GENOTYPE BY ENVIRONMENT INTERACTION FOR OIL QUALITY IN HIGH OLEIC ACID SUNFLOWER LINES

By

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DECLARATION

“I declare that the thesis hereby submitted by me for the degree of Doctor of Philosophy in Agriculture at the University of the Free State is my own independent work, except where otherwise stated. I certify that this work has not been previously submitted by me for any degree at another University/Faculty.

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“The greatest thing in this world is not so much where we are, but in what direction we are moving.” - Oliver Wendell Holmes

This work is dedicated to my husband, Werner,
my mother Martie Coetzee and my father, Nick Coetzee

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LIST OF ABBREVIATIONS

ACP	Acyl carrier protein
A.D.	Anno Domini
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists’ Society
AOM	Active oxygen method
ATP	Adenosine 5’-triphosphate
AV (*p*-AV)	Anisidine value
B.C.	Before Christ
BSA	Bulk segregant analysis
Ca	Calcium
CDP	Cytidine 5’-diphosphate
CH_2	Methylene group
CoA	Coenzyme A
CTAB	Hexadecyltrimethylammonium bromide
CV	Coefficient of variation
DAA	Days after anthesis
DAF	Days after the initiation of flowering
DAG	Diacylglycerol
DALP	Direct amplification of length polymorphism
DNA	Deoxyribonucleic acid
dNTP	2’Deoxynucleoside 5’triphosphate
DTT	Dithiotreitol
E	Environment
EDTA	Ethylene-diaminetetra-acetate
ER	Endoplasmic reticulum
EST	Expressed sequence tag
F_1	First filial generation
F_2	Second filial generation
F_3	Third filial generation

FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organisation of the United Nations
FAS	Fatty acid synthetase
FFA	Free fatty acid
G	Genotype
GCA	General combining ability
GXE	Genotype by environment interaction
H^2	Broad sense heritability
HCO_2	Formic acid
HDL	High-density lipoprotein
IBr	Iodine bromine
INDEL	Insertion-deletion
IP	Induction period
IPCA	Interaction principle component axes
IV	Iodine value
K	Potassium
KCl	Potassium chloride
LDL	Low-density lipoprotein
LG	Linkage group
LLL	Trilinoleoyl-glycerol
LOD	Log-likelihood score
LSD	Least significant difference
MAB	Marker-assisted breeding
MAS	Marker-assisted selection
Mg	Magnesium
$MgCl_2$	Magnesium chloride
mRNA	Messenger ribonucleic acid
MS	Mean square
MUFA	Monounsaturated fatty acid
N	Nitrogen
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
$Na_2S_2O_3$	Sodium thiosulfate

ND	Not detected
N_D	Refractive index measurement value at 589.3 nm (the D Fraunhofer line).
ODS	Oleoyl-phosphatidylcholine desaturase
OLL	Monooleoyl-dilinoleoyl-glycerol
OOL	Dioleoyl-monolinoleoyl-glycerol
OSI	Oxidative stability index
P	Phosphorus
PA	Phosphatidic acid
PAGE	Polyacrylamide gel electrophoresis
PC	Phosphatidylcholine
PCA	Principle component analysis
PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
QTL	Quantitative trait loci
R	Replicates
RAPD	Random amplified polymorphic DNA
REP	Replication
RFLP	Restriction fragment length polymorphism
RH	Fatty acid group
RI	Refractivity index
RIL	Recombinant inbred line
RNA	Ribonucleic acid
ROOH	Hydroperoxide
S	Sulphur
SAD	Stearoyl-acyl carrier protein desaturase
SCA	Specific combining ability
SCAR	Sequence characterised amplified region
SFA	Saturated fatty acid
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
STS	Sequence tagged site
TAG	Triacylglycerol
Taq	*Thermus aquaticus*

TBE	Tris-Boric acid-EDTA buffer
TE	Tris-EDTA buffer
TEMED	*N’N’N’N’*-tetramethylene-diamine
Tris-Cl	Trishydroxymethyl-aminomethane
U	Unit(s)
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
Zn	Zink
σ^2e	Environmental variance component
σ^2g	Genetic variance component
σ^2p	Phenotypic variance component

LIST OF SI UNITS

Ab	Absorbance
α	Alpha
β	Beta
ºC	Degrees Celsius
cm	Centimetre(s)
cM	CentiMorgans
g	Relative centrifugal force
g	Gram(s)
h	Hour(s)
ha	Hectare(s)
kg	Kilogram(s)
l	Litre(s)
m	Metre(s)
μg	Microgram(s)
μl	Microlitre(s)
μm	Micrometre(s)
μM	Micromolar
meq	Milli-equivalents
mg	Milligram(s)
min	Minute(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
mmol	Millimole(s)
M	Molar
N	Normality
ng	Nanogram(s)
nm	Nanometre
pmol	Picomole(s)
psi	Pounds per square inch
pH	Power of hydrogen
r/s	Revolutions per second

s	Second(s)
t	Tonne(s)
V	Volt(s)
v/v	Volume per volume
W	Watt(s)
w/v	Weight per volume
w/w	Weight per weight

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CHAPTER 1

GENERAL INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the fourth most important vegetable oil crop after soybean, palm and edible rapeseed (canola) in world trade. It accounts for approximately 13% of the world's total edible oil production (Paniego *et al*., 2007). In South Africa sunflower is the largest source of vegetable oil. It contributes about 80% to the total oil produced and is followed by soybeans and canola that make up the balance (20%). The sunflower oil market has shown a steady increase of approximately three percent per year in the last few years. About 540 million litre oil is annually consumed and sunflower oil provides half of this quantity.

There are three types of sunflower. These include oilseed sunflower, non-oilseed (or confectionary) sunflower and ornamental sunflower. However, the production of sunflower is mainly devoted to oil extraction (Dorrell and Vick, 1997). The whole seed contains approximately 40% oil and up to 25% protein. The meal left after oil removal is usually used as livestock feed (Paniego *et al*., 2007).

Oilseed sunflower has many potential applications in both the non-food and food industry. Because of its relatively high iodine value of 133 (O'Brien, 2004), traditional sunflower oil is considered a semi-drying oil that can be used in the formulation of paints and for other industrial uses. Sunflower oil is traditionally used for cooking, frying, making salad dressing and margarine production. However, standard sunflower oil is not optimally suited to some potential applications that require a high oxidative stability, for example manufacturing of shelf-stable fried foods. In order to be able to use sunflower oil for industrial frying applications, the oil must be partially hydrogenated (Gupta, 2002). Hydrogenation involves the chemical addition of hydrogen to unsaturated fatty acids by mixing heated oil and hydrogen gas in the presence of a catalyst (O'Brien, 2004). During this chemical treatment, not only are unsaturated fatty acids converted to saturated ones, but many positional and "trans" isomers not normally found in nature, are also produced. There is evidence that the intake of these artificial (*trans*) fatty acids is casually related to the risk of developing heart disease (Stender and Dyerberg, 2004). Consequently, there is an increasing interest within the food industry to produce oil crops with higher amounts of saturated and mono-unsaturated fatty acids in their oils.

Traditional sunflower oil has been a popular vegetable oil for many years. However, it is polyunsaturated with a high linoleic acid and low saturated fatty acid content and the fatty acid composition of traditional sunflower oil is far from being appropriate for specific uses that require high saturation levels in the oil. Fortunately plant breeders have been successful in overcoming limitations of the traditional oil by developing a wide range of novel and healthier oil types (Fernández-Martínez *et al*., 2004). Recent research has lead to the development of high oleic acid sunflower varieties with oil that approach or exceed 89% oleic acid content (Dorrell and Vick, 1997). The high and mid oleic acid sunflower variants were developed through conventional breeding in the 1980s and 1990s and are speciality oils especially useful in food products such as spray oils (snacks and crackers), frying oils and for other products that require an oil with high oxidative stability (O'Brien, 2004). Due to their natural stability, these oils do not need to be hydrogenated in order to be used for these applications (Paniego *et al*., 2007). Additionally, high and mid oleic sunflower oils are considered healthier oils, because they contain no *trans* fatty acids.

The development of healthier sunflower oil types was encouraged by South African breeding companies after breeders rights in America expired. Breeding for high oleic acid sunflower started around 1983 in South Africa (R. Lochner, PANNAR®, personal communication, 2010). High oleic hybrids have been released and seed is commercially available. However, high oleic sunflower oil production started in 2003 and is still in the foundation stage. Only a few farmers are being contracted to plant high oleic acid hybrids.

Sunflower is commercially planted in South Africa in the Free State, North West, Limpopo, Mpumalanga, Gauteng, Western Cape, Eastern Cape and Northern Cape provinces with a total area of 635 800 ha planted during the 2008/2009 season (Dredge, 2010). The Free State and North West provinces are the major sunflower producing areas and constitute 88% of the total area planted in South Africa. These areas of sunflower production vary for climate, weather (rainfall and temperature) and other environmental factors such as intercepted solar radiation, altitude, latitude and soil type. Sunflower growth and development are greatly influenced by the weather and the environment it is grown in. Temperature and the amount of moisture in the soil are the major factors influencing sunflower seed oil composition and especially oleic acid content (Baldini *et al*., 2002). Fatty acid composition of sunflower is also affected by the genotype and its interaction with the environment. Genotype by environment interaction (GXE) has been reported for sunflower oil fatty acid composition (Lajara *et al*., 1990). As a result, the study of GXE interaction for South African sunflower hybrids is necessary in order to select stable and widely adapted hybrids in South African production areas.

Additionally, unusually high temperatures occurring during the seed-filling period have a huge influence on the fatty acid composition of traditional sunflower oil (Rondanini *et al*., 2003). It has been reported that high temperatures lead to an increase in oleic acid and decrease in linoleic acid content and vice versa (Harris *et al*., 1978; Chunfang *et al*., 1996). However, in high oleic acid sunflower, controversy exists regarding the effect of high temperature on oil composition. Differences in reports may be a consequence of different genetic backgrounds used (Salera and Baldini, 1998). Current trends toward increased global temperature (Easterling *et al*., 1997) may increase the probability of occurrence of high temperatures in many regions of the world (Conroy *et al*., 1994). These might also increase the frequency of episodes of high temperatures in warmer climates (Wheeler *et al*., 2000). This change in weather may cause unusually high temperatures during the critical stage of seed maturation which will have an influence on sunflower oil quality. Therefore the effect of temperature during the seed-filling period on oil content and composition in traditional, mid oleic and high oleic sunflower hybrids within South African genetic backgrounds is necessary. This would facilitate breeding strategies focussing on developing stable and widely adapted high oleic and mid oleic hybrids that are less sensitive to large temperature differences.

Since oleic acid content is largely affected by the environment and high oleic acid genes show unstable expression for oleic acid content in different genetic backgrounds, phenotypic selection for the high oleic acid trait may be difficult across different environments and seasons (Demurin and Škorić, 1996). DNA markers are not influenced by the environment and therefore selection for markers linked to the high oleic acid trait will further advance selection for this trait. Identifying molecular markers linked to the high oleic acid trait that can be further developed for use in marker-assisted breeding (MAB) would greatly assist breeding programmes in developing stable mid and high oleic acid breeding lines.

High oleic acid hybrids with comparable yield to that of traditional sunflower oil have recently been developed by South African breeding companies. As a result, local farmers can produce sunflower that has high yield potential as well as the benefits of healthier and more stable oil. Breeding high oleic hybrids with high seed yield, disease tolerance and shorter growth periods are some of the main focal points of sunflower breeding companies. In addition, much research is still needed to improve the stability of oil fatty acid composition.

The aims of this study were to::

- Investigate the effects of genotype, environment and their interaction on the fatty acid composition of traditional, high oleic and mid oleic sunflower hybrids and to make recommendations on the most stable and adaptable hybrids for the sunflower production areas under study.
- Study the effects of a short period of high temperature stress during the seed-filling stage on some yield traits as well as fatty acid composition. Genetic parameters including general and specific combining ability, variance components and heritability were investigated for yield traits and fatty acids.
- Identify putative DNA markers linked to the high oleic acid trait in South African lines that may in future be implemented in high oleic sunflower breeding programmes.
- Investigate a few physical and chemical properties of high oleic, mid oleic and traditional sunflower hybrids and to compare the three oil variants with regard to quality and oxidative stability.

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CHAPTER 2

LITERATURE REVIEW

2.1 Brief history of sunflower

Sunflower originated in northern Mexico and south-western USA and domestication occurred about 3000 B.C. by the Native American Indians (Heiser, 1954). It was of substantial importance to the indigenous population of that region who used the seed for food and medicine (Putt, 1997). Flower petals and oil were used for ceremonial body painting, while dried stalks were used as building material. At the beginning of the 1500s A.D. the arrival of Spanish explorers introduced sunflower to Europe. Nicolás Bautista Monardes (1508-1588) did the first scientific review of American plants and it was the first time that sunflower was mentioned (Grompone, 2005; Paniego *et al.*, 2007). It was popular as an ornamental plant and was later established as an oilseed crop in Eastern Europe (Putt, 1997). In the 18th century, sunflower cultivation spread to Russia and Peter the Great was accredited for this introduction (Semelczi-Kovacks, 1975). Sunflower oil became the main source of vegetable oil in Russia. The first commercial production of sunflower oil occurred in 1830 and since then the crop has steadily grown in importance. The introduction of Russian varieties such as Peredovic, Mennonite and Sunrise that were suitable for mechanical harvesting opened doors for the commercial development of sunflower. The discovery of cytoplasmic male sterility (Leclercq, 1969) and fertility restoration (Kinman, 1970) allowed efficient production of high oil content hybrid seed in the late 1970s that replaced the older varieties (Weiss, 2000). The crop was reintroduced into America in the late 18th century by Ukrainian immigrants. The first commercial use of sunflower was for poultry feed and processing of oil started in 1926. The first official sunflower-breeding programmes in America started during the 1930s using seeds introduced by European immigrants (Putt, 1997). In Argentina, a short-cycle and high oil content variety (Klein) was bred in 1938. Commercial production of oil seed-type sunflower started with the Peredovic variety and other cultivars and since 1966 several research programmes in the USA have sought to improve sunflower hybrids (Grompone, 2005). From then sunflower cultivation steadily increased in both North and South America leading to it being ranked first in the world for sunflower production (Paniego *et al.*, 2007).

Soldatov (1976) identified genotypes with oleic acid contents as high as 80-90% (Fick and Miller, 1997). Pervenets was the first high oleic acid variety developed through conventional breeding. Several breeding programmes included Pervenets in their crosses as the high oleic acid content parent. High oleic oil gained market acceptance, especially for food and industrial purposes where a high level of oxidative stability was required. High oleic sunflower oil became commercially available in Russia in the late 1970s and in the USA in 1985. The development of high oleic sunflower varieties was encouraged by South African breeding companies after breeders' rights in America expired. Breeding for high oleic acid sunflower started around 1983 in South Africa (R. Lochner, PANNAR®, personal communication, 2010).

2.2 Economic importance of sunflower

Sunflower oil production is determined by the production of seed. Total world production of sunflower seed was on average 31 million metric tons during the last few years (Table 2.1). Russia and Ukraine rank first in the world for sunflower production. The European countries account for about 50% of the world production of sunflower. In 2007, South Africa ranked 15th in the world with a seed production of 300000 metric tons (FAO, 2010). High oleic sunflower is commercially produced mainly in the United States and France. High oleic oil contributes less than 5% of the total sunflower production globally with about 300000 metric tons of high oleic oil produced annually.

Table 2.1 World sunflower seed production (in 1000 metric tons)

Area	**2006/2007**	**2007/2008**	**2008/2009**
Russia/Ukraine	11900	10380	14320
European Union	6407	4944	6848
Argentina	3120	4600	3130
China	1850	1800	1850
India	1450	1460	1150
United States	997	1309	1553
Turkey	820	670	850
Rest of Europe	385	295	454
South Africa	300	872	801
Other	2863	2929	3595
Total	**30092**	**29259**	**34551**

(Dredge, 2010; National Sunflower Association, 2010).

2.3 Botanical description

Sunflower belongs to the subtribe Helianthinae, the subfamily Asteroideae and family Compositae (Seiler and Rieseberg, 1997). The genus name of sunflower is derived from two Greek words: *helios* meaning sun and *anthos*, meaning flower (Salunkhe *et al*., 1992; Paniego *et al*., 2007). The genus has a basic chromosome number of n=17 and contains diploid (2n=2x=34), tertaploid (2n=4x=68) and hexaploid (2n=6x=102) species. It includes 12 annual and 36 perennial species (Jan, 1997). The Jerusalem artichoke (*Helianthus tuberosus* L.) is related to sunflower. Sunflower that is commercially cultivated for seed purposes is grouped under *H. annuus* variety *macrocarpus* (Maiti *et al*., 1988). The commercial crop is a predominantly cross-pollinating annual upright plant with a long stem of 1-3 m. It has a terminal flower head (also called a capitulum) that is commonly about 30 cm in diameter. The characteristic of turning its head towards the sun additionally accounts for sunflower's common and botanical name (Seiler, 1997; Paniego *et al*., 2007).

In oilseed cultivars, the sunflower head consists of 700-3000 flowers. The sunflower inflorescence consists of two types of flowers. The outer whorl of flowers, called the ray florets, is sterile and has a display role. The remainder of the flowers, the hermaphroditic disk florets, are arranged in arcs radiating from the centre of the head and these produce the seed. During anthesis the outer whorl of disk flowers opens first and then progresses to the centre of the head at one to four rows per day. Opening of all florets on the head is usually completed within 10-15 days, but individual florets can remain receptive for up to two weeks. Sunflower is generally an open-pollinator and bees are beneficial in transferring pollen from plant to plant that result in cross-pollination. Varieties differ in their dependence on insect pollinators. The older open-pollinated varieties have a seed set of only 15-20% without pollinators, while recent autogamous sunflower hybrids have a seed set of 85-100% without pollinators (Knowles, 1978; Weiss, 2000; Putnam *et al*., 2009).

The sunflower seed (or achene) consists of a kernel and adhering pericarp (or hull). The hull comprises about 21-30% of the final achene weight (Dorrell and Vick, 1997). All achenes develop hulls, even if they are not fertilised. The kernel consists of two cotyledons and an embryo. The embryo contains the oil-rich, large aleurone particles and protein crystals (Knowles, 1978; Salunkhe *et al*., 1992; Seiler, 1997). Accumulation of reserve lipids in the embryo begins several days after the rapid growth of the embryo. Little oil is deposited during the first third of the seed-filling period, but increases to a fairly stable rate that is maintained until close to physiological maturity (Harris *et al*., 1978; Villalobos *et al*., 1996; Connor and Hall, 1997; Rondanini *et al*., 2003; Mantese *et al*., 2006; Dong *et al*., 2007). Physiological maturity of the seed is reached when seed oil percentage and dry weight are at their maximum about 35 days after the initiation of flowering (DAF) (Robertson *et al*., 1978).

2.4 Chemical composition of sunflower seed and oil

The chemical composition of sunflower seed varies widely due to genetic and environmental factors. Proximate chemical compositions of open-pollinated cultivars and hybrid sunflowers are presented in Table 2.2. Data were obtained from a United States Department of Agriculture (USDA) study in 1994 (Gupta, 2002).

Table 2.2 Proximate composition of sunflower seeds

Constituent	Percentage (%)
Hull	20-25
Oil	44-51
Protein	15-25
Fibre residue	15-20
Ash	0.41-0.45

(Weiss, 2000; Gupta, 2002; Paniego *et al*., 2007).

2.4.1 Seed oil content

Oil content in sunflower seed ranges between 25-48%, but can reach 65% depending on the genotype and environmental factors (Salunkhe *et al*., 1992; Weiss, 2000). The kernel (dehulled seed) contains more oil than the whole seed. The kernel contains the highest percentage of oil (87%) followed by the embryo (7.4%).

The oil percentage of whole sunflower achenes depends on both the percentage of oil in the kernel and the proportion of hull (Weiss, 2000). The hull contains a low percentage of oil and is reported to be between 0.4-5.2% by several authors (Salunkhe *et al*., 1992).

2.4.2 Triacylglycerol structure

Sunflower oil mainly contains triacylglycerol (TAG) molecules that represent more than 95% of the total oil weight (Fernández-Martínez *et al.*, 2009). The storage lipid structure fits the general hypothesis for distribution of the fatty acid on the triacylglycerol molecule. Three fatty acids are esterified to the hydroxyl groups of a glycerol backbone (Figure 2.1).

A stereochemical numbering system is used to identify the three positions on the glycerol derivative as *sn*-1, *sn*-2 and *sn*-3 from the top to the bottom with the secondary hydroxyl to the left of the central carbon. Several seed oil analyses have indicated that saturated fatty acids tend to occupy the *sn*-1 position, whereas unsaturated fatty acids are found at the *sn*-2 position. The *sn*-3 position is occupied with variable molecular fatty acid species (Weselake, 2002). In sunflower oil, the TAG molecule has unsaturated fatty acids at all three positions of the glycerol molecule. However, linoleic acid preferentially esterifies the *sn*-2 position.

The predominant form (39%) is monooleoyl-dilinoleoyl-glycerol (OLL) with one molecule of oleic acid and two molecules of linoleic acid esterified to one molecule of glycerol. Dioleoyl-monolinoleoyl-glycerol (OOL) and trilinoleoyl-glycerol (LLL) forms occur in lesser amounts (Dorrell and Vick, 1997).

2.4.3 Fatty acid compositions of the three types of sunflower

Fatty acid contents vary slightly between different reports for traditional, high oleic and mid oleic sunflower oil. This can be attributed to differences in genetic backgrounds used and growing conditions of sunflower plants. However, typical fatty acid compositions of the three types of sunflower are accepted according to the report of Gupta (2002) (Table 2.3). Traditional sunflower oil is characterised by a high concentration of linoleic acid (66-72%), a moderate level of oleic acid (16-20%) and low level of linolenic acid (less than 1%). The saturated fatty acids (SFA), palmitic (C16:0) and stearic (C18:0) acids, account for less than 15% of the fatty acids. Lauric, arachidic, behenic, lignoceric and eicosenoic acids occur in minor percentages (Seiler and Brothers, 1999).

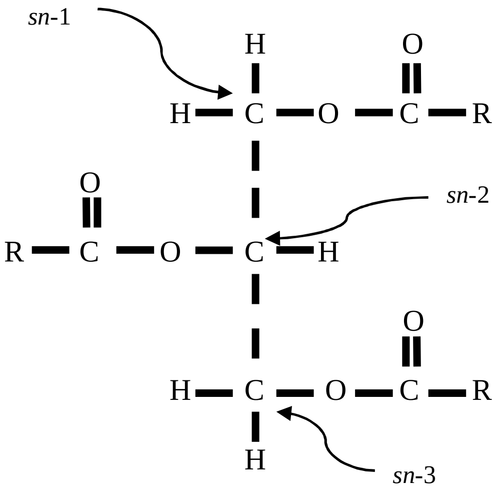

Figure 2.1 **General structure of triacylglycerol.** R is the fatty acyl chain without the carboxyl group (Weselake, 2002).

Table 2.3 **Typical fatty acid composition (%) of traditional, high oleic and mid oleic sunflower oil**

Fatty acid	Traditional (%)	High oleic (%)	Mid oleic (%)
Total SFAs	11-13	9-10	<10
Oleic acid	20-30	80-90	55-75
Linoleic acid	60-70	5-9	15-35
Linolenic acid	<1	<1	<1

SFA: Saturated fatty acid.

Sunflower cultivars with high oleic acid content were introduced during the 1980s. High oleic sunflower oil differs from traditional sunflower oil by a significantly increased oleic acid content to more than 80% (Dorrell and Vick, 1997), a low concentration of linoleic acid (2-9%) and generally less than 10% SFAs. The high level of monounsaturation makes the high oleic oil less susceptible to oxidative degradation than the traditional sunflower oil and therefore the high oleic oil shows potential for applications requiring a high oxidative stability (Seiler and Brothers, 1999).

In early 1995, the initial idea to redesign traditional sunflower oil to contain an oleic acid content of approximately 60% was suggested by the snack food and oil processing industry in the USA. NuSun sunflower oil was developed by F. Miller and B. Vick in Fargo, North Dakota through conventional hybrid breeding. It is referred to as mid oleic oil because it contains higher oleic acid content than traditional sunflower oil but lower oleic acid content than the high oleic variety. The mid oleic oil has a lower SFA content than traditional sunflower oil, but the same SFA content as high oleic oil. NuSun's oil fatty acid profile leads to highly stable oil that does not need to be hydrogenated for commercial use (Gupta, 2002).

2.4.4 Non-acylglycerol components

In addition to oil and protein, sunflower seed contain micro-constituents that include phospholipids, sterols, waxes and tocopherols among others. Phospholipids, also known as phosphadites, are naturally present in all oilseeds and are oil-soluble. These lipids are composed of glycerol esterified with fatty acids and phosphoric acid and comprise about 1% of the lipids. A low phospholipid content is desirable for refined sunflower oil. This is accomplished by chemical or refining processing of the oil. Sterols and sterol esters are essential components of cell membranes. They are natural antioxidants that showed benefits in human nutrition by lowering total and low-density lipoprotein (LDL) cholesterol (Fernández-Martínez *et al*., 2009). Wax and wax like material are mainly present in the seed hull (83% of the total) and are usually less than 1% of the total lipids in the seed. Its content in the crude oil is minimised by de-hulling of seeds before crushing. Waxes are undesirable for salad oils as they give the oil a cloudy appearance when refrigerated. The process of dewaxing is used to reduce the wax content of the oil. Tocopherols are natural fat-soluble compounds that exert an antioxidant action both *in vivo* (vitamin E activity) and *in vitro*. Tocopherols exist in four forms, including, alpha, beta, gamma and delta and each form differs in antioxidant activity. Alpha-tocopherol is the most efficient antioxidant *in vivo*, while gamma-tocopherol is the most powerful antioxidant *in vitro* (Kalmal-Eldin and Appelqvist, 1996). Beta- and delta-tocopherols have intermediate properties (Pongracz *et al*., 1995). Sunflower oil contains a high concentration of alpha-tocopherol (95% of the total tocopherol) that has the highest *in vivo* activity, but the lowest *in vitro* activity, of the four antioxidants (Dorrell and Vick 1997; Gupta, 2002).

2.4.5 Oil quality parameters

Traditionally oil quality was measured primarily based on oil content and fatty acid composition and the ideal fatty acid composition depended on the end-use of the oil (Knowles, 1983; Rondanini *et al*., 2003). However, more recently other components of vegetable oils that influence their nutritional and technological properties are being emphasised by oil chemists and nutritionists (Fernández-Martínez *et al*., 2007). The main parameters defining the quality of oil are 1) fatty acid composition, 2) the distribution of fatty acids within the triacylglycerol molecule and 3) the total content and composition of natural antioxidants, tocopherols and sterols (Fernández-Martínez *et al*., 2004; 2007).

From a nutritional viewpoint, SFAs are regarded as undesirable for human health. Intake of especially lauric, myristic and palmitic acid has a detrimental atherogenic effect by raising both total serum and LDL cholesterol levels (Katan *et al*., 1995). However, individual fatty acids within this group have different effects. Although lauric acid greatly increases total cholesterol, its effect is mostly on HDL cholesterol. Oils rich in lauric acid decrease the ratio of total to HDL cholesterol, while myristic and palmitic acids show little effect on the ratio. Stearic acid reduces the ratio (Mensink *et al*., 2003). Conversely, monounsaturated (oleic) and polyunsaturated (linoleic) fatty acids are hypocholesterolemic (Mensink and Katan, 1989; Kris-Etherton and Yu, 1997). Although linoleic acid is an essential fatty acid, it is more susceptible to oxidation than oleic acid. Therefore, oil rich in oleic acid is preferred as it combines the hypocholestrolemic effect and a greater oxidative stability (Yodice, 1990). From a technological point of view, manufacturing of certain food products, such as margarine, requires solid or semi-solid fats. Since traditional sunflower oil is a liquid at room temperature, the oil needs preceding chemical hardening to change it to a semi-solid state. This is usually obtained by hydrogenation or trans-esterification of the oil that produces harmful *trans* fatty acids (O’Brien, 2004). For these applications, sunflower oil with a high concentration of SFAs is necessary (Pérez-Vich *et al*., 2000).

The stereochemical position of the three fatty acids in the TAG molecule is another important parameter in the nutritional value of oils. The absorption rate of fatty acids is higher when they occupy the central *sn*-2 TAG position than when they are at the external *sn*-1 and *sn*-3 positions (Bracco, 1994). As a result, oils that have undesirable fatty acids at the *sn*-2 position are more atherogenic than those that have similar total fatty acid contents, but distributed at the external *sn*-1 and *sn*-3 positions (Alvarez-Ortega *et al.*, 1997).

Tocopherols are important compounds that have antioxidant activity in sunflower seeds. The antioxidant properties of oil depend on both the total tocopherol content and its composition (Shintani and DellaPenna, 1998). In sunflower, large variation for tocopherol content has been reported (Marquard, 1990; Demurin, 1993). Tocopherol content of sunflower seed is affected by both the genotype and environment (Dolde *et al.*, 1999; Velasco *et al.*, 2002).

Sunflower seed oil quality has been modified by the development of oil with enhanced nutritional and functional properties as well as oil that requires less or no processing for specific end-use markets. Oil quality modifications include breeding for increased linoleic acid content (Miller and Vick, 2001) for special margarine markets, reducing palmitic and stearic acids (Miller and Vick, 1999; Seiler, 2004) for improved nutritional value and increased levels of palmitic acid (Fernández-Martínez *et al.*, 1997) to prevent crystallisation in manufacturing and storage of margarine (Fick and Miller, 1997). Breeding efforts to improve oil oxidative stability also led to the development and characterisation of several sources of modified tocopherol profiles in sunflower (Demurin, 1993; Demurin *et al.*, 1996; Velasco *et al.*, 2004a).

2.4.6 Oil oxidative stability

Lipid oxidation is a major factor for quality deterioration in edible oils and fatty acid foods since it alters their chemical, sensory and nutritional properties (Frankel, 1998). Autoxidation is a major cause of quality losses in crude and refined oils during storage. The rate of oxidation depends on storage conditions, such as temperature and the presence of light, as well as on the availability of soluble and reactive oxygen in the oil's mass (Márquez-Ruiz *et al.*, 2003). Oil oxidative stability and deterioration depend on the initial oil composition, concentration of compounds with antioxidant or pro-oxidant characteristics and degree of processing (Crapiste *et al.*, 1999; Kanavouras *et al.*, 2005). Oxidation of oils occurs at sites of unsaturation (Labuza and Dugan, 1971) and as a result, the rate of oxidation of fatty compounds depends on the number of double bonds and their position (Frankel, 1998). Autoxidation of unsaturated lipids is a series of free radical reactions, initiated and propagated by free radicals reacting with methylene ($-CH_2-$)

groups that are adjacent to double bonds (Figure 2.2). A free radical is an unpaired electron, indicated as a heavy dot in chemical formulas and is a highly reactive species. Autoxidation can be described in terms of initiation, propagation and termination (Stauffer, 1996; Crapiste *et al.*, 1999; Choe and Min, 2006).

Initiation starts when a hydrogen atom departs from the α-methylenic carbon, adjacent to a double bond in a fatty acid (RH) group of the lipid molecule. This reaction may be catalysed by light, heat or metal ions to form a free radical (R•) (reaction 1).

Initiation: $\mathrm{RH} \xrightarrow{\text{initiator}} \mathrm{R}\bullet + \mathrm{H}\bullet$

The resultant alkyl free radical is highly susceptible to attack by atmospheric oxygen and the dissolved oxygen adds to this site and an unstable peroxide free radical is formed (ROO•) (reaction 2). The peroxide free radical abstracts a hydrogen from another methylene group and reacts with the hydrogen to form a hydroperoxide (ROOH) and a new alkyl free radical (reaction 3).

Propagation:

$$\begin{tikzcd}[column sep=small] & \mathrm{R}\bullet + \mathrm{O_2} \rightarrow \mathrm{ROO}\bullet \\ & \mathrm{ROO}\bullet + \mathrm{RH} \rightarrow \mathrm{ROOH} + \mathrm{R}\bullet \arrow[u, bend right=60] \end{tikzcd}$$

These free radicals serve as strong catalysts of further oxidation reactions, hence oxidative degradation of oils become an autocatalytic process. The chain reaction (or propagation) may be terminated by the formation of non-radical products that result from the combination of two radical species (reactions 4-6). In the final stage of oxidation, the hydroperoxides are readily decomposed into aromatic organic compounds, mainly aldehydes, ketones, alcohols and acids. These compounds cause the rancidity condition that ultimately destroys acceptability and usefulness of oils (Sherwin, 1978; Shahidi and Wanasundara, 1996; Stauffer, 1996; Choe and Min, 2006).

Termination: $\mathrm{R}\bullet + \mathrm{R}\bullet \rightarrow \mathrm{RR}$

$\mathrm{R}\bullet + \mathrm{ROO} \rightarrow \mathrm{ROOR}$

$\mathrm{ROO}\bullet + \mathrm{ROO}\bullet \rightarrow \mathrm{ROOR} + \mathrm{O_2}$

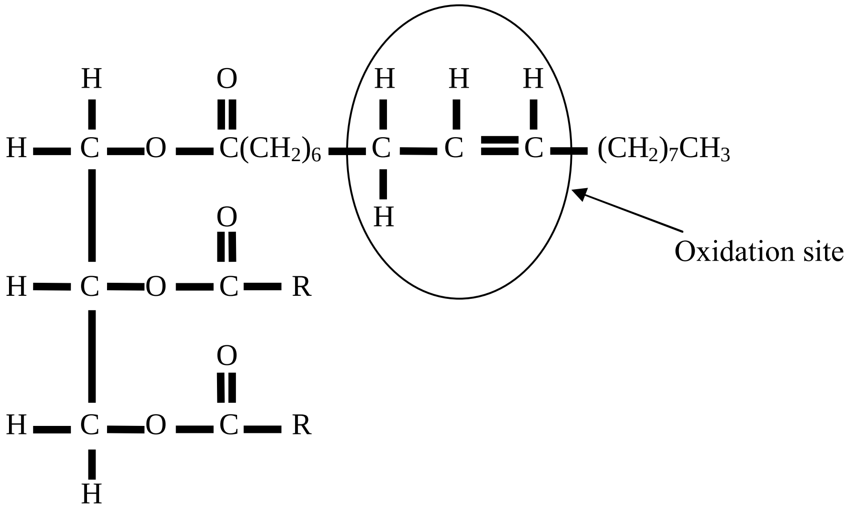

Figure 2.2 Typical unsaturated triglyceride molecule with double-bond linkage and α-methylenic carbon (oxidation site). R: Fatty acid group (Sherwin, 1978).

The progress of oxidation can be studied by the quantification (or measurement) of oxidised TAG monomers, dimers and polymers (Márquez-Ruiz *et al.*, 1996). Martín-Polvillo *et al.* (2004) studied the evolution of oxidation in sunflower oils during long-term storage at room temperature and distinguished two oxidation stages: 1) An induction period which is characterised by slow progress of oxidation and 2) an accelerated (advanced) oxidation stage. During the induction period a significant increase in the monomeric oxidation compounds occur and the oxidised monomers are mainly composed of hydroperoxides during the early oxidation stage (Márquez-Ruiz *et al.*, 1996; Martín-Polvillo *et al.*, 2004). The end of the induction period is defined as the point when a notable shift in the oxidation rate is observed and is clearly characterised by a sharp increase in levels of total oxidation compounds, exhaustion of antioxidants (α-tocopherol) and significant formation of polymerisation products. The length of the induction period depends on the degree of unsaturation. The higher the degree of oil unsaturation, the shorter the induction period and the higher the amount of primary oxidation products accumulated at the end of the induction period (Martín-Polvillo *et al.*, 2004). During the advanced oxidation stage, secondary products are formed. As a consequence, TAG containing oxygenated functions other than the hydroperoxide (epoxy, keto, hydroxy, etc.) starts contributing to the amount of oxygenated TAG monomers. Hydroperoxide functions are therefore not only present in primary oxidation compounds but are also involved in dimeric linkages of polymerisation compounds (Martín-Polvillo *et al.*, 2004).

Various methods are available to measure lipid oxidation and may be divided into two groups. The first group measures primary changes and the second group secondary changes. Primary changes are generally measured by monitoring loss of unsaturated fatty acids, oxygen uptake by weight gain, hydroperoxide values and conjugated diene value. During the early stages of lipid oxidation, edible oils increase in weight as fatty acids combine with oxygen during hydroperoxide formation. Therefore, the increase in weight in a heated sample during storage can be used to determine the induction time of the oil (Shahidi and Zhong, 2005). Secondary changes are followed by quantitation of carbonyl compounds, malonaldehyde and other aldehydes and fluorescence products. The method chosen depends on the nature of the oxidised sample, type of information required, time available and test conditions. Rapid methods have been developed to test the resistance of edible oils to oxidation. The active oxygen method (AOM) is based on the principle that rancidification of fat is greatly accelerated by aeration in a tube held at constant temperature. The Metrohm Rancimat, which assesses the oxidative stability index (OSI), is a rapid automated method and is frequently used due to its ease of use and reproducibility. The OSI and Rancimat tests measure the changes in conductivity of water in which volatile organic acids (mainly formic acid) are trapped, while in AOM, peroxide values are measured. The OSI determines the induction period precisely and is based on analysis of stable secondary products. The AOM, however, does not determine the induction period (is merely related to it) and relies on the analysis of unstable primary reaction products (Shahidi and Wanasundara, 1996; Pike, 2001).

The above mentioned oxidative quality indices have been used in combinations to study lipid oxidation in various vegetable oils. For instance, peroxide value (PV), anisidine value (AV), free fatty acids (FFAs), polar compounds and weight gain (Shahidi and Zhong, 2005) were used to study the oxidative deterioration of crude sunflower oils obtained by either pressing or solvent extraction (Crapiste *et al*., 1999). The oil was stored at different temperatures and varying oxygen concentrations (Crapiste *et al*., 1999). These authors found a positive correlation between polar compound content and PV. In another study Martín-Polvillo *et al.* (2004) investigated the oxidative stability of sunflower oils that differed in their unsaturation degrees during long-term storage at room temperature (25ºC). FFA, PV, ultraviolet (UV) absorbance at K_{270} nm (measures secondary oxidation products such as ethylenic diketones, conjugate ketodienes and dienals) and unsaponifiable matter content was determined in order to evaluate the initial oil quality of the three sunflower oil types. The Rancimat apparatus was used to determine OSI values. Induction periods obtained for the three oils were compared and a significant correlation was found between the length of the induction period and the degree of oil unsaturation. High oleic sunflower oil proved to be twice as stable as traditional sunflower oil during long-term storage.

Smith *et al.* (2007) also compared the oxidative stability of high oleic sunflower oil with those of traditional sunflower, soybean, maize and groundnut oils during storage at 55ºC. Oxidative stability was evaluated by measuring oxygen depletion, head space volatile compound formation and peroxide value. They found that the high oleic sunflower oil had a higher oxidative stability than regular sunflower and soybean oil, but it was similar to those of maize and groundnut oil. Oil stability index and PV have been used to determine oil quality and stability of conventional and high oleic varieties of commercial vegetable oils with and without added antioxidants (Merrill *et al.*, 2008). OSI values obtained in hours at 110ºC for the conventional oils were 5.2 h (sunflower), 7.6 h (soybean), 8.4 h (canola), 9.8 h (maize), 10.9 h (partially hydrogenated soybean) and 14.3 h (oleic safflower oil). OSI values of high oleic variants were 12.9 h (high oleic canola), 16.5 h (high oleic sunflower) and 18.5 h (very high oleic canola), respectively. Additionally, these authors found that the added antioxidants increased the OSI value and the most stable oils were high oleic canola, very high oleic canola, oleic safflower and high oleic sunflower with OSI values of 40.9 h, 48.5 h, 48.8 h and 55.7 h, respectively. Oil stability during use and storage can effectively be improved by the natural antioxidants contained in sunflower oil. Food applications demanding high nutritional values require an improvement of the alpha-tocopherol content and a reduction in the SFAs of the oil (Fick and Miller, 1997; Fernández-Martínez *et al.*, 2007).

2.5 Lipid biosynthesis

In oilseeds fatty acids are synthesised at high rates over a short period and used preferentially for TAG synthesis. Vegetable oil synthesis is divided into two distinct, spatially separated reactions, namely fatty acid biosynthesis in the plastids and lipid biosynthesis in the cytosol (Figure 2.3). More than 20 separate biochemical steps are involved, starting with the first step of fatty acid biosynthesis and ending with TAG production (Schultz and Ohlrogge, 2002).

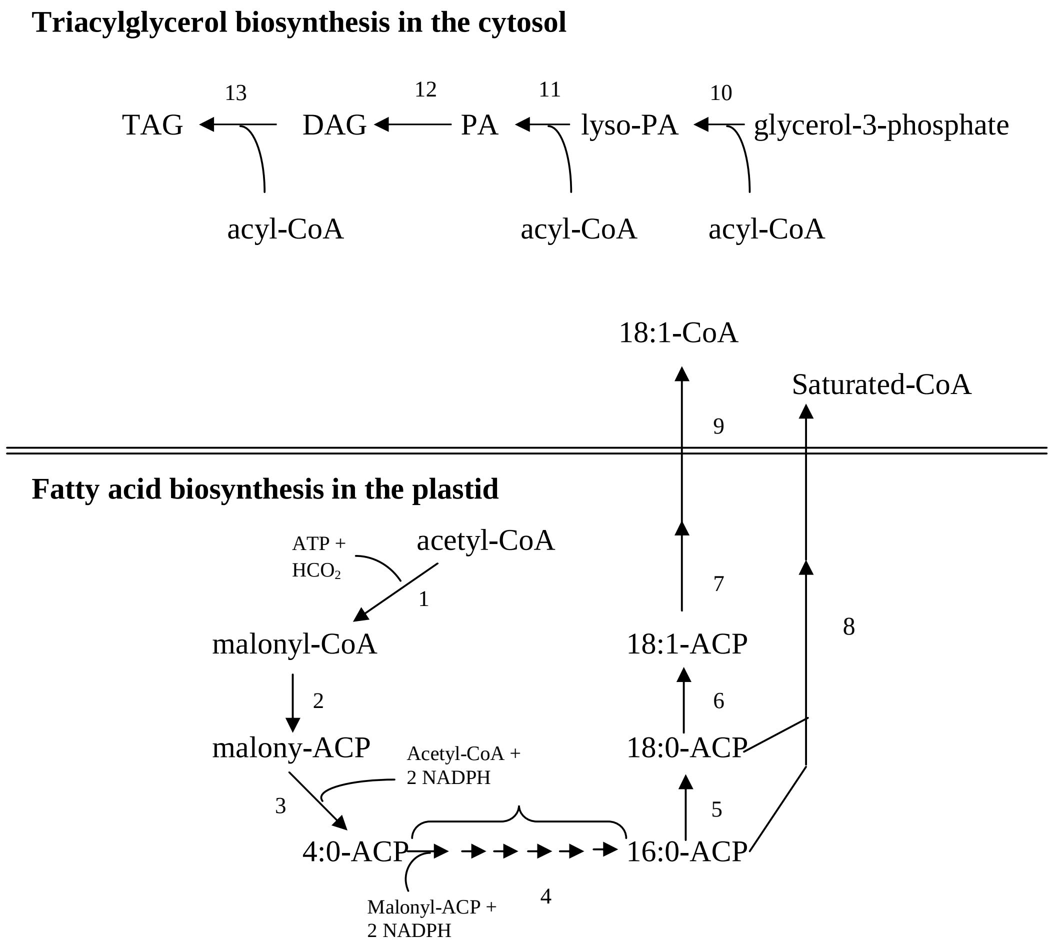

Figure 2.3 Simplified schematic showing the two spatially separate pathways involved in lipid biosynthesis in vegetable oil production.

PA: Phosphatidic acid, DAG: Diacylglycerol, TAG: Triacylglycerol, Enzymes for numbered reactions: (1) Acetyl-coenzyme A (CoA) carboxylase, (2) Malonyl-CoA:acyl carrier protein (ACP) transacylase, (3) β-ketoacyl-acyl carrier protein synthase (KAS III), β-ketoacyl-ACP reductase, β-hydroaxyacyl-ACP dehydrase, Enoyl-ACP reductase, (4) β-ketoacyl-acyl carrier protein synthase (KAS I) β-ketoacyl-ACP reductase, β-hydroaxyacyl-ACP dehydrase, Enoyl-ACP reductase, (5) β-ketoacyl-acyl carrier protein synthase (KAS II) β-ketoacyl-ACP reductase, β-hydroaxyacyl-ACP dehydrase, Enoyl-ACP reductase, (6) Δ9 steroyl-ACP desaturase, (7) Thioesterase (Fat A), (8) Thioesterase (Fat B), (9) Acyl-CoA synthetase; (10) Cytosolic G-3-P acyltransferase, (11) Lyso-phosphatidic acid acyl transferase, (12) Phosphatidic acid phosphatase, (13) Diacylglycerol acyl transferase, ATP: Adenosine 5'-triphosphate, HCO_2: Formic acid, NADPH: Nicotinamide adenine dinucleotide phosphate (Schultz and Ohlrogge, 2002).

Fatty acid biosynthesis in oilseed is initiated with acetyl-coenzyme A (CoA) as substrate and is catalysed by the actions of acetyl-CoA carboxylase and the dissociable fatty acid synthetase (FAS) enzyme complex type III (Harwood, 1996). The sequential actions of FAS III and FAS I produce mainly palmitoyl-acyl carrier protein (palmitoyl-ACP), which is then elongated by two carbon atoms to produce stearoyl-ACP by the FAS II complex. In turn, the stearoyl-ACP is desaturated by the stearoyl-ACP desaturase (SAD) enzyme, which introduces the first double bond in the carbon chain (between carbon atoms 9 and 10) to produce oleoyl-ACP. The acyl-ACPs, palmitoyl-ACP, stearoyl-ACP and oleoyl-ACP are hydrolysed to free fatty acids that can be exported to the cytosol. These are activated to the corresponding acyl-CoAs, by the action of the acyl-ACP thioesterases (Rolletschek *et al.*, 2007). Two types of thioesterases have been identified: Fat A and Fat B (Martínez-Force *et al.*, 2000). Fat A preferentially acts on long chain fatty acids and has a high specificity for 18:1-ACP. In contrast, Fat B exhibits a higher affinity for the saturated 16:0-ACP and 18:0-ACP (Pleite *et al.*, 2006). Although these thioesterases influence the final composition of seed oils (Davies, 1993), fatty acid biosynthesis is also influenced by the interaction of thioesterases with key enzymes such as FAS II and SAD (Martínez-Force and Garcés, 2002).

Once acyl-ACPs are hydrolysed, acyl-CoA synthase incorporates acyl molecules to the pool of acyl-CoA. Oleic acid, once incorporated into phosphatidyl-choline, can be desaturated to linoleic acid and then into α-linolenic acid by the actions of oleoyl-phosphatidylcholine desaturase (ODS) and linoleoyl-phosphatidylcholine desaturase respectively. Both enzymes are membrane-bound and located in the endoplasmic reticulum (ER) (Gray and Kekwick, 1996).

TAG biosynthesis has been proposed to take place by the Kennedy pathway in some species. Fatty acids are sequentially transferred from CoA to positions *sn*-1 and *sn*-2 of glycerol-3-phosphate, resulting in the formation of phosphatidic acid (PA), a central metabolite. Diacyglycerol (DAG) is released by the defosforilation of PA. A third fatty acid is transferred to position *sn*-3 of DAG to form TAG, catalysed by diacylglycerol-acyltransferase.

Most of the fatty acids produced in the plastid are not immediately available for TAG biosynthesis. Instead, in most oilseeds the major flux of acyl chains enter into phosphatidylcholine (PC) pools where further desaturation and hydroxylation occur. The fatty acids from PC may then become available for TAG synthesis by one of two mechanisms (Figure 2.4) (Ohlrogge and Browse, 1995; Thelen and Ohlrogge, 2002):

1) Fatty acids attached to CoA and those on PC may essentially trade places. This acyl exchange probably occurs by the combined reverse and forward reactions of an acyl-CoA:PC acyltransferase (Stymne and Stobart, 1987). The resulting acyl-CoA may then be used as an acyl donor in TAG synthesis.

2) PC can donate its entire DAG portion for TAG synthesis. In many plants, the synthesis of PC from DAG and cytidine 5'-diphosphate (CDP)-choline appears to be rapidly reversible as catalysed by the choline-DAG phosphotransferase. The reversibility of this reaction allows the DAG moiety of PC to become available for TAG synthesis.

The flux of acyl chains in the ER eventually leads to esterification on all three positions of the glycerol to form TAG (Thelen and Ohlrogge, 2002).

Mutations leading to modification of enzymatic activities could occur at several levels (desaturase gene deletion or duplication, transcription levels of these genes or accumulation of desaturase gene transcripts) and therefore several mechanisms may explain the oleic acid accumulation in high-oleic seeds (Lacombe and Bervillé, 2000).

Several studies indicated that in high oleic acid content sunflower, ODS activity was drastically reduced during the critical stages of reserve lipid synthesis, explaining the low linoleic and high oleic acid content in high oleic acid mutants of sunflower (Garcés *et al.*, 1989; Garcés and Mancha, 1991).

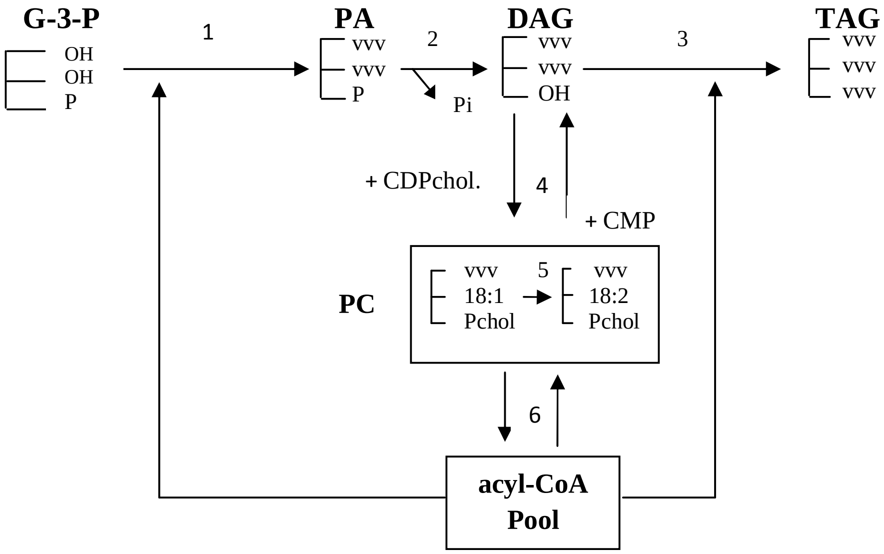

Figure 2.4 The biosynthetic pathway of TAG in developing sunflower seeds.
G-3-P: Glycerol-3-phosphate, PA: Phosphatidic acid, PC: Phosphatidyl-choline, DAG: Diacylglycerol, TAG: Triacylglycerol, CDPchol: Cytidine diphosphocholine, CMP: Cytidine monophosphate, vvv: Fatty acid. Enzymes for numbered reactions: (1) G-3-P and lyso PA-acyltransferases, (2) Phosphatidate-phosphatase, (3) DAG-acyltransferase, (4) CDP choline:DAG-phosphotransferase, (5) Oleoyl PC-desaturase, (6) Lyso PC-acyltransferase, Pi: Phosphatidylinositol, Pchol: Phosphocholine, CoA: Coenzyme A (Triki *et al*., 1999).

Martínez-Rivas *et al.* (2001) reported that the seed specific microsomal ODS encoded by the *FAD2-1* gene was completely active, however, the level of gene expression was drastically reduced. They suggested that the high oleic mutation in sunflower obstructs the regulation of the transcription of the seed-specific *FAD2-1* gene. Other studies revealed that the high oleic phenotype was correlated to a strong reduction in the level of ODS transcript accumulation in the high oleic sunflower embryos during the critical stages of storage lipid biosynthesis (Hongtrakul *et al*., 1998; Lacombe and Bervillé, 2000). The lack in ODS transcript accumulation could be due to a non-expression of the *FAD2-1* gene (Lagravere *et al*., 2000).

2.6 Genotypic and environmental factors influencing seed oil content and composition

2.6.1 Genotypic factors

Sunflower seed oil content and fatty acid composition are significantly influenced by genetic factors. Seed oil content depends on the proportion of hull in the seed. A negative correlation between oil content and hull thickness has been reported by several researchers (Anand and Chandra, 1979; Lajara *et al*., 1990; Denis *et al*., 1994; Weiss, 2000). Cultivars with low hull content contain more oil than those with a high hull content. Therefore, breeding and selection for lower hull percentage resulted in an increase in seed oil percentage (Fick and Miller, 1997). Head size and shape are additional factors that have an influence on these traits (Weiss, 2000). Afzalpurkar and Lakshminarayana (1980) reported that seed from smaller sized heads contained more oil than those from larger heads. Oil content and fatty acid composition also vary with the position of seed within the head. Munshi *et al*. (2003) found that oil content was higher in seed from the peripheral whorls than in those from the middle and central whorls. For fatty acid composition, the linoleic acid and palmitic acid contents increase, while the oleic acid content decreases in seed from the perimeter towards the centre of the head (Fick and Zimmerman, 1973; Zimmerman and Fick, 1973).

2.6.2 Environmental factors

Sunflower oil content and composition is not only influenced by genetic factors, but also by environmental and agronomical factors including planting location, climate, temperature, water regime, planting date and fertiliser and irrigation applications (Salunkhe *et al*., 1992; Seiler and Brothers, 1999; Flagella *et al*., 2002; Izquierdo *et al*., 2002; O’Brien, 2004; Qadir *et al*., 2006).

Robertson *et al*. (1978) found that latitude and average temperature had no significant effect on the oil content of sunflower seed in a study including several locations and years in North America. However, Chunfang *et al*. (1996) reported that both latitude and altitude had an influence on oil content and fatty acid composition and that the higher the latitude, the higher the oil content. They also found that in the same latitudes, sunflower grown in areas of high altitude contained more oil and that the influence of altitude was stronger than that of latitude. These differences may vary with temperature.

The fatty acid composition of sunflower seed varies with planting location and climatic conditions during the growing season (Kinman and Earle, 1964; Cummins *et al*., 1967; Chunfang *et al*., 1996; Qadir *et al*., 2006). In general there exists a correlation between latitude and fatty acid composition. Sunflower seed grown in the northern hemisphere had higher linoleic acid content than seed grown in the southern hemisphere. However, this correlation was only because of the influence of latitude on the climatic conditions (Lajara *et al*., 1990). Temperature is mostly the direct consequence of location and therefore microclimatic conditions may determine acute differences within the same latitude (Chunfang *et al*., 1996).

