Genetic Gain, Advanced Cycle Pedigree Breeding and Correlated Response to Selection under Varying Moisture Conditions in Sunflower

by

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Abstract

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in South Africa and genetic improvement for grain yield and oil-content was initiated in the country in the early 1970s. Commercial production of sunflower in South Africa is done under natural rainfall conditions in areas where frequencies of drought are high hence the requirement for drought tolerant cultivars. An assessment of the genetic gains in seed and oil yield achieved since 1970, the effects of re-cycling inbred lines and strategies for developing drought tolerant sunflower cultivars has not been done for South African sunflower breeding programmes. Two data-sets were used for the genetic gain studies: side-by-side evaluation of historical and current sets of popular cultivars in the same environment under one set of trial management practices; and yield trends in commercial farmers' fields based on annual production estimates. The estimated relative genetic gain for seed yield based on side-by-side trials was 1.5% year^{-1} and the relative gain in seed yield per year under commercial production was 1.9% year^{-1}. The contribution of new cultivars to total seed yield progress in sunflower were 56.3% for the period 1970 to 1989; 23.9% from 1990 to 2009 and the mean over the four decades under consideration from 1970 to 2009 was 41.6%.

Quantifying the usefulness of inbred lines in advanced cycle plant breeding was done using four base breeding populations based on: phenotypic or genetic variability; heterosis; and combining ability. Significant genetic variation was evident for seed yield and oil yield while genetic variability for oil content was low. Genetic advance (GA%), with a 10% selection intensity, was high for seed yield and oil yield for each of the four populations ranging from 36-42% and 38-43%, respectively. The GA% for oil content was low ranging from 1.3% to 5.1% indicating the need to introgress high oil content germplasm in the present breeding populations in advanced cycle pedigree breeding.

Founder parent heterosis (FPH), mid-standard heterosis (MSH) and high standard heterosis (HSH) indicated that some new testcross hybrids from the advanced cycle pedigree breeding populations were performing better than their founder parents in hybrid combination as well as the standard commercial hybrid checks. From variance component analysis, general combining ability (GCA) was predominant over specific

combining ability (SCA) for seed and oil yields indicating that superior hybrids can be identified based on positive and significant GCA effects of the female lines. For oil content, SCA was predominant over GCA indicating that it would be best to select for specific hybrids combinations with high oil content rather than selecting female lines with high GCA effects.

Variable moisture conditions characterise the sunflower production environments in South Africa. Breeding for such environmental conditions requires a combination of strategies including use of secondary traits and developing appropriate test environments. Three secondary traits, head diameter, stem diameter and stay green canopy which are easy to measure in the field were evaluated for their appropriateness for selecting for drought tolerance under three moisture conditions: random stress environments (RSE), managed drought stress environments (MSE) and well watered nonstress environments (NSE). Type A genetic correlations indicated that stay green canopy (SG) had the potential to be used as secondary trait to indirectly improve oil yield under the three moisture conditions. The indirect selection efficiency (ISE) for SG using genetic correlations based on H^2 were 0.79, 0.82 and 0.78 in the RSE, MSE and NSE, respectively, while that using genetic correlation based on h^2 were 0.67, 0.98, and 0.93 in the RSE, MSE and NSE, respectively. In both cases selection in the MSE had the highest efficiency using genetic correlations based on either H^2 or h^2. Estimates of indirect selection based on type B genetic correlations indicated that indirect selection for oil yield (OY) in the MSE and NSE for the target RSE was as effective as direct selection of OY in the RSE based on additive genetic correlations of 0.96 obtained in both selection environments. Overall, the results from the exploratory drought tolerance study should inform the development of breeding strategies to improve drought tolerance and associated yield stability of sunflower cultivars grown in South Africa and associated environments.

Declaration

College of Agriculture, Engineering and Science

I, Godfree Chigeza, declare that:

The research work presented in thesis contains no material which has been accepted for the award of any other degree in any tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis

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Godfree Chigeza (Candidate)

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Dr. Paul Shanahan (Academic Supervisor)

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DEDICATION

To my late loving parents **Simon** and **Lina Chigeza** who highly valued education. In my mother's words 'You will never go wrong with education'

Table of Contents

List of Tables

List of Figures

Introduction and Research Objectives

Worldwide, cultivated sunflower (*Helianthus annuus* L.) is one of the major vegetable oil crops after soybean (*Glycine max* L.), rapeseed (*Brassica napus* L.) and palm (*Elaeis guineensis* L.). Of the edible oils obtained from the three major oilseed crops grown in South Africa, namely: sunflower, soybean and groundnuts (*Arachis hypogaea* L.), 82% are from sunflower (Nel, 2001). Formal public sector sunflower breeding in South Africa has been ongoing since the early 1960s and a F_1 hybrid breeding programme commenced in 1973 (Vermeulen et al., 1979). By the early 1990s, the land area under sunflower production in South Africa was 100% cultivated to hybrids as both public and private breeding programmes constantly release new hybrid cultivars that in turn become the source of elite parental lines for the next generation of improved cultivars.

The amount of progress breeding programmes have been making has been reviewed for a number of commercial and food crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) (Silvey, 1985; Austin, 1999; Duvick and Cassman, 1999; Peng et al., 2000; Adeledo et al., 2003; Fufa et al., 2005; ; Cargin et al., 2009; Lillemo et al., 2010). Compared to other crops of commercial importance, few published reports are available on the genetic gains achieved in sunflower for seed yield, oil content and oil yield. In on farm trials of commercial and near commercial cultivars released between 1983 and 2005 in Argentina, de la Vega et al. (2007) demonstrated a gain in oil yield of 11.9 kg ha^{-1} $year^{-1}$. There is a dearth of documented information on the genetic gains or yield increases achieved in the major crop species in South Africa despite decades of breeding and continued investment in genetic improvement. Quantification of genetic gain is important both from a scientific and economic point of view as clear demonstrations of progress are critical to administrators as well as breeders in order to justify the continued funding of breeding programmes (Gardner, 1987). Furthermore, periodical assessments of genetic gain allow breeders to follow changes in the expression and performance of agronomic and end-use quality traits (Fufa et al., 2005).

As a plant breeding programme matures, re-utilisation of elite inbred parents of obsolete hybrids or hybrids in current use to create new hybrid combinations is inevitable, and the process has been termed advanced cycle pedigree breeding (Lu and Bernado, 2001).

The challenge with this approach is not only to maintain the much needed genetic variability in the base breeding populations but also to assess their usefulness for further genetic advancement as well as developing commercially viable experimental hybrids. In China, 71% of the commercial maize hybrids grown were found to be from four inbred lines or their derivatives (Fan et al., 2008). Similar trends were reported in maize and barley in the USA by Bernado et al. (2000) and Condón et al. (2008), respectively, indicating widespread use of elite inbreds as source populations. The potential impact of advanced cycle pedigree breeding in sunflower is not well understood. In order to determine the potential of a selection programme, genetic variation and other statistics such as heritability, combining ability for populations in advanced cycle pedigree breeding should be determined and monitored to avoid any potential drawbacks such as loss of resistance genes or quality traits.

When breeding for drought tolerance in crops, the aim is to improve yield and stability of cultivars for performance under variable moisture conditions encountered in the target production environments through genetic selection. Two environments are normally considered (Allen et al., 1978), the selection environment or test environment and target production environment which characterise the farmers' production environments where occurrence of drought is highly variable and unpredictable over time and space. Sunflower production in South Africa is largely dependent on natural rainfall, and is consequently affected by droughts of variable and unpredictable intensities which may cause significant yield reduction. The debate on whether selection should be done under optimal conditions with an assumption of yield spill over to the marginal environments or selection should be done under stress managed conditions has been of great interest to breeders and physiologists (Ceccarelli et al., 1998). Genetic variance and heritability of the primary trait, yield (grain, seed or oil) is greatly reduced under stress (Chapman and Edmeades, 1999). In crops like maize, the genetic variance and heritability of secondary traits with strong correlations with yield increase under stress (Chapman and Edmeades, 1999). Theoretical considerations on the amount of improvement of primary trait *y* (yield) obtained by indirect selection for secondary trait *x* (morphological traits or physiological traits) is extensively reviewed by Falconer and Mackay (1996). Equally important, is the reliability and effectiveness of the selection environment or test environment to discriminate among genotypes (Isik and Kleinschmit, 2005).

Understanding and quantifying phenotypic variance, heritability, combining ability, correlated response to selection and reliability of selection environments in a breeding program are cornerstones for greater gains in the target traits and therefore overall genetic gain. However, in sunflower there are a limited number of studies which have attempted to quantify these parameters and how they relate to overall core breeding objectives. The objectives of this study were therefore to:

(i) quantify genetic gain for yield, oil concentration and oil yield in sunflower since the inception of formal sunflower breeding in South Africa;
(ii) estimate heterosis and phenotypic and genotypic variances of sunflower lines from breeding populations in advanced cycle pedigree breeding;
(iii) quantify the importance of general combining ability (GCA) and specific combining ability (SCA); and
(iv) estimate indirect selection efficiency versus direct selection efficiency using secondary traits and selection environments based on type A and type B genetic correlations.

While genetic parameters are population and environment dependent, the methodologies and principles used in this study are not only limited to sunflower breeding but applicable to a wide range of cross pollinated crops where the aim is to quantify breeding progress and genetic parameters of the base breeding populations.

The literature review is presented in Chapter 1. The literature review provides a background on sunflower production trends, constraints and breeding objectives in South Africa. It also provides an overview of the procedures for quantifying genetic gain and the importance of correlated response to selection in a breeding programme with special reference to a mature breeding programme.

Chapter 2 deals with genetic gain in the context of seed yield improvement in sunflower over the past four decades of breeding in South Africa. It also provides a historical overview of sunflower breeding in South Africa and how genetic gain in trial plots translated into improved performance in the farmers' fields.

Chapters 3 and 4 are based on quantifying genetic variability, genetic advance, heterosis and combining ability of lines from breeding populations formed by crossing

good by good inbred lines or their derivatives to develop new inbred lines for use in hybrid combinations.

Chapter 5 explores future selection strategies to stabilise oil yield in sunflower under variable moisture conditions based on proven successes of breeding for drought tolerance in maize and rice using secondary traits and managed selection environments.

The thesis is presented in the form of standalone research chapters, some of which have been already published as research papers as indicated in the footnote on the first page of the relevant chapters or a manuscript in the process of being prepared for submission to a journal and as such there is some repetition of introductory information and references.

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CHAPTER 1

Literature Review

1.1 Sunflower production in South Africa

1.1.1 Sunflower production trends

Sunflower (*Helianthus annuus* L.) is grown in a wide range of climatic conditions. The major producing countries in the world are Russia, Argentina, France, Spain, and United States of America. South Africa is the largest sunflower producer in Africa and it was ranked 12th in the world in 2007 (FAOSTAT, 2008). Sunflower production in South Africa started in the early 1930s with the area under production rising from 107 000 ha in 1956 to 185 000 ha by 1971 (Birch et al., 1978). Total production for the 2012/13 season was estimated to be 505 000 tonnes with an average seed yield of 1000 kg ha^{-1} (SAGIS, 2012). Over the past two decades the annual total production in South Africa has fluctuated between 500 000 to 860 000 tonnes (Beukes, 2003; FAOSTAT, 2008; Dredge, 2011, SAGIS, 2011; SAGIS, 2012), with the mean annual seed yield ranging from 900-1300 kg ha^{-1} (Nel and Bloem, 2007, SAGIS, 2012).

1.1.2 Sunflower production environments in South Africa

Sunflower production in South Africa is mainly dependent on natural rainfall and the production environments differ with regard to the scale and prevalence of biotic and abiotic stresses. In the 2010 and 2011 production seasons, the Northwest and Free State provinces contributed nearly 80% of total sunflower production with the remainder coming from the other seven provinces mainly Gauteng, Limpopo and Mpumalanga (SAGIS, 2011; SAGIS, 2012). The combined production of KwaZulu-Natal, Eastern Cape, Western Cape and Northern Cape provinces contributes less than 1% to total production (SAGIS, 2011; SAGIS, 2012). The Northwest, Free State and Limpopo provinces generally receive low and erratic rainfall and soils are either shallow or sandy and low in carbon content (<1%) (Nel and Bloem, 2007).

1.2 History of sunflower breeding in South Africa

1.2.1 Germplasm acquisition and screening

The early breeding of sunflower is credited to Pustovoit and Jdanov in the former Soviet Union now Russia who increased oil concentration in sunflower seed to above 500 g kg^{-1} on a dry mass basis leading to the development of the modern day sunflower cultivars (Škorić, 1992; Fick and Miller, 1997a). Two major developments which accelerated sunflower hybrid breeding were the discovery of the cytoplasmic male sterile (CMS) system by Leclrecq in 1969 in France, and the fertility restorer lines *(Rf)* by Kinman in 1970 in the United States (Fick and Miller, 1997b). These developments allowed the practical use of heterosis in sunflower and currently all the hybrids being cultivated worldwide are based on only one source of cytoplasmic male sterility (Škorić, 1992).

In South Africa, formal public sector sunflower breeding spans over four decades and it was initiated by the then Department of Agricultural Technical Services, now Agricultural Research Council (ARC), in the late 1960s (Vermeulen, 1979). The major objectives of the breeding programme in its first 10 to 15 years of existence were germplasm acquisition followed by characterisation to determine its value for the local breeding programme (Vermeulen, 1979). Germplasm was acquired from various regions notably Romania, Russia, USA and Canada. Major emphasis in selection was placed on seed yield, adaptability and oil content. A hybrid breeding programme was initiated in 1973 (Vermeulen et al., 1979) and by the early 1990s, hybrids were being grown in the entire area under sunflower cultivation (Vermeulen et al., 1979). The adoption of the hybrid breeding model in South Africa resulted in the initiation of several sunflower breeding programmes by private seed companies most notably Saffola Seed Company, Pioneer Hybrid Seeds, Sensako Seed Company and Pannar Seeds to name a few. Both the public and private breeding programmes have been characterised by a steady release of improved and better performing cultivars that in turn become parental sources of the next generation of improved cultivars.

1.2.2 National cultivar trials

As the number of improved cultivars increased on the market, it became necessary to quantify their performance at national level and the ARC in collaboration with private seed companies initiated comprehensive national sunflower cultivar trials with the first trials conducted in 1975 (Birch et al., 1978). The national sunflower cultivar trials evaluate superior sunflower hybrids from different seed companies in the same trials at a number of locations for performance comparison purposes. The trials have been an important testing platform and information resource for the different stakeholders in the sunflower industry who have specific requirements in order to overcome their production problems and improve profitability. Information from cultivar trials allows farmers to choose cultivars that consistently give high yields under their production environments while on the other hand the processors require cultivars with high oil content. For breeders, the performance of cultivars in national cultivar trials objectively quantifies the progress the breeding programmes are making while it also gives the different seed companies the opportunity to showcase their hybrids.

1.3 Breeding objectives versus production constraints

1.3.1 Oil yield and related traits

The major breeding objectives in sunflower have remained unchanged, revolving around continued improvement for seed yield, percent seed oil content and oil quality (Miller, 1987; Škorić, 1992; Chigeza, 2007). These are the main traits that influence sunflower production and demand by the industry stakeholders. A high seed yield ensures that farmers realise higher profit margins and high oil percentage brings added price premiums to producers, while improved oil quality increases demand by end-users. The oil quality of sunflower is determined mainly by the percent composition of unsaturated fat acids, mainly linoleic acid, a poly-unsaturated fatty acid or oleic acid, a mono-unsaturated fatty acid. Sunflower cultivars with high linoleic acid are generally referred to as ‘conventional’ or ‘standard’ sunflower cultivars (Fernandez-Martinez et al., 1993). ‘High oleic’ sunflower cultivars have oleic-rich oil ranging from 65 to 90% (Burton et al., 2004) and production of high oleic sunflower cultivars in South Africa is still in its infancy stage (van der Merwe et al., 2013).

The mathematical product of seed yield and percent seed oil content is oil yield. Further increases in national sunflower oil production can be achieved by either expanding the area under cultivation or increased oil yield (seed yield and/or percent seed oil content). Expanding the production area will mean pushing sunflower cultivation further into marginal lands resulting not only in increased cost of production but further exploitation of the already over-utilised environment. Increasing oil yield appears a viable option to keep pace with population growth and also minimise further negative impacts on the environment.

1.3.2 Yield stability and reliability

In addition to seed yield and oil concentration, a major breeding consideration for diverse and heterogeneous environments such as those in the tropics is stability/reliability of performance (Guillen-Portal et al., 2003; de la Vega, 2012). Yield stability and reliability are functions of several components and tolerance to biotic and abiotic stresses is one of them. In South Africa, sunflower is not prone to major biotic stresses (DAFF, 2010), but the increasing threat of head diseases caused by *Sclerotinia sclerotiorum* is evident (Flett, 2013). Other diseases of importance include white rust caused by *Albugo tragopogonis*, and brown rust caused by *Puccinia helianthi*. Abiotic stresses which affect stability and reliability of sunflower production in South Africa include high soil temperatures and variable moisture conditions mainly in the major sunflower production areas of the Free State and North-West provinces (Nel, 1998). In contrast, the main causes for production instability in sunflower in Argentina were found to be photoperiod and low temperatures during reproductive phases (de la Vega, 2012), hence the need to quantify variability in the local germplasm.

1.4 Genetic gain and its quantification

The success of a breeding programme is judged by the performance of the cultivars in the target production environment. Genetic gain based on realised improved yield (grain, seed or oil yield) and its return in financial terms forms the basis of investment in breeding and considerable debate exists around whether further progress can be achieved (Austin, 1999; Duvick and Cassman, 1999). Comparisons of genetic gains in crops are complicated as peak performances of cultivars are confounded within the

genotype by environment interaction encountered during evaluation or production (Duvick, 2005; de la Vega, 2012). Advances in statistical computing have resulted in more reliable methods being devised to evaluate cultivar performance across environments and these include mixed models analysis (Frensham et al., 1998).

Several methods are used to measure genetic gain for yield in major crops based on either: (i) side-by-side performance trials of obsolete cultivars and new cultivars under common field conditions (Luque et al., 2006); (ii) historical data from national yield trials (de la Vega et al., 2007; Mackay et al., 2011); and (iii) yield increase in farmers' fields (Tracy, et al., 2004; Lillemo et al., 2010). Each method has its own merits and demerits. Arguments against side-by-side comparisons are that agronomic practices and incidence of pests and disease change with time and therefore the performance of older cultivars under modern growing conditions may be detrimentally affected (de la Vega et al., 2007). For a crop such as sunflower where agronomic practices and inputs have remained relatively stable and moderate (Škorić, 1992; Fick and Miller, 1997b), side by side comparison offers the opportunity to measure yield differences in the same environment enabling the determination of the drivers of improvement (or lack thereof) in performance (Morrison et al., 2000; Ortiz et al., 2002).

A number of studies have reported yield gains in crops such as maize (*Zea mays* L.) (Ci et al., 2011), wheat (*Triticum aestivum* L.) (Cargin et al., 2009; Gourdji et al., 2012; Sanchez-Garcia et al., 2013), rice (*Oryza sativa* L.) (Tabien et al., 2008; Breseghello et al., 2011), barley (*Hordeum vulgare* L.) (Lillemo et al., 2010) and soybean (*Glycine max* L.) (Egli et al., 2008; Jin et al., 2010). For sunflower, progress in oil yield improvement has only been formally reported for Argentina by de la Vega et al. (2007) who demonstrated a gain in oil yield of 11.9 kg $ha^{-1}year^{-1}$ in on farm trial comparisons of commercial and near commercial cultivars released between 1983 and 2005 in Argentina. The scientific literature on genetic gain or yield increases in South Africa is limited despite decades of breeding and continued investment in genetic improvement. Periodical assessments of genetic gain for yield allow analyses of the contributions of different traits to yield gain in space and time. In maize, large gains in early breeding were a result of improving standability, while in recent years gains in yield has been through reduced anthesis silking interval and increased stay green canopy (Niebur et al., 2004).

1.5 Selection methods, genetic variability and heterosis in sunflower

1.5.1 Selection methods and genetic variability

Plant breeding consists of several interlinked steps and selection for the next generation of parents or improved cultivars is the fundamental consideration. The basis for effective selection is the phenotype, applied directly; as in conventional breeding or indirectly using markers linked to the phenotypic trait of interest as in the case of molecular marker breeding (Comstock, 1996). Phenotypic variability of traits permits selection which in turn determines the potential gain that can be realised when optimal selection strategies are applied. Phenotypic variability is determined by the magnitude of genetic and environment components and their interactions (Falconer and Mackay, 1996). In relation to phenotypic variability, the process of hybrid cultivar development was summarised by Dudley and Moll (1969) and restated by Bernado (2008) as: (i) creation of genetic variability by crossing good with good; (ii) selection of the best inbred lines from the crosses through generations of inbreeding; and (iii) combine the best selected new inbred lines into a new and improved cultivar. The crossing of good by good elite inbred parents of already in use commercial hybrids cultivars or of obsolete cultivars to form new breeding populations followed by pedigree selection or single seed descent in breeding programmes is commonly referred to as advanced cycle pedigree breeding (Lu and Bernardo, 2001). Although widely used in a number of crops, advanced cycle pedigree breeding could result in diminished genetic variation among parents leading to a limited choice of parents for crossing to form a breeding population for the next generation. Careful characterization and quantification of the genetic parameters of breeding populations in advanced cycle pedigree breeding therefore becomes necessary to assess their usefulness for further genetic advancement as well for developing commercially viable hybrids.

Studies on phenotypic and genetic variability among traits in advanced cycle pedigree breeding in sunflower based on field performances are limited or not available at all. Substantial genetic diversity and differentiation has been found in open pollinated varieties and composites using molecular markers (Cheres et al., 2000; Yue et al., 2009; Moreno et al., 2013). Although studies on genetic diversity using molecular markers are useful, especially in removing duplications and correcting for possible mis-groupings

(Cheres et al., 2000), they do not provide an indication of how much progress will be achieved when selection is applied. In addition, several studies on genetic diversity using either coancestry data or genetic distance based on molecular markers have generally concluded that diversity or genetic distance is a poor predictor of hybrid performance (heterosis) and combining ability (Cheres et al., 2000; Makumbi et al., 2011). In studies involving crosses between lucerne germplasm (*Medicago sativa*. subsp. *sativa* L.) and (*Medicago sativa* subsp*. falcate* L.), Riday and Brummer (2005) concluded that heterosis cannot be ensured by genetic diversity per se but by usable complementary variation between parents. The usable genetic variation in the parental material determines the "usefulness" of a cross or breeding population and hence effectiveness and progress expected from selection.

1.5.2 Heterosis in sunflower

In sunflower, the cytoplasmic male sterility (CMS) system that involves the use of a CMS line (A), maintainer line (B) and fertility restorer line *(Rf)* has allowed breeders to exploit heterosis through the development of three way and single cross hybrids (Miller et al., 1980; Meena et al., 2013). Heterosis, which is defined as the measure of F_1 performance for the trait of interest in relation to its parental performance (Shull, 1948), has been extensively exploited in cross-pollinated crops although its genetic basis is still not well understood (Lamkey and Edwards, 1998; Flint-Garcia et al., 2009). Several types of heterosis estimates have been proposed. In studies involving cross-pollinated crops such as maize and sunflower, estimation of heterosis for yield and other agronomic traits is either based on mid-parent (MP) or better parent (BP) heterosis and is often expressed as a percentage (Duvick, 1999). The major drawback to these approaches is that their relevance in applied plant-breeding of cross-pollinated crops is limited since inbred lines are normally not the final product the farmers grow. Estimation of heterosis based on MP or BP requires that both parental lines are also included in the trials or planted adjacent to the testcrosses, which may not be practically possible when large number of testcross hybrids are involved.

A third estimate of heterosis, known as standard heterosis (SH), is defined as the superiority of experimental or commercial F_1 hybrids relative to the performance of check hybrids (Makanda et al., 2009). The use of SH has been widely accepted and is based

on the argument that in developing new hybrids, the aim is to surpass the performance of existing commercial cultivars for the trait of interest, thus SH measures the commercial breeding potential of experimental lines and is sometimes referred to as useful heterosis (Meredith and Bridge, 1972). While the argument is practically valid, the check hybrids may not be in anyway related to the experimental hybrids being developed making interpretation of heterosis based on the genetic architecture of the parental populations difficult (Lamkey and Edwards, 1998).

1.6 Heritability and combining ability for yield related traits

1.6.1 Heritability

The ratio of σ^2_G/σ^2_P, known as broad sense heritability (H^2), expresses the extent to which the phenotypic variance (σ^2_P) for a trait is determined by genetic variance (σ^2_G). The ratio informs the breeder as to the extent of non-heritable environmental influences in the phenotypic performance of genotypes in a breeding population (Hallauer and Miranda, 1988). The ratio σ^2_A/σ^2_P, known as narrow sense heritability (h^2), expresses the extent to which phenotypic variation for a trait is determined by additive genetic variance (σ^2_A) and assists the breeder decide on which traits to improve through selection and the appropriate breeding strategy to use (Falconer and Mackay, 1996; Rief et al., 2012). The goal in breeding is to maximise response to selection or genetic gain (ΔG) and that is partly achieved by maximising heritability (Hallauer and Miranda, 1988):

$$\Delta G = ih^2 \sigma_P; \text{ where } i \text{ is the selection intensity.}$$

Factors which affect both H^2 and h^2 are the variances among individuals and families, experimental error of trials (σ^2_e), the magnitude of genotype x environment interaction, and the number of replications (r) and environments (e) included in the trials (Hallauer and Miranda, 1988). The total genetic variance (σ^2_G) is partitioned into additive variance (σ^2_A), dominance variance (σ^2_D) and epistatic variance (σ^2_I). In calculating the heritability, the additive variance (σ^2_A) component is the most important as it is the portion of total genetic variance (σ^2_G) transmitted from parents to offspring and is called the breeding value (Falconer and Mackay, 1996). The non-additive variance which is the sum of the

dominance variance (σ^2_D), which is the interaction between alleles at the same loci, and epistatic variance (σ^2_I), which is the interaction between alleles at different loci, is present in populations of most crops and expressed anew in hybrids but is usually less important in many genetically broad populations (Hallauer and Miranda, 1988).

1.6.2 Combining ability and gene action

Sprague and Tatum (1942) proposed the concept of combining abilities of inbred lines in maize breeding and the concept rapidly spread to all crops including tree species. Combining ability has become one of the most important statistics breeders use to identify potential new inbred lines on the basis of their superior hybrid combinations and the nature of gene action in the trait of interest. Building on the concept of combining ability, a number of genetic models, mating designs and field designs have been developed to obtain genetic information on quantitatively inherited traits (Griffing, 1956; Hallauer and Miranda, 1988, Bernardo, 2002). The mating designs and models have generally partitioned the overall combining ability of lines into general combining ability (GCA) and specific combining ability (SCA). The GCA is generally regarded as a measure of additive gene action while the SCA is equated to the non-additive (dominance and epistatic) effects (Comstock et al. 1949; Reif et al., 2013). The predominance of either additive or non-additive genetic variance in breeding populations will determine the selection strategy to use and type of products to develop. Results from early generation testing in maize indicate that GCA is heritable across generations (Bernardo, 2002).

In sunflower, contrasting results have been obtained on the predominance of either additive or non-additive genetic variances for a number of traits. In a 3 x 10 tester line analysis in sunflower, Miller et al. (1980) found that additive genetic variance accounted for a major portion of the genetic variation for all traits studied except head diameter. The studies by Miller et al. (1980) corroborate earlier conclusions by Sindagi et al. (1979) who found additive genetic effects to be more predominant than non-additive for the traits studied. However, recent studies by Khan et al. (2008) found a greater manifestation of non-additive gene effects in all the traits studied across environments using a 5 x 5 tester line analysis. The different results obtained are perhaps due to the small sizes of populations used in both studies and are therefore specific to the narrow

genetic backgrounds. Recent studies by Reif et al. (2013) using a large sunflower population indicated that additive genetic variance was more predominant than non-additive for seed yield, oil yield and percent oil content and the accuracy of predicting hybrids based on GCA effects could not be further improved by genomic selection methods.

1.7 Selection strategies for variable moisture conditions

1.7.1 Drought tolerance in sunflower

Variable soil moisture environments characterise the sunflower target production environments in South Africa and yield reduction due to drought is common. Developing drought tolerant germplasm should therefore be a priority to stabilise commercial yields. Drought resistance in plants is the phenotypic expression of a number of morphological characteristics and physiological mechanisms, and this is achieved by escape or tolerance (Levitt, 1980). Drought escape involves rapid phenological development and developmental plasticity thereby allowing the plant to complete its life cycle before serious soil and plant water deficits develop (Levitt, 1980; Blum, 1997). Tolerance involves either: (i) dehydration avoidance by maintaining water uptake or reducing water loss (drought tolerance at high water potential); or (ii) desiccation tolerance, which involves osmotic adjustments (drought tolerance at low water potential) (Blum, 1997). Drought tolerance is a complex trait affected by several interacting plant and environmental factors from germination to physiological maturity (Rauf, 2008; Škorić, 2009). Breeders have been less successful in utilising the strategies of drought avoidance and drought tolerance mechanisms in sunflower improvement mainly because not all growth phases of the plant are equally affected by moisture stress. Vannozzi et al. (1999) and identified three important growth stages (GS) as they relate to drought stress in sunflower: (i) early stress, which affects seedling establishment and vegetative growth; (ii) mild, intermittent stress affecting the pre-flowering stages; and (iii) late stress affecting flowering-grain filling period.

1.7.2 Secondary traits associated with drought tolerance in sunflower

Breeding for drought tolerance improves the drought tolerance of the crop at sensitive growth stages and increases yields under drought while maintaining maturity without incurring any yield penalty under optimum conditions (Edmeades et al, 1997). According to Škorić (1992), breeding for drought resistance in sunflower "implies improvement in the efficiency of the root system in terms of water uptake from the soil and utilisation of nutrients, architecture of the main plant parts, time of maturation, and resistance to diseases". In sunflower, morphological traits such as leaf area in the post-flowering period were found to be positively correlated with yield under drought conditions only and genotype by environment interaction contributed to the variability of yield under drought conditions (Vannozzi and Baldini, 1997; Gómez-Sánchez et al*.,* 1999).

