at Kroonstad and Lichtenburg. *Sclerotinia* head rot infestation severely damaged yield at the Delmas locality. Flowering data were collected.

Table 4.70 Medium A-line trials (Group 1) using the R10 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 24.92	1 Lichtenburg 29.71	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.60	100	0.60	0.50	0.55	38.10	76.10	
1	PAN 7033	1.99	129.41	0.70	0.71	0.71	38.50	78.00	118.45
3	PAN 7049	1.92	141.78	0.70	0.84	0.77	39.10	78.00	114.29
4	A37	1.69	117.66	0.62	0.66	0.64	37.40	76.00	100.60
12	PAN 7351	1.68	132.56	0.71	0.74	0.72	41.90	77.00	
27	A30	1.46	102.18	0.50	0.61	0.55	38.00	77.00	86.90
60	A4	1.12	46.09	0.45	0.08	0.27	35.90	73.00	66.67

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.71 Medium A-line trials (Group 2) using the R10 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 37.14	1 Lichtenburg 34.68	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.80	100	0.64	0.58	0.61	37.40	0.00	
5	PAN 7033	2.08	128.80	0.60	0.95	0.77	37.50	0.00	113.66
14	A31	1.99	123.16	0.68	0.80	0.74	35.50	0.00	108.74
15	A13	1.98	117.25	0.75	0.68	0.71	35.90	0.00	108.20
26	PAN 7351	1.83	118.30	0.66	0.77	0.71	41.00	0.00	
42	PAN 7049	1.78	106.66	0.88	0.44	0.66	38.30	0.00	97.27
43	A16	1.71	86.88	0.71	0.37	0.54	39.60	0.00	93.44
51	A23	1.50	91.67	0.46	0.64	0.55	38.00	0.00	81.97

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.72 Medium A-line trials (Group 3) using the R10 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 36.99	1 Lichtenburg 37.28	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.80	100	0.53	0.86	0.69	37.70	0.00	
16	PAN 7049	2.07	110.98	0.50	1.10	0.80	38.20	0.00	108.38
20	PAN 7033	2.05	105.89	0.50	1.00	0.75	37.60	0.00	107.33
21	A26	2.03	108.32	0.55	0.97	0.76	38.80	0.00	106.28
24	PAN 7351	1.91	107.88	0.57	0.93	0.75	39.00	0.00	
52	HA89	1.71	87.09	0.59	0.54	0.56	39.90	0.00	89.53
56	A6	1.59	82.42	0.37	0.81	0.59	35.30	0.00	83.25

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Tables 4.73-4.75 contain the last group of A-lines which represents the late group. All three late trials were planted at Kroonstad, Lichtenburg and Delmas. For the first trial data from Kroonstad and Lichtenburg were used and flowering data collected. For the second late trial all three localities produced successful results. Flowering data were collected. For the third late trial the Lichtenburg locality was the only usable locality. Flowering data were collected.

Table 4.73 Late A-line trials (Group 1) using the R10 tester

Rnk	Hybrid	2 O/a Yield	% Yield	1 Kroonstad 33.71	1 Lichtenburg 38.52	2 Oil t/ha	2 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.80	100	0.45	0.83	0.64	38.40	0.00	
2	PAN 7049	2.26	142.22	0.74	1.00	0.87	40.00	0.00	154.79
3	A15	2.24	130.93	0.56	1.15	0.85	41.20	0.00	153.42
7	PAN 7033	2.08	125.50	0.65	0.88	0.77	38.00	0.00	142.47
27	A24	1.95	95.75	0.43	0.80	0.61	37.40	0.00	133.56
30	A25	1.84	98.08	0.38	0.92	0.65	40.90	0.00	126.03
35	A17	1.66	61.93	0.24	0.58	0.41	36.70	0.00	113.70
52	A9	1.64	76.49	0.28	0.75	0.51	36.50	0.00	112.33
58	PAN 7351	1.46	102.70	0.46	0.85	0.66	41.60	0.00	

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.74 Late A-line trials (Group 2) using the R10 tester

Rnk	Hybrid	3 O/a Yield	% Yield	1 Kroonstad 38.25	1 Lichtenburg 39.03	1 Delmas 36.48	3 Oil t/ha	3 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.90	100	0.51	0.79	0.69	0.66	37.60	75.90	
2	PAN 7033	2.30	129.48	0.67	0.99	0.90	0.85	37.60	76.00	123.66
3	PAN 7049	2.23	128.93	0.64	1.15	0.79	0.86	37.70	76.00	119.89
4	A39	2.13	113.44	0.58	1.05	0.64	0.76	36.90	72.00	114.52
24	PAN 7351	1.86	110.14	0.50	0.88	0.84	0.74	40.80	75.00	
41	A18	1.77	92.53	0.45	0.71	0.68	0.61	35.00	77.00	95.16
51	A22	1.62	83.25	0.51	0.51	0.59	0.53	38.90	74.00	87.10

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.75 Late A-line trials (Group 3) using the R10 tester

Rnk	Hybrid	1 O/a Yield	% Yield	1 Kroonstad 34.43	1 Oil t/ha	1 Oil cont	1 Flow days	% Yield Rel PAN 7351
	Treatments mean	1.60	100	0.62	0.62	42.40	77.80	
2	PAN 7049	2.19	82.46	0.51	0.51	41.90	79.00	141.29
15	A42(HO)	1.88	148.26	0.93	0.93	41.70	76.00	121.29
28	PAN 7033	1.78	113.99	0.71	0.71	41.50	80.00	114.84
34	PAN 7351	1.55	105.20	0.66	0.66	45.10	78.00	
36	A41(HO)	0.92	73.09	0.46	0.46	39.10	76.00	59.35

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

The A41(HO), A4, A10, A7, A23, A6, A38, A30, A22, A5, A8, A29, A36, A16 and A18 A-lines in combination with the R10 tester performed worse than the commercial check PAN 7351 when looking at overall yield. The A-lines A15, A34, A28, A35, A24, A12, A32, A25, A42(HO), A19, A39, A17, A9, A31, A13, A26 and A37 in combination with the R-line tester R10 performed better than the commercial check PAN 7351 when looking at overall yield. Flowering dates were captured on the one trial at the Delmas locality and ranged from 70 days for the A-line A7 in combination with R10 to 81 days for PAN 7049. Most of the South African A-line germplasm combinations with R10 expressed an average flowering date of 76.52 days. The oil content of the A-line combinations with R10 ranged from 35.00% for A18 to 43.60% for A7. The average oil content for all combinations was 38.51%. The R10 R-line produced combinations with low oil content in combination with the A-lines. There were exceptions such as is the case of A18 where the oil content of the A-line contributed more towards the final oil content of the combination. The R-line R10 tended to make the combinations with the A-lines later maturing than was the norm for other R-lines. The genetic distance varied from 0.458 between A12 and R10 to 0.687 between A17 and R10 (Table 4.76).

Table 4.76 Genetic distance and relative yield for each A-line combination with R10

A-line	Genetic distance	% Relative yield	% Oil content
A12	0.4580	132.59	37.50
A32	0.4885	128.50	38.70
A43(HO)	0.5163	No data	No data
A13	0.5248	108.20	35.90
A42(HO)	0.5253	121.29	41.70
A4	0.5333	66.67	35.90
A10	0.5424	72.12	40.60
A6	0.5455	83.25	35.30
A22	0.5455	87.10	38.90
A7	0.5463	81.25	43.60
A24	0.5472	133.56	37.40
A19	0.5472	118.13	41.10
A35	0.5497	141.48	39.20
A36	0.5545	90.16	40.70
A41(HO)	0.5556	59.35	39.10
A5	0.5784	87.98	40.70
A31	0.5885	108.74	35.50
A38	0.5885	86.01	37.70
A25	0.5936	126.03	40.90
A29	0.5946	89.63	39.00
A8	0.6091	89.53	39.90
A39	0.6107	114.52	36.90
A16	0.6133	93.44	39.60
A9	0.6224	112.33	36.50
A30	0.6248	86.90	38.00
A28	0.6291	141.48	38.10
A26	0.6339	106.28	38.80
A15	0.6487	153.42	41.20
A18	0.6500	95.16	35.00
A37	0.6609	100.60	37.40
A34	0.6827	145.93	36.80
A23	0.6869	81.97	38.00
A17	0.6870	113.70	36.70

As shown in Figure 4.10, relative yield from hybrids obtained from crosses made between R10 and 32 A-line females were plotted against the relevant genetic distances as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R10 are shown in Table 4.76. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield (R^2=0.0061; p=0.6705) and oil content (R^2=0.0317; p=0.3296) as well as between yield and oil content (R^2=0.0019; p=0.8129).

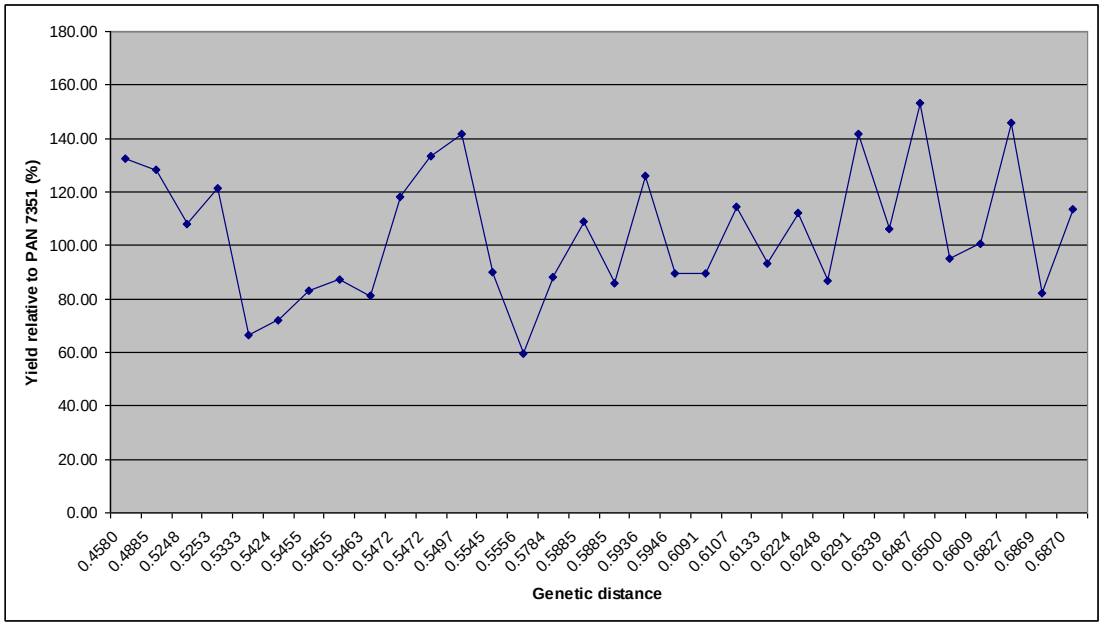

Figure 4.10 Relative yield versus genetic distance of hybrids obtained from crosses with R10 as a male tester crossed to 32 female A-lines.

4.4.1.11 R48 as tester on A-lines

R48 is the closest related to the group of A-lines. Table 4.77 shows the very early and Table 4.78 the early A-lines used in the trials. The very early group was planted at the Delmas, Villiers, Kroonstad and Klerksdorp locality with only the Klerksdorp locality being usable. No flowering data were collected. The early A-lines were planted at Delmas, Villiers, Kroonstad and Klerksdorp localities. The Delmas and Villiers localitites yielded successful trials. Flowering data were collected.

Table 4.77 Very early A-line trials using the R48 tester

	No. of Trials	1		1	1	1	0	
Rnk	Hybrid	O/a Yield	% Yield	Klerksdorp 24.17	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.53	100.00	2.53	0.98	38.76		
1	PAN 7351	3.80	150.34	3.80	1.45	38.20		
7	PAN 7355	3.40	134.49	3.40	1.26	37.10		89.47
10	A7	3.05	120.73	3.05	1.21	39.50		80.26
47	A10	2.00	79.21	2.00	0.76	38.00		52.63

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.78 Early A-line trials using the R48 tester

	No. of Trials	2		1	1	2	2	1	
Rnk	Hybrid	O/a Yield	% Yield	Delmas 15.41	Villiers 13.02	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.83	100.00	1.99	1.68	0.67	36.28	68.31	
1	A19	2.81	153.26	2.72	2.86	1.12	40.20	70.00	112.85
2	PAN 7355	2.63	143.15	2.35	2.83	0.97	37.75	69.00	105.62
4	PAN 7351	2.49	135.69	2.61	2.36	0.90	36.30	68.00	
9	A32	2.14	116.69	2.39	1.90	0.74	34.10	69.00	85.94
13	A34	2.07	112.82	2.65	1.55	0.74	35.15	71.00	83.13
20	A4	2.02	110.39	2.43	1.66	0.71	34.80	69.00	81.12
23	A37	1.97	107.26	1.88	2.01	0.69	35.50	68.00	79.12
29	A38	1.69	92.21	1.70	1.66	0.60	35.80	69.00	67.87
39	A5	1.47	80.22	1.66	1.29	0.52	35.20	68.00	59.04

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.79 includes the early-medium and Table 4.80 the medium A-lines as used in the trials. The early-medium trial was planted at Delmas, Villiers, Kroonstad and Klerksdorp with the Kroonstad locality yielding the only successful trial. No flowering data were collected. The medium trial was planted at Delmas, Villiers, Kroonstad and Klerksdorp. The Delmas and Villiers localities were successful. Flowering data were collected.

Table 4.79 Early-medium A-line trials using the R48 tester

	No. of Trials	1		1	1	1	0	
Rnk	Hybrid	O/a Yield	% Yield	Kroonstad 27.41	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	2.13	100.00	2.13	0.79	37.04		
7	A16	2.65	124.44	2.65	1.09	41.10		116.23
8	A31	2.59	121.66	2.59	1.04	40.20		113.60
18	PAN 7351	2.28	107.19	2.28	0.90	39.30		
21	A28	2.27	106.61	2.27	0.86	38.00		99.56
22	A36	2.27	106.56	2.27	0.83	36.50		99.56
23	A6	2.16	101.56	2.16	0.68	31.20		94.74
24	PAN 7355	2.16	101.18	2.16	0.84	39.10		94.74
29	A29	2.08	97.79	2.08	0.83	40.00		91.23
30	A35	2.08	97.73	2.08	0.63	30.20		91.23

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.80 Medium A-line trials using the R48 tester

	No. of Trials	2		1	1	2	2	1	
Rnk	Hybrid	O/a Yield	% Yield	Delmas 18.50	Villiers 11.33	Oil t/ha	Oil cont	Flow days	% Yield Rel PAN 7351
	Treatments mean	1.83	100.00	1.91	1.76	0.67	36.24	69.69	
2	PAN 7355	2.46	134.17	2.57	2.35	0.91	36.75	69.00	110.31
3	A15	2.38	129.64	2.42	2.33	0.92	38.90	72.00	106.73
5	A8	2.25	122.82	2.58	1.95	0.88	39.10	70.00	100.90
6	PAN 7351	2.23	121.57	2.32	2.14	0.77	34.35	68.00	
23	A13	1.93	105.12	2.07	1.79	0.70	36.15	68.00	86.55
25	A30	1.84	100.27	1.79	1.87	0.68	36.80	68.00	82.51

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.81 includes the medium-late and Table 4.82 the very late A-lines used in the trials. Both the medium-late and very late trials were planted at Delmas, Villiers, Kroonstad and Klerksdorp. In both instances the Delmas and Villiers localitites yielded successful results. Flowering dates were collected in both instances.

Table 4.81 Medium-late A-line trials using the R48 tester

	No. of Trials	2		1	1	2	2	1	
		O/a	%	Delmas	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	17.21	15.42	t/ha	cont	days	PAN 7351
	Treatments mean	1.85	100.00	1.98	1.71	0.69	36.96	68.90	
1	PAN 7355	2.54	137.32	2.60	2.46	0.91	35.75	70.00	117.59
8	PAN 7351	2.16	116.85	2.28	2.03	0.77	35.80	68.00	
9	A25	2.12	114.92	2.07	2.15	0.85	40.35	69.00	98.15
16	A22	2.00	108.28	2.11	1.88	0.76	38.25	71.00	92.59
17	A24	2.00	107.95	2.34	1.68	0.75	37.25	67.00	92.59
46	A39	1.46	78.79	1.68	1.25	0.51	34.65	70.00	67.59

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

Table 4.82 Very late A-line trials using the R48 tester

	No. of Trials	2		1	1	2	2	1	
		O/a	%	Delmas	Villiers	Oil	Oil	Flow	% Yield Rel
Rnk	Hybrid	Yield	Yield	30.36	20.52	t/ha	cont	days	PAN 7351
	Treatments mean	2.35	100.00	2.49	2.21	0.91	38.63	69.12	
5	PAN 7351	2.78	118.45	3.40	2.22	1.12	39.75	69.00	
8	PAN 7355	2.71	115.15	3.23	2.22	1.02	37.15	68.00	97.48
24	A18	2.31	98.11	2.56	2.07	0.92	39.40	70.00	83.09
28	A17	2.26	96.26	2.14	2.36	0.81	36.15	69.00	81.29

Rnk	Rank
O/a yield	Overall yield
Oil t/ha	Oil yield expressed in ton per hectare
Oil cont	Oil content expressed as a percentage
Flow days	Days counted until flowering
% yield rel PAN 7351	The performance of each variety relative to PAN 7351 expressed as a percentage

At the Delmas locality, severe instances of *Sclerotinia* stem and head rot were experienced and some trials had to be discarded. It is also possible that certain combinations performed better in environments with different heat units and rainfall.