According to Flagella *et al*. (2002), different planting dates and water regimes cause different environmental conditions during seed-filling and oil synthesis of sunflower seed and therefore a possible alteration in oil content and fatty acid composition of the seed. Different planting dates may cause flowering and seed development to occur during periods of widely different temperatures, radiation, day length and soil water availability. Both oleic and linoleic acid concentrations of the oil of cultivated sunflower were significantly related to total solar radiation and day length (Seiler, 1983).

Sunflower seeds grown under non-irrigated (rainfed) or limited irrigation conditions contained less oil than those grown under fully irrigated conditions (Göksoy *et al*., 2004). In addition, exposure of sunflower to brief periods of water stress during the seed-filling period reduced oil content, however, this was associated with an increase in the proportion of hull (Hall *et al*., 1989). Furthermore, when water stress conditions occurred during the seed-filling period, an increase in the oleic to linoleic ratio was observed. This could be due to the effect of unfavourable cell water status on the activity of the ODS enzyme. However, Baldini *et al*. (2002) hypothesised that water stress caused accelerated and earlier embryo development and lipid accumulation. This therefore caused a shorter duration of all enzymatic activities, including those of ODS and this could reflect on the final fatty acid composition. Flagella *et al*. (2002) claimed that upon irrigation, the plant tissue temperature is lowered that may cause higher activity of ODS and therefore a lower oleic to linoleic ratio.

Temperature was shown to affect both oil content and composition of sunflower seed during the period of seed development and maturation (Seiler, 1983; Seiler and Brothers, 1999; Rondanini *et al*., 2003; 2006; Qadir *et al*., 2006). High temperature (above 35ºC) during seed development resulted in a reduction in total oil content (Rondanini *et al*., 2003). However, the effect of temperature on oil content was variable. Canvin (1965) found that sunflower grown at a constant temperature of 21°C had a higher oil content than those grown at both higher and lower temperatures. According to Harris *et al*. (1978), oil content decreased as temperature increased. The cause of the reduction in oil content may lie in the greater proportion of the pericarp, due to the shortening of the seed-filling period at high temperatures (Connor and Hall, 1997). This was explained by the fact that oil content decreased with an increase in the proportion of the pericarp (Anand and Chandra, 1979). Unger and Thompson (1982) reported that oil content of seed maturing late in the season (at lower temperatures) was lower compared to seed from sunflower planted earlier that matured during warmer weather. According to Connor and Hall (1997), this supposedly positive effect of temperature on oil content might possibly be associated with small or light seed that could be due to problems with pollination or subsequent seed growth.

The relative proportions of the major unsaturated fatty acids (oleic and linoleic acid) are strongly influenced by the environmental temperature during sunflower seed development (Harris *et al*., 1978). There is an inverse relationship between temperature and the degree of unsaturation of the oil (Qadir *et al*., 2006). Several studies have been conducted in order to clarify how temperature affects the fatty acid composition of plant lipids (Garcés *et al*., 1992; Rondanini *et al*., 2003; 2006; Qadir *et al*., 2006). In controlled environment studies, high temperatures during seed development (especially night temperature) have been found to cause a decrease in the amount of linoleic acid and a corresponding increase in the amount of oleic acid in the oil (Izquierdo *et al*., 2002). Seed maturation during periods of low temperature gave opposite results. The mechanism involved appeared to be the direct effect of temperature on the activity of the desaturase enzymes that are responsible for the conversion of oleic to linoleic acid (Canvin, 1965; Harris *et al*., 1978; Silver *et al*., 1984; Garcés and Mancha, 1991). Therefore, both temperature and genetic effects are mediated by changes in the activity of the microsomal ODS. According to Izquierdo *et al*. (2002) variations in oil fatty acid composition were related to night temperature and maximum temperature during the light period. They suggested that the effect of temperature during the dark period on fatty acid composition was an indication that light or a metabolite associated with the day/night cycle could affect the activity of the ODS enzymes. Environmental factors (that may cause any type of stress) influence the proportions of fatty acids by altering the enzyme activity as well as transport between organelles (Steer and Seiler, 1990) and therefore a thorough understanding of the environmental factors influencing seed development and oil quality is necessary.

2.6.3 Temperature effect on oleoyl phoshatidylcholine desaturase activity

The ODS enzyme is highly regulated by temperature in sunflower seed and according to García-Díaz *et al*. (2002), different mechanisms might be involved in the control of the microsomal ODS activity. These mechanisms include: 1) *de novo* enzyme synthesis or activation of ODS that is stimulated by low temperatures, 2) the rapid and reversible partial inhibition of the pre-existing enzyme at high temperatures and 3) the exchange of oleate and linoleate between TAGs and PC (Canvin, 1965; Garcés *et al*., 1992; Sarmiento *et al*., 1998).

In addition, Martínez-Rivas *et al*. (2001) proposed two separate and independent mechanisms that could be involved in the temperature regulation of ODS activity in developing sunflower seeds: 1) The long-term direct effect of temperature, mostly related to the low thermal stability of the ODS enzyme and 2) the short-term indirect effect of temperature on the availability of oxygen. Higher solubility of oxygen in water at low temperatures may increase the total desaturase activity by increasing the availability of oxygen that acts as co-substrate for oleate desaturation. Both regulation mechanisms are of particular relevance as they act during field growth conditions of sunflower plants. However, temperature does not only regulate ODS activity, but also the amount of oleate (synthesised *de novo* and mobilised from preformed TAG) available as substrate for the enzyme (García-Díaz *et al*., 2002).

2.6.4 Temperature effect on high oleic sunflower

It is reported that in high oleic sunflower seed, fatty acid composition was not affected by climatic conditions (Salunkhe *et al*., 1992). Several researchers reported that oleic acid content showed a great stability in different environments in high oleic genotypes, even if genetic differences were present (Salera and Baldini, 1998). Additionally, in high oleic mutants the oleic and linoleic acid contents were less influenced by temperature than standard genotypes (Flagella *et al*., 2000). However, Champolivier and Merrien (1996) suggested that temperature had an effect on oleic acid content in high oleic sunflower hybrids. Tatini (1995) showed that an increase in temperature from 10-20°C during seed-filling produced an increment from 45-80% of oleic acid content in a high oleic genotype. In contrast, Lagravere *et al*. (2000) found that the high oleic hybrids they studied were insensitive to temperature conditions. The differences between these reports could be related to differences in hybrids studied as well as their genetic backgrounds. Oleic hybrids can be characterised as high or low oleic acid potential hybrids and the largest part of total variation in oleic acid percentage could be due to differences in potential acid percentages of the hybrids (Izquierdo *et al*., 2002). Lagravere *et al*. (2000) suggested that hybrids with low oleic acid potentials could be more sensitive to environmental conditions such as temperature, while hybrids with a higher oleic acid content genetic potential were insensitive to temperature conditions.

2.7 Genotype by environment interaction

Cultivars grown under a wide range of conditions are exposed to different soil types and fertility levels, rainfall and soil moisture conditions, temperatures and agricultural practices. These variables can all be described collectively as the environment. When comparing cultivars in different environments, their performance relative to each other may differ. These differential responses of cultivars across different environments are referred to as GXE interaction (Fehr, 1987; De la Vega and Chapman, 2000). GXE interactions are of interest for both the plant breeder as well as the agronomist for several reasons. When developing cultivars for specific purposes or for specific geographical areas, it is necessary to understand the interaction of the genotypes with predictable environments. The resources for testing genotypes across locations and years can be effectively allocated upon the relative importance of genotype x location, genotype x year and genotype x location x year interactions. The stability of performance of genotypes to various productivity levels among environments can be determined. Environmental stability of genotypes help to determine their suitability for fluctuations in growing conditions that are likely to be encountered (Fehr, 1987). High yield stability refers to the ability of a genotype to perform consistently (whether at high or low yield levels) across a wide range of environments (Annicchiarico, 2002).

A number of statistical procedures can be applied to determine GXE interaction and its relationship to phenotypic stability. The regression technique (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966) has been widely used due to its simplicity and because its information on adaptive response is easily applicable to locations (Annicchiarico, 1997). The principle component analysis (PCA) method that shows the mean squares of the principle components axis has also been used (Gauch and Furnas, 1991). Methods that have been widely used to asses stability include the regression coefficient (Finlay and Wilkenson, 1963), the environmental variance (Lin *et al*., 1986), Shukla's stability variance (Shukla, 1972), Wricke's ecovalence (Wricke, 1962) and univariate and multivariate methods (Akçura *et al*., 2005).

2.7.1 Genotype by environment interaction in sunflower

Sunflower yield and oil quality traits are both dependant on the genotype (cultivar) and its interaction with the environment (Connor and Sadras, 1992; Pereyra-Irujo and Aguirrezábal, 2007). For traditional cultivated sunflower genotypes, yield and oil quality depend largely on the environment that is highly variable among locations (Seiler, 1983; Lajara *et al*., 1990; De la Vega and Chapman, 2000; Radić *et al*., 2008), years (Laureti and Del Gatto, 2000; Mijić *et al*., 2007) and sowing dates within a single year (Unger and Thompson, 1982; Göksoy *et al*., 1998; Radić *et al*., 2008).

GXE interaction was reported for SFA contents after evaluating high palmitic and high stearic acid sunflower mutants under different temperature regimes (Martínez-Force *et al*., 1998). High palmitc and mid stearic acid mutants proved to be more sensitive to temperature differences during seed development than the high stearic acid mutant CAS-3. On the other hand, Fernández-Moya *et al*. (2002) indicated that the stearic acid levels of the high stearic acid mutant CAS-14 were highly dependent on temperature during seed-filling.

Genotype and temperature exert a major effect on the proportions of linoleic and oleic fatty acids during seed-filling and oil deposition (Seiler, 1986; Flagella *et al*., 2002). However, not all genotypes are equally affected by temperature and show strong GXE interactions (Salera and Baldini, 1998). It has been shown that the oleic to linoleic ratio was more stable across environments in high oleic acid content mutants than in traditional sunflower types, high linoleic acid lines or some wild species across environments (Fernández-Martínez *et al*., 1986; Garcés *et al*., 1989; Simpson *et al*., 1989; Seiler, 1992). Some high oleic acid sunflower inbred lines have shown stability in the field over years, whereas the oleic acid percentages varied according to year for others. The instability of mutants may be caused by the presence of modifier (instability) genes that could be the same as those that cause instability in the traditional high linoleic hybrids. During the course of a breeding programme for high oleic acid hybrids, it is necessary to do analyses over several generations in order to select for the most stable genotypes. However, if an unstable line has good agronomic characteristics, a number of hybrids should be made from these genotypes and tested for stability of oleic and linoleic acid contents (Triboï-Blondel *et al.*, 2000). By combining the genetic variability in the response of the fatty acid composition to temperature and the climatic diversity under which sunflower is cultivated, it is possible to obtain sunflower oils with different qualities (Izquierdo and Aguirrezábal, 2008).

2.7.2 Analysis of variance

Phenotypic performances of genotypes in multi-environments can be used to calculate the amount of variation attributed to genotypic effects, environmental effects, GXE effects and experimental error (Basford and Cooper, 1998). In a conventional variety assessment trial where the yield of genotypes (G) is measured in environments (E) over replicates (R), the classic model to analyse the total yield variation contained in GER observations, is the analysis of variance (ANOVA). After removing the replicate effects when combining the data, GXE observations are partitioned into two sources: 1) additive main affects for genotypes and environments and 2) non-additive effects due to GXE (Pillay, 2000). The ANOVA of the combined data expresses the observed (Y_{ij}) mean yield of the i^{th} genotype at the j^{th} environment as:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + \varepsilon_{ij}$$

Where μ is the overall mean, G_i, E_j and GE_{ij} represent the effect of the genotype, environment and genotype x environment interaction, respectively, and ε_{ij} is the average of random errors. The non-additive interaction GE_{ij} implies that an expected value of Y_{ij} depends not only on the separate levels of G and E, but also on their combination (Crossa, 1990; Purchase, 1997). Estimation of the interaction is important to estimate the genotypic effects and optimal allocation of resources. The variance components can be calculated by the estimated mean squares from the observed mean squares in the ANOVA (Pillay, 2000).

The ANOVA has been used in several studies to analyse data obtained for several agronomic and oil quality traits from trials planted in different localities (or environments) and across several growing seasons (Khan *et al*., 2008). For example, Alza and Fernández-Martínez (1997) evaluated six sunflower hybrids in eight environments. The experimental area consisted of two adjacent trials, the first under irrigation and the second under rainfed conditions. Statistical analysis was carried out in two steps. First a separate analysis was done for each environment and secondly, a combined analysis over environments. In the separate analysis highly significant differences were observed for yield (kg/ha), no of seeds per head, seed weight and head diameter between hybrids planted under rainfed and irrigated conditions. According to the combined ANOVA, genotypes showed significant effects for all traits, while GXE interactions were also highly significant for all traits. In another study Denis *et al*. (1994) studied the genetic control of hullability in 36 sunflower hybrids that were produced by a factorial cross of six male sterile and six restorer lines. Hybrids were evaluated in two locations in Spain (one rainfed and one irrigated) and one location (irrigated) in France. The ANOVA of hullability indicated significant genotypic and environmental effects, while the GXE effect was also highly significant. The trial under rainfed conditions in Spain gave a mean hullability of 83% and it was double the value obtained in France (41.5%). The irrigated trial in Spain gave an intermediate value of 60.4%.

Velasco *et al*. (2002) investigated the genetic and environmental variation of tocopherol content and composition in sunflower. A total of 36 hybrids were grown at 13 locations in southern Spain. They reported that both genotypic and environmental effects were highly significant for tocopherol content and composition. The effect of genotype was larger than that of environment for alpha-, beta- and total tocopherol content whereas the effect of environment was greater on gamma-tocopherol content. GXE interaction was significant for alpha-, gamma- and total tocopherol content.

Zobel *et al*. (1988) compared the traditional statistical analysis with additive main effects and multiplicative interaction (AMMI) analysis and showed that these analyses were not always effective in analysing the multi-environment trial data structure. The ANOVA, an additive model, describes main effects effectively and determines if GXE interaction is a significant source of variation. However, it does not provide insight into genotype or environment patterns that give rise to interaction. The multiplicative model of PCA contains no sources of variation for additive genotypic or environmental main effects and also does not analyse interactions effectively. The linear regression method uses environmental means, which are sometimes a poor estimate of environments, such that the fitted lines in most cases account for a small fraction of the total GXE and could be deceptive (Byth *et al*., 1976; Zobel *et al*. 1988; Ariyo, 1999).

2.7.3 Additive main effects and multiplicative interaction analysis

AMMI analysis has been reported to be suitable for depicting adaptive responses and is useful in understanding complex GXE interactions (Gauch and Zobel, 1989). The AMMI model separates the additive variance from the multiplicative (interaction) variance and applies PCA to the interaction portion from the ANOVA analysis to extract a new set of coordinate axes (interaction principle component axes) that account more effectively for the interaction patterns (Shaffi *et al*., 1992). In clarification of GXE interactions, AMMI summarises patterns and relationships of genotypes and environments (Crossa, 1990). The plant breeder is interested in both adaptability (average performance across localities) and stability (consistent performance across environments). The variance across environments can be used for traits such as quality. Cultivar influence is a limitation of stability analysis, because large deviations from the regression do not necessarily imply instability, but it might be that a specific genotype only reacts differently from the rest of the genotypes (Pillay, 2000).

AMMI results are graphed in a highly informative biplot that shows the main and interaction effects for both genotypes and environments on the same scatter plot. Also, the data are partitioned into a pattern rich model, while the noise rich residual is discarded to gain accuracy (Gauch and Zobel, 1996). AMMI combines ANOVA and PCA into a single model with additive and multiplicative parameters. The equation of the AMMI model is:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \Sigma\lambda_n\gamma_{gn}\eta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where Y_{ger} is the yield of genotype g in environment e for replicate r, μ is the overall mean, α_g is genotype mean deviation (mean minus grand mean), β_e is the environment devations, n is the number of PCA axes retained in the model, λ_n is the singular value of PCA axis n, γ_{gn} is the genotype eigenvector values for PCA axis n, η_{en} is the environment eigenvector values for PCA axis n, ρ_{ge} is the AMMI residuals and ε_{ger} is the residual error (Gauch and Zobel, 1996).

Studies on GXE interaction, stability and adaptability analyses have been previously performed in sunflower for various traits across different environments and for consecutive years (De la Vega and Chapman, 2000; Laureti and Del Gatto, 2000; Lúquez *et al*., 2002). These authors assessed stability using Fisher's protected least significant difference (LSD) test (Steel and Torrie, 1993), relative yield (Yau and Hamblin, 1994), Shukla's stability variance statistic (Shukla, 1972), Kang's yield stability statistic (Kang, 1993), Piepho's method of multiple comparison (Piepho, 1995) and PCA (De la Vega and Chapman, 2000). However, the AMMI model was shown to overcome some of the problems with linear regression and proved to be a valuable tool for depicting adaptive responses (Gauch and Zobel, 1989; Gauch, 1993; Annicchiarico, 1997; Ariyo, 1999), improving the probability of successful selection and for analysing GXE interaction with greater precision (Schoeman, 2003).

Leeuwner (2005) used the AMMI model to evaluate the effects of GXE interaction in sunflower and their adaptation at 32 South African locations. The AMMI identified considerable GXE interaction. The first two interaction principle components axes (IPCA) were highly significant ($P<0.001$), but factors responsible for GXE could not be identified. The two IPCAs contributed 34% and 22.5% of the interaction sums of squares respectively. By plotting scores of IPCA1 against that of IPCA2, genotypes and environments grouped together according to the length of their growing season. But, because genotypes and environments could not be described according to the factors responsible for the observed GXE interaction, genotypes could not be recommended for adaptability to specific environments. However, widely adapted cultivars for South African environments were selected.

2.8 Breeding of sunflower to change or improve oil composition

In some cases standard sunflower oil needs additional processing and chemical additives to meet its many potential applications in both the food and non-food industry. The result of this processing is a final product that might have detrimental implications for human health. For common uses such as deep frying or margarine production, sunflower oil needs to be hydrogenated (hardened off) in order to obtain solid or semi-solid fats.

However, this process induces *cis-trans* isomerisation of fatty acids, resulting in the production of *trans*-fatty acids that might be related to heart disease (Willet *et al*., 1993). In addition, sunflower oil is not suited for uses that require a high oxidative stability (Fernández-Martínez *et al*., 2004). Breeding of novel and healthier sunflower oil types with improved oil quality for specific applications has received great attention by plant breeders. Improvement in oil quality has been achieved mainly by modifying the fatty acid composition of the oil (Knowles, 1983; Ivanov *et al*., 1988; Osorio *et al*., 1995; Fernández-Martínez *et al*., 1997; Salas *et al*., 2004; Seiler, 2004 Fernández-Moya *et al*., 2005). Genetic variation of fatty acid composition in sunflower oil has been achieved through conventional breeding and mutagenesis (Fernández-Martínez *et al*., 2007). Sunflower mutants with altered fatty acid compositions that have been developed are summarised in Table 2.4.

Because sunflower fatty acid composition varies with different temperatures during seed development, there has been interest in developing both high linoleic and high oleic types that are temperature insensitive (Knowles, 1983; Miller and Vick, 2001). High oleic lines derived from Pervenets have been shown to be stable under different temperature regimes (Fernández-Martínez *et al*., 1986). Increased concentrations of SFAs are necessary for applications in the food industry that requires plastic fats (for production of margarines and shortenings) without the need of hydrogenation. Oils with higher SFA contents would be naturally more stable than oil from traditional sunflower. A high level of stearic acid is preferred over other SFAs due to its neutral effect on serum lipoprotein cholesterol (Pearson, 1994). Mutagenesis was effective in developing mutants with increased levels of SFAs at the *sn*-1 and *sn*-3 TAG positions where the absorption rate of the fatty acid is lower than at the *sn*-2 position (Bracco, 1994; Alvarez-Ortega *et al*., 1997).

Using mutagenesis of dry seeds through X-ray radiation, Ivanov *et al*. (1988) and Osorio *et al.* (1995) developed single mutants with high palmitic acid contents above 25% (Table 2.4). The double mutant, CAS-12 was developed by mutagenising a high oleic acid line isogenic to the original low oleic acid line used to obtain CAS-5 (Fernández-Martínez *et al*., 1997).

Table 2.4 Sunflower mutants or lines with altered fatty acid composition developed through mutagenesis or germplasm evaluation

Trait	Mutant or line	Reference
High oleic acid	1) Pervenets	Soldatov, 1976
High palmitic acid	1) 275 HP 2) CAS-5	Ivanov *et al*., 1988 Osorio *et al*., 1995
High palmitic acid and increased oleic acid content	1) CAS-12	Fernández-Martínez *et al*., 1997
High palmitic and medium content of palmitoleic acid	1) CAS-37	Salas *et al*., 2004
Medium to high stearic acid	1) CAS-8 2) CAS-4 3) CAS-3	Osorio *et al*., 1995
Very high stearic acid content	1) CAS-14	Fernández-Moya *et al*., 2002
Low stearic acid content	1) LS-1 2) LS-2	Miller and Vick, 1999
Low palmitic acid content	1) LP-1	Miller and Vick, 1999
Low total saturated fatty acids*	1) RS1 2) RS2 3) NMS 2229	Vick *et al*., 2002 Vick *et al*., 2002 Seiler, 2004
High stearic acid on high oleic background*	1) CAS-15	Fernández-Moya *et al*., 2005
High linoleic acid content*	1) 2698-L	Miller and Vick, 2001

*Lines developed with variation in fatty acid compositions through germplasm evaluation and recombination (Fernández-Martínez *et al*., 2007).

CAS-12 showed a high palmitic acid content of above 30% and an increased oleic acid content of about 56%. The high palmitic acid mutants were found to exhibit palmitoleic acid (C16:1) in their seed oil, a fatty acid that is absent in standard sunflower oil. Salas *et al*. (2004) reported on a mutant, CAS-37, with high levels of palmitic acid and presence of palmitolinoleic (C16:2) and asclepic acid (C18:1Δ11). Three mutants with increased stearic acid contents CAS-3, (>25%), CAS-4 (>13%) and CAS-8 (>10%) were isolated after chemical mutagenesis with ethyl methanesulfonate and sodium azide (Osorio *et al*., 1995). Fernández-Moya *et al*. (2002) isolated a very high stearic acid mutant CAS-14 with stearic acid levels above 35% using mutagenesis with sodium azide. Later two more lines, CAS-19 and CAS-20, with medium stearic acid levels were further developed by Pérez-Vich *et al.* (2004) from crossing CAS-3 and a standard sunflower line.

During the last decade, consumers have become concerned about the consumption of SFAs in their diets due to the fact that high levels of saturated fat may contribute to increased blood serum cholesterol. This increases the risk for coronary heart disease (Mensink *et al*., 1994; Willett, 1994). The reduction of total SFA content is an important objective for specific dietary uses. Lowered SFA contents have been developed by using both germplasm evaluation and mutagenesis. Miller and Vick (1999) obtained the LP-1 mutant with lowered palmitic acid content, as well as two low stearic acid mutants, LS-1 and LS-2, using chemical mutagenesis. Similarly Vick *et al*. (2002) selected two germplasm lines, RS1 and RS2, with reduced total SFA from a cultivated sunflower line. For these lines, the decrease in total SFAs was principally obtained by reduction of the stearic acid content. Seiler (2004) also identified low SFA levels in a population of wild *H. annuus.*

Further improvement of oil quality was achieved by recombining several seed oil quality traits in a single phenotype. However, to facilitate this process, traits to be recombined should be inherited independently. Pérez-Vich *et al*. (2002a) studied the relationship between the high palmitic and high stearic acid traits in crosses between the mutant lines CAS-5 and CAS-3. Three genes are involved in the control of high levels of palmitic acid namely *P1, P2* and *P3,* as well as high stearic acid content, namely *Es1, Es2* and *Es3*. One or two genes are, however, involved in the control of low levels of these fatty acids. They reported that complete recombination of these traits was not possible because of an epistatic effect of loci controlling high palmitic acid content on the loci responsible for high stearic acid content. Similarly, these authors investigated the genetic relationships of high stearic and high oleic acid contents involving the high stearic line CAS-3 and the high-oleic acid line HAOL-9. They found that the genetic linkage between the stearic acid locus *Es2* and the oleic acid gene *Ol* hindered complete recombination of the high stearic and high oleic acid levels of the parents (Pérez-Vich *et al*. 2002b). However, Pérez-Vich *et al*. (2002a) also reported that the high palmitic acid and high oleic acid traits were independently inherited.

High temperature processes (e.g. the frying oil industry) need sunflower oil that is highly resistant to thermoxidation, with a low concentration of linoleic acid and a high concentration of *in vitro* oxidants (mainly gamma- and delta-tocopherol). Variation for increased levels of beta- and gamma-tocopherol has been found in collections of cultivated germplasm (Demurin, 1993; Velasco *et al*., 2004b). Demurin *et al*. (1996) found that genes controlling fatty acid levels were independently inherited from those controlling the tocopherol profile. This allowed the combination of different fatty acid and tocopherol profiles (Fernández-Martínez *et al*., 2004). Novel fatty acid and tocopherol traits are usually under embryonic control by a low number of genes that facilitate their management in breeding programmes (Fernández-Martínez *et al*., 2004).

2.9 Inheritance of the high oleic acid trait

In general, genetic modifications that alter the fatty acid profile have been found to be qualitative rather than quantitative. This means that they are controlled by a low number of genes and are less affected by the environment than quantitative traits such as oil content. They are mostly determined by the genotype of the developing embryo with little or no maternal influence. This could be crucial in breeding programmes, since low maternal inheritance allows selection to be carried out at a single seed level (Fernández-Martínez *et al*., 2004).

The study of oleic acid inheritance in sunflower is complex and no general agreement on how oleic acid is inherited has been reached. Several studies have been carried out to explain the inheritance of the high oleic acid content in germplasm derived from the Pervenets variety. Miller and Zimmerman (1983) detected lack of dominance and maternal influence after crossing high oleic plants with a low oleic parent. The first genetic analysis done on the high oleic sunflower mutant, obtained by Soldatov (1976), concluded that the high oleic acid trait was controlled by a dominant or partially dominant single gene that was designated *Ol* (Fick, 1984; Urie, 1984). In a further study Urie (1985) detected the presence of modifiers as well as a reversal of the dominance of the *Ol* gene that could not be explained. A second gene modifying the high oleic acid content was identified by Miller *et al*. (1987) and was designated *Ml*. The authors proposed a model that stated that the high oleic acid trait was expressed in genotypes having one dominant allele of the *Ol* gene combined with the recessive allele *ml* in a homozygous condition (*Ol_mlml*).

Fernández-Martínez *et al*. (1989) and Pérez-Vich *et al*. (2002a) observed three different F_2 segregation patterns (1:3, 7:9 and 37:27) for high oleic and low-intermediate classes. These authors hypothesised that the high oleic character is controlled by three dominant complementary genes, Ol_1, Ol_2 and Ol_3 and according to this model, the high oleic acid trait was only expressed when all three dominant genes were present. However, although the segregation 3 high : 1 low + intermediate (indicating a single dominant gene) was observed by several studies (Fernández-Martínez *et al*., 1989; Schmidt *et al*., 1989; Pérez-Vich *et al*., 2002a) (Table 2.5), further studies demonstrated that the genetic control of the high oleic acid trait was more complex. However, Demurin and Škoric (1996) could not confirm the previous hypothesis and concluded that the *Ol* locus exhibited genetically unstable expression. Fernández *et al*. (1999) postulated a two gene model and suggested that the high oleic content was produced by the recessive allele *ol* and the dominant allele *Ml*, both in a homozygous condition. They also suggested that *Ml* might be a gene complex rather than a single gene.

All the above genetic studies were carried out under non-controlled environments despite of the well-known influence of temperature on the phenotypic expression of oleic acid content. In the first controlled environment study done, Alonso (1988) used growth chambers at three temperatures (10°C, 20°C and 30°C) and detected a large effect of temperature on oleic acid content in different generations. It was concluded that oleic acid content was controlled by the single *Ol* gene that acted either as dominant or partially dominant depending on the temperature (Velasco *et al*., 2000).

Table 2.5 Theoretical genotypic and phenotypic classes for oleic acid (C18:1) levels in crosses segregating for this fatty acid

Line	Genetic model	Expected F_2 genotypes	Frequency	18:1 Phenotype	References
Pervenets selection	One partially dominant gene	*Olol*	1/4	Low	Fick, 1984
		*Ol_*	3/4	High + intermediate	
Pervenets selection	One dominant gene	*Olol*	1/4	Low-intermediate	Urie, 1985
		*Ol_*	3/4	High	Schmidt *et al*., 1989
Pervenets selection	Single dominant gene and recessive modifier	*Olol_ _*	4/16	Low	Miller *et al*., 1987
		*Ol_Ml_*	9/16	Intermediate	
		Ol_mlml	3/16	High	
R 978	Single recessive gene and recessive modifier	*Ol_Ml_, Ol_mlml, olol_Ml_*	15/16	Low + Intermediate	Fernández *et al*. 1999
		olol mlml	1/16	High	
HAOL-9	One dominant gene	$ol_1ol_1OL_2OL_2OL_3OL_3$	1/4	Low-intermediate	Fernández-Martínez *et al*., 1989
		$OL_1\_\ OL_2OL_2OL_3OL_3$	3/4	High	Pérez-Vich *et al*., 2002a
HAOL-9	Two dominant genes	$ol_1ol_1\_\ \_\ OL_3OL_3$ or $Ol_1\_OL_2\_\ OL_3OL_3$	7/16	Low-intermediate	Fernández-Martínez *et al*., 1989
		$OL_1\_\ OL_2OL_3OL_3$	9/16	High	Pérez-Vich *et al*., 2002a
HAOL-9	Three dominant genes	$ol_1ol_1\_\ \_\ \ \_\ \_$ *or* $\_\ \_\ ol_2ol_2\_\ \_$ *or* $\_\ \_\ \ \_\ \_\ ol_3ol_3$	37/64	Low-intermediate	Fernández-Martínez *et al*., 1989 Pérez-Vich *et al*., 2002a
		$OL_1\_\ OL_2\_\ OL_3$	27/64	High	
HAOL-9	One recessive gene	$OL_1\_OL_2OL_2OL_3OL_3$	3/4	Low-intermediate	Fernández-Martínez *et al*., 1989
		$ol_1ol_1OL_2OL_2OL_3OL_3$	1/4	High	Pérez-Vich *et al*., 2002a
LG-27	One recessive gene	*OL_*	3/4	Low	Demurin *et al*., 2000
		ol_1ol_1	1/4	Mid	
HAOL-9	Five genes				Velasco *et al*., 2000

(Fernández-Martínez *et al*., 2004).

In a second controlled environment study, Velasco *et al*. (2000) observed a number of different segregation patterns in the F_1, F_2 and BC_1F_1 generations produced by a cross between a standard inbred sunflower line and a near-isogenic high oleic line. They proposed a genetic model consisting of five genes that explained the segregation patterns. Genes controlling the oleic acid content were designated Ol_1, Ol_2, Ol_3, Ol_4 and Ol_5. They found that genotypes Ol_1Ol_1, $Ol_1ol_1Ol_2Ol_2$ and $Ol_1ol_1Ol_2ol_2$ were high in oleic acid, whereas the genotype ol_1ol_1 had a low oleic phenotype. Conversely, depending on the Ol_3, Ol_4 and Ol_5 genes, the genotype $Ol_1ol_1ol_2ol_2$ could exhibit low, medium or high oleic acid content.

Lacombe *et al*. (2001) reported that the high oleic acid content was directed by two independent loci, a locus carrying the oleHOS allele (that is exclusively correlated to the high oleic acid content status of the genotype) and another locus that carries a suppressor allele (supHOAC) that also directs the high oleic acid trait. The supHOAC allele suppresses the effect of the high oleic acid content allele. Therefore, depending on the supHOAC allele in the segregating population, the high oleic acid trait is controlled by one or two loci. The suppressor allele could disturb the conventional segregating pattern for high oleic to linoleic acid and therefore other observations that reject the dominance of the high oleic acid trait might be due to segregation at this locus.

In short, there is general agreement on the presence of a principle *Ol* gene controlling the high oleic acid content character, but this trait is complex and involves a number of modifying genes whose number and function still need to be determined. Studies by Fernández-Martínez *et al*. (1989), Demurin and Škoric (1996), Velasco *et al*. (2000) and Pérez-Vich *et al*. (2002a) confirmed the reversal of dominance that was mentioned by Urie (1985). Furthermore, interpretative differences in these studies may be due to background genes in the parental lines used, environments utilised to test the segregating generations and the number of modified genes present in the breeding material (Miller, 1992; Miller and Fick, 1997; Triboï-Blondel *et al*., 2000). Finally, the molecular and genetic nature of the high oleic acid trait is still not well understood and this lack of understanding causes difficulties in directing the conversion of traditional linoleic lines into high oleic lines during breeding programmes.

2.10 Advanced breeding for oil crop modification: DNA marker-assisted selection

Conventional plant breeding has always relied on the phenotypic selection of agronomically important traits from the diverse gene pool that is present within a crop species. Traits like height and flower colour are visible and can be easily identified. However, for some traits phenotypic selection has made little progress due to challenges in measuring phenotypes. Traits like seed oil quality can only be measured by sophisticated analytical methods and in addition, the expression of this trait depends on variable environmental conditions and developmental stage. Formerly it was necessary for breeders to develop and analyse each generation before they could measure the specific trait or phenotype and select the best plants. With the introduction of marker-assisted selection (MAS), this has changed and breeders can now select plants that are likely to express the required traits from thousands of progeny even before the plants have matured (Murphy, 2006; Moose and Mumm, 2008).

A prerequisite for MAS is the development of molecular tags. Molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellite or simple sequence repeat (SSR) among others, have been developed for many oil crops. These markers are assembled into genetic maps that are useful in research and commercial breeding programmes. Markers that are tightly linked to the trait of interest can be used to trace the presence of that specific trait in large segregating populations and at young plant stage as part of a crop-breeding programme. In addition, molecular markers might increase the probability of identifying superior genotypes, by focussing on genotypes with the greatest potential, by decreasing the number of progeny that need to be screened phenotypically and by allowing simultaneous improvement of traits that are negatively correlated (Mohan *et al*., 1997; Murphy, 2006; Moose and Mumm, 2008).

Quality traits are quantitative traits (also called polygenic traits) and are controlled by several genes. Regions within a plant genome that contain genes associated with a particular quantitative trait are called quantitative trait loci (QTL). DNA markers are used to construct linkage maps which are used to identify chromosomal regions that contain genes and/or QTL associated with traits of interest. This is called QTL analysis and these maps are then referred to as QTL maps. The principle of QTL mapping is based on genes and markers that segregate via chromosome recombination during meiosis, therefore allowing their analysis in the progeny. Genes and markers that are close together (tightly linked) will be transmitted together from parent to progeny (Collard *et al.*, 2005).

The construction of linkage maps and QTL analysis takes a considerable amount of time and may be expensive. Subsequently, there are alternative methods that can save time and money, especially if resources are limited. One method that can be used to identify markers and tag major QTL is bulk segregant analysis (BSA). The BSA approach, developed by Michelmore *et al.* (1991), is frequently used in gene tagging and is based on the principle of near-isogenic lines. In BSA, two parents that differ in their expression of the targeted trait are crossed to develop a segregating population that are subsequently phenotyped for the targeted trait. Two “bulks” of DNA samples differing for the trait of interest are constructed. Theoretically, the genetic constitution of the two bulks are similar, but for the genomic region associated with the trait. DNA markers are then screened across the two parents and the two bulks. A marker showing a linked polymorphism between parents as well as the bulks is considered putatively linked to the targeted trait or QTL of interest. Afterwards, all individuals of the entire segregating population are genotyped with these polymorphic markers and a localised linkage map can be constructed. QTL analysis can then be performed and the position of the QTL determined. BSA generally prefers high-throughput marker techniques such as RAPD and AFLP that can generate multiple markers from a single DNA preparation (Collard *et al.*, 2005). This method has been applied in several molecular analysis studies for sunflower to map genes related to agronomically important traits such as disease resistance (Lawson *et al*., 1996; Lu *et al*., 2000; Tang *et al*., 2003a), nuclear male-sterility (Chen *et al*., 2006), gamma-tocopherol content (García-Moreno *et al*., 2006), chlorophyll deficiency (Yue *et al*., 2009) and fatty acid composition (Dehmer and Friedt, 1998a; Pérez-Vich *et al*., 2006).

2.10.1 Amplified fragment length polymorphism

AFLP is defined as the selective amplification of restriction fragments from total genomic DNA digests using the polymerase chain reaction (PCR). This technique represents a combination of RFLP and PCR, however, in contrast to the RFLP technique, AFLPs will display the presence or absence of restriction fragments rather than length polymorphisms (Vos *et al*., 1995).

The AFLP technique can be used for DNA samples of any origin with no prior sequence information. Small sequence variations can be detected using only small quantities of genomic DNA. The AFLP technique is extremely efficient because numerous fragments can be analysed simultaneously on a gel. AFLP has the capacity to inspect a great number of loci for polymorphism and the number of polymorphisms detected per reaction is especially high. Markers obtained are reliable and reproducible within and between laboratories and are relatively easy to generate. AFLP can generate a virtually unlimited number of markers by simply varying the restriction enzymes and the number and nature of the selective nucleotides. AFLP has applications for DNA fingerprinting, MAB, the construction of high density molecular maps and the positional cloning of target genes (Blears *et al*., 1998). Although the AFLP technique is powerful and reliable in identifying markers closely linked to genes of interest, it has some disadvantages. AFLP markers are dominant and therefore homozygotes and heterozygotes cannot be distinguished. The technique is technically demanding and due to the number of steps involved it is too laborious to be used in high-throughput screening.

The AFLP technique involves the following basic steps: Genomic DNA is first digested simultaneously using rare and frequent cutting restriction endonucleases that results in fragments with overhanging ends. Double-stranded oligonucleotide adapters, homologous to the 5'-sticky end of the corresponding restriction site, are ligated to the DNA fragments to generate template DNA for amplification. The ligated fragments are amplified by PCR using primers complementary to the adapter and restriction site sequence with additional selective nucleotides at their 3'-end. From the vast number of fragments generated by restriction, only a subset of the template fragments with complementary nucleotides extending beyond the restriction site will be amplified under strict annealing conditions. Genetic polymorphisms are revealed by analysis of amplified fragments on denaturing polyacrylamide gels and comparison of patterns generated for each sample (Vos *et al*., 1995; Blears *et al*., 1998; Agarwal *et al*., 2008).

2.10.2 Genetic markers and linkage map construction in sunflower

The first DNA markers developed in sunflower were RFLPs. Genetic linkage maps based on RFLP markers were reported by several research groups (Jan *et al*., 1993; 1998; Gentzbittel *et al*., 1994; 1995; Berry *et al*., 1995). These included maps based on data of individual F_2 populations as well as composite maps based on data of different mapping populations (Gentzbittel *et al*., 1995; 1999; Berry *et al*., 1996). Maps consisted of 17 or more linkage groups (LG) that presumably corresponded to the 17 haploid chromosomes of sunflower. Genetic distances covered by these maps (1650 cM) were close to the estimated length of the sunflower genome (Gentzbittel *et al*., 1995). Additionally, RFLP maps have been used as tools for mapping agronomic traits and QTL (Leon *et al*., 1995; 1996; 2000; 2001; 2003; Lu *et al*., 1999; Bert *et al*., 2001; Pérez-Vich *et al*., 2002c; Rachid Al-Charaani *et al*., 2002). However, the extensive use of RFLPs and linkage maps in sunflower has been restricted by a lack of public RFLP probes, consequent lack of a dense public RFLP map and the low-throughput nature of RFLP markers (Yu *et al*., 2003).

RAPD markers were used in early genetic studies in sunflower. Several reports indicated high levels of variation with the proportion of polymorphic loci averaging more than 50% for most lines (Lawson *et al*., 1994; Teulat *et al*., 1994). RAPDs have been used for tagging phenotypic loci such as rust (*Puccinina helianthi* Schwein.) and boomrape (*Orobanche cumana* Wallr.) resistance genes (Lawson *et al*., 1998; Lu *et al*., 2000) as well as the development of markers for the high oleic acid trait in sunflower (Dehmer and Friedt, 1998a). RAPD maps have been developed for wild *H. annuus* and *H. petiolaris* Nutt. (Rieseberg *et al*., 1995), based on 212 and 400 RAPD loci respectively. They reported 17 LGs for both species covering 1084 cM and 1761 cM for *H. annuus* and *H. petiolaris*, respectively.

AFLP markers were rapidly adopted in sunflower because of their high multiplex ratio and high reproducibility (Vos *et al*., 1995). They have been previously used to fingerprint elite inbred sunflower lines (Hongtrakul *et al*., 1997), to construct new genetic maps (Flores-Berrios *et al*., 2000) and to increase the density of already existing genetic maps (Gedil *et al*., 2001; Al-Charaani *et al*. 2002). Langar *et al*. (2003) constructed a genetic map with a distance of 2169 cM by combining AFLP and direct amplification of length polymorphism (DALP) markers. However, RAPD and AFLP markers are inadequate for establishing a genome-wide framework for DNA markers for anchoring and cross referencing of genetic maps. Microsatellite markers that are preferred for such purposes have only been recently developed in sunflower. These include SSR and expressed sequence tag (EST) derived single nucleotide polymorphism (SNP) markers (Paniego *et al*., 2007). Several research groups have described the development and characterisation of SSR markers and these added to a total of 2040 markers (Dehmer and Friedt, 1998b; Paniego *et al*., 2002; Tang *et al*., 2002; Yu *et al*., 2002).

The first genetic linkage map based on SSR markers for sunflower was developed by Tang *et al*. (2002). This public reference SSR map was constructed using F_7 recombinant inbred lines (RILs) and 408 polymorphic SSR markers. Yu *et al*. (2003) constructed the first cross-referenced maps by adding 701 new SSR and 89 RFLP or insertion-deletion (INDEL) marker loci onto three populations. Two of these populations were previously used by Gedil *et al*. (2001) and Tang *et al*. (2002). From these maps, Tang *et al*. (2003b) published a composite linkage map of sunflower that integrated 657 loci, covering a distance of 1423 cM.

The AFLP map constructed by Al-Charaani *et al*. (2002) was improved by increasing the number of AFLP markers and integrating 38 SSR markers (Al-Charaani *et al*., 2004). In the new map, 367 AFLP and SSR marker loci were placed in 21 linkage groups that covered 2916 cM. Paniego *et al*. (2007) reported an improvement of this map. They integrated 161 new SSR markers from different sources and this map was cross-referenced to the public SSR map of Tang *et al*. (2002). The use of molecular markers linked to target traits together with MAB will contribute to breeding efficiency for sunflower fatty acid profiles (Fernández-Martínez *et al*., 2007). In addition, since DNA markers are not affected by the environment in which the sunflower plants are grown in, they could be especially vital in breeding programmes for high oleic sunflower.

The molecular basis of modified fatty acid content in the sunflower seed oil has been studied through a QTL and a candidate gene approach. A number of sunflower genes, coding for enzymes involved in the biosynthetic pathway of fatty acids in seeds, have been cloned and their polymorphism studied in cultivated sunflower (Fernández-Martínez *et al*., 2004). The first DNA markers linked to the high oleic acid content in sunflower were identified by Dehmer and Friedt (1998a). These were two RAPD markers linked to the Ol_1 gene. Subsequent studies showed that the Ol_1 gene cosegregated with *FAD2-1*, a seed-specific ODS gene. The *FAD2-1* gene showed strong expression in the normal sunflower type, but weak expression in high oleic acid lines (Hongtrakul *et al*., 1998; Lacombe and Bervillé, 2001; Martínez-Rivas *et al*., 2001). Pérez-Vich *et al*., 2002c reported that the Ol_1-*FAD2-1* locus mapped to LG 14 of the public sunflower genetic map. This locus was found to underlie a major oleic acid QTL that explained 56% of the phenotypic variance for the high oleic acid trait. The physical structure of the *FAD2-1* locus was determined and sequence tagged site (STS) DNA markers diagnostic for the Ol_1 mutation were developed by Schuppert *et al.* (2006a). It was also indicated that *FAD2-1* was silenced by RNA interference and this mechanism underlie the Ol_1 mutation (Schuppert *et al.* (2005). Modifying genes that affect oleic acid content have also been characterised. A minor QTL on LG 8 that showed an epistatic interaction with the major QTL for oleic acid at the *FAD2-1* locus on LG 14 has been identified (Pérez-Vich *et al.*, 2002c). In other studies, Lacombe *et al.* (2001; 2002) identified a locus that suppressed the effect of *FAD2-1*. This was probably through a mechanism of gene silencing. In addition, Schuppert *et al.* (2003. 2006b) identified another ODS gene (*FAD2-2*) on LG 1 that showed an epistatic interaction with the Ol_1-*FAD2-1* locus on LG 14.

Although molecular markers have been identified for some of the fatty acids in preliminary studies (Dehmer and Friedt, 1998a; Hongtrakul *et al.*, 1998; Pérez-Vich *et al.*, 2000; 2002c), these might not be suitable for MAS. Generally, markers should be validated by testing their effectiveness in determining the target phenotype in independent populations and different genetic backgrounds (Collard *et al.*, 2005).

The optimal quality of sunflower oil depends on the end-use of the oil. In general characteristics that are required for salad and cooking oils may be undesirable for other applications such as margarine and shortening that requires semi-solid fats. For the latter a high SFA content is desirable because the semi-solid consistency reduces the need for hydrogenation or transesterification that generates *trans* isomers that are related to heart disease (Willett and Ascherio, 1994). On the other hand, SFA are regarded as detrimental to human health because of their contribution to raising cholesterol levels (Mensink *et al.*, 1994). Accordingly, breeding objectives were aimed at producing healthy oil for direct consumption (salad and cooking oils) by lowering total SFA content. However, at the moment oleic acid is the preferred fatty acid for edible purposes as it combines a hypocholsterolemic effect (Mensink and Katan, 1989) with a much greater oxidative stability than PUFAs. Therefore, selection for mid and high oleic acid content has been a priority in sunflower breeding programmes. Nevertheless, the genetic control of high oleic acid content is not well understood. High oleic acid content was initially identified as a monogenic trait produced by dominant alleles *Ol* (Urie, 1984), but afterwards several modifying genes were identified that affect the *Ol* gene and produce reversal of the expected dominance. This has complicated the practical management of the trait in breeding programmes. Additionally, oleic acid content is largely influenced by the environment and the occurrence of GXE interaction may complicate selection of hybrids that are stable and widely adapted for this trait. However, the development of molecular markers for this trait will contribute to improving breeding efficiency for high oleic sunflower.

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CHAPTER 3

GENOTYPE BY ENVIRONMENT ANALYSIS IN HIGH AND MID OLEIC SUNFLOWER HYBRIDS

3.1 Introduction

Sunflower is the most important oilseed crop in South Africa. The most important cropping areas include the Free State, North West, Limpopo, Mpumalanga and Gauteng provinces (Table 3.1) (Dredge, 2010). These areas of sunflower cultivation vary considerably in soil type, climate and elevation. Together with these environmental factors, water regime and planting date also have a marked influence on the performance of sunflower (Alza and Fernández-Martínez, 1997; Qadir *et al.*, 2006).

Table 3.1 Areas planted and sunflower seed yield for the production seasons 2006/2007, 2007/2008 and 2008/2009

	2006/2007		**2007/2008**		**2008/2009**	
Province	**Area planted (ha)**	**Final yield (ton)**	**Area planted (ha)**	**Final yield (ton)**	**Area planted (ha)**	**Final yield (ton)**
Free State	135000	155000	270000	459000	280000	363000
North West	130000	110000	200000	300000	230000	298000
Limpopo	30000	13500	70000	77000	90000	90000
Mpumalanga	13000	13000	17000	25500	27000	37700
Gauteng	7000	7700	6000	8700	7000	9820
Western Cape	500	300	600	540	700	700
Eastern Cape	150	180	300	360	600	780
Northern Cape	700	1320	400	900	500	1000
Total	**316350**	**300000**	**564300**	**872000**	**635800**	**801000**

(Dredge, 2010).

Cultivars and hybrids grown in multi-environment trials react differently to environmental variations. Plant breeders aim to develop genotypes that are widely adapted over a broad range of environmental conditions. Stable and more adaptive genotypes that show a low degree of fluctuation when grown over a range of environments are selected. The difference in response of genotypes to variable environmental conditions is attributed to GXE interaction (De la Vega and Chapman, 2000). Assessment of GXE interaction is advantageous for the identification of stable genotypes, to increase the efficiency of the breeding programme and to select the best genotypes.

Several studies investigated GXE interaction in sunflower (Schoeman, 2003; Dijanovic *et al*., 2004; Ghafoor *et al*., 2005; Leeuwner, 2005) and other oil crops like sesame (John *et al*., 2001; Boshim *et al*., 2003), linseed (Adugna and Labuschagne, 2002) and Ethiopian mustard (Kassa, 2002). These authors reported that the mean squares for genotypes, environments and GXE were highly significant. This was an indication of a broad range of variation between genotypes, between seasons and genotypes over seasons. The effects of genotype and environment on sunflower seed oil content and composition have been studied by several researchers (Qadir *et al*., 2006; Radić *et al*., 2008). It was reported that oil content and fatty acid composition are significantly influenced by environmental conditions. Piva *et al.* (2000) indicated that especially temperature had a profound effect on the oleic/linoleic acid ratio. In addition, genotypes exhibited different sensitivity to environmental conditions. The high oleic genotypes seemed to be more stable than traditional sunflower cultivars regarding fatty acid composition (Lagravere *et al*., 2000; Laureti and Del Gatto *et al*., 2000; Piva *et al*., 2000). However, some differences may occur in high oleic genotypes due to different genetic backgrounds (Salera and Baldini, 1998; Radić *et al*., 2008).

Since genotype and environment have a large influence on oil content and composition, it is necessary for plant breeders to analyse hybrid lines over several growing locations and seasons in order to test for the occurrence of GXE interaction. This will give an indication of the most stable and widely adapted cultivars or hybrids for these locations regarding oil quality traits.

The aim of this study was to compare oil and fatty acid content of high and mid oleic acid sunflower hybrids against two commonly produced linoleic sunflower hybrids in different environments and seasons and to determine to what extent oil content and fatty acid profiles are influenced by the environment.

3.2 Materials and methods

3.2.1 Plant material

Sunflower seed material was obtained from the PANNAR® sunflower breeding programme and included two standard linoleic, eight high oleic and six mid oleic hybrids (Table 3.2). The oleic type of each hybrid was defined according to its pedigree. Due to confidentiality reasons, the names of hybrids as well as their pedigrees may not be revealed. These high and mid oleic hybrids are not commercialised yet and are still being evaluated across years and localities for agronomic performance, oil quality and disease resistance.

Table 3.2 The 16 sunflower hybrids used to determine the effect of location, year and genotype on oil content and composition

Hybrid number	Oleic type
1	Standard linoleic
2	Standard linoleic
3	High oleic
4	High oleic
5	High oleic
6	High oleic
7	High oleic
8	High oleic
9	High oleic
10	Mid oleic
11	Mid oleic
12	Mid oleic
13	Mid oleic
14	High oleic
15	Mid oleic
16	Mid oleic

3.2.2 Field trials

Three sets of trials were planted over three consecutive seasons. A lattice design (4x4) with three replications was used. A different randomisation was used at each location. Plots consisted of two 6 m rows with a 0.91 m between row spacing. The plant population was 36000 plants per hectare. Normal cultural practices were followed. The 16 hybrids were evaluated in eight different locations from the 2004/2005 to the 2006/2007 season (Table 3.3). A summary of the regional orientation, coordinates and climatic region for each location is listed in Table 3.4. Not all locations and genotypes were planted each season due to unforeseen weather conditions and insufficient seed.

Table 3.3 Mean seed and oil yield, rainfall and temperature data for growing seasons 2004/2005, 2005/2006 and 2006/2007

Season of 2004/2005

Location	Planting date	Harvest date	Mean yield (t/ha)	Mean oil yield (t/ha)	Rain during growing season (mm)*	Average daily temperature (°C)#											
						Maximum						Minimum					
						Nov	Dec	Jan	Feb	Mar	Apr	Nov	Dec	Jan	Feb	Mar	Apr
Kroonstad	15-12-2004	10-05-2005	3.29	1.31	359.8	31.0	29.9	30.0	28.3	25.4	22.4	14.5	15.7	16.8	15.6	13.5	9.6
Delmas	03-12-2004	04-04-2005	2.49	1.08	382.8	29.5	27.9	29.0	29.3	27.1	25.1	16.8	18.0	18.7	17.8	15.4	11.6
Standerton	16-11-2004	10-04-2005	3.46	1.61	470.6	27.4	25.1	25.7	26.0	23.5	20.7	12.2	12.8	14.1	12.5	10.4	7.3
Klerksdorp	26-01-2005	13-06-2005	2.34	0.86	542.0	30.6	28.4	27.8	27.3	24.8	21.4	13.6	14.5	15.2	14.4	11.5	8.5
Lichtenburg	14-12-2004	20-04-2005	2.13	0.93	638.4	32.0	29.8	29.8	29.0	26.8	23.0	15.5	16.4	17.2	16.4	13.8	10.7
Settlers	25-01-2005	03-06-2005	1.13	0.49													

Season of 2005/2006

Location	Planting date	Harvest date	Mean yield (t/ha)	Mean oil yield (t/ha)	Rain during growing season (mm)*	Average daily temperature (°C)#											
						Maximum						Minimum					
						Nov	Dec	Jan	Feb	Mar	Apr	Nov	Dec	Jan	Feb	Mar	Apr
Kroonstad	08-12-2005	02-05-2006	2.17	0.83	203.7	29.2	29.6	26.8	23.4	20.8	20.0	14.1	14.9	16.1	13.3	10.3	7.4
Bloemfontein	25-01-2006	26-06-2006	1.88	0.72	612.3	28.7	30.8	29.0	26.7	25.0	22.7	10.3	14.6	18.0	17.4	12.8	10.0

Season of 2006/2007

Location	Planting date	Harvest date	Mean yield (t/ha)	Mean oil yield (t/ha)	Rain during growing season (mm)*	Average daily temperature (°C)#											
						Maximum						Minimum					
						Nov	Dec	Jan	Feb	Mar	Apr	Nov	Dec	Jan	Feb	Mar	Apr
Kroonstad	23-11-2006	20-04-2007	2.31	0.91	228.1	30.0	31.3	32.7	31.4	29.0	25.2	16.0	18.5	16.9	14.6	11.9	10.2
Bloemfontein	13-11-2006	03-04-2007	1.84	0.77	310.0	27.7	30.7	31.6	32.0	28.3	24.2	13.2	15.4	16.3	15.7	13.1	10.9
Koster	05-01-2007	22-05-2007	1.42	0.56													

*: Total rain for growing season October to May, #: Average daily temperature for growing season November to April. Weather data was not available for Settlers and Koster (University of the Free State weather station, 2010; South African Weather Services, 2010).