Grain yield under water limited conditions is the mathematical product of the amount of water transpired (amount of water available to the plant), water use efficiency (WUE), and harvest index (HI) (Passioura, 1977). Edmeades et al. (1997) classified putative secondary traits associated with drought tolerance in maize into three categories: (i) traits associated with increased water uptake; (ii) traits associated with water use efficiency; and (iii) traits associated with harvest index. Traits associated with increased harvest index were found to be good indicators of yield potential under drought stress (Bolanos and Edmeades, 1993). The traits were also found to be highly heritable, genetically variable, and stable within the period of measurement, not associated with a yield penalty under unstressed conditions, cheaper and faster to measure (Edmeades et al., 1997). The use of these secondary traits in selection indices has been found to increase selection efficiency for drought tolerance in maize (Chapman and Edmeades, 1999). In sunflower little research has been done along this line and more experiments are needed to quantify traits associated with harvest index and how they relate to grain yield.

1.7.3 Selection environments

For secondary traits to be expressed and measured, materials under selection should be grown under stress conditions that simulate the clearly defined stress relevant in farmers' fields (Bänziger et al., 2000). Conducting drought managed trials during the

winter rain free period has been largely used in southern Africa with the aim of developing drought tolerant maize genotypes (Bänziger et al., 2004). Selection for drought tolerance is then done under managed drought stress conditions with emphasis on the: (i) timing of the drought stress with the sensitive growth stage but which can be modified or improved by breeding; (ii) intensity of the drought stress to enable expression of different plant characteristics for yield formation under stress; and (iii) uniformity of the stress applied (Bänziger et al., 2000).

1.7.4 Genetic correlations

Burdon (1977), proposed two types of genetic correlations: type A correlations between two traits measured on the same individuals within the same environment or meaned across environments; and type B correlations between the same trait on the same individuals but measured in different environments. Both estimates of type A and type B genetic correlations are of practical value in quantitative genetics and breeding especially in the use of indirect selection and predicted correlated response to selection either for the trait of interest (type A) or for the target production environment when selection is carried out in a test environment (type B). Type A genetic correlation is particularly important as the genetic variance and heritability of the primary trait in crop improvement, yield is greatly reduced under stress (Chapman and Edmeades, 1999). In crops like maize, the genetic variance and heritability of some secondary traits with strong correlations with yield increase under stress (Bolanos and Edmeades, 1993) and therefore selection for the secondary traits under such conditions theoretically increases the selection efficiency of the primary trait. Likewise type B genetic correlation can be useful in studying genotype by environment interactions between stressed and non-stressed environments (Lu et al., 1999).

1.7.5 Indirect selection and correlated response to selection

Improvement in a trait can be a result of direct or indirect selection. Direct response to selection (R_y) is a function of selection intensity and heritability (H^2 or h^2) of the trait, while indirect response to selection (CR_y) in addition to being a function of selection intensity and heritability is also a function of genetic correlations between the traits *x* and *y* (Falconer and Mackay, 1996). Therefore the amount of improvement or correlated

response to selection (CR_y) for a primary trait *y* (yield) obtained by indirect selection for a secondary trait *x* is given by Falconer and Mackay (1996) as:

$$CR_y = iH_xH_yr_{Gxy}\sigma_{Py}; \text{ based on } H^2 \text{ or } CR_y = ih_xh_yr_{Axy}\sigma_{Py} \text{ based on } h^2$$

Where: *y* and *x* are two traits under consideration; r_{Gxy} is the total genetic correlation between traits *y* and *x*, (i.e. additive and non-additive effects not separated); or r_{Axy} the correlation of breeding values if additive genetic variance is partitioned from total genetic variance; *i* is the selection intensity; H_x *or* h_x is the square root of the broad sense or narrow sense heritability of the secondary trait *x,* respectively; H_y or h_y is the square root of the broad sense or narrow sense heritability of the primary trait *y,* respectively; and σ_{Py} is the square root of the phenotypic variance of the primary trait *y*.

The potential value of indirect selection for a secondary trait *x* compared to direct selection for the primary trait *y* can be computed based on H^2 or h^2 as:

$$CR_y/R_y = r_{Gxy}(i_xH_x/i_yH_y) \text{ or } CR_y/R_y = r_{Axy}(i_xh_x/i_yh_y)$$

Where: R_y is the amount of improvement obtained by direct selection for the primary trait *y*; i_y is the selection intensity for primary trait *y* and all other terms are as previously defined. If the selection intensity is the same for both traits, the indirect selection efficiency (*ISE*) based on H^2 or h^2 is:

$$ISE=CR_y/R_y = r_{Gxy}\, H_x/H_y \text{ or } ISE=CR_y/R_y = r_{Axy}\, h_x/h_y$$

The above formulae are applicable to both type A and type B genetic correlations and in this thesis to distinguish between the two types of genetic correlations and indirect selection efficiency, type B parameters will be denoted by the prime symbol r'_{Gxy}; r'_{Axy} and *ISE'* for total genetic correlation; additive genetic correlation and indirect selection efficiency, respectively.

1.8 Synopsis of the Literature Review

While a review of literature for each subject of study is provided in the chapters of the thesis, a detailed search of the literature indicates that there is limited if any information on the genetic gain, advanced cycle pedigree breeding and correlated response to selection in sunflower under variable moisture conditions in Africa and in particular South Africa despite years of continued investment in sunflower breeding. Quantifying genetic gain is necessary as it forms the basis of continued investment in plant breeding, and the challenge is to establish or maintain a balance between genetic gain and diversity.

The success of hybrid cultivar development using conventional breeding methods has been associated with the widespread use of advanced cycle pedigree breeding and its use in hybrid cultivar development will continue for the foreseeable future. Therefore periodic assessment of the genetic parameters in a breeding programme should be carried out to monitor and rectify if necessary, the potential negative effects of advanced cycle pedigree breeding.

Drought continues to limit crop production in subtropical and tropical environments including sunflower. Current selection strategies of using secondary traits and managed drought environments to improve tolerance to drought have been applied successfully in other crops such as maize and rice but their application in sunflower is still limited.

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CHAPTER 2

Seed yield and associated trait improvements in sunflower cultivars over four decades of breeding in South Africa[1]

Abstract

Genetic improvement for seed yield and oil-content in sunflower (*Helianthus annuus* L.) was initiated in the early 1970s in South Africa. Since then no study has been carried out to assess the progress and contribution of new cultivars to seed yield improvement to justify continued investment into breeding new cultivars. The aim of this study was therefore to quantify the contribution of new cultivars to seed yield and associated traits in sunflower over four decades of breeding in South Africa. Two data-sets were used in this study: (i) side-by-side evaluation of historical and current sets of popular cultivars in the same environment under one set of trial management practices; (ii) yield trends in commercial farmers' fields based on annual production estimates. The absolute genetic gain (yield increase per year) for seed yield in the side-by-side trials ranged from 18 to 32 kg ha^{-1} $year^{-1}$ with a mean of 24 kg ha^{-1} $year^{-1}$. In contrast, the absolute seed yield increase under commercial production was 12 kg ha^{-1} $year^{-1}$. The estimated relative genetic gain for seed yield based on side-by-side trials was 1.5% $year^{-1}$ and the relative gain in seed yield per year under commercial production was 1.9% $year^{-1}$. The contribution of new cultivars to total seed yield progress in sunflower were 56.3% for the period 1970 to 1989; 23.9% from 1990 to 2009 and the mean over the four decades under consideration 1970 to 2009 was 41.6%. Positive increases in genetic gains for oil yield, oil content and number of seeds per head were also obtained although the gain in oil content was relatively low.

Keywords: Absolute genetic gain, breeding decade, relative genetic gain, sunflower, yield increase

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2.1 Introduction

Sunflower (*Helianthus annuus*. L) is a major oil crop contributing 82% of all edible oil in South Africa (Nel, 2001). Annual sunflower production area in South Africa rose from 107 000 ha in 1956 to a peak of 828 000 ha in 1999 (Figure 2.1). The increases in sunflower production area and yield per ha were characterised by fluctuations caused by a combination of factors including seed price, introduction of hybrid cultivars in the late 1970s, drought in the drier regions and Sclerotinia stalk and head rot (*Sclerotinia sclerotiorum*) in cooler areas (Birch et al., 1978). Although not documented, lack of tolerant cultivars to Sclerotinia stalk and head rot in the cooler areas is thought to have pushed sunflower production from cooler areas to the drier and more marginal regions (Liebenburg, *personal communication*). In recent years, approximately 80% of the area annually under sunflower production is mainly concentrated in the low rainfall areas of the North-West and Free State provinces of the country (SAGIS, 2011).

Formal sunflower breeding in South Africa spans more than four decades and hybrid cultivars became available on the market in late 1970s (Birch et al., 1978). The major breeding objectives have remained unchanged, revolving around continued exploitation of heterosis for seed yield, seed oil concentration and oil quality (Chigeza, 2007). Reported mean levels of heterosis for seed yield in sunflower vary from 149-152% (Vrănceanu, 1998), 15.3-288.3% (Kaya, 2005) while Miller (1987) states that sunflower hybrids yield about 50% more than the better open pollinated cultivars (OPCs). Compared with other hybrid crops, the estimated yield advantage of sunflower hybrids over OPCs is more than three times as large as that of maize (Duvick, 1999). Despite the high magnitude of heterosis for seed yield and yield advantage of sunflower hybrids over OPCs, studies to assess genetic progress over time are limited and only a few studies have been reported notably that of Pereira et al. (1999) and de la Vega et al. (2007), both based on sunflower breeding programmes in Argentina. Investment in plant breeding must be considered like any other investment in terms of potential gains versus costs. Periodic assessment of the benefits of continued investment in plant breeding allows for: (i) identification of temporary constraints obstructing further genetic improvement in yield overtime that could be permanent if not investigated and improved; and (ii) prioritisation of traits especially those linked to quality, quantity and a reduction in the impact of crop production on the environment.

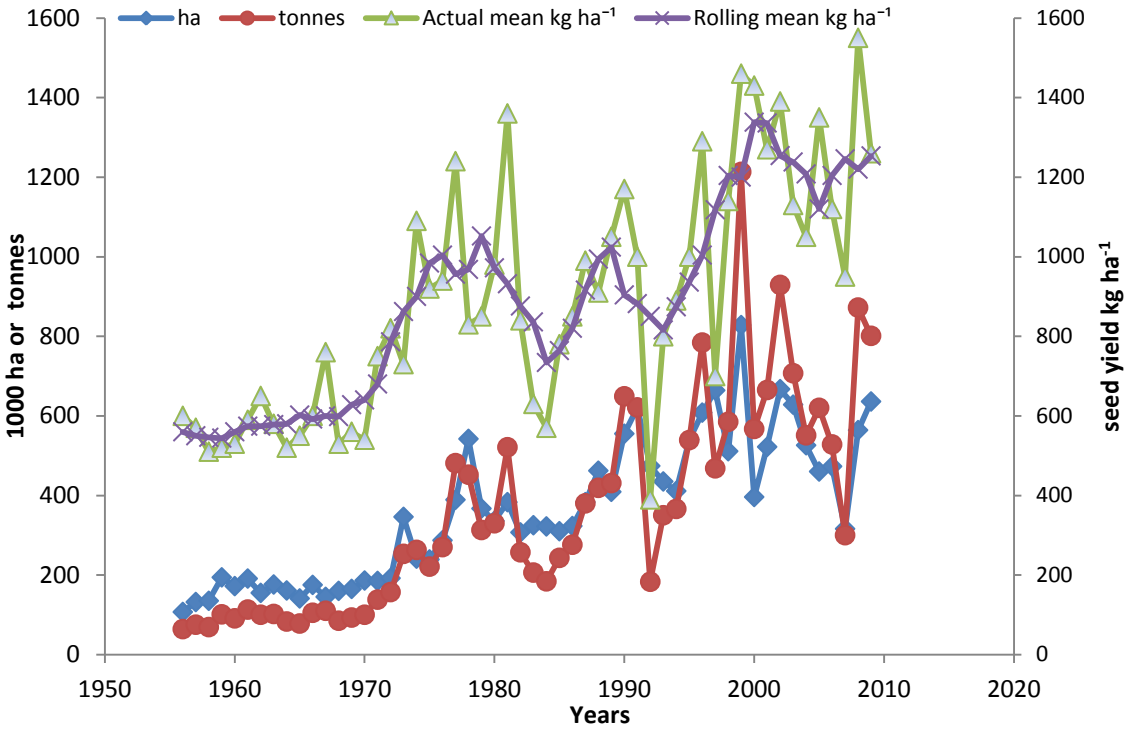

Figure 2.1 Sunflower production trends (ha year^{-1}, tonnes year^{-1}, mean seed yield and five-year rolling mean seed yield) in South Africa from 1956-2009. (Source: SAGIS, 2011).

Several methods are used to measure genetic gain for yield in major crops based on either side-by-side performance trials of obsolete cultivars and new cultivars under common field conditions (Luque et al., 2006) or historical data from national yield trials (de la Vega et al., 2007). Each method has its own merits and demerits. Arguments against side-by-side comparison is that agronomic practices and incidence of pests and disease change with time and therefore the performance of older cultivars under modern growing conditions may be affected (de la Vega et al., 2007). For a crop like sunflower where agronomic practices and inputs have remained relatively stable and moderate (Škorić, 1992; Fick and Miller, 1997), side-by-side comparison offers the opportunity to measure yield differences in the same environment enabling the determination of the drivers of improvement (or lack thereof) in performance (Morrison et al., 2000; Ortiz et al., 2002).

Thus the objectives of the current study were: (i) to explore the changes in seed yield and its associated traits since the formal inception of sunflower breeding in South Africa four decades ago using side-by-side trial evaluation; and (ii) compare and combine the relative genetic gains estimated from side-by-side trial evaluation with those estimated under commercial production to determine the contribution of new cultivars to total yield improvement.

2.2 Methods and materials

Two sets of data were used for the study: (1) field trial data based on side-by-side evaluation of sunflower cultivars at two sites over two years (four environments) under the same agronomic management practices at each environment; and (2) commercial production data over all cultivars based on the Department of Agriculture's crop assessment reports in South Africa for the period 1970-2009 (SAGIS, 2011).

2.2.1 Side-by-side evaluation

2.2.1.1 Genotypes

Twenty five sunflower genotypes released or introduced in South Africa during the period 1975 to 2006 were used in this study (Table 2.1). The genotypes evaluated represented different breeding eras or decades of crop improvement and were also the best performing cultivars widely grown by commercial farmers in South Africa. The 25 genotypes were allocated to their applicable breeding decade as follows: (i) the 1970s, six OPCs released between 1975 and 1979; (ii) the 1980s, six hybrid cultivars released between 1980 and 1989 that were included in the national cultivar trials until the early 1990s; (iii) the 1990s, five hybrid cultivars released between 1990 and 1999 some of which are still included in the national cultivar trials; and (iv) the 2000s, eight hybrid cultivars released between 2000 and 2006 with at least two or more years in the national cultivar trials. For this study, the year the cultivar was first entered in the national sunflower cultivar trials was considered as its year of release (YOR). Remnant seed stored at 4ºC from a series of past national sunflower cultivar trials was used. Seed viability was first tested by germinating seed in the dark at 20/30ºC and the seed used had a minimum germination of 88%. The national sunflower cultivar trials in South Africa,

coordinated by the Agricultural Research Council (ARC), were started in 1975 (Birch et al., 1978) and trials continue to be conducted throughout the sunflower growing regions. Information from national cultivar trials not only allows farmers to choose cultivars that consistently give high yields under their production environments but also to apply similar agronomic practices as those used in research trials.

Table 2.1 List of the sunflower genotypes used in the study

Cultivar	Cultivar Type	Breeding Decade	Year of Release[a]	Year last included in the Cultivar Trials[b]	Source of Seed
KORTUS	OPC	1970	1975	1980 (5)	Eastern Transvaal Co-op
GOR104	OPC	1970	1975	1981 (6)	Gunson SA (Pty) Ltd
F_LEVER	OPC	1970	1975	1979 (4)	Northern Transvaal Co-op
SMENA	OPC	1970	1975	1979 (4)	Northern Transvaal Co-op
GOR33	OPC	1970	1976	1978 (3)	Gunson SA (Pty) Ltd
HV775	OPC	1970	1978	1980 (3)	Agricultural Research Council
SO323	Hybrid	1980	1980	1992 (11)	Saffola Seeds (Pty) Ltd
SO222	Hybrid	1980	1981	1992 (10)	Saffola Seeds (Pty) Ltd
SO210	Hybrid	1980	1984	1992 (4)	Saffola Seeds (Pty) Ltd
CAR1006	Hybrid	1980	1985	1993 (7)	Cargill Seeds (Pty) Ltd
SNK37	Hybrid	1980	1988	1997 (10)	Sensako
AS470	Hybrid	1980	1989	1994 (5)	Asgrow SA/Carnia Seed
HY333	Hybrid	1990	1994	2006 (13)	Africa Pacific Seeds/K2 AgriSeeds
AG8751	Hybrid	1990	1997	2005 (9)	Agricol
PAN7355	Hybrid	1990	1997	2004 (8)	Pannar Seed CO.
HV3037	Hybrid	1990	1997	2005 (9)	Agricol
AG5551	Hybrid	1990	1999	2008 (9)[†]	Agricol
AG8251	Hybrid	2000	2001	2008 (7)[†]	Agricol
DK4040	Hybrid	2000	2001	2008 (6)[†]	Monsanto
DKF68_22	Hybrid	2000	2002	2008 (6)[†]	Monsanto
PAN7034	Hybrid	2000	2004	2008 (2)[†]	Pannar Seed CO.
PAN7033	Hybrid	2000	2005	2008 (3)[†]	Pannar Seed CO.
AG5383	Hybrid	2000	2005	2008 (3)[†]	Agricol
PAN7050	Hybrid	2000	2006	2008 (2)[†]	Pannar Seed CO.
PAN7048	Hybrid	2000	2006	2008 (2)[†]	Pannar Seed CO.

[a]Year of release (YOR) -Year first entered in the national cultivar trials.
[b] Actual years in the national sunflower cultivar trials in brackets.
[†]Cultivar's life span in the national sunflower cultivar trials which have gone past the 2008 season.

2.2.1.2 Trial sites and agronomic practices

The trials were planted at two locations: Potchefstroom and Bothaville, during the December-May 2006/7 and 2007/8 standard seasons, or simply the 2007 and 2008 seasons, respectively. Potchefstroom is situated at 26.745°S, 27.083°E, 1 322 m above sea level in the Northwest Province, and Bothaville is situated at 27.235°S, 26.067°E, 1 276 m above sea level in the Free State Province, South Africa. These two provinces

constitute 80% of total sunflower production area in South Africa (SAGIS, 2011). The weather data for the two locations during the cropping seasons are provided in Figure 2.2. The 2007 season trial at Potchefstroom was planted in the first week of December 2006 and was irrigated to field capacity at planting. Further supplementary irrigation totaling approximately 75 mm was applied at approximately 30, 50 and 70 days after emergence. For the 2008 trial at Potchefstroom, planting was done in the last week of December 2007 and the trial was irrigated to field capacity only at planting. The Bothaville trials were planted in the first week of January of each year (2007 and 2008), and no irrigation was applied.

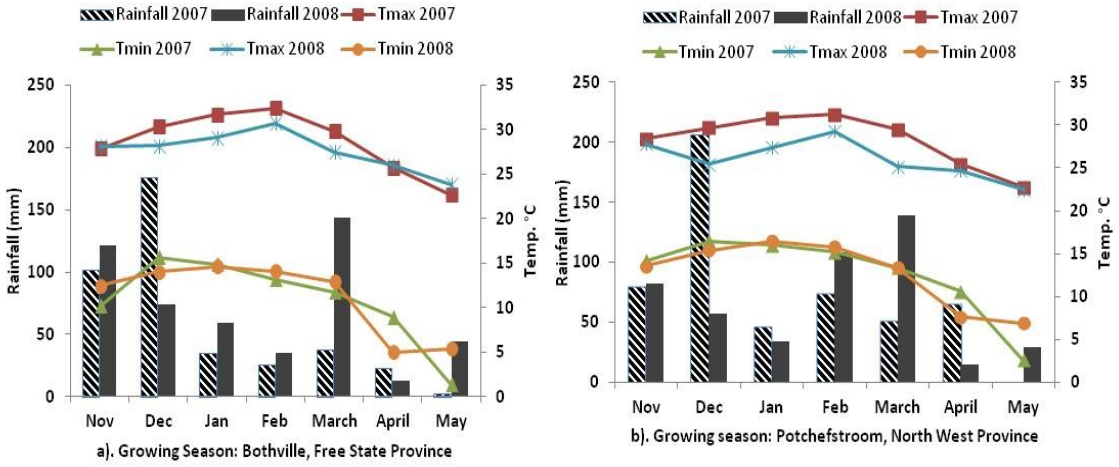

Figure 2.2 Monthly rainfall, maximum and minimum temperatures during the growing seasons: (a) Bothaville, and (b) Potchefstroom. (Source: ARC-Institute for soil, climate and water, 2008).

Trial design was a randomised complete block with two replications. Plots with four rows, each 6 m long, and an inter-row spacing of 0.9 m were used for both years and sites. Intra-row spacing for both years was 0.33 and 0.40 m for Potchefstroom and Bothaville, respectively. Each trial was bordered by four or six rows on either side of the field to eliminate possible border effects. Trials were overplanted using a mechanical planter to ensure uniform plant stand in each plot after thinning. Early seedling vigour was scored subjectively on a 1-5 scale; (1-good to 5-poor) before thinning. As most plots had a score of either 1 or 2, seedling vigour was therefore assumed to be normal and was not considered as a covariate in seed yield calculations. Seedlings were thinned at three weeks after emergence and plant population at Potchefstroom was 32 000 plants ha^{-1}, while that of Bothaville was maintained at 28 000 plants ha^{-1}. Recommended agronomic practices were followed at both sites, include basal application of 150 kg ha^{-1} blended

fertilizer (3N:2P:1K (25) + 0.5% Zn) incorporated into the seedbed before planting. A further 28 kg ha^{-1} N was applied at four weeks after emergence. The fields were kept weed free by pre-emergent herbicide application soon after planting and mechanical weeding during the thinning operations.

2.2.1.3 Data collection

Data were collected on the middle two rows on each plot. The traits measured were: seed yield (kg ha^{-1}), oil content (%), oil yield (kg ha^{-1}), number of seeds (achenes) per head, 1000-seed mass (g),days to 50% flowering, plant height (cm) at early physiological maturity, mid-stem diameter at harvest (cm), and head diameter (cm) at harvest. Heads were harvested by hand at approximately 20% seed moisture. Seed yield was first determined on a plot basis adjusted to 10% seed moisture by weighing seed which had been dried in continuous air flow dryers at 32-35˚C over a period of two weeks until no further loss in mass was recorded. Oil content (%) was determined on 12 g, air-dried seed samples by nuclear magnetic resonance using a Newport Analyzer (Newport-Oxford Instruments Ltd, England). Oil yield (kg ha^{-1}) is the mathematical product of seed yield (kg ha^{-1}) and oil content (%).

2.2.1.4 Statistical analysis

Statistical analyses were performed using REML procedures in GENSTAT version 12 (Payne et al., 2007). With less than five environments, rule 8 of Piepho et al. (2003) was applied and environments were considered as fixed effects, rather than as random effects as the small sample size would have provided unreliable variance components. The fixed effect model used for the analysis was:

$$Y_{ijkr} = \mu + D_i + (D/C)_{ij} + E_k + (DE)_{ik} + E_k(D/C)_{ij} + (E/R)_{kr} + \varepsilon_{ijkr}$$

Where Y_{ijkr} is the phenotypic observation on cultivar *j* released in the breeding decade *i* tested in environment *k*, and replicate *r*; μ is the grand mean; D_i the effect of breeding decade *i*; $(D/C)_{ij}$ the effect of cultivar *j* released in breeding decade *i*; E_k the effect of environment *k* (i.e. location x year combination); $(E/R)_{kr}$ the effect of the replicate *r* nested within environment *k*. The D_i and $(D/C)_{ij}$ constitute the genotype main effects of

the model; E_k the environment main effects; $(DE)_{ik}$ and $E_k(D/C)_{ij}$ the genotype x environment interaction; and ε_{ijkr} the random error term.

Absolute genetic gains for seed yield and other traits were determined from the slope of the regression of the actual mean performance for each trait (ordinate) on year of release (YOR) (abscissa). Relative genetic gain as percent increase per year was obtained by regressing the actual performance of each cultivar relative to the 1970s decade base mean (ordinate) on YOR (abscissa). The 1970s base mean was determined as the mean of the predicted performance (from the absolute genetic gain regression) of all cultivars within the breeding decade 1970.

Phenotypic correlations between seed yield and the other traits were done using cultivar means over environments. In order to quantify changes in correlation between seed yield and other traits due to breeding, the breeding decades were grouped into breeding decades spans as follows: 1970s and 1980s; 1970s, 1980s and 1990s; 1980s and 1990s; 1980s, 1990s and 2000s; 1990s and 2000s; and all four decades from the 1970s to 2000s. The phenotypic correlation analysis was conducted with PROC CORR (SAS Institute, 2010) and the scatter plots of two breeding decades spans, 1970s and 1980s, and 1990s and 2000s, was plotted with PROC SGSCATTER (SAS Institute, 2010).

2.2.2 Contribution of new cultivars to total seed yield increase

The annual national sunflower production data from 1970 to 2009 was used to calculate the seed yield trends per ha under commercial production conditions. These data included total tonnage produced and the area under production over all cultivars in a particular year. Approximate mean national seed yields per ha were computed by dividing total tonnage produced by total area under production in a given year. In order to avoid under or over-estimation as a result of some years having exceptionally poor or good yields due to variable weather conditions, a five-year rolling mean was calculated (Tracy et al., 2004).

Absolute genetic gain per year under farmers' production environment was then calculated as the slope of the regression line of annual yields per ha regressed against year of production. Relative yield increase in the farmers' fields from 1970 to 2009 was

calculated based on the rolling mean of yield obtained during the 1970 production year as baseline, i.e. rolling mean of yield for 1970 was set at 100 and the percentage increase/decrease regressed against the year of production.

The contribution of new cultivars to yield improvement was estimated for the periods 1970 to 1989, 1990 to 2009 and the entire four decades 1970 to 2009. The contribution of new cultivars to yield improvement at a specified interval was calculated as:
Contribution of new cultivars = (% yield progress in cultivars (breeding decades)) divided by (% yield increase in commercial production) (Lillemo et al. (2010).

2.3 Results and Discussion

2.3.1 Side-by-side trial evaluation

2.3.1.1 Growing season conditions

The amount and distribution of rainfall during the growing season varied between locations and years. In 2007, Bothaville received 400 mm during the growing season of which more than 50% was received before sowing. In contrast, of the 490 mm of rainfall during the 2008 growing season, more than 50% was received during flowering and seed filling stage (Figure 2.2a). Potchefstroom received 519 mm during the 2007 growing season of which 50% was received soon after sowing. In the 2008 growing season, the total rainfall at Potchefstroom was 465 mm of which more than 50% was received during the flowering and seed filling stage (Figure 2.2b). Temperatures at both locations were high during the 2007 growing season compared to 2008 with mean maximum temperatures occurring during the flowering to seed filling stages (Figures 2.2a and b).

The trial mean for seed yield increased with the amount of rainfall with Potchefstroom, 2007 recording the highest trial mean of 2 201 kg ha^{-1}. Bothaville, 2007 which received the least amount of rainfall had the lowest trial mean of 1 388 kg ha^{-1}. The high seed yield obtained at Potchefstroom during the 2007 season may also be due to the application of supplementary irrigation during the vegetative and early flowering stages.

2.3.1.2 Genotype and environmental effects

Genotype and environmental effects were highly significant ($p<0.01$) in the pooled analysis over four environments for all the traits indicating that statistical differences occurred among genotypes and among environments (Table 2.2). When genotype is partitioned into decade and cultivars within a decade; decade effects were significant ($p<0.01$) for all the traits except for 1000-seed mass while there was no significant differences among cultivars within a decade for head diameter (Table 2.2). The magnitude of the mean squares for decade and cultivar within decade varied with trait. The decade effect had mean squares 1.5 to 3 times larger than the cultivar within decade effect for agronomic traits such as seed yield, oil yield, oil content and numbers of seeds per head indicating that the variation among decades was greater than mean variation of cultivars within decades. The reverse was true for the morphological traits: 1000-seed mass, stem height and days to flowering. The causes of the differences in the magnitudes of these mean squares are complex and partly due to the effects of genotype, environment and the interaction thereof.

Genotype-environment interaction was significant ($p<0.01$) for percent oil content, stem height and stem diameter only (Table 2.2). These traits are readily affected by environmental factors for example, oil content in the seed is affected by several factors such as moisture availability at seed fill, duration of seed fill, and mean daily temperatures above 25ºC (Škorić, 1992). The overall genotype by environment interaction for oil yield and 1000-seed mass was non-significant; however, the decade by environment component was significant ($p<0.05$).The magnitude of the mean squares of the genotype by environment interaction for oil yield, oil content, and seed yield, number of seeds, stem height, and days to flower was 1.5 to 3 times smaller than the mean square for genotype effect indicating a relatively stable ranking of the cultivars (Gomez and Gomez, 1984). Similar trends have been found in comparisons of old and new maize and wheat cultivars where the magnitude of genotype by environment interaction was either non-significant or 5 to 100 times less than that of either genotype or environment (Ortiz et al., 2002; Fufa et al., 2005; Ci et al., 2011). This alone may not be an indication that genotype by environment does not exist in the target production environments but only reinforces the expectation that cultivars in the same breeding decade are likely to be closely ranked together.