The A-line females A10, A5, A39, A38, A37, A7, A4, A17, A30, A18, A34, A32, A13, A35, A29, A22, A24, A6, A25, A28 and A36 in combination with R48 performed worse than the commercial check PAN 7351 when looking at overall yield. The A-line females A8, A15, A19, A31 and A16 all performed better in combination with R48 than the commercial check PAN 7351 when looking at overall yield. Flowering dates were captured on the one trial at the Delmas locality and ranged from 67 days for the A-line A24 in combination with R48 to

72 days for A15. Most of the South African A-line germplasm combinations with R48 expressed an average flowering date of 69.07 days. The oil content of the A-line combinations with R48 ranged from 30.20% for A35 to 41.10% for A16. The average oil content for all combinations was 37.02%. The R48 R-line produced combinations with low oil content in combination with the A-lines. There are exceptions such as is the case of A25, A31, A19 and A16 where the oil content of the A-line contributed more towards the final oil content of the combination. The genetic distance varied from 0.491 between A22 and R48 to 0.797 between A23 and R48 (Table 4.83).

The relative yield from hybrids obtained from crosses made between R48 and 26 A-line females were plotted against the relevant genetic distances (Figure 4.11) as described in section 4.4.1.1. Certain crosses were not realised and zero values were found. The A-lines in order from smallest to largest genetic distance from R48 are shown in Table 4.83. Simple linear regressions were done to determine whether genetic distance was significantly correlated with yield. (R^2=0.1696; p=0.0362) and oil content (R^2=0.0493; p=0.2754) as well as between yield and oil content (R^2=0.1635; p=0.0405).

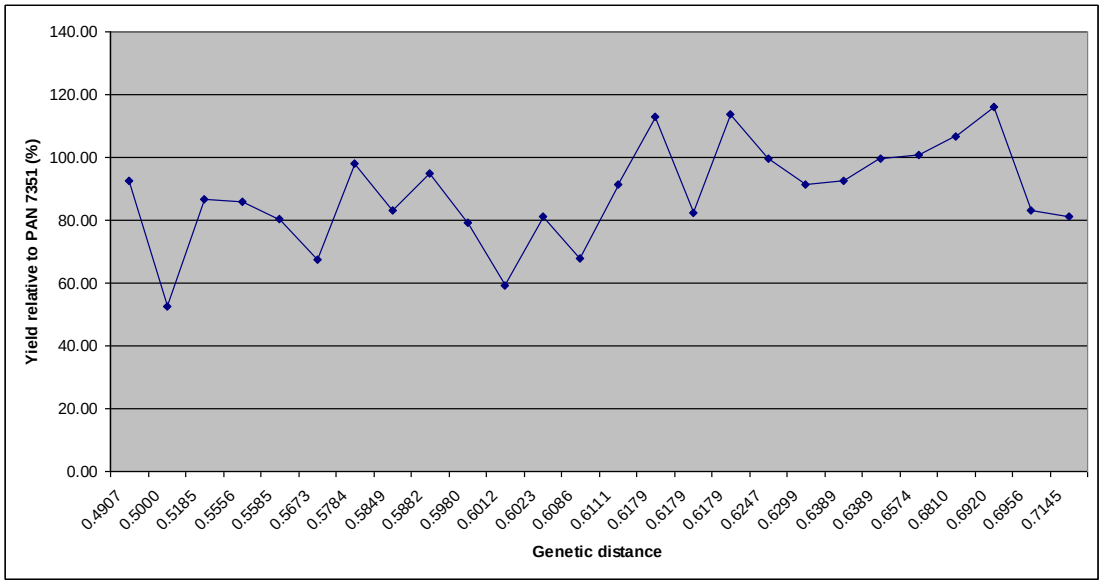

Figure 4.11 Relative yield versus genetic distance of hybrids obtained from crosses with R48 as a male tester with 26 female A-lines.

Table 4.83 Genetic distance and relative yield for each A-line combination with R48

A-line	Genetic distance	% Relative yield	% Oil content
A22	0.4907	92.59	38.25
A10	0.5000	52.63	38.00
A13	0.5185	86.55	36.15
A12	0.5189	No data	No data
A32	0.5556	85.94	34.10
A7	0.5585	80.26	39.50
A39	0.5673	67.59	34.65
A25	0.5784	98.15	40.35
A34	0.5849	83.13	35.15
A6	0.5882	94.74	31.20
A37	0.5980	79.12	35.50
A5	0.6012	59.04	35.20
A4	0.6023	81.12	34.80
A9	0.6061	No data	No data
A38	0.6086	67.87	35.80
A35	0.6111	91.23	30.20
A30	0.6179	82.51	36.80
A19	0.6179	112.85	40.20
A31	0.6179	113.60	40.20
A26	0.6222	No data	No data
A41(HO)	0.6226	No data	No data
A28	0.6247	99.56	38.00
A29	0.6299	91.23	40.00
A36	0.6389	99.56	36.50
A24	0.6389	92.59	37.25
A8	0.6574	100.90	39.10
A42(HO)	0.6792	No data	No data
A15	0.6810	106.73	38.90
A16	0.6920	116.23	41.10
A43(HO)	0.6923	No data	No data
A18	0.6956	83.09	39.40
A17	0.7145	81.29	36.15
A23	0.7970	No data	No data

Table 4.84 indicates the range of genetic distances between the A-lines and the 11 R-lines. The lowest and highest genetic distance is shown. The average of the ranges of the genetic distances calculated between the A-lines crossed to the 11 R-lines was found to be 0.24. No large differences were found between the lines involved in the crosses. The lowest genetic distance (0.44) was found between the A-lines and the R-line R29. The highest genetic distance (0.60) was found between the A-lines and the R-line R47(RM).

Table 4.84 Lowest and highest genetic distances of the A-lines crossed to 11 R-lines

R-line crossed to A-line females	Lowest genetic distance	Highest genetic distance	Range
R44(RM)	0.55	0.78	0.23
R34	0.48	0.70	0.22
R9	0.51	0.74	0.23
R13	0.51	0.77	0.26
R15	0.53	0.79	0.26
R11	0.55	0.76	0.21
R47(RM)	0.60	0.82	0.22
R32	0.50	0.73	0.23
R29	0.44	0.67	0.23
R10	0.46	0.69	0.23
R48	0.49	0.80	0.31
Average			0.24

4.4.2 Combining ability

4.4.2.1 General combining ability

Estimates for the GCA effects of the lines are given in Table 4.85 and the GCA effects of the testers are given in Table 4.86. The GCA of the parental line A34 was the best for relative yield. Other lines which showed a high GCA were A15, A25, A19, A22 and A16. The GCA of the parental lines A41(HO), A42(HO) and A43(HO) were the lowest for relative yield. All three of these lines are high oleic lines.

Table 4.85 General combining ability effects for relative yield in the lines

Line code	Relative yield GCA
A4	-1.49
A5	15.22
A6	4.89
A8	-10.30
A9	-13.42
A10	4.62
A7	-19.54
A12	-24.00
A41(HO)	-49.90
A42(HO)	-44.93
A43(HO)	-45.75
A13	-1.08
A15	32.57
A16	23.02
A17	4.34
A18	9.29
A19	27.14
A22	25.27
A23	7.90
A24	15.17
A25	29.51
A26	-0.23
A28	-2.32
A29	-16.69
A30	-21.82
A31	-8.68
A32	-4.87
A34	32.78
A35	-0.28
A36	-20.67
A37	22.48
A38	11.45
A39	20.33

For the tester lines the GCA of R10 was the highest for relative yield followed by line R29. The lines R13, R32 and R47(RM) were the lowest for GCA (Table 4.85).

According to Rahimi *et al*. (2010), lines with high positive and negative GCA effects could be used to make up crosses with competitive relative yield.

Table 4.86 General combining ability effects for relative yield parameter in the testers

Line code	Relative yield GCA
R9	1.75
R10	33.06
R11	12.34
R13	-29.53
R15	14.81
R29	30.35
R32	-33.35
R34	1.45
R44(RM)	-1.70
R47(RM)	-34.13
R48	4.95

4.4.2.2 Specific combining ability (SCA)

Estimates of SCA for the relative yield characteristic are listed in Appendix 4.

The hybrid A42(HO)/R32 was the best specific combination for relative yield. A6/R34 was the second best specific combination for relative yield. Two more high oleic female lines were also involved as good specific combiners for relative yield in the crosses A43(HO)/R9 and A42(HO)/R29. Crosses which performed well commercially such as A25/R34 (163.32 relative yield) and A38/R34 (145.63 relative yield) also had a good SCA.

4.4.2.3 GCA:SCA ratio

The GCA:SCA ratio was calculated for the relative yield characteristic was 0.99. The GCA:SCA ratio represents an indication of additive or non-additive gene action. The SCA variance was found to be higher than the GCA variance. This translates to the fact that a large part of the total genetic variability associated with the measured traits was the result of non-additive gene action. The ratios between the mean squares of GCA and SCA were found to be positive. The ratio was less than one and indicated that non-additive effects were more important than additive effects for relative yield. The low GCA:SCA ratio is the result of the relatively high SCA effects.

Table 4.87 Variances, standard errors and LSDs of genetic effects

Genetic effects	Variance	Standard error	LSD (0.05)
Difference between lines	0.011	0.106	0.209
Difference between testers	0.004	0.061	0.121
Difference between lines and testers	0.008	0.087	0.171
Difference between SCA	0.109	0.331	0.650
Difference between means	0.062	0.249	0.490
Difference between crosses	0.124	0.353	0.693

LSD Least significant difference

4.4.2.4 Heritability

The broad-sense and narrow-sense heritabilities were calculated for relative yield. The broad-sense heritability was found to be very high (99.99%). The narrow-sense heritability was found to be average (52.98%).

4.5 Discussion

Cheres *et al.* (2000) found that genetic distance could be a predictor of heterosis and resultant hybrid performance within and also between heterotic groups in sunflower. It was found that heterotic groups in sunflower were of significant use. Unfortunately, they also found that diversification was not as distinct as is the case in maize. Yue *et al.* (2009) utilised target region amplification polymorphism (TRAP) markers to determine genetic relationships as well as diversity between public sunflower lines released by the USDA. They were able to discriminate between lines and more importantly also group lines into female and male groups. They found that the data could be used by breeders to more successfully use the lines in question to make better yielding crosses and predict heterosis as was done by Cheres *et al.* (2000).

Sunflower hybrid breeding entails the crossing of a female fertile, male sterile A-line with a male fertile R-line. Pannar Seed (Pty) Ltd aims to keep the two groups separate with regard to heterotic groupings and not breed new lines from crosses between the two groups. This will ensure successful hybrid crosses between the two groups. This could however narrow the germplasm base and create the potential for having branching in the female lines and the hybrid. This study attempted to determine the relationship between genetic distance and seed yield. The relationship between genetic distance and oil content and the relationship between seed yield and oil content were also evaluated. Some lines had inherent characteristics such

as a major contribution towards traits such as high oil content (A25, A16 and R32) and can potentially increase the average oil content of hybrids they are involved in (according to pedigree knowledge and previous trials done with these lines). Crosses were attempted between as many elite A-and R-lines as possible and tested in yield trials.

Unfortunately not all crosses were successful and this led to limited data for some testers and could have distorted the results obtained. REML can compensate for missing plots, but cannot compensate for data where crosses have not been successful. It is however possible to look at results to see if there could be any correlation between genetic distance and yield, genetic distance and oil content and yield and oil content from the available data.

From the data combinations were found which outperformed commercial hybrids based on overall yield. This is the most important factor for South African farmers seeing that no premium is paid for higher oil content yet as is the case in South America. Although high yielding cultivars are in demand, trials are done for oil yield due to the fact that the situation in South Africa might change. New lines are developed yearly and it is important to include these lines in the dendrogram and determine genetic distance.

Specific locations might influence the outcome of trials. A location might have seasonal problems such as drought, *Sclerotinia* infections and even flooding. Severe *Sclerotinia* infections occur regularly in the eastern regions of South Africa and localities such as Delmas and Standerton tend to be more conducive for this type of infection. At the Villiers locality drought played a role in reducing yields. The Klerksdorp location had good rains and this can be seen from the good yields and excellent CV achieved in the trials planted there. Data from the other locations can then still be used in decision making with regard to choosing the best combination. It is important to keep on testing the successful selections to determine repeatability from season to season and location to location.

Later maturing lines or combinations tended to perform better in the field due to the fact that a full growing season could be taken advantage of. Earlier maturing combinations can furthermore also be influenced by external problems such as bird damage and therefore reduce yield. One example is where A7/R44(RM) had a flowering date of 57 days and overall yield of 2.01 t/ha and PAN 7355 had a flowering date of 69 days and an overall yield of 2.89 t/ha. The combination of A16/R44(RM) had a flowering date of 69 days and overall yield of

2.84 t/ha. This trend is generally found in sunflower where the later maturing combinations express higher yields, but exceptions do exist where the genetic influence of the line such as the combination of A29/R44(RM) had a flowering date similar to A16/R44(RM) but only expressed an overall yield of 1.81 t/ha.

According to the graphs drawn between relative yield and genetic distance and the correlation value, yield did not increase with increased genetic distance. Very low correlations were found between genetic distance and yield, between genetic distance and oil content and between relative yield and oil content with the exception of the R-line tester R32 crossed with the A-line females. R32 is known to have high oil content and contributed this characteristic to the combinations it was involved in. This R^2 value was the highest of all R^2 values obtained. The p-value for R32 crossed with the A-lines was 0.0226 which is $p<0.05$ and therefore indicated a significant correlation between relative yield and oil content. This line can be used to elevate the oil content of breeding combinations it is involved in. There were some crosses where $p<0.05$ and the correlations confirmed between genetic distance and relative yield and between relative yield and oil content. This was the case for the A-lines crossed to R48 where the p value was 0.0362 for genetic distance versus relative yield and the p value was 0.0405 for relative yield versus oil content. There does seem to be significant negative correlation between the before mentioned parameters for R48 crossed to the A-lines. The p value for relative yield versus oil content for the R-line R44(RM) crossed to the A-lines was found to be 0.0147 and therefore significant.

Results from this study did not correspond with some of the results found by Cheres *et al*. (2000). They tested 42 female by male (A x R) and 81 female by female (A x B) single cross hybrids. Genetic distance was significantly correlated with seed yield and plant height, but was a poor predictor of hybrid performance. Hybrid performance varied greatly among hybrids with similar genetic distances. In the current study crosses were only made between female by male (A x R) lines and genetic distance was found to be a poor predictor of relative yield. In future, crosses between female by female (A x B) or male by male (R x R) lines should be included in order to better determine whether more significant correlations exist between genetic distance and yield. This can assist in determining the diversity within the groups.

Results from this study however were consistent with results found in a later study done by Gvozdenović *et al*. (2009). A lower number of crosses were tested in their study (20 male lines crossed to three female lines). The lines were tested based on the fact that they were new to the programme. Genetic distance was estimated using SSR markers. Hybrids used in their study had the NuSun® midoleic trait. They found that a significant high correlation existed between genetic distance and mean oil percentage. The midoleic trait being shared between the lines, could have contributed to this. All lines had the same trait and only a limited number of lines were used. Similar to the current study, large genetic distance differences were found between male and female lines and smaller genetic distance differences within the male and within the female lines. They found genetic distance to be a poor predictor of heterosis. This was similar to results found in this study.

The low correlation between genetic distance and relative yield might be explained by the selection of lines crossed with each other and then evaluated for yield. Table 4.84 indicates the lowest and highest genetic distance as well as the range observed for the A-lines crossed to the 11 R-line testers.

The average of the ranges of the genetic distances calculated between the A-lines crossed to the 11 R-lines was 0.24. The possible problem with this value was that no large differences were found between the lines involved in the crosses. This could explain the low correlation between genetic distance and relative yield. More lines should be selected and crossed to get a better representation of the heterotic groups. On the other hand, this may be representative of what most breeding programmes will encounter.

A number of combinations have proven to be high yielding. This will lead to the selection of these lines for further elite testing as well as the further testing of the specific crosses in hybrid testing programmes. The highest yielding combination was A25 (a high yielding, high oil content line) with R34 (a high yielding, *Puccinia* resistant line). Both of these lines can now potentially be used as testers in their own right for new lines to be crossed with. Selections of other lines can be done in a similar fashion. Combinations such as A6 crossed with R34, A15 crossed with R10, A34 crossed with R10 and A38 crossed with R34 can be selected for further testing. R34 and R10 have proven to give good hybrids with the A-line females and can be considered in future for use as R-line male testers. A male line such as R47(RM) did not produce high yielding combinations, but combinations from this line can be

used in problematic downy mildew infested areas. A line such as R13 will in all likelihood not be used again as a male tester due to the fact that combinations with the A-line testers did not yield sufficiently. The female line A16 exhibited the capability for high oil content in most of the combinations it was involved in. The line ranked third with R44(RM) (43.80%), first with R34 (42.50%), first with R9 (45.00%), first with R13 (47.85%), second with R15 (41.30%), third with R11 (45.97%), third with R47(RM) (41.70%), first with R32 (45.60%), second with R29 (44.13%) and first with R48 (41.10%). The exception was with the male line R10 (39.60%) where the combination ranked tenth. A16 can be used to increase oil content in new line breeding introductions. This line was a good general combiner and advantageous for use in higher yielding combinations. South African oil expressors are critical of hybrids having an oil content below 40% and this line could help avoid possible penalties arising from low oil hybrids.