Table 3.4 Summary of the regional orientation, coordinates and climatic region for eight locations planted from seasons 2004/2005 to 2006/2007

Location	Coordinates	Regional orientation	Climatic region
Bloemfontein	29º06'S 26º12'E	West	Dry Highveld Grassland
Delmas	26º08'S 28º41'E	East	Moist Highveld Grassland
Klerksdorp	26º51'S 26º39'E	West	Dry Highveld Grassland
Kroonstad	27º39'S 27º14'E	West	Dry Highveld Grassland
Lichtenburg	26º08'S 26º09'E	West	Dry Highveld Grassland
Settlers	24º57'S 28º32'E	North	Central Bushveld
Standerton	26º55'S 29º14'E	East	Moist Highveld Grassland
Koster	25º52'S 26º54'E	West	Dry Highveld Grassland

(Leeuwner, 2005).

The western region locations are situated in the dry highveld grassland climatic region that is characterised by a precipitation range of about 450 mm in the west to 700 mm at its northern border. The rainy season reaches its maximum during December and January in the north, but during February and March in the west and the south. Locations in the eastern region are located in the moist highveld grassland climatic region that is similar to the dry highveld grassland region, but cooler and wetter due to higher elevation and position relative to rain bearing systems. Precipitation ranges from 600-800 mm per year and the rainy season reaches it maximum during December and January in the east, but during February in the south. Settlers is located in the northern region that occurs in the central bushveld climatic region. This region is characterised by a rather erratic precipitation that ranges from 500-750 mm pear year. The rainy season lasts from approximately November to March, with peak rainfall in January.

During the 2004/2005 growing season trials were planted at six different locations namely Delmas, Standerton, Lichtenburg, Settlers, Klerksdorp and Kroonstad. During the 2005/2006 season, these trials were repeated using the same 16 hybrids and six locations. However, Bloemfontein (Bainsvlei) was included as an additional locality. Because of the high rainfall during the 2005/2006 season (above 600 mm over three months: January, February and March), trials in four of the locations were lost due to the high occurrence of *Sclerotinia* head and stem rot and therefore only seed material harvested at Bloemfontein and Kroonstad was obtained from PANNAR®. To obtain representative data for statistical analyses, more data was needed and another year's trial was planted. The same hybrids were planted, except for two hybrids (8 and 13) that were substituted because of a lack of seed. These were planted in Bloemfontein, Delmas, Kroonstad and another new locality, Koster, during the 2006/2007 season. The other localities were either not planted or trials failed due to drought. Seed material obtained from PANNAR® included two replications from Kroonstad and one replication each from Bloemfontein and Koster. Since at least two replications' data are needed for statistical analyses, seed material obtained from Bloemfontein and Koster could not be used. During harvest, seed from each plot were bulked. A representative seed sample from each plot was obtained from PANNAR® and a random sample of 100 g seed was dehulled and prepared for oil extraction and grounded with a coffee mill.

3.2.3 Oil extraction and fractionation

Total lipid was quantitatively extracted from a ground seed sample (±1 g) from each hybrid according to Folch *et al*. (1957). Chloroform and methanol in a ratio of 2:1 was used. Butylated hydroxy toluene was added to the mixture at a concentration of 0.001% (v/v) which served as an antioxidant. Samples were left overnight in a refrigerator at 4ºC. Each sample was filtered into a separating funnel, distilled water added and the funnel was shaken thoroughly. Samples were left 1 hour for separation of the phases to occur. The lower phase was drained into a flask and the upper phase discarded. The extracted samples were dried under vacuum in a rotary evaporator and further dried in a vacuum oven at 50ºC with anhydrous phosphorus pentoxide as moisture absorbent. The extractable fat content was determined by weighing and expressed as percentage fat (w/w) per 100 g seed material. From the extracted lipid, ±10 mg was weighed for each sample and transferred to new glass vials for methylation. These were stored under a blanket of nitrogen at -20ºC until analysed.

3.2.4 Fatty acid analysis

An amount of ±10 mg of the extracted fat was methylated by using methanol-BF3 (Slover and Lanza, 1979) to prepare fatty acid methyl esters (FAME) for gas chromatographic analysis. These were quantified using a Varian GX 3400 flame ionisation gas chromatograph, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm internal diameter, 0.2 μm thickness). The column temperature was 40-230ºC (hold 2 minutes 3ºC/min; hold 10 minutes). The FAME in hexane (1 μl) were injected into the column by use of a Varian 8200 CX Autosampler with a split ratio of 100:1. Both the injection port and detector were maintained at 250ºC. Hydrogen was used as the carrier gas at 45 psi, while the make-up gas was nitrogen. The chromatograms were recorded using the Varian Star Chromatography Software. Sample FAME identification was made by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19). Fatty acids were expressed as the relative percentage of each individual fatty acid of the total of all fatty acids present in the sample.

3.2.5 Statistical analysis

ANOVA was done on individual trials followed by a combined analysis across localities and years (Agrobase, 2005). This gave an indication of significant differences between measured characteristics for the high oleic, mid oleic and traditional linoleic acid germplasm as well as GXE interactions. The relative contribution of sources of variation was calculated by using the sums of squares. Where significant GXE was detected, further multivariate analysis (AMMI) was done. This gave an indication of the stability of the varieties for oil and fatty acid content across locations and years (Zobel *et al.*, 1998). The AMMI analysis provides a biplot of main effects and the first principle component scores of the interactions (IPCA 1) of both genotypes and environments. The IPCA 1 score is on the vertical axis and the mean yield on the horizontal. Genotypes or environments that appear almost on a perpendicular line of the graph have similar means, while those that fall almost on a horizontal line have similar interaction patterns (Crossa, 1990). High PCA scores (either negative or positive as it is a relative value) indicate the specific adaption of a genotype to certain environments. The more the IPCA score approximates zero, the more stable the genotype is over all respective environments. Genotypes or environments on the right side of the midpoint of the perpendicular line have higher yields than those on the left side. By using IPCA 1 scores of individual environments in conjunction with IPCA scores of the genotype, the adaptability of a genotype is determined by characterisation of environments. Environments can be classified into low or high potential environments (Crossa, 1990).

3.3 Results

With reference to mean seed yield and mean oil yield per hectare, differences were observed among locations and seasons (Table 3.3). During the first season (2004/2005) Standerton and Kroonstad showed the highest mean seed yields of 3.46 and 3.29 t/ha respectively of the six locations planted. In addition, these two locations also showed the highest mean oil yields (1.61 and 1.31 t/ha respectively). Delmas and Klerksdorp showed more or less the same mean yields (2.49 and 2.34 t/ha respectively), but Delmas yielded slightly more oil (1.08 t/ha) than Klerksdorp (0.86 t/ha). Lichtenburg and Settlers showed the lowest mean seed yields (2.13 and 1.13 t/ha respectively) and oil yields (0.93 and 0.49 t/ha) of the six locations planted. In the second season (2005/2006) Kroonstad yielded 2.17 t/ha seed and that was more than 1 t/ha less than what was observed the previous season. Additionally, the mean oil yield (0.83 t/ha) was lower than for the previous season. Bloemfontein showed lower mean seed yield (1.88 t/ha) and mean oil content (0.72 t/ha) than Kroonstad. In the third season (2006/2007) Kroonstad showed more or less the same mean seed yield (2.31 t/ha) and mean oil yield (0.91 t/ha) than the second season. Bloemfontein also showed similar mean seed (1.84 t/ha) and mean oil (0.77 t/ha) yields than observed in the second season. The differences observed in mean seed and oil yields across locations and years showed an association with the amount of rain the different locations received during the growing season.

Representative seed samples for each hybrid, obtained after harvesting trials at the different locations and over the three seasons, were analysed for oil content and fatty acid composition. From the fatty acid analyses, a total of 11 different fatty acids were identified, but not all hybrids contained all 11. The predominant fatty acids were oleic (C18:1), linoleic (C18:2), stearic (C18:0) and palmitic (C16:0) acid. Behenic, arachidic, linolenic, lignoceric and eicosasienoic acid occurred in trace amounts.

3.3.1 Separate analyses of variance for six locations: Season of 2004/2005

Oil content

ANOVA for oil content (Table 3.5) indicated significant mean squares ($P \leq 0.05$) for replication at Kroonstad and Klerksdorp. Genotype mean squares were highly significant at Kroonstad, Delmas and Standerton. Between 59.99-62.48% of the total sum of squares was accounted for by genotypes at these locations. Large variation was observed among rankings of genotypes in the different environments, due to the fluctuation of the genotypes in response to the different environments (Table 3.6). Where significant genotypic variation occurs, genotypes can be genetically improved for the trait of interest. Highly significant differences were observed among some of the genotypes, mostly at Kroonstad, Delmas and Standerton. In general, genotypes did not differ significantly at Lichtenburg and Settlers. Standerton yielded on average the highest oil content (55.86%), while Klerksdorp yielded the lowest oil content (45.91%).

Linoleic acid

In the ANOVA for linoleic acid content (Table 3.7), mean squares for genotype were highly significant at all six locations and 95.81-97.97% of the total variance was accounted for by genotypes at all six locations. Less variation was observed in the rankings of genotypes across six locations than observed for oil content (Table 3.8). The standard linoleic genotypes 1 and 2 ranked first and second respectively across all six locations with mean linoleic acid contents of 65.31% and 60.27% respectively. Values measured for these two genotypes were expected for traditional sunflower genotypes. Of the eight high oleic genotypes, genotype 3, 5, 6, 8 and 14 had low linoleic acid contents as expected for high oleic sunflower. However, high oleic genotypes 4, 7 and 9 had mid-level linoleic acid contents (between 20.64-40.74%) that compared well with the mid oleic genotypes. The mid oleic genotypes had mid-level linoleic acid contents that ranged between 24.94-39.65%. The lowest average linoleic acid content was observed at Kroonstad (26.15%) while Klerksdorp displayed the highest average linoleic acid content (31.18%). Additionally, most hybrids showed higher linoleic acid contents at Kroonstad compared to the other locations.

Table 3.5 Mean squares of oil content for six locations in 2004/2005

Source	Kroonstad		Delmas		Lichtenburg		Standerton		Klerksdorp		Settlers	
	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation
Rep	16.60*	8.79	0.535	0.21	20.99	9.04	1.70	1.53	21.94*	14.53	0.84	0.82
Genotype	15.11**	59.99	20.76**	62.48	9.23	29.82	9.17**	61.89	4.12	20.49	5.44	40.07
Error	3.93	31.22	6.20	37.30	9.46	61.13	2.71	36.59	6.54	64.98	4.02	59.11
Mean	50.12		50.18		48.67		55.86		45.91		52.97	
LSD (Entry)	3.31		4.15		5.13		2.75		4.26		3.34	
CV (%)	3.96		4.96		6.32		2.95		5.57		3.78	

*P≤0.05, **P≤0.01. MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.6 Mean values and rankings of oil content for six locations in 2004/2005

Genotype	**Kroonstad**		**Delmas**		**Lichtenburg**		**Standerton**		**Klerksdorp**		**Settlers**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
1	50.19	10	50.72	6	48.36	11	56.63	5	45.61	11	52.23	12
2	53.98	1	52.98	3	48.42	10	56.26	8	45.25	13	50.88	15
3	51.86	3	45.93	15	50.92	1	56.80	3	44.30	15	52.90	10
4	48.71	13	51.00	5	49.36	7	60.25	1	45.90	9	53.39	7
5	45.11	16	48.66	14	50.04	4	53.43	15	43.40	16	52.82	11
6	49.13	12	50.60	7	47.63	13	54.48	14	45.55	12	53.36	8
7	51.02	7	53.63	2	49.81	6	56.64	4	46.89	3	53.89	5
8	49.65	11	49.23	10	49.95	5	55.22	12	45.05	14	50.38	16
9	47.28	14	49.15	11	45.07	16	55.16	13	45.80	10	53.51	6
10	50.35	9	52.51	4	49.30	8	55.25	11	46.14	6	51.00	14
11	50.83	8	55.68	1	50.21	3	56.20	9	45.91	8	54.26	3
12	51.84	4	45.63	16	50.72	2	55.55	10	47.90	2	54.90	1
13	51.40	6	48.81	12	45.25	15	56.35	6	46.54	4	54.56	2
14	47.05	14	50.00	8	47.29	14	52.21	16	45.99	7	53.15	9
15	52.16	2	49.65	9	47.64	12	56.35	7	46.27	5	52.09	13
16	51.40	5	48.75	13	48.68	9	56.96	2	48.10	1	54.19	4
Average	50.12		50.18		48.67		55.86		45.91		52.97	
LSD (0.05)	3.31		4.15		5.13		2.75		4.26		3.34	

LSD: Least significant difference.

Table 3.7 Mean squares of linoleic acid content for six locations in 2004/2005

Source	**Kroonstad**		**Delmas**		**Lichtenburg**		**Standerton**		**Klerksdorp**		**Settlers**	
	MS	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Rep	2.97	0.05	3.19	0.05	6.27	0.08	8.74	0.16	20.15	0.31	2.32	0.04
Genotype	764.45**	96.92	863.03**	95.81	989.37**	97.97	713.11**	97.60	853.58**	97.57	804.92**	97.10
Error	11.96	3.03	18.62	4.13	9.80	1.94	8.20	2.24	9.28	0.02	11.88	2.87
Mean	26.15		29.09		28.42		26.56		31.18		28.89	
LSD (Entry)	5.77		7.19		5.22		4.77		5.08		5.75	
CV (%)	13.23		14.84		11.02		10.78		9.77		11.93	

**P≤0.01. MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.8 Mean values and rankings of linoleic acid content for six locations in 2004/2005

Genotype	**Kroonstad**		**Delmas**		**Lichtenburg**		**Standerton**		**Klerksdorp**		**Settlers**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
1	62.45	1	65.34	1	68.31	1	62.26	1	67.34	1	66.18	1
2	56.53	2	58.85	2	65.37	2	52.90	2	65.77	2	62.22	2
3	12.16	13	11.99	13	11.05	13	13.43	12	17.39	13	20.26	12
4	26.99	4	36.68	4	34.34	4	31.89	5	32.29	8	29.00	7
5	14.85	12	10.47	14	16.86	12	13.35	13	20.97	12	15.65	13
6	7.28	15	8.18	15	6.95	15	6.83	16	9.27	16	10.36	15
7	26.42	9	40.74	3	28.38	8	21.71	11	34.58	6	26.79	10
8	6.03	16	14.82	12	5.42	16	6.99	15	10.40	15	6.27	16
9	20.64	11	22.15	11	27.20	10	23.59	10	26.46	11	26.14	11
10	27.51	6	25.34	10	29.64	7	25.77	9	32.77	7	30.25	6
11	24.94	10	31.40	9	26.42	11	28.28	8	28.47	10	28.00	8
12	27.14	7	32.42	6	35.00	3	33.00	4	31.81	9	33.43	5
13	31.03	5	35.91	5	32.68	5	34.31	3	39.65	3	33.67	4
14	7.81	14	6.78	16	7.33	14	9.80	14	11.03	14	11.32	14
15	33.94	3	32.35	7	32.34	6	31.31	6	35.57	4	35.76	3
16	32.60	4	31.99	8	27.42	9	29.59	7	35.05	5	26.95	9
Average	26.15		29.09		28.42		26.56		31.18		28.89	
LSD (0.05)	5.77		7.19		5.22		4.77		5.08		5.75	

LSD: Least significant difference.

Oleic acid

Highly significant mean squares (P≤0.01) were observed for replication at Klerksdorp (Table 3.9). Genotype mean squares were highly significant at all six locations and 95.62-97.81% of the variance was contributed by genotypes at all six locations. Small variation was observed in rankings of genotypes across six locations (Table 3.10). The standard linoleic genotypes 1 and 2 ranked sixteenth and fifteenth for oleic acid and this was expected from the negative correlation between oleic and linoleic acid content. The high oleic genotypes (except for genotypes 4 and 7) ranked the highest at all six locations for oleic acid content. The high oleic genotypes (3, 5. 6, 8 and 14) had significantly higher oleic acid percentages than the mid oleic and linoleic acid genotypes. The oleic acid contents of the high oleic genotypes 4, 7 and 9 compared well with mid oleic hybrids with oleic acid contents between 55-75%. However, genotype 7 ranked first in Settlers with an oleic acid content of 82.58%. The mid oleic genotypes ranged between 51.26-63.93%. Generally, genotypes had lower oleic acid contents in Klerksdorp compared to the other five localities. Klerksdorp, as a result, yielded the lowest mean oleic acid content (55.83%), while Kroonstad yielded the highest average oleic acid content (62.58%).

Palmitic acid

Highly significant replication mean squares were evident at Klerksdorp (Table 3.11). Genotype mean squares were highly significant at all six locations and 57.22-93.97% of the total variance was accounted for by genotypes at all locations. Large variation was observed among rankings of genotypes across the six locations for palmitic acid content (Table 3.12). The standard linoleic genotypes 1 and 2 ranked in the top three places for palmitic acid content across all six locations with contents between 4.72-6.46%. The high oleic genotypes, with the exception of genotypes 4, 7 and 9 had the lowest rankings for palmitic acid content. The high oleic genotypes contained on average less than 4% palmitic acid. The mid oleic genotypes showed mid-level palmitic acid contents since these contained less palmitic acid than the traditional linoleic genotypes and more palmitic acid than the high oleic genotypes. The palmitic acid contents of the mid oleic genotypes ranged between 3.74-4.63%. Kroonstad yielded the lowest average palmitic acid content (3.81%), while Standerton yielded the highest (4.47%).

Table 3.9 Mean squares of oleic acid content for six locations in 2004/2005

Source	Kroonstad		Delmas		Lichtenburg		Standerton		Klerksdorp		Settlers	
	MS	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Rep	2.42	0.04	4.03	0.06	3.77	0.05	8.01	0.13	65.623**	0.90	3.08	0.05
Genotype	798.04**	96.72	892.38**	95.62	1059.40**	97.81	773.44**	97.41	935.86**	96.70	869.84**	96.92
Error	13.39	3.24	20.19	4.33	11.60	2.15	9.76	2.46	11.61	2.40	13.63	3.04
Mean	62.58		60.17		60.35		62.13		55.83		59.19	
LSD (Entry)	6.10		7.49		5.68		5.21		5.68		6.16	
CV (%)	5.85		7.47		5.64		5.03		6.10		6.24	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.10 Mean values and rankings of oleic acid content for six locations in 2004/2005

Genotype	**Kroonstad**		**Delmas**		**Lichtenburg**		**Standerton**		**Klerksdorp**		**Settlers**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
1	25.72	16	23.55	16	19.20	16	25.00	16	18.22	16	20.61	16
2	32.29	15	29.48	15	21.73	15	33.20	15	20.15	15	24.27	15
3	76.34	4	76.49	4	77.63	4	74.70	5	70.46	4	67.32	5
4	61.22	11	52.15	13	54.64	13	57.48	11	52.44	10	59.55	10
5	75.25	5	80.04	3	72.68	5	76.31	4	67.57	5	74.35	4
6	81.44	3	81.27	2	82.49	3	82.39	1	77.82	2	75.83	3
7	61.54	10	48.05	14	59.71	9	66.68	6	51.22	13	82.58	1
8	82.84	1	74.45	5	84.02	1	81.92	2	77.74	3	61.33	7
9	68.12	6	66.37	6	60.80	8	64.84	7	59.91	6	62.29	6
10	61.78	8	63.66	7	59.45	10	62.92	8	54.33	9	57.61	11
11	63.93	7	57.86	9	62.26	6	60.66	9	57.97	7	60.11	9
12	61.68	9	57.46	10	54.22	14	56.43	13	56.12	8	54.54	13
13	58.21	12	53.47	12	56.15	12	54.69	14	47.62	14	55.12	12
14	81.67	2	83.31	1	82.72	2	80.01	3	78.56	1	79.16	2
15	53.56	14	57.25	11	56.32	11	57.14	12	51.60	11	51.26	14
16	56.66	13	57.92	8	61.57	7	59.77	10	51.57	12	61.04	8
Average	62.58		60.17		60.35		62.13		55.83		59.19	
LSD (0.05)	6.10		7.49		5.68		5.21		5.68		6.16	

LSD: Least significant difference.

Table 3.11 Mean squares of palmitic acid content for six locations in 2004/2005

Source	**Kroonstad**		**Delmas**		**Lichtenburg**		**Standerton**		**Klerksdorp**		**Settlers**	
	MS	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Rep	0.01	2.46	0.00	0.04	0.05	0.35	0.28	1.94	1.88**	17.45	0.06	0.55
Genotype	0.74**	91.13	1.10**	92.25	1.67**	93.97	1.39**	73.06	0.82**	57.22	1.40**	93.02
Error	0.03	8.41	0.05	7.71	0.05	5.68	0.24	25.00	0.18	25.33	0.05	6.43
Mean	3.81		4.03		4.26		4.46		3.98		4.19	
LSD (Entry)	0.31		0.36		0.38		0.81		0.71		0.37	
CV (%)	4.85		5.31		5.28		10.93		10.72		5.25	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.12 Mean values and rankings of palmitic acid content for six locations in 2004/2005

Genotype	**Kroonstad**		**Delmas**		**Lichtenburg**		**Standerton**		**Klerksdorp**		**Settlers**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
1	4.95	1	5.41	1	5.77	2	6.46	1	4.97	1	5.73	1
2	4.73	2	4.87	2	5.85	1	5.32	2	4.72	3	5.40	2
3	3.31	14	3.28	16	3.52	14	4.70	4	3.46	14	3.80	12
4	3.88	6	4.42	4	4.62	3	4.27	10	4.79	2	4.34	5
5	3.34	13	3.31	15	3.83	12	3.75	15	3.87	10	3.64	13
6	3.29	15	3.38	14	3.59	13	3.66	16	3.46	13	3.46	14
7	3.99	4	4.56	3	4.18	9	4.15	12	4.15	5	3.96	10
8	3.09	16	3.44	12	3.11	16	3.81	14	3.37	15	3.15	16
9	3.81	10	3.93	9	4.60	4	4.55	6	4.03	8	4.17	9
10	3.91	5	3.89	11	4.41	6	4.41	9	4.19	4	4.28	7
11	3.74	11	4.34	5	4.17	10	4.51	7	3.88	9	4.25	8
12	3.83	9	4.01	8	4.57	5	4.46	8	4.05	7	4.52	4
13	3.87	7	4.20	7	4.27	7	4.73	3	4.07	6	4.63	3
14	3.37	12	3.39	13	3.46	15	3.89	13	3.08	16	3.43	15
15	4.05	3	3.89	10	4.21	8	4.56	5	3.81	11	4.33	6
16	3.84	8	4.23	6	4.06	11	4.22	11	3.77	12	3.94	11
Average	3.81		4.03		4.26		4.47		3.98		4.19	
LSD	0.31		0.36		0.38		0.81		0.71		0.37	

LSD: Least significant difference.

Stearic acid

Highly significant replication mean squares were indicated at Klerksdorp (Table 3.13). Genotype mean squares were highly significant at all six locations. Between 60.71-88.31% of the total variance was accounted for by genotypes at all six locations. Large variation was observed among rankings of genotypes across the six locations (Table 3.14). The high oleic genotypes 4 and 7 ranked in the first two places for stearic acid content in Kroonstad and Klerksdorp. However, genotype 7 ranked second in both Lichtenburg and Standerton. Klerksdorp yielded the highest average stearic acid content (6.89%), while Delmas yielded the lowest (4.66%).

3.3.2 Combined analysis of variance across six locations for 2004/2005

The mean squares for environment, genotype and GXE interaction were highly significant for all five sets of data (Table 3.15). Oil content varied from 48.91% (genotype 5) to 52.18% (genotype 11), with a mean of 50.62% (Table 3.16). Significant differences occurred between genotypes for oleic and linoleic acid contents. Genotypes 1 and 2 are standard linoleic genotypes with low oleic (22.05% and 26.69% respectively) and high linoleic (65.32% and 60.27% respectively) acid contents (Table 3.14). The high oleic genotypes 6, 8 and 14 yielded oleic acid contents above 80% and linoleic acid contents of less than 10%. These high oleic genotypes are regarded as high potential oleic acid genotypes with oleic acid contents above the minimum limit of 75% that is recommended for high oleic sunflower. The high oleic genotypes 3, 4, 5, 7 and 9 were considered low potential oleic acid genotypes, since their oleic acid contents were less than the minimum of 75% accepted for high oleic genotypes. The mid oleic genotypes contained oleic acid contents of between 54.21-60.46%. The oleic and linoleic acid contents of the mid oleic genotypes were within the ranges recommended for mid oleic genotypes. With regard to palmitic acid content, the traditional linoleic genotypes ranked first, while the high oleic genotypes with the exception of genotypes 4 and 7 ranked last. A negative association between palmitic and oleic acid content was observed. High oleic genotypes with high oleic acid potentials had the lowest palmitic acid contents. No specific pattern for stearic acid was observed concerning the different oleic types.

Table 3.13 Mean squares of stearic acid content for six locations in 2004/2005

Source	Kroonstad		Delmas		Lichtenburg		Standerton		Klerksdorp		Settlers	
	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation
Rep	0.12	2.27	0.01	0.29	0.07	0.10	0.09	2.08	5.98**	20.62	0.13	1.10
Genotype	0.47**	67.48	0.45**	82.20	0.77**	82.84	0.52**	88.31	2.35**	60.71	1.29**	82.40
Error	0.11	30.27	0.05	17.51	0.08	16.15	0.03	9.60	0.36	18.66	0.13	16.50
Mean	5.16		4.66		5.03		4.80		6.89		5.43	
LSD (Entry)	0.54		0.36		0.46		0.28		1.00		0.60	
CV (%)	6.30		4.68		5.46		3.49		8.72		6.62	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.14 Mean values and rankings of stearic acid content for six locations in 2004/2005

Genotype	Kroonstad		Delmas		Lichtenburg		Standerton		Klerksdorp		Settlers	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	5.05	11	4.16	15	4.91	10	4.42	12	7.38	5	5.58	7
2	5.28	5	4.80	8	5.54	3	4.88	7	7.38	4	6.37	1
3	5.71	3	5.24	1	5.79	1	5.71	1	6.34	12	6.30	3
4	5.74	2	5.04	3	4.83	12	4.58	10	8.53	1	5.27	11
5	4.45	16	4.12	16	4.23	15	4.40	13	5.66	15	4.13	16
6	5.27	7	4.85	7	4.85	11	4.93	6	7.10	8	4.92	14
7	5.75	1	5.02	4	5.76	2	5.32	2	8.05	2	5.87	4
8	5.27	6	4.91	5	5.22	7	5.31	3	6.27	13	5.43	9
9	5.46	4	5.21	2	5.40	4	5.11	4	7.32	7	5.36	10
10	4.84	14	4.88	6	4.60	13	4.79	9	6.71	10	5.70	5
11	5.17	9	4.48	11	5.09	9	4.57	11	7.47	3	5.59	6
12	4.87	13	4.21	14	4.45	14	4.29	16	6.06	14	5.25	12
13	4.93	12	4.49	10	5.14	8	4.98	5	6.51	11	5.10	13
14	4.47	15	4.25	12	4.08	16	4.31	15	5.05	16	4.19	15
15	5.18	8	4.72	9	5.26	6	4.87	8	7.03	9	5.45	8
16	5.13	10	4.23	13	5.37	5	4.40	14	7.33	6	6.35	2
Average	5.16		4.66		5.03		4.80		6.89		5.43	
LSD (0.05)	0.54		0.36		0.46		0.28		1.00		0.60	

LSD: Least significant difference.

Table 3.15 Mean squares of oil and fatty acid contents over environment for six locations in 2004/2005

Source	Mean squares				
	Oil content	**Linoleic acid**	**Oleic acid**	**Palmitic acid**	**Stearic acid**
Environment	570.10**	162.06**	282.04**	2.53**	31.42**
Rep in Environment	10.43*	7.28	14.49	0.38**	1.07**
Genotype	16.04**	4857.49**	5191.75**	6.33**	3.47**
GXE	9.56**	26.19**	27.44**	0.18**	0.48**
Residual	5.48	11.62	13.36	0.10	0.12
CV (%)	4.62	12.01	6.09	7.72	6.62

*P≤0.05, **P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, GXE: Genotype by environment interaction, CV: Coefficient of variation.

Table 3.16 Mean values and rankings of oil and fatty acid contents for six locations in 2004/2005

Genotype	Oil content		Linoleic acid		Oleic acid		Palmitic acid		Stearic acid	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	50.62	9	65.32	1	22.05	16	5.55	1	5.25	12
2	51.30	5	60.27	2	26.69	15	5.19	2	5.71	3
3	50.45	11	14.38	13	73.82	5	3.68	12	5.85	2
4	51.44	3	31.87	6	56.25	12	4.39	3	5.67	4
5	48.91	16	15.36	12	74.37	4	3.62	13	4.50	15
6	50.13	12	8.15	16	80.21	3	3.47	14	5.32	10
7	51.98	2	29.77	8	58.09	10	4.17	8	5.96	1
8	49.91	13	8.32	15	80.59	2	3.33	16	5.40	8
9	49.33	14	24.36	11	63.72	6	4.18	7	5.64	5
10	50.76	7	28.55	9	59.96	8	4.18	6	5.25	11
11	52.18	1	27.92	10	60.46	7	4.15	9	5.40	9
12	51.09	6	32.13	5	56.74	11	4.24	5	4.86	14
13	50.48	10	34.54	3	54.21	14	4.30	4	5.19	13
14	49.28	15	9.01	14	80.91	1	3.44	15	4.39	16
15	50.69	8	33.54	4	54.52	13	4.14	10	5.42	7
16	51.35	4	30.60	7	58.09	9	4.01	11	5.47	6
Average	50.62		28.38		60.04		4.13		5.33	
LSD (0.05)	1.54		2.24		2.40		0.17		0.23	

LSD: Least significant difference.

3.3.3 Separate analyses of variance for two locations: Season of 2005/2006

During this season, the same localities were planted than for the previous season except for Bloemfontein that was a new location. However, due to the high rainfall during this season, trials planted at Delmas, Standerton and Klerksdorp failed due to the occurrence of *Sclerotinia* head and stem rot. Seed obtained from Lichtenburg included only one replication and could therefore not be included in the analyses.

Oil content

Replication and genotypic mean squares were significant at Kroonstad and 45.12% of the total variance was accounted for by genotype (Table 3.17). Large variation was observed among rankings of genotypes in the two environments (Table 3.18). The standard linoleic genotypes 1 and 2 ranked second and third at Kroonstad, while at Bloemfontein these hybrids ranked in the eight and sixth places respectively. The mid oleic genotype 12 ranked first at Kroonstad, but at Bloemfontein it ranked fifth. In Bloemfontein genotype 4 ranked first for oil content, but in Kroonstad it ranked thirteenth. Significant differences were observed among genotypes at Kroonstad, however, at Bloemfontein, only genotypes 6 and 14 differed significantly from genotype 1. Kroonstad yielded an average oil content of 49.02%, while that of Bloemfontein was 42.73%.

Table 3.17 Mean squares of oil content for two locations in 2005/2006

Source	Kroonstad		Bloemfontein	
	MS	**% Variation**	**MS**	**% Variation**
Rep	17.76*	11.35	4.81	2.01
Genotype	9.42*	45.12	11.45	35.80
Error	4.54	43.53	9.94	62.18
Mean	49.01		42.73	
LSD (Entry)	3.55		5.26	
CV (%)	4.35		7.38	

*P≤0.05, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.18 Mean values and rankings of oil content for two locations in 2005/2006

Genotype	Kroonstad		Bloemfontein	
	Mean	Rank	Mean	Rank
1	51.08	2	42.91	8
2	50.94	3	43.25	6
3	48.54	11	44.31	4
4	47.38	13	46.09	1
5	46.35	15	42.61	9
6	49.42	7	39.82	15
7	50.01	5	41.89	13
8	48.93	9	41.90	12
9	47.09	14	41.98	11
10	45.83	16	39.33	16
11	49.39	8	45.45	2
12	52.28	1	43.93	5
13	50.29	4	44.97	3
14	48.17	12	40.08	14
15	49.93	6	42.96	7
16	48.62	10	42.21	10
Average	49.02		42.73	
LSD (0.05)	3.55		5.258	

LSD: Least significant difference.

Linoleic acid

Genotype mean squares were highly significant for both Kroonstad and Bloemfontein. Genotypes contributed 80.30-93.18% of the total variance at both locations (Table 3.19). Slight variation was observed between rankings of genotypes 5-16 across the two locations (Table 3.20). The standard linoleic genotypes 1 and 2 ranked first and second respectively at both Kroonstad and Bloemfontein for linoleic acid content. However, the linoleic acid contents of these two genotypes were 10.2% and 13.61% higher at Bloemfontein than at Kroonstad. The high oleic genotypes (except for genotype 4 at both locations and genotypes 7 and 9 at Bloemfontein) had linoleic acid contents of less than 25.0%. The mid oleic genotypes (except for genotypes 12 and 13 at Bloemfontein) had mid-level linoleic acid contents of between 26.0-38.49%. Genotypes 12 and 13 had slightly higher linoleic acid contents (42.19% and 48.53% respectively) at Bloemfontein. Most genotypes yielded higher linoleic acid contents at Bloemfontein when compared to Kroonstad. As a result, Bloemfontein yielded the highest linoleic acid content (33.97%), while Kroonstad yielded the lowest (27.5%).

Table 3.19 Mean squares of linoleic acid content for two locations in 2005/2006

Source	Kroonstad		Bloemfontein	
	MS	% Variation	MS	% Variation
Rep	148.07	2.46	10.83	0.11
Genotype	643.98**	80.30	1194.35**	93.18
Error	69.12	17.24	43.01	6.71
Mean	27.50		33.97	
LSD (Entry)	13.86		10.94	
CV (%)	30.23		19.30	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.20 Mean values and rankings of linoleic acid content for two locations in 2005/2006

Genotype	Kroonstad		Bloemfontein	
	Mean	Rank	Mean	Rank
1	61.67	1	71.87	1
2	57.61	2	71.22	2
3	14.08	14	11.90	14
4	32.66	3	49.71	3
5	15.08	13	21.72	12
6	8.17	16	6.68	15
7	22.44	12	31.13	10
8	25.00	10	5.99	16
9	22.94	11	34.14	8
10	27.89	7	33.52	9
11	26.00	9	37.37	7
12	32.21	4	42.19	5
13	28.34	6	48.58	4
14	9.36	15	14.33	13
15	27.12	8	38.49	6
16	29.40	5	27.70	11
Average	27.50		33.97	
LSD (0.05)	13.86		10.94	

LSD: Least significant difference.

Oleic acid

Highly significant genotype mean squares were seen at both Kroonstad and Bloemfontein and 79.78-93.02% of the variance was contributed by genotype at both locations (Table 3.21). Slight variation was observed in rankings of some genotypes across the two locations (Table 3.22). Only genotypes 1, 2, 4 and 6 did not differ in their rankings across both locations. The standard linoleic genotypes 1 and 2 had the lowest oleic acid contents as expected and ranked sixteenth and fifteenth respectively at both locations. However, the oleic acid contents of these two genotypes were higher at Kroonstad than at Bloemfontein.

The oleic acid contents of genotypes 1 and 2 were 13.36% and 17.04% higher at Kroonstad than at Bloemfontein. The high oleic genotypes had the highest oleic acid contents as expected and genotypes 3, 6 and 14 had oleic acid contents above 75% at both locations. However, the high oleic genotype 5 had an oleic acid content of 75.67% at Kroonstad, but 67.26% at Bloemfontein. An oleic acid content of above 75% is recommended for genotypes to be regarded as high oleic genotypes. Also, the high oleic genotype 8 had an oleic acid content of 81.22% at Bloemfontein, while at Kroonstad it was 63.90% (less than 75%). This indicates that high oleic genotypes have different oleic acid potentials in different environments. Genotype 4 had an oleic acid content of 55.78% at Kroonstad, while at Bloemfontein it was 35.08%. This genotype is considered a low oleic potential genotype with oleic and linoleic acid contents that compare well with those of mid oleic genotypes. The high oleic genotypes 7 and 9 also had oleic acid contents (between 52.94-66.83%) that compared to the mid oleic genotypes at both locations. The mid oleic genotypes had mid-level oleic acid contents of between 36.92-63.08%. In general, all genotypes (except genotype 8) had higher oleic acid contents at Kroonstad than at Bloemfontein. The average oleic acid content was the lowest at Bloemfontein (52.57%) and the highest at Kroonstad (61.88%). It was observed that Kroonstad promoted higher oleic acid contents, while Bloemfontein promoted higher linoleic acid contents.

Table 3.21 Mean squares of oleic acid content for two locations in 2005/2006

Source	Kroonstad		Bloemfontein	
	MS	% Variation	MS	% Variation
Rep	148.72	2.33	12.43	0.12
Genotype	678.18**	79.78	1291.11**	93.02
Error	76.03	17.89	47.63	6.86
Mean	61.88		52.57	
LSD (Entry)	14.54		11.51	
CV (%)	14.09		13.13	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.22 Mean values and rankings of oleic acid content for two locations in 2005/2006

Genotype	Kroonstad		Bloemfontein	
	Mean	Rank	Mean	Rank
1	26.87	16	13.51	16
2	31.40	15	14.36	15
3	75.14	4	74.75	3
4	55.78	14	35.08	14
5	75.67	3	67.26	5
6	81.31	2	80.08	2
7	66.26	6	54.91	7
8	63.90	7	81.22	1
9	66.83	5	52.94	8
10	61.64	10	52.72	9
11	63.08	8	52.11	10
12	56.91	13	44.02	12
13	61.27	11	36.92	13
14	81.35	1	74.64	4
15	62.03	9	47.46	11
16	60.55	12	59.05	6
Average	61.88		52.57	
LSD (0.05)	14.54		11.51	

LSD: Least significant difference.

Palmitic acid

Highly significant genotype mean squares were observed at Kroonstad and Bloemfontein (Table 3.23). Variance due to genotype contributed 59.46-77.22% of the total variance. Large variation was observed among rankings of some genotypes (4, 6, 8 and 13) across both locations (Table 3.24). Only genotypes 1, 11 and 14 did not differ in their rankings across both locations. The standard linoleic genotypes 1 and 2 ranked in the top three positions for palmitic acid with contents of between 4.99-6.52% at both locations. However, both genotypes contained more palmitic acid at Bloemfontein than at Kroonstad. The high oleic genotypes did not differ significantly from the mid oleic genotypes for palmitic acid content at Kroonstad. However, at Bloemfontein significant differences were observed among the high and mid oleic genotypes for palmitic acid content. The high oleic genotypes contained on average less palmitic acid than the traditional and mid oleic genotypes at both locations. In general, genotypes at Kroonstad contained less palmitic acid than those at Bloemfontein with the exception of genotypes 6 and 8. The average palmitic acid content observed for Kroonstad was 4.51% and 5.23% for Bloemfontein.

Stearic acid

Highly significant replication mean squares were evident at Kroonstad. Genotype mean squares were highly significant at both Kroonstad and Bloemfontein and 83.75-88.85% of the total variance was accounted for by genotypes at both locations (Table 3.25). Large variation was observed among rankings of genotypes in the two locations (Table 3.26). However, only genotypes 4, 11, 15 and 16 did not differ in their rankings at both locations. The standard linoleic acid genotypes 1 and 2 had higher stearic acid contents at Bloemfontein than at Kroonstad. Genotype 1 and 2 contained 2.32% and 1.98% more stearic acid respectively at Bloemfontein than at Kroonstad. These genotypes contained on average less stearic acid than the high oleic genotypes, but more than the mid oleic genotypes. The high oleic genotypes had on average the highest stearic acid contents that ranged between 3.97-8.06% at both locations. The high oleic genotype 4 ranked first in both locations for stearic acid with contents of 5.58% and 8.06% at Kroonstad and Bloemfontein respectively. The mid oleic genotypes had on average the lowest stearic acid contents and ranged between 3.78-7.21% at both locations. In general, all genotypes contained more stearic acid at Bloemfontein than at Kroonstad. The average stearic acid content for Kroonstad was 4.52% and 6.55% at Bloemfontein.

Table 3.23 Mean squares of palmitic acid content for two locations in 2005/2006

Source	Kroonstad		Bloemfontein	
	MS	% Variation	MS	% Variation
Rep	0.22	2.08	0.02	0.01
Genotype	0.83**	59.46	1.59**	77.22
Error	0.27	38.46	0.23	22.77
Mean	4.51		5.23	
LSD (Entry)	0.86		0.81	
CV (%)	11.49		9.25	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.24 Mean values and rankings of palmitic acid content for two locations in 2005/2006

Genotype	Kroonstad		Bloemfontein	
	Mean	Rank	Mean	Rank
1	5.75	1	6.52	1
2	4.99	3	6.26	2
3	3.89	15	4.29	14
4	4.34	9	5.73	5
5	3.97	14	4.75	12
6	4.45	8	4.21	15
7	4.70	5	5.20	8
8	4.77	4	4.54	13
9	4.26	13	5.06	9
10	4.58	7	5.65	6
11	4.27	11	5.01	11
12	5.37	2	5.78	4
13	4.33	10	5.84	3
14	3.72	16	4.12	16
15	4.59	6	5.61	7
16	4.27	12	5.05	10
Average	4.51		5.23	
LSD (0.05)	0.86		0.81	

LSD: Least significant difference.

Table 3.25 Mean squares of stearic acid content for two locations in 2005/2006

Source	Kroonstad		Bloemfontein	
	MS	% Variation	MS	% Variation
Rep	0.28**	5.01	0.12	0.55
Genotype	0.63**	83.75	2.29**	88.85
Error	0.04	11.23	0.14	10.53
Mean	4.52		6.55	
LSD (Entry)	0.34		0.61	
CV (%)	4.54		5.63	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.26 Mean values and rankings of stearic acid content for two locations in 2005/2006

Genotype	Kroonstad		Bloemfontein	
	Mean	Rank	Mean	Rank
1	4.18	13	6.50	10
2	4.78	3	6.76	7
3	5.22	2	6.93	5
4	5.58	1	8.06	1
5	3.97	15	4.47	16
6	4.24	12	6.84	6
7	4.77	5	7.41	2
8	4.62	7	6.53	9
9	4.63	6	6.44	12
10	4.42	10	6.38	13
11	4.78	4	7.01	4
12	3.78	16	6.21	14
13	4.47	9	7.21	3
14	4.04	14	4.82	15
15	4.49	8	6.72	8
16	4.40	11	6.49	11
Average	4.52		6.55	
LSD (0.05)	0.34		0.61	

LSD: Least significant difference.

3.3.4 Combined analysis of variance across two locations for 2005/2006

The mean squares for environment were highly significant for all five data sets (Table 3.27). All four major fatty acids showed highly significant variance for genotype. GXE was significant for linoleic and oleic acid content and highly significant for stearic acid content. Oil content varied between 42.58% (genotype 10) and 48.10% (genotype 12) with a mean of 45.87% (Table 3.28). The standard linoleic genotypes 1 and 2 ranked fifth and fouth respectively for oil content. These genotypes yielded the highest linoleic acid contents (66.77% and 64.42% respectively) as well as the highest palmitic acid contents (6.13% and 5.63% respectively). The high oleic genotypes 3, 6 and 14 can be considered high potential oleic genotypes with high oleic acid contents of 74.95%, 80.70% and 78.00% respectively and linoleic acid contents of less than 13%. The remaining high oleic genotypes 4, 5, 7, 8 and 9 were considered low potential oleic genotypes since their oleic acid contents were less than the recommended 75% for high oleic sunflower. Genotype 4 with its low oleic acid content (45.43%) and relatively high linoleic acid content (41.18%) should be regarded a mid oleic genotype than a high oleic genotype. In addition, this hybrid had the highest stearic acid content (6.82%) of all genotypes.

Table 3.27 Mean squares of oil and fatty acid contents over environments for two locations in 2005/2006

Source	Mean squares				
	Oil content	Linoleic acid	Oleic acid	Palmitic acid	Stearic acid
Environment	948.53**	1005.44**	2079.85**	12.18**	98.31**
Rep in Environment	11.29	79.45	80.57	0.11	0.20
Genotype	13.67*	1709.24**	1827.01**	2.042**	2.34**
GXE	7.19	129.09*	142.27*	0.34	0.58**
Residual	7.24	56.06	61.83	0.25	0.09
CV (%)	5.87	24.36	13.74	10.30	5.39

*P≤0.05, **P≤0.01, Rep: Replication, GXE: Genotype by environment interaction, CV: Coefficient of variation.

Table 3.28 Mean values and rankings of oil and fatty acid contents for two locations in 2005/2006

Genotype	**Oil content**		**Linoleic acid**		**Oleic acid**		**Palmitic acid**		**Stearic acid**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
1	47.00	5	66.77	1	20.19	16	6.13	1	5.34	13
2	47.09	4	64.42	2	22.88	15	5.63	2	5.77	6
3	46.42	8	12.99	14	74.95	3	4.09	15	6.08	3
4	46.73	6	41.18	3	45.43	14	5.04	7	6.82	1
5	44.48	14	18.40	12	71.47	5	4.36	13	4.22	16
6	44.62	12	7.43	16	80.70	1	4.33	14	5.54	9
7	45.95	9	26.79	11	60.59	6	4.95	8	6.09	2
8	45.42	10	15.50	13	72.56	4	4.65	11	5.58	8
9	44.53	13	28.54	10	59.89	7	4.66	9	5.54	10
10	42.58	16	30.70	7	57.18	10	5.12	4	5.40	12
11	47.42	3	30.19	8	57.60	9	4.64	12	5.89	4
12	48.10	1	37.20	5	50.46	12	5.58	3	5.00	14
13	47.63	2	38.46	4	49.10	13	5.08	6	5.84	5
14	44.13	15	11.84	15	78.00	2	3.92	16	4.43	15
15	46.44	7	32.80	6	54.75	11	5.10	5	5.61	7
16	45.42	11	28.55	9	59.80	8	4.66	10	5.45	11
Average	45.87		30.73		57.22		4.87		5.54	
LSD (0.05)	3.11		8.65		9.08		0.58		0.34	

LSD: Least significant difference.

3.3.5 Separate analyses of variance for Kroonstad: Season of 2006/2007

Kroonstad was the only environment analysed the third season. Data obtained for Bloemfontein and Koster included only one replication each and could therefore not be included for statistical analysis. This trial included only 14 entries. Genotypes 8 and 13 from the previous trials were excluded from this trial because of a shortage of seed during planting of trials.

Genotype mean squares were highly significant for linoleic, oleic, palmitic and stearic acid with genotype contributing 59.68-76.72% to the total variance (Table 3.29). Oil content varied between 47.39% (genotype 11) and 38.63% (genotype 5) (Table 3.30). Apart from genotype 11 that was significantly better than genotypes 5 and 9, no significant differences were observed among genotypes for oil content. The standard linoleic genotypes 1 and 2 ranked second and third for oil content (47.33% and 46.49% respectively). In addition, these genotypes ranked second and first respectively for linoleic acid with contents of 47.24% and 50.81% respectively. The high oleic genotypes 3 and 14 had low linoleic acid contents of less than 15% and they had the highest oleic acid contents (80.26% and 77.71% respectively). These two genotypes were regarded as high oleic potential genotypes. The rest of the high oleic genotypes had higher linoleic acid contents of between 20.46-22.19%. The high oleic genotype 4 contained 27.29% linoleic acid and 61.02% oleic acid content. High oleic genotypes 4, 5, 6, 7 and 9 were regarded as low oleic potential genotypes since their oleic acid contents were less than the recommended 75% for high oleic sunflower. Their oleic acid contents (between 61.02-69.33%) were within the range recommended for mid oleic genotypes. The mid oleic genotypes 10, 11, 12, 15 and 16 had mid-level oleic acid contents of between 53.17-65.60%.

The traditional genotypes 1 and 2 yielded the highest palmitic acid contents (5.24% and 5.02% respectively). With the exception of the mid oleic genotype 12 that differed significantly from the high oleic genotypes 3 and 5, no significant differences were observed among high and mid oleic genotypes for palmitic acid content. The high oleic genotype 4 ranked first for stearic acid content (5.64%) and was followed by genotype 3 (5.61%). Some of the genotypes showed significant differences for stearic acid content. On average, the high oleic genotypes contained more stearic acid than the traditional and mid oleic genotypes.

Table 3.29 Mean squares of oil and fatty acid contents for Kroonstad in 2006/2007

Source	**Oil content**		**Linoleic acid**		**Oleic acid**		**Palmitic acid**		**Stearic acid**	
	MS	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Rep	1.41	0.50	66.77	1.79	78.29	2.09	0.20	4.64	0.11	1.58
Genotype	19.18	44.06	422.77**	73.86	429.95**	73.39	0.39**	59.68	0.83**	76.72
Error	12.99	55.50	69.66	24.34	71.83	24.55	0.12	35.69	0.12	21.70
Mean	44.06		27.30		61.83		4.62		4.93	
LSD (Entry)	5.83		14.01		14.22		0.58		0.58	
CV (%)	7.90		30.57		13.71		7.42		6.96	

**P≤0.01, MS: Mean squares, Rep: Replication, LSD: Least significant difference, CV: Coefficient of variation.

Table 3.30 Mean values and rankings of oil and fatty acid contents for Kroonstad in 2006/2007

Genotype	Oil content		Linoleic acid		Oleic acid		Palmitic acid		Stearic acid	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	47.33	2	47.24	2	41.82	13	5.24	1	4.57	9
2	46.49	3	50.81	1	37.73	14	5.02	2	5.18	6
3	44.82	5	8.78	14	80.26	1	4.05	16	5.61	2
4	43.19	11	27.29	6	61.02	10	4.58	9	5.64	1
5	38.63	14	20.46	12	69.33	3	4.14	14	4.43	13
6	44.34	7	21.51	11	67.51	4	4.44	13	5.19	5
7	44.23	8	22.04	10	66.55	5	4.70	6	5.59	4
9	40.14	13	22.19	9	66.18	6	4.64	7	5.60	3
10	45.78	4	23.56	8	65.60	7	4.57	9	4.92	7
11	47.39	1	26.29	7	63.40	8	4.75	5	4.45	12
12	44.11	9	35.89	4	53.72	11	5.00	3	4.13	14
14	41.65	12	12.04	13	77.71	2	4.09	13	4.48	11
15	43.98	10	27.66	5	61.58	9	4.56	10	4.79	8
16	44.72	6	36.43	3	53.17	12	4.96	4	4.53	10
Average	44.06		27.30		61.83		4.62		4.93	
LSD (0.05)	5.84		14.01		14.22		0.58		0.58	

LSD: Least significant difference.

3.3.6 Combined analysis of variance for Kroonstad across three years (2004-2007)

Kroonstad was the only locality that had enough data for analysis across three consecutive years. Trials planted at the rest of the localities either failed due to the occurrence of Sclerotia disease, or localities were not planted due to unforeseen circumstances. The mean squares for environment were highly significant for oil content as well as palmitic and stearic acid contents, with environment contributing 20.45-41.19% to the total variance. The mean squares for genotype were highly significant for all five data sets. For the two major fatty acids, linoleic and oleic acid, genotype contributed 85.63% and 85.40% respectively to the total variance, while genotype contributed only 44.64% and 52.46% to the total variance for palmitic and stearic acid. Only 22.35% of the total variance was accounted for by genotype for oil content. The mean squares for GXE interaction were significant for linoleic and oleic acid contents (Table 3.31).

The standard conventional genotypes 1 and 2 yielded the highest oil contents (49.54% and 50.47% respectively). However, genotype 2 was not significantly better than six of the other genotypes (Table 3.32). The two standard linoleic genotypes yielded the highest linoleic acid contents (57.12% and 54.98% respectively) and these were significantly better than the rest of the genotypes. These also yielded the lowest oleic acid contents (31.47% and 33.4% respectively) of all genotypes as expected for traditional sunflower genotypes. High oleic genotypes 3, 6 and 14 ranked in the top three positions for oleic acid content. These genotypes were considered high oleic potential genotypes with oleic acid contents of 77.25%, 76.75% and 80.24% respectively and low linoleic acid contents of less than 13%. The rest of the high oleic genotypes (4, 5, 7 and 9) were considered low oleic potential genotypes with oleic acid contents that ranged between 59.34-73.42%. The linoleic acid contents of these genotypes ranged between 16.80-28.98%.

The standard linoleic genotypes 1 and 2 ranked first and second and yielded palmitic acid contents of 5.31% and 4.91% respectively. The high oleic genotypes contained on average the lowest palmitic acid contents, while the standard linoleic genotypes contained on average the highest palmitic acid content. Significant differences were observed among some of the genotypes for palmitic and stearic acid content. Genotype 4 ranked first for stearic acid content (5.65%) and was followed by genotype 3 (5.51%). On average, the high oleic genotypes had the highest stearic acid contents and were followed by the standard linoleic and mid oleic genotypes.

Table 3.31 Mean squares of fatty acid contents for Kroonstad across three years

Source	Oil content		Linoleic acid		Oleic acid		Palmitic acid		Stearic acid	
	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation	MS	% Variation
Environment	427.93**	41.19	1.76	0.01	1.82	0.01	7.12**	27.66	4.53**	20.45
Rep in Environment	11.68	3.37	28.88	0.62	31.78	0.66	0.21	2.43	0.16	2.22
Genotype	35.72**	22.35	1832.24**	85.63	1909.17**	85.40	1.77**	44.64	1.79**	52.64
GXE	5.46	6.84	55.66*	5.20	59.63*	5.33	0.15	7.43	0.14	8.47
Residual	6.99	26.25	30.42	8.53	32.03	8.60	0.12	17.85	0.09	16.20
CV (%)	5.55		20.14		9.18		7.93		6.23	

*P≤0.05, **P≤0.01, MS: Mean squares, Rep: Replication, GXE: Genotype by environment interaction, CV: Coefficient of variation.

Table 3.32 Mean values and rankings of fatty acid contents for Kroonstad across three years

Genotype	Oil Content		Linoleic acid		Oleic acid		Palmitic acid		Stearic acid	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	49.54	2	57.12	1	31.47	14	5.31	1	4.60	11
2	50.47	1	54.98	2	33.47	13	4.91	2	5.08	5
3	48.41	7	11.67	13	77.25	2	3.75	13	5.51	2
4	46.43	11	28.98	6	59.34	9	4.27	8	5.65	1
5	43.36	14	16.80	11	73.42	4	3.81	12	4.28	13
6	47.63	9	12.32	12	76.75	3	4.06	11	4.90	6
7	48.42	6	23.63	9	64.78	6	4.46	4	5.37	3
9	44.84	13	21.93	10	67.04	5	4.23	10	5.23	4
10	47.32	10	26.32	7	63.01	8	4.35	7	4.73	9
11	49.20	4	25.74	8	63.47	7	4.25	9	4.80	8
12	49.41	3	31.74	4	57.44	11	4.73	3	4.26	14
14	45.62	12	9.74	14	80.24	1	3.73	14	4.33	12
15	48.69	5	29.57	5	59.06	10	4.40	5	4.82	7
16	48.25	8	32.81	3	56.79	12	4.35	6	4.69	10
Average	47.68		27.38		61.68		4.33		4.87	
LSD (0.05)	2.48		5.18		5.31		0.32		0.28	

LSD: Least significant difference.