Table 2.2 Wald statistics (mean squares) of seed yield and related traits of sunflower cultivars evaluated over four environments.

Fixed term	d.f.	Grain yield (kg ha^{-1})	Oil - content (%)	Oil-yield (kg ha^{-1})	Seeds head^{-1}	1000 seed mass (g)	Stem Height (cm)	Stem Diameter (cm)	Head Diameter (cm)	Days 50% flowering
Rep (Env)	4	15.9**	4.4	13.1*	15.8**	1.5	5.8	7.2	2.6	0.9
Env	3	134**	20.5**	148.1**	20.2**	75.6**	395.0**	155.8**	235.1**	25.4**
Genotype	**24**	**145.3*** **	**319.5*** **	**195.6*** **	**147.6*** **	**58.1*** **	**324.1*** **	**149.5*** **	**74.6*** **	**196.7*** **
Decade	3	95.5**	228.6**	136.4**	99.9**	2.5	68.2**	81.0**	53.8**	35.4**
Cultivar (Decade)	21	49.6**	91.2**	58.9**	47.3**	55.5**	255.9**	68.4**	20.8	161.3**
Env.Genotype	**72**	**83.4**	**149.8*** **	**87.9**	**86.6**	**75.9**	**135.9****	**135.9****	**66.3**	**79.3**
Env.Decade	9	13.8	35.2**	20*	13.3	18.6*	21.9*	20.3*	13	15.4
Env.Cultivar (Decade)	63	69.8	114.3**	68.3	74	57.4	114**	115.6**	53.4	63.9
Error	96	153376	1.8	24335	36352	56.2	197.8	0.1	2.9	5.1
CV (%)		22.9	3.4	22.9	23	10.9	8.3	18.6	14.4	3.3

*p<0.05.
** p<0.01.
†Genotype = Breeding decades and cultivars released within decade; CV = Coefficient of variation (%).

2.3.1.3 Absolute genetic gain and response to the environment

The mean for seed yield at each environment increased with the breeding decades, with cultivars in the 1970s decade having the lowest mean (Figure 2.3). The mean of cultivars released in the 1970s and evaluated at Bothaville, 2007 was 1 110 kg ha^{-1} compared to 1 646 kg ha^{-1} for the hybrid cultivars released in the 2000s. Potchefstroom, 2007, which received supplementary irrigation, had the highest seed yield with the mean of OPCs released in the 1970s at 1 835 kg ha^{-1}, while the mean of hybrid cultivars released in the 2000s was 2 742 kg ha^{-1} (Figure 2.3). Similar trends were observed in other traits in which the OPCs released in 1970 had lower means than the new cultivars for most traits under consideration except 1000-seed mass.

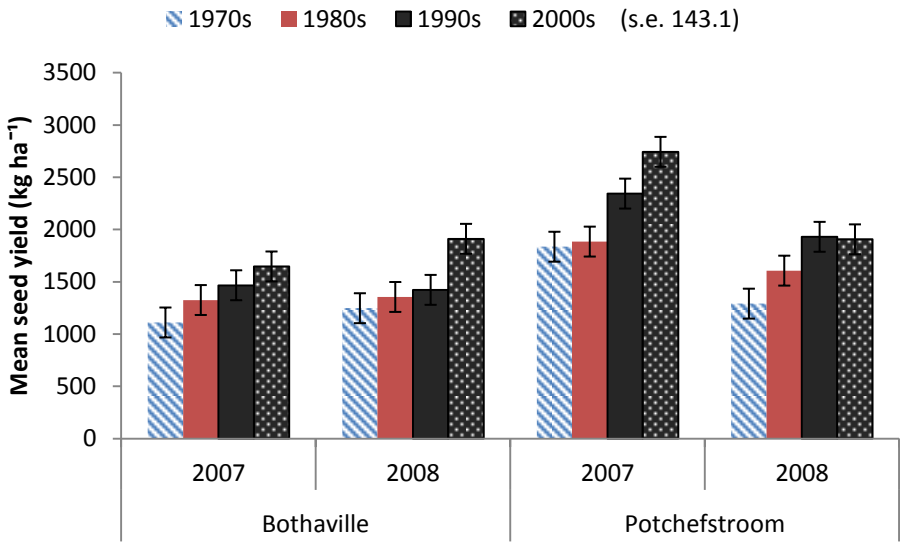

Figure 2.3 Decade mean (breeding period) for seed yield at four environments.

The absolute genetic gain for seed yield ranged from 18 kg ha^{-1} year^{-1} (Bothaville, 2007) to 32 kg ha^{-1} year^{-1} (Potchefstroom, 2007; Table 2.3). The mean absolute gain across environments was linearly related with the breeding decade at 24 kg ha^{-1} year^{-1} (Figure 2.4). The magnitude of absolute genetic gain for seed yield was higher at Potchefstroom, 2007 than Bothaville, 2007 probably because of the supplementary irrigation applied and slightly higher plant population density used at Potchefstroom, 2007. Pereira et al. (1999) and de la Vega et al. (2007) reported that in Argentina seed yield in sunflower increased at a rate of 49 kg ha^{-1} year^{-1} from early 1970s to mid-1990s.

The lower rate of absolute seed yield gain obtained in this study compared to results by Pereira et al. (1999) and de la Vega et al. (2007) maybe due to the lower plant populations density used in South Africa. The recommended plant populations density of 28 000-40 000 plants ha^{-1} in South Africa have remained unchanged since the 1970s (Nel and Venter, 2008) and is lower than that recommended by Škorić (1992) of 55 000 to 65 000 plants ha^{-1}.

The absolute genetic gain for oil yield followed similar trends to those of seed yield and ranged from 9 kg ha^{-1} $year^{-1}$ (Bothaville, 2007 season) to 16 kg ha^{-1} $year^{-1}$ (Potchefstroom, 2007) (Table 2.3). The mean absolute genetic gain for oil yield across environments was 12 kg ha^{-1} $year^{-1}$ (Figure 2.4). A similar rate of increase in oil yield was found by de la Vega et al. (2007) in Argentina, using data of 49 conventional sunflower hybrids based on unbalanced long term data from national cultivar trials from 1983 to 2005.

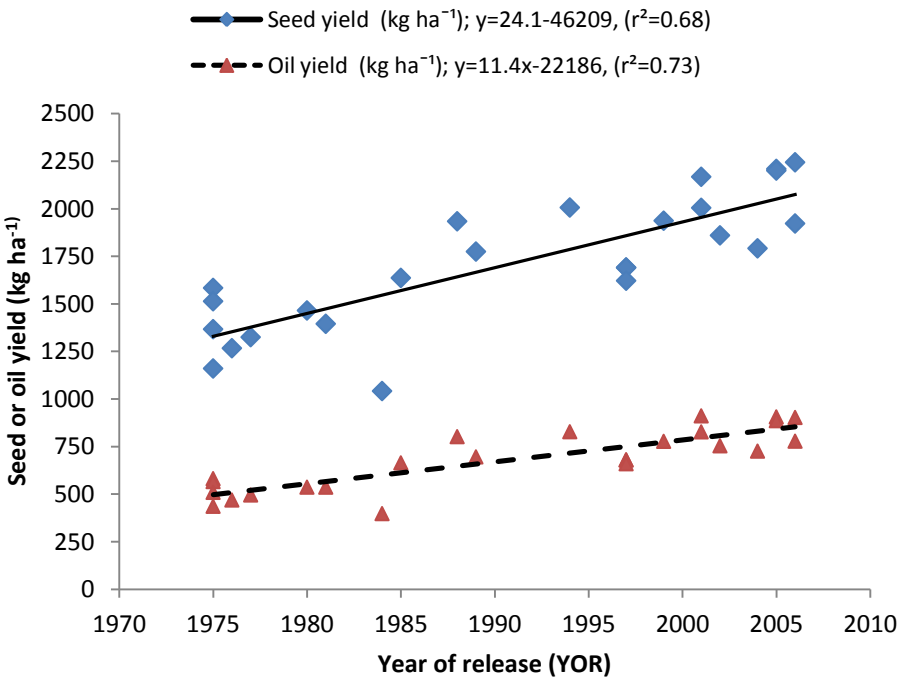

Figure 2.4 Absolute genetic gain for seed yield and oil yield across all environments. Seed yield per cultivar over four environments regressed on year of release (YOR).

Although absolute genetic gain of oil content increased with breeding decade, its magnitude was not influenced by the amount of rainfall or supplementary irrigation

applied indicating that other factors such as temperature are also important. The absolute genetic gain for oil content ranged from 0.09% year^{-1} (Potchefstroom, 2008) to 0.17% year^{-1} (Bothaville, 2008), with a mean of 0.12% year^{-1} (Table 2.3). When mean oil content of the cultivars was plotted against YOR, a fitted quadratic model showed an increase in oil content in cultivars up to the 1990 breeding decade and a leveling off in oil content increase in the 2000 breeding decade (Figure 2.5). For all other traits, except 1000-seed mass absolute genetic gains showed a linear trend with YOR indicating genetic improvements of the traits under various environmental conditions over time (Table 2.3). The most notable traits which had significant ($p<0.01$) improvements over time were number of seeds per head, head and stem diameter (Table 2.3). When only hybrid cultivars released in the 1980s, 1990s and 2000s breeding decades were considered, a slight increase in absolute genetic gain for seed yield of 27 kg ha^{-1} year^{-1} and oil yield 12.2 kg ha^{-1} year^{-1} was observed while oil content dropped to 0.09% year^{-1} (Table 2.3).

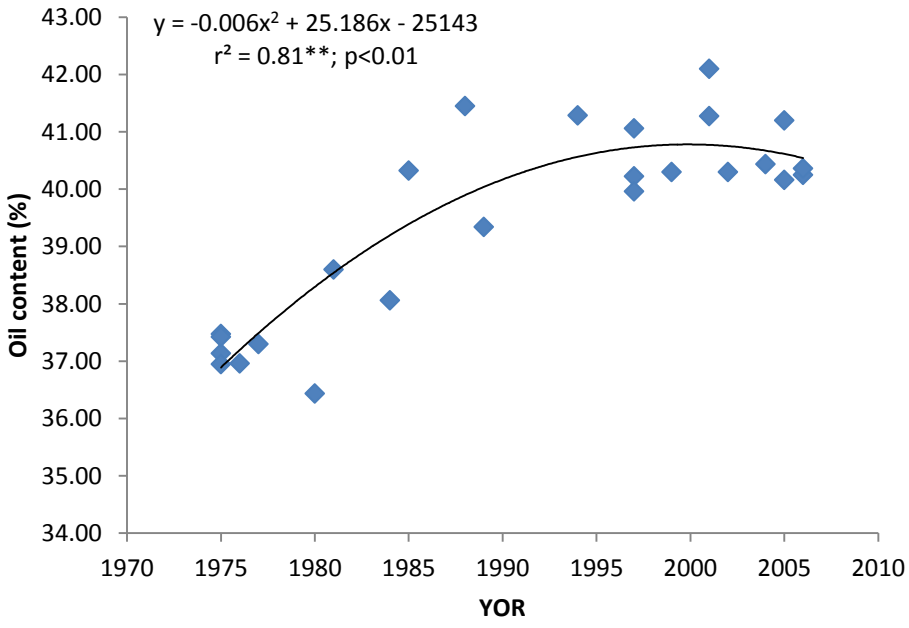

Figure 2.5 Absolute genetic gain for oil content across all environments. A quadratic model was fitted rather than a bi-lineal model.

Table 2.3. Environment mean, absolute genetic gain (*b*) and r^2 for seed yield and related traits at each environment and across environments.

		Seed yield (kg ha^{-1})	Oil content (%)	Oil yield (kg ha^{-1})	Seeds head^{-1}	1000-seed mass (g)	Plant height (cm)	Stem diameter (cm)	Head diameter (cm)	Days to 50% flowering
Bothaville 2007 season	**Mean**	1404±145.9	39.6±0.8	559±58.9	805±101.7	62.5±6.1	135.6±10.9	1.307±0.2	9.0±1.4	67±1.9
	b$_t$	18.2	0.1	8.6	11.0	-0.04	0.6	0.01	0.1	0.1
	r²	0.6**	0.4**	0.6**	0.6**	0.0	0.1	0.2*	0.4**	0.1
Bothaville 2008 season	**Mean**	1520±279.2	38.8±1.0	594±107.4	788±136.3	69.4±6.0	186±10.2	1.9±0.2	13.2±1.3	68.8±1.6
	b	23.1	0.2	11.3	11.1	0.1	1.2	0.0	0.1	0.0
	r²	0.4*	0.6**	0.5**	0.3*	0.0	0.2*	0.5**	0.5**	0.0
Potchefstroom 2007 season	**Mean**	2239±259.6	40.0±1.0	903±111.2	935±118.4	75.3±4.6	180.3±10.5	2.0±0.3	13.6±1.0	68.1±1.4
	b	32.4	0.1	15.8	14.2	-0.1	0.3	0.0	0.1	0.1
	r²	0.6**	0.6**	0.6**	0.7**	0.0	0.1	0.3*	0.5**	0.4**
Potchefstroom 2008 season	**Mean**	1691±374.8	39.35±1.0	668±146	796±174	66.9±4.4	174±8.0	1.6±0.2	11.2±1.1	66.8±1.4
	b	22.6	0.1	10.1	12.2	-0.1	0.8	0.0	0.0	0.1
	r²	0.3*	0.4**	0.4*	0.3*	0.0	0.3*	0.2*	0.2*	0.1
Across Environments	**Mean**	1713±139.8	39.5±0.5	681±55.7	831±67.7	68.5±2.7	169±5.0	1.7±0.1	11.8±0.6	67.7±0.8
	b	24.1	0.1	11.5	12.1	-0.0	0.7	0.0	0.1	0.1
	r²	0.7**	0.7**	0.7**	0.7**	0.0	0.2*	0.5**	0.7**	0.2*
	***b*†**	26.8	0.1	12.2	13.1	0.0	0.9	0.02	0.1	0.1
	r²†	0.6**	0.4**	0.5**	0.6**	0.0	0.2*	0.5**	0.6**	0.2

*p<0.05, ** p<0.01.
† Only for the hybrid cultivars, 1980, 1990 and 2000 breeding decades.

Response to the environment was estimated by regressing mean seed yield of cultivars within a decade against the environmental means, genotype responses from low yielding to high yielding sites were higher in the 1990 and 2000 breeding decades, indicating that newer cultivars were more responsive to favourable conditions (Figure 2.6). As there were no pests and diseases observed in the trials, the performance of hybrid cultivars released in the 2000s compared to cultivars released in the 1970s and 1980s is an indication that there are more tolerant to 'uncontrolled abiotic stresses' (Duvick, 2005).

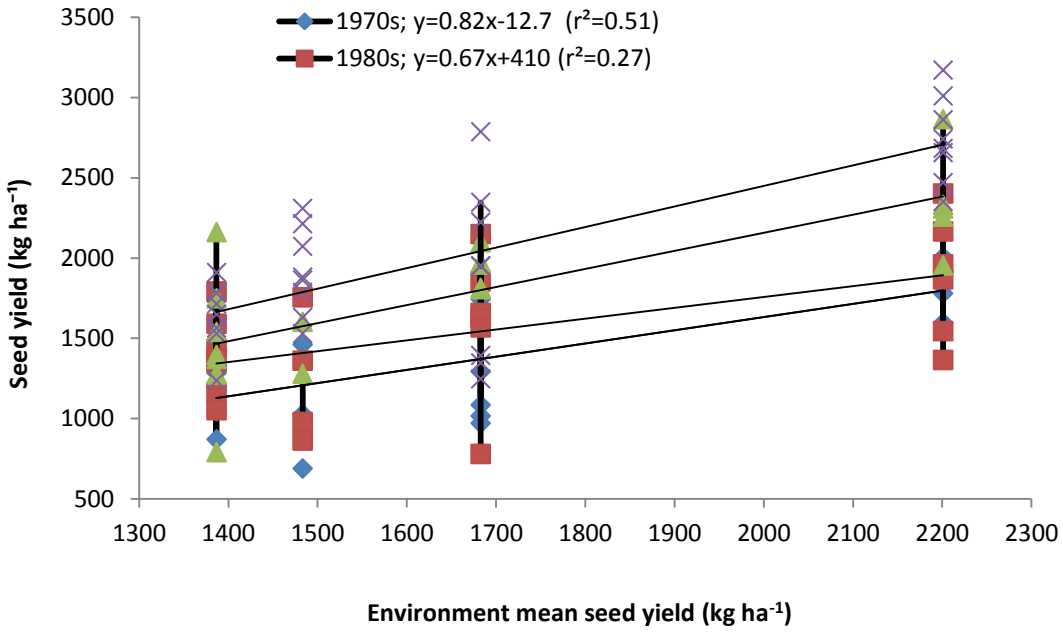

Figure 2.6 Mean seed yield of cultivars released over four decades regressed on the mean of the environment (trial mean).

2.3.1.4 Relative genetic gain and estimation of genetic yield gain

Since the overall national sunflower seed production is collectively based on all cultivars in use during that year, the relative genetic gain per year was calculated using the predicated mean of the OPCs released in the 1970s as the baseline. The estimated relative genetic gain for seed yield, oil content and oil yield are 1.52% year^{-1}, 0.33% year^{-1} and 2.31% year^{-1}, across all decades and cultivars, respectively (Figure 2.7). The estimate of genetic gain for seed yield is within the limits of most crops reported in the literature. In soybean estimates of relative genetic gain of 1.0 to 1.35% year^{-1} have been reported in the mid-western USA of (Egli, 2008). Relative genetic gain in wheat in the UK

was reported to be 1.2% year^{-1} (Shearman et al., 2005) while estimates of relative genetic gain in rice *(Oryza sativa)* in Brazil was 1.44% year^{-1} (Breseghello et al., 2011). Much higher relative genetic gains have also been reported such as the 6.7% year^{-1} in wheat grain yield in Brazil by Cargnin et al. (2009) and 4.4% year^{-1} in semi-determinate cowpeas by Kamara et al. (2011), amongst other studies. Estimates of genetic gain for oil content are on the lower side in this study probably due to genotype x environment interaction (Table 2.2). The relative genetic gain per year for oil yield is high due to selecting for both seed yield and oil content.

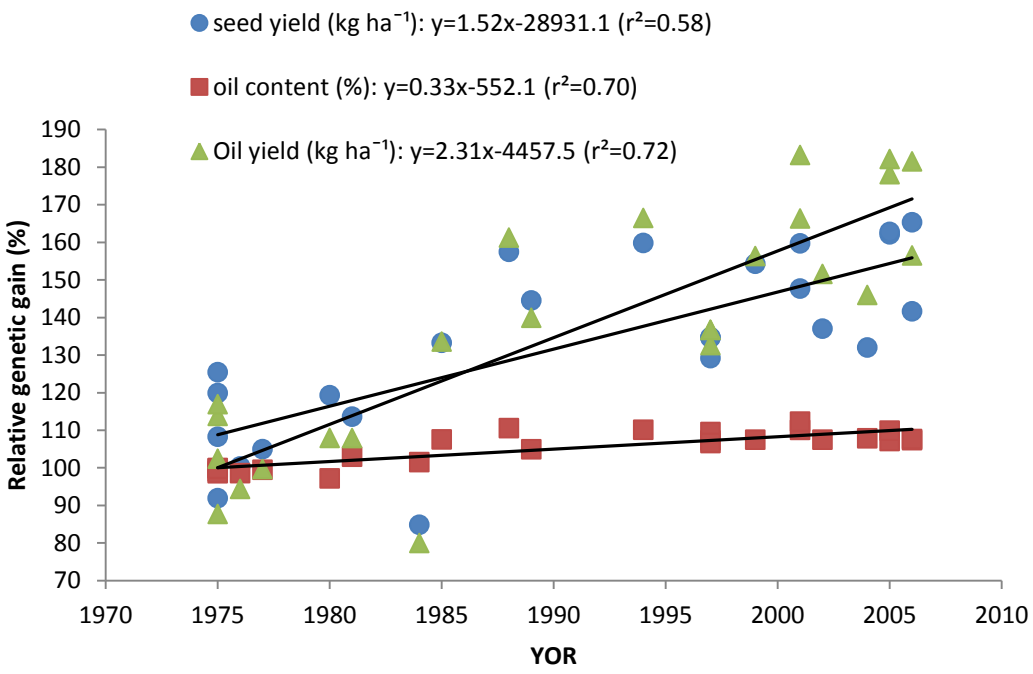

Figure 2.7 Relative genetic gains for seed yield, oil content and oil yield regressed on year of release. Relative genetic gain calculated based on predicted value for the 1970 decade as baseline.

Although only four environments were used in this study, this did not negatively affect the objective to establish yield trends over time mainly because the selected genotypes had been extensively evaluated as indicated by the number of years that had each been included in the national cultivar trials (Table 2.1). Pereira et al. (1999) using one site with four replications over two years found that most response variables were affected by cultivar x year interaction but both seed and oil yield of sunflower were positively associated with year of release in the two year study. Similarly, using two sites over two

years, Donmez et al. (2001) found genetic gains in grain yield to be as prominent for new wheat cultivars evaluated in the Great Plains as in the other regions and the gains were comparable to other studies where larger number of sites were used.

2.3.1.5 Phenotypic correlations between seed yield and other traits

The phenotypic correlations between seed yield and other traits were largely positive and highly significant ($p<0.001$) for most of the traits except 1000-seed mass when all the four decades from the 1970s to 2000s are considered (Table 2.4). When each decades span was considered, high and significant ($p<0.001$) correlation between seed yield and oil yield ($r = 0.99$) was obtained in all the decades spans (Table 2.4). Škorić (1992) similarly obtained a high correlation ($r = 0.99$) between seed yield and oil yield. The high correlation between seed yield and oil yield has also resulted in a steady increase in oil yield per year despite the increase in oil content leveling in the 2000s breeding era. The correlation between seed yield and number of seeds per head when each decades span was considered was significant ($p<0.001$) with only minimal variation in magnitude ranging from $r = 0.92$, for 1970s and 1980s decades span to $r = 0.98$ for 1980s, 1990s and 2000s decades span (Table 2.4). Similar magnitudes in correlations between seed yield and number of seeds per head were obtained Razi and Assad (1999) in well-irrigated and limited-irrigated conditions. The number of seeds per head is a function of floret differentiation, flowering, fertilization and grain set. Cantagallo and Hall (2002) found that light stress during floret differentiation interval irreversibly reduce seed number and yield. Seed yield and oil content were significantly ($p<0.05$) correlated in all breeding decade group spans except the 1990s and 2000s decades span explaining the observed slowing down in genetic gain in oil content in the cultivars from the 2000 breeding decade. Days to flowering was correlated with seed yield when all cultivars were considered for the entire study period and for the breeding decades' span of 1970s and 1980s, indicating that the OPC and early hybrids which were high yielding were also late maturing. This is partly because some of the commercial sunflower production in South Africa during the 1970s and 1980s took place in high potential and cooler regions which permitted cultivation of full season cultivars. The 1000-seed mass was non-significantly correlated with seed yield in all the breeding decade groups even when Kortus, an OPC which is generally large seeded was excluded. Similar results were obtained by Alba et al. (1979) who recorded a positive non-significant correlation between seed yield and 1000-seed mass. Although seed

mass is regarded as one of the significant seed yield components in sunflower (Vannozzi, et al., 1999), selection for high oil content in sunflower tends to reduce seed size and percentage hull due to kernels adhering tightly to the hull (Roath et al., 1985).

Table 2.4 Phenotypic correlation coefficients between seed yield (kg ha^{-1}) and other traits based on cultivar means across four environments for the entire breeding period and breeding decades groups.

	Breeding decades groups					
Trait	**1970-2000 (n = 25)**	**1970-1990 (n = 17)**	**1970-1980 (n = 12)**	**1980-2000 (n = 19)**	**1980-1990 (n = 11)**	**1990-20000 (n = 13)**
Oil content (%)	0.80***	0.76***	0.65*	0.71***	0.72**	0.34
Oil yield (kg ha^{-1})	0.99***	0.99***	0.98***	0.99***	0.99**	0.99***
Seeds $head^{-1}$	0.97***	0.94***	0.92***	0.98**	0.98**	0.94**
Days to flower	0.43*	0.48*	0.33	0.38	0.58	0.19
Stem height (cm)	0.41*	0.34	0.25	0.33	0.29	-0.03
1000-seed mass (g)	0.11	0.25	0.2	0.09	0.22	0.07
Stem diameter (cm)	0.71***	0.59**	0.37	0.69***	0.59*	0.37
Head diameter (cm)	0.78***	0.67**	0.39	0.67**	0.53	0.62*

*p<0.05, ** p<0.01, *** p<0.001.
n = number of cultivars in a breeding decades groups.

A scatter plot of the relationship between the means for two breeding decades spans 1970s to 1980s and 1990s to 2000s, shows generally, greater spread of the means for the former decades span compared to the latter (Figure 2.8). This may indicate that although seed yield increased with breeding period, the variation among the hybrid cultivars for the traits of interest was reduced. Reduction in genetic variation in hybrid breeding programmes is common due to recycling of elite inbred lines as the breeding programme matures, a process known as advanced cycle pedigree breeding (Yu and Bernado, 2004).

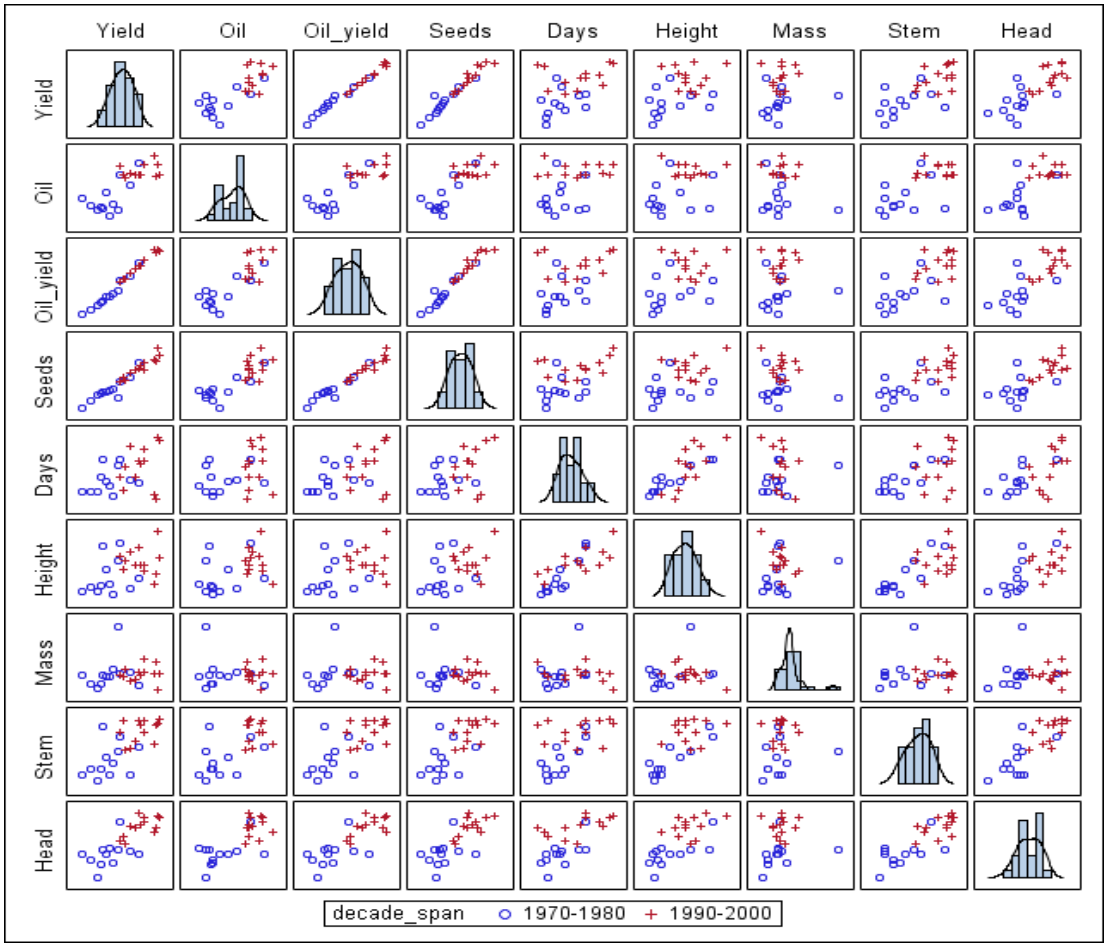

Figure 2.8 Matrix of scatter plots and frequency distributions (diagonal) of means across four environments for seed yield and eight associated traits of cultivars released within two-decade spans. Yield = Seed yield (kg ha^{-1}); Oil = Oil content (%); Oil_yield = Oil yield (kg ha^{-1}); Seeds = No. seeds per head; Days = Days 50% flowering; Height = Stem height at maturity (cm); Mass = 1000-seed mass (g); Stem = Stem diameter (cm); Head = Head diameter (cm).

2.3.2 Contribution of new cultivars to genetic gain

Yield of sunflower in South Africa rose from 538 kg ha^{-1} in 1970 to 1 259 kg ha^{-1} in 2009 representing an absolute yield gain of 12 kg ha^{-1} year^{-1} (r^2 = 0.35, $p<0.001$) (Figure 2.9). The absolute genetic gain in the commercial production environment is lower than those in the side by side trials due to erratic rainfall in combination with high soil variability which usual results in yields varying from 0 to 3 000 kg ha^{-1} in individual fields (Nel and Bloem, 2007). The calculated relative gain in seed yield per year from 1970 to 2009 was

1.91% year^{-1}. The procedure of Lillemo et al. (2010) was used to calculate the contribution of new cultivars to genetic gain under commercial production (Table 2.5).

Table 2.5 †Estimation of contribution of new cultivars to yield increase under commercial production 1970-2009 in South Africa.