In the Pannar Seed (Pty) Ltd sunflower programme there are distinct R-line male groups and distinct female A-line groups. Lines are therefore bred to be different and pedigrees tend not to overlap between these groups. It is possible that an R-line as male parent in general might not be suited for a specific locality. It can be deduced that combinations made with the R-line in question could be good or bad in respect to the R-line's adaptability in a specific country or environment. It is also important to consider the background of the various lines when making combinations, seeing that lines obtained from the USA and used in South Africa might not perform as well as lines developed in South Africa. The dendrogram can therefore be a valuable tool in predicting the most successful crosses between R- and A-lines, but other factors such as maturity and genetic origin should be taken into account. The dendrogram used in this study can be used for the possible prediction of future crosses. This can be done through the selection of closely related lines for the development of new lines and, for instance, confirm whether lines from different heterotic groups are not incorrectly being used and in so doing mixing the heterotic groups. This can be combined with genetic distances to determine those crosses. The lines found in cluster A generally have a common line in their background (R41) and these lines are known to combine specifically well with lines from cluster J according to previous yield tests done not shown in this study. There are differences between lines in the clusters A and J and different combinations could be tested but it would be safe to assume that new lines which are introduced into these two groups have the potential to produce successful, good yielding hybrid combinations. It should be possible to group a new line into one of the clusters, for instance if a new line is accessed from the

USDA to determine where it cluster in relation to the other germplasm with a distinct USA background (clusters C, D, E and K). Cluster E contained the exception of the female A-line A24 which clustered with this set of R-lines. Cluster G contained two R-lines in the A-line cluster: R35 and R7. These three lines would most likely contribute poorly when used in hybrid combinations due to the fact that they cluster too closely to the heterotic parent group which they would not be expected to cluster in.

Differences were found in the GCA effects between parents for relative yield. Results indicated that the female A-line A34 was the best general combiner for relative yield. Lines A15, A25, A19, A22 and A16 were good general combiners as well. These lines could be used as sources for the development of new A-lines and developing new good general combiners. The data achieved from the GCA analysis confirmed known pedigree data that lines A19, A16, A25 and A34 are good general combiners. They are successful lines used in the Pannar Seed (Pty) Ltd programme. The R-lines R10 and R29 could be used to breed new R-lines with high GCA.

Differences were found for the SCA effects between the crosses for the relative yield characteristic measured. Lines involved in the crosses of A6/R34, A25/R34 and A38/R34 could be used to produce high yielding specific combinations. The A25/R34 hybrid was the highest yielding combination. Positive SCA effects were found for 194 of the crosses. Negative SCA effects were found for 169 of the crosses. Most of the successful combinations were attributed to SCA effects.

Results from this study showed that the SCA effect was greater which indicated non-additive gene action. Merinkovic (1993) found that non-additive gene effects controlled seed yield and Putt (1966) found that non-additive gene effects controlled the inheritance of among others, seed weight. Karasu *et al*. (2010) found that GCA effects needed to be stable to determine whether additive or non-additive played a greater role. They found in certain characteristics such as number of seeds per head, 1 000 seed weight and seed yield, non-additive effects were more important than other types of genetic effects. The same result was found for relative yield in this study.

Narrow-sense heritability is of importance to the plant breeder because it measures the relative significance of the additive portion of the genetic variance that can be transmitted to

the next generation of offspring. The narrow-sense heritability was found to be average (52.98%) for relative yield. The average narrow-sense heritability was caused by equal parts additive effects and dominant gene actions in the relative yield characteristic.

Since correlations between genetic distance and relative yield, genetic distance and oil content and relative yield and oil content were low, genetic distance in this study tended to be a poor predictor of seed yield in the A-line females crossed with R-line males. It is worthwhile to look at more lines and repeat some of the trials to determine an even more accurate set of data from the trials. Crossing the R-lines with R-lines and the B-lines with other B-lines through the use of gibberelic acid (or even B-lines onto A-lines) to determine whether enhanced diversity might be achieved within the groups could be an added option. Another option would be to select lines based solely on genetic distance and cross those with the largest genetic distance with each other as well as those with the smallest genetic distance. The resulting crosses could be evaluated in yield trials and a possible correlation could be established.

The lines used in this study basically clustered into two major groups namely the R-lines and the A-lines. In Pannar Seed (Pty) Ltd the breeders normally cross these two groups with each other. The resultant crosses normally produce competitive yielding hybrids which confirms the correct usage of the heterotic groups. The next step would be to attempt to increase the effectiveness or distinctness (diversity) of the lines within the groups which might lead to enhanced crosses between the groups.

4.6 References

Agrobase, 2000. User's Guide and Command Reference. Agronomic software Inc. R3N 054. Canada

Anscombe, F.J., 1948. The validity of comparative experiments. Journal of the Royal Statistical Society 111: 181-211.

Betrán, F.J., Ribaut, J.M., Beck, D. and Gonzalez de León, D., 2003. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. Crop Science 43: 797-806.

Camlin, M.S., 2000. Possible future roles for molecular techniques in the identification and registration of new plant cultivars. International symposium on molecular markers for characterizing genotypes and identifying cultivars in horticulture. Acta Horticulturae (ISHS) 546, Montpellier, France. pp. 289-296.

Chaudhary, R.C., 1982. Introduction to Plant Breeding. Oxford and IBH Publishing Co. PVT. LD. New Delhi-Bombay. pp. 267.

Cheres, M.T., Miller, J.F., Crane, J.M. and Knapp, S.J., 2000. Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. Theoretical and Applied Genetics 100: 889-894.

Cooke, R.J. and Reeves, J.C., 2003. Plant genetic resources and molecular markers: variety registration in a new era. Plant Genetic Resources: Characterization and Utilization 1: 81-87.

Crow, J.F., 1999. Dominance and overdominance. In: The genetics and exploitation of heterosis in crops. Eds. Coors, J.G. and Pandey, S. American Society of Agronomy and Crop Science Society of America, USA. pp. 49-58.

Diggle, P. J., 1992. Multivariate regression analysis for categorical data. Journal of the Royal Statistical Society 54: 28-29.

Fehr, W.R., 1987. Heritability. In: Principles of cultivar development: Theory and technique. Ed. Fehr, W.R. McMillan Publishing Company, New York. pp. 95-105.

Gvozdenović, S., Saftić-Panković, D., Jocić, S. and Radić, V., 2009. Correlation between heterosis and genetic distance based on SSR markers in sunflower (*Helianthus annuus* L.). Journal of Agricultural Sciences 54: 1-10.

Karasu, A., Oz, M., Sincik, M., Goksoy, A.T. and Turan, Z.M., 2010. Combining ability and heterosis for yield and yield components in sunflower. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38: 259-264.

Kelly, A.M., Cullis, B.R., Gilmour, A.R., Eccleston, J.A. and Thompson, R., 2009. Estimation in a multiplicative mixed model involving a genetic relationship matrix. Genetics Selection and Evolution 41: 1-9.

Leeuwner, D.V., 2005. Genotype x environment interaction for sunflower hybrids in South Africa. MSc dissertation. University of Pretoria. pp. 16-19.

McCulloch, C.E., 1997. Maximum likelihood algorithms for generalized linear mixed models. Journal of the American Statistical Association 92: 162-170.

Merinkovic, R., 1993. Combining ability of some inbred sunflower (*Helianthus annuus* L.) lines. The Indian Journal of Genetics and Plant Breeding 53: 299-304.

Miller, J.F., Hammond, J.J. and Roath, W.W., 1980. Comparison of inbred vs single-cross testers and estimation of genetic effects in sunflower. Crop Science 20: 703-706.

Paterson, S. and Lello, J., 2003. Mixed models: getting the best use of parasitological data. Trends in Parasitology 19: 370-375.

Payne, R.W., 2009. GenStat. Wiley Interdisciplinary Reviews: Computational Statistics 1: 255-258.

Putt, E.D., 1966. Heterosis, combining ability and predicted synthetics from a diallel cross in sunflowers (*Helianthus annuus* L.). Canadian Journal of Plant Science 46: 59-67.

Rahimi, M., Rabiei, B., Samizadeh, H. and Ghasemi, A.K., 2010. Combining ability and heterosis in rice (*Oryza sativa* L.) culitvars. Journal of Agricultural Science and Technology 12: 223-231.

Singh, R.K. and Chaudhary, B.D., 1979. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India. pp. 191-200.

Smith, J.S.C., Hoeft, E., Cole, G., Lu, H., Jones, E.S., Wall, S.J. and Berry, D.A., 2009. Genetic diversity among sunflower inbreds and hybrids: assessing probability of ancestry and potential for use in plant variety protection. Crop Science 49: 1295-1303.

Sprague, G.F., 1983. Heterosis in maize: Theory and practice. In: Heterosis. Ed. Frankel, R. Springer-Verlag, Berlin, pp. 48-70.

Sprague, G.F. and Tatum, L.A., 1942. General versus specific combining ability in a single cross of corn. American Journal of the Society of Agronomists 34: 923-932.

Tang, S., Yu, J.K., Slabaugh, M.B., Shintani, D.K. and Knapp, S.J., 2002. Simple sequence repeat map of the sunflower genome. Theoretical and Applied Genetics 105: 1124-1136.

Turkec, A. and Goksoy, A.T., 2006. Identification of inbred lines with superior combining ability for hybrid sunflower (*Helianthus annuus*) production in Turkey. New Zealand Journal of Crop and Horticultural Science 34: 7-10.

Yu, J., Mangor, J., Thompson, L., Edwards, K.J., Slabaugh, M.B. and Knapp, S.J., 2002. Allelic diversity of simple sequence repeats among elite inbred lines of cultivated sunflower. Genome 45: 652-660.

Yu, J., Tang, S., Slabaugh, M.B., Heesacker, A., Cole, G., Herring, M., Soper, J., Han, F., Chu, W., Webb, D.M., Thompson, L., Edwards, K.J., Berry, S., Leon, A.J., Grondona, M., Olungu, C., Maes, N. and Knapp, S.J., 2003. Towards a saturated molecular genetic linkage map for cultivated sunflower. Crop Science 43: 367-387.

Yue, B., Cai, X., Vick, B.A. and Hu, J., 2009. Genetic diversity and relationships among 177 public sunflower inbred lines assessed by TRAP markers. Crop Science 49: 1242-1249.

Zhang, L.S., Le Clerq, V., Li, S. and Zhang, D., 2005. Establishment of an effective set of simple sequence repeat markers for sunflower variety identification and diversity assessment. Canadian Journal of Botany 83: 66-72.

Chapter 5
Conclusions

An important process in sunflower breeding programmes is the identification of potential parental combinations to produce hybrids with superior yield. The development of hybrids could be expensive and time consuming. This limits the number of hybrids which can be tested from all available crosses. The use of genetic distances for the prediction of hybrid yields has been seen as a possible tool. The efficiency of hybrid breeding programmes could be enhanced if the inbred lines could be evaluated and the best crosses predicted prior to field evaluation. This study attempted to establish a dendrogram (through the use of SSRs) for commercial sunflower lines (A-, B- and R-lines) to determine the heterotic group layout in the Pannar Seed (Pty) Ltd germplasm (which represents a large variety of germplasm). It was also done to determine whether the groupings established in the dendrogram could be of value to be used as a predictor for the best performing combinations between A- and R-lines in the context of South African germplasm as well as to determine whether correlations exist between oil content and dry relative yield and genetic distance and oil content.

The genetic distances between A16, A23, A8 and R47(RM) were the largest as well as those between A23 and R48. The average genetic mean between the 49 R-lines was calculated to be 0.5129. The average genetic mean between the 44 female lines were found to be 0.5992. Two main clusters were established in the dendrogram, namely the R-line male group and the A- and B-line female group. The AMOVA data established that most of the diversity was attributed to variation within the populations. This indicated that the screened lines were very diverse. The genetic distance between A34 and R10 was one of the largest observed (0.683) in the current data set and these two lines also had the highest GCA values for relative yield. The combination with the highest SCA value [A42(HO)/R32] had a large genetic distance of 0.62.

Eleven elite, high yielding male R-lines were identified and crossed to 33 elite A-lines. No or small significant correlations were found between genetic distance and relative yield, between relative yield and oil content and between genetic distance and oil content. The average genetic distance of the selected lines were 0.24 and might explain the low correlation between crosses for genetic distance versus relative yield. A different result might have been

established if more genetically distant lines were used. The only significant correlations were found for the A-lines crossed with R48 and then for genetic distance versus relative yield as well as relative yield versus oil content, for the A-lines crossed with R32 for relative yield and oil content and for relative yield versus oil content for the A-lines crossed to R44(RM). The reason why this set of lines was selected was due to their capacity specifically for high yield and not for being genetically unrelated. The R-lines are more related and from similar backgrounds. A16 has been proven to be a successful contributor to high oil content seeing that in all the male combinations it was involved in, it had one of the highest oil rankings with the exception of the combination with R10.

The conclusion can be made that there was no relationship between genetic distance and relative yield, genetic distance with oil content and relative yield with oil content. It does seem to indicate that genetic distance was a poor indicator of relative yield in this set of material. The genetic distance data did however indicate relationships between the lines as shown in the dendrogram. The R-lines clustered together and the A- and B-lines clustered together. There were exceptions and the R-lines which clustered with the A-line group and vice versa would probably not make good combinations due to their genetic similarity within the specific cluster it was involved in. As expected, the R-lines clustered into one group and the A- and B-lines into the other which confirmed the groupings found in the genetic material of Pannar Seed (Pty) Ltd. Specific backgrounds also clustered together within the R-line and A- and B-line group. Examples of these include the R41 type lines and the high oleic lines. Previous studies also attempted to use SSRs to determine genetic distance in sunflower and establish correlations between genetic distance and heterosis. In these studies low correlations were found and suggestions were made to evaluate specific hybrid combinations and specific traits separately. This information could be used as a starting point for the make-up of potential crosses and this can be tested in yield trials. No time would be wasted in the making-up of crosses which are potentially low yielding. Crosses could be made within the R-line group and within the A- and B-line group to attempt to produce novel germplasm within the lines and the focus should be more about line development than hybrid development through the use of genetic distance data.

As was mentioned before, results of this study confirmed that genetic distance did not correlate with relative yield. Studies have shown in crops species such as maize, pepper,

alfalfa and wheat that a low correlation existed between genetic distance and yield or heterosis. Although genetic distance has been known to be a poor predictor of relative yield or heterosis, better results could be achieved if hybrid combinations for each tester and trait could be analysed separately. It will be possible to plan more field trials for sunflower heterotic performance if prior information on genetic distance of inbreds are used. Reliable genetic distances can be obtained by using more molecular markers. This set of markers should involve ones associated with quantitative trait loci for specific traits. It is important to note that the GCA and SCA of a number of inbreds have been studied and elite lines can now be selected to be used for the production of specific high yielding hybrids. These inbreds can be tested on larger plot sizes to confirm their yield potential and to determine their stability over different climatic conditions. Selected parents with desirable performance and combining ability can be involved in multiple crossing systems to recombine various productivity parameters. Parents such as A16 with superior oil content coupled with good yield performance and combining ability for other important economic traits could be included in different cross combinations to produce stable productive hybrids with enhanced oil quality for commercial release.

Summary

Breeders would prefer to be able to predict the outcome of crosses prior to the production and testing of lines derived from them in field trials. One way to do this would be to find a correlation between the genetic distances of inbreds and relative yield obtained from the hybrids. The aim of this study was to determine whether the SSR based dendrogram can be of value as a predictor for the best performing combinations between A- and R-lines in the context of South African germplasm as well to determine whether correlations existed between oil content and relative yield and genetic distance and oil content. The study analysed 93 inbred lines, consisting of 49 R-, 40 A- and four B-lines which were planted in a glasshouse at Greytown, KwaZulu-Natal (South Africa). Two experiments were performed on the sets of lines, namely SSR analysis to establish genetic distance and a dendrogram and the second was to establish yield trials with a subset of the lines. A dendrogram was constructed using 55 SSR markers widely distributed over the entire sunflower genome. The objective was to establish genetic distances of the lines involved in order to determine heterotic groups in the hybrid breeding programme. SSR cluster analysis separated lines into two main groups, one included A- and B-lines (females) and the other R-lines (males). The groupings represented the breeding history and basic heterotic pattern of sunflower. Genetic similarities were lower overall for female (A) line x restorer (R) line crosses than for A x A or R x R. The highest level of dissimilarity was found between A9 and A40 and the second highest between A23 and R8. The lowest level of dissimilarity was found between R14 and R41 and the second lowest between R42(HO) and R43(HO).

The second experiment included crossing of 33 A-lines to 11 R-lines to produce F_1 hybrid seed. However, not all crosses were successful. The successful hybrids were planted as lattice designs in a total of 133 trials on six locations. The yield of the hybrids was calculated and expressed relative to the yield of the commercial hybrid PAN 7351 which was included in all trials. Relative yield and oil percentage was calculated. The line x tester analysis was used to determine the combining ability of the inbred lines and to determine if there existed correlations between genetic distance and relative yield, between genetic distance and oil percentage and between relative yield and oil percentage. The highest yielding combination was A25/R34. Low to no correlations were found between genetic distance and relative yield, between genetic distance and oil percentage and between relative yield and oil percentage.

Exceptions were significant correlations found between relative yield and oil percentage for the A-lines crossed to R32, for genetic distance versus relative yield and for relative yield versus oil percentage for the A-lines crossed to R48 and for relative yield versus oil content for the A-lines crossed to R44(RM). The female line A16 exhibited high oil content in most of the combinations it was involved in. The average genetic distance for the lines involved in the yield trials was 0.24. This indicated low differences between the lines used and a possible reason for the low correlations found between combinations. Differences were found in GCA effects with A34 being the best general combiner for relative yield. The combinations A6/R34 and A25/R34 were the combinations with the best SCA effects for relative yield. According to the GCA:SCA ratio the SCA was greater which indicated non-additive gene action. Narrow-sense heritability was found to be 52.98% for relative yield. This was caused by equal parts additive effects and dominant gene actions.