3.3.7 Stability analyses for genotype performance

3.3.7.1 Additive main effects and multiplicative interaction analysis for one year over six locations (season of 2004/2005)

Oil content

The AMMI analysis of oil content revealed that the IPCA axis 1 and IPCA axis 2 explained 40.52% and 24.37% of the GXE interaction respectively (Table 3.33). Environments and GXE interaction showed highly significant variation for oil content (Table 3.34). The standard linoleic genotypes 1 and 2 as well as the high oleic genotypes 4, 5, 8 and 9 were the most stable and widely adapted genotypes for oil content across all six locations (Figure 3.1). Genotypes are stable when their IPCA 1 scores are close to zero. However, the high oleic genotypes 6 and 14 as well as the mid oleic genotypes 13 and 15 were relatively stable across environments. From the stable genotypes, only the standard genotype 1, the high oleic genotype 4 and the mid oleic genotype 15 had average or above average oil content. Genotype 11 had the highest oil content in the combined ANOVA (Table 3.16), but was not significantly better than genotypes 2, 4, 7, 10, 12, 15 and 16.

Settlers, Lichtenburg and Klerksdorp showed similar interactions and were the most stable environments for oil content. However, only Settlers and Standerton occurred in the high potential area of the biplot. Kroonstad and Standerton showed similar interactions but were less stable locations. Delmas was an average potential location, but was a highly unstable for oil content. As a result, Delmas may have a high probability for GXE interaction. Klerksdorp, Lichtenburg and Kroonstad occurred in the low potential area of the biplot and were less favourable environments for oil content.

Table 3.33 Contribution of IPCA 1 and IPCA 2 to the total variation for GXE interaction for one year (2004/2005) over six locations

Trait	% GXE explained		Cumulative %
	IPCA 1	IPCA 2	
Oil content	40.52	24.37	64.89
Linoleic acid	48.90	22.18	71.07
Oleic acid	49.45	22.87	72.32
Palmitic acid	46.63	25.58	72.21
Stearic acid	62.97	28.53	91.50

IPCA: Interactive principle component analysis, GXE: Genotype by environment interaction.

Table 3.34 Mean squares of oil and fatty acid contents for the AMMIs for six locations for season 2004/2005

Source	Mean squares				
	Oil content	Linoleic acid	Oleic acid	Palmitic acid	Stearic acid
Environments	570.07**	162.03**	282.08**	2.53**	31.41**
Reps in environments	10.43	7.28	14.49	0.38	1.07
Genotype	16.03	4857.48**	5191.68**	6.33**	3.46**
GXE	9.56**	25.19**	27.44**	0.18**	0.48**
IPCA 1	15.29**	50.55**	53.57**	0.32**	1.19**
Residual	5.47	11.62	13.36	0.10	0.12
CV (%)	4.62	12.01	6.09	7.72	6.62

$^{**}P \leq 0.01$, AMMI: Additive main effects and multiplicative interaction, Reps: Replications, GXE: Genotype by environment interaction, IPCA: Interactive principle component analysis, CV: Coefficient of variation.

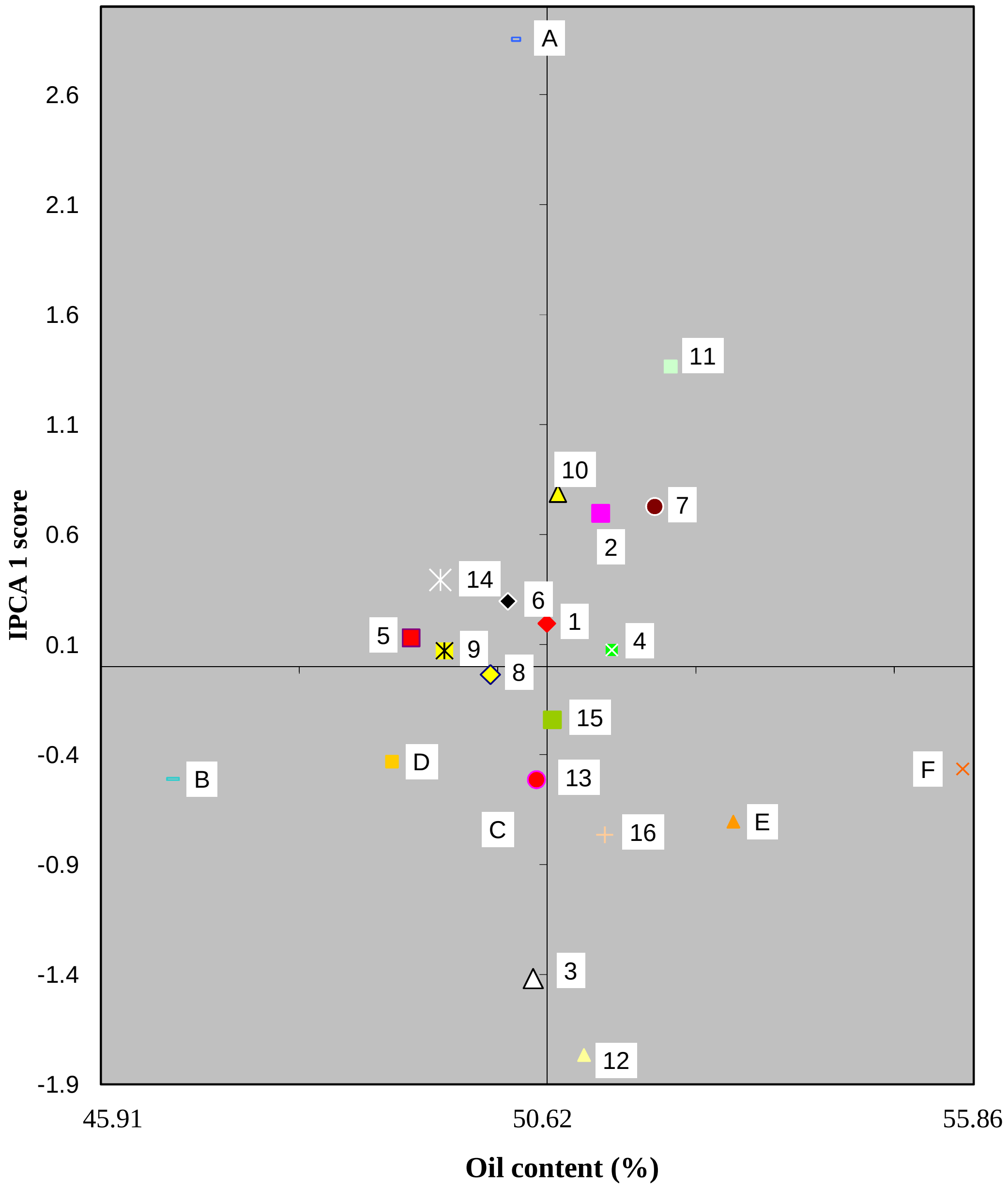

Figure 3.1 AMMI biplot-1 for the 2004/2005 season for oil content showing means of genotypes (1-16) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). A: Delmas, B: Klerksdorp, C: Kroonstad, D: Lichtenburg, E: Standerton, F: Settlers.

Linoleic acid

The IPCA axis 1 and IPCA axis 2 explained 48.90% and 22.18% of the GXE interaction respectively (Table 3.33). Genotypes and environments showed highly significant variation for linoleic acid content (Table 3.34). Additionally, highly significant GXE interaction was observed. The standard linoleic genotypes 1 and 2 were close in relation to each other, indicating similar genetic backgrounds (Figure 3.2). They had the highest linoleic acid percentages, significantly higher than the remaining 14 genotypes (Table 3.16) as expected for the traditional sunflower genotypes. However, genotype 1 was more stable and widely adapted than genotype 2 across all six environments. The high oleic genotype 6 and the mid oleic genotypes 12 and 13 were relatively stable genotypes for linoleic acid content. From these, genotype 6 had a low linoleic acid (as expected for the high oleic genotypes) whereas the mid oleic genotypes had above average linoleic acid contents. The rest of the genotypes showed variation for linoleic acid and were unstable across environments.

Klerksdorp was the most stable location and was a favourable environment for linoleic acid content. Delmas and Standerton were average potential locations, but unstable environments for linoleic acid percentage. Kroonstad was relatively stable, but unfavourable. Lichtenburg and Settlers showed similar interactions, but was relatively unstable locations. Genotype 7 was specifically adapted to Delmas.

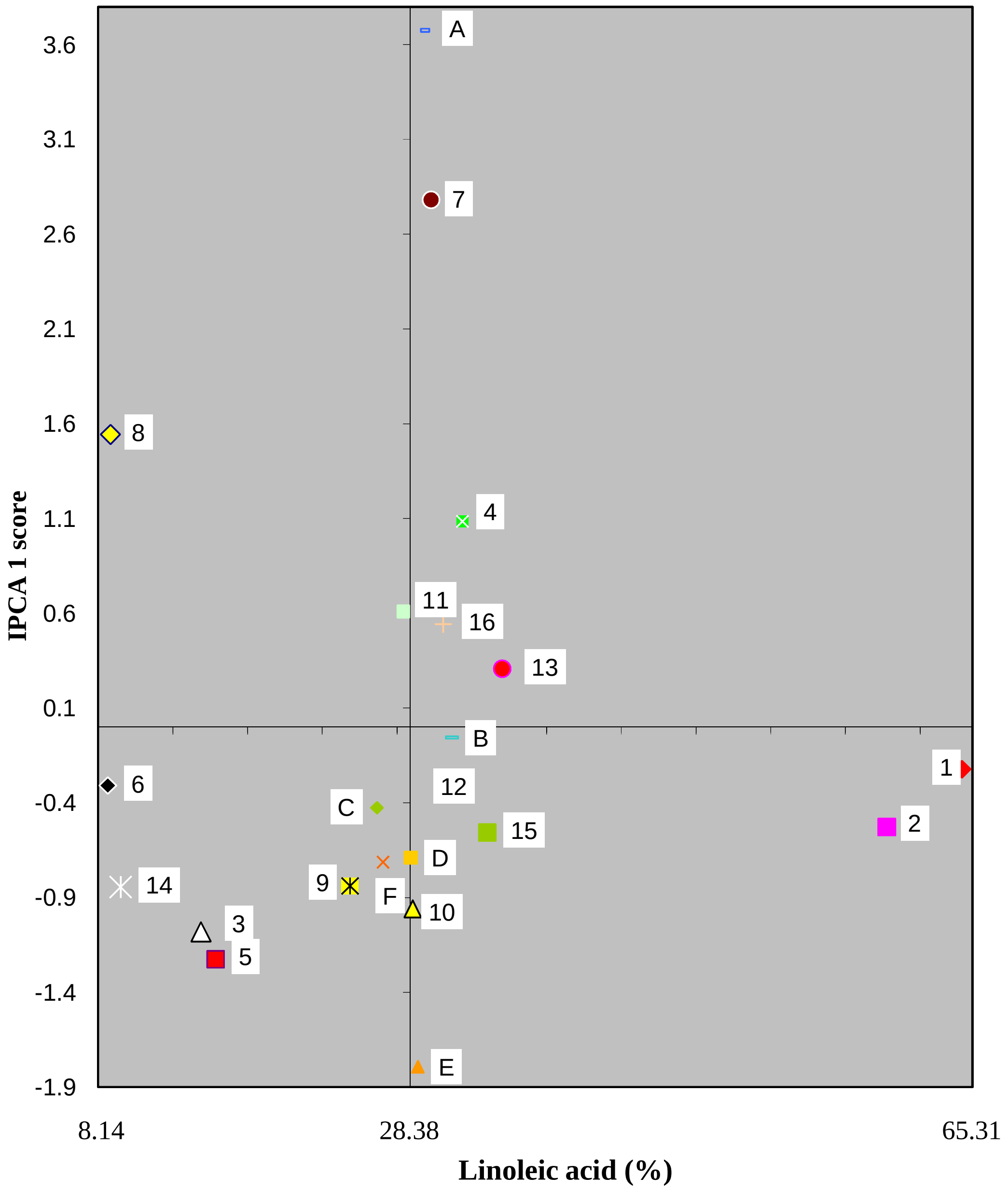

Figure 3.2 **AMMI biplot-1 for the 2004/2005 season for linoleic acid content showing means of genotypes (1-16) and environments plotted against their respective scores of the first interaction principle component (IPCA-1).** A: Delmas, B: Klerksdorp, C: Kroonstad, D: Lichtenburg, E: Standerton, F: Settlers.

Oleic acid

The IPCA axis 1 and IPCA axis 2 explained 49.45% and 22.87% of the GXE interaction respectively (Table 3.33). Genotypes and environments showed highly significant variation for oleic acid content (Table 3.34). Additionally, highly significant GXE interaction was observed. The standard linoleic genotypes 1 and 2 were closely grouped as expected for these two genotypes. They contained the lowest oleic acid contents because of the negative correlation between oleic and linoleic content. From these, genotype 1 was more stable than genotype 2 for oleic acid content and showed good adaptability across the six environments (Figure 3.3). As observed for linoleic acid content, the high oleic genotype 6 and the mid oleic genotypes 12 and 13 have shown relatively good stability for oleic acid content. The rest of the genotypes showed variation for oleic acid content and were unstable across the six environments.

Klerksdorp and Kroonstad were the most stable environments relating to oleic acid content, however, only Kroonstad occurred in the high potential area of the biplot and this would be the location to select from. Delmas, Lichtenburg and Standerton were unstable locations for oleic acid content but, they showed average potentials for oleic acid content. Settlers was a more favourable environment, but was an unstable location for oleic acid content.

Palmitic acid

The IPCA axis 1 and IPCA axis 2 explained 46.63% and 22.58% of the GXE interaction respectively (Table 3.33). Genotypes and environments showed highly significant variation for palmitic acid content (Table 3.35). Highly significant GXE interaction was also observed. The standard linoleic genotypes 1 and 2 grouped in the high potential area of the biplot (Figure 3.4). These two genotypes had above average palmitic acid contents. Genotype 2 was stable and widely adapted across all six locations for palmitic acid content, but genotype 1 was unstable. The high oleic genotypes 8 and 9 were the most stable and widely adapted, followed by genotypes 5, 6 and 14. The rest of the high oleic genotypes (3, 4 and 7) were unstable. Except for genotype 4, all high oleic genotypes had below average palmitic acid contents. The mid oleic genotypes grouped together and showed more or less average palmitic acid contents. From these, genotypes 10, 11 and 12 were the most stable mid oleic genotypes for palmitic acid content.

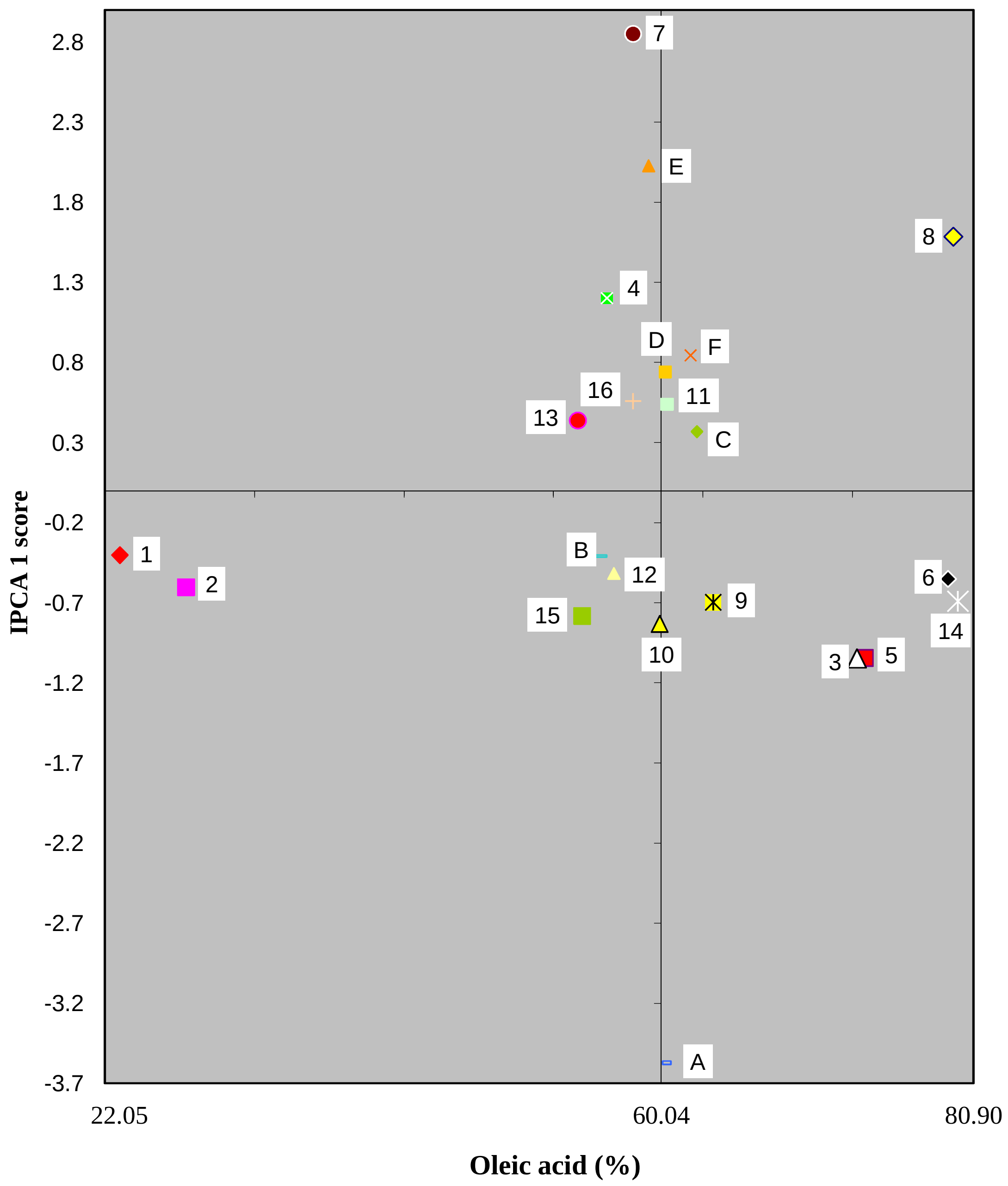

Figure 3.3 AMMI biplot-1 for the 2004/2005 season for oleic acid content showing means of genotypes (1-16) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). A: Delmas, B: Klerksdorp, C: Kroonstad, D: Lichtenburg, E: Standerton, F: Settlers.

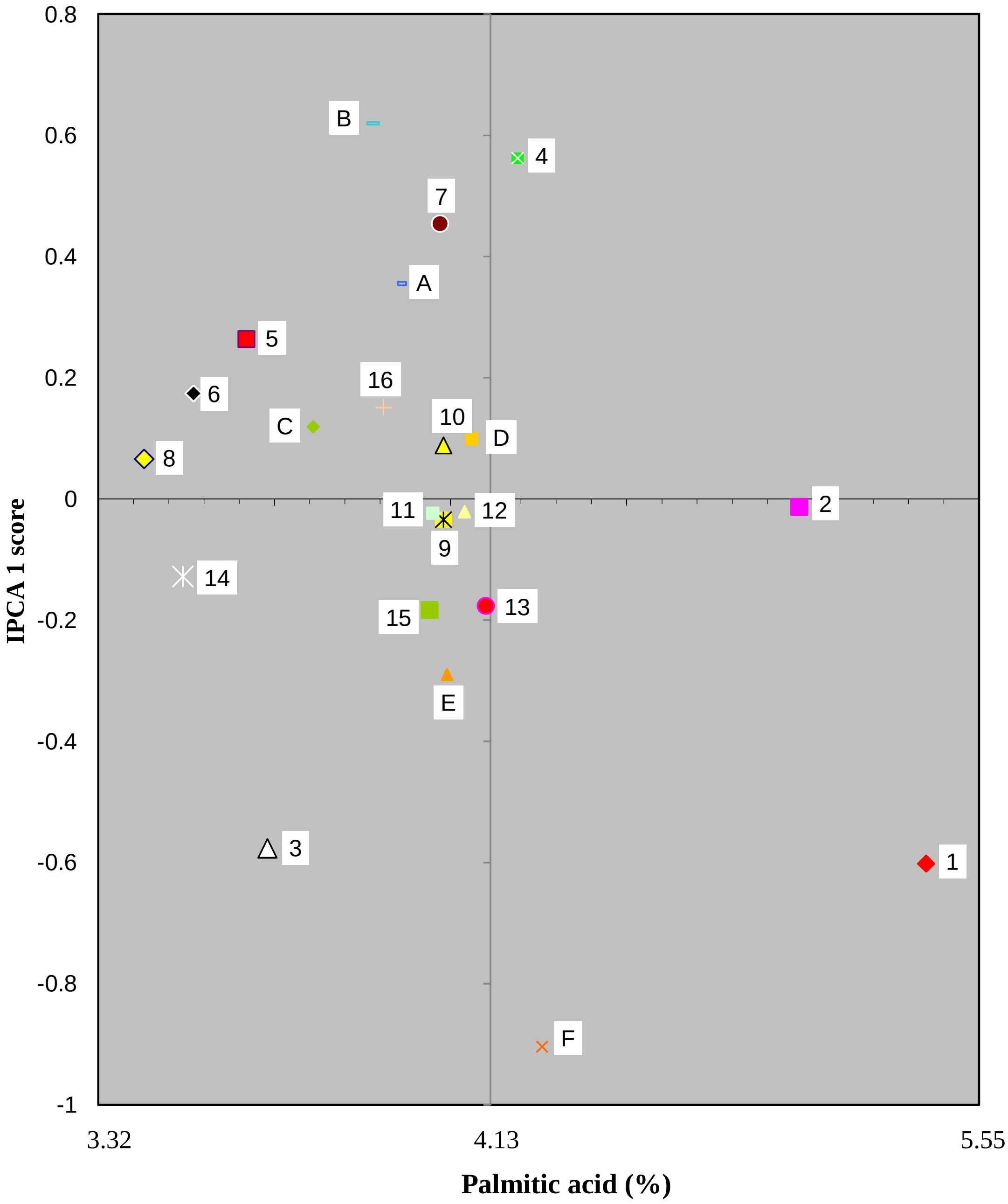

Figure 3.4 AMMI biplot-1 for the 2004/2005 season for palmitic acid content showing means of genotypes (1-16) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). A: Delmas, B: Klerksdorp, C: Kroonstad, D: Lichtenburg, E: Standerton, F: Settlers.

Klerksdorp and Settlers were the most unstable environments for palmitic acid content. However, from these Settlers was a more favourable environment for palmitic acid content. Kroonstad and Lichtenburg showed similar interaction and these two locations were the most stable. However, Lichtenburg was a more favourable environment for palmitic acid content, while Kroonstad was a less favourable environment.

Stearic acid

The IPCA axis 1 and IPCA axis 2 explained 62.97% and 28.53% of the GXE interaction respectively (Table 3.33). Genotypes and environments showed highly significant variation for stearic acid content (Table 3.34). Additionally, highly significant GXE interaction was observed. The high oleic genotypes 6 and 9 as well as the mid oleic genotypes 10 and 15 were the most stable and widely adapted genotypes for stearic acid content across all six environments analysed (Figure 3.5). However, from these only genotypes 9 and 15 had above average stearic acid contents. The rest of the genotypes were unstable for stearic acid content.

Kroonstad and Standerton were the most stable locations for stearic acid content. However, Kroonstad was a less favourable environment for stearic acid content. Klerksdorp was the most favourable location for stearic acid content, but it was highly unstable. Lichtenburg was a relatively stable location, but a less favourable environment for stearic acid content. Delmas and Settlers occurred in the low potential are of the biplot and were unstable locations for stearic acid content.

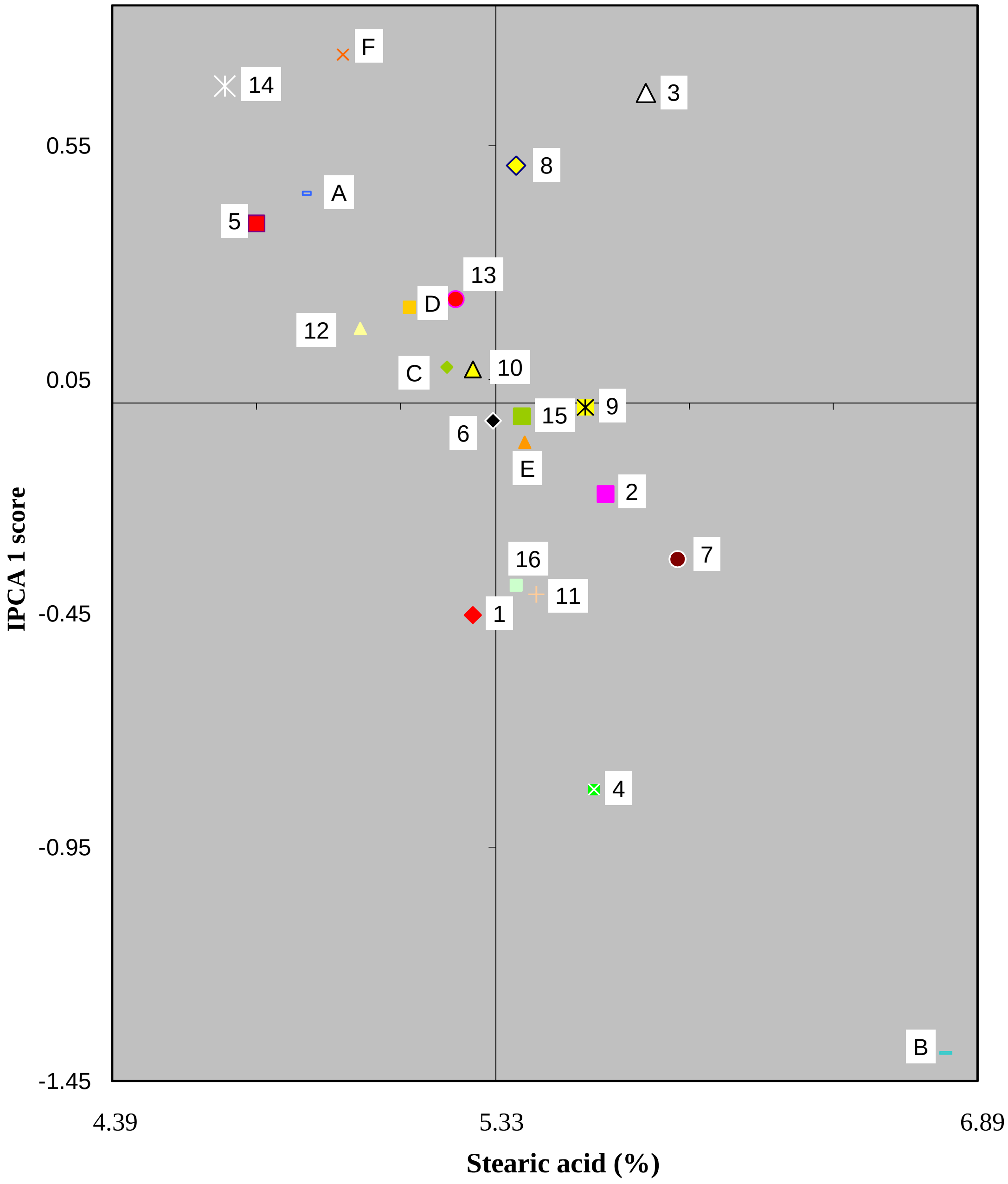

Figure 3.5 AMMI biplot-1 for the 2004/2005 season for stearic acid content showing means of genotypes (1-16) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). A: Delmas, B: Klerksdorp, C: Kroonstad, D: Lichtenburg, E: Standerton, F: Settlers.

3.3.7.2 Additive main effects and multiplicative interaction analysis over three years for Kroonstad (seasons of 2004 to 2007)

Oil content

IPCA axis 1 explained 68.33% of the GXE interaction and IPCA axis 2 31.67% (Table 3.35). Genotype and environment indicated highly significant variation for oil content (Table 3.36). However, GXE interaction was non-significant for oil content across the three seasons. The standard linoleic genotypes 1 and 2, the high oleic genotypes 4, 6 and 7 and the mid oleic genotype 15 were the most stable and widely adapted genotypes for oil content across the three seasons at Kroonstad (Figure 3.6). The standard genotypes 1 and 2 had the highest oil contents across the three seasons; however, genotype 2 was not significantly better than the high oleic genotypes 3 and 7, and the mid oleic genotypes 11, 12, 15 and 16 (Table 3.32). The high oleic genotypes 5, 9 and 14 showed below average oil contents and were unstable genotypes. The high oleic genotype 10 had an average oil content, but was highly unstable.

The year effect was not consistent for Kroonstad. During the first season (2004/2005) Kroonstad was a stable and high potential location. However, during the second season (2005/2006) it was a high potential but unstable location. In the third season (2006/2007) it was a low potential and unstable location and selection of adapted genotypes for Kroonstad during that season would be inefficient. In an unstable environment, more interaction occurs among genotypes and the environment and therefore it would be difficult to select adapted genotypes.

Table 3.35 Contribution of IPCA 1 and IPCA 2 to the total variation for GXE interaction for three years (2004/2005) and one location

Trait	% GXE explained		Cumulative %
	IPCA 1	IPCA 2	
Oil content	68.33	31.67	100.00
Linoleic acid	85.54	14.46	100.00
Oleic acid	84.45	15.55	100.00
Palmitic acid	59.92	40.08	100.00
Stearic acid	62.52	37.48	100.00

IPCA: Interactive principle component analysis, GXE: Genotype by environment interaction.

Table 3.36 Mean squares of fatty acid contents for the AMMIs for Kroonstad for three seasons

Source	Mean squares				
	Oil content	Linoleic acid	Oleic acid	Palmitic acid	Stearic acid
Environments	427.93**	1.76	1.82	7.12**	4.53**
Reps within environments	11.68	28.88	31.78	0.21	0.16
Genotype	35.72**	1832.24**	1909.20**	1.77**	1.79**
GXE	5.46	55.66*	59.62*	1.25	0.14
IPCA 1	6.93	88.43**	93.50**	0.16	0.17*
IPCA 2	3.75	17.44	20.09	0.13	0.12
Residual	6.99	30.42	32.03	0.12	0.09
CV (%)	5.55	20.14	9.18	7.93	6.23

*P ≤0.05, **P≤0.01, AMMI: Additive main effects and multiplicative interaction, Reps: Replications, GXE: Genotype by environment interaction, IPCA: Interactive principle component analysis, CV: Coefficient of variation.

Linoleic acid

IPCA axis 1 explained 85.54% of the GXE interaction (Table 3.35). Highly significant variation occurred among genotypes for linoleic acid content across the three years (Table 3.36). In addition, significant GXE interaction was also observed. The standard linoleic genotypes 1 and 2 showed the highest linoleic acid contents as expected. However, these genotypes were highly unstable for linoleic acid content across the three seasons analysed at Kroonstad (Figure 3.7). The high oleic genotypes 4, 7 and 9 as well as the mid oleic genotype 11 were the most stable and widely adapted genotypes for linoleic acid content. The high oleic genotypes occurred in the low potential area of the biplot as expected for high oleic genotypes with low linoleic acid contents. The mid oleic genotypes 10, 12, 15 and 16 showed average to above average linoleic acid contents. However, these were unstable genotypes for linoleic acid content. Kroonstad was an average potential and unstable location for linoleic acid across all three seasons.

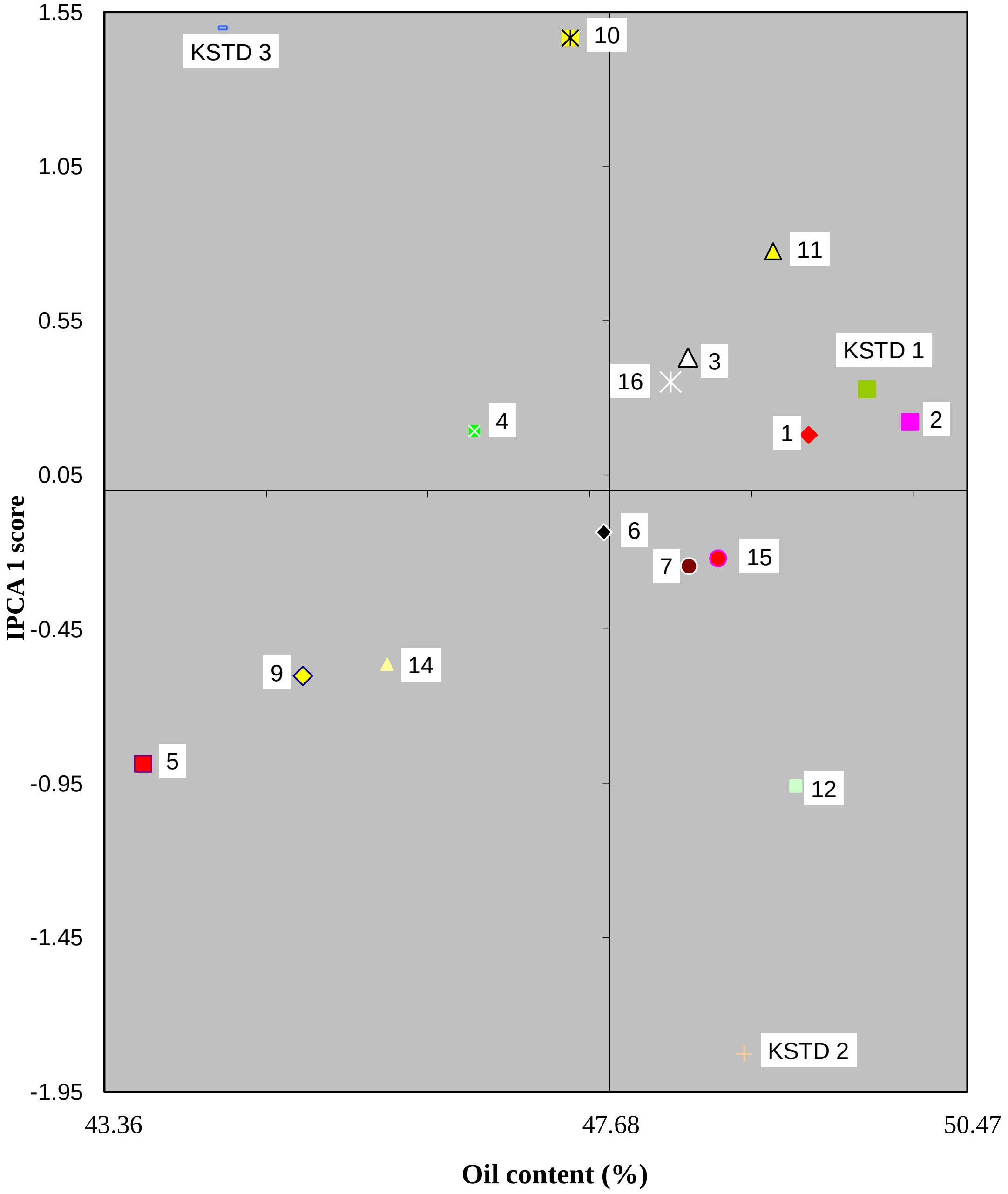

Figure 3.6 AMMI biplot-1 for seasons 2004 to 2007 for oil content showing means of genotypes (1-16, without 8 and 13) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). KSTD 1: Kroonstad season 1, KSTD 2: Kroonstad season 2, KSTD 3: Kroonstad season 3.

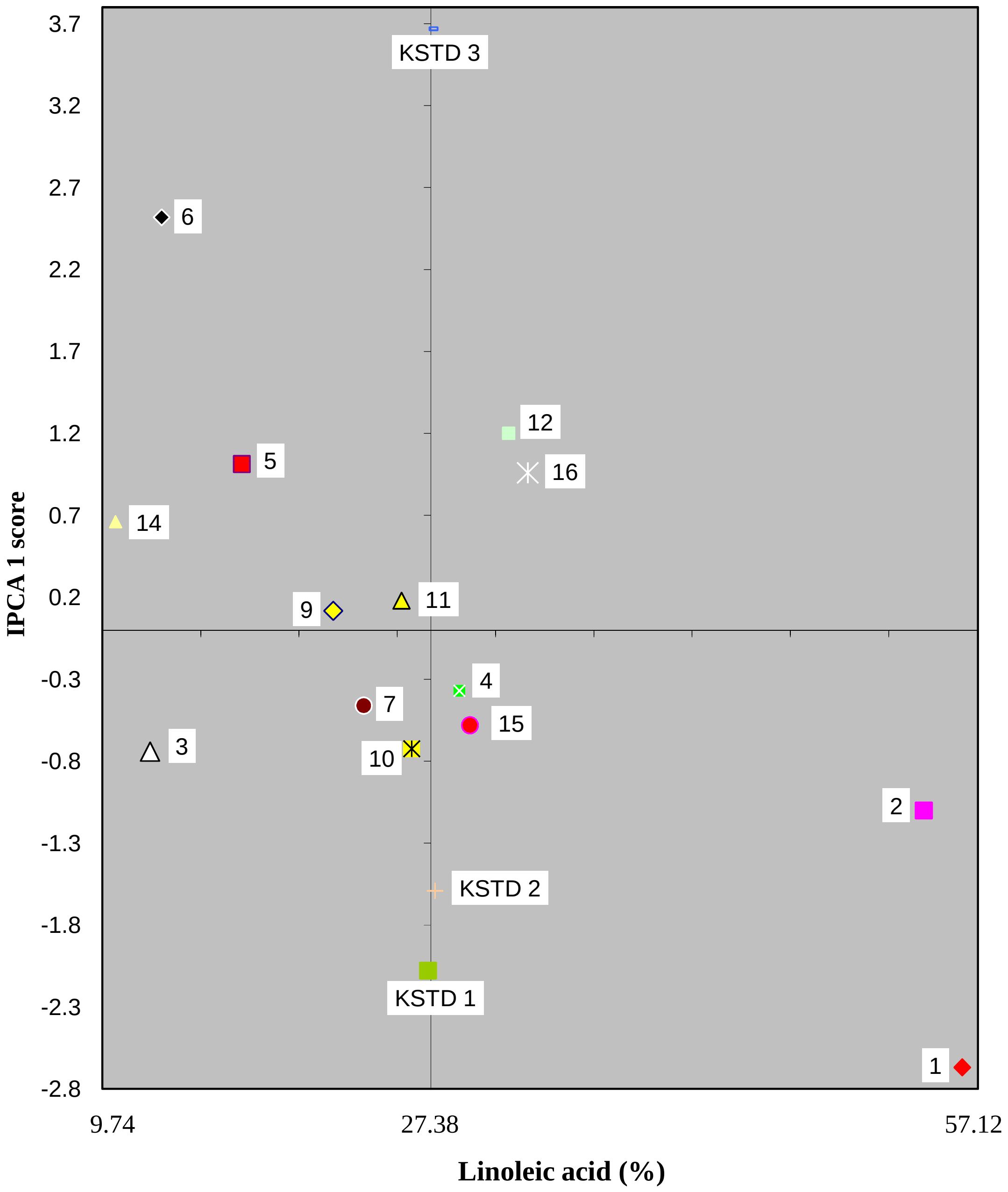

Figure 3.7 AMMI biplot-1 for seasons 2004 to 2007 for linoleic acid content showing means of genotypes (1-16, without 8 and 13) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). KSTD 1: Kroonstad season 1, KSTD 2: Kroonstad season 2, KSTD 3: Kroonstad season 3.

Oleic acid

IPCA axis 1 explained 84.45% of the GXE interaction (Table 3.35). Highly significant variation was observed between genotypes for oleic acid content and GXE interaction was also significant (Table 3.36). The standard linoleic genotypes 1 and 2 occurred in the low potential area of the biplot for oleic acid content (Figure 3.8). This was the result of the negative correlation between oleic and linoleic acid content. Genotype 2 showed better stability for oleic acid then genotype 1, nonetheless, both were unstable for oleic acid content. These two genotypes showed the same instability for oleic acid content as observed for linoleic acid content. The high oleic genotypes 4, 7, and 9 as well as the mid oleic genotype 11 were the most stable and adapted genotypes for oleic acid content across the three seasons at Kroonstad. This observation was in correlation with that observed for linoleic acid content. The high oleic genotypes 3, 5, 6 and 14 occurred in the high potential area of the biplot as expected for high oleic genotypes. However, these genotypes were unstable for oleic acid content. The mid oleic genotypes 10, 12, 15 and 16 had more or less average oleic acid contents. These were also unstable for oleic acid content. This observation was in correlation with that observed for linoleic acid content. Kroonstad was an average potential and unstable location for oleic acid percentage across the three seasons.

Palmitic acid

IPCA axis 1 explained 59.92% of the GXE interaction and IPCA axis 2 40.08% (Table 3.35). Genotype and environment showed highly significant variation for palmitic acid content (Table 3.36). However, GXE interaction was non-significant. The standard linoleic genotypes 1 and 2 occurred in the high potential area of the biplot and had the highest palmitic acid contents across all three seasons at Kroonstad (Figure 3.9). However, these genotypes were unstable for palmitic acid content. The high oleic genotypes 3, 5 and 7 as well as the mid oleic genotypes 10 and 15 were the most stable and widely adapted genotypes for palmitic acid content. From these, genotypes 7, 10 and 15 had above average palmitic acid percentages. Except for genotype 7, all high oleic genotypes had below average palmitic acid contents. The mid oleic genotypes 9, 11 and 16 had more or less average palmitic acid contents and these, together with genotype 12 that occurred in high potential area of the biplot, were unstable genotypes for palmitic acid content.

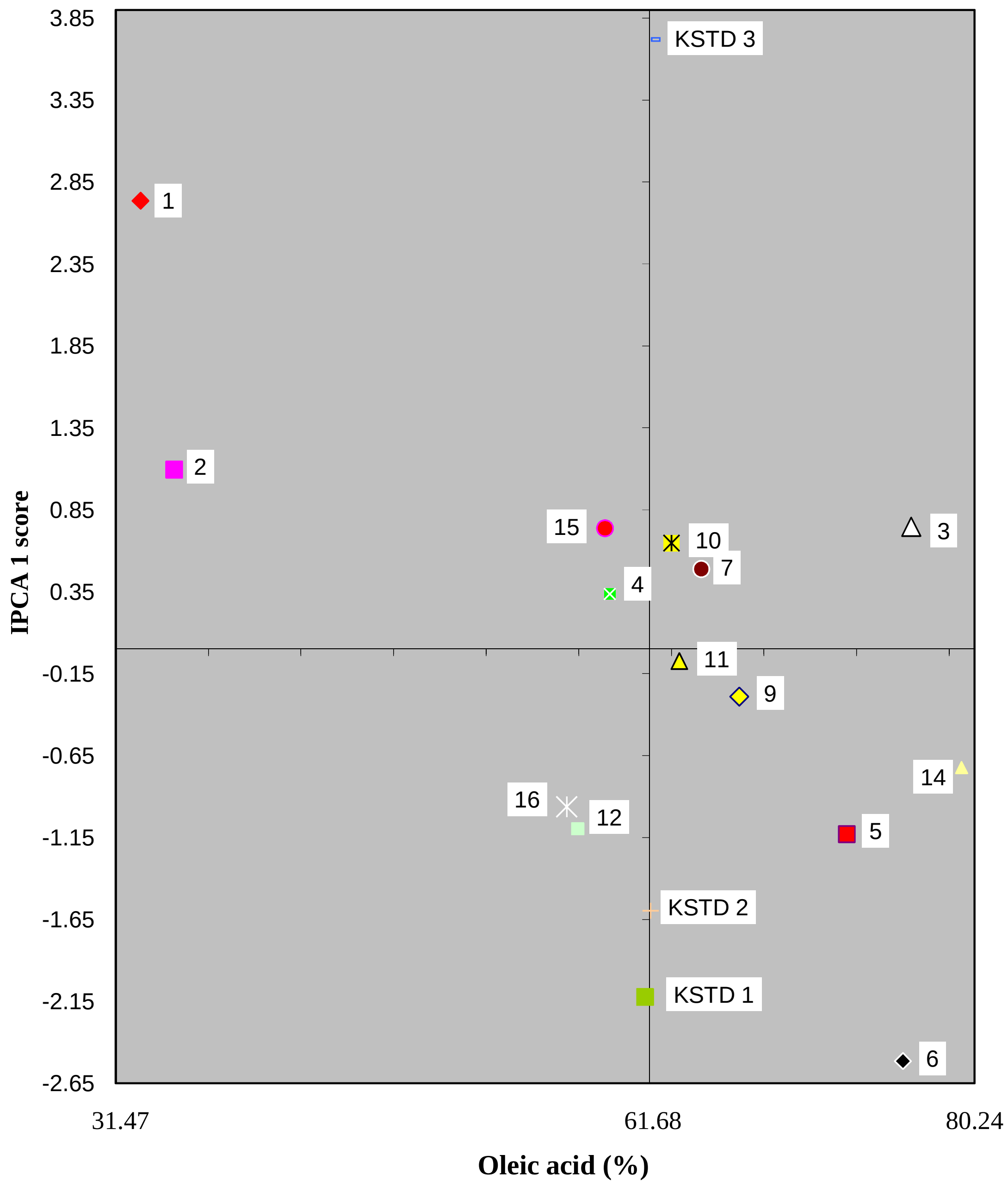

Figure 3.8 AMMI biplot-1 for seasons 2004 to 2007 for oleic acid content showing means of genotypes (1-16, without 8 and 13) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). KSTD 1: Kroonstad season 1, KSTD 2: Kroonstad season 2, KSTD 3: Kroonstad season 3.

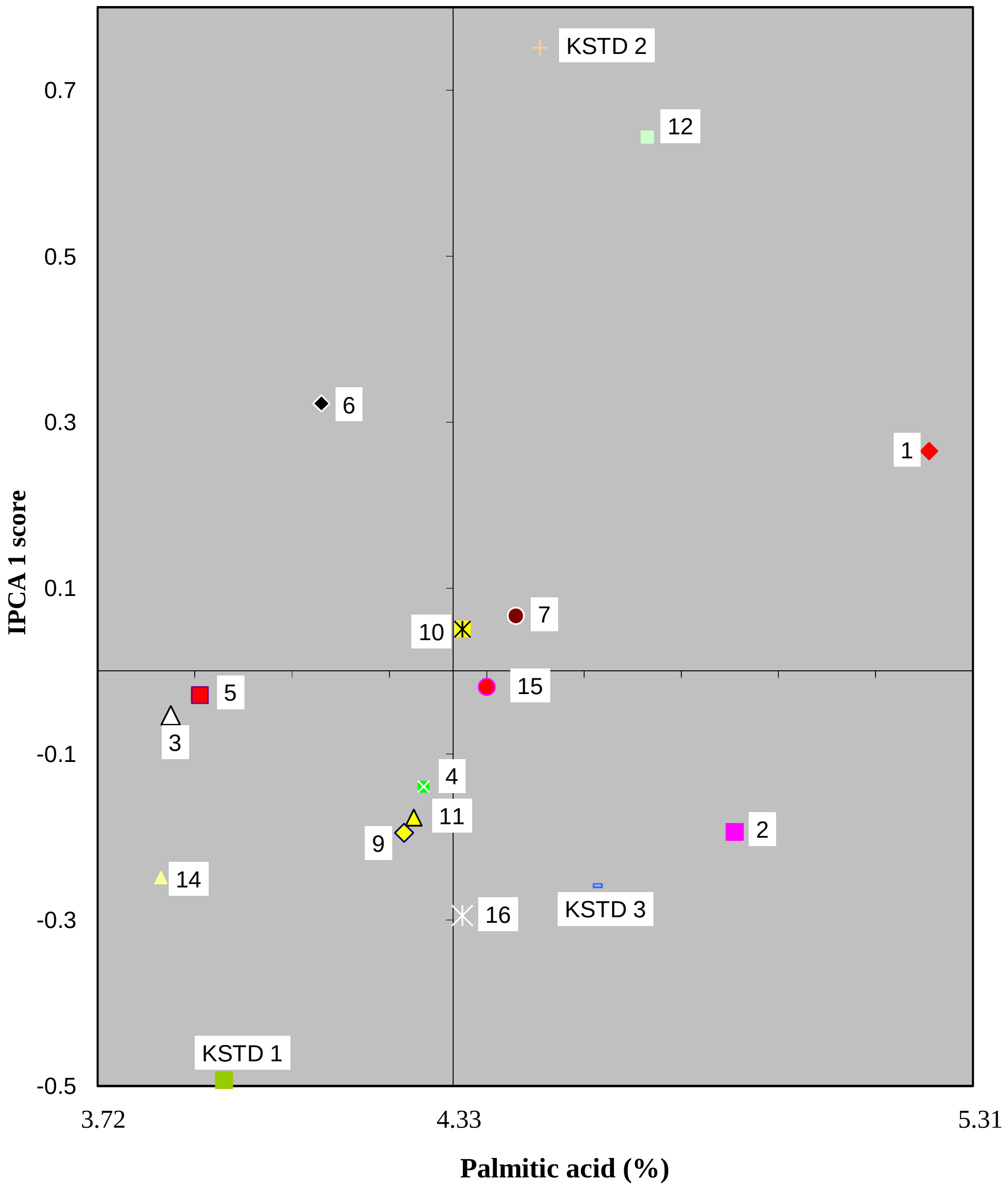

Figure 3.9 AMMI biplot-1 for seasons 2004 to 2007 for palmitic acid content showing means of genotypes (1-16, without 8 and 13) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). KSTD 1: Kroonstad season 1, KSTD 2: Kroonstad season 2, KSTD 3: Kroonstad season 3.

During the first season (2004/2005), Kroonstad was a less favourable environment for palmitic acid content. But, in both the second (2005/2006) and third (2006/2007) seasons, Kroonstad was more favourable for palmitic acid content. In general Kroonstad was an unstable location for palmitic acid content across all three seasons.

Stearic acid

IPCA axis 1 explained 62.52% of the GXE interaction and IPCA axis 2 37.48% (Table 3.35). Genotype and environment showed highly significant variation for stearic acid content (Table 3.36). However, GXE interaction was non-significant. The standard linoleic genotypes 1 and 2 showed good stability and adaptability across the three seasons at Kroonstad (Figure 3.10). However, genotype 1 had below average stearic acid content, while genotype 2 had above average stearic acid content. The high oleic genotypes 3, 5 and 14 were stable genotypes across the three seasons. However, from these only genotype 3 had above average stearic acid content. The high oleic genotypes 4, 6, 7 and 9 had above average stearic acid contents, but were unstable genotypes. Genotype 4 had the highest stearic acid content of all genotypes. The mid oleic genotypes 10, 12 and 15 were relatively stable, but genotypes 9, 11 and 16 were unstable. From all mid oleic genotypes, only genotype 9 had above average stearic acid content.

In the first season (2004/2005) Kroonstad was a highly favourable environment for stearic acid content and was a relatively stable location. However, in the second season (2005/2006) Kroonstad was a less favourable environment and a highly unstable location for stearic acid content. In the third season (2006/2007) Kroonstad was again a more favourable environment for stearic acid content, but was a highly unstable location.

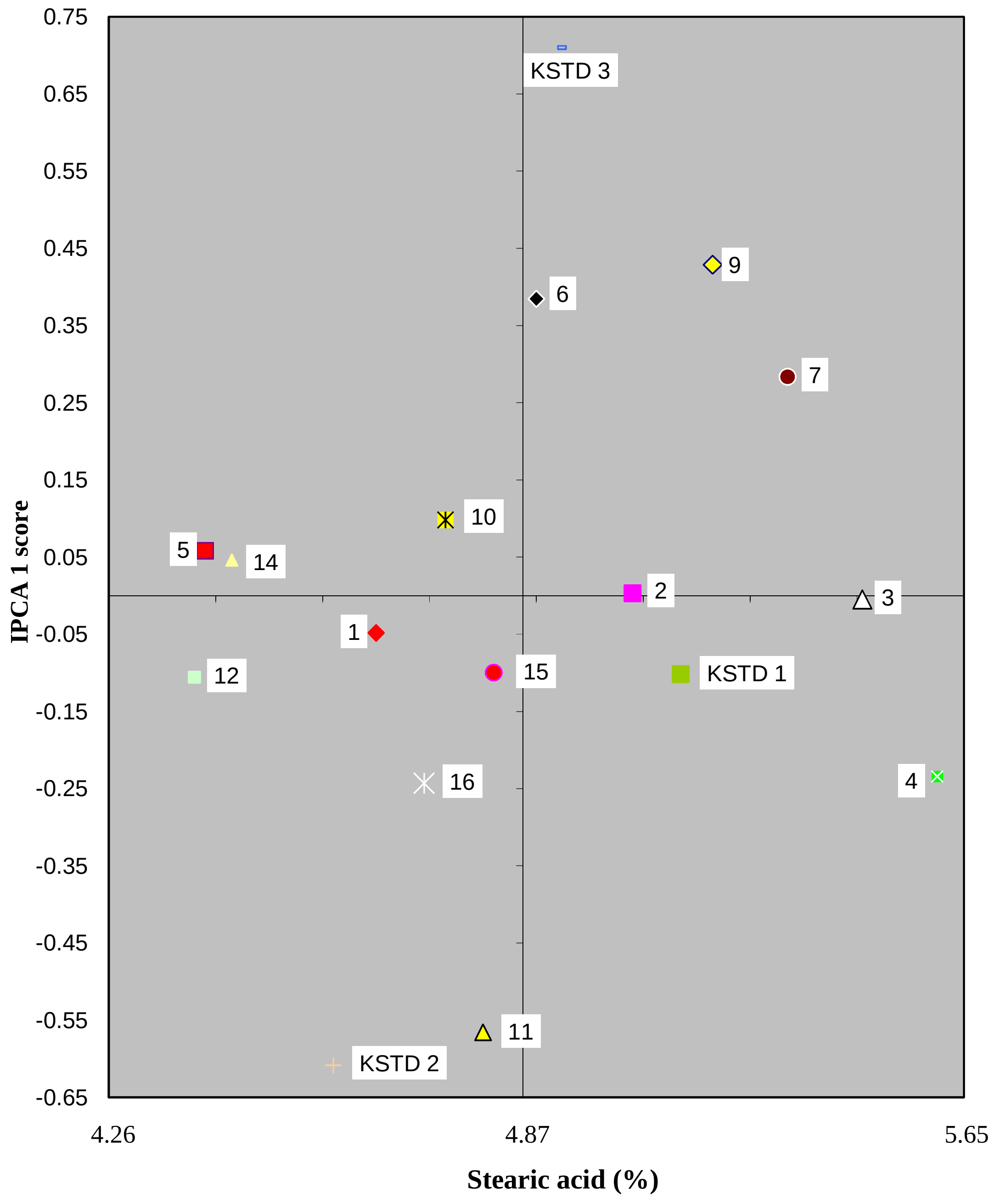

Figure 3.10 AMMI biplot-1 for seasons 2004 to 2007 for stearic acid content showing means of genotypes (1-16, without 8 and 13) and environments plotted against their respective scores of the first interaction principle component (IPCA-1). KSTD 1: Kroonstad season 1, KSTD 2: Kroonstad season 2, KSTD 3: Kroonstad season 3.