	Year Period		
Parameters	**1970**	**1990**	**2009**
% Relative genetic gain of breeding decade (Trial results)	108.5	142.6	151.1
Five-year rolling mean (kg ha^{-1}) (commercial prodn)	640	996	1246
Types of cultivars grown	50% OPC; 50% hybrids	100% hybrids	100% hybrids
Time period under consideration	**% yield increase in breeding decades**	**% yield increase under commercial production**	**% contribution of cultivars in the breeding decades**
1970-1989 (OPC to hybrids)	31.3	55.6	56.3
1990-2009 (Hybrid era, marginal areas)	6	25.1	23.9
Entire period (1970-2009)	39.2	94.7	41.4

†Method of calculation was based on that used by Lillemo et al. (2010).

The estimated contribution of new cultivars to total seed yield progress in commercial production was estimated to be 56.3% during the period from 1970 to 1989 and 23.3% from 1990 to 2009 with a mean increase of 41.4% during the entire four decades under consideration (Table 2.5). Much of the increase in yield from 1970 to 1979 is a result of early exploitation of heterosis from OPC to hybrids (Miller, 1987; Duvick, 1999). The reduced genetic gain contribution of new cultivars in the second part of the period under study maybe due to two factors; (i) a shift in the production environment from the high potential area to semi-arid regions of the North-West and Free State Provinces; and (ii) the rate of cumulative gain from selection decrease with time due to narrowing of the genetic base (Meridith, 2000). In the USA, reduction in genetic progress in cotton (*Gossypium hirsutum* L.) was associated with narrowing of the genetic base due to a decline in public germplasm enhancement programs (Meridith, 2000).

The amount of progress breeding programmes have been making with regard to yield has been a subject of considerable debate and questions have been asked whether further progress can be achieved (Duvick and Cassman, 1999). In the UK, the minimum

contribution of genetic improvement in yield for cereals and oil crops since 1982 was estimated to be 88% with negligible contribution from agronomic changes (Mackay et al., 2011). Estimates of the contribution through genetic improvement in maize have ranged from 53% in China (Ci et al., 2011) to 60% in the US (Duvick, 2005). In Norway, the yield increase ascribed to the use of new barley cultivars from 1980-2008 was estimated at 78% (Lillemo et al., 2010). The comparatively lower rates of genetic gain obtained in this study is in contrast with the theory that if farmers do not improve their agronomic practices but continue to adopt new cultivars one would expect the contribution of plant breeding to be close to 100% (Lillemo, personal communication). In South Africa, agronomic improvement in sunflower production has remained static and nitrogen application in sunflower is a standard practice (Nel and Bloem, 2007), indicating the possibility of other contributory factors limiting the increase in sunflower yields under commercial production in South Africa. In maize, increased yield stability achieved through selection for stress tolerance of morpho-physiological traits rather than increased yield potential has been regarded as a major contributor to gains in grain yield (Tollenaar and Lee, 2002).

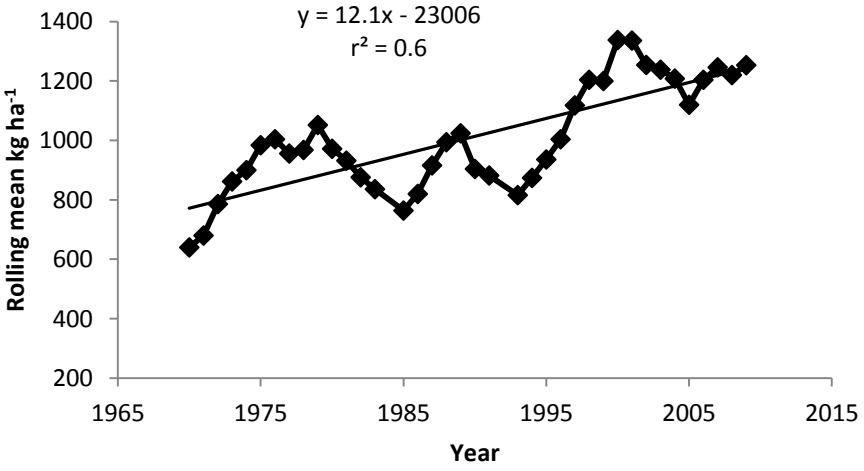

Figure 2.9 Absolute genetic in seed yield (kg ha^{-1} yr^{-1}) from 1970 to 2009 based on five-year rolling means of the national sunflower production statistics (Source: SAGIS, 2011).

The standard procedure for hybrid cultivar development involves mating two inbreds and the resultant testcross hybrids are evaluated in multi-environmental trials (Vrănceanu, 1998). A complimentary approach would require inbred yield testing rather

than the testcross hybrids (Troyer and Wellin, 2009). Only the best performing inbreds will then be used for hybrid development for further testing. As pointed out by Troyer and Wellin (2009), inbred yield testing permits better sampling of stress in the production environments and better phenotyping of traits as inbreds are more susceptible to stress than hybrids allowing incorporation of other technologies such as molecular marker assisted selection.

2.4 Conclusions

Excluding cultivars released after 2004, the average life span in the national sunflower cultivar trials of the cultivars used in study the ranged from three to 13 years with a mean of seven years (Table 2.1). A cultivar's lifespan in the national sunflower cultivar trials is indicative of its minimum life span in the commercial market and therefore its popularity. The cultivars used in this study represent progressive adoption of genetically improved new cultivars which resulted in a positive genetic gain over the four decades considered. When results from side-by-side trials under similar agronomic conditions are combined with commercial production data the contribution of new cultivars was estimated to be 41.4% with the residual percentage attributable to other factors. This study has revealed that genetic gain in oil content has been relatively low and is slowing down (Figure 2.5), which means that the increase in oil yield during the past decade was mainly due to the increased seed yield ha^{-1}. Phenotypic correlations of traits indicate that seed mass in this study was not significantly correlated with seed yield. To obtain further increase in grain yield ha^{-1}, breeders will need to focus on increasing seed mass while maintaining or increasing the number of seeds per head. This will mean developing cultivars which can withstand abiotic stresses during critical periods such as the flowering and grain filling stages by exploiting morpho-physiological traits associated with maintaining good water and nutrient supply to the sink source. Strategies such as inbred yield testing (Troyer and Wellin, 2009) offers the possibility to compliment the current approaches as this will allow greater sampling of the production environments and hence better understanding of plant morpho-physiological traits affecting seed yield heterosis.

Unlike gains from other investments which may become exhausted, investment in genetic gain is cumulative if proper breeding strategies are applied. Parents (inbred lines) and their hybrid progeny (cultivars) developed and released in one breeding era

are used as parents for the next breeding era. The imperative for the future is to maintain the rate of genetic gain despite declining genetic variation to meet the combined challenges of climatic changes and burgeoning human population.

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CHAPTER 3

Advanced cycle pedigree breeding in sunflower. I: Genetic variability and testcross hybrid performance for seed yield and other agronomic traits[2]

Abstract

As a plant breeding programme matures there is a general tendency to recycle elite inbred parents, a strategy known as advanced cycle pedigree breeding. The challenge with this approach is not only to maintain genetic variability in the base breeding populations but also their usefulness for further genetic advancement in developing experimental hybrids with better performance than commercial hybrids on the market. To assess the genetic variability and usefulness of breeding populations in sunflower (*Helianthus annuus* L.), 109 new inbred lines across four breeding populations Pop1, Pop2, Pop3 and Pop4 in an advanced cycle pedigree breeding scheme were testcrossed to two testers to form testcross hybrid (TCH) groups: $Pop1_{TC}$, $Pop2_{TC}$, $Pop3_{TC}$ and $Pop4_{TC}$. Moderate to high genetic variability along with high heritability were also obtained for seed yield and oil yield within and across all TCH groups. Heritability for oil content was low to high ranging from 0.36 ($Pop2_{TC}$) to 0.81 ($Pop1_{TC}$). The calculated genetic advance GA% assuming 10% selection intensity for seed yield ranged from 36% ($Pop2_{TC}$) to 42% ($Pop1_{TC}$), while that of oil yield ranged from 38% ($Pop3_{TC}$) to 43% ($Pop1_{TC}$). The GA% for oil content ranged from 1.3% ($Pop2_{TC}$) to 5.1% ($Pop1_{TC}$). To quantify the commercial potential of experimental TCHs, founder parent heterosis (FPH), mid-standard heterosis (MSH) and high standard heterosis (HSH) were calculated. Out of a total of 218 experimental TCHs evaluated, 28 had positive FPH, MPH and HSH values for oil yield representing 13% selection intensity as is usually applied in early generation testing.

Keywords: Genetic variability, *Helianthus annuus*, Heterosis, Testcross hybrids

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3.1 Introduction

The crossing of related parental lines of commercial cultivars or obsolete cultivars to form new breeding populations followed by pedigree selection or single seed descent in breeding programmes is commonly referred to as advanced cycle pedigree breeding (Lu and Bernardo, 2001). The strategy usually involves recycling elite inbred lines as way of avoiding linkage drag and thereby conserving gains already achieved in the past (Condón et al., 2008). While the avoidance of linkage drag is a necessity especially in mature plant breeding programmes, an undesirable feature of advanced cycle pedigree breeding is the narrowing of the genetic base (Yu and Bernardo, 2004). Further challenges to this approach in crops such as sunflower is whether the variability and correlations of traits in the base breeding population consequent to advanced cycles of pedigree breeding will enable future genetic gains through direct and indirect selection of traits.

Despite the successes and potential bottle necks of advanced cycle breeding recorded in other crops notably maize (*Zea mays* L.) (Gethi et al., 2002; Yu and Bernardo, 2004), studies on heterosis, phenotypic and genetic variability among traits in advanced cycle pedigree breeding in sunflower based on field performances are limited or not available at all. Genetic diversity studies of cultivated sunflower germplasm using molecular markers found substantially higher diversity and genetic differentiation among sunflower groups (Cheres et al., 2000, Yue et al., 2009). Although studies on genetic diversity using molecular markers are useful especially in removing duplications and correcting for possible mis-groupings (Cheres et al., 2000), they do not provide an indication of how much progress will be achieved when selection is applied. In addition, several studies on genetic diversity using either coanstery data or genetic distance based on molecular markers have generally concluded that diversity or genetic distance is a poor predictor of hybrid performance (heterosis) and combining ability (Cheres et al., 2000; Makumbi et al., 2011). In studies involving crosses between lucerne germplasm (*Medicago sativa* subsp. *sativa* L.) and (*Medicago sativa* subsp*. falcate* L.), Riday and Brummer (2005) concluded that heterosis cannot be ensured by genetic diversity *per se* but by usable complementary variation between parents. The usable genetic variation in the parental material determines the “usefulness” of a cross or breeding population and hence effectiveness and progress expected from selection. Schnell and Utz (1975) cited by

Utz et al. (2001) introduced the usefulness criterion (U_p) for identifying promising crosses. When selection is by truncation and constant selection intensity is applied across breeding populations, U_p, is a function of genetic variance of testcrosses and heritability of the trait under study in the specific breeding population (Utz et al., 2001; Tabanao and Bernardo, 2005).

Increasing oil yield per unit area is an important objective in sunflower breeding (Kaya, 2005; Škorić et al., 2007) and exploiting heterosis is a common approach to achieving this although the genetic basis thereof is not well understood (Gallais, 1988; Riday and Brummer, 2005; Škorić et al., 2007). The hybrid breeding scheme which involves the use of a cytoplasmic male sterility (CMS) line (also known as A-line), a maintainer line (B-line) and a fertility restorer line (Rf-line), has allowed breeders to exploit heterosis in sunflower through the development of three-parent single-cross hybrids of the form $(P_i \times P_j) \times T_k$, where P_i is the A-line, P_j is the B-line and T_k is the Rf-line (Miller et al., 1980). The level of heterosis expressed in the hybrids is commonly used as an indication/measure of the breeding value/potential of new inbred lines. There are two commonly used estimates of heterosis: (i) mid-parent heterosis (MPH) which is the relative performance of a hybrid compared to the mean of its parents; and (ii) better parent heterosis (BPH), which is the performance of the hybrid compared to the mean of its better parent (Betrán et al., 2003). Although MPH or BPH for seed yield and oil yield in sunflower have been reported, their application in practical sunflower breeding has been limited as evaluation for seed and oil yield in male fertility restorer lines *per se* is rarely carried out. This is because the female parents in sunflower are single headed, while the male parents have multiple heads that enhance longer periods of pollen availability to the females (Duvick, 1999). The choice between hybrid and open-pollinated cultivars depends on the magnitude of heterosis (Gallais, 1988). However, most studies indicate that in cross pollinated crops such as maize and sunflower, hybrid cultivars always outperform their parental lines for quantitatively controlled traits such as seed yield by wide margins from 50-400%, based on MPH or BPH (Betrán et al., 2003; Kaya, 2005; Flint-Garcia et al., 2009; Makumbi et al., 2011). These high levels of heterosis mean BPH and MPH are not definitive for determining which inbred lines are likely to be retained in the breeding programme. Flint-Garcia et al. (2009) found that a higher BPH does not necessarily translate into a high yielding or adapted hybrid as the magnitude of heterosis depends on the relative performance of the inbred parents.

Furthermore, Smith (1997) noted that the costs involved versus the usability of information obtained by evaluating new inbred lines and corresponding hybrids in balanced sets was a major operational drawback of BPH or MPH. Thus heterosis studies in cross-pollinated crops based on BPH or MPH would be more appealing for studies intended to identify sources of tolerance or resistance to biotic or abiotic stresses in the inbred lines rather than yield performance *per se.*

Patnaik et al. (1990) and Geleta and Labuschagne (2004) used the term standard heterosis (SH), to define the superiority or yield advantage of experimental F_1 hybrids or testcross hybrids (TCHs) relative to the performance of the commercial check in rice (*Oryza sativa* L.), and peppers (*Capsicum annuum* L.), respectively. If more than one commercial check is used in the trials, calculation of SH can be based on the mean of all checks i.e. mid-standard heterosis (MSH), or based on the best commercial check i.e. high standard heterosis (HSH). The use of SH is based on the argument that in developing new hybrids, the aim is to surpass the performance of existing commercial cultivars for the trait of interest, thus SH measure the commercial breeding potential of experimental lines and is sometimes referred to as useful heterosis (Meredith and Bridge, 1972). While the argument is both practically and economically valid, the check may not be in anyway related to the experimental TCHs being developed thereby extending the standard interpretation of heterosis as being based on the heterotic performance of the progeny relative to their parental lines.

When developing new inbred lines from a narrow genetic base such as bi-parental breeding populations the objective is to develop new inbreds with higher potential than the founder parental lines when testcrossed to the same testers. This performance, termed founder parent heterosis (FPH), provides a measure of genetic progress and also information on gains for traits that may have been already improved in founder parents or have reached a plateau especially when inbred parents are recycled. Since the founder parents are related to the new inbreds, FPH compliments SH if the genetic background of the inbreds is to be interpreted. In addition to interpretation of heterosis based on genetic progress and background, as only two founder parents are involved per bi-parental population, the operational costs of assessing FPH are usually negligible compared to BPH or MPH. For example, if 50 new inbreds are developed from a population and crossed to two testers, 100 experimental TCHs plus the 50 new inbreds

and the two testers (152 genotypes) will have to be evaluated to estimate BPH or MPH. For FPH estimation, only four founder parent testcross hybrids two founder parents x two testers will need to be evaluated along with the 100 experimental TCHs (104 genotypes).

As pointed out by Nyoka (2005), an effective plant breeding programme aims not only to produce cultivars but also to generate and document information that will allow further optimisation and changes in subsequent breeding cycles. Ideally this information should not be based on 'borrowed' genetic parameters/measurements but based on the germplasm in the breeding programme and the target production environment in which the cultivars will be grown. Thus the objectives of this study were to:

(i) assess the genetic variability and usefulness of breeding populations for seed yield and other agronomic traits via experimental testcross hybrids (TCHs) of inbred lines derived through an advanced cycle pedigree breeding scheme; and

(ii) determine the commercial breeding potential of new sunflower inbred lines, derived through an advanced cycle pedigree breeding scheme, via experimental testcross hybrids (TCHs) by quantifying founder parent heterosis (FPH), mid-standard heterosis (MSH), and high standard heterosis (HSH).

To the best knowledge of the authors no previously published studies have used the term “founder parent heterosis” and draw pertinent conclusions regarding the merits of advanced cycle pedigree breeding in sunflower.

3.2 Materials and methods

3.2.1 Genetic material

Four base breeding populations (BBPs) of sunflower were formed by crossing elite founder parental lines during the 1997 to 1999 seasons at the Agricultural Research Council's Grain Crops Institute (ARC-GCI), Potchefstroom, South Africa. The BBP were formed as follows: (a) Population1 (Pop1), a cross between H52xKB61; (b) Population2 (Pop2), a cross between HA89xKB16; (c) Population3 (Pop3), a cross between HA89xKB61; and (d) Population4 (Pop4), a cross between HA89xKB189. All the elite founder parental lines used in establishing the BBP were maintainer lines (B-lines). H52, purportedly originating from Romanian germplasm, is an inbred line that was developed

by ARC-GCI in the late 1970s, and HA89 is a public line released by USDA-ARS in 1972. These two inbred lines are widely used in sunflower breeding programmes around the world as measured by their inclusion in number of studies e.g. Miller et al. (1980) and Yue et al. (2009) to mention a few. The other elite founder parental lines KB61, KB16 and KB189 are inbred lines derived from different composite populations developed by the ARC-GCI in the early 1990s using multiple source germplasm including versions of H89 and H52. Different versions of the KB series elite founder parental inbred lines exist and have extensively been used in South African sunflower breeding programmes (Barend 2008, personal communication). After selfing the F_1, plants in the subsequent F_2 generation were allowed to undergo random mating within each population with selection starting at the $F_{2/3}$ generation followed by selfing for three generations to produce S_3 inbred lines. The resultant S_3 lines (B-lines) were converted to CMS lines by backcrossing to form S_3CMS lines (A-lines) during the 2003 to 2006 seasons.

Advancement to each stage of selection was based on adequate quantities of seed for both the CMS and maintainer lines during the backcrossing stages. A total of 109 S_3CMS lines and their corresponding maintainer lines with adequate quantities of seed were developed across the four BBPs as follows: Pop1 had 44 lines, Pop2 had 12 lines, Pop3 had 26 lines and Pop4 had 27 lines. Each of the 109 S_3CMS lines along with the CMS versions of the founder parental lines: H52CMS, HA89CMS, KB61CMS, KB16CMS, KB189CMS and H55CMS, were testcrossed to two male fertility restorer lines RP865 (T1) and RP953 (T2) to produce a total of 218 experimental TCHs and 12 founder parental testcross hybrids (FP_{TC}). Although the maintainer version of H55 was not used to form any of the BBP, it was included as it is a widely used inbred line in South Africa and Europe (van der Merwe 2008, personal communication).

The 218 experimental TCHs were grouped according to their original BBP (Table 3.1). In addition to the 218 experimental TCHs, other genotypes included for evaluation were the 12 FP_{TC}, the four BBPs ($F_{2/3}$ generation of Pop1, Pop2, Pop3, Pop4 maintained for three seasons through isolated open pollination and mass selection for good plant aspect i.e. plants with large heads and free from leaf diseases) and six commercial hybrid check cultivars (AGSUN8251, AGSUN5551, PAN7033, PAN7355, Mydelo, DKF 68-22) to make up a total of 240 genotypes (Table 3.1).

3.2.2 Test environments and experimental design

The 240 entries were evaluated in an alpha (0, 1) design with two replications in three environments: Potchefstroom early (POTCH06), planted at the end of November in 2006; Potchefstroom late (POTCH07), planted the first week of January 2007; and Bothaville (BTV07), planted in the second week of January 2007. Potchefstroom (26.745S, 27.083E) is situated in the Northwest Province, and Bothaville (27.235S, 26.67E) is located in the Free State Province, South Africa. The Northwest and Free State provinces contribute 80% of the total area planted to sunflower annually (SAGIS, 2011). The plant population for the POTCH06 and POTCH07 trials was 36 000 plants ha^{-1}, while that for BTV07 was 28 000 plants ha^{-1}. Irrigation was applied to field capacity at planting and thereafter applied at approximately 30 day intervals at POTCH06 until physiological maturity, while POTCH07 received light irrigation at planting to enable even germination. For BTV07 no irrigation was applied. Experimental units were two row plots, 5 m long and 0.9 m wide while within intra-row spacing for the Potchefstroom trials was 0.33 m and 0.4 m for the Bothaville trial. Plots were overplanted and then thinned at 4 weeks after emergence. Recommended agronomic practices were followed at both trials, include basal application of 150 kg ha^{-1} fertilizer [3N:2P:1K (25)] incorporated into the seedbed before planting. A further 28 kg ha^{-1} N was applied at four weeks after emergence. The fields were kept weed free by both pre-emergence herbicide application soon after planting and mechanical weeding during the thinning operations.

Table 3.1. Description of genotypes used in the study

Group	Number of genotypes	Description of the materials
$Pop1_{TC}$	88	Experimental TCHs formed by crossing two male testers T1 and T2 to 44 S_3CMS lines developed from Pop1. S_3CMS inbred lines coded Pop1-1, Pop1-2…1-44 and their TCHs as Pop1-1_T1, Pop1-2_T1…1-44_T1; Pop1-1_T2, Pop1-2_T2…1-44_T2.
$Pop2_{TC}$	24	Experimental TCHs formed by crossing two male testers T1 and T2 to 12 S_3CMS lines developed from Pop2. S_3CMS inbred lines coded Pop2-1, Pop2-2…2-12 and their TCHs as Pop2-1_T1,Pop2-2_T1…2-12_T1; Pop2-1_T2,Pop2-2_T…2-12_T2
$Pop3_{TC}$	52	Experimental TCHs formed by crossing two male testers T1 and T2 to 26 S_3CMS lines developed from Pop3. S_3CMS inbred lines coded Pop3-1, Pop3-2…3-26 and their TCHs as Pop3-1_T1, Pop3-2_T1…3-26_T1; Pop3-1_T2, Pop3-2_T2…3-26_T2.
$Pop4_{TC}$	54	Experimental TCHs formed by crossing two male testers T1 and T2 to 27 S_3CMS lines developed from Pop4. S_3 CMS inbred lines coded Pop4-1, Pop4-2, 4-27 and their TCHs as Pop4-1_T1, Pop4-1_T1…4-27_T1; Pop4-1_T2, Pop4-2_T2…4-27_T2.
FP_{TC}	12	Founder parent TCHs formed by crossing two male testers T1 and T2 to six founder parental inbred lines: H55, H52, HA89, KB61, KB16 and KB189.
BBP	4	$F_{2/3}$ generation of Pop1, Pop2, Pop3, and Pop4 maintained through isolated open pollination and mass selection for three seasons.
Checks	6	Commercial hybrid checks: AGSUN8251, AGSUN5551, PAN7033, PAN7355, Mydelo and DKF 68-22.
Total	240	

3.2.3 Agronomic traits recorded

The traits measured were: seed yield (kg ha^{-1}); percent oil content and oil yield (kg ha^{-1}). Seed yield was first determined on a plot basis and adjusted to 10% seed moisture by weighing seed which had been dried in continuous air flow dryers at 32-35˚C over a period of two weeks until no further loss in mass was recorded. Percent oil content was determined on 12 g, air-dried seed samples by nuclear magnetic resonance using a Newport Analyzer (Newport-Oxford Instruments Ltd, England). Oil yield (kg ha^{-1}) was obtained by multiplying seed yield by percent oil content.

3.2.4 Statistical analysis

In order to address the objectives of the study various statistical models were applied to each of the stated specific objectives.

3.2.4.1 Genetic variability and usefulness of base breeding populations

Genetic variability within and across the TCH groups for seed yield, oil content and oil yield was assessed by combined analyses over environments excluding hybrid checks, FP_{TC} and BBP groups. The analysis was done using the PROC MIXED procedures in SAS (SAS Institute 2010) treating the TCHs within each group as random effects and the groups and environments as fixed effects to obtain estimates of TCH (σ^2_{TC}) and TCH x environment interaction (σ^2_{TCE}) variance components which are equivalent to genotype (σ^2_g) and genotype x environment interaction (σ^2_{ge}) variance components, respectively. Calculation of broad sense heritability (*H*) was done on a testcrosses mean basis (Hallauer and Miranda, 1988) for each TCH group and across the TCH groups. Genotypic coefficient of variation (GCV%) and phenotypic coefficient of variation (PCV%) for each TCH group and across groups were calculated as: GCV% = 100 x σ_g/mean TCH group; PVC% = 100 x σ_p/mean TCH group. The σ_g and σ_p were estimated as the square root of the genotypic (σ^2_g) and phenotypic (σ^2_p) variances, respectively; where: $\sigma^2_p = \sigma^2_g + \sigma^2_{ge} + \sigma^2_e$; and σ^2_e = the error variance. The across group GCV% and PVC% was likewise calculated combining all the TCHs across the groups. Expected genetic advance (GA%) was calculated for the three traits and expressed as a percentage of the trait mean: GA% (TCH group) = 100 x $iH\sigma_p$/mean TCH group, assuming a 10% selection intensity then $i = 1.76$ (Becker, 1984), while the combined or across GA% was calculated based on the grand mean of all TCHs. The usefulness criterion of a particular TCH group (U_p) was calculated based on the method given by Tabanao and Bernardo (2005) as: $U_p = \mu + k_p\sigma^2_{TC}\sigma_p$; where: μ is the mean of TCH group; and k_p is the standardized selection differential also known as selection intensity *i* (Becker, 1984). Calculation of U_p was also based on a 10% selection intensity.

3.2.4.2 Determining the commercial breeding potential of new inbred lines

Since seed yield, total oil content and oil yield per unit area are the primary selection traits in sunflower (Škorić, 1992), the breeding potential of new inbred lines in testcross combination were determined based on FPH, MSH and HSH for the three traits. Analysis of variance over the three environments was performed using the PROC MIXED procedures in SAS (SAS Institute, Cary, NC, 2010). For the purpose of comparing the commercial worth and improvements in new inbred lines, the environments and genotypes (experimental TCHs; FP_{TC}; BBPs and hybrid checks) were considered fixed, while replications and incomplete blocks within replications were considered random. Significance differences between the least square mean values (lsmeans) here referred to as means of the experimental TCHs check hybrids and founder parent TCHs for seed yield, oil content and oil yield were calculated using the PDIFF option of the LSMEANS statement (SAS Institute, 2010). The FPH within each TCH group was calculated as:

FPH = (mean TCH within a group-mean best FP_{TC} corresponding group/mean best FP_{TC} corresponding group) x 100.

Where: mean TCH within a group is the mean across the three environments for each testcross hybrid within a group; and the mean best FP_{TC} is the mean across the three environments of the best testcross hybrid of the four founder parent testcross hybrids in each corresponding group.

MSH = (mean TCH-mean all hybrid checks)/mean all hybrid checks x 100, and

HSH = (mean TCH-mean best hybrid check)/mean best hybrid check x 100.

3.3 Results and discussion

3.3.1 Genetic variability and usefulness of base breeding populations

Testcross variance component analysis across the three environments indicated a highly significant and positive ($p<0.01$) genetic variance within and across the TCH groups for seed yield and oil yield (Table 3.2). Their magnitudes were 3-10 times greater than their standard errors showing that there is adequate genetic variation within and across TCH groups for seed yield and oil yield.

Table 3.2 Variance components, broad sense heritability, genotypic and phenotypic coefficient of variation, expected genetic gain and usefulness criteria for seed yield, oil content and oil yield of each TCH group and across all TCH groups

Trait	Parameters	TCH group/Genotype group				
		$Pop1_{TC}$	**$Pop2_{TC}$**	**$Pop3_{TC}$**	**$Pop4_{TC}$**	**Across TCHs**
Seed Yield[‡]	**Mean (kg ha^{-1})**	**1936^{b}**	**2000^{ab}**	**1940^{b}**	**1860^{c}**	**1904**
	S.E.	18.4	35.2	23.9	23.5	173
	σ^2_{TC}=σ^2_g	2.57±0.48**	2.26±0.82**	1.93±0.45**	1.97±0.45**	2.23±0.26**
	σ^2_{TCE}=σ^2_{ge}	0.76±0.19**	0.51±0.36	-0.16±0.2	0.12±0.17	0.36±0.10**
	σ^2_e	1.67±0.15	2.02±0.34	2.35±0.27	1.76±0.20	1.89±0.11
	σ^2_g /σ^2_{ge}	3.38	4.43	0.00	16.42	6.2
	H	0.83	0.82	0.83	0.86	0.84
	GCV %	26.2	23.75	22.63	23.85	24.44
	PCV%	28.77	26.29	24.83	25.79	26.71
	GA%	41.97	37.77	36.31	38.83	39.36
	U_p	252.16	208.85	163.41	166.19	
Oil content	**Mean (%)**	**39.9^{c}**	**40.1^{b}**	**40.5^{a}**	**40.0^{bc}**	**39.9**
	S.E.	0.05	0.09	0.06	0.06	0.44
	σ^2_{TC}=σ^2_g	1.64±0.31**	0.26±0.23	0.65±0.24*	1.49±0.41**	1.22±0.17**
	σ^2_{TCE}=σ^2_{ge}	0.68±0.14**	0.81±0.31*	1.14±0.23**	1.04±0.24**	0.89±0.101**
	σ^2_e	0.95±0.09	1.16±0.20	0.87±0.1	1.22±0.14	1.04±0.06
	σ^2_g /σ^2*ge*	2.41	0.32	0.57	1.43	1.37
	H	0.81	0.36	0.55	0.73	0.72
	GCV %	3.21	1.26	1.99	3.05	2.75
	PCV%	3.57	2.11	2.68	3.57	3.24
	GA%	5.09	1.32	2.61	4.59	4.12
	U_p	44.25	40.51	41.37	43.87	
Oil yield[‡]	**Mean (kg ha^{-1})**	**773^{b}**	**801^{ab}**	**789^{b}**	**747^{c}**	**763**
	S.E.	7.4	14.2	9.7	9.5	70.1
	σ^2_{TC}=σ^2_g	0.43±0.08**	0.39±0.14**	0.35±0.08**	0.33±0.08**	0.38±0.04**
	σ^2_{TCE}=σ^2_{ge}	0.13±0.03**	0.08±0.06	-0.01±0.03	0.04±0.03	0.06±0.02**
	σ^2_e	0.28±0.02	0.31±0.05	0.39±0.05	0.29±0.03	0.31±0.02
	σ^2_g /σ^2*ge*	3.31	4.88	0.00	8.25	6.33
	H	0.83	0.84	0.84	0.85	0.84
	GCV %	26.68	24.77	23.45	24.44	25.09
	PCV%	29.28	27.1	25.54	26.56	27.39
	GA%	42.78	39.87	37.9	39.58	40.45
	U_p	16.94	15.04	12.61	11.64	

*, ** Significance levels at p<0.05 and p<0.01, respectively for variance components
Means sharing a letter in their superscript are not significantly different at the p< 0.05 level
[‡] x10^5 for the variance components and U_p
σ^2_g = genotypic variance components; σ^2_{ge} = genotype by environment interaction variance component; σ^2_e = error; σ^2_g/σ^2_{ge} = genotype and genotype by environment interaction ratio for variance components; *H* = broad sense heritability; *GCV%*, *PVC%* = Genetic and phenotypic coefficient of variation as a percentage, respectively; *GA%* = Genetic advance as a percentage; and *Up* = Usefulness criterion

Genotypic variance for oil content was high in $Pop1_{TC}$ and $Pop4_{TC}$, moderate in $Pop3_{TC}$, and low and non-significant in $Pop2_{TC}$ (Table 3.2) and this is also illustrated by the density distribution plots (Figure 3.1, 3.2, and 3.3). In maize, Yu and Bernardo (2004) found that genetic variance for most traits they studied were lost during advanced cycle pedigree breeding except for that of grain yield suggesting that during selection breeders tend to focus more on yield variability than any other trait resulting in, consciously or unconsciously, the narrowing of genetic variability in other traits. High genetic variability found within and across the TCH groups for seed yield and oil yield permits selection for seed yield improvement despite using elite older inbred lines as base parents in advanced cycle pedigree breeding. Gethi et al. (2002) found that older generation inbred lines were still widely used in developing new inbred lines in maize in the USA. Equally an inspection of the list of germplasm used by Yue et al. (2009) reveals that out of the 65 maintainer lines released publicly in the USA between 1971-2005, 18 inbred lines (28%) had HA89 in their background (HA89 is one of the founder parents used in this study) but still substantial genetic variability was detected using molecular markers.