Opsomming

Telers verkies om in staat te wees om die uitkoms van kruisings te voorspel voordat die toetsing en produksie van basters in die veld plaasvind. Een manier om dit te bewerkstellig is om `n korrelasie tussen genetiese afstande van ingeteelde lyne en opbrengs van basters verkry vanuit die lyne, te bepaal. Die doel van die studie was om te bepaal of `n mikrosatelliet gebaseerde dendrogram effektief gebruik kan word om die beste presterende kombinasies tussen A- en R-lyne binne die konteks van Suid-Afrikaanse kiemplasma te voorspel, asook om te bepaal of daar ‘n korrelasie bestaan tussen oliepersentasie en relatiewe opbrengs asook tussen genetiese afstand en oliepersentasie. Die studie het 93 ingeteelde lyne ingesluit, wat bestaan het uit 49 R-, 40 A- en vier B-lyne wat in `n glashuis in Greytown, Kwazulu-Natal (Suid-Afrika) geplant is. Twee eksperimente is op die stel lyne uitgevoer, naamlik mikrosatelliet analises om genetiese afstand te bepaal en `n dendrogram op te stel en tweedens is `n subset van hierdie lyne gebruik om opbrengsproewe te doen. `n Dendrogram is opgestel deur 55 mikrosatelliet merkers wat oor die hele genoom versprei is, te gebruik. Die doel was om genetiese afstande van die lyne te bepaal met die oog om heterotiese groepe in die baster teelprogram te bepaal. Mikrosatelliet analises het die lyne in twee hoofgroepe verdeel, met een groep wat die A- en B-lyne (wyfies) bevat het en die ander die R-lyne (mannetjies). Hierdie groeperings dui die telingsgeskiedenis asook die basiese heterotiese tendense van sonneblom aan. Genetiese ooreenkomste was oor die algemeen laer vir wyfie (A) lyn x hersteller (R) lyn kruisings as in die geval van A x A of R x R. Die kleinste ooreenkomste is tussen A9 en A40 gevind en die tweede kleinste tussen A23 en R8. Die grootste ooreenkomste is tussen R14 en R41 gevind en die tweede grootste tussen R42(HO) en R43(HO).

In die tweede eksperiment is 33 A-lyne met 11 R-lyne gekruis om F_1-bastersaad te produseer. Nie al die kruisings het egter gerealiseer nie. Die suksesvolle basters is in `n rooster ontwerp in 133 proewe oor ses lokaliteite geplant. Die opbrengs van die basters is bereken en relatief tot die opbrengs van die kommersiële baster PAN 7351, wat in al die proewe ingesluit is, uitgedruk. Relatiewe opbrengs asook oliepersentasie is bereken. Die lyn x toetser analise is gebruik om kombineervermoë van die ingeteelde lyne te bereken en om vas te stel of daar korrelasies tussen genetiese afstand en relatiewe opbrengs bestaan, tussen genetiese afstand en oliepersentasie asook tussen relatiewe opbrengs en oliepersentasie.Die kombinasie met die

beste opbrengs was A25/R34. Lae tot geen korrelasies is tussen genetiese afstand en relatiewe opbrengs, tussen genetiese afstand en oliepersentasie en tussen relatiewe opbrengs en oliepersentasie gevind. Uitsonderings was betekenisvolle korrelasies wat vir genetiese afstand teenoor oliepersentasie vir die A-lyne gekruis met R32 gevind is asook tussen genetiese afstand en relatiewe opbrengs en vir relatiewe opbrengs teenoor olie persentasie vir die A-lyne gekruis met R48. Betekenisvolle korrelasies is vir relatiewe opbrengs teenoor olie-opbrengs vir die lyne gekruis met R44(RM) gevind. Die wyfielyn A16 het hoë olieinhoud getoon in kombinasie met die R-lyne wat gebruik is. Die gemiddelde genetiese afstand van die lyne wat ingesluit is in die opbrengsproewe was 0.24. Die waarde dui op kleiner verskille tussen die lyne en is `n moontlike rede vir die lae korrelasies tussen die kombinasies. Verskille is vir die GCA effekte met A34 gevind wat die beste algemene kombineerder vir relatiewe opbrengs was. Die kombinasies A6/R34 en A25/R34 was die kombinasies met die beste SCA effekte vir relatiewe opbrengs. Indien die GCA:SCA verhouding in ag geneem word, was SCA groter wat op nie-additiewe geenaksie dui. Nou-oorerfbaarheid is bereken as 52.98% vir relatiewe opbrengs. Dit word deur gelyke dele additiewe effekte en dominante geenaksies veroorsaak.

Appendices

Appendix 1: Primer sequences of primers used in the study

Primer	Forward primer sequence	Reverse primer sequence
ORS 297	TGCAAAGCTCACACTAACCTG	GTGTCTGCACGAACTGTGGT
ORS 307	CAGTTCCCTGAAACCAATTCA	GCAGTAGAAGATGACGGGATG
ORS 316	TGGCGTCTTCATAGCATCAG	GAGATTTGAGCTTCGTGTTGC
ORS 331	TGAAGAAGGGTTGTTGATTACAAG	GCATTGGGTTCACCATTTCT
ORS 342	TGTTCATCAGGTTTGTCTCCA	CACCAGCATAGCCATTCAAA
ORS 366	AACCAACTGAGCATTCTTGTGA	GCGCTAGGTTAAAGAGGACAAA
ORS 371	CACACCACCAAACATCAACC	GGTGCCTTCTCTTCCTTGTG
ORS 381	CCAACGGTGATGTAACTAGGAA	GTTCTCCTGGATAGCTCGACA
ORS 407	TGGCTAGGATTGCTTCATCA	TTTGCTTGCGCTTCTTACCT
ORS 420	TCATGGTGTTTGGTTTGTGTC	TGCCAAATTCCTCTTCTTTCT
ORS 428	TGCATTCATGATCAAAGGTTG	CATCACATCATTATCATCTCATCATG
ORS 437	GACGTCTTCACAGTTCAAATAACG	GCATCGACTCTGTTCTTCTCG
ORS 456	CCAAGGAATTCTAACAAGAGTTTAAG	GATTTCTCACTTCACTCCTCTATGC
ORS 457	TGCATACCCAATCTACCAGCTA	AAGACGAAGGTGCAACCAGT
ORS 483	CCGAACAACAATCTCCACAA	GGTTTAGGTGTCGCATCACA
ORS 502	ATCCCAACAGACGCCATTAT	AACATTGGAGGGAGCCAATA
ORS 505	GTGCGTTGGCTCTTATGGAT	AGTGATGGCATTCCCAATTT
ORS 543	CCAAGTTTCAGTTACAATCCATGA	GGTCATTAGGAGTTTGGGATCA
ORS 561	CTTTGCACGTTGGTCATCAT	ACCAGCACCTTCCTCAACTG
ORS 621	CGCCTTATGCTGAGAGGAAA	CCTGAAGCGAAGAAGAATCG
ORS 630	TGTGCTGAGGATGATATGCAG	GCACGACCCGGATATGTAAC
ORS 650	TTAAGGGAAGCGTGATCTTCA	CCCATGATTGAGTTAGGGTGTT
ORS 656	TCGTGGTAAGGGAAGACAACA	ACGGACGTAGAGTGGTGGAG
ORS 665	GCACATGAGGTATGGATCTCCT	TGCAAATACAACTCGGGAAA
ORS 674	ACATGAGGGCAAAACAGACA	GCACAAAGACAACCACACCA
ORS 687	ACCGTTACACTTATTGGTTATTTCATT	GGGGTTTGTTGTTCTGTTTTG
ORS 716	CCCCACAACCCATAGCCTAA	GAACTAACCGCCATCCAAGA
ORS 733	TATGAGTTGGCAAGGGCTTC	GGACTCCAACGAGAGAATCAGT
ORS 735	TGCTAACCTGAAACCCCATC	ACTGAAAAAACAGAACAAGGAGGT
ORS 750	TCCATACCCACTGGCTGGAT	TGGAAGGATACTCTTTGCGTTG
ORS 761	GGTGCCGGTGTTTCTCTTAC	ACGGTTGCGATCACTTGTTA
ORS 778	CAACCAATCAATCCCACAAA	TGTTCACGCTTACACACATAATTG
ORS 785	CAAAATACCCAGGTCAAAGCA	CCTAGCTTATGGGACGTATGGA
ORS 837	TGAAGGGCAATGGGATAGAAATA	TGAGATTTAGGTAGCGTGCAGAC
ORS 857	ACATCCGAACGAAGGACAATC	CAAGAAAGTATGTCACCCAATAGCA
ORS 878	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC
ORS 885	AATGTAAGCCAGAATCCCAAATG	CCTTCGTCGTTTCCAAGTAGATT
ORS 887	TCGAAAACGACTAATCCAACTTTC	GAGCATGAACAAGAATTGACACA
ORS 894	TTTCCTCATGATCCCGATTCTAT	TGCATTACCTAATTTCTAGTGGGTTT
ORS 925	ATGATTCTAAGTTGCGGTAGTGC	GTTGGGTTTAAGTTGTTGCTTCC
ORS 938	ACCAACTCCCATGCAACCTAA	GCGTTCTCACCGTTCTAACACTT
ORS 1024	GGGAAGTGGGCTTGTCTATGTAT	AACACACCGAAATCACCTATGAA
ORS 1036	CCCTTTCACTTCCTATTTTCTATTCA	CTAAGAGGGGTCGGTATGATTTC
ORS 1041	AAACAAACCTTAATGGGGTCGTA	ATATTGGCTGGTTGATGCTGAT
ORS 1065	ACCGCTGTCAACACCTTAAACTC	GGCTGGGAATCAACTGCTACTAC
ORS 1079	TACGACTGACGATTCCATTTCTC	AACTGGATTTCACAGGGAGTGTT
ORS 1085	GACCTCAAGGCATGCTAACACTC	ACTAAGTGTGTGGACGGGGAAA
ORS 1114	AGATGGTGGCAGGAGAGTTAAAG	GCAGAAACAGATCAGGAGGGTAT
ORS 1141	CGCATATGGGAACGTATACACAC	TGAAACTGAACACAAGGCATACA
ORS 1161	CAACTACGTCACGATACTCGCC	GGAGCTGAAGCTGAAGACAAATC
ORS 1179	GATTCGGAGCTGTTAGGAGGTAG	AAACGGGAAGCAAGAATAGAACA
ORS 1222	GGCATTGTTGTCATTTCATCTCT	ATCATGTCGGAATAGCTTGTTGA
ORS 1227	TTCATCCAAATGCCTAACCAAGT	ACTCTTCATCTGCCATCACCAT
ORS 1245	GAAGTGGAGCAATGTTGGTGA	CGCCAAGATATTAGTGTGATGATT
ORS 1248	TGTCCGATCTACCATCTGAAATC	TTAGAGCGAAATCTAGTTACATGAGTG

Appendix 2: Rogers distance matrix of the 93 inbred sunflower lines

Line code	A1	A2	A3	A4	B4
A1					
A2	0.4974				
A3	0.1920	0.4904			
A4	0.6415	0.5550	0.6859		
B4	0.6364	0.6012	0.6920	0.1111	
A5	0.5371	0.6082	0.6125	0.4288	0.4327
A6	0.6124	0.5273	0.6712	0.0341	0.0673
A7	0.5635	0.3628	0.6082	0.6415	0.5506
A8	0.3771	0.4057	0.4444	0.6859	0.6612
A9	0.4950	0.5163	0.5000	0.6526	0.6406
A10	0.5992	0.3523	0.6818	0.6375	0.6818
R1	0.6734	0.6012	0.7290	0.6222	0.6364
R2	0.6154	0.5918	0.6271	0.6192	0.5860
R3	0.7401	0.7094	0.7197	0.6163	0.6745
R4	0.6457	0.6154	0.6944	0.7526	0.7383
R5	0.6265	0.5654	0.6913	0.6458	0.6466
R6	0.6987	0.6176	0.7290	0.7718	0.7200
R7	0.5531	0.5446	0.5648	0.5444	0.5521
A11	0.6020	0.5510	0.6633	0.1585	0.2500
A12	0.5163	0.4623	0.5472	0.4886	0.4907
B41(HO)	0.4314	0.4038	0.4811	0.4091	0.3981
B42(HO)	0.3679	0.3654	0.4623	0.5538	0.5716
B43(HO)	0.3942	0.3725	0.4904	0.5651	0.5824
R8	0.7145	0.6320	0.7642	0.5667	0.5809
R9	0.6931	0.6102	0.7239	0.6674	0.6895
R10	0.6574	0.5069	0.7315	0.5333	0.5885
R11	0.7333	0.6248	0.7075	0.5568	0.6484
R12	0.6717	0.6100	0.7247	0.5680	0.5781
R13	0.7256	0.5588	0.7500	0.5581	0.5729
R14	0.6931	0.6863	0.7428	0.6674	0.6673
R15	0.7566	0.6538	0.7685	0.6189	0.6247
R16	0.7265	0.6390	0.7636	0.6415	0.6521
R17	0.7230	0.7420	0.7807	0.6100	0.6365
R18	0.6648	0.6569	0.6956	0.5568	0.5189
R19	0.6786	0.6908	0.7471	0.5000	0.5258
R20	0.6524	0.6077	0.7265	0.5911	0.5456
R21	0.6030	0.6489	0.6814	0.6347	0.6235
R22	0.6827	0.6320	0.7315	0.7222	0.7475
R23	0.7148	0.6801	0.7568	0.6748	0.7012
R24	0.6956	0.6667	0.7264	0.6477	0.6698
R25	0.6339	0.6107	0.6836	0.6106	0.6339
R26	0.6031	0.6124	0.5893	0.6530	0.6503
R27	0.6710	0.6872	0.7265	0.6274	0.6339
R28	0.6845	0.6648	0.7197	0.6859	0.7224
R29	0.6481	0.5566	0.7222	0.5222	0.5455
R30	0.5585	0.5679	0.6717	0.5848	0.6154
R31	0.5364	0.5429	0.5969	0.5970	0.5944
R32	0.5421	0.5551	0.5541	0.5682	0.6111
R33	0.6086	0.5660	0.6759	0.5222	0.5545
R34	0.5969	0.5755	0.6827	0.6304	0.6042
R35	0.5569	0.5258	0.6242	0.5667	0.4794
R36	0.7037	0.5566	0.6852	0.5111	0.5703
R37	0.7197	0.6538	0.7500	0.6556	0.6389
R38	0.5893	0.6542	0.6201	0.5590	0.5773
R39	0.6012	0.5824	0.6987	0.6214	0.5969
R40	0.6592	0.6321	0.6339	0.6103	0.5812
A13	0.6339	0.5729	0.6895	0.4556	0.4091

A14	0.5600	0.6735	0.6000	0.6628	0.7157
A15	0.5072	0.5473	0.5856	0.6302	0.6077
A16	0.5043	0.5352	0.5160	0.6829	0.6769
A17	0.5446	0.6250	0.5849	0.7159	0.6389
A18	0.5069	0.5865	0.5472	0.7159	0.6204
A19	0.6271	0.5163	0.6271	0.5889	0.5091
A20	0.4641	0.4852	0.5019	0.5621	0.6129
A21	0.4512	0.5481	0.5185	0.5444	0.5648
A22	0.4142	0.3748	0.5068	0.5859	0.5157
R41	0.6987	0.6923	0.7290	0.6748	0.6734
R42(HO)	0.6528	0.6765	0.7522	0.6416	0.5893
R43(HO)	0.6592	0.6923	0.7568	0.6526	0.6086
R44(RM)	0.7055	0.6487	0.7728	0.6466	0.6500
R45	0.6296	0.6484	0.6667	0.6859	0.6769
R46	0.6487	0.7373	0.6993	0.6477	0.6609
R47(RM)	0.6750	0.7673	0.7256	0.6674	0.6417
A23	0.4753	0.4659	0.5543	0.7093	0.7500
A24	0.6617	0.5635	0.6364	0.6222	0.6364
A25	0.4706	0.4700	0.5294	0.5879	0.5840
A26	0.4092	0.4692	0.4024	0.6022	0.5654
A27	0.4597	0.4608	0.4528	0.5568	0.6415
A28	0.5599	0.5729	0.6271	0.6163	0.5679
A29	0.5558	0.5465	0.6170	0.7761	0.7420
A30	0.5346	0.4717	0.5093	0.6859	0.6157
A31	0.5068	0.4717	0.4630	0.6304	0.6157
A32	0.5278	0.4340	0.6019	0.4637	0.4339
A33	0.5359	0.4902	0.5481	0.6560	0.6956
A34	0.6698	0.4528	0.6509	0.4742	0.4420
A35	0.6019	0.5472	0.6574	0.5748	0.4885
A36	0.4327	0.4245	0.4630	0.5748	0.5157
A37	0.6275	0.5673	0.5686	0.2857	0.2885
A38	0.6296	0.5755	0.6481	0.1444	0.1455
A39	0.5918	0.6444	0.6295	0.4318	0.3962
R48	0.7075	0.5962	0.6887	0.6023	0.5833
R49	0.6513	0.5541	0.7371	0.4052	0.4745
A40	0.7116	0.6780	0.7524	0.5000	0.4898