3.4 Discussion

High and mid oleic acid sunflower hybrids, together with two traditional sunflower hybrids, were planted in field trials and these were analysed for variance across locations and years. Significant differences were observed between measured traits for the high and mid oleic, and traditional sunflower genotypes. Additionally, genotypes responded differently between locations and years and environmental conditions had a large influence on the performance of genotypes. Significant GXE interactions were observed and consequently further multivariate analysis was done in order to determine the stability and adaptability of genotypes across locations and years for oil and fatty acid content.

An almost linear association between sunflower seed yield and rainfall was observed. Generally, sunflower seed and oil yield decreased in locations and years with lower rainfall. This was in agreement with findings in literature (Göksoy *et al*., 2004; Weiss 2000). In the first season, Standerton, Delmas and Kroonstad received fairly high rainfalls that resulted in the high mean seed and oil yields observed for these locations. However, Klerksdorp and Lichtenburg received much more rain than Kroonstad, Delmas and Standerton, but showed lower seed and oil yields than these three locations. This observation may be explained by the fact that in locations with high rainfall, low radiation (because of the cloudy weather) and/or waterlogging conditions might have occurred. Waterlogging has a direct effect on a range of physiological processes such as respiration and photosynthesis. Under waterlogging conditions the plant shuts down its stomata in order to reduce transpiration as a survival mechanism. Consequently, plant growth and seed development are restricted that may result in lower seed set and as result in lower seed and oil yields. Grassini *et al*. (2007) reported that higher than normal rainfall during seed-filling resulted in reductions of sunflower grain yield. Therefore, in Klerksdorp and Lichtenburg, high rainfall might have caused restriction in growth and flower development and consequently lower seed set. This might have caused lower seed and oil yields.

In the second and third seasons, the lower seed and oil yield observed for Kroonstad, compared to the first season, was because of the lower rainfall Kroonstad received during these seasons. However, this association could not be applied to Bloemfontein. Although Bloemfontein received almost 300 mm rain less during the third season compared to the second season, the mean seed and oil yields remained more or less the same for the two seasons.

Genotypes used in this study were characterised as standard linoleic, mid oleic and high oleic acid genotypes according to their pedigrees and genetic backgrounds. The standard linoleic genotypes 1 and 2 performed as expected for traditional sunflower with regard to linoleic and oleic acid content. However, the high oleic genotypes showed differences in their response to oleic and linoleic acid contents among different trials. It has been previously reported that high oleic genotypes are less variable for oleic acid content than traditional sunflower genotypes (Lagravere *et al.*, 2000; Laureti and Del Gatto, 2000; Piva *et al.* 2000). However, the high oleic acid genotypes have shown large variation for linoleic and oleic acid content among genotypes and across environments. The low linoleic acid contents observed for the high oleic genotypes was a result of the negative correlation between oleic and linoleic acid content. Linoleic and oleic acid contents are complementary and the increase in one results in a more or less similar decrease of the other (Lajara *et al.*, 1990). From the eight high oleic genotypes analysed genotype 6 was the most stable genotype for oleic and linoleic acid content across locations. The other high oleic genotypes (3, 4, 5, 7 and 14) were highly unstable and showed large variation across locations for oleic and linoleic acid content. Radíc *et al.* (2008) also reported significant differences in oleic acid contents of high oleic acid hybrids among different locations (Serbia and Argentina). Salera and Baldini (1998) reported unstable oleic acid contents of high oleic acid hybrids across different environments. From the analysis at Kroonstad, across years, the high oleic genotype 9 showed good stability for oleic and linoleic acid content.

According to literature, the high oleic mutation is partially dominant and oleic acid content is under the control of the allelic pair OL/ol (Tatini, 1995). Whatever the allelic combinations might be (OL/OL, OL/ol or ol/ol), the expression of the trait is influenced by the environment (especially temperature) during seed maturation. Variations in the oleic acid content may either be a function of the allelic combination or temperature. However, Fernández-Matínez *et al.* (1993) and Triboï-Blondel *et al.* (2000) reported the presence of modifier factors (or genes) that may cause unstable expression of the *Ol* gene (Demurin and Skoric, 1996). These modifier factors come from the linoleic parent used to produce high oleic acid hybrids and cause a reduction of high oleic acid content to intermediate levels in high oleic hybrids. Lagravere *et al.* (2000) have reported that high oleic genotypes may have different oleic acid potentials and that genotypes with high oleic acid potentials are more stable than those with low oleic acid potentials. It is therefore possible that the unstable high oleic genotypes (3, 4, 5, 7 and 14), that perform as high oleic genotypes in some environments but as mid oleic genotypes in others, or over different seasons, may contain these modifier genes that are influenced by the environment genotypes are grown in.

The most important environmental effects that had an influence on the performance of genotypes for oil content and fatty acid composition were rainfall, temperature and planting date. The drier environments (less rain received) as well as the warmer environments had higher oleic acid contents than those that received more rain or that had cooler weather during the growing season. For example, Kroonstad received 203.7 mm rain during the growing season compared to the 612.3 mm rain Bloemfontein received. Therefore Kroonstad was a much drier location than Bloemfontein and this could explain the higher oleic acid content observed at Kroonstad and the higher linoleic acid content observed at Bloemfontein. Baldini *et al.* (2000; 2002) also observed an increase in oleic acid content when water stress occurred during seed-filling, while Flagella *et al.* (2002) observed a decrease in oleic acid content when irrigation was applied. However, in another report it was indicated that a severe water deficit during the seed-filling period caused a reduction in oleic acid content of 10-16% in traditional hybrids and a concomitant increase of linoleic acid content (Roche *et al.*, 2006).

In addition, different planting dates resulted in trials maturing at different temperatures that had a large influence on the fatty acid composition of genotypes. It is well known that when high temperatures occur during sunflower maturation, the seed may contain less oil and the oleic/linoleic acid ratio is higher (Piva *et al.*, 2000). Trials planted in the beginning of the season (beginning of November-middle of December) resulted in maturation of the sunflower plants during February-March when high temperatures occurred. However, later planting dates (end of January) resulted in maturation of plants when mean daily temperatures were lower. For example, in the first season, locations planted in the beginning of the season showed the highest average oleic acid content (above 60%) per locality, while locations planted later in the season (Klerksdorp and Settlers) showed the lowest average oleic acid contents (less than 60%). In addition, the high oleic genotypes generally showed higher oleic acid contents in localities planted earlier. A similar response was observed for linoleic acid content. The traditional and some of the high and mid oleic genotypes had higher linoleic acid contents in locations planted later in the season (Klerksdorp and Settlers) than in locations planted earlier (Kroonstad and Delmas). Lichtenburg was an exception. This location was planted early, but some of the traditional, high and mid oleic genotypes had higher linoleic acid contents than in Klerksdorp and Settlers. This might have been the result of the higher rainfall at Lichtenburg (638.4 mm) that was the highest rainfall of all locations. The high rainfall might have contributed to the higher linoleic acid contents observed at Lichtenburg.

Also, in the second season, Kroonstad (planted at the beginning of December) was planted earlier than Bloemfontein (end of January) and as result, genotypes planted at Kroonstad generally had higher oleic acid contents (average of 61.88%) compared to those planted at Bloemfontein (52.57%). This effect of planting date on fatty acid composition was in agreement with the findings of Unger and Thompson (1982), Flagella *et al.* (2002) and Qadir *et al.* (2006). According to these authors, early (or spring) plantings accumulated higher oleic acid content in comparison with later (autumn) plantings that accumulated higher linoleic acid contents.

It can therefore be accepted that the unstable high oleic acid genotypes that showed high oleic acid contents in some locations (but lower oleic acid content in others) may be a consequence of high temperatures occurring during maturation or less rainfall received at locations. In addition, these genotypes may contain modifying factors (genes) that make them more susceptible to environmental influences.

It was difficult to characterise specific genotypes as stable for all oil traits measured and therefore genotypes that have shown good yield and stability are discussed separately for each trait. Genotypes that have shown stability across most of the locations and seasons for oil content and that had average to above average oil contents included the standard linoleic genotype 1, the high oleic genotypes 6 and 8, and the mid oleic genotype 15. The linoleic genotype 1 has shown stability across most locations and seasons for linoleic acid content and had a high linoleic acid content. The high oleic genotypes that have shown stability across most locations and seasons for oleic acid content and had high oleic acid potentials (oleic acid contents of above 75%) included the high oleic genotypes 6, 8 and 14.

The high oleic genotypes 5 and 9 have shown stability across most locations and seasons for palmitic acid content and had average to low palmitic acid contents. A negative association between oleic and palmitic acid content was observed and this indicated that the higher the oleic acid content, the lower the palmitic acid content. This association appeared to be genetic and not related to environmental influences. This negative correlation in high oleic genotypes is generally observed in literature (Martín-Polvillo *et al.*, 2004; Márquez-Ruiz *et al.*, 2008; Merrill *et al.*, 2008; Codex Alimentarius Commission, 2009).

Stearic acid showed no consistent association with any of the other fatty acids. This was not in agreement with the report of Lajara *et al.* (1990). These authors observed an inverse correlation between oleic and stearic acid content in traditional sunflower. Genotypes that have shown stability across most of the locations and seasons for stearic acid content and that had average to above average stearic acid contents included the high oleic genotype 9 and the mid oleic genotype 10.

Across locations in the first season, all locations were unstable for oil content and only Standerton and Settlers were favourable environments for oil content. Kroonstad has shown stability for all fatty acid traits measured. However, Kroonstad was a more favourable environment for oleic acid content and a less favourable environment for linoleic, palmitic and stearic acid content. Klerksdorp has shown stability for oleic and linoleic acid content with average potential for linoleic acid and low potential for oleic acid content. Lichtenburg was only stable for palmitic acid content. The rest of the environments were unstable for all oil traits measured. At Klerksdorp, replication showed significant variation for oil content, palmitic and stearic acid content. This might have resulted from the position of replications in the field trial. It may happen that some replications are planted at the border of a trial that is exposed to different environmental influences for example more wind, sunlight radiation and warmer temperatures. This resulted in a different response of these replications compared to others planted in the middle of the field trial.

Sunflower oil quality is dependent on the genotype and its interaction with the environment. For traditional sunflower genotypes, oil quality depends largely on the environment, which is highly variable among years, locations and sowing dates within a single year. For stable high oleic sunflower, the genotype can be the main determinant of oil composition (Flagella *et al.*, 2002). Analyses of the high oleic genotypes over several generations will permit selection of the most stable genotypes (Triboï-Blondel *et al.*, 2000). It is recommended that the unstable high oleic genotypes be selected against in the breeding programme.

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CHAPTER 4

GENETIC ANALYSIS OF SEED OIL QUALITY AND RELATED TRAITS IN SUNFLOWER EXPOSED TO HEAT STRESS DURING SEED-FILLING

4.1 Introduction

Sunflower has an optimum temperature range for normal growth and development. This specific temperature is dependent on both the genotype and growth and developmental stage of the specific genotype. When temperatures exceed the optimal range, temperature stress is caused, which interferes with the plants' performance (Škorić, 2009). High temperature stress occurring during growth and development of plants causes major yield losses in crops (Burkhanova *et al*., 2001). Heat stress induces reduction in the duration of plant growth and development that consequently leads to fewer and smaller plant organs. Reduced light perception and a shortened life cycle, in turn, influence processes like transpiration and may lead to a reduction in yield (Amutha *et al*., 2007). Additionally, high temperatures during reproductive development often negatively impact fertilisation, seed-filling and seed composition (Rondanini *et al*., 2003; Hall, 2004).

Constant high temperatures or brief periods of very high temperatures have different effects on sunflower seed growth and oil quality. Sunflower oil quality is related to seed oil content and fatty acid composition and defines the oil's value for industry (Rondanini *et al*., 2003). Constant high temperatures during grain growth modify oil content and fatty acid composition (Harris *et al*., 1978; Rawson *et al*., 1984; Lajara *et al*., 1990). High temperatures during the entire growing season or applied from anthesis to maturity can increase oleic acid content and decrease linoleic acid in standard sunflower cultivars (Canvin, 1965; Chunfang *et al*., 1996; Triboï-Blondel *et al*., 2000). This increase in the oleic/linoleic acid ratio (unsaturation ratio) may be caused in part by inhibition of the ODS enzyme, involved in the synthesis of linoleic acid from oleic acid during seed development. High temperatures strongly inhibit ODS enzyme activity and oleic acid accumulates (Garcés and Mancha, 1991; Garcés *et al*., 1992; Sarmiento *et al*., 1998). Brief periods of heat stress during the seed-filling period in sunflower produced seed with lower oil content and altered fatty acid composition, thus lower commercial quality. According to Rondanini *et al*. (2003) the period from 12-19 days after anthesis (DAA) showed the greatest sensitivity to heat stress regarding grain weight responses, while the period from 19-26 DAA showed the greatest sensitivity to oil quality. The threshold temperature for seed growth responses is 35°C. With current trends toward increased global temperatures, sunflower breeders may be challenged with defining a suitable breeding strategy in environments where heat stress may occur. To achieve this, reliable information on heritability and gene effects on oil quality and related traits under brief periods of heat stress during the critical seed-filling period is necessary.

The aim of this study was to estimate genetic parameters using 12 F_1 high and mid oleic hybrids from a hierarchal cross (presented by Comstock and Robinson, 1952 as *Experiment 1*) of four male and 12 different female lines. These hybrids were evaluated under heat stress conditions for grain oil quality and related traits.

4.2 Materials and methods

4.2.1 Plant material

Seed material from 12 F_1 hybrids were obtained from the PANNAR® breeding station in Delmas, South Africa. These included mid and high oleic hybrids (Table 4.1) and were generated by a hierarchical cross of four male inbred lines with 12 different female inbred lines. Due to confidentiality reasons, names of hybrids and their pedigrees may not be revealed.

4.2.2 Glasshouse trial

The heat stress and control trials were planted in the glasshouse at the University of the Free State (Bloemfontein). The F_1 seed were sown on 19 February 2007 in polystyrene seedling trays with Hygrotech seedling mix. This method was used to promote optimal germination. Seedlings were transplanted 15 days later in 9 l black nursery planting bags. The bags were filled with Bainsvlei type red soil with fertilizer N:P:K [3:2:1 (25) + 0.5% Zn] and Curaterr® (2 ml per plant). Two trials were compiled: the first one was a control and the second the temperature treatment trial. The two trials were planted next to each other and a randomised complete block design with three replications and eight plants per replication was used for each trial.

Table 4.1 Hierarchical cross of male parents A, B, C and D with 12 different females

Male parent	Oleic type	Female parent	Oleic type	Hybrid
A	High oleic	*a*	High oleic	1
A	High oleic	*b*	Low oleic	2
A	High oleic	*c*	Low oleic	3
B	High oleic	*d*	High oleic	4
B	High oleic	*e*	High oleic	5
B	High oleic	*f*	Low oleic	6
C	Low oleic	*g*	High oleic	7
C	Low oleic	*h*	High oleic	8
C	Low oleic	*i*	High oleic	9
D	Low oleic	*j*	High oleic	10
D	Low oleic	*k*	High oleic	11
D	Low oleic	*l*	High oleic	12

Plants were irrigated using computerised drip irrigation pipe lines. Each plant received 350 ml water daily. The minimum and maximum temperature of the glasshouse was set at 18°C and 25°C respectively. Day length was set at 14 hours daylight.

4.2.3 Temperature treatment

All plants reached 50% anthesis between 22 April 2007 and 9 May 2007. Each plant was labelled at 50% anthesis. Heat treatment for each plant started at 15 DAA (when flowering of the entire sunflower head was at 50%) and plants were treated in heat cabinets for 10 consecutive days. Treatment temperatures were set at 24°C (minimum) and 36°C (maximum). The humidity was 60% and the light intensity 100%. Daylight was set at 14 hours. All plants in the cabinets were watered daily by hand. After treatment, plants were returned to the glasshouse to complete their growth cycles. All seed were harvested on 29 June 2007.

4.2.4 Sunflower head and seed traits

For the total of 576 (288 heat treated and 288 control) plants harvested, the following traits were measured for each plant:

1. Head diameter was measured in centimetre over the length of the head with a measuring tape.

2. The total number of filled (non-sterile) seeds per head was hand counted for each head.
3. Twenty-five seed weight was determined in gram on a laboratory scale on 25 non-sterile seeds, randomly selected from each head.
4. The sterile area of the head was measured in centimetre over the sterile area of the head with a measuring tape.

4.2.5 Seed oil extraction and fatty acid analysis

Twenty seeds were randomly selected from the outer ring of each sunflower head. The 20 seeds for each of the eight plants per entry per replication were bulked. Lipid extraction and fatty acid analyses were done for each of the bulked seed samples according to the methods described in Chapter 3.

4.2.6 Statistical analysis and genetic parameters

ANOVA was performed for the sunflower head and seed traits as well as for the four major fatty acids using Agrobase (2005). Male and female interaction effects as well as estimates of variance components for each trait and for each temperature trial were based on the ANOVA presented in Singh and Chaudhary (1979). The following formulae were used for estimation of genetic ($\sigma^2 g$), environmental ($\sigma^2 e$) and phenotypic ($\sigma^2 p$) variances:

$$\sigma^2 g = (MS_2 - MS_1)/r$$

$$\sigma^2 e = MS_1$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

Where MS_2 and MS_1 are estimates of hybrid mean square and error mean square respectively and r is the number of replicates.

Estimates of broad sense heritability based on F_1 mean basis were calculated for each trait in both temperature treatments. It is the ratio of genotypic to phenotypic variance:

$$H^2 = \sigma^2 g / \sigma^2 p$$

4.3 Results

The oleic status of seed from the F_1 offspring was determined based on the fatty acid analysis (Table 4.2). Hybrids that contained more than 70% oleic acid were considered high oleic hybrids, while the mid oleic hybrids contained between 50%-70% oleic acid. Hybrids that contained less than 50% oleic acid were regarded as low oleic (linoleic) hybrids. In the first cross, where the high oleic male parent A was crossed with the high oleic female parent *a*, the F_1 offspring (hybrid 1) contained an intermediate level of oleic acid and was regarded a mid oleic hybrid (Tables 4.1 and 4.2). However, when this male was crossed with low oleic females *b* and *c*, the resulting hybrids (2 and 3) contained mid and low oleic acid levels respectively. When the high oleic male parent B was crossed with the high oleic female parent *d*, the resulting hybrid 4 contained a high oleic acid level and was therefore regarded a high oleic hybrid. Male B crossed with high oleic females *e* and *f* produced hybrids 5 and 6 with intermediate levels of oleic acid. Crosses made between the low oleic male parent C with three different high oleic acid females (*g*, *h* and *i*) all resulted in hybrids (7, 8 and 9) containing low levels of oleic acid. In crosses where the low oleic male parent D was crossed with three different high oleic females (*j*, *k* and *l*), one hybrid (11) with an intermediate oleic acid level and two hybrids (10 and 12) with low oleic acid levels resulted.

Table 4.2 Oleic acid types of the F_1 offspring from the hierarchal cross between the male and female inbred lines

Entry	Oleic type
1	Mid oleic
2	Mid oleic
3	Low oleic
4	High oleic
5	Mid oleic
6	Mid oleic
7	Low oleic
8	Low oleic
9	Low oleic
10	Low oleic
11	Mid oleic
12	Low oleic

4.3.1 Analysis of variance for sunflower head and seed traits

Head diameter

Highly significant differences were observed between replications and treatments for head diameter (Table 4.3). No significant differences were observed between hybrids. Significant differences were observed between treatments and the heat treatment caused a significant average increase in head diameter. The interaction between hybrids and treatments were not significant and this indicated that all hybrids responded similarly to the heat stress treatment.

Total number of filled seeds per sunflower head

Significant differences were observed between replications for number of filled seeds per head. Additionally, highly significant differences were observed between hybrids. This indicated that selection can be made for improved number of filled seeds per head. No significant differences were observed between treatments, however, significant differences were observed for interactions of hybrids with treatments. This indicated that some hybrids were more sensitive to the heat stress treatment (Table 4.3).

Twenty-five seed weight

Significant differences were observed between hybrids for twenty-five seed weight indicating genetic variability for this trait. Highly significant differences were observed for treatments and the heat treatment caused a significant decrease in twenty-five seed weight. No significant differences were observed for the interaction between hybrids and treatments for twenty-five seed weight, indicating that all hybrids responded similarly to the heat stress treatment (Table 4.3).

Head sterile centre diameter

Highly significant differences were observed between hybrids for head sterile area indicating genetic variability for this trait. Highly significant differences were observed for treatments with heat treatment causing a significant increase in sterile centre diameter. This led to a smaller, non-sterile area of the head and as a result, less filled seeds.

Table 4.3 Mean squares of sunflower head traits, seed traits and major fatty acid composition for 12 sunflower F_1 hybrids analysed at two temperature treatments

	Head and seed traits					Oil major fatty acids			
Source	**Head diameter**	**Number of seeds**	**25-seed weight**	**Head sterile centre diameter**	**Oil content**	**C16:0**	**C18:0**	**C18:1**	**C18:2**
Replications	7.25**	22614.23*	0.10	0.78**	25.04	0.05	0.33	63.90	65.80
Hybrids	2.87	34325.01**	0.09*	0.77**	21.23*	2.60**	4.95**	1203.03**	1196.48**
Treatments	26.22**	8236.28	0.34**	5.94**	667.83**	16.87**	42.72**	2355.03**	3738.26**
Hybrids x treatments	2.74	12910.76*	0.07	0.18	14.67	0.54	0.28	101.29**	94.88**
Residual	1.37	4974.98	0.03	0.12	9.43	0.29	0.29	22.79	22.95

*P≤0.05, **P≤0.01.

No significant differences were observed for the interaction between hybrids and treatments for twenty-five seed weight indicating that all hybrids responded similarly to the heat stress treatment (Table 4.3). The sterile centres of sunflower heads could be a result of low self-fertility, since insect pollination did not occur in the glasshouse.

4.3.2 Analysis of variance for sunflower oil and major fatty acid content

Oil content

Variation among hybrids was significant for oil content (Table 4.3). Additionally, highly significant differences were observed between treatments. No significant differences were observed for the interaction between hybrids and treatments for oil content and this indicated that hybrids responded similarly to heat stress.

Palmitic (C16:0) and stearic (C18:0) acid

Highly significant differences were observed between hybrids for both palmitic and stearic acid percentage, indicating genetic variability for these fatty acids (Table 4.3). Highly significant differences occurred between treatments for both palmitic and stearic acid percentage. No significant differences were observed for the interaction between hybrids and treatments for palmitic and stearic acid content. This indicated that hybrids responded similarly to the heat stress treatment.

Oleic acid (C18:1) and linoleic acid (C18:2)

Highly significant differences were observed between hybrids and treatments for both oleic and linoleic acid percentage (Table 4.3). This is due to the negative correlation between oleic and linoleic acid. Significant interactions between hybrids and treatments indicated that both fatty acids in hybrids were highly sensitive to the heat stress treatment.

4.3.3 Mean values for sunflower head and seed traits

Head diameter

Within the control, significant differences were observed between some of the hybrids for head diameter (Table 4.4). Hybrids 1, 2 and 10 ranked in the first three positions for head diameter, but these were not significantly different from each other and from hybrids 3, 7, 8, 11 and 12. Hybrids 4, 5, 6 and 9 had significantly smaller head sizes than hybrids 1, 2, and 10. No significant differences were observed between hybrids for head diameter in the heat treatment. The average of the heat treatment was significantly higher than that of the control value. The heat treated plants showed an average increase of 1.21 cm in head diameter (Table 4.5). All hybrids except for hybrids 1 and 10 showed an increase in head diameter after heat treatment. From these, the high oleic hybrid 4, the mid oleic hybrid 6 and the low oleic hybrid 9 showed significant increases in head diameter after heat treatment (Table 4.5).

Total number of filled seeds per head

Within the control, significant differences occurred between some of the hybrids (Table 4.6). Hybrid 10 ranked first, but it was not significantly different from hybrids 2, 7 and 8. The remaining hybrids had significantly smaller numbers of filled seed per head than hybrid 10. Significant differences were also observed between individual hybrids within the heat treatment. Hybrids 9 and 10 ranked first and second, but these were not significantly different from each other and from hybrid 8. The remaining hybrids had significantly smaller numbers of filled seeds per head than hybrids 9 and 10. The change in rankings of hybrids indicated that the hybrids responded differently to the heat stress treatment and interaction occurred between hybrids and treatments. The total number of filled seeds per head varied between 108 and 465 for hybrids for both the control and the heat treatment. In general, hybrids showed an average decrease of 21.39 seeds after heat treatment; however this decrease was non-significant (Table 4.7). All hybrids showed a decrease in filled seeds per head after heat treatment, except for hybrids 4, 8 and 9 that showed an increase. Hybrid 9 showed a significant increase in filled seeds after heat treatment (Table 4.7).

Table 4.4 Mean values and rankings of 12 sunflower F_1 hybrids for head diameter analysed at two temperature treatments

	Control			Heat treatment	
Rank	**Hybrid**	**Average***	**Rank**	**Hybrid**	**Average***
1	1	8.80a	1	6	9.26a
2	10	8.78a	2	3	9.16a
3	2	8.58a	3	8	9.14a
4	8	8.06ab	4	2	9.05a
5	3	8.04ab	5	9	8.96a
6	12	7.84ab	6	12	8.83a
7	7	7.84ab	7	4	8.56a
8	11	7.26ab	8	11	8.36a
9	9	6.43b	9	7	5.20a
10	5	6.35b	10	10	8.17a
11	4	5.53b	11	1	8.04a
12	6	5.51b	12	5	7.78a
LSD (0.05) = 1.92					

*Averages followed by the same letter in the same column did not differ significantly at P≤0.05. LSD: Least significant difference.

Table 4.5 Mean values and differences between 12 sunflower F_1 hybrids and treatments for head diameter

Hybrid	Control	Heat treatment	Difference and significance level
1	8.80	8.04	0.76
2	8.58	9.05	0.46
3	8.04	9.16	1.11
4	5.53	8.56	3.03*
5	6.35	7.78	1.43
6	5.51	9.26	3.75*
7	7.84	8.20	0.36
8	8.06	9.14	1.08
9	6.43	8.96	2.53*
10	8.78	8.18	0.60
11	7.26	8.36	1.10
12	7.84	8.83	0.99
Average	**7.42**	**8.630**	**1.21***

*P≤0.05.

Table 4.6 Mean values and rankings of 12 sunflower F_1 hybrids for total number of filled seeds per head analysed at two temperature treatments

	Control			Heat treatment	
Rank	**Hybrid**	**Average***	**Rank**	**Hybrid**	**Average***
1	10	465.16a	1	9	413.01a
2	2	324.87ab	2	10	372.70a
3	7	311.53ab	3	8	332.54ab
4	8	304.77ab	4	12	251.88b
5	11	291.94b	5	2	236.79b
6	12	287.81b	6	4	235.50b
7	3	262.23b	7	7	229.97b
8	1	248.39bc	8	11	220.72bc
9	5	242.42bc	9	3	206.36bc
10	9	208.05bcd	10	5	179.15bc
11	6	138.74cd	11	1	163.80bc
12	4	121.97d	12	6	108.79c
LSD (0.05) = 115.92					

*Averages followed by the same letter in the same column did not differ significantly at $P \leq 0.05$. LSD: Least significant difference.

Table 4.7 Mean values and differences between 12 sunflower F_1 hybrids and treatments for total number of filled seeds per sunflower head

Hybrid	Control	Heat treatment	Difference and significance level
1	248.39	163.80	84.59
2	324.87	236.79	88.08
3	262.23	206.36	55.87
4	121.97	235.50	113.53
5	242.42	179.15	63.27
6	138.74	108.79	29.95
7	311.53	229.97	81.56
8	304.77	332.54	27.77
9	208.05	413.01	204.96*
10	465.16	372.70	92.70
11	291.94	220.72	71.22
12	287.81	251.88	35.93
Average	**267.32**	**245.93**	**21.39**

*$P \leq 0.05$.

Twenty-five seed weight

Significant differences were observed between some hybrids within the control (Table 4.8). Hybrid 1 ranked first for twenty-five seed weight; however, this hybrid did not differ significantly from hybrids 2 and 3. The remaining hybrids had significantly lower twenty-five seed weights than hybrid 1. No significant differences were observed between individual hybrids of the heat treatment. The average twenty-five seed weight varied between 0.65 g to 1.39 g between hybrids for both the control and the treatment. The average of the heat treatment was significantly lower than the control. The heat treatment resulted in an average decrease of 0.13 g for twenty-five seed weight (Table 4.9). Except for hybrids 4, 6 and 9 which showed a non-significant increase in twenty-five seed weight, most of the hybrids showed a decrease. From these, hybrids 1 and 3 showed a significant reduction in twenty-five seed weight after the heat treatment.

Head sterile centre diameter

Significant differences were observed between individual hybrids of both the control and the heat treatment for head sterile centre diameter (Table 4.10). In the control treatment, hybrids 2, 8, 9 and 10 had relatively large sterile centre diameters and these did not differ significantly from each other. Hybrid 11 had the smallest sterile centre diameter, but it was not significantly different from seven other hybrids. In the heat treatment, hybrid 8 had the largest sterile centre diameter and it was not significantly different from hybrids 3, 6 and 9. In both treatments, hybrids 1, 4 and 11 had relatively small head sterile centre diameters. Large variation was observed between hybrids for head sterile centre diameter. The average sterile centre diameter varied between 1.46 cm and 2.66 cm for hybrids of the control and between 1.73 cm and 3.27 cm for the heat treatment. The heat treatment showed an average significant increase of 0.58 cm in sterile centre diameter compared to the control. All 12 hybrids showed an increase for this trait after heat treatment and from these seven were significant (Table 4.11). Hybrid 1 showed a small and insignificant increase, while hybrid 5 showed the largest increase in sterile centre diameter.

Table 4.8 Mean values and rankings of 12 sunflower F_1 hybrids for twenty-five seed weight analysed at two temperature treatments

Rank	Control Hybrid	Average*	Rank	Heat treatment Hybrid	Average*
1	1	1.39a	1	4	0.93a
2	3	1.18ab	2	2	0.93a
3	2	1.13ab	3	6	0.85a
4	7	0.99b	4	1	0.83a
5	11	0.93bc	5	11	0.79a
6	5	0.84bc	6	12	0.79a
7	10	0.83bc	7	3	0.76a
8	6	0.82bc	8	5	0.74a
9	12	0.81bc	9	7	0.72a
10	8	0.80bc	10	9	0.70a
11	4	0.70bc	11	8	0.70a
12	9	0.65c	12	10	0.69a
LSD (0.05) = 0.30					

*Averages followed by the same letter in the same column did not differ significantly at $P \leq 0.05$. LSD: Least significant difference.

Table 4.9 Mean values and differences between 12 sunflower F_1 hybrids and treatments for twenty-five seed weight

Hybrid	Control	Heat treatment	Difference and significance level
1	1.39	0.83	0.56*
2	1.13	0.92	0.20
3	1.18	0.76	0.42*
4	0.70	0.93	0.23
5	0.84	0.74	0.10
6	0.82	0.85	0.03
7	0.99	0.72	0.27
8	0.80	0.70	0.10
9	0.65	0.70	0.05
10	0.83	0.69	0.14
11	0.93	0.79	0.14
12	0.81	0.79	0.02
Average	**0.92**	**0.79**	**0.13***

*$P \leq 0.05$.

Table 4.10 Mean values and rankings of 12 sunflower F_1 hybrids for head sterile centre diameter analysed at two temperature treatments

Rank	Control Hybrid	Average*	Rank	Heat treatment Hybrid	Average*
1	8	2.66a	1	8	3.27a
2	2	2.36ab	2	9	2.87ab
3	10	2.19abc	3	6	2.79ab
4	9	2.16abc	4	3	2.66ab
5	6	1.86bcd	5	5	2.61bc
6	7	1.83bcd	6	10	2.53bc
7	12	1.75cd	7	7	2.51bc
8	3	1.72cd	8	2	2.47bc
9	1	1.69cd	9	4	2.19cd
10	4	1.63cd	10	11	2.17cd
11	5	1.50d	11	12	1.88cd
12	11	1.46d	12	1	1.73d
LSD (0.05) = 0.58					

*Averages followed by the same letter in the same column did not differ significantly at $P \leq 0.05$, LSD: Least significant difference.

Table 4.11 Mean values and differences between 12 sunflower F_1 hybrids and treatments for head sterile centre diameter

Hybrid	Control	Heat treatment	Difference and significance level
1	1.69	1.73	0.05
2	2.37	2.47	0.11
3	1.72	2.66	0.95*
4	1.63	2.19	0.57
5	1.50	2.61	1.11*
6	1.87	2.79	0.93*
7	1.83	2.51	0.68*
8	2.66	3.27	0.62*
9	2.16	2.87	0.71*
10	2.19	2.53	0.35
11	1.46	2.18	0.72*
12	1.75	1.88	0.13
Average	**1.90**	**2.48**	**0.58***

* $P \leq 0.05$.

4.3.4 Mean values for sunflower oil and major fatty acid content

Oil content

Within the control and heat treatments respectively, significant differences were observed between some individual hybrids for oil content that would allow selection for this trait under normal and heat stress conditions (Table 4.12). In the control treatment, hybrid 4 ranked first, but it was not significantly different from hybrids 1, 3, 5 and 9. The remaining hybrids did not differ significantly from each other. In the heat treatment hybrids 1, 4, 3 and 5 ranked in positions 7-10. Hybrid 9 ranked in the second position but it was only significantly different from hybrids 11 and 12 that ranked in the last two positions for oil content. Additionally, hybrid 8 that ranked first in the heat treatment was only significantly different from hybrids 5, 11 and 12. However, in the control, hybrid 8 ranked seventh and was only significantly different from the top two hybrids (3 and 4). The average oil content of the control was 28.19%, while that of the heat treatment was 22.10%. The heat stress treatment resulted in a significant decrease of 6.09% in oil content (Table 4.13). All 12 hybrids showed a reduction in oil content after heat treatment, but only seven hybrids showed a significant decrease in oil content. These included hybrids 1, 3, 4, 5, 6, 11 and 12. Of these, hybrid 4 showed the highest decrease in oil content (11.80%). Hybrids 8 and 10 showed a slight decrease in oil content.

Palmitic acid (C16:0)

Within the control and heat treatments respectively, significant differences occurred between individual hybrids for palmitic acid content (Table 4.14). In the control treatment, the low oleic hybrids 3, 7, 8, 9, 10 and 12 ranked in the first six positions and these did not differ significantly for palmitic acid content. The high and mid oleic hybrids had significantly lower palmitic acid contents than the low oleic hybrids with the exception of hybrid 3 that did not differ from mid oleic hybrids 2 and 6. The high and mid oleic hybrids contained less than 4% palmitic acid, while the low oleic acid hybrids contained more or less 5% palmitic acid. A similar tendency was observed in the heat treatment. Low oleic hybrids 3, 7, 8 and 12 ranked in the first four positions and did not differ significantly from each other. These contained more than 5% palmitic acid. The high and mid oleic acid hybrids, with the exception of hybrid 1, ranked in the last five positions with oleic acid contents of 5% and less (Table 4.15). The average of the heat treatment was significantly higher than the control. The heat treatment showed an average increase of 0.97% in palmitic acid.

Table 4.12 **Mean values and rankings of 12 sunflower F_1 hybrids for oil content analysed at two temperature treatments**

	Control			Heat treatment	
Rank	**Hybrid**	**Average***	**Rank**	**Hybrid**	**Average***
1	4	33.95a	1	8	26.05a
2	3	30.46ab	2	9	24.77ab
3	1	29.54abc	3	10	23.17abc
4	5	29.19abc	4	2	22.75abc
5	9	29.06abc	5	6	22.71abc
6	6	27.99bc	6	7	22.69abc
7	8	27.50c	7	3	22.65abc
8	2	27.31c	8	4	22.15abc
9	11	26.70c	9	1	21.52abc
10	7	26.31c	10	5	20.46bcd
11	12	25.43c	11	11	19.69cd
12	10	24.89c	12	12	16.62d
LSD (0.05) = 5.05					

*Averages followed by the same letter in the same column did not differ significantly at $P \leq 0.05$, LSD: Least significant difference.

Table 4.13 **Mean values and differences between 12 sunflower F_1 hybrids and treatments for oil content**

Hybrid	Control	Heat treatment	Difference and significance level
1	29.54	21.52	8.02*
2	27.13	22.75	4.56
3	30.46	22.65	7.80*
4	33.95	22.15	11.80*
5	29.19	20.46	8.73*
6	27.99	22.71	5.28*
7	26.31	22.69	3.62
8	27.50	26.05	1.45
9	29.06	24.77	4.29
10	24.89	23.17	1.72
11	26.70	19.69	7.01*
12	25.43	16.62	8.81*
Average	**28.19**	**22.10**	**6.09***

*$P \leq 0.05$.

Table 4.14 Mean values and rankings of 12 sunflower F_1 hybrids for palmitic acid content analysed at two temperature treatments

	Control			Heat treatment	
Rank	**Hybrid**	**Average***	**Rank**	**Hybrid**	**Average***
1	9	5.34a	1	12	6.26a
2	8	5.19a	2	3	5.77ab
3	10	5.02a	3	8	5.71ab
4	12	4.97a	4	7	5.46ab
5	7	4.78ab	5	1	5.33b
6	3	3.99bc	6	10	5.21bc
7	2	3.91bc	7	9	5.10bc
8	6	3.63c	8	2	5.00bc
9	1	3.52c	9	6	4.84bcd
10	5	3.42c	10	5	4.66bcd
11	4	3.23c	11	4	4.37cd
12	11	3.16c	12	11	4.07d
LSD (0.05) = 0.8926					

*Averages followed by the same letter in the same column did not differ significantly at P≤0.05, LSD: Least significant difference.

Table 4.15 Mean values and differences between 12 sunflower F_1 hybrids and treatments for palmitic acid content

Hybrid	Control	Heat treatment	Difference and significance level
1	3.52	5.33	1.81*
2	3.91	5.00	1.09*
3	3.99	5.77	1.78*
4	3.23	4.38	1.14*
5	3.45	4.66	1.21*
6	3.63	4.84	1.22*
7	4.78	5.46	0.68
8	5.19	5.71	0.52
9	5.34	5.10	0.23
10	5.02	5.21	0.19
11	3.16	4.07	0.91*
12	4.97	6.26	1.29*
Average	**4.18**	**5.15**	**0.97***

*P≤0.05.

Eight of the 12 hybrids showed a significant increase in palmitic acid after heat treatment. Low oleic acid hybrids 7, 8, 9 and 10 showed small and insignificant changes in palmitic acid content after heat treatment.

Stearic acid (C18:0)

Significant differences were observed between hybrids for stearic acid content in both treatments (Table 4.16). In both treatments hybrids 1, 3, 4 and 5 ranked in the first four positions for stearic acid content. The mid oleic hybrid 1 ranked first and was not significantly different from the low oleic hybrid 3 in the control and the mid oleic hybrid 5 in the heat treatment. Hybrids 2, 7, 10, 11 and 12 ranked in positions 5-9 in both treatments and did not differ significantly from each other. Hybrids 9, 6 and 8 ranked in positions 10, 11 and 12 respectively in both treatments. No significant differences were observed between these hybrids except for hybrid 9 that differed significantly from hybrid 8 in the heat treatment. The average of the heat treatment was significantly higher than the control (Table 4.17). The heat treatment caused an average increase of 1.56% in stearic acid. All 12 hybrids showed a significant increase in stearic acid after heat treatment. Hybrid 5 showed the largest increase (2.53%) in stearic acid content.

Oleic acid (C18:1)

Significant differences were observed between individual hybrids for both treatments (Table 4.18). The high oleic acid hybrid 4 ranked first in both the control and heat treatments and had a significantly higher oleic acid content than all 12 hybrids in the control treatment. In the heat treatment, the value of hybrid 4 was not significantly higher than mid oleic acid hybrids 1, 5 and 11. Mid oleic acid hybrids 1, 5 and 11 ranked in the second, third and fourth positions in both treatments. Some of the mid oleic acid hybrids showed significant differences for oleic acid content. The low oleic acid hybrids ranked in the last six positions for oleic acid content, however, significant differences were observed among some of these. The change in rankings of the low oleic hybrids indicated that these responded differently to the heat stress treatment and an interaction occurred between hybrids and treatments. The average oleic acid percentage varied between 25.10% and 74.18% for all hybrids for both the control and heat treatment (Table 4.19). The average of the heat treatment was significantly higher than the control. The heat treatment caused an average increase of 11.44% in oleic acid. Of the 12 hybrids, seven showed a significant increase in oleic acid percentage after heat treatment.

Table 4.16 Mean values and rankings of 12 sunflower F_1 hybrids for stearic acid content analysed at two temperature treatments

	Control			Heat treatment	
Rank	**Hybrid**	**Average***	**Rank**	**Hybrid**	**Average***
1	1	7.37a	1	1	8.95a
2	3	6.75ab	2	5	8.33ab
3	4	5.93bc	3	3	7.98bc
4	5	5.80bc	4	4	7.18cd
5	12	5.49cd	5	7	7.07d
6	10	5.47cde	6	10	7.04d
7	11	5.40cde	7	11	6.80de
8	2	5.29cde	8	12	6.77de
9	7	5.10cdef	9	2	6.65de
10	9	4.80def	10	9	6.57de
11	6	4.59ef	11	6	5.94ef
12	8	4.24f	12	8	5.44f
LSD (0.05) = 0.88					

*Averages followed by the same letter in the same column did not differ significantly at P≤0.05, LSD: Least significant difference.

Table 4.17 Mean values and differences between 12 sunflower F_1 hybrids and treatments for stearic acid content

Hybrid	Control	Heat treatment	Difference and significance level
1	7.37	8.95	1.58*
2	5.29	6.65	1.36*
3	6.75	7.98	1.24*
4	5.93	7.18	1.25*
5	5.81	8.33	2.53*
6	4.59	5.94	1.35*
7	5.10	7.07	1.98*
8	4.24	5.44	1.21*
9	4.80	6.57	1.76*
10	5.45	7.04	1.58*
11	5.40	6.80	1.40*
12	5.50	6.77	1.27*
Average	**5.52**	**7.06**	**1.56***

*P≤0.05.

Table 4.18 **Mean values and rankings of 12 sunflower F_1 hybrids for oleic acid content analysed at two temperature treatments**

	Control			Heat treatment	
Rank	**Hybrid**	**Average***	**Rank**	**Hybrid**	**Average***
1	4	74.18a	1	4	73.57a
2	11	66.06b	2	1	69.71ab
3	5	63.92bc	3	5	69.43ab
4	1	60.79bc	4	11	66.72abc
5	6	56.64cd	5	6	64.24bc
6	2	50.40d	6	2	60.74c
7	3	36.57e	7	10	52.24d
8	8	34.84e	8	9	49.45de
9	9	29.52ef	9	8	47.84de
10	10	28.74ef	10	12	46.94de
11	12	26.53f	11	7	46.52de
12	7	25.10f	12	3	43.13e
LSD (0.05) = 7.85					

*Averages followed by the same letter in the same column did not differ significantly at $P \leq 0.05$. LSD: Least significant difference.

Table 4.19 **Mean values and differences between 12 sunflower F_1 hybrids and treatments for oleic acid content**

Hybrid	Control	Heat treatment	Difference and significance level
1	60.79	69.71	8.92*
2	50.40	60.74	10.34*
3	36.57	43.13	6.56
4	74.18	73.57	0.61
5	63.92	69.43	5.51
6	56.64	64.24	7.60
7	25.10	46.52	24.42*
8	34.84	47.84	13.00*
9	29.52	49.45	19.93*
10	28.74	52.24	23.50*
11	66.06	66.72	0.66
12	26.53	46.94	20.41*
Average	**46.11**	**57.55**	**11.44***

*$P \leq 0.05$.

All hybrids, except for the high oleic hybrid 4, showed an increase in oleic acid after heat treatment. Additionally, the mid oleic acid hybrid 11 showed only a slight increase in oleic acid content. For these hybrids the heat treatment did not have a major influence on the oleic acid content.

Linoleic acid (C18:2)

Significant differences were observed between individual hybrids for both treatments (Table 4.20). The low oleic acid hybrids ranked in the first six positions in both the control and the heat treatment. In the control treatment, significant differences were observed between hybrid 7 and 3 and between hybrid 7 and 8. However, in the heat treatment, no significant differences were observed among all linoleic acid hybrids. The change in rankings of the low oleic hybrids indicated that these responded differently to the heat stress treatment. The mid oleic hybrids 2 and 6 ranked in the seventh and eighth positions in both treatments and did not differ significantly from each other. Significant differences were observed between some of the remaining mid oleic acid hybrids in both treatments. The high oleic acid hybrid 4 ranked in the last position with the lowest linoleic acid content in both treatments (less than 15%).

The average linoleic acid content varied between 14.21% and 63.12% in the control treatment, while in the heat treatment the linoleic acid averages were lower and varied between 12.46% and 40.93% for all 12 hybrids (Table 4.21). The average of the heat treatment was significantly lower than the control. The heat treatment caused an average decrease of 14.41% in linoleic acid compared to the control. Of the 12 hybrids, 10 showed a significant decrease in linoleic acid after heat treatment. Hybrids 4 and 11 showed only slight decreases in linoleic acid content after heat treatment. For these hybrids, temperature stress did not have a major influence on the linoleic acid content.

The heat treatment had a significant influence on the oleic and linoleic acid contents of most hybrids. It resulted in a significant increase in oleic acid and simultaneously a significant reduction in the linoleic acid contents of most hybrids (Tables 4.19 and 4.21). Hybrid 10 showed the second highest increase in oleic acid (23.50%) and at the same time the highest decrease in linoleic acid (25.66%).

Table 4.20 Mean values and rankings of 12 sunflower F_1 hybrids for linoleic acid content analysed at two temperature treatments

	Control			Heat treatment	
Rank	Hybrid	Average*	Rank	Hybrid	Average*
1	7	63.12a	1	3	40.93a
2	12	61.48ab	2	8	39.17a
3	10	59.55ab	3	7	39.13a
4	9	59.38ab	4	12	38.14a
5	8	54.57bc	5	9	37.00a
6	3	50.86c	6	10	33.88a
7	2	38.54d	7	2	25.46b
8	6	33.71de	8	6	23.03bc
9	1	26.49ef	9	11	19.74bcd
10	5	25.21f	10	5	15.47cd
11	11	23.49f	11	1	13.27d
12	4	14.21g	12	4	12.46d
LSD (0.05) = 7.87					

*Averages followed by the same letter in the same column did not differ significantly at P≤0.05, LSD: Least significant difference.

Table 4.21 Mean values and differences between 12 sunflower F_1 hybrids and treatments for linoleic acid content

Hybrid	Control	Heat treatment	Difference and significance level
1	26.49	13.26	13.23*
2	38.54	25.46	13.08*
3	50.86	40.93	9.94*
4	14.21	12.46	1.75
5	25.20	15.47	9.73*
6	33.71	23.03	10.68*
7	63.12	39.13	23.99*
8	54.57	39.17	15.40*
9	59.38	37.00	22.38*
10	59.55	33.88	25.66*
11	23.49	19.74	3.74
12	61.48	38.13	23.34*
Average	**42.55**	**28.14**	**14.41***

*P≤0.05.

4.3.5 General and specific combining ability means of F_1 sunflower hybrids for sunflower head and seed traits

The means of F_1 hybrids within the crossing pairs were used to determine the general (GCA) and specific combining ability (SCA) of the parents for the traits studied.

Head diameter

Male A showed the best GCA of all males in the control treatment, however, it was not significantly better than males C and D (Table 4.22). Male B showed the lowest GCA in the control treatment and was significantly lower than males A and D. Therefore, when selecting parents for head diameter under normal temperature conditions, all crosses made with males A, C and D can be considered. For heat treatment, the four males performed the same with regards to GCA and male B did not differ significantly from the other males. As a result, when selecting parents to produce F_1 hybrids that will perform good for this trait under high temperature conditions, male B could be considered even though it had a poor GCA under normal temperature conditions.

Number of filled seeds per head

Male D showed the best GCA of the males in the control treatment, however, it was not significantly better than males A and C (Table 4.22). Male B showed the lowest GCA and was significantly lower than male D. Female *j* was significantly higher than all females with regard to SCA for this trait and crosses of male D with female *j* produced F_1 hybrids with significantly increased number of filled seeds per sunflower head in the control treatment. In the heat treatment the rankings changed and male C showed the best GCA, but it was only significantly better than male B. Female *i* showed the best SCA in a cross with male C and was significantly better than most of the other females with regard to this trait. Under high temperature conditions, male C crossed with females *h* and *i* would give the best results. However, when selecting parents to produce F_1 hybrids that will perform well for this trait, male D x female *j* would be the best choice for normal and high temperature conditions.

Table 4.22 General and specific combining ability means of 12 sunflower F_1 hybrids for agronomic traits analysed at two temperature treatments

			Sunflower head and seed traits							
			Head diameter		**Number of filled seeds per head**		**Twenty-five seed weight**		**Sterile centre diameter**	
Males	X	Females	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
A		*a*	8.80	8.04	248.39	163.80	1.39	0.82	3.10	3.24
		b	8.58	9.05	324.87	236.69	1.36	0.92	5.02	5.71
		c	8.04	9.16	262.23	206.36	1.18	0.76	3.03	5.75
Mean			8.48	8.75	278.50	202.28	1.31	0.84	3.71	4.90
B		*d*	5.53	8.56	121.96	235.50	0.70	0.93	2.33	4.16
		e	6.35	7.78	242.42	179.15	0.84	0.74	2.27	5.66
		f	5.51	9.26	138.74	108.79	0.82	0.85	3.06	6.41
Mean			5.80	8.53	167.70	174.48	0.79	0.84	2.56	5.41
C		*g*	7.84	8.20	311.53	229.97	0.99	0.72	3.06	5.65
		h	8.06	9.13	304.76	332.54	0.79	0.70	6.15	8.71
		i	6.43	8.96	208.06	413.01	0.65	0.70	4.24	7.11
Mean			7.44	8.76	274.78	325.17	0.81	0.71	4.48	7.16
D		*j*	8.78	8.17	465.17	372.70	0.83	0.69	3.93	5.34
		k	7.26	8.36	291.94	220.72	0.93	0.79	2.03	3.94
		l	7.84	8.83	287.81	235.21	0.80	0.79	2.92	2.99
Mean			7.96	8.45	348.31	276.21	0.86	0.76	2.96	4.09
LSD (0.05)			2.08	1.87	112.66	111.50	0.37	0.27	1.63	2.06

LSD: Least significant difference.

Twenty-five seed weight

Male A showed the best GCA in the control treatment and was significantly better than the other three males for this trait (Table 4.22). Female *a* showed the best SCA in a cross with male A and was significantly better than the other females in crosses with their respective males. However, in the heat treatment, the male parents did not differ significantly from each other with regard to GCA for this trait. In addition, no significant differences for SCA were observed between all females in all crosses for twenty-five seed weight. Under normal temperature conditions, male A x female *a* and *b* would be the choice, while for high temperature conditions, any of the parent crosses can be chosen. However, when selecting for F_1 hybrids that would perform well for this trait under normal and high temperature conditions, male A x female *b* would be the choice.

Head sterile centre diameter

Crosses made with male B produced F_1 hybrids that showed the lowest average sterile centre diameters under control treatment conditions (Table 4.22). Male B was the best male parent with regards to this trait and showed the lowest GCA. However, male B was only significantly better than male C for this trait. Crosses made between male A x female *b*, and male C x females *h* and *i* produced F_1 hybrids that had significantly larger head sterile centres than the rest and these crosses should not be considered if the breeder aims to decrease this trait under normal temperature conditions. The heat treatment previously caused a significant mean increase in head sterile centre diameter (Table 4.9). Male D was the best male parent with regards to this trait and showed the weakest GCA. Male D was also significantly better than male C. When selecting parents to produce F_1 hybrids with decreased head sterile centre under heat treatment conditions, the best crosses would be male A x female *a*, and male D x females *k* and *l*.

4.3.6 General and specific combining ability means of F_1 sunflower hybrids for sunflower seed oil composition

Oil content

Male B showed the best GCA in the control treatment for oil content, but it was not significantly different from the other males for this trait (Table 4.23). Female *d* in a cross with male B showed significantly better SCA than females *g*, *j*, *k* and *l* in their respective crosses.

Table 4.23 General and specific combining ability means of 12 F_1 sunflower hybrids for seed oil composition at two temperature treatments

		Sunflower seed oil composition									
		Oil content		**C16:0**		**C18:0**		**C18:1**		**C18:2**	
Males X	Females	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
A	*a*	29.54	21.52	3.52	5.33	7.37	8.95	60.79	69.71	26.49	13.26
	b	27.31	22.75	3.91	5.00	5.30	6.65	50.40	60.74	38.54	25.46
	c	30.46	22.65	3.99	5.77	6.78	7.98	36.57	43.13	50.86	40.93
Mean		29.10	22.31	3.80	5.37	6.48	7.86	49.25	57.86	38.63	26.55
B	*d*	33.95	22.15	3.27	4.37	6.09	7.18	74.18	73.57	14.21	12.46
	e	29.19	20.46	3.45	4.66	5.80	8.33	63.92	69.43	25.20	15.47
	f	27.99	22.71	3.62	4.84	4.59	5.94	56.64	64.24	33.71	23.03
Mean		30.38	21.77	3.45	4.63	5.49	7.15	64.91	69.08	27.38	16.99
C	*g*	26.31	22.69	4.78	5.46	5.10	7.07	25.10	46.51	63.12	39.13
	h	27.50	26.05	5.19	5.70	4.24	5.44	34.84	47.84	54.57	39.17
	i	29.06	24.77	5.33	5.10	4.80	6.56	29.52	49.45	59.38	37.00
Mean		27.62	24.50	5.10	5.42	4.71	6.36	29.82	47.94	59.02	38.43
D	*j*	24.89	23.17	5.02	5.21	5.46	7.04	28.74	52.24	59.55	33.88
	k	26.70	19.69	3.16	4.07	5.40	6.80	66.06	66.72	23.49	19.74
	l	25.43	16.62	4.97	6.26	5.49	6.77	26.53	46.94	61.48	38.13
Mean		25.67	19.83	4.38	5.18	5.45	6.87	40.44	55.30	48.17	30.59
LSD	**(0.05)**	6.76	2.88	0.70	1.12	0.86	0.90	9.88	5.56	9.65	6.31

LSD: Least significant difference.

Therefore when the breeder selects parents to produce F_1 hybrids with high oil content under normal temperature conditions, the choice of parents would be male B crossed with females *d* and *e*, male A crossed with females *a* and *c* and male C crossed with female *i*. Under heat stress conditions, male C showed the best GCA for oil content. However, male C was only significantly better than male D. Female *h* in a cross with male C showed the best SCA and it was significantly better than the rest of the females (except for female *i*) in their respective crosses. As a result, when selecting parents to produce F_1 hybrids with high oil contents under heat stress conditions, the best choices would be male C with females *h* and *i*. In both the control and the heat treatment male D showed the lowest CGA as well as low SCA used in crosses with some of the females.