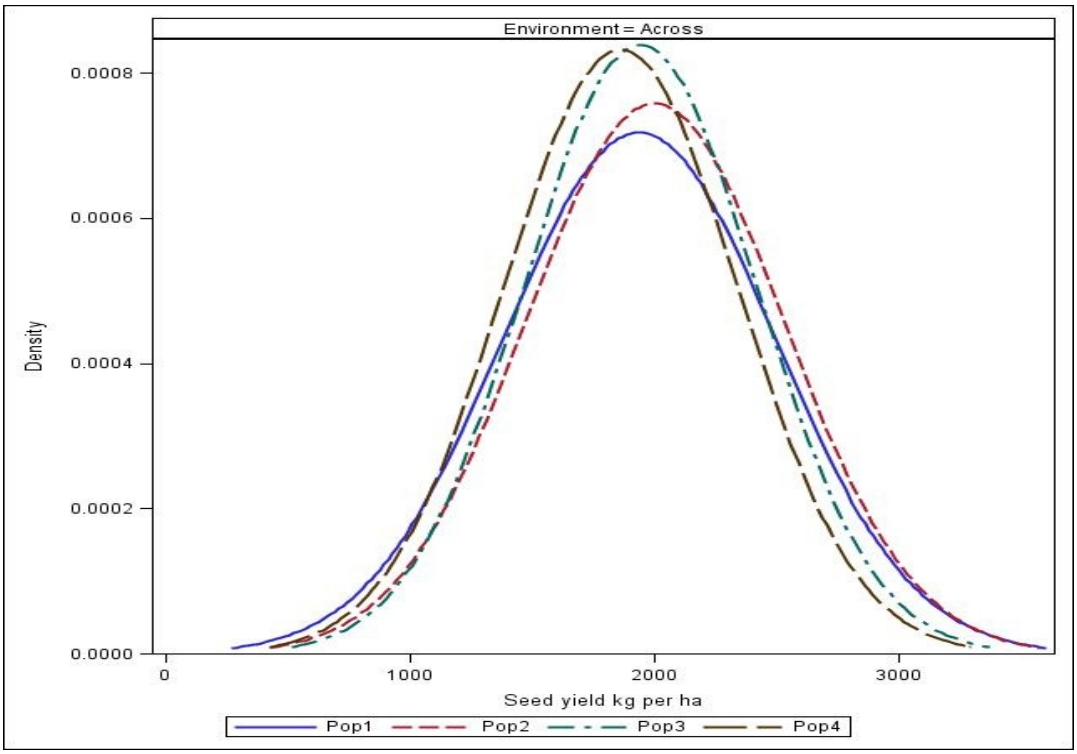

Figure 3.1 Density distribution for seed yield (kg ha^{-1}) of experimental testcross hybrids (TCHs) from four base breeding populations (Pop1 = $Pop1_{TC}$; Pop2 = $Pop2_{TC}$; Pop3 = $Pop3_{TC}$; Pop4 = $Pop4_{TC}$)

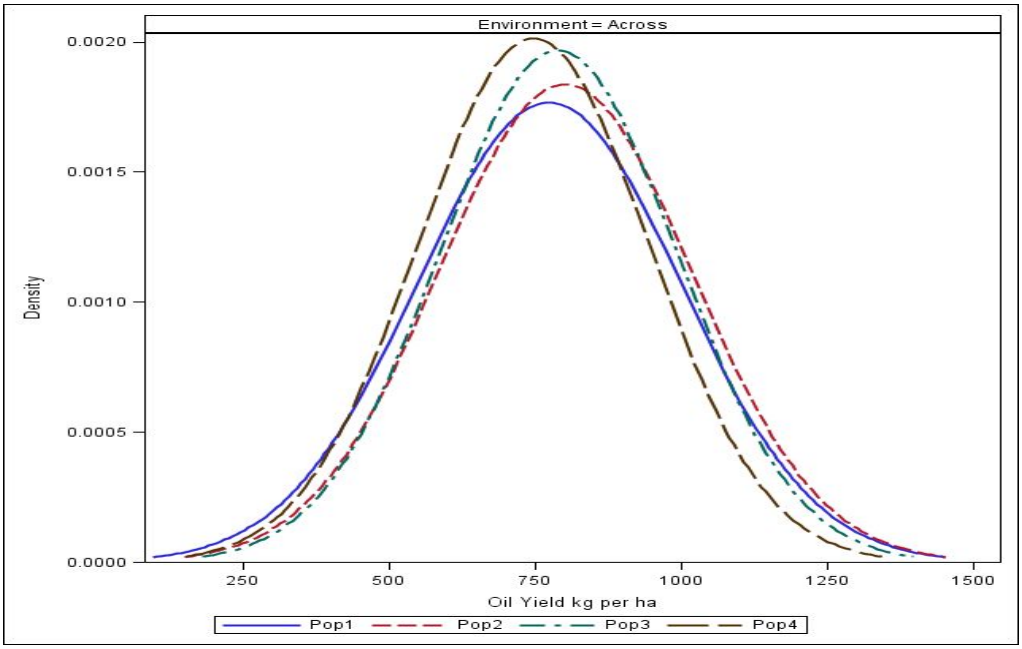

Figure 3.2 Density distribution for oil yield (kg ha^{-1}) of experimental testcross hybrids (TCHs) from the four base breeding populations (Pop1 = $Pop1_{TC}$; Pop2 = $Pop2_{TC}$; Pop3 = $Pop3_{TC}$; Pop4 = $Pop4_{TC}$)

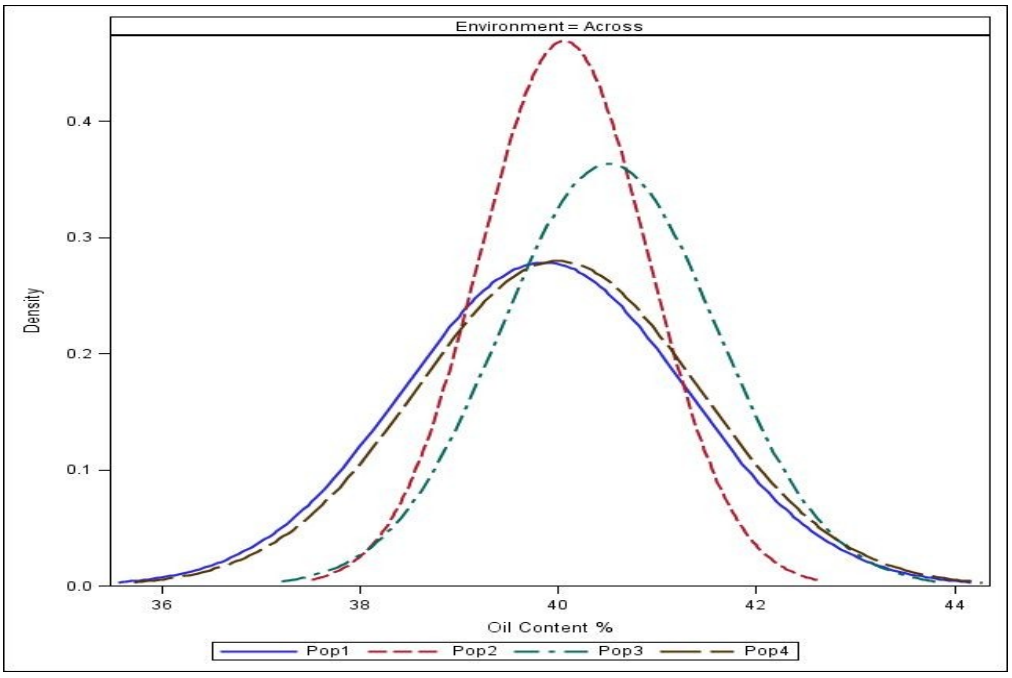

Figure 3.3 Density distribution for oil content (%) of the experimental testcross hybrids (TCHs) from the four base breeding populations (Pop1 = $Pop1_{TC}$; Pop2 = $Pop2_{TC}$; Pop3 = $Pop3_{TC}$; Pop4 = $Pop4_{TC}$)

Genotype by environment interaction for oil content was significant in all the four TCH groups as evidenced by the small ratios of σ^2_g/σ^2_{ge} indicating high sensitivity to environmental variation of TCHs from both groups for oil content. The significant genotype environment interaction for oil content detected in all the four TCH groups requires that evaluation of TCHs for oil content determination be carried out in more than one environment for selection to be effective. Genotype by environment interaction for seed yield and oil yield was only significant ($p<0.01$) in $Pop1_{TC}$ and across the TCH groups. This is evident from the large ratios of σ^2_g/σ^2_{ge} for $Pop2_{TC}$ and $Pop4_{TC}$ in contrast to $Pop1_{TC}$. $Pop3_{TC}$ had negative genotype by environment variance for seed yield and oil yield and hence were equated to zero as they cannot be interpreted by themselves (Dudley and Moll, 1969).

Heritability was high and of similar magnitude for both seed and oil yield in all the TCHs groups (Table 3.2) mainly due to the fact that oil yield is a product of seed yield and oil content and high correlation close to a unit between seed yield and oil yield is common (Škorić, 1992; Chigeza et al., 2012). Although estimates of heritability are population specific (Dudley and Moll, 1969), the magnitude of heritability for seed yield and oil yield within and across the TCHs in this study are similar to those obtained with other genotypes in other environments (Sujatha et al., 2002; Seneviratne et al., 2004; Sridhar et al., 2006). Low to high heritability for oil content was obtained with $Pop2_{TC}$ having the lowest heritability (Table 3.2). Other than $Pop2_{TC}$ which had low heritability the other TCH groups and across TCH had heritability within the range obtained by Sujatha et al, 2002) and Seneviratne et al. (2004).

Genotypic and phenotypic coefficients of variations were high for seed yield and oil yield, and low for oil content. The GVC% and PVC% for seed yield and oil yield were within the range to those obtained by Seneviratne et al. (2004), but substantially lower than those obtained by Sujatha et al.(2002). The GVC% and PVC% were low for oil content in all the TCHs groups but were of similar magnitude to those obtained by Razi and Asad (1997) for $Pop1_{TC}$ and $Pop4_{TC}$. Compared to other studies such as those by Sujatha et al. (2002) and Seneviratne et al. (2004), the GVC% and PVC% of the TCH groups for oil content were 2-6 times lower. Expected genetic gains based on 10% selection intensity expressed as a percentage of the mean of each corresponding TCH group and across TCHs were also high for seed yield and oil yield indicating the potential gain to be made

through selection for the two traits. Of the four TCH groups, $Pop1_{TC}$ exhibited the highest expected genetic gain for the three traits in testcross performance and it had also the highest U_p for all the traits (Table 3.2). Similar genetic advances for seed yield in random-mating sunflower populations at lower selection intensity of 20% were reported by Syed et al. (2004) indicating that more genetic advance will be achieved in unselected populations. These results are in agreement by conclusions drawn by Helms et al. (1997), where the population with the largest genetic variance would be expected to produce a greater proportion of high yielding inbreds in testcross combinations if the mean of different populations are not significantly different.

Expected genetic advance for oil content was least in $Pop2_{TC}$ and is consistent with the genetic theory that the lower the genetic variability and heritability the lower the response to selection. Furthermore, Chigeza et al. (2012) observed that sunflower breeding in South Africa has generally focused more on seed yield than oil content leading to a general slowdown in genetic gain for oil content with very little having been done to introduce high oil content germplasm in the base breeding populations.

3.3.2 Breeding potential of new inbred lines

The mean values across environments for the TCHs, FP_{TC} and hybrid check groups were significantly different for the three traits. The means for the hybrid check group for seed yield and oil yield were significantly ($p<0.05$) higher than the mean for all the experimental TCH groups except $Pop2_{TC}$ where it was non-significantly higher. This was expected as the hybrid check group was comprised of the best hybrid cultivars widely tested and recommended for commercial production while the TCH groups consisted of both high and low yielding cultivars as indicated by the ranges of means (Table 3.3). In all four TCH groups, there were TCHs that had means higher than the best hybrid check. The mean for the best TCH for seed yield within $Pop1_{TC}$, $Pop2_{TC}$, $Pop3_{TC}$ and $Pop4_{TC}$ were 3116 kg ha^{-1}, 2891 kg ha^{-1}, 2767 kg ha^{-1}, and 2975 kg ha^{-1}, respectively compared to 2586 kg ha^{-1} for the best hybrid check (Table 4.3). The combined seed yield for all FP_{TC} groups was significantly ($p<0.05$) lower than all the experimental TCH groups indicating better performance of experimental TCHs over the founder parents when testcrossed to the same testers. Mean oil content was highest in $Pop3_{TC}$, followed by $Pop2_{TC}$. Oil content was highly significant ($p<0.05$) and better in all the TCH groups than

either the hybrid check or the FP_{TC} groups (Table 3.3), indicating a high frequency of TCHs with higher oil content than the mean of the hybrid checks and FP testcrosses respectively. There was no significant difference between the mean oil content of FP_{TC} group with that of hybrid check group (Table 3.3).

In cross pollinated crops such as maize and sunflower, progenies are evaluated for their performance when crossed with an inbred tester and breeders generally tend to retain base breeding populations with high mean performance and large genetic variances on testcross basis to increase the chances of developing superior recombinants (Bernardo and Nyquist, 1998). Based on the means for seed yield and oil yield, $Pop2_{TC}$ would be the desirable population for inbred development as its mean for seed yield and oil yield is the highest among the testcross populations and the pairwise differences of the means for seed yield and oil yield is not statistically different from those of the hybrid checks group (Table 3.3).

The mean estimates of FPH for seed yield, oil content and oil yield were positive for $Pop2_{TC}$, $Pop3_{TC}$ and $Pop4_{TC}$ but negative for $Pop1_{TC}$ indicating that in general the founder parents for $Pop1_{TC}$ were also high yielding when testcrossed to the same testers (Table 3.3). Although mean FPHs were negative for $Pop1_{TC}$ there were some experimental TCHs within this group that outperformed the best FP_{TC} by as much as 42.2% for seed yield, 4.9% for oil content and 48.1% for oil yield (Table 3.3). Compared to BPH and MPH, which gives higher heterosis in cross pollinated crops (Betrán et al., 2003; Kaya, 2005; Makumbi et al., 2011), thereby encouraging the retention of low yielding inbreds (Troyer and Wellin, 2009), the FPH approach provides more realistic and usable heterosis estimates which allows elimination of low yielding inbreds. Furthermore, the FPH also allows benching marking breeding progress based on the performance of founder parents in testcross combinations

Table 3.3 LSMean values (means), range and heterosis for seed yield, oil content and oil yield for the genotypic groups used in the study

	TCH group/Genotype group					
	$Pop1_{TC}$ (n=88)	**$Pop2_{TC}$ (n=24)**	**$Pop3_{TC}$ (n=52)**	**$Pop4_{TC}$ (n=54)**	**FP_{TC} (n=12)**	**Checks (n=6)**
Descriptive statistics	**Seed yield (kg ha^{-1}) (Trial mean over 3 environments=1904±173)**					
LSMeans	1936^{b}	2000^{ab}	1940^{b}	1860^{c}	1625^{d}	2108^{a}
Range	847-3116	1136-2891	852-2767	976-2975	1194-2191	1629-2586
Heterosis (%)						
FPH-Mean	-11.7	24.1	29.3	4.0		
-Range	-61.3-+42.2	-29.5-+79.4	-43.2-+84.5	-45.4-+66.3		
MSH-Mean	-8.2	-5.1	-8.0	-11.8		
-Range	-59.8-+47.8	-46.1-+37.2	-59.6-+31.3	-53.7-+41.2		
HSH-Mean	-25.1	-22.7	-25.0	-28.1		
-Range	-67.2-+20.5	-56.1-+11.8	-67.1-+7.0	-62.2-+15.1		
Descriptive statistics	**Oil content (%) (Trial mean over 3 environments=39.9±0.44)**					
LSMeans	39.9^{c}	40.1^{b}	40.5^{a}	40.0^{bc}	39.5^{d}	39.5^{d}
Range	36.2-42.0	38.2-41.4	37.7-42.7	34.9-41.9	37.4-40.7	38.8-41.1
Heterosis %						
FPH-Mean	-0.3	0.2	8.2	4.7		
-Range	-9.5-+4.9	-4.5-+3.4	0.6-14.1	-8.8-+9.8		
MSH-Mean	0.9	1.5	2.6	1.3		
-Range	-8.3-+6.3	-3.2-+4.7	-4.6-+8.2	-11.7-+6.2		
HSH-Mean	2.5	3.0	4.2	2.8		
-Range	-6.9-+7.9	-1.7-+6.3	-3.1-+9.9	-10.4-+7.9		
Descriptive statistics	**Oil yield (kg ha^{-1}) (Trial mean over 3 environments=763±70.1)**					
LSMeans	773^{b}	801^{ab}	789^{b}	747^{c}	647^{d}	834^{a}
Range	348-1301	439-1182	346-1155	405-1255	475-879	648-1005
Heterosis%						
FPH-Mean	-12.0	25.0	35.8	8.1		
-Range	-60.4-+48.1	-31.4-+84.5	-40.4-+98.9	-41.4-+81.7		
MSH-Mean	-7.3	-4.0	-5.5	-10.5		
-Range	-58.3-+56	-47.3-+41.7	-58.5-+38.4	-51.5-+50.4		
HSH-Mean	-23.0	-20.3	-21.5	-25.7		
-Range	-65.4-+29.5	-56.2-+17.6	-65.5-+14.9	-59.7-+24.9		

Means sharing a superscript letter are not significantly different at the $p< 0.05$ level

Mean estimates of MSH and HSH were negative for seed yield and oil yield for all the TCH groups though range of MSH and HSH for seed yield indicated that the best TCH was 47.8% and 20.5% better than the mean for the hybrid check group and best hybrid check, respectively. The mean MSH and HSH were positive for all the TCHs groups for oil content indicating a high frequency of TCHs with higher oil content than the mean of hybrid check group and best check hybrid, respectively (Table 3.3). When selecting TCHs for further testing and subsequent release, TCHs with positive HSH for oil yield are usually retained. A total of 28 TCHs had positive HSH for oil yield which is equivalent

to 12.8% selection intensity when all the 218 TCHs are considered, thereby falling within the range of 10-15% selection intensity normally applied in early generation testing (Hallauer and Miranda, 1988).

In order to relate the level of improvement and commercial worth in the founder parents and their experimental TCHs, respectively the respective values of FPH, MSH and HSH for seed yield and oil content were expressed as percentages of their sum total for the top five TCHs in each group based on oil yield as selection criterion (Figure 3.4).

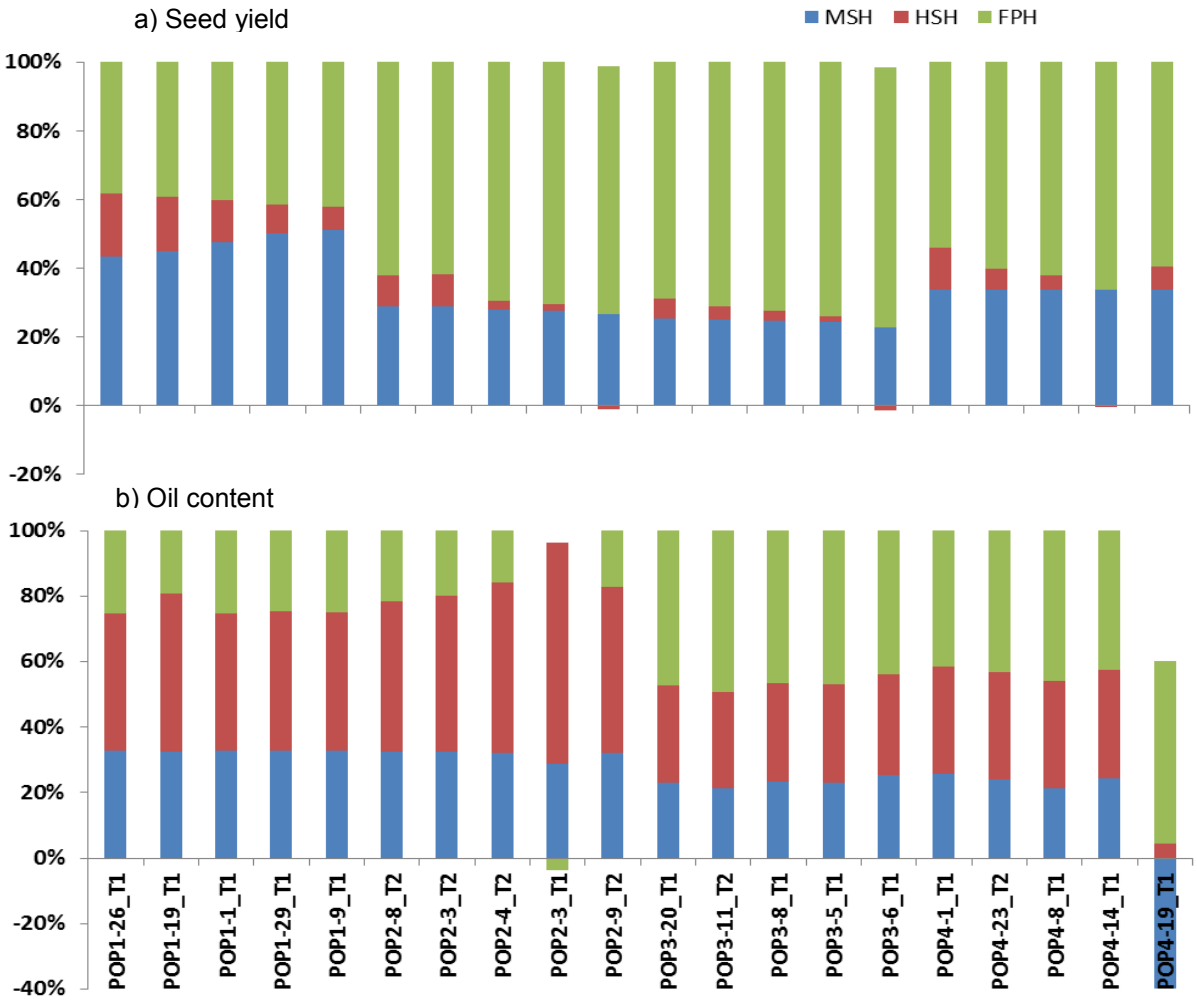

Figure 3.4 Mid-parent heterosis (MSH), high standard heterosis (HSH) and founder parent heterosis (FPH) for (a) seed yield and (b) oil content, expressed as percentages of their sum total for the top five TCHs of each group

The top five TCHs from the group $Pop1_{TC}$ had the highest MSH and HSH for seed yield compared to the top five TCHs from other groups (Figure. 3.4a). The top five TCHs from $Pop2_{TC}$, $Pop3_{TC}$ and $Pop4_{TC}$ had high positive FPH for seed yield indicating that the hybrid progeny (FP_{TC}) from crossing the founder parents with the same testers were not as high yielding as their experimental TCH progenies (S_3CMS x Tester 1 or 2) whereas the reverse was true for the top five of $Pop1_{TC}$ which had low FPH. For oil content, the top five experimental TCHs in the groups $Pop1_{TC}$ and $Pop2_{TC}$ had a high HSH and low FPH compared to $Pop3_{TC}$ and $Pop4_{TC}$ (Figure 3b). A low FPH is an indication that the founder parents may already have been considerably improved for that trait and as the founder parents become more improved so do the later selfed generations derived from them and hence a gradual decrease in recorded heterosis (Troyer and Wellin, 2009).

3.4 Conclusions

This is one of the few studies in sunflower in which multiple breeding populations in advanced cycle pedigree breeding have been used to estimate genetic variability and heterosis. Theoretically advanced cycle breeding improves the population mean at the expense of reducing genetic variability (Yu and Bernardo, 2004). In this study the usable genetic variance to permit selection was not reduced for seed yield and oil yield and the mean of the TCH groups were significantly better than the FP_{TC} confirming the potential value of advanced pedigree cycle breeding in sunflower. In contrast the usable genetic variance for oil content ranged from moderate to low with $Pop2_{TC}$ having the lowest genetic variance indicating the need to broaden the current level of genetic variance for oil content to sustain long-term genetic gains.

The application of genetic parameters such as those related with genetic variability and heterosis can be regarded as a two-stage selection process with independent selection gains. Selection approaches using genetic variability concentrate on selecting the best TCH group based on the potential genetic advance and usefulness criterion hence selection gain depends on the σ^2_{TC} and *H* of that group. Further studies to partition σ^2_{TC} into additive, dominance and epistatic genetic variances will shed more light on the predominant gene action in advanced cycle breeding in sunflower. The second step of using heterosis concentrates on selecting within a TCH group and selection gain depends on the number of TCHs outperforming the founder parent testcrosses and

hybrid checks. On the basis of the results from this study it was concluded that using FPH in combination with MSH and HSH to determine heterosis is more relevant in cross pollinated crops such as sunflower if the principle aim is to develop inbred lines that are have a high probability of outperforming both their founder parents in testcross combinations and commercial checks. Furthermore the FPH approach is cost effective as only the testcrosses of founder parents and TCHs are included in the trials compared to evaluating inbreds in balanced sets of TCHs at a higher cost as in BPH or MPH (Smith, 1997). High performances of some of the lines in testcross combinations from this study indicate that generating heterosis is not dependent on genetic diversity *per se* but usable genetic variation (Riday and Brummer, 2005).

As it is not currently obvious that genetic variance in advanced cycle pedigree breeding has been exhausted or not, the procedures described in this study are valuable if one wishes to develop new elite lines based on sound genetic parameters. The genetic parameters calculated in this study provide the breeder with an insight into which parental lines to cross to form future base breeding populations and hence maximize resources by only concentrating on using parental lines with the highest probability of producing superior progeny.

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CHAPTER 4

Advanced cycle pedigree breeding in sunflower. II: Combining ability for oil yield and its components[3]

Abstract

Combining ability is one of the most important statistics breeders use to identify superior inbred lines on the basis of their performance in hybrid combinations. The objectives of this study were: (i) to quantify the importance of general combining ability (GCA) and specific combining ability (SCA) variances for seed yield, oil content and oil yield; and (ii) estimate GCA and SCA effects of seed yield, oil content and oil yield of inbred lines developed from advanced cycle pedigree breeding populations in sunflower. A total of 109 female S_3 cytoplasmic male sterile (CMS) lines from four bi-parental populations in advanced cycle pedigree breeding were crossed with two testers to form 218 testcross hybrids (TCHs). The TCHs were then evaluated in three environments. Variance component analysis results showed predominance of σ^2_{GCA} over σ^2_{SCA} for seed yield and oil yield indicating that superior TCHs can be identified based on positive and significant GCA effects of the female lines. For oil content, σ^2_{SCA} was predominant over σ^2_{GCA} indicating that selecting for TCHs with high oil content would be best among line x tester combinations and not among female S_3CMS lines *per se*. The relative proportion of GCA and SCA effects in the best five TCHs in each breeding population also confirmed the predominance of GCA effects over SCA effects for seed yield and oil yield while for oil content both GCA and SCA effects appear to be important, with SCA effects having more influence than GCA. The best selection strategy would therefore be to capture the GCA in the early stages of inbreeding and then SCA for the few unique combinations when lines are almost fixed.