Line code	A5	A6	A7	A8	A9
A5					
A6	0.3869				
A7	0.5654	0.5814			
A8	0.6432	0.6320	0.5716		
A9	0.7030	0.6102	0.5784	0.2727	
A10	0.6447	0.6310	0.4515	0.4545	0.4825
R1	0.6129	0.5865	0.6549	0.6612	0.7042
R2	0.6333	0.5788	0.6710	0.5976	0.6745
R3	0.6260	0.6339	0.7055	0.7927	0.7200
R4	0.5962	0.7006	0.6956	0.6759	0.7358
R5	0.5777	0.6210	0.5296	0.6175	0.7151
R6	0.6660	0.7012	0.5901	0.6000	0.6860
R7	0.6753	0.4974	0.5506	0.5545	0.4455
A11	0.4373	0.2041	0.5279	0.6300	0.6473
A12	0.5228	0.4385	0.5352	0.5556	0.5784
B41(HO)	0.4949	0.3431	0.4906	0.4352	0.4790
B42(HO)	0.5962	0.4974	0.4717	0.3426	0.4049
B43(HO)	0.6102	0.5170	0.4811	0.3302	0.4409
R8	0.5773	0.5622	0.6481	0.7685	0.7568
R9	0.5396	0.6490	0.6956	0.6549	0.7358
R10	0.5784	0.5455	0.5463	0.6091	0.6224
R11	0.6679	0.5846	0.6346	0.6509	0.6698
R12	0.5820	0.5397	0.5980	0.6437	0.6987
R13	0.6173	0.5373	0.6487	0.7428	0.7402
R14	0.6269	0.6246	0.7064	0.7805	0.8037
R15	0.7660	0.6150	0.7119	0.7105	0.7889
R16	0.6432	0.6224	0.7265	0.7133	0.7539
R17	0.6798	0.6115	0.6882	0.8000	0.7878
R18	0.5403	0.5073	0.5500	0.7145	0.6742
R19	0.5666	0.4946	0.6217	0.7471	0.7257
R20	0.5773	0.5555	0.5918	0.6457	0.6944
R21	0.5508	0.6089	0.5684	0.5599	0.6599
R22	0.6314	0.6908	0.6604	0.7315	0.7870
R23	0.6220	0.6418	0.7402	0.8191	0.7679
R24	0.5884	0.6073	0.7090	0.7830	0.7874
R25	0.5061	0.5830	0.6786	0.6524	0.6938
R26	0.5877	0.6266	0.5596	0.6717	0.6786
R27	0.6477	0.6098	0.6905	0.7130	0.7012
R28	0.5666	0.6846	0.6920	0.6769	0.7339
R29	0.5809	0.5359	0.6574	0.5091	0.6430
R30	0.5364	0.5902	0.6245	0.4487	0.6339
R31	0.5509	0.6000	0.5679	0.6037	0.7173
R32	0.5044	0.5856	0.6245	0.5438	0.5969
R33	0.5716	0.5070	0.6457	0.6455	0.6430
R34	0.5901	0.5743	0.6247	0.5976	0.6200
R35	0.5944	0.4974	0.4975	0.5038	0.5157
R36	0.6987	0.5096	0.6247	0.7157	0.7248
R37	0.6270	0.5954	0.6578	0.7315	0.7543
R38	0.5474	0.5493	0.6391	0.6578	0.6365
R39	0.5122	0.5711	0.5203	0.6381	0.7151
R40	0.5734	0.5737	0.5481	0.7133	0.6200
A13	0.3241	0.4493	0.5481	0.6769	0.7472
A14	0.5700	0.6667	0.6673	0.5490	0.6248
A15	0.5170	0.5734	0.4849	0.5429	0.6147
A16	0.6271	0.6487	0.5321	0.3612	0.4703
A17	0.6509	0.6863	0.5987	0.5185	0.5969
A18	0.6509	0.6667	0.5610	0.4815	0.5599
A19	0.5136	0.5000	0.4950	0.5430	0.6315
A20	0.5044	0.5607	0.4213	0.5456	0.6080

A21	0.5138	0.5170	0.6176	0.5741	0.5969
A22	0.4883	0.5070	0.3771	0.3157	0.4679
R41	0.6151	0.6320	0.7119	0.7846	0.8074
R42(HO)	0.6243	0.5420	0.6391	0.7591	0.8012
R43(HO)	0.6408	0.5634	0.6578	0.7753	0.8166
R44(RM)	0.6408	0.6071	0.6691	0.7543	0.7771
R45	0.6222	0.6365	0.6827	0.5818	0.6430
R46	0.5875	0.6046	0.7134	0.7955	0.7423
R47(RM)	0.6169	0.6046	0.6820	0.8218	0.7493
A23	0.6940	0.6744	0.6556	0.2935	0.4565
A24	0.6617	0.5936	0.7055	0.5521	0.5679
A25	0.5170	0.5173	0.4287	0.5577	0.5840
A26	0.5642	0.5307	0.5228	0.3273	0.3339
A27	0.5769	0.5700	0.5743	0.4340	0.5094
A28	0.6407	0.5910	0.5784	0.5248	0.6018
A29	0.6292	0.7284	0.6320	0.4846	0.6820
A30	0.6592	0.6032	0.5809	0.2636	0.3248
A31	0.6086	0.5840	0.5809	0.2455	0.3727
A32	0.5851	0.4301	0.4975	0.5000	0.5406
A33	0.4852	0.6346	0.5814	0.4057	0.5232
A34	0.5413	0.4385	0.5704	0.6204	0.6617
A35	0.5666	0.4948	0.4790	0.5727	0.6067
A36	0.5944	0.5359	0.3864	0.3273	0.4182
A37	0.5019	0.2755	0.6542	0.6154	0.6705
A38	0.4302	0.1154	0.6086	0.6545	0.7067
A39	0.2955	0.4373	0.5596	0.7050	0.7471
R48	0.6012	0.5882	0.5585	0.6574	0.6061
R49	0.5271	0.4128	0.6432	0.6031	0.6333
A40	0.3903	0.4652	0.6222	0.8136	0.8721

Line code	A10	R1	R2	R3	R4
A10					
R1	0.5568				
R2	0.6220	0.6339			
R3	0.5992	0.3364	0.6381		
R4	0.6788	0.5346	0.4765	0.6037	
R5	0.6469	0.5266	0.5448	0.5036	0.4067
R6	0.6416	0.6067	0.6084	0.4885	0.3771
R7	0.4939	0.5545	0.5927	0.6067	0.7938
A11	0.5467	0.6900	0.5873	0.7346	0.7143
A12	0.5000	0.6944	0.4673	0.7080	0.7453
B41(HO)	0.4767	0.6759	0.6061	0.7105	0.7170
B42(HO)	0.4767	0.6271	0.5253	0.7358	0.6981
B43(HO)	0.5000	0.6201	0.5163	0.7402	0.7115
R8	0.6530	0.6019	0.4185	0.5994	0.4692
R9	0.5953	0.5556	0.4648	0.5969	0.4786
R10	0.5424	0.5133	0.5563	0.6430	0.5599
R11	0.5984	0.5635	0.6082	0.6107	0.5446
R12	0.6031	0.5577	0.5758	0.6175	0.4950
R13	0.6040	0.6226	0.4006	0.5918	0.3942
R14	0.6713	0.5918	0.4245	0.5987	0.3679
R15	0.6447	0.6549	0.3796	0.6129	0.5566
R16	0.6757	0.6521	0.4248	0.6836	0.4327
R17	0.7434	0.6705	0.4852	0.6557	0.4852
R18	0.5899	0.5189	0.5302	0.5069	0.5729
R19	0.6211	0.5635	0.5371	0.4761	0.5799
R20	0.5621	0.5599	0.5179	0.5876	0.5784
R21	0.5810	0.5084	0.4515	0.5654	0.5567
R22	0.6757	0.5278	0.5531	0.6111	0.4234
R23	0.6985	0.6271	0.4648	0.5228	0.4444
R24	0.6713	0.6132	0.4151	0.5824	0.3774
R25	0.6045	0.5901	0.4142	0.6154	0.5283
R26	0.6064	0.6082	0.6434	0.5232	0.5824
R27	0.6727	0.5901	0.4530	0.5160	0.4950
R28	0.7068	0.5430	0.6109	0.6133	0.4487
R29	0.5083	0.5636	0.4769	0.7248	0.5463
R30	0.5818	0.5043	0.5438	0.6247	0.5232
R31	0.6159	0.5648	0.4673	0.6364	0.5228
R32	0.5434	0.6296	0.5278	0.6734	0.5824
R33	0.5879	0.6273	0.5157	0.6224	0.5926
R34	0.5197	0.5067	0.5157	0.6291	0.5000
R35	0.5538	0.7219	0.5497	0.7976	0.5901
R36	0.5227	0.5455	0.4703	0.6067	0.4722
R37	0.6477	0.6204	0.4327	0.5969	0.4444
R38	0.7263	0.5842	0.5302	0.6151	0.4220
R39	0.6272	0.4878	0.5636	0.5920	0.4117
R40	0.6705	0.5945	0.5521	0.6308	0.5068
A13	0.6477	0.7182	0.5836	0.6951	0.6920
A14	0.6750	0.5490	0.7327	0.7006	0.7000
A15	0.7263	0.6147	0.7878	0.7301	0.6418
A16	0.5879	0.6951	0.7769	0.7472	0.6549
A17	0.5519	0.6574	0.6524	0.6777	0.6509
A18	0.5201	0.6574	0.6339	0.6710	0.6509
A19	0.6674	0.6182	0.5109	0.7042	0.7037
A20	0.5984	0.6703	0.6660	0.6796	0.6503
A21	0.6220	0.6759	0.6154	0.6777	0.6759
A22	0.5341	0.6612	0.6109	0.7679	0.6574
R41	0.6788	0.5994	0.4167	0.5876	0.3796
R42(HO)	0.6597	0.5446	0.3748	0.5893	0.3962
R43(HO)	0.6674	0.5648	0.3957	0.5969	0.4074

R44(RM)	0.6500	0.5691	0.4605	0.6129	0.3426
R45	0.6530	0.6248	0.5357	0.7042	0.4395
R46	0.6395	0.5000	0.5045	0.3917	0.4904
R47(RM)	0.6798	0.5455	0.4993	0.4179	0.5743
A23	0.5467	0.6848	0.6819	0.7717	0.6848
A24	0.5455	0.5818	0.5248	0.5769	0.6457
A25	0.5732	0.6224	0.6993	0.6968	0.6373
A26	0.5651	0.6497	0.6109	0.6745	0.6154
A27	0.5116	0.7170	0.6012	0.7214	0.6887
A28	0.6220	0.6042	0.5472	0.6993	0.6061
A29	0.6246	0.6446	0.5693	0.7220	0.6040
A30	0.5909	0.7248	0.6200	0.7497	0.7290
A31	0.6023	0.7067	0.6018	0.7315	0.6827
A32	0.5114	0.6885	0.4976	0.7381	0.6852
A33	0.6397	0.7333	0.6553	0.7729	0.7115
A34	0.5424	0.6271	0.5876	0.7030	0.6792
A35	0.6705	0.7067	0.6679	0.7381	0.7222
A36	0.5682	0.6612	0.6273	0.7860	0.7315
A37	0.7955	0.6923	0.4974	0.7205	0.7157
A38	0.7386	0.5885	0.5563	0.6993	0.6710
A39	0.6597	0.6792	0.5824	0.6245	0.7145
R48	0.5000	0.6944	0.5741	0.7636	0.5849
R49	0.5621	0.5783	0.4993	0.6654	0.5246
A40	0.6625	0.6224	0.6299	0.6374	0.6911

Line code	R5	R6	R7	A11	A12
R5					
R6	0.3175				
R7	0.6745	0.7612			
A11	0.6539	0.7246	0.5973		
A12	0.6283	0.7012	0.5068	0.3900	
B41(HO)	0.6382	0.6457	0.4815	0.3367	0.4057
B42(HO)	0.5962	0.6944	0.4630	0.4898	0.3113
B43(HO)	0.6075	0.6792	0.4811	0.5000	0.3173
R8	0.5617	0.5160	0.6296	0.4667	0.5163
R9	0.5339	0.5506	0.6457	0.6476	0.5138
R10	0.3521	0.5133	0.6364	0.5373	0.4580
R11	0.5269	0.5704	0.5660	0.6222	0.5455
R12	0.5109	0.5157	0.6169	0.5512	0.6166
R13	0.4761	0.4006	0.6673	0.5521	0.5263
R14	0.5458	0.5585	0.6742	0.5833	0.5481
R15	0.6104	0.6154	0.6061	0.6476	0.5327
R16	0.5127	0.5654	0.6951	0.5900	0.5253
R17	0.5038	0.5378	0.6801	0.6113	0.5411
R18	0.5723	0.6434	0.4761	0.5521	0.5577
R19	0.6376	0.6760	0.5704	0.5313	0.5936
R20	0.5228	0.5809	0.5438	0.5632	0.5610
R21	0.4867	0.5169	0.5375	0.6139	0.5962
R22	0.4901	0.5346	0.6111	0.6503	0.5918
R23	0.6258	0.6314	0.6895	0.6197	0.5515
R24	0.5483	0.5679	0.6956	0.5833	0.5167
R25	0.6030	0.5994	0.6037	0.5273	0.4555
R26	0.6194	0.6528	0.5729	0.6298	0.6102
R27	0.5913	0.6432	0.6592	0.6476	0.5207
R28	0.4395	0.5563	0.6248	0.6646	0.6432
R29	0.5217	0.6182	0.6091	0.4900	0.4444
R30	0.5129	0.5413	0.6802	0.5946	0.6061
R31	0.5382	0.5759	0.6179	0.5762	0.5396
R32	0.6635	0.6407	0.5994	0.5000	0.5377
R33	0.6308	0.5951	0.5430	0.4600	0.4352
R34	0.6351	0.6248	0.4951	0.5173	0.4815
R35	0.6472	0.6315	0.5364	0.4973	0.4883
R36	0.5333	0.6224	0.5339	0.5500	0.5809
R37	0.5012	0.5203	0.6827	0.5918	0.4623
R38	0.5559	0.6125	0.6459	0.6118	0.6128
R39	0.4739	0.5333	0.6630	0.5720	0.5741
R40	0.5424	0.5472	0.6424	0.6173	0.4512
A13	0.5672	0.6448	0.6769	0.4800	0.5556
A14	0.6437	0.7398	0.7059	0.6809	0.6973
A15	0.6532	0.7230	0.5403	0.6248	0.6516
A16	0.6036	0.6157	0.6248	0.6473	0.5716
A17	0.6703	0.6710	0.6432	0.7116	0.6887
A18	0.6333	0.6339	0.6617	0.7116	0.6509
A19	0.5721	0.6976	0.5381	0.5673	0.4697
A20	0.5698	0.6753	0.6407	0.5483	0.4478
A21	0.7024	0.7753	0.5506	0.5891	0.4245
A22	0.5721	0.6364	0.5248	0.5300	0.4512
R41	0.5542	0.5666	0.6802	0.5918	0.5377
R42(HO)	0.5414	0.5113	0.6270	0.5597	0.5647
R43(HO)	0.5339	0.5203	0.6457	0.5660	0.5704
R44(RM)	0.4987	0.5061	0.6753	0.5660	0.5515
R45	0.5399	0.5406	0.6818	0.6273	0.6457
R46	0.6429	0.6147	0.6295	0.6489	0.6418
R47(RM)	0.6813	0.6724	0.6365	0.6035	0.6320
A23	0.6594	0.6167	0.6058	0.7040	0.7222

A24	0.6169	0.6472	0.6067	0.6700	0.5278
A25	0.6339	0.6731	0.5096	0.5313	0.4902
A26	0.6563	0.6727	0.6406	0.5646	0.5506
A27	0.6546	0.7050	0.5377	0.5729	0.4615
A28	0.6055	0.6679	0.6042	0.5273	0.5531
A29	0.5593	0.6346	0.6446	0.7770	0.6013
A30	0.6878	0.7000	0.5455	0.6600	0.4907
A31	0.6333	0.6455	0.5818	0.6400	0.4907
A32	0.5812	0.7224	0.5976	0.4000	0.3426
A33	0.7301	0.7188	0.6578	0.6122	0.5936
A34	0.6660	0.7728	0.6086	0.5400	0.5463
A35	0.6308	0.7406	0.6703	0.4800	0.4537
A36	0.5812	0.6794	0.5364	0.5500	0.4444
A37	0.7275	0.8192	0.6609	0.4271	0.5455
A38	0.6151	0.7472	0.5521	0.2900	0.5253
A39	0.6641	0.7377	0.6862	0.4184	0.5096
R48	0.6610	0.6870	0.5901	0.5102	0.5189
R49	0.5357	0.6630	0.6042	0.4993	0.4783
A40	0.6543	0.6680	0.7857	0.5000	0.5938