Palmitic acid (C16:0)

Male C showed the best GCA in the control treatment and was significantly better than the other three males for palmitic acid content (Table 4.23). Females *g*, *h* and *i* showed significantly better SCA in crosses made with male C compared to the crosses made with males A and B. Therefore, when the breeder aims to increase palmitic acid content under normal temperature conditions, the choice of parents would be male C crossed with females *g*, *h* and *i*, and male D crossed with females *j* and *l*. Under heat treatment conditions, the male parents did not differ significantly for GCA with regard to this trait. However, the cross between male D x female *l* produced F_1 hybrids that showed a significantly increased palmitic acid content on average when compared to crosses made between male B x females *d*, *e* and *f*.

Stearic acid (C18:0)

Male A showed the best GCA in the control treatment and was significantly better than the other three males for stearic acid content (Table 4.23). Variation occurred between the females with regard to SCA, however, females *a* and *c* crossed with male A showed the best SCA, significantly better than for most of the crosses. Therefore, when the breeder aims to increase stearic acid content under normal temperature conditions, the choice of parents would be male A with females *a* and *c*. Under heat treatment conditions, male A also showed the best GCA and was significantly better than males C and D for stearic acid content. Female *a* in a cross with male A showed the best SCA and was significantly better than the rest of the females (except for female *e* x male B) in their respective crosses. When selecting parents to produce F_1 hybrids with higher stearic acid contents under heat treatment conditions, the best crosses would be male A x female *a*.

Oleic acid (C18:1)

Male B showed the best GCA in the control treatment and was significantly better than the other three males for oleic acid content (Table 4.23). Significant variation occurred between females with regards to SCA, however, female *d* in a cross with male B had the highest SCA and was significantly better than most of the females (except for female *k* x male D) in their respective crosses. Therefore, the parents of choice when selecting for high oleic acid content under normal temperature conditions would be male B x female *d*. Under high temperature conditions, male B also showed the best GCA and was significantly better than the other three males for oleic acid content. Significant variation occurred between females with regard to SCA. However, female *d* in a cross with male B again had the highest SCA but was not significantly better than females *a*, *e* and *k* in their respective crosses. When selecting parents to produce F_1 hybrids with high oleic acid content under heat treatment conditions, the best crosses would be male A x female *a*, male B x females *d* and *e* and male D x female *k*.

Linoleic acid (C18:2)

Male C showed the best GCA in the control treatment and was significantly better than the other three males for linoleic acid content (Table 4.23). Females *g*, *h* and *i* showed significantly better SCA in crosses made with male C compared to the crosses made with females and males A and B. Also, females *j* and *l* in crosses with male D showed significantly better SCA compared to crosses made with females and males A and B. When selecting parents to produce F_1 hybrids with high linoleic acid contents under normal temperature conditions, male C with female *g* would be the choice. Although the average linoleic acid contents of the F_1 hybrids were less under high temperature treatment conditions, male C also showed the best GCA and was significantly better than the other three males for linoleic acid content. Female *c* in a cross with male A showed the best combining ability of the females in their respective crosses. However, the combining ability for this female was not significantly better than females *g*, *h* and *i* in crosses with male C, and female *l* in a cross with male D. When selecting parents to produce F_1 hybrids with high linoleic acid content under heat treatment conditions, the best crosses would be male A x female *c*, male C x females *g*, *h* and *i* and male D x female *k*.

4.3.7 Estimates of variance components and broad sense heritability for sunflower head and seed traits

Head diameter

The environmental variance ($\sigma^2 e$) proportion contributed most to the phenotypic (total) variation in both the control and the heat treatments, indicating high impact of the environment on head diameter (Table 4.24). A low heritability estimate of 38% was observed for head diameter in the control treatment. A negative heritability estimate (-0.14) was observed for head diameter in the heat treatment. The negative heritability estimate was a result of the negative genotypic variance. Head diameter was highly sensitive to high temperature. The high temperature treatment resulted in large, but even-sized heads for all hybrids and therefore a low estimate of genetic variance was observed for this trait. In addition, the significant differences between replications might have contributed to the low genetic variance component.

Number of filled seeds per head

The genotypic variance proportion contributed most to the phenotypic variation in both the control and the heat treatments for number of filled seeds per head (Table 4.24). Although environmental variance contributed less to the total variance, the environment had some impact on this trait. Intermediate levels of heritability were observed for number of filled seeds per head in the control (60%) and heat treatment (59%).

Twenty-five seed weight

The environmental variance proportion contributed slightly more to the total variation in both the control and the heat treatments, indicating impact of the environment on twenty-five seed weight (Table 4.24). A low heritability estimate of 47% was observed for twenty-five seed weight in the control treatment. A negative heritability estimate (-0.07) was observed for twenty-five seed weight in the heat treatment. Seed weight was highly sensitive to high temperatures. The high temperature treatment caused a reduction in seed weight to an extent where no genetic variability was observed between hybrids. This might have contributed to the low genetic variance component estimate observed for twenty-five seed weight.

Table 4.24 Variance components and broad sense heritability for head and seed traits of 12 sunflower F_1 hybrids analysed at two temperature treatments

	Head diameter		**Number of filled seeds per head**		**Twenty-five seed weight**		**Head sterile centre diameter**	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
σ^2g	0.92	-0.15	6625.79	6221.42	0.043	-0.00	1.16	2.18
σ^2e	1.51	1.21	4400.45	4310.77	0.048	0.02	0.93	1.47
σ^2p	2.423	1.06	11026.24	10532.20	0.09	0.02	2.08	3.65
H^2	**0.38**	**-0.14**	**0.60**	**0.59**	**0.47**	**-0.07**	**0.56**	**0.60**

σ^2g: Genotypic variance component, σ^2p: Phenotypic variance component, σ^2e: Environmental variance component, H^2: Broad sense heritability.

Head sterile centre diameter

The genotypic variance proportion contributed mostly to the total variation in both the control and the heat treatments for head sterile centre diameter (Table 4.24). However, although environmental variance contributed less to the total variance, the environment had some impact on this trait. Intermediate levels of heritability were observed for head sterile centre diameter in the control (56%) and heat treatment (60%) respectively.

4.3.8 Estimates of variance components and broad sense heritability for sunflower seed oil and major fatty acid content

Oil content

The environmental variance proportion contributed predominantly to the phenotypic variation in the control treatment, indicating high impact of the environment on oil content (Table 4.25). Under heat stress conditions the environmental variance proportion was much lower and the genotypic variance component contributed predominantly to the phenotypic variation in the heat treatment. As a result, under heat stress conditions the environment had a smaller effect on oil content than under normal temperature conditions. The high impact of environment under normal temperature conditions resulted in the low broad sense heritability estimate (5%) observed for oil content under these conditions. However, under heat stress conditions an intermediate level of heritability (63%) was observed for oil content.

Palmitic acid (C16:0)

The genotypic variance proportion contributed mostly to the phenotypic variation in palmitic acid content (Table 4.25). However, under heat stress, the environmental variance component contributed most to the total variance and palmitic acid was sensitive to the high temperature environment. In addition, the low genotypic variance estimate indicated there was small genetic variability for palmitic acid in the heat treatment. The reason for this is that the heat treatment caused an increase in palmitic acid content to an extent where hybrids might have reached their maximum potential for this fatty acid. Relatively high broad sense heritability was observed for palmitic acid content in the control (78%) while in the heat treatment, the estimate was low (35%).

Table 4.25 Variance components and broad sense heritability for oil and major fatty acid contents of 12 sunflower F_1 hybrids analysed at two temperature treatments

	Oil content		**C16:0**		**C18:0**		**C18:1**		**C18:2**	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
σ^2g	0.85	4.88	0.61	0.23	0.71	0.86	303.95	115.97	297.08	117.99
σ^2e	15.85	2.88	0.17	0.43	0.25	0.28	33.82	10.79	32.31	13.82
σ^2p	16.70	7.75	0.77	0.67	0.96	1.14	337.77	126.74	329.40	131.81
H^2	**0.05**	**0.63**	**0.78**	**0.35**	**0.74**	**0.75**	**0.90**	**0.91**	**0.90**	**0.90**

σ^2g: Genotypic variance component, σ^2p: Phenotypic variance component, σ^2e: Environmental variance component, H^2: Heritability.

Stearic acid (C18:0)

The genotypic variance proportion contributed most to the phenotypic variation in both the control and the heat treatments for stearic acid content (Table 4.25). Relatively high levels of heritability were observed for stearic acid content in the control (74%) and heat treatment (75%) respectively.

Oleic acid (C18:1)

Estimates of genetic variance were much larger than estimates of environmental variances for oleic acid content in both temperature treatments (Table 4.25). This indicated that genotypic variance contributed largely to the phenotypic variance and that the environment had almost no effect on the trait within each treatment. High levels of heritability were observed for oleic acid content in both the control (90%) and heat treatment (91%).

Linoleic acid (C18:2)

The genotypic variance proportion contributed most to the phenotypic variation in both the control and the heat treatments for linoleic acid content (Table 4.25). Within each treatment, the environment had almost no effect on linoleic acid content and the trait exhibited a large amount of genetic variability. High levels of heritability were observed for linoleic acid content in both the control (90%) and heat treatment (90%).

4.4 Correlations

4.4.1 Correlations between agronomic traits

In the control treatment strong positive and significant correlations were observed between head diameter and number of filled seeds per head as well as between head diameter and twenty-five seed weight (Table 4.26). In the heat treatment, the same positive correlations were observed, however, the correlation between head diameter and number of filled seeds per head was weaker. (Table 4.27) The reason for this could be because the heat treatment resulted in an average decrease in the number of seeds per head and simultaneously a significant increase in the average head diameter (Tables 4.5 and 4.7). Head sterile centre diameter did not show any strong correlations with the other agronomical traits in both treatments.

Table 4.26 **Correlation matrix obtained for sunflower head and seed traits and oil composition from 12 F_1 sunflower hybrids of the control treatment**

	NFSH	HD	25-SW	HSCD	Oil content	C16:0	C18:0	C18:1
HD	0.83**							
25-SW	0.29	0.66**						
HSCD	0.28	0.29	-0.18					
Oil content	-0.16	0.03	0.14	-0.15				
C16:0	0.25	0.09	-0.34*	0.43*	-0.40*			
C18:0	-0.12*	0.19	0.63**	-0.45**	0.16	-0.40*		
C18:1	-0.43*	-0.30	0.12	-0.32	0.34*	-0.89**	0.28	
C18:2	0.43	0.29	-0.14	0.34*	-0.34*	0.89**	-0.32	-0.99**

*P≤0.05, **P≤0.01, HD: Head diameter, NFSH: Number of filled seeds per head, 25-SW: Twenty-five seed weight, HSCD: Head sterile centre diameter.

Table 4.27 **Correlation matrix obtained for sunflower head and seed traits and oil composition from 12 F_1 sunflower hybrids of the heat treatment**

	NFSH	HD	25-SW	HSCD	Oil content	C16:0	C18:0	C18:1
HD	0.47*							
25-SW	0.28	0.70**						
HSCD	0.32	0.31	-0.11					
Oil content	0.52**	0.32	0.22	0.62**				
C16:0	0.05	0.03	-0.26	0.09	-0.14			
C18:0	-0.37*	-0.28	-0.15	-0.48*	-0.43*	0.00		
C18:1	-0.48**	-0.29	0.13	-0.33	-0.27	-0.60**	0.34*	
C18:2	0.52*	0.31	-0.10	0.39*	0.31	0.55**	-0.45**	-0.99**

*P≤0.05, **P≤0.01, HD: Head diameter, NFSH: Number of filled seeds per head, 25-SW: Twenty-five seed weight, HSCD: Head sterile centre diameter.

4.4.2 Correlations between seed oil traits

In the control treatment, significant and negative correlations were observed between oil content and palmitic acid as well as between oil content and linoleic acid (Table 4.26). However, these correlations were relatively weak. Oil content was significantly and positively correlated with oleic acid content. In the heat treatment a significant and negative correlation was observed between oil content and stearic acid content. This correlation was, however, positive and non-significant in the control treatment (Table 4.27). The reason for this difference might be due to the significant decrease in oil content (Table 4.13) and simultaneous significant increase in stearic acid content (Table 4.17) in the heat treatment. Oil content was negatively correlated with palmitic and oleic acid content, but these correlations were non-significant.

Strong negative and significant correlations were observed between palmitic and oleic acid, as well as between oleic and linoleic acid in the control treatment. The strong positive and significant correlation between palmitic and linoleic acid may be a result of the strong negative correlation between oleic and linoleic acid. In the heat treatment, the same correlations were observed; however, some were weaker than in the control treatment. The weaker negative correlation between palmitic and oleic acid may be because the heat treatment resulted in significant average increases in both palmitic (Table 4.15) and oleic (Table 4.19) acids. Similarly, the weaker positive correlation between palmitic and linoleic acid was because of the increase in palmitic acid, but significant average decrease in linoleic acid (Table 4.21) in the heat treatment.

4.4.3 Correlations between agronomic and seed oil traits

In the heat treatment, relatively strong and highly significant positive correlations were observed between oil content and number of filled seeds per head as well as between oil content and head sterile centre diameter. However, in the control treatment these correlations were negative, weak and non-significant. A relatively strong and significantly positive correlation was observed between stearic acid and twenty-five seed weight in the control treatment. However, this correlation was weak, non-significant and negative in the heat treatment. The reason for this difference might be due to the significant average increase in stearic acid (Table 4.17) and simultaneous significant decrease in twenty-five seed weight (Table 4.9) in the heat treatment. Significant and negative correlations were observed between number of filled seeds per head and oleic acid content in both the control and heat treatment. The significant and negative correlation between number of filled seeds per head and oleic acid content may be a result of the negative correlation between oleic and linoleic acid, since linoleic acid was positively correlated with number of filled seeds per head. The last correlation was significant in the heat treatment. Significant and negative correlations were observed between number of filled seeds per head and stearic acid content in both treatments. However, these correlations were relatively weak. The reason for the negative correlation between number of filled seeds per head and stearic acid content may result from the significant negative correlation between stearic acid and linoleic acid, since linoleic acid was positively correlated with number of filled seeds per head. Significant and negative correlations were observed between head sterile centre diameter and stearic acid content in both treatments. The reason for the significant and negative correlation between head sterile centre diameter and stearic acid content may result from the negative correlation between stearic and linoleic acid, since linoleic acid was significantly and positively correlated with head sterile centre diameter.

4.5 Discussion

Brief periods of temperatures higher than 35ºC have a direct and sometimes unfavourable effect on oil quality in sunflower. The most sensitive period for modification in oil quality is from 19-26 DAA, the period of rapid TAG accumulation (Rondanini *et al.*, 2003). Applying a heat stress period of 10 consecutive days at temperatures of 24ºC minimum and 36ºC maximum during the period of 15-25 days after 50% anthesis in sunflower was done to examine the responses of hybrids with regard to some head and seed characteristics as well as oil quality.

Significant genetic variability was observed between hybrids for most traits and this indicated that hybrids can be genetically improved for the specific trait. Where significant interactions occurred between hybrids and treatments, hybrids differed in their sensitivity to the environment and this complicates breeding for the specific trait.

Heat treatment resulted in a significant increase in the average head diameter. However, all hybrids responded the same in the two treatments since no significant interaction occurred between hybrids and treatments for this trait. This increase in head diameter with higher temperature was contradictory to reports on the effect of drought stress on head diameter. Rauf (2008) reported that drought stress during the vegetative phase of sunflower resulted in a reduction in head diameter. Jaleel *et al.* (2009) also pointed out that water stress reduced head diameter. The reason why head diameter was not reduced by heat stress might be that hybrids were more tolerant to high temperatures for this trait. Genetic variability was low in the control treatment, while no genetic variability was seen in the heat treatment. The low broad sense heritability estimate (38%) observed for head diameter in the control treatment was in agreement with low broad sense heritability estimates reported. Çamaş and Esendal (2006) reported that in safflower, head diameter had a low broad sense heritability of 21%. According to Miller and Fick (1997) broad sense heritability estimates of 22% and 44% were reported by Kloczowski (1975) and Pathak (1974) for sunflower. The low genetic variation, together with the low heritability estimates indicate that no progress from selection will be made for head diameter. Selection for this trait is not advised in breeding programmes to improve yield under both normal and high temperature environments.

Heat treatment resulted in a decrease in the number of filled seeds per head, however, it was non-significant. Although genetic variability was seen in both temperature treatments, the significant interaction between hybrids and treatments, as well as the intermediate level of heritability (±60%) will make selection for this trait under both temperature environments difficult. Ayub (2001) reported a low broad sense heritability of -6% for number of seed per head. Seed number per head has the largest direct influence on seed yield and is determined, among others, by self-compatibility and environmental factors at flowering and pollination (Škorić *et al.*, 2007).

The average twenty-five seed weight was significantly reduced by the heat treatment. Seed weight is sensitive to short, but severe heat stress. Rondanini *et al.* (2003) reported that temperatures above 35°C significantly reduced final seed weight. This decrease observed in seed weight might be due to a reduction of the duration of seed growth under temperature stressed conditions. No interaction between hybrids and treatments was observed and this indicated that hybrids performed similarly to heat stress. Although some genetic variability was seen between hybrids in the control treatment, no significant genetic variation was seen between hybrids in the heat treatment. A low broad sense heritability estimate of 47% was observed for twenty-five seed weight in the control treatment and was in the range reported. According to Miller and Fick (1997) broad sense heritability estimates of between 30-66% were reported for seed weight (Pathak, 1974; Shabana, 1974; Kloczowski, 1975; Zhao-Cheng *et al.*, 1988). The low heritability estimates also indicated that this trait was sensitive to the environment and therefore selection for twenty-five seed weight is not advised in breeding programmes to improve yield under both normal and high temperature environments.

Heat treatment resulted in a significant increase in the average head sterile centre diameter and no significant interaction was seen between hybrids and treatments. Significant genetic variation was seen between hybrids in both treatments, however, heritability estimates was of intermediate level for both treatments. Selection for reduced head sterile centres in both normal and high temperature environments will not be effective. Cytoplasmic male sterility or self-incompatibility may play an important role in hybrids for the sterile area of the head. Fernández-Martínez *et al.* (1992) reported that the high oleic mutant alleles may be associated with self-compatibility and found that the high oleic acid hybrids had a consistently lower self-compatibility. Since self-compatibility is an important objective in sunflower breeding, the association of *Ol* alleles with lower levels of self-compatibility is obstructive in the development of high oleic hybrids. However, since the level of self-compatibility vary with genetic background, it is possible to breed for high oleic self-compatible hybrids with the use of favourable genetic backgrounds.

Results obtained from this study showed that the broad sense heritability estimates for head diameter, number of filled seeds per head and twenty-five seed weight were higher for normal conditions than for heat-stressed conditions. Alza and Fernández-Martínez (1997) also reported that estimates of narrow sense heritability were generally higher in non-stressed conditions than in water-stressed conditions for the same yield-related traits. Additionally, heritability estimates are unique and belong to the specific plant population in a specific experiment.

Oil content and major fatty acid composition was significantly modified after exposure to heat stress. Heat treatment resulted in a significant decrease in oil content. This decrease in oil content with an increase in temperature was in accordance with other reports (Harris *et al.*, 1978; Silver *et al.*, 1984; Salunkhe *et al.*, 1992; Jasso de Rodriquez *et al.*, 2002; Rondanini *et al.*, 2003). It was reported that high temperature during seed development can reduce oil content with up to 16% (Weiss, 2000). Temperatures higher than 35ºC during the early seed-filling stage changes the embryo growth pattern that results in higher pericarp:embryo ratios (Rondanini *et al*., 2003). This may contribute to reductions in seed oil content, since hull percentage is negatively correlated with oil content (Lajara *et al*., 1990; Weiss, 2000). However, the effect of temperature on oil content is variable and higher oil content at higher temperatures has been reported (Ahmad and Hassan, 2000; Qadir *et al*., 2006).

All hybrids responded similarly to both treatments for oil content. Significant variation was observed between hybrids in both temperature treatments indicating the potential for genetic improvement for oil content. The low heritability estimate (5%) observed in the control treatment indicated that no progress from selection will be made for oil content. However, the heritability estimate observed in the heat treatment was intermediate (63%) and selection for oil content in high temperature conditions might be more effective than in normal temperature conditions. Heritability estimates vary considerably for oil content in sunflower. This variation is probably due to different genetic backgrounds, environmental effects and methods of estimation (Miller and Fick, 1997). Broad sense heritability ranging from 62-68% has been reported for sunflower (Abd-Elkreem *et al*., 1983). A high broad sense heritability of 96% has been reported by Ayub (2001).

Heat treatment resulted in a significant increase in palmitic acid content. This observation was in agreement with the report of Rondanini *et al*. (2003) who observed an increase in palmitic acid content after exposure to temperatures above 35ºC during the early seed-filling period. However, all hybrids responded the same to both treatments for this fatty acid. Highly significant genetic variation was observed between hybrids in both temperature treatments. The relatively high heritability estimate (78%) seen in the control treatment, indicated that selection for palmitic acid will be effective under normal temperature conditions. However, it is not advised in high temperature environments, since palmitic acid content was sensitive to heat.

The average stearic acid content was significantly increased by the heat treatment. This was in agreement with the findings of Rondanini *et al*. (2003) who reported an increase in stearic acid content after exposure to temperatures above 35ºC during 19-26 DAA. Conversely, Martínez-Force *et al*. (1998) reported that stearic acid content progressively decreased with an increase in temperature in sunflower. Significant variation was seen between hybrids in both temperature treatments. Also, the relatively high heritability in both temperature conditions makes selection for stearic acid effective in both normal and high temperature environments. From a nutritional point of view, a reduction in SFA (palmitic and stearic acid) content of traditional sunflower oil might result in healthier edible oil by lowering the risk of cardiovascular disease (Kris-Etherton and Yu, 1997; Seiler, 2004). However, from a technological point of view an increase in SFA content is necessary for many industrial uses, for example production of shortenings and margarine (Fernández-Martínez *et al*., 2004).

The heat treatment resulted in a significant increase in the oleic acid content of most hybrids. On average, increases between 8.92% and 24.42% were observed. Simultaneously, linoleic acid content was significantly reduced by the heat treatment. Some hybrids showed a reduction of up to 25% in linoleic acid. Hybrids responded differently to the two temperature treatments for both fatty acids. Some hybrids were more sensitive to temperature treatment than others. However, the high oleic acid hybrid showed less sensitivity to the high temperature conditions. High heritability estimates were observed for both oleic and linoleic fatty acids in both temperature treatments. Therefore, selection for oleic or linoleic acid content would be effective in breeding programmes. In addition, the largest part of the total variation in oleic acid percentage observed in the control and heat treatments respectively was due to differences in potential oleic acid percentage between the 12 hybrids. Therefore, hybrid selection for a specific temperature environment is important to obtain a specified oil quality.

A negative correlation was observed between oleic and linoleic acid profiles for both temperature treatments. For most hybrids, as oleic acid increased, a simultaneous reduction in linoleic acid was observed. Previous reports indicated that during seed maturation high temperatures (and especially high night temperatures) modify the oleic/linoleic ratio in standard sunflower cultivars by increasing the oleic acid content and/or reducing the linoleic acid content (Harris *et al*., 1978; Triboï-Blondel *et al*., 2000; Izquierdo *et al*., 2002; Rondanini *et al*., 2003).

However, it was reported that in high oleic acid mutants, the oleic and linoleic acid contents were less influenced by temperature than for standard genotypes (Garcés *et al*., 1989; Lagravère *et al*., 2000; Izquierdo *et al*., 2002). Results obtained for the high oleic hybrid (hybrid 4) was in accordance with these reports. The heat treatment had no significant influence on both oleic and linoleic fatty acid percentages for the high oleic acid hybrid. The reason for this could be due to a different influence of temperature on the ODS activity or the high oleic *Ol* alleles could be associated with genes at other loci for adaptation to high temperature conditions (Fernández-Martínez *et al*., 1992).

The different temperature treatments had an influence on the combining ability of the males and females especially for agronomical traits. Selecting for larger head diameter may result in higher seed yield because head diameter, among other characters, is positively correlated with yield (Fick and Miller, 1997). However, it was previously reported that smaller sunflower heads (less than 20 cm in diameter) carry seeds with up to 40% more oil than seed from larger heads (30 cm in diameter) (Afzalpurkar and Lakshminarayana, 1980). Therefore, when selecting for yield, larger heads should be considered, but when selecting for oil content, smaller head diameters should be selected.

Number of filled seeds per head is positively correlated with seed yield (Fick and Miller, 1997). Therefore, selecting parents for increased number of filled seeds per head would result in an indirect selection for increased seed yield. Seed weight is also reported to be positively correlated with seed yield (Fick and Miller, 1997). Therefore, selecting parents for increased twenty-five seed weight would result in an indirect selection for increased seed yield. As a result, parents should be carefully selected to produce hybrids that will perform the best for specific agronomical traits and/or oil fatty acid contents in areas where high temperatures may occur during the growing season and especially heat stress periods during the early seed-filling period.

Characteristics like head diameter, filled seed number per plant and seed weight are generally correlated with sunflower yield (Fick and Miller, 1997). Consequently, when selecting parents to produce hybrids with increased yield components like number of filled seeds per head, there is a good chance for increasing yield at the same time. Although significant correlations were observed between the agronomical and oil quality traits, these were not strong. Therefore, when selecting traits for increased yield, the fatty acid composition will not be influenced to a large extend. Also, when selecting for oleic, linoleic or palmitic acid content, the agronomical traits will not be largely influenced.

Relationships between oil and fatty acid contents were in agreement with other reports. A negative correlation between oil content and palmitic acid has been reported by Velasco *et al*. (2007) in sunflower and by Möllers and Schierholt (2002) in rapeseed. This negative correlation suggests that selection for higher oil content would automatically lead to a reduction in palmitic acid content. The correlation between oil content and oleic acid content was inconsistent at different temperatures. A significant positive correlation was observed in the control, but it was negative and weak in the heat treatment. A positive correlation between oil content and oleic acid content has been reported by Fernández-Martínez *et al*. (1993) and by Velasco *et al.* (2007) in sunflower. Selection for higher oil content would result in an increase in oleic acid content under normal temperature conditions. However, under heat stress conditions, selection for higher oil content would result in a reduction in oleic acid content. The relationship between oil content and stearic acid content was inconsistent at different temperatures. Under normal temperatures, this correlation was positive and non-significant. Conversely, under heat stress conditions it was negative and significant. As result, selection for both traits is feasible under normal temperatures, but under heat stress conditions selection for higher oil content would result in a reduction in stearic acid content. This observation was in accordance with Velasco *et al*. (2007) who reported an inconsistent correlation between oil content and stearic acid content. The strong and highly significant correlations observed between palmitic and oleic acid content in both temperature treatments were in agreement with reports by Ebrahimi *et al*. (2008) for sunflower and by Möllers and Schierholt (2002) for rapeseed. Selection for increased oleic acid content would lead to a reduction in palmitic acid content. Significant negative correlations between oleic and linoleic acid content were in accordance with other reports for sunflower (Lagravère *et al*., 2004; Qadir *et al*., 2006; Ebrahimi *et al*. 2008) and for rapeseed (Möllers and Schierholt, 2002).

Correlations between some traits were weaker in the heat treatment than in the control treatment. These were explained by the different responses of the particular traits to temperature treatment. The correlations between traits should be taken into account when parents are selected for a specific trait.

Variation between hybrids in response to high temperature can be used to select temperature stable hybrids for specific traits. The most stable hybrids (less sensitive to heat stress) that have shown small differences between the two temperature treatments for head diameter include the low oleic hybrids 7 and 10 and the mid oleic hybrid 2. The high oleic hybrid 4 was highly sensitive to heat stress for head diameter and showed a significant increase (3.03 cm) in head diameter. For number of filled seeds per head, the mid oleic hybrid 6 and the low oleic hybrids 8 and 12 were the most stable hybrids. However, the low oleic hybrid 9 was the most sensitive hybrid and showed a significant increase (204.96) in number of filled seeds per head. The low oleic hybrids 9 and 12 as well as the mid oleic hybrid 6 were the most stable hybrids for twenty-five seed weight. Conversely, the mid oleic hybrid 1 and the low oleic hybrid 3 were the most sensitive hybrids that showed significant reductions in twenty-five seed weight after heat stress. For head sterile centre diameter, mid oleic hybrids 1 and 2 as well as the low oleic hybrid 12 were the most stable hybrids. In addition, the high oleic hybrid 4 and the low oleic hybrid 10 were relatively stable across the two treatments for this trait. The mid oleic hybrid 5 was the most sensitive hybrid for heat stress and showed a large and significant increase (1.11 cm) in head sterile centre diameter.

With regard to oil quality characteristics, low oleic hybrids 8 and 10 were the most stable hybrids for oil content. The rest of the hybrids were sensitive and showed large reductions in oil content under heat stress. The high oleic hybrid 4 showed the largest decrease in oil content (11.80%) and was highly unstable for oil content. Low oleic hybrids 7, 8, 9 and 10 were the most stable hybrids for palmitic acid content. The rest of the hybrids (mostly the mid and high oleic hybrid) were sensitive to the heat stress and showed significant increases in palmitic acid content. All hybrids showed more or less the same sensitivity to heat stress. However, the mid oleic hybrid 5 was the most sensitive of all to heat stress and showed a large and significant increase (2.53%) in stearic acid content. For oleic acid content, the high oleic hybrid 4 and mid oleic hybrid 11 were the most stable hybrids between the two temperature treatments. Mid oleic hybrids 1 and 2 as well as the low oleic hybrids 7, 8, 9, 10 and 12 were the most unstable hybrids and showed large and significant increases in oleic acid content. With regard to linoleic acid, the high oleic hybrid 4 and mid oleic hybrid 11 were again the most stable hybrids. The rest of the hybrids showed significant decreases in linoleic acid content. The low oleic hybrids 10 and 12 were the most unstable hybrids with large reductions in linoleic acid content (25.66% and 23.34% respectively).

Although the high oleic hybrid 4 showed stability for oleic acid content under heat stress conditions, this hybrid did not perform well with regard to agronomical traits. This hybrid showed a significant increase in head diameter that indirectly resulted in the significant decrease in oil content observed for this hybrid. It was previously reported that larger heads resulted in increased hull content (Pustovoit, 1966) and since hull content is negatively correlated with seed oil content (Dennis *et al*., 1994), the larger head size resulted in lower oil content.

This knowledge on how sunflower hybrids respond to heat stress with regard to head and seed characteristics and oil quality may assist breeders in determining the correct breeding methods and selection criteria for environments where high temperatures may occur during critical growth stages and seed-filling periods.

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CHAPTER 5

IDENTIFICATION OF MOLECULAR MARKERS LINKED TO THE HIGH OLEIC ACID TRAIT IN SUNFLOWER

5.1 Introduction

Traditional sunflower oil is economically important because it is used in the food, feed and chemical industries. Sunflower oil is characterised by a high content of polyunsaturated fatty acids and most common varieties contain about 65-70% linoleic, 20% monounsaturated (oleic) and 10-14% saturated (palmitic and stearic) fatty acids. Breeding efforts in sunflower have focussed on modifying the proportions of fatty acid in the seed oil in order to increase its suitability for potential applications such as deep frying. Soldatov (1976) developed the first stable high oleic acid variety 'Pervenets" through chemical mutagenesis and further selections for high oleic acid in several generations. High oleic acid lines have been derived from Pervenets. High oleic varieties typically contain 80-90% oleic acid and low linoleic acid contents of between 5-9% (Gupta, 2002).

Many studies have been conducted to explain oleic acid accumulation in high oleic sunflower (Lacombe and Bervillé, 2000a; Lacombe *et al*., 2001; 2009). Oleic acid is produced from stearic acid by stearoyl-acyl carrier protein desaturase and subsequently desaturated into linoleic acid by ODS. In high oleic acid sunflower ODS activity is drastically reduced (Shanklin and Cahoon, 1998) that results in an increased oleic acid content of sunflower seeds. Further studies revealed a correlation between the high oleic acid phenotype and a strong reduction of ODS transcript accumulation in high oleic acid seeds during the critical stages of storage lipid synthesis (Kabbaj *et al*., 1996; Hongtrakul *et al*., 1998a. Lacombe and Bervillé, 2000a). It was suggested that the mutation that has occurred in Pervenets affected the structure of the high oleic specific ODS region. In a later study, Lacombe *et al*. (2009) demonstrated that the ODS allele is organised in two sections. The first is present in both high and low oleic acid genotypes and carries a normal ODS gene. However, the second section is specific to the high oleic acid genotype and carries duplications of the ODS gene. By studying messenger ribonucleic acid (mRNA) accumulation in high and low oleic acid seeds these authors revealed that the Pervenets mutation was dominant and induced ODS mRNA down-regulation. In addition, they found that ODS small interfering RNA (that is characteristic of gene silencing) accumulated only in high oleic acid seeds. Therefore it was concluded that the mutation was associated with ODS duplications that led to gene silencing of the ODS gene. As a result silencing of the ODS gene resulted in accumulation of oleic acid in high oleic seed (Lacombe *et al*. 2009).

On the other hand, many studies also have been conducted to study the genetics of the high oleic acid trait in sunflower. However, inheritance of the high oleic acid trait is complex and is not well understood. Contradictory results were obtained that are concerned with 1) the high oleic acid trait that behaved as dominant, recessive or intermediate, 2) the different number of genes and modifiers involved deduced from these studies and 3) the presence of some maternal effects. In addition, oil composition is largely influenced by the environment the plants are grown in as well as the genetic background of genotypes used. These factors may explain differences observed in previous genetic studies (Lacombe and Bervillé, 2000b).

Several studies demonstrated the importance of molecular markers in the genetic analysis of sunflower. The development of molecular markers has largely contributed to the establishment of saturated molecular maps. A number of linkage maps using various molecular markers, including RFLP, RAPD, AFLP and SSR markers have been published for sunflower (Berry *et al.*, 1995; Gentzbittel *et al.*, 1995; Gedil *et al.*, 2001; Tang *et al.*, 2002; Yu *et al.*, 2003; Rachid Al-Chaarani *et al.*, 2004). In general, most of these maps had 17 or more linkage groups that presumably corresponded to the haploid chromosome number (2n=2x=34) of cultivated sunflower. A unified consensus molecular genetic map, integrating independently developed linkage maps, has recently been published for cultivated sunflower (Paniego *et al.*, 2007). This framework covered 96% of the full sunflower genome. Molecular genetic linkage maps provide molecular breeders with a dense genome-wide framework of DNA markers that allow the mapping of qualitative traits and the localisation of factors underlying quantitative traits (Lu *et al*., 2007).

The application of DNA markers to sunflower breeding has been mainly focussed on the development of markers for some agronomic traits and disease resistance. RFLP, AFLP and SSR analyses have been applied in several studies to map QTL related to seed oil content (Leon *et al*., 1995; Mokrani *et al*., 2002; Bert *et al*., 2003; Rachid Al-Chaarani *et* *al*., 2004), seed weight (Mestries *et al*., 1998; Mokrani *et al*., 2002; Bert *et al*., 2003), days to flowering and photoperiod response (Leon *et al*., 2001; Mokrani *et al*., 2002; Rachid Al-Chaarani *et al*., 2004) and plant height, stem and head diameter (Rachid Al-Chaarani *et al*., 2004). QTL have been identified that showed association with *Sclerotinia sclerotiorum* (Lib) de Bary (Mestries *et al*., 1998; Rönicke *et al*., 2005), *Diaporthe helianthi* Munt-Cvet et al. (Bert *et al*., 2002), *Plasmopara halstedii* (Farl.) Berl. et de Toni. (Rachid Al-Chaarani *et al*., 2001; Kusterer *et al*., 2004) and *Phoma macdonaldii* Boerema (Rachid Al-Chaarani *et al*., 2001). In addition, the BSA approach (Michelmore *et al*., 1991.) has been applied in several molecular analysis studies to map genes related to disease resistance (Lawson *et al*., 1996; Lu *et al*., 2000; Kusterer *et al*., 2002; Tang *et al*., 2003), chlorophyll deficiency (Yue *et al*., 2009a), nuclear male sterility (Chen *et al*., 2006), fertility restoration (Kusterer *et al*., 2002; 2004) and gamma-tocopherol content (García-Moreno *et al*., 2006).

On the other hand, only a few molecular studies related to sunflower seed oil composition have been published. The molecular basis of modified fatty acid composition in sunflower seed oil has been studied through both QTL analysis and a candidate gene approach. Several genes coding for enzymes in the fatty acid biosynthetic pathway of seed oil have been cloned and their polymorphism studied (Hongtrakul *et al*., 1998a; 1998b; Lacombe and Bervillé, 2000a; Pérez-Vich *et al*., 2002). Genes encoding ODS are logical candidates for the *Ol* mutation. Marker studies related to high oleic acid content in sunflower began with the identification of two RAPD markers linked to the Ol_1 gene (Dehmer and Friedt, 1998). Later studies demonstrated that the Ol_1 gene cosegregated with the ODS gene, *FAD2-1*, that is strongly expressed in low oleic and weakly expressed in high oleic lines (Hongtrakul *et al*., 1998a). The Ol_1-*FAD2-1* locus mapped to LG 14 of the public sunflower genetic map and was found to underlie a major oleic acid QTL explaining 56% of the phenotypic variance for oleic acid (Pérez-Vich *et al*., 2002; Schuppert *et al*., 2006).

In breeding programmes for high oleic acid sunflower, oleic acid contents of seeds are usually determined using gas chromatography. Since environmental effects strongly influence seed oil composition (Harwood, 1996) it is difficult to differentiate between several high oleic acid alleles in high oleic acid genotypes or between homozygous or heterozygous plants for the high oleic acid trait. As a result, genotypic selection using linked molecular markers would be independent from the environment and is therefore a more reliable method (Pérez-Vich *et al.*, 2000). The aim of this study was to identify molecular markers linked to the high oleic acid trait, using the BSA method in conjunction with AFLP and SSR analysis.

5.2 Materials and methods

5.2.1 Plant material and glasshouse trial

A F_1 hybrid was developed from a cross between a high oleic line and traditional linoleic line from the PANNAR® breeding programme. Due to confidentiality reasons the names of the breeding lines may not be revealed. The F_1 hybrids were planted in a field trial at the Delmas breeding station and the F_2 seed were collected during harvest. The F_2 plant population was grown in the glasshouse at the University of the Free State in Bloemfontein from September 2006 to January 2007. The F_2 seed were initially sown in polystyrene seedling trays with Hygromix seedling growth medium (Hygrotech). This method was used to promote an optimal germination percentage. Seedlings were transplanted in 8 l planting pots. Pots were filled with Bainsvlei type red soil with N:P:K fertiliser [3:2:1 (25) + 0.5% Zn] and Curaterr® (2 ml per plant). A total of 80 F_2 plants were grown under normal conditions in the glasshouse. The minimum and maximum temperature was set at 15°C and 26°C respectively. Plants were watered daily by hand. Kompel Hydroponic fertiliser (6.5% N, 2.7% P, 13% K, 7% Ca, 2.2% Mg and 7.5% S) was applied every second week at 5 ml/l water. Red spider mite infestation was controlled by spraying plants every two weeks, alternatively with Red Spider Spray (Wonder) and AbamecPlus (R.T. Chemicals). Young leaves were collected from single plants after each plant formed 10 leaves. Leaves were rinsed with sterile distilled water and placed in sterile tubes and freeze-dried in a VirTis AdVantage freeze-drier for approximately four days. The freeze-dried samples were stored in a freezer at -80ºC for DNA isolation. Each F_2 sunflower plant's head was covered with transparent pollination bags to prevent cross pollination. After maturity, F_3 seed from each F_2 plant was harvested and seeds threshed out separately.

5.2.2 Seed oil extraction and fatty acid analysis

In order to do phenotyping of the individual plants of the segregating mapping population, five seeds from each parental plant and F_3 seed harvested from the 78 individual self-pollinated F_2 plants were prepared for oil fatty acid analysis. Five seeds instead of the 10 seeds generally analysed per plant (Bilyeu *et al.*, 2005; Yue *et al.*, 2009b), were used due to the high cost of fatty acid analysis. Nonetheless, five F_3 seeds proved to be sufficient to discriminate between three oleic acid groups: 1) the high oleic group that included individuals with mean oleic acid contents of above 85%, 2) the low oleic group that included individuals with mean oleic acid contents of less than 35% 3) the mid oleic group that included individuals that showed segregation for oleic acid and with oleic acid contents that ranged from 35-84% (Table 5.1).

Total lipids were quantitatively extracted from single ground seed samples by adding 5 ml hexane as solvent to the sample. Samples were thoroughly shaken for 1 min and left overnight at room temperature. Each sample was filtered into a 10 ml glass vial and the hexane was evaporated under a stream of nitrogen. Oil samples were stored under a blanket of nitrogen at -20ºC until analysed. Oil fatty acid analysis was done according to the method described in Chapter 3. The oleic and linoleic acid contents of the five single seeds of each plant were determined based on the fatty acid analyses. The phenotypes of the two parents (high oleic and low oleic) were confirmed and the 78 F_2 individuals were classified into the three oleic acid groups (high oleic, low oleic and mid oleic) according to the oleic and linoleic acid contents of their F_3 progeny.

5.2.3 Deoxyribonucleic acid (DNA) isolation

The freeze-dried leaf material was homogenised using a Qiagen TissueLyser. A small piece of leaf material together with two stainless steel ball bearings (5 mm diameter each) were placed into a 2 ml Eppendorf tube and grinded to fine powder for 30 seconds at 30 r/s.

Total genomic DNA from the parental lines and F_2 population was isolated from the homogenised leaf material using the hexadecyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al.*, 1984). CTAB DNA isolation buffer was prepared by mixing together SABEX water, 100 mM Tris-Cl (trishydroxymethyl-aminomethane) at pH 8.0, 20 mM EDTA (ethylene-diaminetetra-acetate) at pH 8.0, 1.4 M NaCl, 2% (w/v) CTAB and 0.2% (v/v) β-mercaptho-ethanol. CTAB buffer (750 μl) was added to each Eppendorf tube (containing ± 250 μl powdered leaf material) and incubated at 65°C in a water bath for 1 h. Chloroform:isoamylalchohol (500 μl) at a ratio of 24:1 (v/v) was added to each tube (containing the extraction suspension) and mixed.

Table 5.1 Mean and range for oleic and linoleic acid contents of sunflower seed oil of the parental lines and F_2 individuals used in the high and low oleic acid bulks (based on values of five F_3 seeds) from a cross between high and low oleic parents

	Mean and range of fatty acid content*			
	C18:1		**C18:2**	
Parent or F_2 individuals	**Mean**	**Range**	**Mean**	**Range**
Parents				
High oleic	86.05	85.52-86.60	2.56	2.08-3.69
Low oleic	21.47	19.12-24.17	63.46	59.44-66.39
F_2 individuals				
High oleic group				
1	85.52	82.90-86.64	2.74	1.76-4.54
2	89.57	89.19-90.81	1.60	0.98-2.51
3	89.52	88.16-90.59	1.32	0.87-2.23
4	87.91	84.42-89.83	2.85	1.28-6.86
5	90.58	90.29-91.15	0.99	0.55-1.71
6	89.24	88.10-89.60	1.01	0.81-1.32
7	89.02	86.71-90.56	0.75	0.63-0.89
8	87.42	84.33-90.06	0.99	0.70-1.25
9	87.91	86.08-89.39	0.55	0.38-0.81
10	90.72	88.25-92.87	0.68	0.50-0.88
Low oleic group				
1	34.74	28.85-40.03	52.27	48.32-55.48
2	33.85	28.30-41.72	51.53	43.89-57.81
3	29.05	16.97-46.45	59.16	41.63-71.74
4	30.62	13.85-39.99	57.45	48.13-74.81
5	19.46	17.02-22.74	67.57	64.66-70.13
6	32.30	28.67-35.26	55.28	53.20-57.32
7	27.36	13.11-40.26	55.58	45.49-68.53
8	31.88	18.17-47.12	57.57	43.05-71.15

*Fatty acids were expressed as percentages of total fatty acids, C18:1: Oleic acid, C18:2: Linoleic acid.

Tubes were centrifuged for 5 min at 12000 *g* to separate the DNA from the cell debris, proteins and chloroform:isoamylalchohol. DNA from the aqueous phase was precipitated using isopropanol (500 µl) at room temperature and left to stand for 20 min at room temperature. The sample tubes were centrifuged for 5 min at 12000 *g*. The supernatant was discarded and the tubes drained. The precipitate was washed by adding 70% (v/v) ice-cold ethanol (500 µl) and left to stand for 20 min at room temperature. Tubes were again centrifuged for 5 min at 12000 *g* and the supernatant discarded. The DNA pellet was left to air dry for ±1 h at room temperature. The air-dried pellets were resuspended in 200 µl TE (10 mM Tris-Cl at pH 8.0 and 1 mM EDTA at pH 8.0) buffer overnight at 4°C. The following day, 100 µg/ml DNAse-free RNase A was added to each sample tube and tubes were incubated at 37°C in a water bath for ±2 h. DNA was extracted with 0.75 M ammonium acetate and 200 µl chloroform:isoamylalchohol at a ratio of 24:1 (v/v) and centrifuged for 5 min at 12000 *g*. DNA from the aqueous phase was precipitated with 100% ice-cold ethanol (500 µl) and left overnight at 4°C. The following morning the tubes were centrifuged for 15 min at 12000 *g* and the supernatant was discarded afterwards. The DNA was washed twice with 70% (v/v) ice-cold ethanol (500 µl) by centrifuging for 10 min each time and the supernatant discarded afterwards. The sample tubes were left open at room temperature for the DNA pellets to dry. After ±2 h, the air-dried samples were resuspended overnight at 4°C in TE buffer at pH 8.0.

The quantity and quality of the DNA were estimated with agarose gel electrophoresis. An 0.8% (w/v) agarose gel was prepared and electrophoresis of the DNA was done for 1 h at 80 V in 1x UNTAN buffer (40 mM Tris-Cl and 2 mM EDTA, adjusted to pH 7.4 with acetic acid). DNA was visualised under UV light using ethidium bromide staining. DNA concentrations of samples were determined using a Helios Spectronic Unicam spectrophotometer. Absorbencies were measured at A260 nm and A280 nm and the ratio A260/A280 gave an indication of DNA purity. The DNA concentration was determined from the A260 nm reading and the genomic DNA from each sample was diluted to a working solution of 200 ng/µl.

5.2.4 Bulk segregant analysis

For BSA, equal amounts of genomic DNA from 10 individuals of the high oleic acid group and eight individuals of the low oleic acid group were respectively pooled to form the high oleic- and low oleic-bulk samples. Selection of individual F_2 plants for the bulks was based on the seed oil analysis of their F_3 progenies (Table 5.1). The DNA concentration of each individual constituting the bulks were 20 ng/µl for the high oleic-bulk and 25 ng/µl for the low oleic-bulk in order to create bulks with end-concentrations of 200 ng/µl each. Both bulks, together with the parents were used to identify markers showing linked polymorphisms between the four samples. These polymorphic markers were further used to analyse individual F_2 plants to determine linkages between AFLP markers and the high oleic acid trait.

5.2.5 Amplified fragment length polymorphism analysis

AFLP analysis was performed according to Vos *et al.* (1995) with modifications (Herselman, 2003). Adapters and primers used, together with their sequences, are given in Table 5.2. These were synthesised by Integrated DNA Technologies Inc. (Coralville, IA, USA). Primers were named “E” and “M” for *Eco*RI and *Mse*I, respectively, followed by a number representing a code for the three selective nucleotides, as indicated in Table 5.2. Oligonucleotides used for adapters were purified using polyacrylamaide gel electrophoresis (PAGE). Adapters were prepared by mixing equimolar amounts of both strands. These were heated for 10 min in a water bath at 65ºC. After the 10 min, the water bath was switched off and the adapters were left to cool down overnight to room temperature in the water bath.

Restriction digestion and adapter ligation

Genomic DNA from the two parental lines, the two bulks, the 18 high and low oleic individuals used to construct the bulks and 62 mid oleic F_2 individuals were digested using two restriction enzymes, *Mse*I and *Eco*RI. For each sample, 10 µl DNA (±2 µg) was digested using 4 U *Mse*I (New England Biolabs) and 1x *Mse*I-buffer [50 mM NaCl, 10 mM Tris-Cl, 10 mM $MgCl_2$ and 1 mM dithiotreitol (DTT) at pH 7.9]. Samples were placed in the water bath for 5 h at 37ºC. After the 5 h, 5 U *Eco*RI (Roche Diagnostics) and NaCl (to a final concentration of 100 mM) were added to the samples and the DNA was further digested overnight at 37ºC.

Table 5.2 Adapters and primers used for AFLP analysis to identify and map markers linked to the high oleic acid trait in a F_2 segregating population of sunflower

Enzyme	Adapter / primer	Sequence (5'-3')
*Eco*RI	Adapter-F	CTCGTAGACTGCGTACC
	Adapter-R	AATTGGTACGCAGTCTAC
*Mse*I	Adapter-F	GACGATGAGTCCTGAG
	Adapter-R	TACTCAGGACTCAT
*Eco*RI	Primer+0	GACTGCGTACCAATTC
*Mse*I	Primer+0	GATGAGTCCTGAGTAA
*Eco*RI	Primer+3	GACTGCGTACCAATTC*ANN* *ANN* = AAC (E32), AAG (E33), ACA (E35), ACC (E36), ACG (E37), ACT (E38), AGC (E40), AGG (E41)
*Mse*I	Primer+3	GATGAGTCCTGAGTAA*NNN* *NNN* = AAC (M32), ACA (M35), ACG (M37), AGT (M42), ATA (M43), CAA (M47), CAC (M48), CAG (M49), CAT (M50), CCA (M51), CCC (M52), CCG (M53), CCT (M54), CGA (M55), CGC (M56), CGG (M57), CGT (M58), CTA (M59), CTC (M60), CTG (M61), CTT (M62), GAA (M63), GAC (M64), GCA (M67), GCT (M70), GGA (M71), GGC (M72), TCG (M85), TCT (M86), TGA (M87), TGC (M88), TGT (M90)

Oligonucleotide adapters (Table 5.2) were ligated to the ends of the restriction fragments by adding a 10 µl mixture containing *Mse*I-adapter (50 pmol), *Eco*RI-adapter (5 pmol), 0.4 mM adenosine 5'-triphosphate (ATP), 1x T4 DNA Ligase buffer (66 mM Tris-Cl at pH 7.6, 6.6 mM $MgCl_2$, 10 mM DTT and 66 mM ATP) and 1 U T4 DNA Ligase (USB Corporation). Samples were incubated overnight in a water bath at 16ºC.

Pre-selective and selective amplifications

All PCR amplification reactions were performed in a DYAD™ Peltier Thermal Cycler. The pre-selective amplification step was included to reduce background smears (that result from mismatched amplification) in the AFLP pattern. A mixture containing 1x Promega DNA polymerase buffer (10 mM Tris-Cl at pH 9.0, 50 mM KCl and 0.1% (v/w) Triton X-100), 2 mM $MgCl_2$, 200 µM of each 2'deoxynucleoside 5' triphosphate (dNTP), 30 ng *Mse*I-primer+0, 30 ng *Eco*RI-primer+0 (Table 5.2) and 1 U GoTaq® Flexi DNA polymerase (Promega) was prepared. A volume of 45 µl of the mixture was added to 5 µl restriction/ligation solution. Pre-selective amplification started with a 5 min denaturation step at 94ºC that was followed by 30 cycles of the following cycle profile: a 30 s DNA denaturation step at 94ºC, a 1 min annealing step at 56ºC and a 1 min elongation step at 72ºC. A final elongation step at 72ºC for 10 min followed the last PCR cycle.

The pre-selective amplification fragments were separated on a 1.5% (w/v) agarose gel in order to determine the purity and quantity of the amplified DNA. Gel electrophoresis was performed at 80 V for 1 h using 1x UNTAN buffer. Ethidium bromide was added to the gel solution in order to visualise the fragments under UV light. DNA fragment sizes were determined by comparison with marker DNA fragments (an *Eco*RI/*Hin*dIII digest of lambda DNA). The pre-selective DNA was diluted with 1x TE buffer according to the observed quantity (1:15 to 1:30) and served as templates for the selective amplification reaction.

For selective amplification, a mixture containing 1x *Taq* polymerase buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 100 µg/ml Bovine serum albumin, 30 ng *Mse*I-primer+3, 30 ng *Eco*RI-primer+3 (Table 5.2) and 0.75 U GoTaq® Flexi DNA polymerase was prepared. A volume of 15 µl of the mixture was added to 5 µl diluted pre-selective DNA. Selective amplification started with a 5 min denaturation step at 94ºC, which was followed by 9 cycles of the following cycle profile: a 30 s DNA denaturation step at 94ºC, a 30 s annealing step at 65ºC, and a 1 min elongation step at 72ºC. The annealing temperature was subsequently reduced by 1ºC per cycle. The next cycle profile included: a 30 s denaturation step at 94ºC, a 30 s annealing step at 56ºC and a 1 min elongation step at 72ºC. This profile was repeated for 25 cycles followed by a final elongation step for 2 min at 72ºC.

5.2.6 Simple sequence repeat analysis

SSR analysis was conducted following the procedures of Tang *et al*. (2002). Primers used together with their forward and reverse sequences are given in Table 5.3. Primer sequences were supplied by PANNAR® (Biotechnology division in Greytown). SSR primers were selected based on the published sunflower map of Paniego *et al*. (2007). Since previous reports (Pérez-Vich *et al*., 2000; 2002) indicated that the high oleic acid trait mapped to LG 14, 10 SSR markers, spread over the entire length of LG 14, were selected for analysis. Genomic DNA from each sample (parental lines and entire F_2 population) was diluted to a working solution of 50 ng/μl. PCR was performed using a 10 μl reaction mixture containing 4 μl genomic DNA (0.2 μg), 1x *Taq* polymerase buffer, 2 mM $MgCl_2$, 200 μM of each dNTP, 30 ng of each forward and reverse primer (Table 5.3) and 0.5 U GoTaq® Flexi DNA polymerase. Amplification started with a 3 min denaturation step at 95ºC, followed by 1 cycle with the following profile: 30 s denaturation at 94ºC, 30 s annealing at 64ºC and 30 s elongation at 72ºC. The annealing temperature was decreased 1ºC per cycle in subsequent cycles until reaching either 58ºC or 59ºC (Table 5.3) depending on the primer pair. The next cycle profile included: 30 s denaturation at 94ºC, 30 s annealing at 58ºC and 30 s elongation at 72ºC. The profile was repeated for 32 cycles with a final elongation for 10 min at 72ºC.

5.2.7 Polyacrylamide gel electrophoresis

In order to visualise the amplification products (either AFLP or SSR), fragments were separated on 5% (w/v) denaturing polyacrylamide gels. The gel matrix consisted of 19:1 acrylamide:bis-acrylamide solution, 7 M urea, 1x TBE buffer (89 mM Tris-Cl, 89 mM boric acid and 2 mM EDTA), *N’N’N’N’*-tetramethylene-diamine (TEMED) and 0.22% (w/v) ammonium persulfate. The gel solution was carefully stirred and cast between two clean and treated glass plates. DNA products were mixed with equal volumes of formamide loading dye [98% de-ionised formamide, 10 mM EDTA at pH 8.0, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cyanol].