Key words: General combining ability (GCA); Helianthus annuus, narrow sense heritability; Specific combining ability (SCA)

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4.1 Introduction

Mature plant breeding programmes are guided by decisions made at different stages, and the application of quantitative genetics in decision-making is crucial to maximise breeding techniques such as choosing the initial parents to form the base breeding populations, number of testers to use and when to testcross. Advanced cycle pedigree breeding, a strategy which involves recycling of existing elite inbred lines is widely used to produce better performing hybrids in maize (*Zea mays* L.) (Hallauer, 1990; Lu and Bernado, 2001). The strategy involves mating elite inbred lines within the same heterotic group to form a base breeding population from which the next generations of inbred lines are developed from. Although genetic diversity is reduced by this approach, the prevailing popularity of widely adapted hybrids indicates farmers' past concerns about genetic diversity have, lessened over time (Troyer, 2006). Zhang et al. (2000) cited by Fan et al. (2008) found that 71% of commercial maize hybrids grown in China came from four inbred lines or their derivatives. Similar trends were reported in maize and barley (*Hordeum vulgare* L.) in the USA by Bernado et al. (2000) and Condón et al. (2008) respectively, indicating widespread use and success of advanced cycle pedigree breeding. For a crop like sunflower whose heterotic groups and patterns are loosely defined (Cheres et al., 2000), the effect of advanced cycle pedigree breeding on important genetic properties like combining ability and gene action is not known.

Combining ability is one of the most important statistic breeders use to identify new inbred lines with potential on the basis of their superiority in hybrid combinations. The combining ability of a line is partitioned into general combining ability (GCA) and specific combining ability (SCA). The GCA is generally regarded as a measure of additive gene action while the SCA is equated to the non-additive (dominance and epistatic) effects (Comstock et al., 1949). Selecting for both parents with high GCA maximises additive genetic variance thereby increasing narrow sense heritability, an important statistic used to quantify response to selection (Falconer and Mackay, 1996). Previous studies have also found additive genetic effects to generally be predominant and more important than non-additive genetic variance in the expression of quantitatively inherited traits in previously unselected germplasm hence past population improvement in various crop species has relied on recurrent selection methods to exploit additive gene action (Sprague and Tatum, 1942; Comstock et al., 1949). Methods such as reciprocal

recurrent selection (Comstock et al., 1949) devised to exploit both additive and dominance genetic variance are rarely used in sunflower hybrid breeding because of their long term nature and, therefore inbreeding methods such as pedigree selection are the more commonly preferred methods (Miller and Fick, 1997). In the absence of dominance and epistasis, additive genetic variance between genotypes decreases linearly with inbreeding in bottlenecked populations such as bi-parental populations (Falconer and Mackay, 1996). Thus theoretically, a high reduction in additive genetic variance when recycled elite lines are used as base parents to form bi-parental breeding populations is expected and therefore warrants investigations to determine whether further gains in hybrid performance based on exploiting GCA are attainable.

A number of mating designs have been developed which enable the estimation of GCA and SCA variances and effects (eg the diallel mating design (Griffith, 1956); the North Carolina design II (Comstock et al., 1949); line by tester (Singh and Chaudhary, 1977)). In hybrid breeding programs involving either large numbers of inbred lines or where cytoplasmic male sterile (CMS) systems can be exploited such as in sunflower, for practical convenience the line by tester mating design is commonly preferred (Miller et al., 1980; Khan et al., 2008). The added advantage of line by tester mating design over other designs such as the diallel is its ability to accommodate a large number of genotypes at a given time for identification of potential parents and superior combinations without sacrificing information such as combining ability and type of gene effects (Singh and Chaudhary, 1977). In a 3 x 10 tester line analysis in sunflower, Miller et al. (1980) found that additive genetic variance accounted for a major portion of the genetic variation for all traits studied except head diameter. This study corroborates earlier conclusions by Sindagi et al. (1979) who found additive genetic effects to be more predominant than non-additive on traits studied but differ from recent studies by Khan et al. (2008) who found a greater manifestation of non-additive gene effects in all the traits studied across environments using a 5 x 5 tester line analysis. Recently, Reif et al. (2013) found prediction of hybrid performance based on GCA effects to be high in sunflower for grain yield, oil yield and oil content indicating the importance of additive genetic variance for these three traits. In studies involving morphological traits, petiole length and total leaf area per plant Hladni et al. (2008), found non-additive component of genetic variance to be more important than additive and the ratios of GCA/SCA for both traits was less than a unit. These contradictory results points to the necessity of

evaluating local germplasm under target production environments as results from other studies using different sets of germplasm and environments, though important, may not have a direct application to local conditions. Moreover, as most of the studies mentioned above involved the use of divergent germplasm and there are few studies on advanced cycle pedigree breeding in sunflower to merit the use of GCA or SCA in line development. The objectives of this study were therefore to: quantify the importance of GCA and SCA variances for seed yield, oil content and oil yield of lines developed from advanced cycle pedigree breeding populations; and estimate GCA and SCA effects of seed yield, oil content and oil yield of inbred lines developed from advanced cycle pedigree breeding populations.

Compared to other studies of line by tester analysis in sunflower, the study presented here provides by far the largest line by tester analysis to estimate the relative importance of additive and non-additive genetic variance in determining seed yield, oil content and oil yield.

4.2 Materials and methods

4.2.1 Genetic material and field evaluation

The genetic material and field evaluation procedures were described in Chapter 3, but are repeated here for convenience. Female cytoplasmic male sterile (CMS) lines were developed from four base breeding populations (BBPs) in advance cycle pedigree population herein referred as breeding populations. The BBPs were formed by crossing elite maintainer lines (B-lines) with each other as follows: H52xKB61 (Pop1); HA89xKB16 (Pop2); HA89xKB61 (Pop3); and HA89xKB189 (Pop4) (Table 4.1 and Figure. 4.1). Parental inbred line H52 purportedly developed by ARC is of Romanian origin, while HA89 is a public line released by USDA-ARS. These two old elite inbred lines are still widely used in sunflower breeding programmes as parents in some commercial hybrids around the world or as parents for breeding populations (Jan et al., 2004; Jan 2006). Parental lines KB61, KB16 and KB189 are inbred lines derived from different composite populations developed by the ARC-GCI using multiple source germplasm including versions of H89 and H52 (B. Greyling, personal communication).

Table 4.1 Number of S_3CMS lines developed from four base breeding populations crossed to two testers (T1 and T2) to form experimental testcross hybrids (TCHs)

Testers	Base Breeding Populations				
	Pop1	Pop2	Pop3	Pop4	Subtotal (Tester/half-sib TCHs)
RP865 (T1)	44	12	26	27	109
RP953 (T2)	44	12	26	27	109
Subtotal (Line/half-sib TCHs)	88	24	52	54	
Total TCHs					218

Naming of inbred lines and TCHs:

S_3CMS inbred lines developed from Pop1 were coded as Pop1-1, Pop1-2…1-44 and their TCHs as Pop1-1_T1, Pop1-2_T1…1-44_T1; Pop1-1_T2, Pop1-2_T2…1-44_T2.
S_3CMS inbred lines developed from Pop2 were coded Pop2-1, Pop2-2…2-12 and their TCHs as Pop2-1_T1,Pop2-2_T1…2-12_T1; Pop2-1_T2,Pop2-2_T…2-12_T2
S_3CMS inbred lines developed from Pop3 were coded Pop3-1, Pop3-2…3-26 and their TCHs as Pop3-1_T1, Pop3-2_T1…3-26_T1; Pop3-1_T2, Pop3-2_T2…3-26_T2.
S_3 CMS inbred lines developed from Pop4 were coded Pop4-1, Pop4-2, 4-27 and their TCHs as Pop4-1_T1, Pop4-1_T1…4-27_T1; Pop4-1_T2, Pop4-2_T2…4-27_T2.

After selfing the F_1 of each cross, plants in the subsequent F_2 generation were allowed to undergo random mating within each population with selection starting at the $F_{2/3}$ generation followed by selfing for three generations to produce S_3 inbred lines. The resultant S_3 maintainer lines (B-lines) were converted to CMS lines by backcrossing to form S_3CMS lines (A-lines) during the 2003 to 2006 seasons. Advancement to each generation during the backcrossing stage was based on adequate quantities of seed for both the CMS and maintainer lines. A total of 109 S_3CMS lines and their corresponding maintainer lines with adequate quantities of seed were developed across the four BBPs as follows: Pop1 had 44 lines, Pop2 had 12 lines, Pop3 had 26 lines and Pop4 had 27 lines (Figure 4.1). Each of the 109 S_3CMS lines were then testcrossed to two male fertility restorer lines (*Rf*) as testers; RP865 (T1) and RP953 (T2) to produce a total of 218 experimental testcross hybrids (TCHs) (Table 4.1). Both the T1 and T2 were developed by pedigree breeding method from crosses of two independent commercial hybrids by licensed *Rf* lines of American and Australia origin hence tester T1 belongs to the American heterotic group while T2 belongs to the Australian heterotic group.

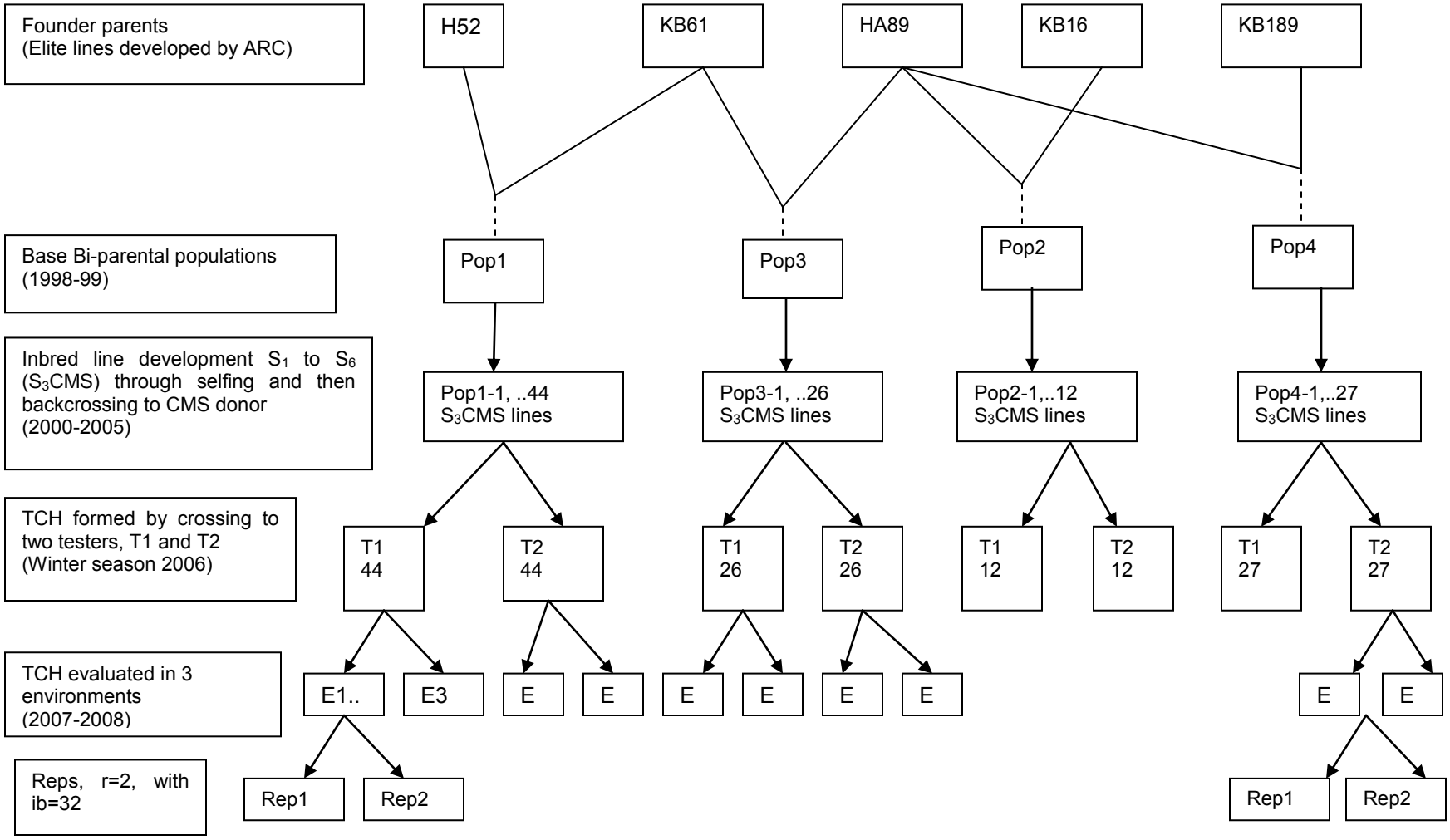

Figure.4.1. Schematic diagram for the development and evaluation of the testcross hybrids (TCHs)

4.2.2 Test environments, experimental design and agronomic traits recorded.

The 218 experimental TCHs along with six commercial checks and testcrosses of the original parental lines (founder parent testcross hybrids/genetic checks) were evaluated in an alpha (0, 1) design with two replications in three environments. The three environments were Potchefstroom early (POTCH06), planted at the end of November in 2006; Potchefstroom late (POTCH07), planted the first week of January 2007; and Bothaville (BTV07), planted in the second week of January 2007. Potchefstroom (26.745S, 27.083E) is situated in the Northwest Province, and Bothaville (27.235S, 26.67E) is located in the Free State Province of South Africa and the two provinces are the major sunflower production environments in South Africa contributing 80% of the total area planted to sunflower annually (SAGIS, 2011). Experimental units and agronomic practices were as described in Chapter 3.

The agronomic traits measured were: seed yield (kg ha^{-1}), percent oil content and oil yield (kg ha^{-1}). Seed yield was determined on a plot basis, adjusted to 10% seed moisture, by weighing seed which had been dried in continuous air flow dryers at 32-35˚C over a period of two weeks until no further loss in mass was recorded. Percent oil content was determined on 12 g, air-dried seed samples by nuclear magnetic resonance using a Newport Analyzer (Newport-Oxford Instruments Ltd, England). Oil yield (kg ha^{-1}) was obtained as the product of seed yield and percent oil content.

4.2.3 Statistical analysis

Combining ability analyses of 218 TCHs across and within the breeding populations for seed yield, oil content and oil yield were done excluding hybrid checks and founder parent testcross. The phenotypic value y_{ijkl} was partitioned into the following components of a mixed linear model:

$$y_{ijkl} = \mu + T_i + L_j + (TL)_{ij} + E_l + (TE)_{il} + (LE)_{jl} + (TLE)_{ijl} + E_k(E_l) + \varepsilon_{ijkl}$$

where y_{ijkl} is the phenotypic observation of the TCH of the i^{th} tester and the j^{th} CMS line/female line across breeding populations or within a breeding population, evaluated in the k^{th} replication, and l^{th} environment; μ is the grand mean; T_i is the fixed effect of the tester i,; L_j is the general combining ability effect (GCA_j) of female line j; $(TL)_{ij}$ the specific combining ability effect (SCA_{ij}) of the TCH involving tester i and female line j; E_k the effect of the environment k; $(TE)_{il}$ the

interaction between effects of tester *i* and environment *l; (LE)$_{jl}$* the interaction effects between GCA of line *j* and environment *l;* (TLE)$_{ijk}$ the interaction effects between SCA of cross between *i x j* and environment *l*; $R_k(E_l)$ the effect of the replicate *k* nested within the environment *l*; and ε_{ijkl} is the random error term. Environments and testers effects were considered fixed while the effects of the female lines and replications within environment were considered random. Variance components estimates for GCA of female lines (σ^2_{GCA}); SCA of TCHs (σ^2_{SCA}) and the respective interactions with the environment; ($\sigma^2_{GCA\ x\ E}$) and ($\sigma^2_{SCA\ x\ E}$) were determined for each breeding population and across breeding populations using the mixed model procedure PROC MIXED, Method = TYPE3 in SAS (SAS Institute, 2010).

Narrow sense heritability (h^2) on the basis GCA effects of the lines for each breeding population and across breeding populations was computed according to Grieder et al. (2012) as:

$$h^2 = \frac{\sigma^2_{\mathrm{GCA}}}{\sigma^2_{\mathrm{GCA}} + {\sigma^2_{\mathrm{SCA}}}/{\mathrm{T}} + {\sigma^2_{\mathrm{GCA\ x\ E}}}/{\mathrm{E}} + {\sigma^2_{\mathrm{SCA\ xE}}}/{\mathrm{ET}} + \sigma^2_{\varepsilon}/ETR}$$

Where: E is the number of environments, T the number of testers and R the number of replications.

Additive (σ^2_A) and dominance (σ^2_D) components of variance were calculated according to Singh and Chaudhary (1977) as:

$$\sigma^2_{GCA} = \left[\frac{1+F}{4}\right]\sigma^2_A \text{ , and } \sigma^2_{SCA} = \left[\frac{1+F}{2}\right]\sigma^2_D$$

Where: F is the inbreeding coefficient, and for S_3 (F_4) lines F=0.875 (Hallauer and Miranda, 1988). The average degree of dominance ṕ was approximated as:

$$\acute{\rho} = \sigma^2_A/\sigma^2_D$$

If ṕ≥1 gene action is predominantly additive and ṕ≤1 gene action is predominantly non-additive (dominance and interactions).

The GCA and SCA effects for seed yield, oil content and oil yield were estimated using the method of Singh and Chaudhary (1977). The significance of GCA_j and SCA_{ij} effects of the j^{th} female line and its interaction with the i^{th} tester were determined by two-tailed t-tests, where

$t = GCA_j/SE_{GCA}$ and $t = SCA_{ij}/SE_{SCA}$ were considered significant if the ratio was more than two standard errors from zero (Wu and Matheson, 2004). Proportional contribution of testers (% T SS), lines (% GCA SS) and their interaction (% SCA SS) to total genetic variance were calculated according to Singh and Chaudhary (1977).

4.3 Results

4.3.1. Mixed model analysis of variance: mean squares

The TCHs or genotypes (G) mean squares (MS) were highly significant ($p<0.01$) across the three environments for each breeding population and across the breeding populations for seed yield, oil content and oil yield (Table 4.2). The genotype SS were partitioned into SS for tester (T), female lines (GCA), and tester by female lines (SCA), across and within breeding populations. The GCA MS were highly significant ($p<0.01$) for seed yield and oil yield across and within breeding populations, while GCA MS for oil content were significant only in Pop4 (Table 4.2). The T MS for the three traits were significant in two populations, Pop1 and Pop2. The SCA MS were highly significant across and within breeding populations for all the three traits studied except for Pop2 (Table 4.2).

The GxE MS was significant for seed and oil yield in Pop1 and across the breeding populations. The TxE and GCAxE MS were significant for seed yield and oil yield in Pop1, Pop2 and across breeding populations. The SCAxE MS were significant only in Pop1 for seed and oil yield, while for oil content they were significant in all the breeding populations with Pop2 being the exception. The %SS for T, GCA and SCA indicated that GCA had the largest contribution to total SS followed by SCA for all the traits except oil content in Pop2 (Table 4.2).

Table 4.2. Mean squares (MS) and degrees of freedom (DF) for the line by tester analyses based on a mixed linear model for seed yield, oil content and oil yield in sunflower across the three environments

		Breeding populations									
Trait		**Across breeding populations**		**Pop1**		**Pop2**		**Pop3**		**Pop4**	
Seed yield (kg ha $^{-1}$)	**Source of variation**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**
	Genotypes (G)	**217**	1578151**	**87**	1837508**	**23**	1658879**	**51**	1355009**	**53**	1374838**
	Testers (T)	**1**	4117457*	**1**	12360921**	**1**	5576131**	**1**	554048	**1**	161943
	Female (GCA)	**108**	2502107**	**43**	2798636**	**11**	2605061**	**25**	2300713**	**26**	2353190**
	SCA	**108**	609807**	**43**	599105**	**11**	356583	**25**	429041**	**26**	418275**
	G x Environment (E)	**434**	259282**	**174**	316938**	**46**	304802	**102**	203980	**106**	199327
	TxE	**2**	1220502**	**2**	2305005**	**2**	969813**	**2**	142563	**2**	177957
	GCA x E	**216**	292939**	**86**	363897**	**22**	380067*	**50**	223511	**52**	210094
	SCA x E	**216**	215281	**86**	220097*	**22**	169081	**50**	187454	**52**	187727
	Error	**644**	189116	**256**	167280	**69**	202126	**152**	235027	**158**	176213
	% T SS		1.2		7.7		14.6		0.8		0.2
	%GCA SS		78.9		75.3		75.1		83.2		84
	%SCA SS		19.2		16.1		10.3		15.5		14.9
Oil content (%)	**Genotypes (G)**	**217**	10.3**	**87**	12.0**	**23**	4.3	**51**	7.0**	**53**	12.2**
	Testers (T)	**1**	17.1	**1**	127.8**	**1**	45.8**	**1**	5.5	**1**	0.35
	Female (GCA)	**108**	12	**43**	11.7	**11**	4	**25**	8.1	**26**	17.6*
	SCA	**108**	8.6**	**43**	9.7**	**11**	0.8	**25**	6.1**	**26**	7.2**
	G x Environment (E)	**434**	2.9**	**174**	2.3**	**46**	2.8**	**102**	3.1**	**106**	3.3**
	TxE	**2**	5.2	**2**	6.5	**2**	5.7*	**2**	2	**2**	3.27
	GCA x E	**216**	3.7**	**86**	2.3	**22**	3.9*	**50**	4.3**	**52**	3.53
	SCA x E	**216**	2.4**	**86**	2.3**	**22**	1.4	**50**	2.0**	**52**	3.0**
	Error	**644**	1.04	**256**	0.9	**69**	1.2	**152**	0.9	**158**	1.22
	% T SS		0.8		12.2		46.2		1.5		0.05
	%GCA SS		57.9		48		44.7		56.8		70.91
	%SCA SS		41.7		40		9.1		42.8		28.89

*, ** Significant at p<0.05 and p<0.01 level, respectively
% T SS = percent tester sum of squares; % GCA SS = percent GCA sum of squares; % SCA SS = percent SCA sum squares

Table 4.2 (continued)

		Breeding populations									
		Across breeding populations		Pop1		Pop2		Pop3		Pop4	
Trait	**Source of variation**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**
Oil yield (kg ha^{-1})	**Genotypes (G)**	**217**	268367**	**87**	303388**	**23**	277612**	**51**	245844**	**53**	235163**
	Testers (T)	**1**	797085**	**1**	2632283**	**1**	1153759**	**1**	71650	**1**	26184
	Female (GCA)	**108**	414611**	**43**	442149**	**11**	414168**	**25**	416825**	**26**	397265**
	SCA	**108**	114043**	**43**	105032**	**11**	61406	**25**	80175**	**26**	77863**
	G x Environment (E)	**434**	43690**	**174**	51962**	**46**	46066	**102**	36222	**106**	36145
	TxE	**2**	209634**	**2**	344518**	**2**	145937*	**2**	28640	**2**	30301
	GCA x E	**216**	48959*	**86**	58474*	**22**	54583	**50**	40472	**52**	39025
	SCA x E	**216**	36696	**86**	38167*	**22**	28470	**50**	32411	**52**	33144
	Error	**644**	30949	**256**	27551	**69**	30288	**152**	39151	**158**	28891
	% T SS		1.4		10		18.1		0.6		0.2
	%GCA SS		76.9		72		71.4		83.1		82.9
	%SCA SS		21.1		17.1		10.6		16		16.2

*, ** Significant at p<0.05 and p<0.01 level, respectively
% T SS = percent tester sum of squares; % GCA SS = percent GCA sum of squares; % SCA SS = percent SCA sum squares

4.3.2 Mixed model analyses: variance components

The σ^2_{GCA} of the female lines were significant for seed yield and oil yield in three out of the four breeding populations and across breeding populations except Pop2 (Table 4.2). The σ^2_{SCA} were significant in three out of the four breeding populations for seed and oil yield except in Pop2. The $\sigma^2_{GCA\ x\ E}$ and $\sigma^2_{SCA\ x\ E}$ were largely significant for oil content in at least three out of the four populations except Pop1 for $\sigma^2_{GCA\ x\ E}$ and Pop2 for $\sigma^2_{SCA\ x\ E}$. Narrow-sense heritability, h^2 was moderate to high for both seed yield and oil yield and low for oil content (Table 4.2). The degree of dominance $\acute{\rho}$ was greater than one ($\acute{\rho}$>1) for seed yield and oil yield in all four breeding populations while for oil content $\acute{\rho}$ was less than one ($\acute{\rho}$<1) in three of the four breeding populations, the exception being Pop4 (Table 4.2).

4.3.3 Combining ability effects

The GCA and SCA effects of the S_3CMS lines from the four breeding populations were largely within the same range for seed yield and oil yield but for oil content, Pop2 had a narrow range of SCA effects compared to the other three breeding populations (Figures 4.2, 4.3 and 4.4). For both traits the median for GCA effects was high in Pop2 while the median for SCA effects was equal to that of the mean in all the breeding populations (Figures 4.2, 4.3 and 4.4).

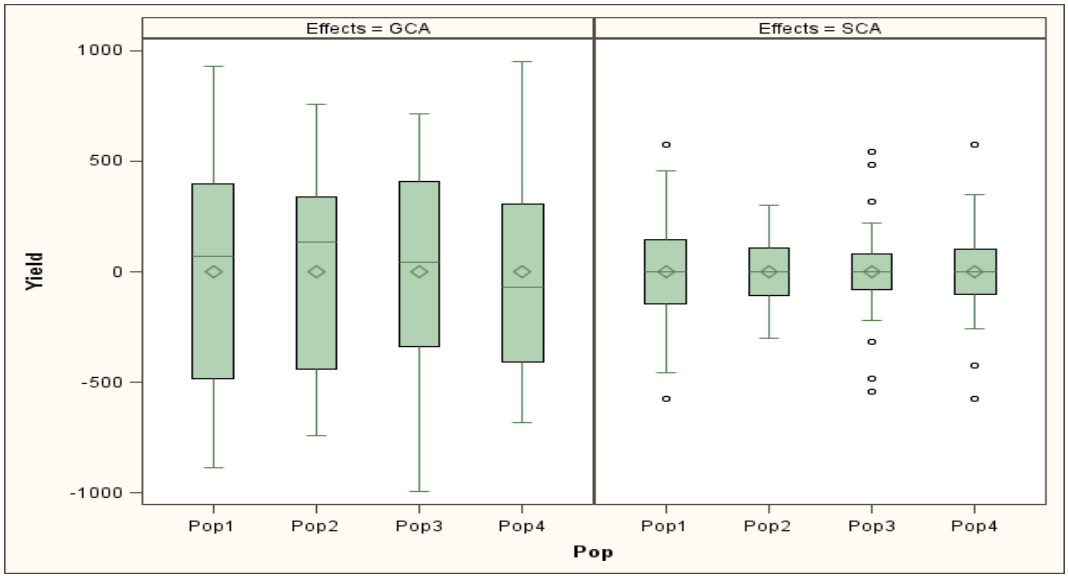

Figure 4.2 Range and median for GCA and SCA effects for seed yield (kg ha $^{-1}$) of the S_3CMS lines in the four breeding populations/TCH groups. (Yield = seed yield (kg ha^{-1}))

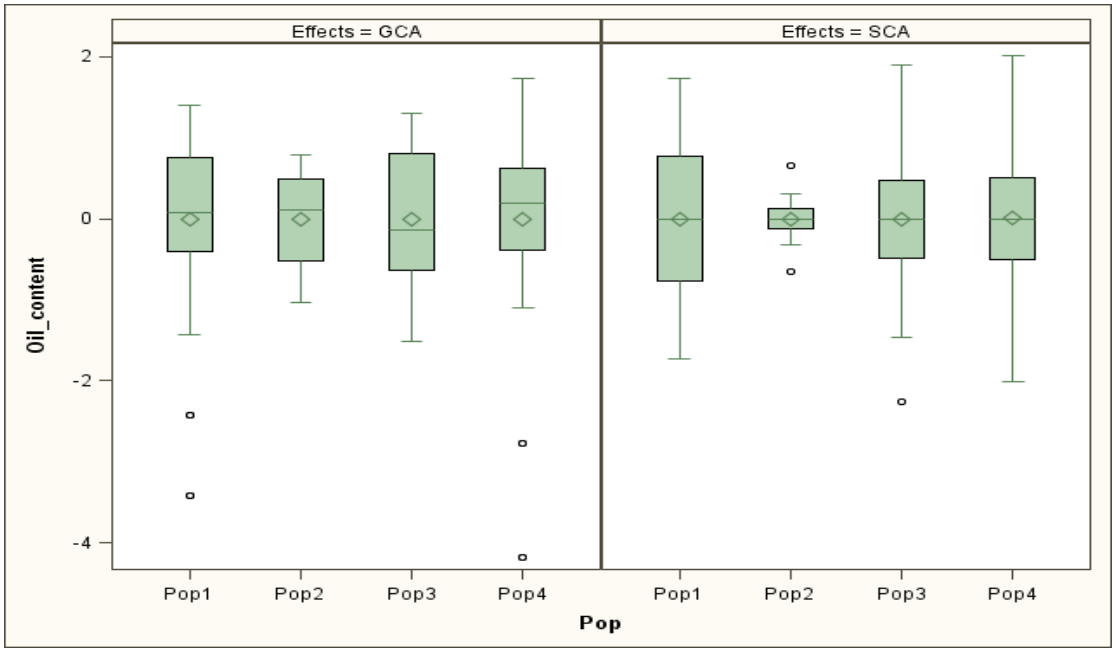

Figure 4.3 Range and median for GCA and SCA effects for oil content (%) of the S_3CMS lines in the four breeding populations/TCH groups. ((Oil_content = Oil content %))

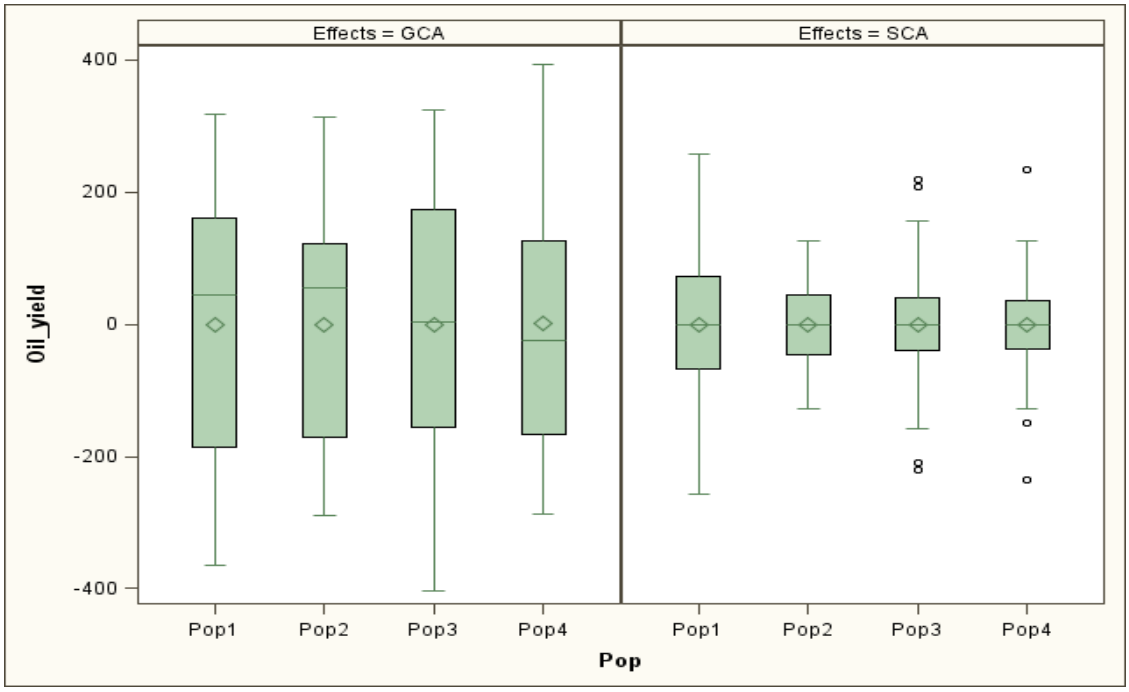

Figure 4.4 Range and median for GCA and SCA effects for oil yield (kg ha^{-1}) of the S3CMS lines in the four breeding populations/TCH groups (Oil_yield = Oil yield (kg ha^{-1}))

Of the 109 S_3CMS lines across the four breeding populations, 33 had positive and significant GCA effects for oil yield (Table 4.3). The S_3CMS lines: P4_1CMS, P2_3CMS, P3_20CMS, P1_19CMS and P3_8CMS had the highest positive and significant ($p<0.05$) GCA effects (Table 4.3). Of the 218 TCHs only 28 had positive and significant SCA effects for oil yield.