Line code	B41(HO)	B42(HO)	B43(HO)	R8	R9
B41(HO)					
B42(HO)	0.2963				
B43(HO)	0.3396	0.0377			
R8	0.5849	0.6604	0.6698		
R9	0.6390	0.6767	0.6609	0.3182	
R10	0.5556	0.5253	0.5163	0.4907	0.4790
R11	0.6731	0.6538	0.6569	0.4423	0.3868
R12	0.5840	0.6531	0.6590	0.5271	0.4555
R13	0.5673	0.6635	0.6961	0.2448	0.3917
R14	0.7212	0.7212	0.7353	0.2570	0.2805
R15	0.6698	0.6767	0.6705	0.4409	0.5138
R16	0.6920	0.6852	0.6792	0.4420	0.3957
R17	0.6810	0.6935	0.6973	0.4118	0.4205
R18	0.6609	0.6417	0.6542	0.4468	0.4993
R19	0.6872	0.6750	0.6882	0.4852	0.4705
R20	0.6742	0.6484	0.6320	0.5232	0.3509
R21	0.5980	0.5913	0.5741	0.4901	0.4018
R22	0.6862	0.6981	0.6827	0.3774	0.2736
R23	0.7616	0.7428	0.7570	0.3440	0.3981
R24	0.7308	0.7115	0.7255	0.3051	0.3025
R25	0.6484	0.6553	0.6487	0.3937	0.2402
R26	0.6173	0.6750	0.6784	0.5192	0.4126
R27	0.7497	0.6931	0.7064	0.4641	0.4239
R28	0.6617	0.7543	0.7402	0.5253	0.2938
R29	0.5648	0.5093	0.5000	0.3611	0.3586
R30	0.5987	0.5679	0.5596	0.5163	0.3440
R31	0.5541	0.5679	0.5500	0.4528	0.3748
R32	0.6132	0.6201	0.6705	0.5163	0.5704
R33	0.5185	0.5741	0.6038	0.2938	0.3957
R34	0.5741	0.5370	0.5755	0.3771	0.4142
R35	0.4112	0.4668	0.4880	0.4352	0.5438
R36	0.6574	0.6389	0.6509	0.5136	0.4512
R37	0.6792	0.6792	0.6923	0.3371	0.3277
R38	0.6609	0.6224	0.6346	0.4660	0.5113
R39	0.6407	0.6154	0.6270	0.4765	0.4370
R40	0.6734	0.6549	0.6673	0.4790	0.5136
A13	0.5185	0.6457	0.6553	0.5346	0.5691
A14	0.6700	0.7100	0.6939	0.7200	0.6248
A15	0.5954	0.5830	0.5746	0.7131	0.6248
A16	0.5068	0.4883	0.4786	0.7290	0.6339
A17	0.6390	0.5943	0.5865	0.7119	0.6528
A18	0.6012	0.5566	0.5481	0.7119	0.6528
A19	0.5876	0.5438	0.5377	0.6987	0.6500
A20	0.5541	0.4358	0.4442	0.6648	0.6390
A21	0.5635	0.5000	0.4904	0.7119	0.6339
A22	0.3679	0.3494	0.3396	0.6481	0.6734
R41	0.7264	0.7264	0.7404	0.2711	0.2805
R42(HO)	0.6609	0.6417	0.6542	0.2859	0.3724
R43(HO)	0.6862	0.6553	0.6679	0.3182	0.3843
R44(RM)	0.7025	0.6836	0.6968	0.2780	0.2994
R45	0.6759	0.6574	0.6415	0.4722	0.4142
R46	0.7621	0.7621	0.7817	0.4686	0.4627
R47(RM)	0.7300	0.7496	0.7693	0.4756	0.5215
A23	0.5415	0.5556	0.5114	0.8415	0.6829
A24	0.6759	0.6271	0.6201	0.6549	0.5228
A25	0.5196	0.4804	0.4900	0.6569	0.6935
A26	0.4117	0.4234	0.4503	0.6710	0.6314
A27	0.3750	0.4135	0.4020	0.7019	0.5647
A28	0.5623	0.4512	0.4597	0.5599	0.5944

A29	0.6299	0.4945	0.4843	0.6193	0.6347
A30	0.5000	0.4630	0.4717	0.7407	0.6642
A31	0.5370	0.4074	0.3962	0.7222	0.6271
A32	0.4444	0.3704	0.3774	0.5901	0.6247
A33	0.5192	0.5192	0.5294	0.7474	0.6679
A34	0.5472	0.5472	0.5577	0.6390	0.6365
A35	0.5185	0.5185	0.5094	0.6642	0.6432
A36	0.4537	0.3981	0.3868	0.7315	0.6920
A37	0.5392	0.5784	0.6100	0.6346	0.7104
A38	0.4537	0.5648	0.5849	0.5784	0.6660
A39	0.5359	0.6128	0.6444	0.5840	0.6006
R48	0.6226	0.6792	0.6923	0.5258	0.6176
R49	0.5680	0.5865	0.6006	0.5136	0.4463
A40	0.5938	0.6535	0.6567	0.5521	0.6042

Line code	R10	R11	R12	R13	R14
R10					
R11	0.5541				
R12	0.4945	0.5440			
R13	0.4031	0.5170	0.4383		
R14	0.5302	0.4289	0.5019	0.3013	
R15	0.5456	0.5141	0.5524	0.3917	0.3462
R16	0.3679	0.5515	0.4654	0.3629	0.2547
R17	0.4250	0.5070	0.4372	0.3020	0.2859
R18	0.4949	0.5327	0.5523	0.4555	0.3813
R19	0.5773	0.5622	0.5320	0.4923	0.4231
R20	0.4630	0.5610	0.4320	0.4897	0.4616
R21	0.5260	0.5251	0.4739	0.4823	0.4616
R22	0.5068	0.4340	0.5506	0.4301	0.2145
R23	0.6154	0.4761	0.5894	0.3942	0.1604
R24	0.5515	0.4397	0.5113	0.2981	0.0481
R25	0.5549	0.5045	0.4808	0.4013	0.3269
R26	0.6673	0.5283	0.5138	0.5366	0.4786
R27	0.6290	0.3817	0.5894	0.5115	0.2994
R28	0.4679	0.4786	0.4812	0.4735	0.3981
R29	0.3794	0.4245	0.5279	0.4597	0.4528
R30	0.4277	0.4686	0.4754	0.4852	0.4493
R31	0.5203	0.4855	0.5339	0.4756	0.4100
R32	0.5623	0.6224	0.6308	0.4975	0.4878
R33	0.5315	0.4314	0.5242	0.4245	0.3302
R34	0.5588	0.4761	0.5127	0.4811	0.2547
R35	0.5727	0.5755	0.5581	0.4666	0.4409
R36	0.4860	0.4975	0.3945	0.3962	0.3465
R37	0.4858	0.4126	0.4969	0.3558	0.1226
R38	0.5421	0.4949	0.6332	0.4431	0.3491
R39	0.5060	0.4975	0.5346	0.4547	0.3208
R40	0.4787	0.4195	0.5672	0.4761	0.3868
A13	0.5248	0.6956	0.5805	0.5069	0.6390
A14	0.6935	0.6200	0.6339	0.7449	0.6973
A15	0.6487	0.6473	0.6180	0.7546	0.6846
A16	0.6133	0.6107	0.6612	0.7591	0.7591
A17	0.6870	0.7641	0.6172	0.6801	0.6801
A18	0.6500	0.7641	0.6172	0.6801	0.6801
A19	0.5472	0.6082	0.6302	0.6648	0.6648
A20	0.5456	0.5718	0.5741	0.6767	0.6269
A21	0.6567	0.6553	0.6190	0.6731	0.6553
A22	0.5455	0.6132	0.5958	0.6836	0.7239
R41	0.5389	0.4289	0.5111	0.3147	0.0000
R42(HO)	0.5396	0.4468	0.4264	0.2843	0.1442
R43(HO)	0.5296	0.4692	0.4370	0.2981	0.1604
R44(RM)	0.4901	0.4169	0.4808	0.2955	0.0566
R45	0.6339	0.5377	0.4769	0.4880	0.3937
R46	0.6968	0.4974	0.5910	0.4974	0.3137
R47(RM)	0.7352	0.5438	0.6269	0.5758	0.4020
A23	0.6869	0.6859	0.6840	0.7727	0.7718
A24	0.5472	0.5541	0.5581	0.5729	0.5541
A25	0.5936	0.6100	0.5969	0.6800	0.6273
A26	0.6339	0.7145	0.5710	0.6578	0.6742
A27	0.5893	0.6058	0.6422	0.7059	0.6731
A28	0.6291	0.6648	0.5630	0.6082	0.6012
A29	0.5946	0.6791	0.7012	0.5854	0.6423
A30	0.6248	0.6981	0.6098	0.6956	0.6956
A31	0.5885	0.5943	0.6164	0.7145	0.7050
A32	0.4885	0.6578	0.5987	0.5635	0.6201
A33	0.7145	0.7033	0.6779	0.7353	0.7425

A34	0.6827	0.6320	0.5919	0.6320	0.6346
A35	0.5497	0.6390	0.6351	0.6578	0.6390
A36	0.5545	0.6132	0.6552	0.6862	0.7428
A37	0.6609	0.6873	0.6576	0.6273	0.6700
A38	0.5885	0.6245	0.5781	0.5987	0.6434
A39	0.6107	0.6993	0.6024	0.5954	0.6032
R48	0.5531	0.5743	0.5610	0.5551	0.5743
R49	0.4951	0.4949	0.3878	0.4735	0.5062
A40	0.6837	0.6354	0.6469	0.6596	0.6458

Line code	R15	R16	R17	R18	R19
R15					
R16	0.3309				
R17	0.4083	0.2378			
R18	0.5424	0.5039	0.4948		
R19	0.5577	0.5189	0.5086	0.1827	
R20	0.5628	0.4438	0.4993	0.3629	0.3509
R21	0.5962	0.5284	0.4897	0.4611	0.4735
R22	0.4786	0.4049	0.3987	0.4830	0.5258
R23	0.4057	0.3333	0.3506	0.4031	0.3843
R24	0.3173	0.2453	0.2595	0.4109	0.4231
R25	0.5163	0.4352	0.4529	0.4730	0.4205
R26	0.6417	0.5893	0.5141	0.4452	0.3795
R27	0.4314	0.4259	0.4660	0.3912	0.3465
R28	0.5944	0.4357	0.4006	0.5842	0.5962
R29	0.4630	0.4273	0.4974	0.5515	0.5635
R30	0.5352	0.4673	0.5411	0.5307	0.5045
R31	0.5490	0.4673	0.4634	0.4710	0.4503
R32	0.6107	0.5531	0.6222	0.4519	0.4503
R33	0.4537	0.4364	0.4730	0.3654	0.4151
R34	0.4537	0.3885	0.4493	0.4284	0.4811
R35	0.5438	0.5612	0.5167	0.4572	0.5585
R36	0.3401	0.3430	0.3987	0.5138	0.4949
R37	0.4057	0.3333	0.3122	0.3843	0.4409
R38	0.5167	0.4597	0.4878	0.4100	0.4827
R39	0.5556	0.4951	0.4705	0.4025	0.4377
R40	0.4975	0.4794	0.4083	0.4731	0.5729
A13	0.6457	0.5703	0.5884	0.5918	0.5987
A14	0.6646	0.7131	0.7163	0.6746	0.7524
A15	0.6653	0.7327	0.7115	0.6415	0.6793
A16	0.6963	0.7472	0.7655	0.6995	0.7446
A17	0.7239	0.6827	0.6954	0.6461	0.6801
A18	0.7239	0.6827	0.6954	0.6653	0.6872
A19	0.5716	0.6248	0.6846	0.6176	0.6648
A20	0.6100	0.6592	0.6210	0.6046	0.6698
A21	0.5799	0.6524	0.6628	0.6648	0.6553
A22	0.5994	0.6339	0.6968	0.6012	0.7000
R41	0.3396	0.2500	0.2859	0.3813	0.4340
R42(HO)	0.3558	0.3585	0.2817	0.3598	0.3750
R43(HO)	0.3679	0.3586	0.2807	0.3843	0.3774
R44(RM)	0.3654	0.2870	0.2545	0.4001	0.4151
R45	0.6154	0.5157	0.4205	0.6176	0.6125
R46	0.5294	0.4712	0.5020	0.3379	0.2692
R47(RM)	0.5784	0.5385	0.5215	0.3922	0.3173
A23	0.7471	0.7007	0.7466	0.7607	0.7526
A24	0.5506	0.5315	0.5474	0.6132	0.5635
A25	0.6346	0.7090	0.6782	0.5773	0.6746
A26	0.7055	0.6588	0.7038	0.6270	0.7043
A27	0.6032	0.6201	0.6516	0.6705	0.7160
A28	0.6222	0.6430	0.7038	0.5987	0.6503
A29	0.5693	0.6866	0.6110	0.6944	0.7015
A30	0.5969	0.6315	0.7327	0.7050	0.7257
A31	0.6339	0.6497	0.7423	0.6578	0.6786
A32	0.6222	0.5885	0.6077	0.5541	0.5868
A33	0.7859	0.7119	0.7793	0.6935	0.7202
A34	0.6415	0.6759	0.7006	0.6058	0.6391
A35	0.6710	0.6612	0.6461	0.4975	0.5302
A36	0.6920	0.7133	0.7423	0.6767	0.7283
A37	0.5936	0.6731	0.6773	0.6400	0.6073
A38	0.6197	0.6406	0.6435	0.5446	0.5327

A39	0.7064	0.6578	0.6686	0.5481	0.5551
R48	0.5824	0.5068	0.5536	0.5840	0.6102
R49	0.5592	0.4539	0.4224	0.6082	0.6031
A40	0.6809	0.6633	0.6430	0.6298	0.6118

Line code	R20	R21	R22	R23	R24
R20					
R21	0.3907				
R22	0.4512	0.3857			
R23	0.5944	0.5246	0.3309		
R24	0.4830	0.4899	0.2428	0.1132	
R25	0.3748	0.3857	0.3440	0.3654	0.2885
R26	0.5232	0.5465	0.5283	0.4264	0.4397
R27	0.5364	0.5111	0.4000	0.2105	0.2711
R28	0.4648	0.4448	0.2846	0.5296	0.4264
R29	0.4605	0.4920	0.4722	0.5346	0.4811
R30	0.3654	0.3302	0.3843	0.5490	0.4493
R31	0.3426	0.3419	0.3148	0.4926	0.4195
R32	0.4975	0.4783	0.5352	0.5446	0.5096
R33	0.4926	0.4969	0.3679	0.3586	0.3585
R34	0.4623	0.4854	0.3494	0.3333	0.3019
R35	0.5413	0.5563	0.5833	0.5228	0.4692
R36	0.4345	0.5672	0.4512	0.4352	0.3302
R37	0.4740	0.4808	0.2938	0.2222	0.1321
R38	0.5327	0.4968	0.4503	0.4528	0.3462
R39	0.4901	0.4437	0.4142	0.4302	0.3302
R40	0.6037	0.6011	0.4975	0.4630	0.3774
A13	0.6104	0.5690	0.6457	0.6364	0.6226
A14	0.7073	0.6313	0.6800	0.6946	0.6735
A15	0.6490	0.6044	0.6275	0.6908	0.6873
A16	0.7173	0.6049	0.6920	0.7913	0.7616
A17	0.6811	0.5845	0.7308	0.7145	0.7019
A18	0.6811	0.5845	0.7308	0.7145	0.7019
A19	0.5802	0.5557	0.6710	0.6920	0.6415
A20	0.6760	0.6136	0.6622	0.6434	0.6176
A21	0.6753	0.6326	0.6617	0.6339	0.6132
A22	0.6339	0.5714	0.6944	0.7383	0.7075
R41	0.4716	0.4716	0.2290	0.1574	0.0472
R42(HO)	0.4522	0.4427	0.3182	0.2453	0.1538
R43(HO)	0.4370	0.4530	0.3123	0.2593	0.1509
R44(RM)	0.4716	0.4623	0.2290	0.1944	0.0849
R45	0.5784	0.5284	0.4722	0.4420	0.3654
R46	0.4897	0.5211	0.4878	0.2282	0.2647
R47(RM)	0.5596	0.5378	0.5647	0.2859	0.3627
A23	0.6949	0.6615	0.6927	0.7906	0.7444
A24	0.5203	0.5805	0.5994	0.5784	0.5377
A25	0.6908	0.6358	0.6765	0.6248	0.6100
A26	0.7030	0.6532	0.7450	0.6617	0.6484
A27	0.6836	0.6685	0.6321	0.6836	0.6635
A28	0.6475	0.5969	0.6154	0.6086	0.6107
A29	0.7210	0.5927	0.6040	0.6339	0.6243
A30	0.6685	0.6781	0.7407	0.6759	0.6792
A31	0.6457	0.5987	0.7315	0.6920	0.6887
A32	0.5969	0.5829	0.6827	0.6179	0.6226
A33	0.7108	0.6685	0.7859	0.7256	0.7059
A34	0.6339	0.6771	0.6956	0.6673	0.6346
A35	0.6339	0.5896	0.6457	0.6179	0.6415
A36	0.6642	0.6193	0.7130	0.7753	0.7453
A37	0.7026	0.6813	0.7719	0.6320	0.6300
A38	0.5919	0.6368	0.7173	0.6592	0.6270
A39	0.5679	0.5182	0.6107	0.6201	0.6058
R48	0.6151	0.6123	0.6390	0.5660	0.5769
R49	0.4993	0.5599	0.5666	0.5203	0.4710
A40	0.6374	0.5884	0.6224	0.6707	0.6354