Table 5.3 Primers used for SSR analysis to identify and map markers linked to the high oleic acid trait in a F_2 segregating population of sunflower

Primer name	Sequence (5’-3’)	Annealing temperature
ORS301-F	CGTGACCTGTGAAACACCAA	58ºC
ORS301-R	CGATAACCGTGTGAATCGTC	58ºC
ORS307-F	CAGTTCCCTGAAACCAATTCA	58ºC
ORS307-R	GCAGTAGAAGATGACGGGATG	58ºC
ORS391-F	AGACTGGAGGGTATGGAGAGC	58ºC
ORS391-R	GCTCGGTTAAGGAGGGAGAAA	58ºC
ORS398-F	CACGTCCTAAATTAAGTAGGAACGA	58ºC
ORS398-R	CCAAGACCTCCGTTGAGCTAT	58ºC
ORS578-F	CTCTCAATCCCTAAAGTCCCT	58ºC
ORS578-R	TGGTGGATGTGGTTGTTGAT	58ºC
ORS694-F	CCTGGAACTGAACCGAGAAC	58ºC
ORS694-R	GCCGTGAAACAGAGAGAGGA	58ºC
ORS782-F	CCATGACGTGTTTGTGTGTGT	58ºC
ORS782-R	GCCAAGCGTACATCAGACTTT	58ºC
ORS1079-F	TACGACTGACGATTCCATTTCTC	59ºC
ORS1079-R	AACTGGATTTCACAGGGAGTGTT	59ºC
ORS1086-F	TTGTTTGTCGCACACTCAAGATT	59ºC
ORS1086-R	ATTATCGGCACATCTTTCCATTT	59ºC
ORS1206-F	GTTAAATCTGGTCATGTGCTTGC	59ºC
ORS1206-R	ACAGAATCAGGAACCATCTTCCA	59ºC

A 100 base pair and 25 base pair DNA ladder (Promega) was used to compare AFLP and SSR fragment sizes respectively. Prior to loading DNA products on the gel, these, together with the ladder, were denatured for 5 min at 95ºC in the thermal cycler and immediately put on ice afterwards. PAGE was performed at a constant power of 80 W for approximately 2 h for AFLP and 1:10 h for SSR. The same 1x TBE buffer used in preparing the gel matrix was used as running buffer.

5.2.8 Silver staining of polyacrylamide gels

The AFLP and SSR patterns in the polyacrylamide gel were visualised using the silver staining procedure described in the Silver Sequence™ DNA Sequencing System manual supplied by Promega. After staining, the gel on the glass plate was left to air-dry overnight. Photographs of the gel were taken by placing photographic paper (Ilford, Multigrade IV RC de lux) face to face with the gel and underneath the glass plate and exposing it to dim white light for approximately 22 s.

5.2.9 Identification of polymorphisms

The BSA strategy was used to target genes/QTL linked to the high oleic acid trait by establishing a linkage relationship between the phenotype and the polymorphic AFLP and SSR markers. Screening of AFLP and SSR primer pair combinations for polymorphic markers that show putative linkage to the high oleic acid trait was conducted with DNA samples from the two parental lines (one showing high oleic acid content and one showing low oleic acid content) and the two bulk samples (Table 5.1). A fragment showing presence in the high oleic parent and high oleic bulk and absence in the low oleic parent and low oleic bulk and vice versa was considered putatively linked to the high oleic acid trait.

The high oleic and low oleic parents and high oleic and low oleic DNA bulks were screened for linked polymorphism using 160 randomly selected *Eco*RI/*Mse*I primer combinations and 10 SSR primer pairs (Tables 5.2 and 5.3). Informative primer combinations that generated informative polymorphisms either in coupling or repulsion phase between the parents and the bulks and were used to screen the parents and the 18 high and low oleic individuals that were used to constitute the two bulks. Informative primers identified after screening the parents and bulk individuals were tested on the remaining 60 F_2 individuals form the segregating population.

5.2.10 Statistical analysis

Linked DNA fragments in both coupling and repulsion phase with the high oleic acid trait were identified and polymorphic fragments were dominantly scored for presence (1) or absence (0). A binary matrix reflecting these fragments was generated for each genotype. For phenotypic trait scoring, the average oleic acid content of the five seeds per individual was used. Linkage analysis of the putative markers was conducted using the software MAPMAKER/EXP version 3.0 (Lander *et al.*, 1987). The Haldane mapping function was used to determine the genetic distances between linked markers in centiMorgans (cM) from the recombination fractions. Interval mapping was used to link putative markers. A minimum log-likelihood score (LOD) of 3.0 with a maximum recombination frequency (theta) of 0.5 was employed. A coding system where the high oleic parent was seen as the homozygous parent and thus coded as B was used. QTL analysis was conducted using MAPMAKER/QTL version 1.1 (Lander and Botstein, 1989). Maps were drawn using Mapchart version 2.2 (Voorrips, 2002). The position of QTL was determined with interval mapping and a minimum LOD score of 3.0 was used for determination of significance. The percentage variation explained by the QTL was estimated at the maximum-likelihood QTL position.

5.3 Results

5.3.1 Phenotypic analysis of the F_2 population (F_3 seed) for oleic acid content

The mean oleic and linoleic acid percentage in the parental lines (high oleic and low oleic) and the F_2 individuals of the mapping population obtained from a cross between the two parents are shown in Table 5.4. The oleic acid contents of the F_3 seeds were assigned to three phenotypic classes: high oleic acid, low oleic acid and intermediate oleic acid. The oleic acid content of the high oleic bulk individuals ranged between 85.52-90.72%, while the oleic acid content of the low oleic bulk individuals ranged between 19.46-34.74%. The oleic acid contents of the mid oleic F_2 individuals ranged between 37.26-84.39%.

Table 5.4 Mean oleic and linoleic acid contents of sunflower seed oil of the high oleic acid and low oleic acid parents and F_2 individuals (based on values of F_3 seeds) from a cross between the two parents

		Fatty acid content*			
		C18:1		**C18:2**	
Parent or F_2 individuals	**Number of individuals**	**Mean**	**Range**	**Mean**	**Range**
Parents					
High oleic	1	86.05	85.52-86.60	2.56	2.08-3.69
Low oleic	1	21.47	19.12-24.17	63.46	59.44-66.39
F_2 individuals					
High oleic bulk individuals	10	88.74	85.52-90.72	1.35	0.55-2.85
Low oleic bulk individuals	8	29.91	19.46-34.74	57.05	51.83-67.57
Mid-oleic individuals	60	60.79	37.26-84.39	26.97	5.56-48.78

*Fatty acids were expressed as percentages of total fatty acids, C18:1: Oleic acid, C18:2: Linoleic acid.

5.3.2 Amplified fragment length polymorphism analysis

Analysis of the high and low oleic parents and the two bulks with a total of 160 *Eco*RI/*Mse*I primer combinations, identified a total of 9058 loci, of which 41 (0.45%) were polymorphic between the two bulks (Table 5.5). A maximum of 104 and a minimum of 24 loci, with an average of 56.61 loci, were detected by a single primer pair combination. From the total of 160 primer combinations tested, 32 (20%) displayed linked polymorphisms between the two parental lines and two bulk samples and from these only 14 (8.75%) primers generated linked polymorphisms between parents and 18 individuals of the two bulks. A maximum of 21 polymorphisms per primer pair were scored between the two parental lines, with an average of 8.67 polymorphisms per primer pair. The 14 primer combinations that were informative between the two parents and individuals from the bulks were tested on the rest of the mid oleic population to confirm co-segregation of fragments with the high oleic acid trait.

5.3.3 Simple sequence repeat analysis

Analysis of the two parental lines and the two bulk samples with 10 primer pairs identified a total of 12 loci. Of the 10 primer pairs tested, three (25%) generated polymorphisms between the two parents, while two (16.6%) generated polymorphisms between the two parents and two bulks. The two primer pairs that were informative between the individuals from the bulks were then tested on the rest of the mid oleic population to confirm co-segregation of the fragments with the high oleic acid trait.

5.3.4 Linkage analyses of putative markers related to the high oleic acid trait

Data obtained from 14 AFLP two SSR primer pairs that were informative between the two parents and 18 high and low oleic individuals from the segregating population used to construct the bulk samples (and that were screened on the entire population), were used to order markers into a linkage group (Figure 5.1). The linkage group was constructed from the information from 16 putative AFLP and four SSR markers using MAPMAKER/EXP. Markers linked both in coupling and repulsion phase with the oleic acid trait were used for linkage mapping. The putative AFLP markers were named using the universal code of the primer combinations.

Table 5.5 AFLP analysis data obtained using *Eco*RI/*Mse*I primer combinations

	***Eco*RI/*Mse*I reactions**
Primers tested	160
Total number of fragments detected	9058
Total polymorphisms	1417
Informative primers between parents	89%
Informative primers between bulks	20%
Average fragments per primer pair	56.61
Maximum fragments per primer pair	104
Minimum fragments per primer pair	24
Maximum polymorphisms per primer pair	21
Polymorphic fragments in parents	15.31%
Polymorphic fragments in bulks	0.45%
Average polymorphisms per primer (parents)	8.67
Average polymorphisms per primer (bulks)	0.26

Of the total of 20 markers used, 19 markers (15 AFLP and 4 SSR) mapped to a single linkage group (Table 5.1). Since all four tested SSR markers mapped to this linkage group and since they mapped to LG 14 of sunflower during several previous studies (Pérez-Vich *et al*., 2000; 2002; Paniego *et al.*, 2007), it was assumed that the linkage group obtained in this study was the same as LG 14 of these reports. One marker was unlinked. Putative LG 14 covered a distance of 147.2 cM. Distances between adjacent markers on putative LG 14 ranged from 2.6-29.0 cM. A clustering of SSR markers ORS1086.2, ORS307.1 and ORS307.2 was observed on putative LG 14.

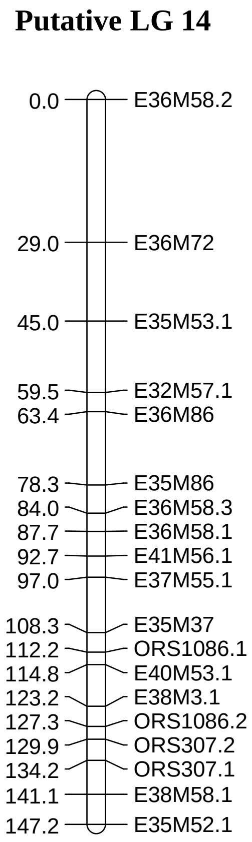


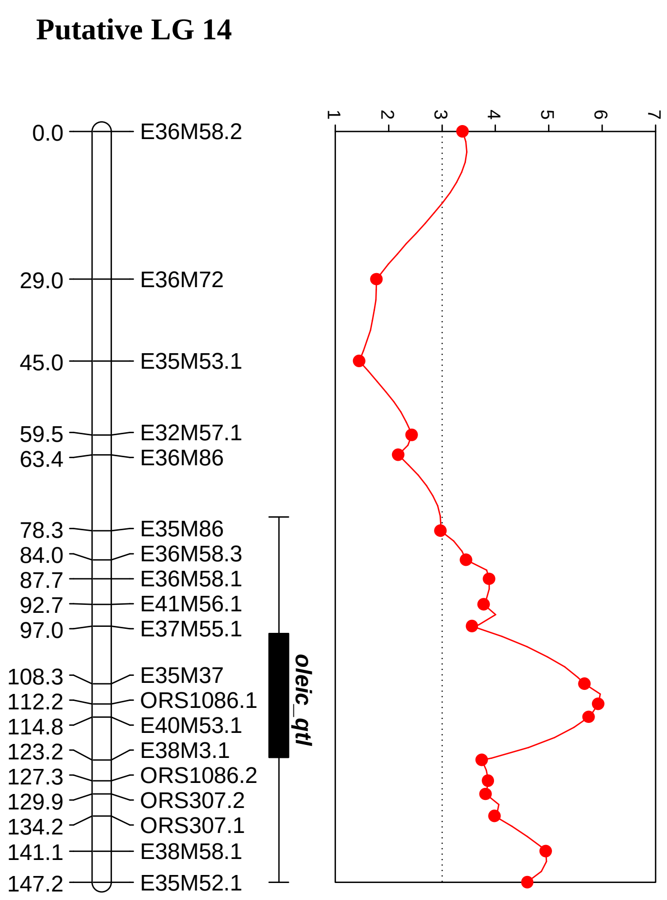
Figure 5.1 AFLP and SSR marker order of putative linkage group 14 of sunflower based on a segregating F_2 population. Names of markers are shown on the right and their map position (cM) on the left. LG: Linkage group. Codes used for marker names are given in Table 5.2.

5.3.5 Identification of putative quantitative trait loci for high oleic acid genes

Data obtained from the linkage analysis were used in MAPMAKER/QTL in order to identify putative QTL linked to the high oleic acid genes. Putative QTL were identified based on LOD scores higher than 3.0. The LOD profile identified the most likely position for the QTL in relation to the linkage group. Putative LG 14 was scanned using free, dominant and additive genetics. One major QTL was identified on putative LG 14 with a LOD score of 5.97 (Figure 5.2) using additive genetics. The QTL detected on putative LG 14 explained 35.0% of the phenotypic variance for high oleic acid in the segregating population. The putative QTL was detected between flanking marker loci E37M55.1 and E38M3.1 with a genetic distance of 26.2 cM. Two AFLP marker loci, E35M37 and E40M53.1 with LOD scores of 5.67 and 5.79 respectively mapped within the QTL region, 6.5 cM apart. In addition to AFLP markers, one SSR marker, ORS1086.1 with a LOD score of 5.93, also mapped within the QTL region. This SSR marker mapped in the middle of the two AFLP marker loci, 3.9 cM from E35M37 and 2.6 cM from E40M53.1.

5.4 Discussion

The AFLP and SSR techniques in conjunction with the BSA method were successful in targeting markers located in specific chromosomal regions that are linked to the high oleic acid trait in high oleic sunflower. AFLP analysis was conducted since markers obtained are reliable and reproducible and AFLP has the capacity to inspect an entire genome for polymorphism and the number of polymorphisms detected per reaction is high (Vos *et al.*, 1995). AFLP analysis was furthermore selected for this study in an attempt to increase the marker density on the targeted chromosomes in order to improve QTL analysis for the trait of interest, namely high oleic acid. However, AFLP markers are unsatisfactory for anchoring and cross-referencing of linkage maps and therefore their use is limited in breeding programmes. Consequently SSR analysis was employed to overcome these constraints and to determine whether there was a relationship between the QTL map obtained in this study and QTL previously reported for the high oleic acid trait. This was the first publicly available report of using SSR markers in QTL analysis to characterise the genomic regions for the high oleic acid trait.



Putative LG 14

Figure 5.2 Linkage map of putative linkage group 14 indicating the chromosomal regions containing the quantitative trait loci (QTL) associated with the high oleic trait and the relative distances of the markers from the QTL. LG: Linkage group. The linkage map of putative LG 14 is on the left. Marker names and their genetic distances (cM) are indicated at the right and left sides of the linkage map respectively. The QTL likelihood-profile for the high oleic acid trait is given on the right. LOD scores and map distances are indicated on the horizontal and vertical axes respectively. The bar graph in the middle of the figure represents the standard 1 LOD (shaded region) and/or 2 LOD support intervals for the oleic QTL region.

AFLP markers used in this study that were also used in a previous study are E32M57 and E35M52 (Pérez-Vich *et al*., 2002). Although a number of SSR markers have been used in previous studies to develop linkage maps in sunflower, none of these markers have previously been linked to the high oleic acid trait in sunflower. Since major QTL for oleic acid content in sunflower have previously been mapped to LG 14 (Pérez-Vich *et al*., 2000; 2002), SSR markers used in this study were selected from LG 14 of genetic linkage maps previously been constructed for sunflower (Tang *et al*., 2002; Yu *et al*., 2003; Paniego *et al*., 2007).

Fatty acid analysis of F_3 seeds of the F_2 population from a cross between a high and a mid oleic acid parent demonstrated the existence of three oleic acid groups: one with low oleic acid content (19.46-34.74%), one with high oleic acid content (85.52-90.72%) and a third (intermediate) group formed by individuals whose oleic acid content ranged from 37.26-84.39%. According to these values, individual F_2 plants were classified into three groups namely the high oleic, low oleic and mid oleic groups. High oleic- and low oleic-DNA bulks were formed by pooling equal amounts of genomic DNA from 10 high oleic and eight low oleic individuals respectively. By making bulks, all loci were randomised except for the region containing the high oleic acid trait. After screening the parents and the two bulks with a total of 160 *Eco*RI/*Mse*I primer combinations and 10 SSR primer pairs, 20 markers putatively linked to the high oleic acid trait were identified.

A linkage group for the F_2 population segregating for the high oleic acid trait was constructed using information from 16 putative AFLP and four putative SSR markers. Seven of these markers were linked in repulsion phase and 13 in coupling phase. From the 20 putative markers, 19 (15 AFLP and four SSR) mapped to a single linkage group and covered a distance of 147.2 cM. Linkage analysis generated only a single linkage group since BSA, being a gene targeting approach, generates only a few linkage groups on the targeted regions with a limited number of markers. Distances between adjacent markers ranged from 2.6-29.0 cM. Large distances were observed between E36M58.2 and E36M72, between E36M72 and E35M53.1 as well as between E35M53.1 and E32M57.1. The large distances observed between these markers might be due to lack of markers within these regions and also probably because these markers were on the distal end of the chromosome.

The use of SSR markers, that are highly polymorphic in mapping populations, made it possible to determine whether there existed a relationship between the previously mapped QTL and QTL found in the present study. Both SSR markers that showed linked polymorphisms between the parental lines and individuals of the bulks, mapped with AFLP markers to the same linkage group after being tested on the entire mapping population. Since both these SSR markers previously mapped to LG 14 in sunflower (Tang *et al*., 2002; Yu *et al*., 2003; Paniego *et al*., 2007), they could be used to anchor the current linkage group to LG 14 of previous maps.

QTL analysis was performed to detect an association between the high oleic acid phenotype and markers. A major QTL with a LOD score of 5.97 was identified on putative LG 14 and explained 35.0% of the variation for this trait. The LOD score and phenotypic variance explained by this QTL was low in comparison with previous reports for high oleic acid trait in sunflower. Pérez-Vich *et al*. (2000) identified a major high oleic acid QTL on LG 14 with a peak LOD score of 33.8 and this QTL explained 83.9% of the variation. Additionally, they identified minor QTL on LG 8 with a peak LOD of 2.39 and that explained 0.6% of the variation. In another study, Pérez-Vich *et al.* (2002) constructed a linkage map with AFLP and RFLP markers and identified QTL affecting oleic acid content on LGs 1, 8 and 14. These QTL together accounted for 58.4% of the phenotypic variance. Individual QTL accounted for 24.5% (LG 1 with peak LOD 8.5), 9.6% (LG 8 with peak LOD 3.1) and 56.5% (LG 14 with peak LOD 19.7). In a later study**,** Kusterer *et al*. (2004) identified two QTL (located at the same position) for both linoleic and oleic acid content on linkage group A. The first QTL (LOD 3.19) explained 28.4% while the second QTL (LOD 3.79) accounted for 27.6% of the variation. These two QTL together explained 56.0% of the phenotypic variation for oleic acid content. Linkage group A of this map might correspond to LG 14 of Pérez-Vich *et al.* (2002), however, it still needs to be proven.

Accordingly, there should be other major and/or minor QTL with smaller effects that contribute to the high oleic acid trait, that were not detected in the present study. Reasons for this are that with the BSA approach it is possible that some major and minor QTL with smaller effects might not be detected and that the size of the segregating population was too small. By increasing the population size and by using whole genome QTL analysis instead of the BSA approach it is more likely to detect QTL with smaller effects (Haley and Andersson, 1997). In addition, the profound influence of the environment (Collard *et al.*, 2005) on the expression of the high oleic acid trait as well as the fact that the sunflower trial was planted in the glasshouse (one environment and for one year) may have had an influence on the low number of QTL detected.

Selection for the high oleic acid trait based on the two markers flanking the oleic acid QTL region (E37M55.1 and E38M3.1) would be more reliable than selection based on one single marker detected within the QTL region. However, the genetic distance between the two flanking markers is large (26.2 cM) and there is a chance for recombination to occur within the QTL region. However, since the SSR marker ORS1086.1 mapped within the QTL region, this marker should be more useful and reliable than the AFLP markers in high throughput screening for the high oleic acid trait in a breeding programme.

Although the BSA technique has some limitations, this approach was successful in this study since 19 out of 20 identified markers mapped to a single linkage group, putative LG 14, and the QTL related to the high oleic acid trait also mapped to this linkage group. In addition, phenotyping of five F_3 seeds instead of 10 or more seeds was effective to accurately map the QTL.

In future research, additional markers should be tested for linkage with the high oleic acid trait in the population used in this study in order to develop a more saturated linkage group. In addition, SSR markers identified by Lacombe *et al.* (2009), that showed linkage with the Pervenets mutation, should be screened on the parents and on the entire population in order to detect polymorphisms between high and low oleic acid genotypes. If linked polymorphisms are detected, these should be included in QTL analyses. If these markers also map to putative LG 14 and within the high oleic acid QTL region, it is possible that QTL identified in this study could be genetically linked to the Pervenets mutation explained by Lacombe *et al*. (2009).

Additional major and/or minor QTL influencing the high oleic acid trait should also be mapped. Furthermore, markers identified in this study should be validated by testing their effectiveness in determining the target phenotype in different populations from different genetic backgrounds and by testing in different environments and years since environment has a profound influence on fatty acid composition in sunflower. For these purposes it might be necessary to develop an "eternal" mapping population such as recombinant inbred lines (RILs) or doubled haploid lines in order to conduct replicated trials across different locations and years.

Although the AFLP marker technique was a reliable and stable and generated a high number of markers in a short period of time, AFLP markers are dominant, multicopy and unsatisfactory for anchoring and cross referencing linkage maps. In addition, AFLP analysis is expensive, time-consuming and technologically demanding, and therefore the use of AFLP markers for large scale screening in breeding programmes is limited. This constraint may be overcome by converting AFLP markers into sequence characterised amplified region (SCAR) or STS markers that are technically simpler, less expensive and more efficient (Tang *et al*., 2003; Collard *et al*., 2005). Several studies reported the successful conversion of AFLP markers to SCAR markers that were associated with agronomic traits in *B. juncea*, cowpea and Ponkan mandarin (Negi *et al*., 2000; Boukar *et al*., 2004; JinPing *et al*., 2009). As a result, the AFLP markers detected in this study may need to be converted to single copy, co-dominant SCAR markers to facilitate their wider use in MAS in sunflower breeding.

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CHAPTER 6

PHYSICOCHEMICAL AND OXIDATIVE STABILITY CHARACTERISTICS OF HIGH AND MID OLEIC SUNFLOWER SEED OIL

6.1 Introduction

Sunflower oil is the major product of seed processing that contributes approximately 80% to the total value of the crop. The oil is mostly used as a cooking medium, but is often used in salad oil and to manufacture margarine, shortening and other products. Sunflower oil has a desirable fatty acid composition for use as edible oil. It is a polyunsaturated oil with a relatively low total saturated fatty acid content. However, because of its high unsaturation level, sunflower oil is highly susceptible to oxidation (Salunkhe *et al*., 1992).

Oxidative stability refers to the resistance of oils to lipid oxidation during processing and storage (Guillen and Cabo, 2002). Different types of oxidation occur and these include autoxidation, photo-oxidation, thermal oxidation and hydrolytic oxidation (O’Brien, 2009). The consequences of lipid oxidation are the formation of off-flavours and odours, associated with rancidity, as well as loss of functional and nutritional value (Nawar, 1996; Stauffer, 1996; Grompone, 2005). Oxidative stability is therefore an important indicator to determine oil quality and predict oil shelf-life (Pike, 2001; Martín-Polvillo *et al*., 2004; Choe and Min, 2006).

Oil oxidative stability depends on various factors including oil composition, minor antioxidant or pro-oxidant compound content and degree of processing. Oil storage conditions and packaging also affect the rate of oxidation. Oil storage in light or dark conditions, temperature and oxygen availability may influence the oil’s oxidative stability (Crapiste *et al*., 1999; Abramovic and Abram, 2005). These factors interactively affect the oxidation of oil and it is difficult to differentiate between their individual effects (Choe and Min, 2006). Autoxidation can be retarded by storing oil in the dark, refrigeration or freezing, vacuum packaging and packing under an inert gas to exclude oxygen. The addition of antioxidants to oil is highly preferable to suppress oxidation (Merrill *et al*., 2008) because the above storing conditions are not always applicable and only a small amount of oxygen in the oil is necessary to initiate and maintain oxidative processes (Naz *et al*., 2004).

The degree of unsaturation of fatty acids is one of the most important parameters that influence oil oxidative stability (Holman and Elmer, 1947; Merrill *et al*., 2008). Much research has focussed on improving oil stability and nutritional quality by altering the fatty acid composition. This was achieved through conventional breeding, coupled with mutagenesis in some cases. Oils that have been developed by decreasing the polyunsaturated (linoleic and linolenic) fatty acids and increasing the monounsaturated (oleic) acid content have improved oxidative stability compared to traditional oils (Liu and White, 1992; Neff *et al*., 1994; Hu *et al*., 2006; Smith *et al*., 2007). These include soybean (Patil *et al*., 2007), canola and rapeseed (Auld *et al*., 1992; Spasibionek, 2006), safflower (Knowles *et al*., 1965) and sunflower oil (Soldatov, 1976).

Oxidative degradation is directly related to the polyunsaturated fatty acid content of the oil (Neff *et al*., 1994). Oxidation essentially takes place at the double-bond (unsaturation) sites in triglyceride molecules (Sherwin, 1978; Stauffer, 1996). Consequently the higher the number of double bonds in the triglyceride structure (the higher the unsaturation level of fatty acids), the more susceptible the oil is to oxidative degradation and the higher the rate of oxidation (Gray, 1978; Sherwin, 1978; Kamal-Eldin, 2006). As a result, polyunsaturated oil is more susceptible to oxidation than monounsaturated oil.

The high monounsaturation makes high oleic sunflower oil much less susceptible to oxidative degradation than traditional sunflower oil with high polyunsaturation (Dorrell and Vick, 1997). For that reason, sunflower oils with high oleic acid contents and low linoleic acid levels should prove more resistant to oxidation than traditional sunflower oil. The aim of this study was to evaluate and compare the physicochemical properties along with the oxidative stability of traditional and high oleic sunflower hybrids developed and grown in South Africa.

6.2 Materials and methods

6.2.1 Seed material

The seed analysed in this study were obtained from a PANNAR® breeding trial planted in Lichtenburg in 2008. The trial layout was the same as trials described in Chapter 3. Seed were harvested in May 2009. Seed material included three traditional (linoleic), three high oleic and one mid oleic hybrid entry with three replications for each entry. Due to confidentiality reasons the names of hybrids may not be revealed. Once received, seed were vacuum-sealed and stored at 4ºC until needed for oil extraction.

6.2.2 Seed oil extraction and fatty acid analysis

Crude seed oil was extracted using a Soxhlet extraction procedure at a rate of six to eight drops per minute with diethyl ether as solvent. Sunflower achenes were ground with a coffee grinder and more or less 70 g of sample was weighed into each thimble. Thimbles were placed in a vacuum oven at 50ºC and left overnight. Phosphorus pentoxide was used as moisture absorbent. The following morning, thimbles were placed in the Soxhlet apparatus and the oil extracted from the dried sample using diethyl ether as solvent. After extraction, the diethyl ether was evaporated on a water bath and the oil weighed. Flasks were left overnight in the refrigerator and the oil filtered the following morning to remove waxes. The filtered oil was again placed in the vacuum oven for two hours at 50ºC. The oil was cooled to room temperature and poured into individual dark bottles. Oil samples were stored with nitrogen in the headspace. Fatty acid analysis was done according to the methods described in Chapter 3.

6.2.3 Physicochemical properties

Refractivity index (RI) was determined according to the Association of Official Analytical Chemists' (AOAC) Official Method 921.08 (2000) with an Abbé automatic digital refractometer from ATAGO® (Model RX 5000α). Two droplets of the extracted lipid were placed on the glass prism of the sample chamber. The RI-readings were done in triplicate at a temperature of 40°C. After each reading, the prism was wiped with 100% ethanol and dried with clean, dry, soft tissue paper. Distilled water was used as a blank (with a known RI-value of 1.33061) and the blank was used to calibrate the refractometer after every seventh sample.

Iodine value (IV) was determined according to AOAC Official Method 920.158 (2000). A sample of 0.5 g lipid, from the Soxhlet extraction, was weighed into a 250 ml Erlenmeyer flask. A volume of 10 ml chloroform was added and the flask shaken until the lipid dissolved. Then 25 ml of the Hanus iodine bromine solution (0.1 M IBr in glacial acetic acid) was added and allowed to stand for 30 min in the dark. After the 30 min, 10 ml of a 15% potassium iodide solution and 100 ml distilled water was added and the flask was shaken. The sample was titrated with 0.1 N sodium thiosulfate. As the yellow colour became faint, a few drops of starch indicator were added and when the blue colour disappeared, the endpoint was reached. The IV was expressed as the number of gram iodine absorbed by 100 g of lipid. IV is determined by the formula (Jacobs, 1958):

$$\text{Iodine/100 g fat} = \frac{[(\text{difference of ml blank} - \text{ml of sample}) \times \text{N of } Na_2S_2O_3) \times 126.91]}{\text{Weight of sample} \times 10}$$

FFA content was determined according to the method described by Pearson (1973). A sample of 0.5 g lipid, from the Soxhlet extraction, was weighed into a 250 ml glass beaker. A solvent was prepared by mixing 20 ml of ethanol [95% (v/v)] with 1 ml phenolphthalein indicator [1% (v/v) in alcohol]. The mixture was neutralised by adding 0.1 N NaOH from a buret. The solvent was added to the oil sample and titrated with 0.1 N NaOH, while constantly being stirred, until the sample's colour turned pink and the pink colour persisted for at least 15 s. FFA was calculated as free oleic acid on a percentage basis: If titration = V ml of 0.1 N of NaOH and W = weight of the sample (g), then

$$\text{FFA (as oleic acid \%)} = (V \times 0.0282 \times 100)/W$$

6.2.4 Determination of oxidative stability

PV of the extracted oils was determined according to the AOAC Official Method 965.33 (2000). A sample of 1.0 g oil was weighed into a 250 ml glass Erlenmeyer flask. A solution of acetic acid and chloroform (3:2) was prepared and 30 ml of this solution was added to the sample. Thereafter, 0.5 ml saturated potassium iodide solution was added to the sample and stirred for precisely 1 min. After 1 min, 20 ml distilled water was added to the sample to stop the reaction. A 1% (w/v) starch indicator was prepared and 0.5 ml of the starch indicator was added to the sample. The sample was titrated with 0.01 N sodium thiosulfate until a specific colour reaction was observed. The reading on the buret was taken and the value was used in the formula to calculate the peroxide value. The peroxide value is expressed in terms of milli-equivalents (meq) peroxide per 1000 g of sample:

$$\text{PV} = (S \times N \times 1000) / \text{weight of sample}$$

where S is the titration value of the sample and N is the normality of sodium thiosulfate solution.

The *p*-anisidine value (*p*-AV) was determined according to the method described by Hamilton and Hamilton (1992). A sample of 0.5 g oil was weighed into a 25 ml volumetric flask. The oil was dissolved with isooctane and the solution was diluted to volume with a disposable pasteur pipet. The spectrophotometer was calibrated at 350 nm absorbency with isooctane as blank. The absorbance (*Ab*) of the fat solution was measured at 350 nm with a Thermo Spectronic spectrophotometer (Genesys™ 10 series). The *ρ*-anisidine was prepared by weighing 0.0625 g anisidine into a 25 ml volumetric flask and adding 25 ml of glacial acetic acid to the flask. The solution was stirred to dissolve the anisidine. Exactly 5 ml of the fat solution and 1 ml of the anisidine reagent was pipetted into a glass test tube. The blank contained 5 ml isooctane solvent and 1 ml anisidine reagent. Tubes were shaken and left for 10 min. Thereafter, the blank was used to calibrate the spectrophotometer and the absorbance (*As*) of the fat solution with anisidine was measured. The colour was quantitated and converted to AV. The *p*-AV is given by the formula:

$$p\text{-AV} = [25 \times (1.2As - Ab)] / m$$

where *As* is the absorbance of the lipid solution after reaction with the *p*-anisidine reagent, *Ab* is the absorbance of the lipid solution and *m* is the mass (g) of the lipid.

The OSI measurements were done at Antioxidants Aromas and Fine Chemicals (PTY) LTD in Richards Bay. The oxidative stability instrument from Omnion was used according to the American Oil Chemists' Society (AOCS) Official Method Cd 12b-92. The oil sample was held in a thermostated bath and oxidation was carried out at 110ºC. A stream of purified air was passed through the lipid at a rate of 2.5 ml/s. The effluent air from the lipid sample was bubbled through a vessel containing deionised water. The conductivity of the water was continuously measured as the effluent air contained volatile organic acids (swept from the oxidising oil) which increased the conductivity of the water as oxidation proceeded. The conductivity of the water was monitored by a computer. The time it took for the oil sample to begin a rapid increase in conductivity determines the induction period endpoint (Pike, 2001). The time-based end point was determined by a computer that calculated the maximum of the second derivative of the conductivity with respect to time.

6.2.5 Statistical analyses

ANOVA and LSD tests were conducted to identify differences among means using Agrobase (2005).

6.3 Results

6.3.1 Oil and fatty acid content

The mean seed oil content varied between 35.65-36.56% among the traditional, high oleic and mid oleic oil types (Table 6.1). On average, the three oil types did not differ significantly for oil content even though the traditional oil contained 0.6% more oil than the high oleic oil. The traditional hybrid 3 contained the highest percentage of oil (38.08%) and this was significantly higher than the oil contents for the traditional hybrid 1 and the high oleic hybrid 6. The mid oleic hybrid 7 had, on average, a similar oil content as the traditional hybrids, but was not significantly different from any other hybrid.

The predominant fatty acids detected were palmitic (C16:0), stearic (C18:0), oleic (C18:1c9) and linoleic acid (C18:2c9,12). Palmitoleic (C16:1), α-linolenic (C18:3c9,12,15), arachidic (C20:0), eicosenoic (C20:1c11), eicosadienoic (C20:2c11,14), behenic (C22:0) and lignoceric (C24:0) acid were detected in minor percentages in all oils, while vaccenic acid (C18:1c7) was only detected in the traditional and mid oleic oils. Insignificant percentages of myristic acid (C14:0) were detected in a small number of oil samples. Only major fatty acids palmitic, stearic, oleic and linoleic, occurring at levels more than 0.5%, are presented and discussed (Table 6.1).

Averages for palmitic acid content ranged between 3.40-6.28% among the three oil types (Table 6.1). Significant differences were observed between oil types for palmitic acid content. The traditional oil contained on average 2.88% and 1.47% more palmitic acid than the high oleic and mid oleic oils respectively. The palmitic acid content of traditional hybrid 3 was significantly higher than all other hybrids. However, the individual high oleic hybrids did not differ significantly for palmitic acid content.

Table 6.1 Mean oil and major fatty acid contents of traditional, high oleic and mid oleic sunflower oil types

Oil type	**Hybrid**	**Oil content**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**MUFA**	**PUFA**	**SFA**	**PUFA/SFA**
Traditional	1	34.46b	5.91b	3.81a	26.95b	61.96b	27.12b	62.02b	10.86b	5.71a
	2	36.20ab	6.22b	3.83a	22.66c	65.97a	22.82c	66.04a	11.14b	5.93a
	3	38.08a	6.70a	3.89a	19.12c	69.04a	19.26c	69.10a	11.64a	5.94a
Mean		*36.25*	*6.28*	*3.84*	*22.91*	*65.66*	*23.07*	*65.72*	*11.21*	*5.86*
High oleic	4	35.51ab	3.27c	4.07a	84.08a	6.61c	84.31a	6.71c	8.98de	0.76b
	5	36.78ab	3.25c	3.62a	81.67a	9.45c	81.93a	9.55c	8.53e	1.12b
	6	34.66b	3.68c	4.14a	81.94a	8.28c	82.18a	8.43c	9.39cd	0.90b
Mean		*35.65*	*3.40*	*3.94*	*82.56*	*8.11*	*82.81*	*8.23*	*8.97*	*0.93*
Mid oleic	7	36.56ab	4.81d	3.57a	53.77d	36.37d	53.95d	36.42d	9.63c	3.78c
LSD (0.05)		2.93	0.47	0.60	3.77	3.74	3.77	3.74	0.47	0.37

LSD: Least significant difference, C16:0: Palmitic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, SFA: Saturated fatty acids. Mean values followed by the same letter did not differ significantly at $P \leq 0.05$.

A highly significant and negative correlation was observed between palmitic and oleic acid (Table 6.2). The higher the oleic acid, the lower the palmitic acid content. This negative correlation was evident in the traditional and high oleic sunflower oil types. The traditional oil contained more palmitic acid than the high oleic oil. The strong and highly significant positive correlation between palmitic and linoleic acid can be attributed to the strong negative correlation between oleic and linoleic acid. Although both palmitic and stearic acid contributed to the SFA content, only palmitic acid showed a strong and significantly positive correlation with SFA content.

Stearic acid content varied between 3.57-3.94% among the three oil types (Table 6.1). No significant differences were observed between individual hybrids (and as result, between the three oil types) for stearic acid content. A significant and negative correlation between stearic acid and oil content was observed (Table 6.2). However, this correlation was only observed for high oleic and mid oleic hybrids. As oil content increased, stearic acid content decreased. Although significant variation occurred between the traditional hybrids for oil content, their stearic acid contents were more or less the same and the correlation appeared to be positive between stearic acid and oil content.

Oleic acid content varied between 19.12-26.95% among the traditional hybrids (Table 6.1). The traditional hybrid 1 showed significantly higher oleic acid content (26.95%) than the other two traditional hybrids (22.66% and 19.12% respectively). The high oleic oil types contained the highest oleic acid content (82.56%) which was significantly higher than in the traditional (22.91%) and mid oleic (53.77%) oils. No significant differences were observed among the high oleic hybrids for oleic acid content. The oleic acid content of the mid oleic oil was significantly higher than that of the traditional oil. Linoleic acid content varied between 61.96-69.04% among the traditional hybrids (Table 6.1). The traditional hybrid 1 showed a significantly lower linoleic acid content than the other two traditional hybrids. This observation together with the significantly higher oleic acid content for this hybrid was a result of the highly negative correlation between oleic and linoleic acid content (Table 6.2). The traditional oil contained on average significantly higher linoleic acid content (65.66%) than the high oleic (8.11%) and mid oleic (36.37%) oils. No significant differences were observed among the high oleic hybrids for linoleic acid content. However, the average linoleic acid content of the mid oleic oil was significantly different from that of the high oleic oil.

Table 6.2 Significant correlations between oil content, fatty acids and physicochemical properties

	Oil content	C16:0	C18:0	C18:1	C18:2	SFA	MUFA	PUFA	PUFA/SFA	RI
C16:0	0.29									
C18:0	-0.65*	-0.21								
C18:1	-0.20	-0.98**	0.15							
C18:2	0.21	0.98**	-0.16	-1.00**						
SFA	0.07	0.94**	0.15	-0.94**	0.93**					
MUFA	-0.20	-0.94**	0.15	1.00**	-1.00**	-0.94**				
PUFA	0.21	0.98**	-0.16	-1.00**	1.00**	0.93**	-1.00**			
PUFA/SFA	0.21	0.98**	-0.21	-0.99**	0.99**	0.91**	-0.99**	0.99**		
RI	0.23	0.98**	-0.19	-0.99**	0.99**	0.92**	-0.99**	0.99**	0.99**	
IV	0.20	0.91**	-0.25	-0.94**	0.94**	0.82**	-0.94**	0.94**	0.96**	0.94**

**P≤0.01,*P≤0.05, C16:0: Palmitic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, RI: Refractive index, IV: Iodine value.

Fatty acids palmitoleic, oleic, eicosenoic and vaccenic contributed to the monounsaturated fatty acid (MUFA) content of each hybrid. However, palmitoleic, eicosenoic and vaccenic acids occurred in minor percentages (data not shown) and their contributions to the MUFA content can be omitted. As a result, the MUFA averages of hybrids and three oil types correlated well with their oleic acid contents (Table 6.1). MUFA content showed a strong and significantly positive correlation with oleic acid and strong and significantly negative correlation linoleic acid (Table 6.2). Additionally, the strong and significantly negative correlation between MUFA and palmitic acid was a result of the significantly negative correlation between palmitic and oleic acid content.

Fatty acids linoleic, eicosadienoic and linolenic contributed to the total polyunsaturated fatty acid (PUFA) content of each hybrid. Since eicosadienoic and linolenic acids occurred in minor percentages (data not shown) in each hybrid, their contributions to the PUFA content can be ignored. As result PUFA contents of hybrids (and three oil types) were related to their linoleic acid contents (Table 6.1). Therefore, PUFA was positively correlated with linoleic acid and negatively correlated with oleic acid content (Table 6.2). The strong and significantly negative correlation between MUFA and PUFA was a result of the strong and significantly negative correlation between oleic and linoleic acid. Additionally, the strong and significant correlation between PUFA and palmitic acid was a result of the significantly positive correlation between palmitic and linoleic acid.

Myristic, palmitic, stearic, arachidic, behenic and lignoceric acids contributed to the total SFA content of each hybrid. However, palmitic and stearic acids contributed the most to the SFA content and the minor percentages of the other SFAs can be ignored. The three oil types differed significantly for total SFA content. The traditional oil contained on average 11.21% SFA, the high oleic oil 8.97% and the mid oleic oil 9.63% SFAs. Significant differences were observed among some individual hybrids as well as between the three oil types for SFA content. The traditional hybrid 3 contained a significantly higher SFA content than the other two traditional hybrids. Also, significant differences were observed between high oleic hybrids 5 and 6 for SFA. The significantly higher total SFA content of the high oleic hybrid 6 may be attributed to slightly higher contents of palmitic and stearic acids for this hybrid. The average SFA content of the traditional oil (11.21%) was significantly higher than that of the high oleic (8.97%) and mid oleic (9.63%) oils. This observation could be explained by the significantly higher palmitic acid contents observed for the traditional hybrids since the stearic acid contents did not differ significantly between all individual hybrids (Table 6.1). Additionally, SFA content showed a strong and significantly negative correlation with MUFA (oleic acid) content (Table 6.2). Therefore, as the oleic acid content increased, SFA content decreased. The negative correlation was mainly attributed to the strong negative correlation between palmitic and oleic acid. The strong and significantly positive correlation between SFA and PUFA (linoleic acid) content was a result of the strong and significantly positive correlation between palmitic acid and PUFA content.

The three oil types differed significantly in their ratios of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA). The high average PUFA/SFA ratio of the traditional oil (5.86) was attributed to the significantly higher PUFA (linoleic acid) content of the oil, while the low PUFA/SFA ratio of the high oleic oil (0.93) was a result of both the significantly lower PUFA content and SFA contents of the oil. The PUFA/SFA ratio for the mid oleic oil (3.78) was significantly different from that of both the traditional and high oleic oils. The reason for this is because of the significant differences between the mid oleic oil and the other two oils with regard to linoleic acid content. The ratio of PUFA/SFA showed strong and significantly positive correlations with PUFA (linoleic acid) content, but it was negatively correlated with MUFA (oleic acid) content (Table 6.2). The strong and significantly positive correlation of PUFA/SFA with SFA content was a result of the strong and significantly positive correlation of PUFA/SFA with palmitic acid content.

6.3.2 Physicochemical properties

The three oil types differed significantly in their RI values. Traditional hybrids had the highest RI values, followed by the mid oleic and high oleic hybrids (Table 6.3). Of the traditional hybrids, hybrid 1 was significantly different from the other two, while for the high oleic hybrids, hybrid 5 was significantly different from the other two. Similarly to the RI values, the traditional oil had on average the highest IV (104.23), followed by the mid oleic (101.33) and high oleic (85.31) oil (Table 6.3). The traditional and mid oleic oil did not differ significantly, but both were significantly different from the high oleic oil. No significant differences were observed between traditional hybrids and between high oleic hybrids within each oil type.

Table 6.3 Mean values for physical and chemical tests of traditional, high oleic and mid oleic sunflower oil types

Oil type	**Hybrid no.**	**Refractive index (N_D 40°C)**	**Iodine value**	**Free fatty acid (% oleic acid)**	**Peroxide value (meq/1000 g)**	***p*-Anisidine value**
Traditional	1	1.467142b	105.64a	0.32a	10.33bc	4.55a
	2	1.467563a	102.23a	0.37a	12.32b	2.15ab
	3	1.467824a	104.81a	0.41ab	17.34a	2.22ab
Mean		*1.467510*	*104.23*	*0.37*	*13.33*	*2.97*
High oleic	4	1.462098d	83.86b	0.32a	3.17d	0.44b
	5	1.462420c	86.78b	0.33a	4.10d	0.78b
	6	1.462090d	85.29b	0.62b	3.19d	0.27b
Mean		*1.462203*	*85.31*	*0.34*	*3.49*	*0.50*
Mid oleic	7	1.464854e	101.33a	0.32a	8.18c	2.27ab
LSD (0.05)		0.0003	4.33	0.28	2.83	2.85

LSD: Least significant difference. Mean values followed by the same letter did not differ significantly at $P \leq 0.05$. N_D: Refractive index measurement value at 589.3 nm (the D Fraunhofer line).

Highly significant and positive correlations were observed between RI and linoleic acid (PUFA) content and between IV and linoleic (PUFA) content (Table 6.2). These correlations were expected since both RI- and IV-values are indicative of the level of unsaturation of an oil. The higher the unsaturation level, the higher the RI- and IV-values. Additionally, the higher the MUFA (oleic acid) content, the lower the RI- and IV-values.

FFA content ranged between 0.32-0.62% (Table 6.3). These values were below the limit of 2% and indicated that the oil of all seven hybrids was of good initial oil quality.

When considering the oxidation indexes, the three oil types differed significantly for average PV (Table 6.3). The high oleic oil had on average a significantly lower PV than the traditional and mid oleic oils. Between the traditional hybrids, hybrid 3 had a significantly higher PV (17.34 meq/1000 g) than the other two hybrids (10.33 and 12.32 meq/1000 g). No significant differences were observed between the high oleic hybrids for PV values. The PV of the mid oleic hybrid 7 (8.18 meq/1000 g) was not significantly different from the PV of the traditional hybrid 1 (10.33 meq/1000 g). The PV of the mid oleic oil was on average significantly lower than that of the traditional oil.

The traditional oil showed on average the highest *p*-AV of 2.97 and was followed by the mid oleic oil with a *p*-AV of 2.27. The high oleic oil had the lowest average *p*-AV of 0.50. However, the three oil types did not differ significantly for *p*-AV. No significant differences were observed between individual hybrids for *p*-AV apart from for the traditional hybrid 1 that had a significantly higher *p*-AV than all three high oleic hybrids.

6.3.3 Determination of oil oxidative stability and prediction of oil shelf life

Only hybrids with PVs of 10 meq/1000 g and less (Table 6.3) were used for oxidative stability analysis and therefore only hybrids 1, 4, 5, 6 and 7 were included for OSI determination. A PV of less than 10 meq/1000 g indicates that oil oxidation has not occurred yet and therefore that the oil is of good quality. Only the high oleic hybrids were analysed for variation because there were three entries for this oil type.

Table 6.4 Mean oxidative stability index and shelf life values for traditional, mid oleic and high oleic sunflower oils

Oil type	Hybrid	OSI at 110°C (hours)	Shelf life (months)
Traditional	1	2.70	0.96
Mid oleic	7	4.10	1.45
High oleic	4	10.38a	3.69
	5	9.02a	3.21
	6	5.05a	1.79
Mean		*8.15*	*2.90*
LSD (0.05)		6.24	2.21

OSI: Oil oxidative stability index, LSD: Least significant difference.

The traditional and mid oleic oils contained only one entry each and could therefore not be analysed for variation.

The traditional, mid oleic and high oleic oils differed in their OSI times and as a result in their predicted shelf life times (Table 6.4). This observation was a result of the different unsaturation levels of the oils. The traditional hybrid 1 showed the lowest OSI value (2.70 OSI/h) with a corresponding predicted shelf life of one month. The mid oleic hybrid 7 had a slightly higher value (4.10 OSI/h) than hybrid 1 with a predicted shelf life of one and a half months. The high oleic hybrids had the highest OSI values and together they had an average predicted shelf life of three months. Among the three high oleic hybrids, hybrid 4 performed the best of all hybrids and showed the highest OSI value (10.38 OSI/h) with a corresponding predicted shelf life of four months. Hybrid 5 was less stable than hybrid 4 with a predicted shelf life of three months while hybrid 6 had a predicted shelf life of two months. Although the three high oleic hybrids differed in their OSI values and predicted shelf lives, the differences were non-significant.

6.4 Discussion

The mean seed oil content varied between 35.65-36.56% for the three oil types and no significant differences were observed among these for oil content. Therefore, the high and mid oleic hybrids compared well to the traditional hybrids with regard to oil content. Values obtained in this study are in accordance with oil contents reported by several researchers (Rondanini *et al*., 2003; Radić *et al*., 2008). Reported oil contents in whole sunflower seed varied between 34-52% (Salunkhe *et al*., 1992).

According to the fatty acid analysis, three oil groups could be distinguished from the seven sunflower hybrids. They included the traditional (PUFA) oil with a high linoleic acid content (65.66%) and low oleic acid content (22.91%), the high oleic (MUFA) oil with a high oleic acid content (82.56%) and low linoleic acid content (8.11%) and the mid oleic oil with 53.77% oleic and 36.37% linoleic acid. The extent of variation obtained through traditional plant breeding for sunflower seed oil is evident when comparing fatty acid composition of the three oil types.

The three oil types differed significantly for palmitic acid content with traditional oil containing the highest percentage, followed by the mid oleic oil and the high oleic oil. It was evident that as oleic acid content of the hybrids increased, the palmitic acid content decreased and this observation was confirmed by the significantly negative correlation between palmitic and oleic acid content. The high oleic oil contained on average 2.9% less palmitic acid than the traditional sunflower oil. This tendency is generally observed for high oleic acid sunflower oil (Martín-Polvillo *et al*., 2004; Márquez-Ruiz *et al*., 2008; Merrill *et al*., 2008; Codex Alimentarius Commission, 2010). Stearic acid did not vary significantly between the three oil types and was not correlated with oleic acid content as was the case with palmitic acid. However, a negative correlation was observed between stearic acid and oil content of the high oleic hybrids. Therefore, when selecting high oleic hybrids with increased oil content, a reduction in stearic acid content would result. In general, fatty acid contents of the high oleic and traditional oils used in this study were consistent with data reported by other researchers (Martín-Polvillo *et al*., 2004; Márquez-Ruiz *et al*., 2008; Merrill *et al*., 2008) for traditional and high oleic sunflower oil.

High intake of saturated fatty acids has proven to elevate total and low density lipoprotein LDL cholesterol in the blood that increases the risk of heart disease. Several studies showed that by substituting dietary SFAs with PUFAs, the LDL-cholesterol concentrations are reduced in the blood plasma (Mensink and Katan, 1989; Kris-Etherton and Yu, 1997; Kris-Etherton *et al*., 2004). Thus, by increasing the dietary PUFA/SFA ratio by including linoleic oils in the diet may be advantageous in reducing total plasma cholesterol (Jackson *et al*., 1984). However, both high density lipoprotein (HDL) cholesterol and LDL-cholesterol is reduced. Increasing evidence is showing that MUFA also reduce LDL-cholesterol and plasma TAG, but without adversely affecting the HDL-cholesterol levels in the blood (Mattson and Grundy, 1985; Kris-Etherton *et al.*, 1999; Roche, 2001). Recommendations for a preference of MUFA over PUFA for replacing SFA in the diet initially stemmed from a metabolic study by Mattson and Grundy (1985). However, much controversy later evolved over the recommendation for restriction of specific types of dietary fat in the prevention of coronary heart disease. Katan *et al.* (1997) suggested that a diet in which the total fat content is held constant and that is enriched with MUFA will offer better protection against coronary heart disease than a low-fat diet. It was reported that high intakes of linoleic acid were associated with increased susceptibility of LDL-cholesterol to oxidation. This could lead to increased risks of cancer and accelerated ageing (Grundy, 1997). The high oleic oils may therefore play an important role in decreasing the PUFA intake and may help in reducing these health concerns.

From an oil quality perspective, the PUFA/SFA ratio is a measure of the extent of polyunsaturation and is therefore taken as a measure of an oil's tendency to experience autoxidation (Farhoosh *et al.*, 2008). Oils with the highest levels of unsaturation are most likely to experience autoxidation. The significantly different RI and IV values between the traditional and high oleic sunflower oils can be attributed to differences in their unsaturation degrees. RI and IV increases with the number of double bonds present in the fatty acid. As a result, the polyunsaturated oils had higher RI and IV values than the monounsaturated oils. This was confirmed by the highly significant positive correlations between linoleic acid (PUFA) content and RI and IV respectively. The significantly lower RI value observed for the traditional hybrid 1 compared to traditional hybrids 2 and 3 was attributed to the significantly lower linoleic acid (and total PUFA) content observed for this hybrid. The observed RI and IV values fell within the range recommended by the Codex standards (Codex Alimentarius Commission, 2010) for the three sunflower oil types.

The FFA content of an oil is an important quality parameter in oil crops (Moschner and Biskupek-Korell, 2006). FFA production is generated from lipid hydrolysis by chemical or enzymatic action (Robertson *et al.*, 1984; Frega *et al.*, 1999). Improper handling of seed that damages it and unsuitable storage conditions, such as high temperature and humidity, may cause a considerable increase in the FFA content in the crude oil (Moschner and Biskupek-Korell, 2006; O'Brien, 2009). Therefore, the significantly higher FFA value observed for the high oleic hybrid 6 may be a result of the larger amount of damaged seeds (from harvesting) present in the seed sample received. However, results obtained in this study showed that FFA contents of all hybrids (and as result, the three oil types) were less than the threshold maximum value of 2% FFA set for crude sunflower seed oil. The three sunflower oils were therefore of good quality.