Table 4.3 Mean for oil yield across three environments ranked in descending order of the top 30 TCHs along with the GCA and SCA effects and ranks of their S_3CMS lines

		Effects (kg ha^{-1})		Rank[a]	
TCH	**Mean (kg ha^{-1})**	**GCA**	**SCA**	**GCA effect**	**SCA effect**
P1_26CMSxT1	1300.5	256.0*	246.0*	9	2
P4_1CMSxT1	1254.6	365.4*	90.7	1	35
P1_19CMSxT1	1199.5	319.7*	81.4	4	42
P2_8CMSxT2	1181.7	203.1*	229.9*	18	4
P2_3CMSxT2	1181.1	341.9*	90.5	2	36
P1_1CMSxT1	1173.5	242.7*	132.2*	10	21
P3_20CMSxT1	1153.2	338.2*	16.5	3	92
P1_29CMSxT1	1126.1	93.2	234.5*	38	3
P4_23CMSxT2	1122.7	231.8*	142.2*	13	15
P1_9CMSxT1	1120.5	185.8*	136.2*	22	20
P3_11CMSxT2	1115.9	138.2*	229.0*	30	5
P3_8CMSxT1	1111.8	297.6*	15.7	5	93
P1_38CMSxT1	1096.8	207.0*	91.3	16	34
P3_5CMSxT1	1096.5	279.6*	18.4	7	89
P1_31CMSxT2	1094.7	284.4*	61.5	6	50
P3_6CMSxT1	1090.2	239.6*	52.1	11	56
P4_8CMSxT1	1089.2	205.7*	85.0	17	38
P1_2CMSxT2	1088.3	127.8*	211.8*	33	7
P4_14CMSxT1	1073.7	185.8*	89.4	23	37
P2_4CMSxT2	1073.6	162.4*	162.5*	27	10
P4_19CMSxT1	1069.5	163.7*	107.3*	26	29
P4_15CMSxT2	1068.5	237.7*	82.1	12	40
P1_10CMSxT2	1056.2	272.6*	35.0	8	76
P1_16CMSxT1	1050.6	92.8	159.2*	39	11
P1_18CMSxT1	1042.4	149.1*	94.8	28	32
P1_22CMSxT1	1040.9	128.5*	114.0*	32	25
P2_9CMSxT2	1036.7	130.8*	157.2*	31	12
P1_35CMSxT1	1023.2	173.5*	51.2	25	58
P1_21CMSxT1	1021.6	74.2	148.9*	42	13
P3_13CMSxT1	1020.8	198.4*	23.9	20	82
Trial mean	**773.6**				
S.E.	**114.6**	**63.5**	**55.0**		
Probability of being among the top 30 TCHs[b]				**0.8**	**0.47**

* Significant at $p<0.05$ level

[a] Ranks of the GCA and SCA effects for oil yield of the respective S_3CMS lines of each TCH

[b] Probability of selecting a TCH in the top 30 based on rankings of the rankings of the GCA or SCA effects of the respective S_3CMS lines

The five TCHs with the highest SCA effects were: P4_25CMSxT2, P1_26CMSxT1, P1_29CMSxT1, P2_8CMSxT2 and P3_11CMSxT2. The top five TCHs with the highest oil yield were: P1_26CMSxT1, P4_1CMSxT1, P1_19CMSxT1, P2_8CMSxT2 and P2_3CMSxT2

(Table 4.3). Of these top five yielders only two TCHs P1_26CMSxT1 and P2_8CMSxT2 were among the top five TCHs with the best SCA effects while three S_3CMS lines: P4_1CMS, P2_3CMS and P1_19CMS were among the top five lines with the best GCA effects and had their TCHs in the top five performers for oil yield (Table 4.3). Overall, the probability of correctly selecting the top 30 TCHs (i.e. a 14% selection intensity), with highest mean oil yield if selection was based on the GCA and SCA effects of their respective parents was 0.80 and 0.47, respectively (Table 4.3).

The proportion of the GCA and SCA effects for seed yield expressed as percentages of their respective sum totals for each of the top five TCHs in each group based on oil yield as selection criterion indicated a preponderance of GCA effects for seed yield in 18 of the 20 TCHs across the breeding populations. The two exceptions were the crosses: P1_29CMS x T1 and P3_11CMS x T2 in which the proportion of SCA effects was higher than the GCA effects (Figure 4.5). For oil content the proportion of SCA effects was slightly higher than the GCA effects for three of the top five TCHS based on oil yield in each of Pop1, Pop3 and Pop4 (Figure 4.6).

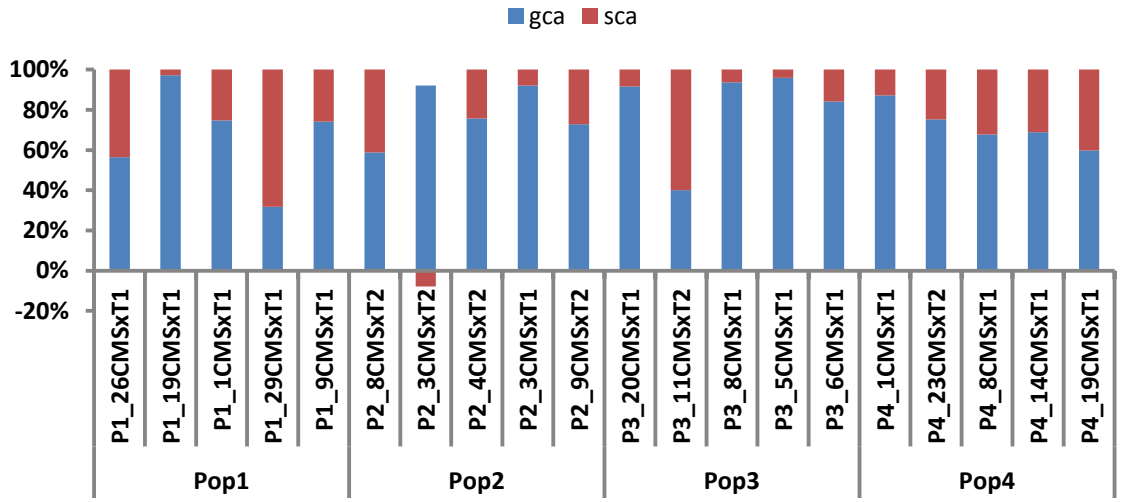

Figure 4.5 GCA and SCA effects for seed yield expressed as percentages of their sum total for the five TCHs of each group.

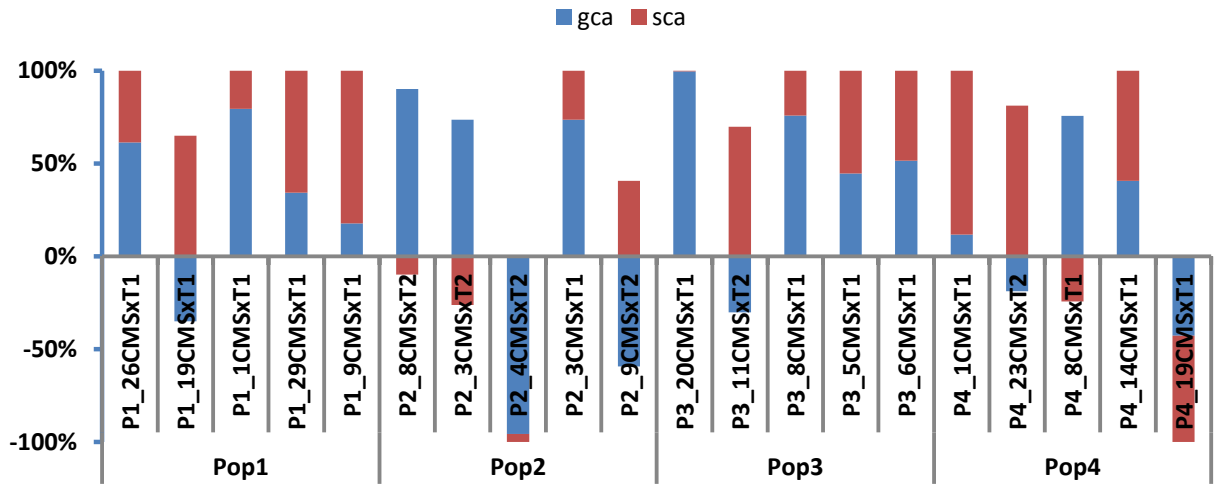

Figure 4.6 GCA and SCA effects for oil content expressed as percentages of their sum total for the top five TCHs of each group.

4.4 Discussion

4.4.1. Analysis of variance and variance component analysis

The results obtained in this study indicated that MS for genotype were significant for the three traits indicating substantial variation between the TCHs across and within breeding populations. The GCA and SCA, MS were significant in two or more of the breeding populations for the three traits indicating the importance of both additive and non-additive gene action in the inheritance of seed yield, oil yield and oil content. Based on the SS of the crosses, GCA effects had the largest contribution indicating the predominance of additive gene effects for both traits although to a lesser extent for oil content. These results are in agreement with those of Volotovich et al. (2008), but are in contrast to those of Ortis et al. (2005) who found non-additive gene action to be more important for seed yield while additive gene action was predominant for oil content. The magnitude of the ratio of % GCA SS of female lines to % T SS ranged from 5-420 for seed yield, 4-414 for oil yield and 1-1418 for oil content with Pop2 always having the lowest ratio and Pop4 having the highest for all three traits. That the % GCA SS for female lines were generally larger and in some instances much larger than that of the tester (restorer) lines is not surprising given that in sunflower breeding, CMS lines with high SCA values are usually converted to restorer (male) lines (Škorić, 1992). Also seed yield improvement *per se* of male sunflower lines is rarely practiced (Duvick, 1999), but rather the selection of male lines is based on oil content, stand ability and duration of pollen production. However, considering the low number of testers used

in this study further research with larger number of male testers is warranted to validate the ratios of % GCA SS of female lines to % T SS determined in this study.

The variance component analysis presented a predominance of σ^2_{GCA} over σ^2_{SCA} for seed yield and oil yield indicating that superior TCHs can be identified based on positive and significant GCA effects of the female lines (Melchinger et al., 1987). For oil content σ^2_{SCA} was predominant over σ^2_{GCA} indicating that selecting for TCHs with high oil content should be based on specific line x tester combinations and not on the average performance of female S_3CMS lines across all their respective crosses. Similarly, Hladni et al. (2008), found the ratio of $\sigma^2_{GCA}/\sigma^2_{SCA}$ to be around 0.33 for oil content and concluded that non-additive genetic variance was more predominant for oil content. As genetic variance is a function of both gene action and genotypic frequencies, the ratio of σ^2_A/σ^2_D, which may be equated to $\sigma^2_{GCA}/\sigma^2_{SCA}$ in the presumed absence of epistasis also depends on allelic frequencies between parental lines and genetic diversity in the base breeding populations (Reif et al., 2007). A high ratio of σ^2_A/σ^2_D observed for seed yield and oil yield indicate that a higher proportion of the genetic variance for these traits results from additive gene effects while a lower ratio of σ^2_A/σ^2_D for oil content indicate that much of the genetic variance is a result of non-additive gene effects.

Narrow sense heritability, as a measure of breeding values for the parents was high for seed yield and oil yield indicating that improvement of these traits for these populations is feasible through conventional selection methods such as pedigree or recurrent selection despite the fact that the base breeding populations were formed from elite recycled inbred parental lines. The narrow sense heritability values for seed yield (0.73-0.81) and oil yield (0.71-0.79) were considerably higher than those obtained by Andarkhor et al. (2012) of 0.21 and 0.29 for seed yield and oil yield, respectively. Generally low narrow sense heritability was obtained for oil content in three out of the four breeding populations, the exception being Pop4. Consequently, continued conventional selection for oil content will only be effective in Pop4, while introgressing germplasm with high oil content will be an option for the other three breeding populations before attempting further selection. The low narrow sense heritability obtained in this study are in contrast to that obtained by Reif et al. (2013) who found high broad sense heritability for oil content of above 0.85 indicating that narrow sense heritability was also likely to be high. The low narrow sense heritability for oil content in the breeding populations studied may also reflect similar scenarios in the private sector breeding programmes in South Africa in which elite inbred

lines are continuously recycled and hence a general slowdown in genetic gain for oil content over the past four decades (Chigeza et al. 2012).

4.4.2 Combining ability effects

Positive and negative significant GCA and SCA effects for seed yield, oil content and oil yield indicated that across and within the breeding populations some S_3CMS lines had good general and/or specific combining ability, while others were poor in this respect (Figures 4.2,4.3 and 4.4). The S_3CMS lines: P4-1CMS, P2-3CMS, P3-20CMS, P1-19CMS and P3-8CMS had the highest positive and significant ($p<0.05$) GCA effects indicating that regardless of the fact that the breeding populations were in advanced cycle pedigree breeding one would find at least one or more lines with productive GCA effects for oil yield across the breeding populations. Similarly, the five TCHs with the highest SCA effects were from all four breeding populations indicating that useful SCA effects for oil yield was generally evenly spread across breeding populations. Overall, the probability of correctly selecting the top 30 TCHs (i.e. 14% selection intensity) with the highest mean oil yield if selection is based on GCA and SCA effects of their respective parents was 0.80 and 0.47, respectively (Table 4.3). Therefore selecting the top performing TCHs would be ideally based on selecting female lines with the best GCA+SCA Reif et al. (2013) found that hybrid prediction based on available information on GCA effects was high and additional approaches using genomic selection could not increase the prediction of intergroup hybrid performance. Hallauer and Miranda (1988) noted that non-additive gene effects in maize on average are small but important in a few unique combinations. In this study the best performing TCH, P1_26CMSxT1 had the 9th highest ranked GCA effect of its female parent but the SCA effect of the cross was the second highest. Similarly, TCH P1_29CMSxT1 was ranked 8th overall in mean performance and while the GCA effect of its female parent was ranked a lowly 38th, the SCA effect of the cross was ranked 3rd (Table 4.3).

In the four breeding populations, the ranges for GCA effects were wider compared to SCA effects for seed yield and oil yield (Figures 4.2 and 4.4), corroborating the high ratio of σ^2_A/σ^2_D obtained for these two traits which is also an indirect estimate of genetic variability (Reif et al., 2007). For oil content, the range for the SCA effects was wider than that of the GCA effects in each of three of the four breeding populations, the exception being Pop2 indicating less variability of additive genetic variance and greater variability of non-additive genetic variance for this trait in these breeding populations (Figure 4.3). In terms of the top five TCHs in each

breeding population, the relative proportion of GCA and SCA effects confirmed that GCA effects predominated over SCA effects for seed yield while for oil content both GCA and SCA effects appear to be almost equally important, with SCA effects having slightly more influence than GCA. The relative magnitude of SCA effects are an important consideration in determining when to testcross and evaluate, i.e. early or late generation testing versus inbred yield testing (Troyer and Wellin, 2009).

4.5 Conclusions

Results from this study indicated that despite breeding populations being in advanced cycle pedigree breeding, significant GCA and SCA variance components and effects were present in the breeding populations. The GCA variance component and effects were predominant for seed yield and oil yield therefore allowing early generation testing and selection to be applied in these breeding populations. For oil content in which non-additive gene action is more important than additive, early generation testing will be of limited value as the performance of TCHs in early generation testing will not be guaranteed in the subsequent later generations of more highly fixed lines. Equally, if estimates of GCA variances and effects for the founder parents are known to be predominant, any resultant inbred progenies from such breeding populations are likely themselves to express favourable magnitudes of GCA effects. Theoretically this should allow for yield testing of the inbred lines with low magnitude of SCA effects to eliminate potential parents with low seed yield and other agronomic defects which may impact negatively on hybrid seed production in the event the lines are commercialised. The best selection strategy for the development of the inbred lines would appear to be to capture the GCA effects in the early stages of inbreeding thereby concentrating additive gene action and then SCA effects for the unique combinations when the lines are almost fixed. The S_3CMS female lines identified in this study with consistently high GCA effects namely: P4_1CMS, P2_3CMS, P3_20CMS, P1_19CMS P1_26CMS and P3_8CMS are not only useful as parents of hybrids based on the potential of their current TCHs but could also be used as founder parents for the next advanced cycle pedigree breeding scheme.

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CHAPTER 5

Combining ability and correlated response to selection for oil yield in sunflower (*Helianthus annuus*) under contrasting moisture environments

Abstract

Sunflower (*Helianthus annuus* L.) production in South Africa depends on natural rainfall which is variable and unpredictable, hence the need to develop drought tolerant sunflower genotypes to stabilise production under random stress environments. An exploratory research study was undertaken to determine the effectiveness of using moisture stress selection environments and three secondary traits, head diameter (HD), stem diameter (SD) and stay green canopy (SG) in developing drought tolerant sunflower cultivars with high oil yield (OY) in the presence of drought stress. A total of 84 testcross hybrids (TCHs) developed by crossing two testers to 42 female cytoplasmic male sterile (CMS) inbred lines were evaluated in nine environments for oil yield and other agronomic traits. The environments were classified as random drought stress environments (RSE), managed drought stress environments (MSE) and non-stress environments (NSE). Type A and type B genetic correlations based on broad sense heritability (H^2) and narrow sense heritability (h^2), respectively were estimated to quantify indirect selection efficiency. Type A genetic correlations indicated that SG had the potential to be used as a secondary trait to indirectly improve OY. The indirect selection efficiency (ISE) for OY via SG using genetic correlation based on H^2 were 0.79, 0.82 and 0.78 in the RSE, MSE and NSE, respectively and genetic correlation based on h^2 were 0.67, 0.98, and 0.93 in the RSE, MSE and NSE, respectively. In both cases, selection in the MSE had the highest ISE. Estimates of indirect selection based on type B genetic correlation indicated that indirect selection for OY in the MSE and NSE for the RSE was as effective as direct selection for OY in the RSE based on the additive genetic correlations of 0.96 obtained in both selection environments. Overall, the results from this exploratory study should inform the development of breeding strategies to improve the drought tolerance of sunflower cultivars grown in South Africa and associated environments.

Key words: Additive genetic correlations, broad sense heritability, drought tolerance, general combining ability (GCA), genotypic correlation, indirect selection efficiency (ISE), narrow sense heritability. Specific combining ability (SCA)

5.1 Introduction

Sunflower (*Helianthus annuus* L.) in South Africa is mainly cultivated under natural rainfall conditions without supplemental irrigation. This is sometimes referred to as natural or random stress environments as they are characterised by variable and limited soil moisture availability for plant growth leading to a reduction in seed and oil yield. Variable and limited soil moisture conditions in natural rainfall environments are subject to periods of drought and the effects of global climate change with predicted decreases in precipitation in some areas of the world will further exacerbate the situation. Drought ranks second after soil infertility as the major prevalent abiotic stress factor and the greatest source of uncertainty limiting crop productivity in the tropics (Edmeades et al., 1997). Breeding cultivars tolerant to drought rather than to escape drought stabilises yield in random stress environments (Edmeades et al., 1997) though the methodologies that facilitate good selection progress remain a challenge. According to Bänziger et al. (2004), good selection progress in developing drought tolerant cultivars can be achieved through: (i) precise evaluation and identification of genetic variation in traits conferring drought tolerance under conditions relevant to the target production environments; and (ii) use of highly discriminating, phenotyping environments relevant to the target environments.

Precise evaluation and identification of traits conferring drought tolerance under conditions relevant to the target environments requires knowledge of plant growth stages from germination to physiological maturity as drought tolerance is a complex trait affected by several interacting plant and environmental factors. Gardner (1995) and Vannozzi et al. (1999) related three general growth stages (GS) in sunflower to oil yield and stress tolerance screening methodologies. The first growth stage (GS1), from planting to floral initiation is the most delicate and sensitive to stress but usually, if good germination occurs, early stress affects vegetative growth stage with minimum impact on oil yield (Gardner, 1995). The second growth phase (GS2), a transitional phase from vegetative to reproductive growth including the pre-flowering and flowering stages determines the final seed yield of the crop (Gardner, 1995). Drought stress at this stage affects head size and number of seeds per head (Gardner, 1995; Škorić, 2009). The third and final growth stage (GS3) commences soon after completion of flowering and is mainly from the grain filling stage to physiological maturity. Drought stress at this stage

affects seed mass (Gardner, 1995) and final oil content of the seed (Škorić, 2009). In most plant breeding programmes, yield (grain, seed or oil yield) is the primary trait of interest and as such screening methodologies that target traits at early stress that promote survival are less likely to be useful than methodologies applied at late stress that target traits affecting productivity such as flowering and grain-filling (Edmeades, et al., 1997).

The use of discriminatory phenotyping environments to consistently evaluate large numbers of genotypes and identify those with traits relevant for drought tolerance is affected by a number of climatic factors such as the duration of rain-free period/s, evapotranspiration demand and temperatures during the growing season. Drought managed trials conducted during the relatively rain-free winter months have been largely used in southern Africa with the aim of developing drought tolerant maize genotypes (Bänziger et al., 2004); however, for sunflower there is a dearth of information on the use of managed stress environments to develop drought tolerant sunflower genotypes.

The effectiveness of the traits and environments used to select for improved drought tolerance in crops in the presence of genotype by environment interactions, particularly those which cause changes in rank order of the genotypes, is a function of the heritabilities of and genetic correlations between the chosen traits within the selection environments, and between the selection environments and the target environments (Rosielle and Hamblin, 1981; Atlin and Frey, 1989). Burdon (1977) proposed two types of genetic correlations: type A between two traits measured on each genotype within the same environment or meaned across environments; and type B between the same trait measured on each genotype in different environments or meaned across genotypes in different environments. Both types of genetic correlations are of practical value in breeding either for predicting the correlated response to indirect selection for the trait of interest (type A), or for predicting the performance of a genotype for the trait of interest in the target production environment when selection is carried out in a test environment (type B).

It is generally accepted that heritability for yield under stress environments is lower than under optimal environments (Edmeades et al., 1999) and that heritabilities of secondary traits are higher than for yield under drought stress in maize (Edmeades et al., 1999;

Bänzinger et al., 2000). However, recent studies in other crops indicate that there is no consistency in the magnitude of heritabilities in relation to the level of stress for yield and secondary traits (Atlin et al., 2004; Zhao et al., 2006; Songsri et al., 2008; Leiser et al., 2012). In cases where there is no consistency in the magnitude of heritability in relation to level of stress, the success of selection based on correlated response is a function of type B genetic correlations which determine the similarity in genotypic rankings between test environments and target production environments (Isik and Kleinshmit, 2005).

The expected mean performance of selected genotypes in target production environments is either a result of direct or indirect response to selection (Falconer and Mackay, 1996). Besides direct selection for yield, a number of traits have been recommended for use in selecting for drought tolerance in sunflower (Rauf and Sadaqat, 2007; Rauf, 2008; Škorić, 2009) but only a few have been evaluated and used in practical breeding programmes. The limited application of putative traits for drought tolerance in sunflower may be due to cost and time restrictions on the number of genotypes that can be sampled for selection purposes, and/or the relationships between the putative traits for drought tolerance and yield are difficult to quantify in the field. In crops such as maize, use of secondary traits for improving drought tolerance under severe stress significantly improved overall selection efficiency by more than 20% (Edmeades et al., 1997). The criteria for identifying useful secondary traits were summarised by Edmeades et al. (1997) as those that: (i) show genetic variability and correlation with yield under stress conditions and target production environments; (ii) have higher heritability than yield under stress conditions; and (iii) are cost effective and less time consuming to measure than grain yield eg. anthesis silking interval.

As not all growth phases of the plant are equally affected by moisture stress, in the absence of practical genetic and physiological data to comprehend drought tolerance in plants, yield *per se* and yield stability under moisture stress are often used as traits to quantify drought tolerance (Rauf, 2008). The genetic basis of yield stability can be examined in terms of the interaction between the test environments and general combining ability (GCA) and specific combining ability (SCA) as estimates of additive and non-additive gene action, respectively (Lee et al., 2003). The usefulness of secondary traits contributing to greater oil yield in sunflower and their combining ability

estimates have not been determined under variable moisture stress conditions and as such exploratory research to establish their value as selection criteria is essential.

The broad objective of this study was, therefore, to quantify the effectiveness of selection under managed drought stress and non-stressed conditions for the target environments under natural rainfall (random stress). The specific objectives were to: (i) quantify the importance of GCA and SCA in selecting for oil yield under variable soil moisture stress conditions; (ii) estimate the value of predetermined secondary traits in improving oil yield under drought; (iii) estimate genetic correlations between managed stress environments and random stress environments; and (iv) estimate response to selection for oil yield in random stress environments by selecting in managed stress and non-stress environments.

5.2 Methods and materials

5.2.1 Genetic material

A set of 50 cytoplasmic male sterile (CMS) lines or A-lines representing elite sunflower lines developed by the Agricultural Research Council (ARC), South Africa during the period 1995-2005 were crossed to two male fertility restorer lines *(Rf)* as testers, RP865 (T1) and RP953 (T2) using a line x tester design. The testcross hybrids (TCHs) were formed by crossing the two tester lines to CMS lines using time isolation during the 2006 winter season at Makhathini Research Station in KwaZulu-Natal Province, South Africa. Of the potential 100 TCHs, 84 TCHs (42 CMS lines x 2 testers) had enough seed for multi-location trials. The CMS lines were coded AP for A parent as; AP1-1 to AP1-42 and the TCHs as AP-1/T1 to AP1-42/T1 if crossed to T1, and AP1-1/T2 to AP1-42/T2 if crossed to T2.

5.2.2 Test environments and management of moisture stress

The 84 TCHs and six commercial checks were exposed to four moisture stress environments between November, 2006 and October, 2007 for the 2007 season's trials, and five moisture stress environments between November, 2007 and October, 2008 for the 2008 season's trials all at three locations: Bothaville, Makhathini and Potchefstroom.

Planting at Makhathini was done during the winter season, while planting at Bothaville and Potchefstroom was done during the summer season. Climatic data during the two cropping seasons for the three locations is provided in Table 5.1.

Table 5.1 Climatic data for the three locations used in the study

		2007 Season				2008 Season			
Location	Period	ET_0 (mm day^{-1})	T_{min} ^0C	T_{max} ^0C	Rainfall (mm)	ET_o (mm day^{-1})	T_{min} ^0C	T_{max} ^0C	Rainfall (mm)
Makhathini (KwaZulu-Natal Province, 27.24169S, 32.19668E, 75.2masl)	Planting season (May-October)	2.9	12.7	28.4	117.8	3.2	13.5	28.3	77.5
	Reproductive stage (July-Aug)	2.7	10.0	27.8	5.8	2.9	12.0	28.1	2.5
Bothaville (Free State Province, 27.235S, 26.67E, 1335masl)	Planting season (Nov-April)	5.7	12.4	29.7	398.3	5.4	12.2	28.3	446.2
	Reproductive stage (Jan-Feb)	6.4	14.0	32.1	60.2	5.4	14.3	29.9	94.0
Potchefstroom (Free State Province, 26.745S, 27.083E, 1347masl)	Planting season (Nov-April)	5.8	14.3	29.2	519.0	4.8	13.7	26.7	436.1
	Reproductive stage (Jan-Feb)	6.7	15.6	31.0	118.5	5.1	16.2	28.4	142.9

ET_0 = Reference evapo-transpiration (mm day^{-1})
T_{min} = Mean minimum temperature (^0C)
T_{max} = Mean maximum temperature (^0C)

The nine environments in this study were determined by the combinations of moisture stress level at each location and date of planting (season). Three moisture stress levels were used to classify the nine environments into: (i) random stress environments (RSE), consisting of four trials exposed to natural rainfall during the normal summer season for sunflower production (November/December through to May). The RSE in this study was considered as representative of the target production environment as most sunflower production in South Africa is conducted under natural rainfall conditions; (ii) managed stress environments (MSE), two trials grown during the rain-free winter season (May to October) were exposed to managed drought stress by withdrawing irrigation 3-4 weeks before flowering until physiological maturity; and (iii) well watered or non-stress environments (NSE), two trials grown in the winter season and one during the summer season received supplementary irrigation according to an irrigation schedule from planting to physiological maturity.