Line code	R25	R26	R27	R28	R29
R25					
R26	0.4013				
R27	0.3981	0.4006			
R28	0.3814	0.4786	0.5242		
R29	0.4673	0.5566	0.4420	0.5067	
R30	0.3722	0.5666	0.4880	0.4648	0.2753
R31	0.3509	0.5163	0.4433	0.3839	0.4234
R32	0.5138	0.6295	0.5258	0.6500	0.5185
R33	0.3957	0.5635	0.3704	0.5381	0.4636
R34	0.3932	0.5515	0.4074	0.5315	0.4339
R35	0.5666	0.5396	0.5784	0.6769	0.5219
R36	0.4907	0.5987	0.4740	0.4927	0.4794
R37	0.3962	0.4949	0.3216	0.4740	0.4907
R38	0.4827	0.5654	0.4478	0.5465	0.5258
R39	0.4117	0.4641	0.3995	0.4515	0.4679
R40	0.5043	0.5352	0.4512	0.5151	0.5612
A13	0.5506	0.6031	0.6432	0.6224	0.4636
A14	0.6146	0.5646	0.5873	0.6471	0.6275
A15	0.6294	0.5746	0.6686	0.5596	0.6513
A16	0.6129	0.5937	0.7290	0.6018	0.5976
A17	0.5893	0.5858	0.6270	0.7055	0.6364
A18	0.5893	0.5858	0.6270	0.7055	0.6364
A19	0.5136	0.7283	0.6734	0.6539	0.6157
A20	0.6031	0.6051	0.6383	0.6475	0.6197
A21	0.5515	0.6622	0.6271	0.6753	0.6179
A22	0.6895	0.6648	0.7012	0.7133	0.5455
R41	0.3396	0.4786	0.2938	0.4092	0.4630
R42(HO)	0.3558	0.4590	0.3654	0.5019	0.5000
R43(HO)	0.3585	0.4692	0.3771	0.4926	0.5278
R44(RM)	0.3396	0.4786	0.3333	0.4277	0.4395
R45	0.5068	0.4880	0.5456	0.4521	0.5455
R46	0.4216	0.3111	0.2212	0.6051	0.6154
R47(RM)	0.4314	0.3209	0.2788	0.6365	0.6295
A23	0.6637	0.6385	0.7500	0.6000	0.6710
A24	0.4975	0.6314	0.5160	0.5836	0.6000
A25	0.6124	0.6073	0.6444	0.6968	0.6538
A26	0.6475	0.5654	0.6339	0.6903	0.6612
A27	0.6128	0.6128	0.6767	0.6082	0.6038
A28	0.5944	0.6220	0.5389	0.7200	0.5067
A29	0.6394	0.6194	0.6523	0.6646	0.5193
A30	0.6802	0.6811	0.6364	0.7224	0.5727
A31	0.6617	0.6905	0.6086	0.6860	0.5364
A32	0.5876	0.6408	0.5809	0.7133	0.5545
A33	0.6224	0.6339	0.7186	0.7025	0.6887
A34	0.6364	0.5955	0.6176	0.7821	0.5833
A35	0.5691	0.5842	0.5623	0.6588	0.6091
A36	0.6154	0.6459	0.6642	0.6406	0.6000
A37	0.6391	0.7020	0.5928	0.7641	0.6346
A38	0.6032	0.6503	0.6037	0.6745	0.5636
A39	0.5429	0.6051	0.6270	0.6553	0.6415
R48	0.6553	0.6583	0.5987	0.6710	0.5463
R49	0.4740	0.5377	0.5246	0.4836	0.4443
A40	0.6430	0.6479	0.6986	0.7728	0.6020

Line code	R30	R31	R32	R33	R34
R30					
R31	0.3037				
R32	0.4383	0.4169			
R33	0.4765	0.4302	0.4352		
R34	0.4673	0.4370	0.4605	0.2545	
R35	0.6032	0.5784	0.5438	0.4703	0.4339
R36	0.5413	0.4950	0.5809	0.4636	0.4273
R37	0.5163	0.4487	0.5189	0.3519	0.3148
R38	0.4057	0.5157	0.4468	0.4786	0.4906
R39	0.3907	0.4481	0.4950	0.5430	0.4157
R40	0.6037	0.6357	0.5666	0.4727	0.4909
A13	0.4765	0.5599	0.5278	0.6000	0.6679
A14	0.5773	0.6473	0.6700	0.7059	0.6542
A15	0.5777	0.6169	0.6810	0.6679	0.6173
A16	0.5574	0.6820	0.7265	0.6430	0.6679
A17	0.5421	0.6459	0.7264	0.6642	0.6271
A18	0.5421	0.6459	0.7264	0.6642	0.6271
A19	0.5901	0.6197	0.6364	0.6248	0.5860
A20	0.5672	0.6263	0.7069	0.6407	0.6357
A21	0.6176	0.6407	0.6887	0.6271	0.6154
A22	0.5111	0.5851	0.6339	0.6091	0.6067
R41	0.4597	0.4210	0.4975	0.3426	0.2685
R42(HO)	0.5045	0.4383	0.5551	0.3774	0.3396
R43(HO)	0.5044	0.4302	0.5566	0.3889	0.3704
R44(RM)	0.4503	0.4277	0.5044	0.3241	0.2500
R45	0.5784	0.5160	0.6222	0.5521	0.4976
R46	0.5536	0.5141	0.5000	0.4904	0.4615
R47(RM)	0.6098	0.5718	0.5525	0.5481	0.5096
A23	0.5304	0.6167	0.6333	0.6710	0.7007
A24	0.5160	0.5876	0.5741	0.5545	0.5794
A25	0.6392	0.6660	0.6738	0.6442	0.5936
A26	0.5944	0.6913	0.6567	0.6157	0.5612
A27	0.6198	0.5824	0.6346	0.5377	0.5755
A28	0.5642	0.6290	0.6407	0.4612	0.4860
A29	0.5734	0.6319	0.6421	0.6593	0.6166
A30	0.6247	0.7240	0.6524	0.6364	0.5885
A31	0.5321	0.6222	0.5969	0.5636	0.6248
A32	0.6314	0.6407	0.6271	0.5091	0.5885
A33	0.6679	0.6846	0.6705	0.6226	0.6484
A34	0.6685	0.6648	0.6390	0.5926	0.6086
A35	0.5944	0.6660	0.7012	0.5455	0.6612
A36	0.5851	0.5919	0.5994	0.5909	0.6339
A37	0.6775	0.6882	0.5882	0.6250	0.6538
A38	0.6407	0.6314	0.6111	0.5612	0.6067
A39	0.5743	0.5302	0.5000	0.5566	0.6082
R48	0.6107	0.6836	0.5189	0.6111	0.5691
R49	0.5377	0.5481	0.6450	0.5448	0.5357
A40	0.6194	0.6633	0.6667	0.6020	0.6503

Line code	R35	R36	R37	R38	R39
R35					
R36	0.5588				
R37	0.4049	0.3796			
R38	0.5138	0.5704	0.4528		
R39	0.5109	0.5588	0.3981	0.2428	
R40	0.5067	0.5315	0.3889	0.2830	0.3727
A13	0.5976	0.6521	0.6296	0.5868	0.5812
A14	0.6275	0.6373	0.7200	0.6473	0.6222
A15	0.6264	0.6391	0.7327	0.6373	0.5737
A16	0.5195	0.6769	0.7290	0.6553	0.6539
A17	0.6247	0.6364	0.7333	0.5743	0.6012
A18	0.5876	0.6364	0.6956	0.5743	0.5944
A19	0.6291	0.5794	0.5994	0.5987	0.6042
A20	0.6007	0.5969	0.6056	0.5802	0.5474
A21	0.6617	0.6111	0.6827	0.6082	0.6500
A22	0.4922	0.6794	0.6574	0.6012	0.5951
R41	0.4512	0.3586	0.1204	0.3491	0.3333
R42(HO)	0.4572	0.3491	0.2453	0.4179	0.3748
R43(HO)	0.4858	0.3241	0.2407	0.4289	0.3957
R44(RM)	0.4327	0.3586	0.1574	0.3679	0.3241
R45	0.5636	0.5067	0.4142	0.5729	0.5084
R46	0.5910	0.5385	0.3436	0.4876	0.4032
R47(RM)	0.6295	0.6346	0.4327	0.5607	0.4845
A23	0.6927	0.6739	0.7797	0.6718	0.7195
A24	0.6976	0.5157	0.5648	0.5773	0.5951
A25	0.5328	0.5769	0.6176	0.5973	0.5718
A26	0.4741	0.6860	0.6710	0.5585	0.5783
A27	0.5228	0.5660	0.6132	0.6705	0.6981
A28	0.6042	0.6588	0.6920	0.5421	0.5630
A29	0.5720	0.6766	0.6653	0.7076	0.6393
A30	0.4765	0.6157	0.6296	0.6553	0.6903
A31	0.5129	0.6157	0.6389	0.5824	0.6903
A32	0.4157	0.5794	0.5926	0.5729	0.6175
A33	0.5824	0.7075	0.7308	0.7523	0.7232
A34	0.4975	0.5346	0.6604	0.6224	0.6425
A35	0.5067	0.6703	0.6296	0.5729	0.5151
A36	0.5038	0.6612	0.6944	0.6201	0.6248
A37	0.5647	0.6032	0.6569	0.5646	0.6173
A38	0.5521	0.5472	0.6524	0.5585	0.5605
A39	0.5352	0.6484	0.5755	0.6269	0.5748
R48	0.5531	0.5716	0.5660	0.6102	0.6179
R49	0.5472	0.4381	0.4993	0.6358	0.5696
A40	0.6837	0.7143	0.6020	0.6298	0.6523

Line code	R40	A13	A14	A15	A16
R40					
A13	0.6084				
A14	0.6640	0.6863			
A15	0.6121	0.5910	0.4764		
A16	0.5836	0.6612	0.5000	0.3436	
A17	0.6524	0.5623	0.4700	0.5660	0.5278
A18	0.6271	0.5438	0.4700	0.5660	0.4907
A19	0.5291	0.5364	0.7255	0.4897	0.5339
A20	0.4969	0.5296	0.6146	0.3529	0.3747
A21	0.6222	0.5185	0.6100	0.4039	0.4790
A22	0.6042	0.4703	0.6275	0.3628	0.3976
R41	0.3796	0.6271	0.6973	0.6908	0.7636
R42(HO)	0.3962	0.6365	0.6884	0.6490	0.7377
R43(HO)	0.4259	0.6549	0.6946	0.6614	0.7426
R44(RM)	0.3864	0.6407	0.7246	0.6980	0.7333
R45	0.5042	0.6563	0.6176	0.6224	0.7612
R46	0.5385	0.6513	0.6458	0.6884	0.8122
R47(RM)	0.5481	0.6391	0.6430	0.6755	0.8000
A23	0.7333	0.7471	0.6023	0.5101	0.4645
A24	0.4836	0.5909	0.6765	0.6794	0.6951
A25	0.5141	0.5551	0.5918	0.2173	0.3243
A26	0.5381	0.6472	0.5562	0.4634	0.4703
A27	0.5755	0.5635	0.5918	0.5446	0.4314
A28	0.6133	0.6381	0.6614	0.6147	0.6497
A29	0.6366	0.5920	0.6248	0.6270	0.5873
A30	0.6018	0.6430	0.6373	0.4993	0.4067
A31	0.5769	0.5976	0.5784	0.4852	0.3339
A32	0.5769	0.5521	0.6667	0.6609	0.5521
A33	0.7188	0.6365	0.5714	0.5620	0.5163
A34	0.6339	0.5809	0.6200	0.6738	0.6734
A35	0.5406	0.6067	0.6667	0.5840	0.5157
A36	0.5315	0.5497	0.5686	0.4590	0.3794
A37	0.6102	0.5192	0.6979	0.6846	0.7282
A38	0.5993	0.4909	0.6908	0.6077	0.6769
A39	0.6289	0.3679	0.6700	0.5920	0.6836
R48	0.5203	0.5185	0.6600	0.6810	0.6920
R49	0.5102	0.6109	0.6418	0.6834	0.7528
A40	0.6068	0.4592	0.6702	0.6539	0.7013

Line code	A17	A18	A19	A20	A21
A17					
A18	0.0370				
A19	0.6574	0.6204			
A20	0.5182	0.4805	0.4185		
A21	0.5849	0.5660	0.3056	0.3560	
A22	0.5556	0.5185	0.3727	0.3099	0.4259
R41	0.6862	0.6862	0.6524	0.6151	0.6432
R42(HO)	0.6513	0.6513	0.6107	0.6051	0.6082
R43(HO)	0.6648	0.6648	0.6296	0.6125	0.6154
R44(RM)	0.6931	0.6931	0.6777	0.6358	0.6567
R45	0.6642	0.6642	0.6976	0.7055	0.7197
R46	0.6935	0.6935	0.7378	0.6980	0.6801
R47(RM)	0.7006	0.7006	0.7448	0.7274	0.6872
A23	0.6000	0.5778	0.5109	0.6355	0.5652
A24	0.6204	0.6019	0.4182	0.5709	0.4352
A25	0.5490	0.5294	0.3750	0.2033	0.3333
A26	0.4765	0.4395	0.5497	0.4067	0.5068
A27	0.5455	0.5070	0.4880	0.4397	0.4220
A28	0.5833	0.5833	0.6430	0.6123	0.5969
A29	0.6755	0.6476	0.6420	0.5986	0.6272
A30	0.6019	0.5648	0.4794	0.4876	0.4722
A31	0.5648	0.5278	0.4727	0.4623	0.4630
A32	0.6019	0.5648	0.5521	0.5179	0.5278
A33	0.5936	0.6128	0.5610	0.5692	0.5743
A34	0.5943	0.5943	0.6111	0.6339	0.6321
A35	0.6204	0.6019	0.6182	0.5111	0.6019
A36	0.4444	0.4074	0.4339	0.4530	0.4630
A37	0.6667	0.6471	0.5359	0.6000	0.5490
A38	0.6734	0.6549	0.5091	0.5944	0.5438
A39	0.6442	0.6442	0.5472	0.5692	0.5943
R48	0.7145	0.6956	0.6179	0.6270	0.6390
R49	0.7191	0.7191	0.6381	0.6303	0.6635
A40	0.6979	0.6979	0.5714	0.5541	0.6224

Line code	A22	R41	R42(HO)	R43(HO)	R44(RM)
A22					
R41	0.7105				
R42(HO)	0.6459	0.1604			
R43(HO)	0.6642	0.1759	0.0189		
R44(RM)	0.7055	0.0741	0.1484	0.1642	
R45	0.7091	0.4049	0.4409	0.4512	0.4049
R46	0.7378	0.3077	0.3039	0.3173	0.3462
R47(RM)	0.7448	0.3942	0.3333	0.3558	0.4231
A23	0.4891	0.7768	0.7442	0.7580	0.7630
A24	0.6248	0.5438	0.5635	0.5716	0.5691
A25	0.2692	0.6150	0.5536	0.5660	0.6490
A26	0.3588	0.6617	0.6717	0.6963	0.6759
A27	0.4528	0.6604	0.7404	0.7522	0.6862
A28	0.5545	0.6086	0.5987	0.6247	0.5599
A29	0.5420	0.6496	0.5902	0.6115	0.5958
A30	0.3612	0.6827	0.7119	0.7290	0.6895
A31	0.3727	0.6920	0.7025	0.7197	0.6987
A32	0.4703	0.6271	0.5610	0.5691	0.5784
A33	0.5138	0.7282	0.7398	0.7570	0.7352
A34	0.5833	0.6415	0.5743	0.5918	0.5987
A35	0.5182	0.6457	0.5987	0.6061	0.6154
A36	0.3703	0.7475	0.7402	0.7568	0.7173
A37	0.5840	0.6569	0.6200	0.6373	0.6712
A38	0.5364	0.6500	0.5465	0.5666	0.6265
A39	0.5729	0.5918	0.6391	0.6459	0.6484
R48	0.4907	0.5635	0.6032	0.6038	0.5773
R49	0.6304	0.5154	0.4968	0.5179	0.5036
A40	0.5687	0.6327	0.5597	0.5762	0.6272

Line code	R45	R46	R47(RM)	A23	A24
R45					
R46	0.5500				
R47(RM)	0.6006	0.1154			
A23	0.5761	0.7841	0.8038		
A24	0.5315	0.5840	0.6154	0.5326	
A25	0.7115	0.6429	0.6680	0.5889	0.6801
A26	0.5339	0.6557	0.6820	0.5000	0.5588
A27	0.6226	0.7915	0.7915	0.4192	0.4717
A28	0.5497	0.6775	0.6916	0.6819	0.6406
A29	0.5746	0.7175	0.7256	0.7031	0.6820
A30	0.6636	0.7545	0.7807	0.4674	0.5067
A31	0.6455	0.7448	0.7711	0.4565	0.5067
A32	0.6612	0.6705	0.6583	0.6630	0.6157
A33	0.6673	0.7173	0.7046	0.4515	0.6767
A34	0.6549	0.6640	0.6738	0.7444	0.7012
A35	0.7157	0.6897	0.6461	0.7065	0.6885
A36	0.6545	0.7955	0.7833	0.5326	0.5885
A37	0.7474	0.6401	0.6503	0.7955	0.6538
A38	0.6836	0.6198	0.6198	0.7471	0.6612
A39	0.6459	0.6150	0.6150	0.7889	0.5849
R48	0.5876	0.6248	0.6124	0.7970	0.6389
R49	0.3588	0.6147	0.6295	0.6623	0.5472
A40	0.7218	0.6489	0.6326	0.7841	0.6939

Line code	A25	A26	A27	A28	A29
A25					
A26	0.4179				
A27	0.5000	0.4478			
A28	0.6320	0.4545	0.6459		
A29	0.6035	0.6446	0.6374	0.5461	
A30	0.4519	0.3182	0.4811	0.5182	0.6146
A31	0.4327	0.3909	0.4340	0.5133	0.5946
A32	0.5481	0.5000	0.5943	0.4067	0.6246
A33	0.5784	0.4623	0.4608	0.6365	0.6319
A34	0.5784	0.6086	0.6635	0.4883	0.6523
A35	0.5481	0.5612	0.6698	0.5521	0.6646
A36	0.4231	0.4067	0.3962	0.5182	0.5646
A37	0.5918	0.5333	0.6200	0.6198	0.6062
A38	0.5647	0.5951	0.6295	0.5794	0.6720
A39	0.6073	0.6434	0.5962	0.6082	0.7180
R48	0.5784	0.6222	0.6442	0.6247	0.6299
R49	0.6764	0.5734	0.6190	0.5721	0.5666
A40	0.5929	0.6857	0.6771	0.6782	0.6731