The oxidative status of the oil samples was evaluated considering both primary and secondary oxidation. The progress of oxidation can be distinguished into two stages (Martín-Polvillo *et al*., 2004). The first stage is characterised by a slow progress of oxidation or the induction period (IP) and the second stage is an accelerated stage of oxidation (secondary oxidation). PV measures the hydroperoxide content of an oil sample and is a useful index for the early stage oxidation. PV reaches a maximum during the progress of oxidation that is followed by a notable decrease in PV. During this stage (secondary oxidation) the decomposition rate of hydroperoxides exceeds the rate of their formation (Martín-Polvillo *et al*., 2004). The *p*-AV test assesses secondary oxidation by estimating the amount of unsaturated aldehydes generated during the decomposition of hydroperoxides (Shahidi and Zhong, 2005). The *p*-AV is a reliable indicator of oxidative rancidity in oils (Van der Merwe *et al*., 2003).

The number of peroxides present in oil is an index of its primary oxidative level and consequently of its tendency to go rancid. The higher PVs observed for the traditional oil (>10 meq/1000 g) might be a consequence of peroxides or hydroperoxides formed during seed storage before oil extraction and during oil extraction itself. Higher PVs observed for traditional hybrids indicated that these were more sensitive to oxygen during storage and extraction and were therefore less stable to oxidation than the high oleic oils. Since PUFAs are more susceptible to oxidation than MUFAs and SFAs, the higher PVs observed for the traditional hybrids was a consequence of their higher unsaturation levels. Therefore, the significantly higher PV observed for the traditional hybrid 3, when compared to traditional hybrid 1, may be attributed to the significantly higher linoleic acid (and PUFA) content for this hybrid. The significantly lower PVs observed for the high oleic hybrids (≤4.1 meq/1000 g) were attributed by their significantly lower PUFA/SFA ratios. According to the Codex standard 210-1999 (Codex Alimentarius Commission,

2010) a maximum PV value of 10 meq/1000 g oil is acceptable for good oil quality. The high oleic and mid oleic hybrids showed PVs lower than the recommended standard (Codex Alimentarius Commission, 2010) and these were therefore considered to be of good quality.

The high oleic oil had the lowest average *p*-AV (0.50) and was followed by the mid oleic oil (2.27) and traditional oil (2.97). However, the three oil types did not differ significantly for average *p*-AV. The reason for the higher *p*-AVs of the traditional and mid oleic hybrids may be because of their higher unsaturation levels. According to White (1995), an anisidine value of between 1-10 mmol/kg is acceptable for well-refined oils, while this level might even be higher for fresh PUFA oils. Monounsaturated oil is expected to have lower *p*-AVs than PUFA oils.

The moderately low PV- and *p*-AV-values observed for all three oil types indicated that oxidation was in the induction stage that is characterised by a slow progress of oxidation. Martín-Polvillo *et al.* (2004) observed that high oleic sunflower oil had longer IPs than traditional sunflower oil. Hydroperoxide formation was slower in high oleic sunflower oil than in the traditional oil. The reason for this is that the length of the induction period depends on the unsaturation degree of an oil and the higher the level of unsaturation, the shorter the induction period. Consequently secondary oxidation is likely to occur more rapidly in traditional sunflower oil than in high oleic sunflower oil.

To estimate their oxidative stability, the three sunflower oil types were evaluated for OSI. Since OSI measures the resistance of lipids to oxidation, OSI duration is positively associated with oil stability (Guillen and Cabo, 2002). In addition, OSI is useful for quality control of oils (Shahidi and Zhong, 2005). The high oleic sunflower oil proved to be the most stable of the three oil types with an average OSI of 8.15 h, followed by the mid oleic oil (4.10 h) and traditional sunflower oil (2.70 h). Variation in initial OSI values between traditional and high oleic sunflower oil have been found in several studies (Márquez-Ruiz *et al.*, 2008; Merrill *et al.*, 2008). According to their initial oil stability analysis results, the traditional sunflower oil had significantly lower OSI values (7.4 h at 100ºC and 5.2 h at 110ºC) than the high oleic sunflower oil (20.1 h at 100ºC and 16.5 h at 110ºC). Dobarganes *et al.* (1993) also observed that induction time was positively correlated with the unsaturation degree of oils and that the high oleic sunflower oil

(80.8% oleic and 6.5% linoleic aicd) showed significantly higher induction times compared to the traditional sunflower oil (22.3% oleic and 65.0% linoleic acid). The distinct OSI values observed for the three oil types can be attributed to differences in the unsaturation degrees of the three oils.

OSI values were used to estimate predicted oil shelf lives. The predicted shelf life of the high oleic sunflower was on average three months, while for traditional sunflower oil it was one month. As a result, the monounsaturated high oleic oils were three times more stable to oxidation than the traditional sunflower oil. Results obtained in this study supported observations of Smith *et al.* (2007) who reported that high oleic sunflower oil had greater oxidative stability than traditional sunflower and soybean oil with high PUFA contents. Among the three high oleic hybrids, hybrid 4 performed the best of all hybrids and showed the highest OSI value (10.38 OSI/h) with a corresponding predicted shelf life of four months. The reason for this could be the high oleic acid content of 84.08% and the low linoleic acid content (6.61%) observed for hybrid 4.

Other oils that are high in oleic acid content include olive oil (78%) and variants of regular safflower (77%), canola (78%), peanut (76%) and soybean (79-68%) oil (Gunstone, 2005). Despite their similar oleic acid contents, some of these oils showed variation in their OSI values in a study by Merrill *et al.* (2008). The high oleic canola oil (71.3% oleic acid), with a lower oleic acid content than safflower oil (72.8%) was more stable (OSI of 18.5 h) than the safflower oil (OSI of 14.3 h). In addition, the high oleic sunflower oil (76.2% oleic acid) was less stable (OSI of 16.5 h) than the high oleic canola oil. These differences can be explained by the tocopherol profiles and levels present in the oils. Oils of similar fatty acid composition, those having higher amounts of tocopherols, have greater stability (Normand *et al*., 2006).

In conclusion, the oil of the high oleic sunflower hybrids proved to be of good quality with low PVs and *p*-AVs. In addition, the high oleic oils had considerably better oxidative stabilities than the traditional sunflower oil. Among the high oleic hybrids, hybrid 4 had the highest oleic and lowest linoleic acid content. This resulted in the low RI and IV values observed for this hybrid. In addition it had the lowest FFA content and PV that indicated good oil quality and oxidative stability. Hybrid 4 indicated the best resistance to oxidation (highest OSI) with the longest predicted shelf life (four months) of the three high oleic acid hybrids. As a result, this hybrid should be considered in high oleic acid sunflower breeding programmes for developing high oleic acid sunflower lines with high quality oil that will show better resistance to autoxidation.

6.5 Oxidative stability of commercial vegetable oils

6.5.1 Introduction

Vegetable oils, the most commonly used cooking media, are beneficial and popular due to their cholesterol lowering effect. However, there are some important issues regarding their well-judged use, that are largely ignored by consumers and the medical society (Naz *et al.*, 2004). In contrast to highly saturated and stable animal fats, unsaturated vegetable oils are more readily reactive to oxygen. In addition, the fatty acid composition and presence of antioxidants in oils also influence oil oxidation. Oil quality and nutritional losses may arise from reaction with atmospheric oxygen (autoxidation) or by hydrolytic reactions catalysed by lipases. The effects of hydrolytic reactions can be minimised by cold storage and careful packaging. However, oxidation is an autocatalytic series of reactions that needs low activation energy and cannot be stopped by lowering temperature (Stauffer, 1996).

Cold pressed high oleic sunflower oil is available on the shelves of various local supermarkets. In order to compete with other cooking oils (traditional sunflower, canola and olive oil), the high nutritional value and oxidative stability of the oil is mostly used to promote high oleic sunflower oil. In addition, the oil is less expensive than olive oil. The fatty acid composition of high oleic oil compares well with olive oil and this, together with the lower price, should make the high oleic sunflower oil more attractive to the general consumer.

The aim of this study was to evaluate the oxidative stability of four commercially produced cooking oils using a simple accelerated oxidation test, the Schaal oven test, and to compare the “recently” produced high oleic sunflower oil with other standard cooking oils commercially used in South Africa.

6.5.2 Materials and methods

Commercial cooking oils were taken from the shelf in a local supermarket and were evaluated for oxidative stability. Oils selected for this study were traditional sunflower oil, cold-pressed high oleic sunflower oil, canola oil and palm oil. Traditional sunflower and canola oil were selected due to their common use as cooking oils and the high relative reaction rates of their unsaturated fatty acids with oxygen. High oleic sunflower oil was included to compare the oxidative stability of this oil with that of the commonly used cooking oils. Palm oil was included due to its high resistance to oxidation, so that a comparison could be made between reaction rates of oils with different inherent stabilities.

A duplicate oil sample of 60 g of each type of cooking oil was weighed in 250 ml glass beakers with free access to air (McGinley, 1991). Oil samples were placed in a heat cabinet with the temperature set at 63ºC. On day zero, the oil was evaluated for initial quality and stability. Fatty acid composition together with free fatty acid content, RI, IV, PV and *p*-AV values were analysed according to methods described previously.

In order to follow the oxidation rate in each oil sample, these were periodically analysed for PV, *p*-AV and RI. Measurements were taken daily for the first two weeks, after that these were taken three times a week until all oil samples reached the required oxidation point. A single reaction criterion was not enough to account for the oxidative changes at various stages. The keeping quality from a PV point of view was determined as the number of days needed for PV to reach a value of 100 meq/1000 g oil (Hertzman *et al*., 1988). Keeping quality from a RI point of view was determined as the number of days for RI to show an increase of 0.001 units from the initial value at day zero. Both methods were used because the claim was made that the RI gives a more precise indication of the end of induction period compared to the PV (McGinley, 1991).

6.5.3 Results

Fatty acid composition

The initial fatty acid analysis results of the four commercial oils before heat storage at 63ºC are shown in Table 6.5. Traditional sunflower, high oleic sunflower and canola oil contained between 3.78-5.80% palmitic acid. However, palm oil contained 40.62% palmitic acid. Traditional sunflower, high oleic sunflower and palm oil contained between 3.99-5.47% stearic acid. Canola oil showed a slightly lower stearic acid content of 2.05%. The different oils showed large variation for oleic and linoleic acid content.

Table 6.5 Initial fatty acid composition (as percentage of total fatty acids) and oil analysis results of four commercial vegetable oils before oil oxidation as well as the number of days for the oils to show the first phase of oxidation

Measurement	Traditional sunflower oil	High oleic sunflower oil	Canola oil	Palm oil
Fatty acid composition (%)				
C14:0	0.03	ND	0.06	0.95
C16:0	5.80	3.78	4.45	40.62
C16:1	ND	0.05	0.18	0.15
C18:0	5.47	5.20	2.05	3.99
C18:1	22.66	74.18	59.63	43.56
C18:2	64.60	15.28	21.76	10.08
C18:3	0.09	0.05	8.98	0.18
C20:0	0.31	ND	0.47	ND
C20:1	0.11	0.15	1.39	0.13
C22:0	0.72	1.02	0.05	ND
PUFA/SFA ratio	5.16	1.49	4.24	0.22
IV (g I_2/100g oil)	104.75	89.26	100.39	56.13
FFA (% oleic acid)	0.31	0.71	0.21	0.19
PV_0 (meq/1000 g oil)	3.01	9.36	3.47	0.98
#Time (days)	9	21	9	58
p-AV	10.71	1.33	1.26	8.82
RI_0 (40ºC)	1.46737	1.46276	1.46577	1.45857
RI_1 (40ºC)	1.46874	1.46394	1.46682	1.45981
*Time (days)	17	29	14	66

C14:0: Myristic acid, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C18:0: Stearic acid, C18:1 Oleic acid, C18:2: Linoleic acid, C18:3: Linolenic acid, C20:0: Arachidic acid, C20:1: Eicosenoic acid, C22:0: Behenic acid, PUFA: Polyunsaturated fatty acids, SFA: Saturated fatty acids, IV: Iodine value, FFA: Free fatty acids, PV_0: Initial peroxide value at day zero, *p*-AV: ρ-Anisidine value, RI_0: Initial refractive index at day zero, RI_1: Refractive index with a difference of ≥ 0.001, ND: Not detected. $^{\#}$Time in days for PV to reach a maximum value of 100 meq/1000g, *Time in days for RI to differ with 0.001 units from RI_0.

The traditional sunflower oil contained the highest percentage of linoleic acid (64.60%) of all four oils and 22.66% oleic acid. High oleic sunflower oil had oleic and linoleic acid contents of 74.18% and 15.28%. Canola oil contained 59.63% oleic and 21.76% linoleic acid. Palm oil contained 43.65% oleic and 10.08% linoleic acid. With regard to minor fatty acids, canola oil contained 8.98% linolenic acid and 1.39% eicosenoic acid, while the other three oils contained minor amounts of these fatty acids that can be ignored. Small amounts of behenic acid were detected in the high oleic sunflower oil (1.02%) and traditional sunflower oil (0.72%). However, this fatty acid occurred in minor amounts for canola and palm oil.

The traditional sunflower oil had the highest PUFA/SFA ratio of 5.16 and was followed by canola oil with a ratio of 4.24 (Table 6.5). The relatively high PUFA/SFA ratio observed for the traditional sunflower oil can be attributed to the high linoleic acid content and relatively low total SFA content of this oil. However, for canola oil, both linoleic and linolenic acids contributed to the PUFA and resulted in a high PUFA content. In addition, canola oil contained less SFA and therefore it had a relatively high PUFA/SFA ratio (4.24). The low PUFA/SFA ratio of high oleic sunflower oil (1.49) was a result of the low PUFA (linoleic acid) content of the oil. Palm oil showed the lowest PUFA/SFA ratio (0.22) of the four oil types. The high palmitic (SFA) content and low linoleic acid (PUFA) content of this oil contributed to the low PUFA/SFA ratio.

Initial oil quality and influence of high temperature storage on oil quality and oxidative stability

Before the oil samples were placed in the heat cabinet for heat storage at 63°C, they were tested for initial oil quality. The IV differences observed between the four oil types were a result of differences in their unsaturation levels. The traditional sunflower and canola oil had the highest IVs (104.75 and 100.39 respectively) and were the result of their high PUFA contents (Table 6.5). The high oleic sunflower oil had a lower PUFA content that resulted in the lower IV (89.26) observed for this oil. Palm oil had the lowest IV (56.13) since this oil had the lowest PUFA content. The high oleic sunflower oil had the highest FFA value (0.71% oleic) and was followed by the traditional sunflower (0.31% oleic) and canola (0.21% oleic) oils (Table 6.5). The palm oil had the lowest FFA value (0.19% oleic).

The traditional sunflower and canola oils showed similar initial PVs (PV_0) of 3.01 and 3.47 meq/1000 g oil respectively (Table 6.5). The high oleic sunflower oil had a relatively high PV_0 of 9.36 meq/1000 g oil, while the palm oil showed the lowest PV_0 of 0.98 meq/1000 g oil. The initial *p*-AVs observed for canola and high sunflower oil were low (1.26 and 1.33 respectively). However, the traditional sunflower and palm oil had relatively high initial *p*-AVs of 10.71 and 8.82 respectively. The initial RI-values (RI_0) for the four oil types were in the ranges recommended by the Codex standards (Codex Alimentarius Commission, 2010).

After placing the oil samples in the heat cabinet, PV, *p*-AV and RI-values were periodically measured for all four oils until the oil reached a maximum PV of 100 meq/1000 g oil and the oil showed an RI-value increase of 0.001 units from the RI_0 value. The number of days it took for the different oils to reach 100 meq/1000 g oil differed with their levels of unsaturation. Both the traditional sunflower and canola oil with the highest PUFA/SFA ratio took only nine days to reach 100 meq/1000 g (Table 6.5 and Figure 6.1). For both the traditional and canola oil, the PV showed a sharp increase from the second day of heat storage. The high oleic sunflower oil with a lower PUFA/SFA ratio reached 100 meq/1000 g after 21 days of heat storage. This oil showed a gradual increase in PV from the first day of heat storage. Palm oil with the lowest PUFA/SFA ratio took the longest of the four oils and reached 100 meq/1000 g after 58 days of heat storage. Palm oil showed constant PVs during the first two weeks of heat storage, however, from day 17 the PV showed a sharp increase.

With regard to the RI-values, canola oil was the fastest of the four oils and reached a RI increase of 0.001 units after 14 days of heat storage (Table 6.5 and Figure 6.2). The traditional sunflower oil took 17 days to reach the same unit increase. The high oleic sunflower oil was much slower and took 29 days to show a RI increase of 0.001 units. The palm oil was the slowest and took 66 days to reach the same RI unit increase.

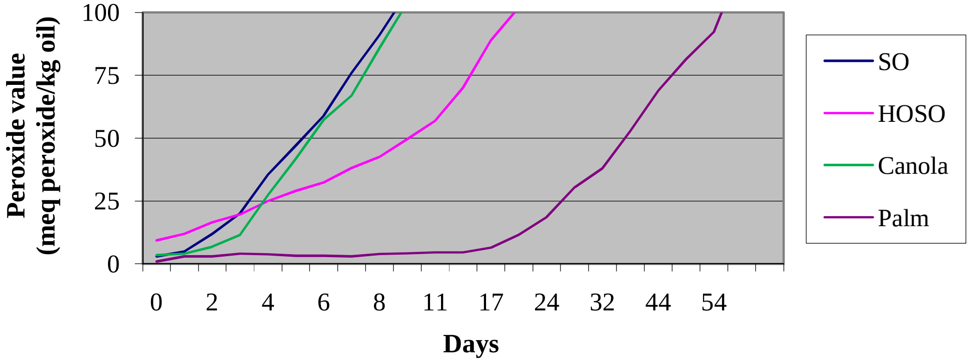

Figure 6.1 Graph indicating number of days for peroxide value to reach maximum of 100 meq peroxide/1000 g oil for traditional sunflower, high oleic sunflower, canola and palm oil. SO: Traditional sunflower oil, HOSO: High oleic sunflower oil.

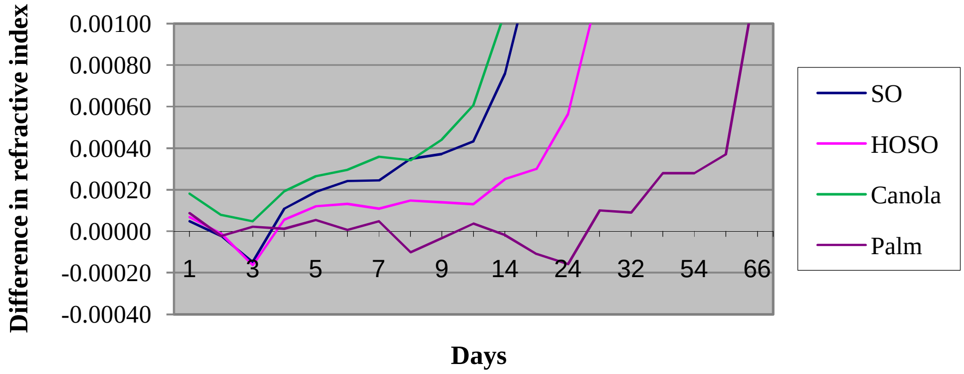

Figure 6.2 Graph indicating number of days for refractive index to reach a value difference of 0.001 for traditional sunflower, high oleic sunflower, canola and palm oil. SO: Traditional sunflower oil, HOSO: High oleic sunflower oil.

The *p*-AVs remained constant during the early stages of oxidation for all four oils (Figure 6.3). Measurements for *p*-AV were taken until the PV of an oil reached its maximum value of 100 meq/1000 g. The *p*-AVs for the traditional sunflower oil remained more or less constant with values ranging between 10-13. The high oleic sunflower oil also showed stable *p*-AVs of between 0-1.7 but *p*-AV gradually increased from day nine to reach a value of 3.5 at day 21. The *p*-AV of palm oil remained more or less stable during the first three weeks with an average value of 9.0. However, palm oil showed a slow but systematic increase in *p*-AV from 10.9 (day 24) to 17.4 (day 58). Only canola oil showed a sharp increase in *p*-AV from day four (5.7) to day nine (27.3).

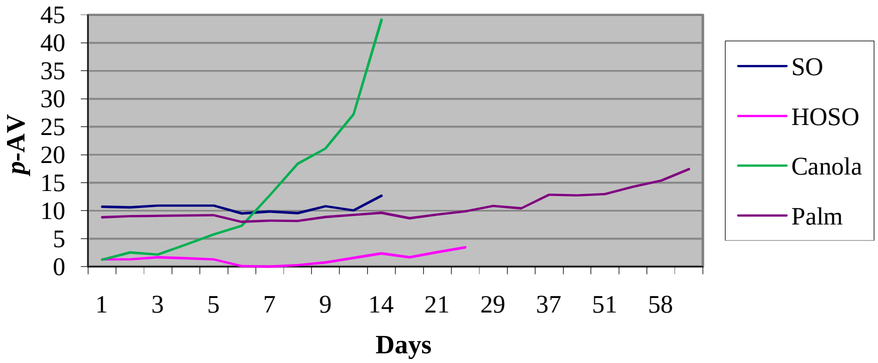

Figure 6.3 Graph indicating gradual increase in *p*-anisidine value measured for traditional sunflower, high oleic sunflower, canola and palm oil. SO: Traditional sunflower oil, HOSO: High oleic sunflower oil.

6.5.4 Discussion

Large variation was observed between the four oil types for fatty acid composition. Traditional sunflower oil is generally characterised as a linoleic oil with linoleic acid contents between 48.3-74.0% and low oleic acid content of less than 39.4% (Codex Alimentarius Commission, 2010). The linoleic acid content of the traditional sunflower oil used in this study was 64.60%, while the oleic acid content was 22.66%. Canola oil had a higher oleic acid content (59.63%) than the traditional sunflower oil, but a lower linoleic acid content (21.76%). However, the canola oil had the highest linolenic acid content (8.98%) of all four oil types. The traditional sunflower, high oleic sunflower and palm oil contained minor percentages of linolenic acid. The contents of major fatty acids were in the ranges recommended for canola oil (Friedt and Snowdon, 2009; Codex Alimentarius Commission, 2010). Although both traditional sunflower and canola oil are widely used for cooking oil, salad oil and in making margarine, canola oil is preferred by health-conscious consumers because it has the lowest saturated fatty acid content of all major edible vegetable oils (Raymer, 2002).

High oleic sunflower oil is characterised by its high oleic acid content (74.18%) and low linoleic acid content (15.28%). However, the oleic acid content showed a lower level than the minimum limit required for high oleic sunflower oil. According to literature, high oleic sunflower oil should not contain less than 75% oleic acid and the linoleic acid content should preferably be less than 10% (Gupta, 2002; Codex Alimentarius Commission, 2010). The lower oleic acid content measured for the high oleic sunflower oil might be the result of the high oleic hybrid seed delivered at the oil press. If high oleic acid hybrids are unstable for the oleic acid trait, they would vary in their oleic acid contents across planting locations (environments) and years, since environment has a large influence on oil fatty acid composition. This would result in seed batches that vary for oleic acid content. As a result, high oleic seed delivered at the oil press might have had lower oleic acid contents than the minimum required limit because of the environmental influence on these hybrids.

Palm oil is the commodity oil richest in palmitic acid with an average content of 44-45% (Lin, 2002). Eighty percent of the oil is mainly used in food (as cooking oil, margarine, and shortenings), while the remaining 20% are used as oleochemicals to replace mineral oils in various industries (pharmaceutical, cosmetics, detergents, lubricants and plastics)

(Soh *et al*., 2009). Palm oil used in this study contained 40.62% palmitic, 3.99% stearic, 43.56% oleic and 10.08% linoleic acid and these values were in the ranges recommended by the Codex Alimentarius Commission (2010) for palm oil.

IV gives an indication of the number of double bonds in the oil and therefore quantifies the degree of unsaturation in the fatty acids (Oderinde *et al*., 2009). The IV is thus a reflection of the susceptibility of an oil to oxidation. The higher IVs observed for the traditional sunflower and canola oil were attributed to the high unsaturation levels of these oils and as a result these were expected to show the lowest oxidative stability. The high oleic sunflower oil had a lower IV since it is a monounsaturated oil with a lower unsaturation level. The saturated palm oil with the lowest IV was expected to show the highest oxidative stability. The IVs of all four oils were in the ranges recommended by the Codex standards (Codex Alimentarius Commission, 2010) for each oil respectively.

FFA and PV are the most frequently determined quality indices during oil production, marketing and storage (Thomaidis and Georgiou, 1999). All four oils showed low FFA values (less than 1.0%) and were below the limit of 2%. The low FFA values observed indicated absence of hydrolytic alteration and that the oil was of good quality.

The traditional sunflower and canola oil showed low PV_0 at the beginning of heat storage. In spite of their low initial PVs, these oils were the most susceptible to oxidation and both oils reached the maximum PV of 100.0 meq/1000 g after only nine days of heat storage. The low oxidative stability of the traditional sunflower oil might be attributed to its high PUFA/SFA ratio and high linoleic acid content. For canola oil, both linoleic and linolenic acid contents contributed to the total PUFA content for this oil. This, together with the low SFA content, resulted in the high PUFA/SFA ratio that contributed to the low oxidative stability for canola oil. The relatively high initial PV_0 of 9.36 meq/1000 g observed for the high oleic sunflower oil may be because the high oleic oil was cold-pressed and therefore may contain impurities that have an effect on the stability of the oil. However, the PV_0 was still below the recommended value. The high oleic oil reached a maximum PV of 100 meq/1000 g after 21 days of heat storage. Regardless of the higher initial PV of the high oleic sunflower oil, it was more than twice as stable as the traditional sunflower and canola oil when PV is considered. This resistance to oxidation may be attributed to the lower PUFA/SFA ratio as well as the high monounsaturated fatty acid (mostly oleic acid) content of the high oleic sunflower oil. Palm oil had a low initial PV_0 of 0.98 meq/1000 g. The oil showed the highest resistance to oxidation of all four oils and the maximum PV value of 100.0 meq/1000 g was reached after 58 days of heat storage. The high oxidative stability of palm oil was expected due to the high total SFA content, low PUFA/SFA ratio and hence, its inherent stability.

The *p*-AV, an indicator of secondary oxidation (aldehyde content) remained constant during the early stages of oxidation for all four oils. However, as oxidation progressed over time, the *p*-AV gradually increased from day eight on for the traditional sunflower, high oleic sunflower and palm oil. On the other hand, canola oil showed a sharp increase in *p*-AV from day four on. The reason for this sudden increase in *p*-AV for canola oil may be explained by the fact that two oxidation stages can be distinguished. The first period is characterised by a slow progression of oxidation, also named the IP and is followed by the second stage of accelerated oxidation (Márquiz-Ruiz *et al.*, 2003; Martín-Polvillo *et al.*, 2004). During the IP, the PV increases to a maximum point, while the AV remains constant. However, when the secondary oxidation stage starts, the peroxides formed during the IP are broken down to secondary oxidation products and as result, the PV decreases while the *p*-AV increases. The reason for the stable *p*-AVs observed for traditional sunflower, high oleic sunflower and palm oil may be because for these oils, oxidation was still in the IP and oxidation did not reach the accelerated stage yet. Alternatively, canola oil might have had a shorter IP and secondary oxidation may have started at day four.

As oxidation of oil samples progressed over time, the RI values increased. Autoxidation is a complex series of chemical reactions that causes a decrease in the total unsaturated content of the oil due to abstraction of hydrogen adjacent to a double bond and the formation of free radicals (Stauffer, 1996). Since RI-values are related to the degree of unsaturation of oils, RI-values increase upon oxidation (Martín-Polvillo *et al.*, 2004). According to the RI_1-values, canola oil showed the shortest IP with a RI-value difference of 0.001 that was reached after two weeks. Canola oil was followed by the traditional sunflower oil with the RI difference that was reached after 17 days. The high oleic sunflower showed a notably longer IP with the RI difference that was reached after approximately four weeks. The palm oil was the most stable oil with the longest IP of more than nine weeks.

In conclusion, the Schaal oven test approach was successful in determining keeping quality and oxidative stability of the four commercial cooking oils. Both methods used to determine keeping quality, PV and RI, gave equal indications of the endpoint of IP. Although PV reached the endpoint of IP first, the tendency of PV and RI the reach the endpoint of IP was similar for the four different oils.

The traditional sunflower and canola oils showed the lowest oxidative stability and were followed by the high oleic sunflower and palm oil. Traditional sunflower and canola oil showed similar oxidative stability, regardless of their notably different fatty acid compositions. The reason for this was because traditional sunflower and canola oil showed similar unsaturation levels (as measured by their PUFA/SFA ratios and IVs) and therefore the same tendencies towards oxidative rancidity. High oleic sunflower oil was more resistant to oxidation and was almost twice as stable as the polyunsaturated oils. Palm oil was the most resistant to oxidation of all four oils analysed and this high stability was attributed to the low level of linoleic acid and high level of total SFA content.

Since the high oleic sunflower oil that is currently available on the shelf is cold-pressed, the oil may contain a higher level of natural antioxidants that is usually removed during the refining steps of a conventional oil processing procedure. As a result, the high oleic oil may contain acceptable shelf stability without added synthetic antioxidants. The natural antioxidants, together with the high MUFA content, may additionally provide health benefits to consumers in disease prevention (Parker *et al.*, 2003).

In order to establish valid and reliable comparisons between different oils, it is necessary to follow the progress of oxidation until the end-point of accelerated stage is reached, because differences may exist in the evolution of oxidation for the different oils. Also, the different oils vary in their content of minor components that have pro-oxidant or antioxidant activity. Therefore it is necessary to evaluate their content and changes because of their decisive contribution to the course of oxidation (Martín-Polvillo *et al.*, 2004).

In the first part of this study it was shown that the high oleic sunflower hybrid seed used in the PANNAR® breeding programme was of good oil quality and showed considerably better oxidative stability than oil of traditional sunflower hybrids. It was then decided to compare results obtained with commercial oils that are available on the shelf for the consumer. The commercially available high oleic sunflower oil proved to be of good quality with noticeably better oxidative stability. However, this high oleic sunflower oil that was randomly taken from the shelf had an oleic acid content that was below the minimum requirements for this oil type. It is not acceptable to label an oil “high oleic” if the oleic acid content of the oil is below the expected requirements for this type. The reason for this is that high oleic sunflower oil production and commercialisation is still in the foundation stage and it is necessary to develop stringent quality control for high oleic sunflower. Additionally, quick methods for testing the fatty acid content of seed batches delivered at the oil press should be put in place (for example by use of near magnetic resonance spectroscopy instruments). Finally, breeding of stable high oleic sunflower hybrids that will produce seed with invariable and high oleic acid contents across seed batches is necessary to produce oil that will comply with the standards set for high oleic sunflower oil.

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CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Sunflower oil has unique properties that are popular with consumers who use the oil for cooking, frying and for salad dressing. High oleic sunflower oil is becoming more attractive to health-conscious consumers as well as for restaurants. This oil type has all the appeal of traditional sunflower oil, while at the same time it is more stable and does not need to be hydrogenated for industrial frying applications. However, high oleic sunflower oil production and commercialisation is still in the foundation stage and much research is still needed to produce high oleic sunflower oil that complies with the standards set for this oil type.

One of the aims of this study was to investigate the effects of genotype, environment and their interaction on oil quality traits of traditional, high oleic and mid oleic acid sunflower genotypes and to identify the most stable and widely adapted genotypes for these traits. Sunflower seed oil content and fatty acid composition have shown significant differences in relation to genotype, environment and year effect. Environmental factors that were found to have a large influence on genotypic performance for these traits included rainfall, temperature and planting date. In general, higher oil yields were observed in environments that received more rain during the growing season. However, too much rain resulted in lower oil yields that could be attributed to either the occurrence of low radiation, low temperatures, diseases or waterlogging conditions. Rainfall also had a significant influence on the fatty acid profiles and especially the oleic and linoleic acid contents. Generally, genotypes grown in drier and warmer environments showed higher oleic and lower linoleic acid contents than those grown in environments that received more rain and had cooler temperatures during the growing season. Different planting dates caused flowering and seed development to occur during periods of widely different temperatures, radiation and day length. The highest oleic acid content was observed for early plantings when seed maturation occurred during high mean daily temperatures. Later plantings caused seed maturation to occur when the mean daily temperatures were lower. As a result, genotypes showed higher linoleic acid contents in later plantings.

Analyses of variance indicated highly significant GXE interaction for all oil quality traits over locations and seasons. As a result it was necessary to do stability analyses. In general, the traditional linoleic genotypes (genotypes 1 and 2) had high linoleic and low oleic acid contents and were relatively stable for these fatty acids over six locations during the first season (2004/2005). However, both these genotypes were unstable for oleic and linoleic acid content across years. The traditional genotype 1 was stable across locations for oil content while both traditional genotypes showed stability across years at one location (Kroonstad). The high oleic genotypes showed large variation for oleic and linoleic acid content across locations and years. However, of the eight high oleic genotypes analysed, genotypes 6, 8 and 14 were the most stable for oleic acid content across locations and years and showed oleic acid contents of above 75%. The high oleic genotypes 6 and 8 and the mid oleic genotype 15 were the most stable for oil content and showed average to above average oil contents. The high oleic genotypes 5 and 9 were the most stable for palmitic acid content, while the high oleic genotype 9 and the mid oleic genotype 10 showed stable stearic acid contents over locations and years. Genotypes that showed stability for the particular traits should be considered for commercial hybrid seed production. High oleic genotypes 6 and 8 that showed stability for both oil content and oleic acid content with average to above average contents of these traits are highly recommended for commercialisation.

Sunflower hybrids have different oleic acid potentials in their oil. In high oleic sunflower, the potential oleic acid content varies between 70-90%. High oleic hybrids with lower oleic acid potentials could be more sensitive to environmental factors such as temperature and soil water availability. The variability in oleic acid content observed for the high oleic acid genotypes across locations and years can possibly be attributed to the presence of modifying factors in their genetic backgrounds. These modifiers are inherited from either one or both parental lines used in hybrid production and have an effect on the expression of the high oleic acid genes. Modifiers may be alleles that suppress the effect of the *Ol* allele on the high oleic acid trait. However, it is possible to breed for stable and widely adapted high and mid oleic acid genotypes. High oleic acid inbred lines that are used in hybrid crosses should be analysed across locations and generations for stability of the high oleic acid trait. Lines that show stability for oleic acid content should then be used for hybrid production. Hybrids obtained from crossing stable inbred lines should then be analysed across locations and seasons for stable oleic and linoleic acid contents. It is recommended that genotypes that show unstable expression for this trait be selected against in the breeding programme.

Most parts of South Africa where sunflower is produced, is semi-arid and therefore erratic weather conditions may occur during the cropping season that has a large effect on agronomical and oil quality properties of sunflower. It is therefore necessary that hybrids are produced that will perform well under heat and drought stress conditions and that show stability for yield-related and oil quality traits. In order to assess the effect of heat stress on these traits, plants of high oleic, mid oleic and traditional sunflower hybrids were exposed to a maximum temperature of 36ºC for a brief period during the most critical stage of storage lipid biosynthesis. Heat stress resulted in a significant increase in head diameter and head sterile centre diameter, but number of filled seeds per head and twenty five-seed weight was reduced. These traits are generally correlated with sunflower seed- and oil yield and therefore heat stress would result in a reduction in oil yield. In general, the most stable hybrids for head and seed traits were the low oleic hybrids 10 and 12 and the mid oleic hybrid 2.

Unfortunately, the high oleic hybrid 4 did not perform well with regard to agronomical traits. This hybrid was highly sensitive to the heat stress and showed a significant increase in head diameter that resulted in the increases observed for number of filled seeds per head and twenty five-seed weight. Although increases in these traits were observed, it was not the ideal since it was previously reported that larger heads resulted in increased percentage of hull that ultimately results in decrease of oil content, since oil content and hull percentage is negatively correlated. This was proved by the significant reduction in oil content observed for hybrid 4. The oil content of this hybrid was reduced with 11.8%, the largest observed among all hybrids analysed. Also, the larger head size resulted in an increase in the sterile centre of the head.

Heat stress significantly modified oil content and fatty acid composition in both traditional and mid oleic hybrids. Oil content showed a significant reduction of 6.09% on average. Palmitic, stearic and oleic acid contents were significantly increased by the heat stress, however, linoleic acid content was significantly reduced. The low oleic hybrids 8 and 10 showed good stability for oil and palmitic acid content. The high oleic hybrid 4 and the mid oleic hybrid 11 were the most stable hybrids for oleic and linoleic acid content. These should be considered for production in areas where high temperatures may occur. Where significant differences were observed between hybrids for seed and oil quality traits, it is possible to genetically improve these traits through selection. However, heritability estimates of these traits also contribute to the effectiveness of selection for a trait.

Estimates of broad sense heritability were higher under normal temperature conditions than under heat stress conditions for head diameter, number of filled seeds per head and twenty five-seed weight. However, these estimates were generally low to intermediate under both normal temperatures and heat stress conditions and indicated that these traits were sensitive to the environment. As a result, chances for improvement of these traits through selection would be complicated. Oil content and palmitic acid showed inconsistent heritability estimates between treatments and therefore it would be difficult to improve upon these traits under heat stress conditions. However, stearic, oleic and linoleic acid contents had high heritability estimates under both normal temperature and heat stress conditions and indicated that it would be possible to improve upon these traits through selection. For oil quality purposes, oleic and linoleic acids are the most important fatty acids because these contribute almost 90% to the total fatty acid composition of sunflower oil. Since these traits showed high heritability estimates, breeding to improve these traits would be relatively simple under different temperature conditions. However, the inconsistent heritability estimates observed for oil content under different temperatures are challenging in producing hybrids with high oil contents.

Although significant correlations were observed between some agronomical and oil quality traits, these were low. Therefore, when selecting for head and seed traits, the fatty acid composition would not necessarily be affected and vice versa. Care should be taken when selecting for traits that show inconsistent correlations at different temperature conditions. The negative correlation between oil content and oleic acid content under heat stress conditions is problematic for the production of high oil yielding high oleic acid hybrids.

Unfortunately, during this trial only one potential high oleic acid hybrid was identified and analysed and therefore no general conclusion on the effect of heat stress on the stability of oleic acid content could be made. It is recommended that more high potential oleic acid hybrids should be included in this type of study since different high oleic hybrids have shown variation in stability across different environments and seasons. In future research the inheritance of the high oleic acid trait in South African genetic backgrounds needs to be investigated. Additionally, the number of genes involved as well as the presence of modifier genes in the parental germplasm need be established. Knowledge of the genetic control and inheritance of high oleic acid content can help breeding programmes to focus on developing stable high oleic acid breeding lines that can be used to develop superior F_1 hybrids with stable oleic acid contents across different environments and seasons.

Since the environment plays a major role on oil fatty acid composition, it is difficult using conventional phenotypic evaluation, to differentiate between different high oleic acid alleles in high oleic acid genotypes or between homozygous and heterozygous plants for this trait. However, genotypic selection for the high oleic acid trait with the use of molecular markers and MAS would be more reliable in breeding programmes, since these markers are not influenced by the environment. Although molecular markers linked to the high oleic acid trait have been identified in previous reports, the development of markers targeting this trait in a South African genetic background was necessary. Furthermore, these previously mapped markers are not suitable for high throughput and reliable analysis in breeding programmes.

A F_2 segregating population was developed from a cross between a high oleic and low oleic acid parent. The F_2 individuals and their two parents were screened for polymorphism using AFLP and SSR markers and a linkage group was constructed based on 19 putative markers. At first, AFLP makers were identified in order to ensure a high density of markers in the targeted chromosome region(s). SSR markers were included to correlate or anchor the linkage group obtained in this study with LG 14 of the public sunflower map. One major QTL (LOD 5.97), controlling oleic acid content, was identified on putative LG 14. This QTL explained 35.0% of the phenotypic variation for the high oleic acid trait. In addition, results of the QTL analysis indicated that the QTL controlling this trait was due to an additive effect. Since only one QTL, explaining 35% of the variation, was detected, other major and/or minor QTL that contribute to the phenotypic variation for this trait might exist that were not identified in this study. Reasons for this are the small population size used and because only one trial was planted in one environment. Also the BSA approach was used to target the gene of interest and whole genome mapping was not done.

From the 19 putative markers that mapped to putative LG 14, five mapped within the high oleic acid QTL region. The two AFLP markers flanking the oleic QTL region (E37M55.1 and E38M3.1) as well as the SSR marker, ORS1086.1, can be used for MAS. However, these need to be validated for their reliability in predicting the phenotype. In order for the AFLP markers to be useful in MAS, these need to be converted to SCAR markers that can be used in high throughput screening for the high oleic acid trait. However, the SSR marker ORS1086.1 should be reliable and useful in the breeding programme. Furthermore, because of the large distance (26.2 cM) between the flanking markers, there is a chance for recombination to occur within the QTL region and therefore it is necessary to identify and map more markers on LG 14 that are linked to the high oleic acid trait.

It is recommended that the identified markers should be tested for their effectiveness in determining the target phenotype in different populations and in different genetic backgrounds. Additionally, these should also be tested in different locations and years since uncontrolled environmental effects could create large phenotypic variation without any genetic basis for the effects. In order to validate markers across locations and years, it will be necessary to develop an immortal mapping population such as a RIL or doubled haploid population. Future research should concentrate on marker saturation of the targeted chromosome. In addition, the size of the mapping population should also be increased to allow detection of additional major and minor QTL.

The last aim of this study focussed on oil quality and oxidative stability properties of the seed oil of high oleic, mid oleic and traditional sunflower hybrids. Oil quality refers to the nutritional and functional properties of oil; however, it is a relative concept that depends on the end-use of the oil. The keeping quality of oil is the length of time that the oil resists significant change of its acceptable properties. Oil content and fatty acid composition are regarded as major oil quality parameters and fatty acid content and the level of unsaturation play a key role in the keeping quality of oil.

The high and mid oleic sunflower hybrids had similar oil contents than the traditional sunflower hybrids. Mean seed oil content varied between 35.65-36.56% for the three hybrid types and was relatively low compared to oil contents observed for hybrids in Chapter 3. Mean oil content varied between 45.9-52.97% for genotypes studied in Chapter 3. Since oil content is largely influenced by the environment, the lower oil contents observed may have resulted from unfavourable conditions like heat or drought stress occurring during the field trial.

The high oleic, mid oleic and traditional sunflower hybrids differed significantly in their fatty acid contents. High oleic hybrids contained on average 82.56% oleic acid (8.11% linoleic acid), the mid oleic hybrid 53.77% oleic acid (36.37% linoleic acid) and traditional hybrids 65.66% linoleic acid (22.91% oleic acid). High oleic hybrids contained on average 3% less palmitic acid than the traditional sunflower oil. This correlation was genetic and contributed to the lower SFA content of high oleic sunflower oil. The PUFA/SFA ratio as well as RI and IV values were indicative of unsaturation levels and as a result oils' tendency to undergo autoxidation. Decreased levels of unsaturation (linoleic acid) will result in increased levels of oxidative stability. Therefore the high and mid oleic sunflower oils with their lower levels of unsaturation should be more resistant to oxidation than the traditional sunflower oil. This conjecture was verified by determining the oxidative quality and stability of the three oil types.

The initial oxidative status of the three oil types was evaluated by measuring PV and *p*-AV values. The significantly lower PV and *p*-AV values observed for the high oleic sunflower oil confirmed that this oil type was more stable than the traditional sunflower oil. For the traditional oil, autoxidation already started during seed storage and was in the induction period. Additionally, oxidative stability of the three oil types were assessed under accelerated oxidation conditions, using the OSI test. The high oleic sunflower oil was the most stable oil with the highest OSI value. Oil shelf life was estimated from the OSI values. The high oleic sunflower oil showed the longest predicted shelf life of three months, while the traditional sunflower oil had a predicted shelf life of one month. The mid oleic oil showed better oxidative stability than the traditional sunflower oil with a predicted shelf life of one and a half months. The considerably better oxidative stability of the high oleic sunflower oil was attributed to its low level of unsaturation. Among the three high oleic acid hybrids analysed, hybrid 4 showed the best initial oil oxidative status and had the best resistance to oxidation. It had the longest predicted shelf life of four months. This hybrid should be considered in breeding programmes for developing high oleic acid hybrids with excellent oil oxidative stability.

Oil of the high oleic sunflower hybrids from PANNAR® showed good oil quality and excellent oxidative stability compared to oil of traditional sunflower hybrids. It was decided to investigate the keeping quality and oxidative stability of commercially available high oleic sunflower oil and compare it with traditional sunflower, canola and palm oil. The high oleic sunflower oil showed outstanding oxidative stability and was more than twice as stable as the traditional sunflower and canola oils. This resistance to oxidation was attributed to the low PUFA/SFA ratio and unsaturation level of the oil. The traditional sunflower and canola oil showed similar oxidative stability due to their similar levels of unsaturation. Palm oil was the most resistant to oxidation of all four oils tested and this high oxidative stability was attributed to the high SFA content and low PUFA/SFA ratio of the oil.

Vegetable oils obtained from the shelf of local supermarkets vary in their content of minor components that have pro-oxidant or antioxidant activity since these oils were obtained through different extraction and processing procedures. Therefore making a comparison between these oils is not exactly valid. Therefore, for future research it is recommended that the same test is repeated on the same oil samples, including olive oil. In order to make more valid comparison between the different oil types, fresh crude oil samples should be obtained that did not go through some sort of processing (degumming, refining, dewaxing or hydrogenation). These processes alter oil composition and structure that will ultimately contribute to differences in keeping quality and oxidative stability.

Although the cold-pressed high oleic sunflower oil, that is currently commercially available, proved to be of high quality with good oxidative stability, its oleic acid content of 74.18% is below the minimum level (75%) recommended for sunflower oil to be regarded as high oleic acid oil. The precise reason for this is unclear, but the instability of high oleic acid hybrids for oleic acid content might contribute to the low level observed. However, high oleic acid sunflower hybrids with oleic acid contents that exceed 80% have been identified in this study and are promising for the production and commercialisation of high oleic sunflower oil that will conform to requirements for high oleic sunflower oil.

This study made a valuable contribution towards high oleic acid sunflower breeding in South Africa, since it was possible to identify high oleic acid hybrids that showed stability and adaptability for the high oleic acid trait in different environments and under different temperature conditions. High heritability estimates, observed from genetic analyses for oleic and linoleic acid contents, will contribute to further development of high oleic sunflower hybrids. Additionally, molecular markers linked to the high oleic acid trait have been identified that may possibly be implemented in breeding programmes in order to select more accurately for the high oleic acid trait. High oleic sunflower seed oil has superior oil quality and oxidative stability properties to traditional sunflower oil and the South African hybrids will meet the demands for more healthy and stable sunflower oil.

SUMMARY

High oleic acid sunflower hybrids have been available on the market for a few years, but research on the stability of these genotypes for oil quality traits in South African production areas is limited. General aims of this study were to compare oil quality traits of high oleic sunflower against traditional sunflower genotypes in different environments, over seasons and under heat stress conditions and to identify possible genetic markers related to the oleic acid trait that would be useful in breeding programmes.

Combined ANOVAs of oil, linoleic, oleic, palmitic and stearic acid content of 16 genotypes tested in a total of nine trials over eight locations and three seasons showed highly significant differences between genotype, environment and GXE. Significant GXE suggested differential response of genotypes across testing locations and years and the need for stability analyses. Stability analyses were performed for all oil traits using AMMI. Stability analyses indicated that high oleic genotypes 6 and 8 could be considered stable across environments and seasons for oil and oleic acid content and genotype 9 for palmitic and stearic acid content.

Sunflower oil quality is affected by genotype and environmental conditions with temperature as a major influence. The effect of heat stress on seed yield and oil quality traits was investigated by applying a maximum temperature of 36ºC to plants during the critical seed-filling stage. This information was needed to define a breeding strategy to further improve seed oil quality in environments where heat stress may occur. All traits measured were significantly influenced by heat stress. The low heritability estimates observed for seed-related yield traits indicated that genetic improvement for these traits would be difficult. Oil and linoleic acid contents were significantly reduced by heat, while oleic acid content was significantly increased in traditional hybrids. The oleic acid content of the high oleic acid hybrid was unaffected, but this hybrid showed the largest decrease in oil content. High heritability estimates observed for oleic and linoleic acid content indicated that it is possible to genetically improve these traits under different temperature conditions.

Since oleic acid content is influenced by the environment, this trait showed instability across environments. Genotypic selection for this trait using linked markers is independent from the environment. A segregating population comprising 78 F_2 individuals was obtained from a cross between a high oleic acid and traditional sunflower line. AFLP and SSR markers were used to identify and map QTL, associated with the high oleic acid trait, putatively to LG 14 of the sunflower consensus map. A major QTL controlling the level of oleic acid was identified that explained 35% of the phenotypic variance for this trait. Markers linked to this trait were identified that could be useful in MAB.

Physicochemical properties and oxidative stability of seed oil of high (82.6% oleic) and mid oleic acid (53.8% oleic) sunflower hybrids were compared with those of traditional sunflower (65.7% linoleic and 22.9% oleic). Oxidative stability was evaluated by measuring PV, *p*-AV and OSI values. High oleic acid oil had the best oxidative stability. Since oxidative stability was related to the unsaturation level of oil, the high oleic oil with significantly reduced linoleic acid content had the highest resistance to oxidation. Keeping quality of commercially available vegetable oils, including high oleic sunflower oil was evaluated using the Schaal oven test. Oil samples, placed in a heat cabinet at 63ºC were periodically examined for keeping quality by determining PV, *p*-AV and RI values. High oleic sunflower oil was twice as stable as traditional sunflower and canola oils.

Keywords: AMMI, ANOVA, broad sense heritability, high oleic acid, fatty acids, molecular markers, *Helianthus annuus* L., oxidative stability, temperature stress

OPSOMMING

Hoë-oleïensuur sonneblombasters is al vir ‘n geruime tyd kommersieel beskikbaar, maar byna geen navorsing is gedoen om die stabiliteit van hierdie genotipes ten opsigte van olie-kwaliteitseienskappe in Suid-Afrikaanse produksiegebiede te ondersoek nie. Die doel van hierdie studie was om die olie-kwaliteitseienskappe van Suid-Afrikaanse hoë-oleïensuur sonneblomgenotipes met dié van tradisionele sonneblomgenotipes te vergelyk en om die effek van verskillende omgewings, seisoene en temperatuurstoestande op hierdie genotipes te bepaal. ‘n Verdere doel was om moontlike molekulêre merkers, wat aan die hoë-oleïeneienskap gekoppel is en in teelprogramme gebruik kan word, te identifiseer.

Die gekombineerde variansie-analises vir palmitiensuur, steariensuur, oleïensuur, linoliensuur en olie-inhoud het aangetoon dat vir ‘n totaal van 16 genotipes, wat in nege proewe in agt lokaliteite oor ‘n tydperk van drie jaar ontleed is, daar hoogs betekenisvolle verskille vir genotipe, omgewing en genotipe-omgewing interaksie was. Betekenisvolle genotipe-omgewing interaksie het aangetoon dat genotipes verskillend in verskillende omgewings en oor jare gereageer het en stabiliteitsontledings was dus nodig. Die stabiliteit van al die olie-eienskappe is deur middel van additiewe hoofeffek en multiplikatiewe en interaksie analise (AMMI) bepaal. Hierdie ontledings het aangetoon dat die hoë-oleïensuur genotipes 6 en 8 hoogs stabiel oor omgewings en seisoene was vir olie- en oleïensuurinhoud, terwyl genotipe 9 stabiel vir palmitiensuur- en steariensuurinhoud was.

Sonneblomolie-kwaliteit word grootliks deur die omgewing beïnvloed en temperatuur speel die belangrikste rol. Die effek van hittestremming op saadopbrengs- en olie-kwaliteiteienskappe is bestudeer deur sonneblomplante aan ‘n maksimum temperatuur van 36ºC gedurende die mees kritieke stadium van lipiedbiosintese bloot te stel. Hittestremming het ‘n betekenisvolle invloed op al die eienskappe gehad. Lae oorerflikhede van saadopbrengs-verwante eienskappe dui daarop dat genetiese vordering vir hierdie eienskappe nie haalbaar is nie. Vir die tradisionele sonneblombasters is ‘n betekenisvolle afname in linoliensuur waargeneem, terwyl oleïensuurinhoud betekenisvol toegeneem het. Die oleïensuurinhoud van die hoë oleïenbaster was onveranderd, maar hierdie baster het ‘n beduidende afname in olie-inhoud getoon. Die hoë oorerflikhede vir oleïensuur en linoliensuur dui daarop dat dit wel moontlik sal wees om hierdie eienskappe geneties onder verkillende temperatuurtoestande te verbeter.

Omdat oleïensuurinhoud grootliks deur omgewingstoestande beïnvloed word, is hierdie eienskap onstabiel oor verskillende omgewings. Deur van genetiese merkers wat aan die hoë-oleïeneienskap gekoppel is, gebruik te maak, kan seleksie op die genotipe gedoen word en die omgewing speel dus nie ‘n rol nie. ‘n F_2 segregerende populasie, bestaande uit 78 plante, is verkry nadat ‘n hoë-oleïen ingeteelde lyn met ‘n tradisionele sonneblomteellyn gekruis is. AFLP (geamplifiseerde fragment lengte polimorfisme)- en SSR (eenvoudig herhalende volgorde) analises is gebruik om QTL (kwantitatiewe eienskap lokusse), wat aan die hoë-oleïeneienskap gekoppel is, te identifiseer en waarskynlik op LG 14 van die konsensus sonneblomkaart te karteer. ‘n Hoof QTL, wat die hoë-oleïensuur inhoud reguleer, is geïdentifiseer en verklaar 35% van die fenotipiese variasie vir hierdie eienskap. Die geïdentifiseerde merkers, wat aan die hoë-oleïeneienskap gekoppel is, kan vir merker-ondersteunende teling gebruik word.

Die fisies-chemiese- en oksidatiewe stabiliteitseienskappe van die saadolie van hoë-oleïensuur (82.6% oleïensuur) en mid-oleïensuur (53.8% oleïensuur) sonneblombasters is met dié van tradisionele sonneblom (65.7% linoliensuur en 22.9% oleïensuur) vergelyk. Oksidatiewe stabiliteit is bepaal deur peroksied-, *p*-ansidien- en olie stabiliteitsindeks (OSI) waardes te meet. Olie van die hoë-oleïensuurbasters het die beste weerstand teen oksidasie getoon. Omdat die vetsuur-onversadigingsvlak van olie ‘n aanduiding van oksidatiewe stabiliteit is, het die hoë-oleïenolie, wat die laagste onversadigingsvlak gehad het, die meeste weerstand teen oksidasie getoon. Die oksidatiewe stabiliteit van kommersiële kookolies, insluitende dié van hoë-oleïen sonneblom olie, is deur middel van die Schaal oondtoets geëvalueer. Olie monsters is in ‘n hittekabinet by 63ºC geplaas en peroksied-, *p*-ansidien- en refraksie indekswaardes is gereeld geneem om te bepaal hoe lank dit vir die olie neem om te oksideer. Die oksidatiewe stabiliteit van die hoë-oleïen sonneblom olie was twee maal hoër as dié van tradisionele sonneblom- en kanola-olie.

Kernwoorde: AMMI, ANOVA, oorerflikheid in die breë begrip, hoë oleïensuur, vetsure, molekulêre merkers, *Helianthus annuus* L., oksidatiewe stabiliteit, temperatuurstremming