Line code	A30	A31	A32	A33	A34
A30					
A31	0.1818				
A32	0.4182	0.4067			
A33	0.4906	0.4528	0.6226		
A34	0.5556	0.5556	0.4074	0.5962	
A35	0.5091	0.4909	0.3091	0.6415	0.5000
A36	0.3545	0.3182	0.4455	0.4811	0.5463
A37	0.5865	0.5481	0.4712	0.5700	0.5096
A38	0.6636	0.6157	0.4818	0.6509	0.4722
A39	0.6578	0.6295	0.5069	0.6248	0.5359
R48	0.6179	0.6179	0.5556	0.7115	0.5849
R49	0.6304	0.6667	0.5812	0.6289	0.5481
A40	0.7830	0.7524	0.6503	0.6222	0.5180

Line code	A35	A36	A37	A38	A39
A35					
A36	0.5000				
A37	0.5288	0.5769			
A38	0.5430	0.5455	0.1731		
A39	0.5635	0.5918	0.5600	0.5094	
R48	0.6111	0.6389	0.5980	0.6086	0.5673
R49	0.6242	0.6510	0.5429	0.4448	0.5868
A40	0.6707	0.6911	0.5745	0.5102	0.4792

Line code	R48	R49	A40
R48			
R49	0.5549		
A40	0.6354	0.6245	

Appendix 3: Eigen values of the 93 inbred sunflower lines

Code	Eigenvalue	Percentage	Cumulative %
A1	4.26442224	27.0304	27.0304
A2	1.76240545	11.1712	38.2016
A3	1.13887062	7.2188	45.4204
A4	0.99163502	6.2856	51.706
B4	0.9036716	5.7280	57.434
A5	0.7597076	4.8155	62.2495
A6	0.71787562	4.5503	66.7998
A7	0.70079695	4.4421	71.2419
A8	0.60146743	3.8125	75.0543
A9	0.57796763	3.6635	78.7178
A10	0.54151766	3.4325	82.1503
R1	0.53132924	3.3679	85.5181
R2	0.47348941	3.0013	88.5194
R3	0.431832	2.7372	91.2566
R4	0.39733182	2.5185	93.7751
R5	0.38818381	2.4605	96.2357
R6	0.35400778	2.2439	98.4796
R7	0.32929757	2.0873	> 100%
A11	0.30536763	1.9356	> 100%
A12	0.28426965	1.8019	> 100%
B41(HO)	0.2480116	1.5720	> 100%
B42(HO)	0.24216097	1.5350	> 100%
B43(HO)	0.2112387	1.3390	> 100%
R8	0.20912165	1.3255	> 100%
R9	0.19540001	1.2386	> 100%
R10	0.18144665	1.1501	> 100%
R11	0.16548858	1.0490	> 100%
R12	0.14934215	0.9466	> 100%
R13	0.14470152	0.9172	> 100%
R14	0.12108823	0.7675	> 100%
R15	0.11362799	0.7202	> 100%
R16	0.10154721	0.6437	> 100%
R17	0.08971624	0.5687	> 100%
R18	0.08104332	0.5137	> 100%
R19	0.07509366	0.4760	> 100%
R20	0.06173163	0.3913	> 100%
R21	0.05599581	0.3549	> 100%
R22	0.04805227	0.3046	> 100%
R23	0.03938789	0.2497	> 100%
R24	0.03340337	0.2117	> 100%
R25	0.02593203	0.1644	> 100%
R26	0.02270271	0.1439	> 100%
R27	0.01852158	0.1174	> 100%
R28	0.01603944	0.1017	> 100%
R29	0.01313201	0.0832	> 100%
R30	0.00512007	0.0325	> 100%
R31	0.00161754	0.0103	> 100%

R32	0.00062237	0.0039	> 100%
R33	0.00000000	0.0000	> 100%
R34	-0.00341706	-0.0217	> 100%
R35	-0.00370318	-0.0235	> 100%
R36	-0.00688700	-0.0437	> 100%
R37	-0.00741544	-0.0470	> 100%
R38	-0.01287759	-0.0816	> 100%
R39	-0.01332018	-0.0844	> 100%
R40	-0.01815359	-0.1151	> 100%
A13	-0.02057307	-0.1304	> 100%
A14	-0.02464977	-0.1562	> 100%
A15	-0.02799463	-0.1774	> 100%
A16	-0.03450008	-0.2187	> 100%
A17	-0.03565330	-0.2260	> 100%
A18	-0.03833396	-0.2430	> 100%
A19	-0.04194118	-0.2658	> 100%
A20	-0.04507090	-0.2857	> 100%
A21	-0.04656674	-0.2952	> 100%
A22	-0.05115852	-0.3243	> 100%
R41	-0.05331096	-0.3379	> 100%
R42(HO)	-0.05612736	-0.3558	> 100%
R43(HO)	-0.06100933	-0.3867	> 100%
R44(RM)	-0.06179900	-0.3917	> 100%
R45	-0.06953329	-0.4407	> 100%
R46	-0.07137520	-0.4524	> 100%
R47(RM)	-0.07386839	-0.4682	> 100%
A23	-0.08120576	-0.5147	> 100%
A24	-0.08332592	-0.5282	> 100%
A25	-0.08571399	-0.5433	> 100%
A26	-0.08812506	-0.5586	> 100%
A27	-0.09137423	-0.5792	> 100%
A28	-0.09444793	-0.5987	> 100%
A29	-0.09718462	-0.6160	> 100%
A30	-0.09995200	-0.6336	> 100%
A31	-0.10435821	-0.6615	> 100%
A32	-0.10613561	-0.6728	> 100%
A33	-0.11433839	-0.7247	> 100%
A34	-0.12054236	-0.7641	> 100%
A35	-0.12971110	-0.8222	> 100%
A36	-0.13634589	-0.8642	> 100%
A37	-0.14272510	-0.9047	> 100%
A38	-0.15060590	-0.9546	> 100%
A39	-0.15994122	-1.0138	> 100%
R48	-0.16943441	-1.0740	> 100%
R49	-0.18107843	-1.1478	> 100%
A40	-0.23456795	-1.4868	> 100%

Appendix 4 Specific combining ability effects for relative yield characteristic

F_1 hybrids	Relative yield
A4/R44(RM)	-60.9360
A4/R34	-63.0940
A4/R9	29.2780
A4/R13	51.0770
A4/R15	-2.5190
A4/R11	18.9560
A4/R47(RM)	59.3800
A4/R32	-27.7900
A4/R29	13.1450
A4/R10	-30.0250
A4/R48	12.5280
A5/R44(RM)	-9.5210
A5/R34	22.5700
A5/R9	35.2330
A5/R13	-48.8190
A5/R15	13.0960
A5/R11	-4.7500
A5/R47(RM)	21.8850
A5/R32	28.9340
A5/R29	-6.9500
A5/R10	-25.4210
A5/R48	-26.2570
A6/R44(RM)	1.5170
A6/R34	83.2990
A6/R9	-69.7690
A6/R13	44.4490
A6/R15	-4.7460
A6/R11	5.9980
A6/R47(RM)	-33.8870
A6/R32	-33.6670
A6/R29	6.8580
A6/R10	-19.8230
A6/R48	19.7710
A9/R44(RM)	30.3270
A9/R34	-50.6610
A9/R9	16.5010
A9/R13	-21.1810
A9/R15	47.5640
A9/R11	22.6180
A9/R47(RM)	-16.0770
A9/R32	-15.3570
A9/R29	13.3580
A9/R10	27.5670
A9/R48	-54.6590
A10/R44(RM)	44.2050
A10/R34	39.4770
A10/R9	-70.0010
A10/R13	43.3770
A10/R15	1.5220
A10/R11	-2.4230
A10/R47(RM)	30.6910
A10/R32	-33.8990
A10/R29	-0.1940
A10/R10	-30.6840
A10/R48	-22.0710
A7/R44(RM)	24.2560
A7/R34	-45.5420

A7/R9	-46.3400
A7/R13	-14.5620
A7/R15	54.1630
A7/R11	7.9170
A7/R47(RM)	-8.4580
A7/R32	-9.7380
A7/R29	5.9770
A7/R10	2.6070
A7/R48	29.7200
A8/R44(RM)	15.9020
A8/R34	-54.7860
A8/R9	29.6870
A8/R13	-23.8050
A8/R15	7.6890
A8/R11	29.9740
A8/R47(RM)	-18.2020
A8/R32	-19.4820
A8/R29	-9.7360
A8/R10	1.6430
A8/R48	41.1160
A12/R44(RM)	-37.9250
A12/R34	61.6370
A12/R9	-40.3810
A12/R13	-9.1030
A12/R15	67.3620
A12/R11	-51.4730
A12/R47(RM)	-5.9990
A12/R32	-5.7790
A12/R29	6.8360
A12/R10	58.4060
A12/R48	-43.5810
A41(HO)/R44(RM)	-12.0220
A41(HO)/R34	-15.6800
A41(HO)/R9	-15.4780
A41(HO)/R13	16.8000
A41(HO)/R15	-28.0350
A41(HO)/R11	-26.0710
A41(HO)/R47(RM)	20.9040
A41(HO)/R32	21.1240
A41(HO)/R29	46.0690
A41(HO)/R10	11.0680
A41(HO)/R48	-18.6780
A42(HO)/R44(RM)	-16.9920
A42(HO)/R34	-19.1500
A42(HO)/R9	-18.9480
A42(HO)/R13	10.8300
A42(HO)/R15	-33.5050
A42(HO)/R11	-30.5410
A42(HO)/R47(RM)	16.4340
A42(HO)/R32	96.5240
A42(HO)/R29	70.7490
A42(HO)/R10	-51.2520
A42(HO)/R48	-24.1480
A43(HO)/R44(RM)	-15.6690
A43(HO)/R34	-18.3270
A43(HO)/R9	79.6960
A43(HO)/R13	12.1540
A43(HO)/R15	-33.1820
A43(HO)/R11	-29.7170
A43(HO)/R47(RM)	17.7570

A43(HO)/R32	16.4770
A43(HO)/R29	43.5630
A43(HO)/R10	-50.4280
A43(HO)/R48	-22.3250
A13/R44(RM)	-59.3430
A13/R34	67.6690
A13/R9	32.0510
A13/R13	44.4390
A13/R15	-76.3560
A13/R11	22.7980
A13/R47(RM)	-28.4170
A13/R32	-28.6970
A13/R29	-2.7920
A13/R10	11.0970
A13/R48	17.5510
A15/R44(RM)	6.6710
A15/R34	11.1830
A15/R9	10.4260
A15/R13	24.3040
A15/R15	-35.0120
A15/R11	8.2530
A15/R47(RM)	-61.0630
A15/R32	31.3870
A15/R29	-22.9070
A15/R10	22.6720
A15/R48	4.0850
A16/R44(RM)	9.8260
A16/R34	13.2180
A16/R9	-6.7600
A16/R13	27.3480
A16/R15	-7.2870
A16/R11	-97.9930
A16/R47(RM)	22.2320
A16/R32	34.9420
A16/R29	9.1070
A16/R10	-27.7630
A16/R48	23.1300
A17/R44(RM)	21.1310
A17/R34	-68.9170
A17/R9	21.7460
A17/R13	49.6740
A17/R15	-15.0420
A17/R11	11.8030
A17/R47(RM)	18.0670
A17/R32	-34.6130
A17/R29	-21.9070
A17/R10	11.1820
A17/R48	6.8750
A18/R44(RM)	3.0110
A18/R34	30.2330
A18/R9	-8.1340
A18/R13	36.4140
A18/R15	-21.9120
A18/R11	21.4630
A18/R47(RM)	10.2370
A18/R32	-38.5630
A18/R29	-24.1670
A18/R10	-12.3080
A18/R48	3.7250
A19/R44(RM)	11.5500

A19/R34	36.8610
A19/R9	-2.4560
A19/R13	-61.2380
A19/R15	-15.3230
A19/R11	19.1310
A19/R47(RM)	-6.5240
A19/R32	26.7350
A19/R29	-17.1790
A19/R10	-7.1900
A19/R48	15.6340
A22/R44(RM)	9.0180
A22/R34	6.6400
A22/R9	9.6420
A22/R13	1.7300
A22/R15	-13.2650
A22/R11	9.9690
A22/R47(RM)	13.2540
A22/R32	31.1040
A22/R29	-28.9810
A22/R10	-36.3520
A22/R48	-2.7580
A23/R44(RM)	21.6770
A23/R34	49.6390
A23/R9	20.3010
A23/R13	35.9790
A23/R15	-0.3860
A23/R11	3.6680
A23/R47(RM)	37.6630
A23/R32	-38.1770
A23/R29	-29.7720
A23/R10	-24.1130
A23/R48	-76.4790
A24/R44(RM)	11.1160
A24/R34	48.2980
A24/R9	-4.1800
A24/R13	-48.2720
A24/R15	0.5630
A24/R11	4.1570
A24/R47(RM)	27.8920
A24/R32	-44.4480
A24/R29	-22.6730
A24/R10	20.2070
A24/R48	7.3400
A25/R44(RM)	12.4050
A25/R34	67.2270
A25/R9	11.2290
A25/R13	10.2070
A25/R15	-12.7680
A25/R11	0.2170
A25/R47(RM)	9.2810
A25/R32	-60.2890
A25/R29	-34.4040
A25/R10	-1.6640
A25/R48	-1.4410
A26/R44(RM)	28.4100
A26/R34	-64.8480
A26/R9	24.3850
A26/R13	30.7730
A26/R15	5.1270
A26/R11	6.2320

A26/R47(RM)	-28.2630
A26/R32	49.4960
A26/R29	8.2020
A26/R10	8.3310
A26/R48	-67.8460
A28/R44(RM)	12.4560
A28/R34	-62.7620
A28/R9	14.6600
A28/R13	-31.2820
A28/R15	16.1730
A28/R11	14.0670
A28/R47(RM)	-26.1780
A28/R32	-27.9580
A28/R29	13.4070
A28/R10	45.6170
A28/R48	31.8000
A29/R44(RM)	14.6300
A29/R34	-48.3890
A29/R9	5.5040
A29/R13	-16.4080
A29/R15	14.6870
A29/R11	19.8210
A29/R47(RM)	-12.3040
A29/R32	-12.5850
A29/R29	-10.9390
A29/R10	8.1400
A29/R48	37.8440
A30/R44(RM)	30.5190
A30/R34	-42.7600
A30/R9	-42.5570
A30/R13	-11.7790
A30/R15	8.4360
A30/R11	30.5300
A30/R47(RM)	-7.1750
A30/R32	-8.9560
A30/R29	-1.0500
A30/R10	10.5390
A30/R48	34.2530
A31/R44(RM)	22.2040
A31/R34	-55.9040
A31/R9	2.1880
A31/R13	-24.4230
A31/R15	13.2210
A31/R11	19.1360
A31/R47(RM)	-20.8200
A31/R32	-21.1000
A31/R29	-5.9350
A31/R10	19.2350
A31/R48	52.1980
A32/R44(RM)	15.1290
A32/R34	-59.2100
A32/R9	20.2130
A32/R13	-29.2290
A32/R15	10.3460
A32/R11	16.8900
A32/R47(RM)	-24.6250
A32/R32	-25.4060
A32/R29	19.9700
A32/R10	35.1890
A32/R48	20.7330

A34/R44(RM)	-12.6900
A34/R34	18.1210
A34/R9	37.6740
A34/R13	19.9320
A34/R15	-12.1930
A34/R11	-1.6890
A34/R47(RM)	-21.4840
A34/R32	1.1850
A34/R29	-24.0990
A34/R10	14.9700
A34/R48	-19.7260
A35/R44(RM)	-60.6430
A35/R34	32.7290
A35/R9	-64.5990
A35/R13	-33.8210
A35/R15	24.4640
A35/R11	19.7880
A35/R47(RM)	-28.7170
A35/R32	49.9830
A35/R29	-4.1920
A35/R10	43.5770
A35/R48	21.4310
A36/R44(RM)	16.8960
A36/R34	-42.9120
A36/R9	25.1600
A36/R13	-12.9320
A36/R15	28.2730
A36/R11	-55.3030
A36/R47(RM)	-8.8280
A36/R32	-8.1080
A36/R29	-5.0430
A36/R10	12.6470
A36/R48	50.1500
A37/R44(RM)	-5.9020
A37/R34	28.9000
A37/R9	8.8620
A37/R13	-56.0800
A37/R15	8.9250
A37/R11	6.6590
A37/R47(RM)	6.7440
A37/R32	37.8740
A37/R29	-2.4810
A37/R10	-20.0620
A37/R48	-13.4380
A38/R44(RM)	10.0400
A38/R34	67.6020
A38/R9	-36.1050
A38/R13	-45.0470
A38/R15	-3.7530
A38/R11	-9.1480
A38/R47(RM)	15.6170
A38/R32	29.9260
A38/R29	8.1420
A38/R10	-23.6190
A38/R48	-13.6560
A39/R44(RM)	-81.2520
A39/R34	27.6400
A39/R9	-8.7280
A39/R13	28.4900
A39/R15	-6.3250

A39/R11	-10.9410
A39/R47(RM)	28.9840
A39/R32	38.9240
A39/R29	10.0190
A39/R10	-3.9920
A39/R48	-22.8180