The MSE and NSE trials at Makhathini were planted adjacent to each other but separated by a 20 m wide strip of a commercial sunflower hybrid to minimize lateral

movement of water between the NSE and the MSE trials. Soil moisture status was monitored in the MSE and NSE trials during the 2008 season, but not in the 2007 season using the gravimetric method (Black, 1965) by sampling the soil at 25-30 cm depth at five random positions for each trial on a weekly basis (Figure 5.1).

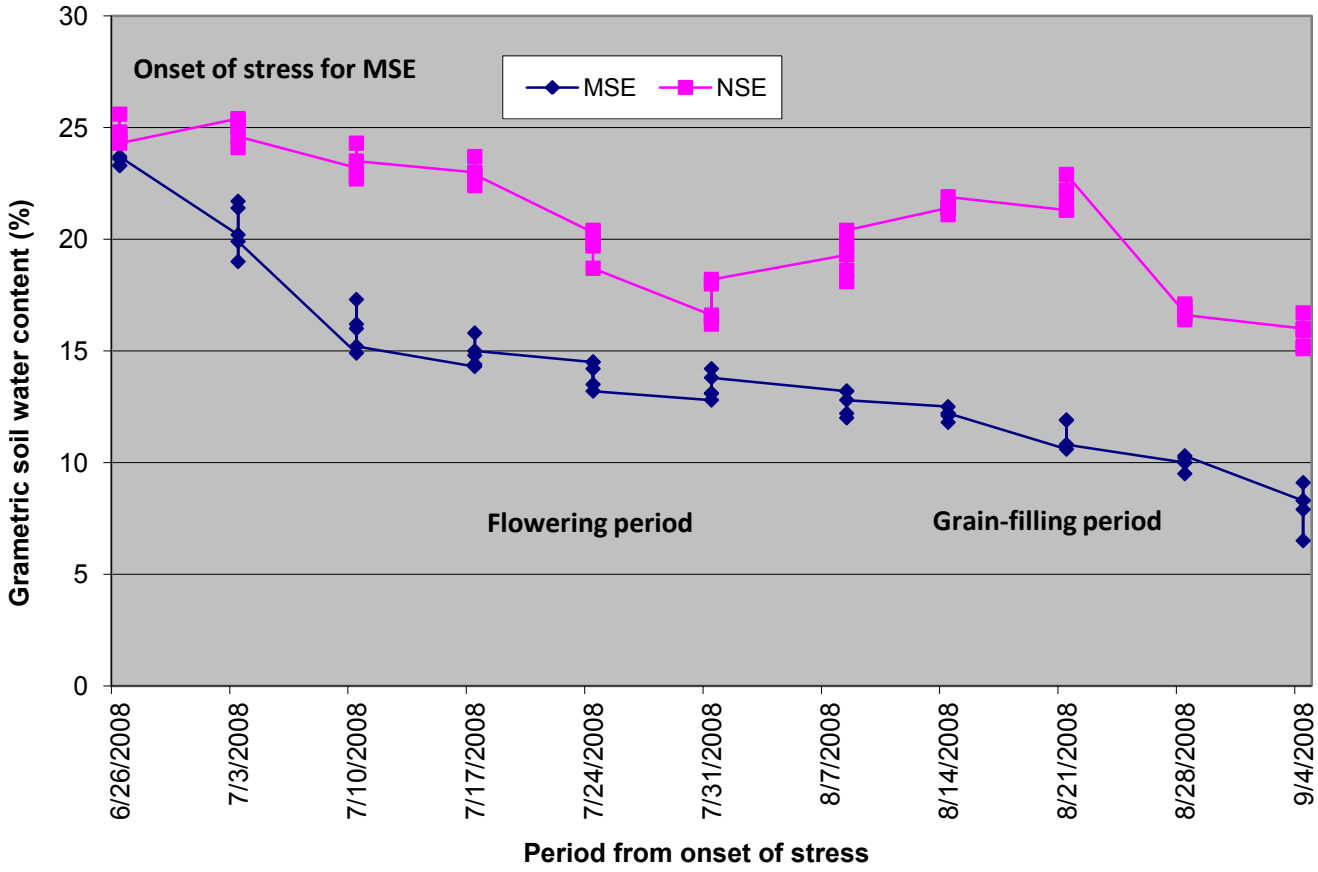

Figure 5.1 Gravimetric soil water content in the MSE and NSE trials across replications during the 2007/08 season at Makhathini Research Station

The RSE trials at Potchefstroom were irrigated once to field capacity soon after planting to enable uniform germination in both years, while that at Bothaville were only planted when enough moisture to enable good germination was available.

5.2.3 Experimental design

An alpha (0, 1) design with two replications was used for all nine trials and experimental units were two row plots, 5 m long and 0.9 m wide with an intra-row spacing of 0.33 m providing for a population density of 36 000 plants ha^{-1}. Plots were overplanted and then thinned at 4 weeks after emergence. Recommended agronomic practices were followed for both trials and basal fertiliser and topdressing were applied as per soil analysis recommendations for each trial. The trials were kept weed free by application of pre-emergence herbicide, Dual Gold, (*S-metolachlor*) soon after planting and by mechanical weeding during the thinning operations.

5.2.4 Traits recorded

The traits measured were: seed yield (kg ha^{-1}) (SY); percent oil content (OC); 1000-seed mass (g) (WT); number of seeds per head (SH); days to 50% flowering (FW); plant height (cm) at full grain fill (PH); mid-stem diameter (cm) at harvest (SD); head diameter (cm) at harvest (HD); and stay green canopy (SG) at full grain fill visually scored on a rating scale from 1-5; where; 1 = 0-20% green leaves, and 5 = 80-100% green leaves. Seed yield was first determined on a plot basis and adjusted to 10% seed moisture by weighing the seed which had been dried in continuous air flow dryers at 32-35˚C over a period of two weeks until no further loss in mass was recorded. Percent oil content was determined on 12 g samples of seed by nuclear magnetic resonance using a Newport Analyzer (Newport-Oxford Instruments Ltd, England). Oil yield (kg ha^{-1}) (OY) was obtained by multiplying seed yield by percent oil content.

5.2.5 Statistical analysis of data

5.2.5.1 Means, combining ability effects, and heritability estimates

Phenotypic data of the TCHs and checks were first analysed to obtain the best linear unbiased estimators (BLUES) for each genotype using the META suite of Vargas et al. (2013) in SAS 9.2 (SAS Institute, 2010) based on the statistical model:

$$Y_{ijkl} = \mu + G_i + E_j + R_k\,(E_j) + B_l\,(E_jR_k) + (GE)_{ij} + \varepsilon_{ijkl}$$

Where: Y is the phenotypic measurement of the trait of interest; μ is the mean; G_j is the effect of the i^{th} TCH or hybrid check; E_j is the effect of the j^{th} environment; R_k is the effect of the k^{th} replication; B_l is the effect of the l^{th} incomplete block; $(GE)_{ij}$ is the interaction terms for genotype and environment; and ε_{ijkl} is the random error term.

To compute the general and specific combining ability effects of the CMS lines, the hybrid check genotypes were excluded from the analysis and G_i in the above statistical model was partitioned into:

$$G_i = GCA_f + GCA_m + SCA_{fm}$$

Where: GCA_f is the general combining ability (GCA) effect of the f^{th} CMS female line (f=1,2..,42); GCA_m is the GCA effect of m^{th} male tester line (n=1,2); and SCA_{fm} the specific combining ability (SCA) of the TCH involving tester line m and female line f. Variance components estimates due to GCA of female lines (σ^2_{GCAf}), SCA of TCHs (σ^2_{SCA}) and their respective interactions with the environment (σ^2_{GCAfxE} and σ^2_{SCAxE}) were estimated across trials within each moisture stress level using the mixed model analyses PROC MIXED, Method=TYPE3 procedures in SAS (SAS Institute 2010). All effects in the combining ability model were declared random except μ, and GCA_m.

To calculate narrow sense heritability (h^2) for each stress level, the estimated variance components associated with GCA and SCA effects were used according to Grieder et al. (2012) as:

$$h^2 = \frac{\sigma^2_{GCAf}}{\sigma^2_{GCAf} + {\sigma^2_{SCA}}/{T} + {\sigma^2_{GCAf\ x\ E}}/{E} + {\sigma^2_{SCA\ xE}}/{ET} + \sigma^2_{\varepsilon}/ETR}$$

Where: E is the number of environments; T the number of testers; and R the number of replications.

Broad sense heritability (H^2) for each stress level was estimated as:

$$H^2 = \frac{\sigma^2_{GCAf} + \sigma^2_{SCA/T}}{\sigma^2_{GCAf} + {\sigma^2_{SCA}}/{T} + {\sigma^2_{GCAf\ x\ E}}/{E} + {\sigma^2_{SCA\ xE}}/{ET} + \sigma^2_{\varepsilon}/ETR}$$

Where the terms are as for the narrow sense heritability equation.

5.2.5.2 Type A and B genotypic and additive genetic correlations

Phenotypic correlations between traits ($r_{P(x,y)}$) were determined from TCH means across trials within a stress level or between stress levels (RSE, MSE and NSE). To distinguish between genetic correlations based on H^2 and h^2, genetic correlation based on H^2 is referred to as genotypic correlation and that based on h^2 is referred to as additive genetic correlation.

Type A genetic correlations were based on genetic variances across the trials within the same environments (type A). Type A genotypic correlation (r_G) between two traits *x* and *y* measured on the same genotype within or meaned across the environments were computed according to Holland (2006) as:

$$r_{G(x,y)} = \frac{Cov_{(x,y)}}{\sqrt{\sigma^2_{G(x)}\ \sigma^2_{G(y)}}}$$

Where: $r_{G(x,y)}$ is the Type A genotypic correlations between two traits *x* and *y*; $Cov_{(x,y)}$ is the genetic covariance between traits *x* and *y* meaned within or across environments; and $\sigma^2_{G(x)}$ and $\sigma^2_{G(y)}$ are the genetic variances of traits *x* and *y* meaned within or across environments.

The type A additive genetic correlation between two traits *x* and *y* was calculated based on the covariance model derived by Wu and Matheson (2006) as:

$$r_{A(x,y)} = \frac{Cov_{GCA(x,y)}}{\sqrt{\sigma^2_{GCA\ (x)}\ \sigma^2_{GCA(y)}}}$$

Where: $r_{A(x,y),}$ is the type A additive genetic correlation between the female line GCA effects of trait *x* and *y*; $Cov_{GCA(x,y)}$ is the covariance between the female line GCA effects for trait *x* and *y* meaned for each TCH within or across environments; and $\sigma^2_{GCA(x)}$ and $\sigma^2_{GCA(y)}$ are the variances of the female line GCA effects for trait *x* and *y* meaned for each TCH within or across environments.

Type B genotypic correlation ($r'_{G(x,x^*)}$) between the same trait *x and* x^* measured on the same genotype in different environments was calculated, assuming no environmental covariance between the TCHs means, according to Cooper et al. (1996) as:

$$r'_{G(x,x*)} = \frac{r'_{p(x,x*)}}{\sqrt{H_x^2 H_{x*}^2}}$$

Where: $r'_{G(x,x^*)}$ is the type B genotypic correlation coefficients between the same trait *x* and x^* measured in different environments; $r'_{P(x,x^*)}$ is the phenotypic correlation coefficient between TCH means for the same trait at the different environments pairs; and H^2_x and $H^2_{x^*}$ are the broad sense heritabilities of the same trait at the different environments.

Type B additive genetic correlation ($r'_{A\,(x,x^*)}$), between the same trait measured in different stress levels were calculated, assuming no environmental covariance between the GCA effects, according to Lu et al. (1998) as:

$$r'_{A(x,x*)} = \frac{r''_{P(GCAx,GCAx*)}}{\sqrt{h_x^2 h_{x*}^2}}$$

Where: $r'_{A(x,x^*)}$ is the type B additive genetic correlation coefficient between the same trait measured in different stress treatments; $r''_{P(GCAx,GCAx^*)}$ is the Pearson correlation coefficient between the GCA effects of same trait in different environments; h^2_x and $h^2_{x^*}$ are narrow sense heritabilities of the same trait in different stress treatments.

5.2.5.3 Indirect response to selection based type A genetic correlation

Indirect response to selection based on secondary traits is synonymous with indirect selection efficiency based on type A r_G and r_A. Genotypic indirect selection efficiency (ISE_G) and additive genetic indirect selection efficiency (ISE_A) for selection practiced on secondary traits to improve oil yield in the different stress levels, assuming the same selection intensity, was estimated according to Falconer and Mackay (1996):

$$ISE_G = r_G\sqrt{H_x^2/H_{OY}^2}\text{ , and }ISE_A = r_A\sqrt{h_x^2/h_{OY}^2}$$

Where: H^2_x and h^2_x are, respectively the broad and narrow sense heritability of the secondary trait, in this study: SD, HD and SG; H^2_{OY} and h^2_{OY} are the broad sense and

narrow sense heritabilities, respectively for oil yield (OY); and all other terms are as previously described.

$$ISE_G = r_G\sqrt{H_S^2/H_{RS}^2}$$

5.2.5.4 Indirect response to selection based on type B genetic correlations

Type B genotypic (*ISE'$_G$)* and additive genetic (*ISE'$_A$)* indirect selection efficiency in the MSE and NSE for the RSE were compared with direct selection in the RSE according to Falconer and Mackay (1996) as:

$$ISE'_G = r'_G\sqrt{H_x^2/H_{x(RSE)}^2} \text{ and } \quad ise'_A = r'_A\sqrt{h_x^2/h_{x(RSE)}^2}$$

Where: H^2_x and h^2_x are the broad and narrow sense heritabilities, respectively of the trait of interest in MSE or NSE; $H^2_{x\ (RSE)}$ and $h^2_{x\ (RSE)}$ are the broad sense and narrow sense heritabilities of the same trait under the RSE target environments, respectively; and all other terms are as previously defined. For indirect selection to be more efficient than direct selection, the square root of heritability in the test environments should be greater than the square root of heritability in the target environments (Falconer and Mackay, 1996).

5.3 Results and discussion

5.3.1 Soil moisture monitoring

Clear differences in the soil moisture content during the reproductive stage were observed at Makhathini in the MSE and NSE trials during the 2007/08 season. Although there was a decrease in gravimetric water content in the NSE trials during peak flowering, there were no noticeable stress symptoms as irrigation was done on a weekly basis. Only 77.5 mm was received during the growing season and of this 2.5mm was received during the flowering and grain filling stages (Table 5.1 and Figure 5.1).

5.3.2 Combined analysis of trials within and across environments

5.3.2.1 Means and ranges of genotypes for oil yield

The lowest trial mean for oil-yield of 417.1 kg ha^{-1} was obtained under MSE at Makhathini Research Station during the 2008 season, while the highest mean oil yield of 1045.5 kg ha^{-1} was also obtained at Makhathini in the NSE during the 2007 season (Table 5.2). The mean oil yield across the four trials in the RSE ranged from 516 to 823.1 kg ha^{-1} and there were no significant differences between the best TCH and the best commercial check hybrid. When trials in RSE are considered individually, the best TCHs out-yielded the best commercial checks indicating that trials in the RSE are better stratified according to the mean yield levels (Windhausen et al., 2012).

Table 5.2 Mean and range for oil yield recorded across the nine environments grouped into three moisture stress environments

				Oil yield (kg ha^{-1})			
Stress environment	**Location**	**Trial name**	**Planting month**	**Mean**	**Range (TCHs)**	**Range (Checks)**	**$LSD_{0.05}$**
RSE	Potchefstroom	PT07A-RS	2006-11-28	498.5	316.1-672.5*	395.6-536.0	85.4
	Potchefstroom	PT08A-RS	2007-11-19	552.6	123.1-1170.5*	217.4-796.9	284.7
	Bothaville	BTV07A-RS	2006-12-05	518.2	245.0-914.3*	391.2-657.3	80.3
	Bothaville	BTV08A-RS	2007-12-18	494.9	130.3-963.6*	266.4-567.1	216.4
		Across		516.0	278.4-823.1	322.7-648.2	206.4
MSE	Makhathini	MAK07B-MS	2007-05-07	482.4	214.6-740.3*	303.1-576.0	135.3
	Makhathini	MAK08B-MS	2008-05-12	417.1	115.9-868.0*	213.7-562.6	168.4
		Across		449.7	197.2-796.0*	263.1-565.5	209.9
NSE	Makhathini	MAK07B-NS	2007-05-07	1045.5	405.6-1491.3	938.1-1363.0	232.8
	Makhathini	PT08A-NS	2007-11-19	801.3	213.6-1378.2	562.5-1123.3	258.0
	Potchefstroom	MAK08B-NS	2008-05-12	922.1	425.7-1461.7*	718.9-1087.5	159.5
		Across		923.0	530.1-1325.0	804.5-1190.1	252.7

RSE = random stress environments; MSE = managed drought stress environments; NSE = non-stress environments; Mean = mean of all testcross hybrids plus hybrid checks; TCHs = Testcross hybrids; $LSD_{0.05}$ = least significant differences at 0.05 probability; *Best TCH significant different from best check hybrid ($p<0.05$)

Mean oil-yield for the TCHs across trials in the MSE ranged from 197.2-796.0 kg ha^{-1} and the mean of the commercial check hybrids ranged from 263.1-565.7 kg ha^{-1}. There were TCHs that performed significantly ($p<0.05$) better than the commercial check hybrids in the MSE, eg. the yield difference between the best TCH (796.0 kg ha^{-1}) and the best commercial check (565.5 kg ha^{-1}) under MSE was 230.5 kg ha^{-1}. The overall mean for the three NSE trials was 923.0 kg ha^{-1}; the TCHs ranged from 530.1-1325.0 kg ha^{-1} and the commercial hybrid checks ranged from 804.5-1190.1 kg ha^{-1}. The

only significant difference between the best TCH and the best commercial hybrid was obtained in one NSE trial, namely MAK08B-NS.

5.3.3 General and specific combining ability effects of female lines for oil yield

The meaned GCA and SCA effects for oil yield of trials within the three stress environments were highly correlated with the mean oil yield of the TCHs in the respective stress environment with the exception of the female line SCA effects with T2 in the RSE (Figure 5.2). The Pearson squared correlation coefficient (R^2) of GCA effects with oil yield was always higher than that for SCA effects in all three stress environments indicating that prediction of hybrid performance based on GCA effects in this sunflower population would be effective (Reif et al., 2013). The top 20 TCHs for oil yield in the RSE, which is the target production environment, were obtained from 14 female parents crossed to the two testers of which six (AP1-1, AP1-3, AP1-7, AP1-10, AP1-15 and AP1-22) were crossed to both testers (Table 5.3).

For continued improvement of oil yield under drought stress, female lines with high and significant GCA effects are likely to contribute to further genetic progress if used as parents in advanced cycle pedigree breeding or recurrent selection for drought tolerance (Hallauer and Miranda, 1988). In the NSE both GCA and SCA effects made significant contributions to oil yield in the TCHs (Figure 5.2), and more specifically a high number of crosses with T1 had significant SCA effects and ranked among the best 20 TCHs in the NSE (Table 5.3).

The frequency of the top 20 TCHs in the RSE occurring in the other test environments, MSE and NSE, is an important indicator of how effective the test environments are in identifying genotypes with both high stability and performance (Isik and Kleinschmit, 2005). Of the best 20 TCHs selected in the RSE, 12 TCHs (60%) would have been selected in the MSE and 10 TCHs (50%) in the NSE. If selection was done under MSE only nine TCHs (45%) would have been selected in the NSE (Table 5.3)

Table 5.3 Means, GCA and SCA effects for oil yield (kg ha^{-1}) of the top 20 TCHs in RSE, MSE and NSE ranked in descending order under the target environment, RSE.

TCH[1]	Mean (kg ha^{-1}) RSE	MSE	NSE	GCA effects (kg ha^{-1}) RSE	MSE	NSE	SCA effects (kg ha^{-1}) RSE	MSE	NSE	Rank[2]
AP1-2 /T2	823.1	575.4	747.7	207.8*	183.9*	52.1	67.9	-85.6*	-248.8	1 (17, 63)
AP1-15 /T2	780.2	609.5	1176.9	226.3*	174.6*	236.8*	6.5	-42.2	-4.3	2 (12, 15)
AP1-10 /T2	753.8	653.4	1265.8	180.7*	151.5*	363.4*	25.7	24.8	-41.9	3 (6, 5)
AP1-4 /T2	724.9	389.2	1122.4	51.6	-65.7	74.5	125.9*	-22.1	103.5*	4 (55, 20)
AP1-1 /T2	721.7	585.4	996.8	165.4*	125.3*	110.2	8.8	-17.0	-57.8	5 (16, 33)
AP1-15 /T1	702.7	634.9	1150.9	226.3*	174.6*	236.8*	-6.5	42.2	4.3	6 (8, 17)
AP1-22 /T2	696.3	606.6	1291.2	152.8*	136.7*	340.5*	-4.0	-7.2	6.3	7 (13, 2)
AP1-11 /T1	689.3	618.4	1275.2	117.1	114.5*	312.98*	89.3*	85.8*	52.6	8 (9, 3)
AP1-40 /T2	689.0	540.2	1138.5	136.2	67.7	105.6	5.5	-4.6	88.5	9 (22, 19)
AP1-6 /T2	677.8	515.0	947.4	111.4	-35.0	-77.2	19.0	72.9*	80.3	10 (25, 38)
AP1-25 /T2	667.7	459.9	1178.0	79.5	-28.5	195.9*	40.8	11.3	37.8	11 (37, 14)
AP1-3 /T1	662.3	515.5	1001.7	136.6	18.9	50.5	42.9	78.5*	41.5	12 (24, 32)
AP1-24 /T2	657.5	503.6	907.4	-18.6	-93.6	-84.7	128.7*	120.2*	47.7	13 (29, 45)
AP1-7 /T1	649.4	702.0	1092.1	127.4	190.8*	107.7	39.2	93.1*	74.7	14 (3, 23)
AP1-3 /T2	641.1	417.5	953.4	136.6	18.9	50.5	-42.9	-78.5*	-41.5	15 (47, 37)
AP1-22 /T1	639.6	562.1	1244.0	152.8*	136.7*	340.5*	4.0	7.2	-6.3	16 (18, 6)
AP1-1 /T1	639.4	560.4	1077.7	165.4*	125.3*	110.2	-8.8	17.0	57.8	17 (19, 26)
AP1-10 /T1	637.8	544.9	1315.0	180.7*	151.5*	363.4*	-25.7	-24.8	41.9	18 (21, 1)
AP1-7 /T2	635.7	590.7	977.3	127.4	190.8*	107.7	-39.2	-77.2	-74.7	19 (14, 36)
AP1-5 /T1	628.6	646.4	1035.7	97.5	206.2*	132.2	48.3	22.1	-6.3	20 (7, 31)
AP1-2 /T1	622.7	687.7	1210.6	207.8	183.9*	52.1	-67.9	85.6*	248.8*	21 (4, 9)
AP1-34 /T1	611.8	388.9	1192.3	77.2	122.1*	137.2	51.8	-151.4*	145.4*	24 (56, 13)
AP1-9 /T2	603.2	802.8	987.2	5.2	149.9*	-10.9	50.5	175.9*	53.7	25 (1, 34)
AP1-5 /T2	596.5	661.1	1082.9	97.5	206.2*	132.2	-48.3	-22.1	6.3	26 (5, 25)
AP1-26 /T2	596.5	490.9	1271.7	67.7	21.1	253.4*	-18.6	-7.3	74.0	27 (32, 4)
AP1-28 /T2	584.6	612.9	946.8	-12.1	-20.3	-47.9	49.3	156.1*	50.3	28 (10, 39)
AP1-17 /T2	582.4	554.6	1094.8	29.1	84.3	-5.8	5.9	-6.8	156.3*	29 (20, 22)
AP1-11 /T2	575.2	505.8	1204.6	117.1	114.5*	312.9*	-89.3	-85.8*	-52.6	31 (28, 11)
AP1-34 /T2	572.8	750.6	936.2	77.2	122.1*	137.2	-51.8	151.4*	-145.4*	33 (2, 41)
AP1-19 /T1	523.4	365.7	1221.4	-22.1	-95.8	32.7	62.7	43.4	278.9*	36 (61, 7)
AP1-32 /T2	501.0	585.9	1219.4	-40.4	47.7	-20.4	-6.0	61.1	295.4*	41 (15, 8)
AP1-37 /T1	437.9	234.5	1156.0	-81.4	-117.6*	-22.9	36.5	-66.1	269.1*	54 (82, 16)
AP1-42 /T1	409.6	404.5	1210.5	-117.5	-64.3	18.4	44.2	50.7	282.4*	66 (50, 10)
AP1-12 /T1	388.0	612.6	1195.9	-69.6	79.9	244.5*	-25.2	114.5*	41.6	72 (11, 12)
Mean	515.1	447.6	927.0							
S.E.				78.6	56.3	98.6	41.5	37.1	54.1	
Freq. of occ. (RSE)[3]				20/20	12/20	10/20				
Freq. of occ. (MSE)[3]					20/20	9/20				

[1]TCHs, Testcross hybrids, the letters and digits before (/) are the female parent code
[2]Rank, rank order based on descending oil yield in the random stress (RSE). Numbers in brackets are the ranks in the MSE and NSE, respectively
[3]Frequency of occurrence of the top 20 TCHs being among the top 20 TCHs in each of the other stress environments
*Significant GCA or SCA effects at $p<0.05$

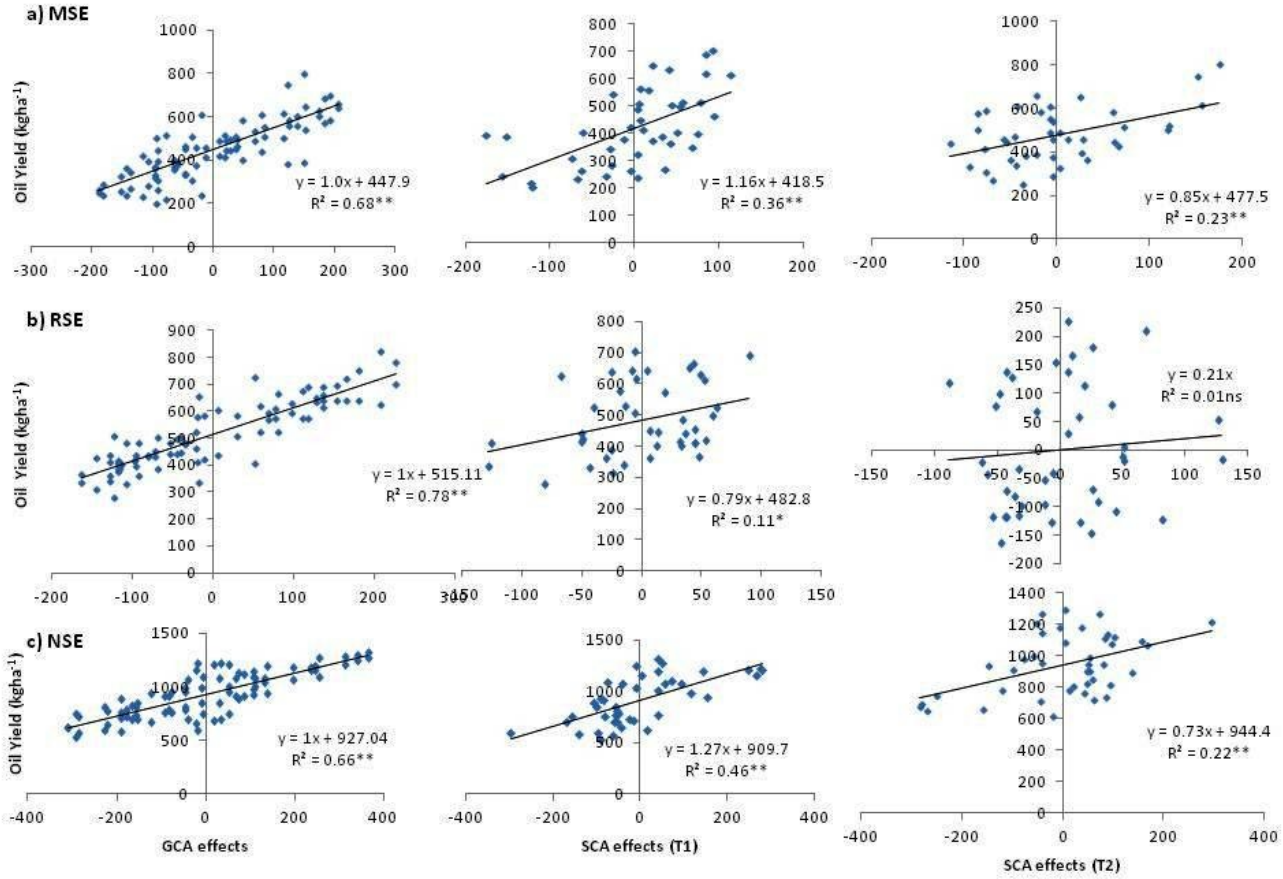

Figure 5.2 Linear relationships between oil yield (kg ha^{-1}) with the GCA and SCA effects in the (a) managed drought stress environments (MSE) (b) random stress environments (RSE) and (c) non-stress environments (NSE), respectively

5.3.4 Heritability within and across the three different stress environments

Broad sense heritabilities (H^2) and narrow sense heritabilities (h^2) were calculated for all the traits in each of the three stress environments and across all three stress environments (Figure 5.3).

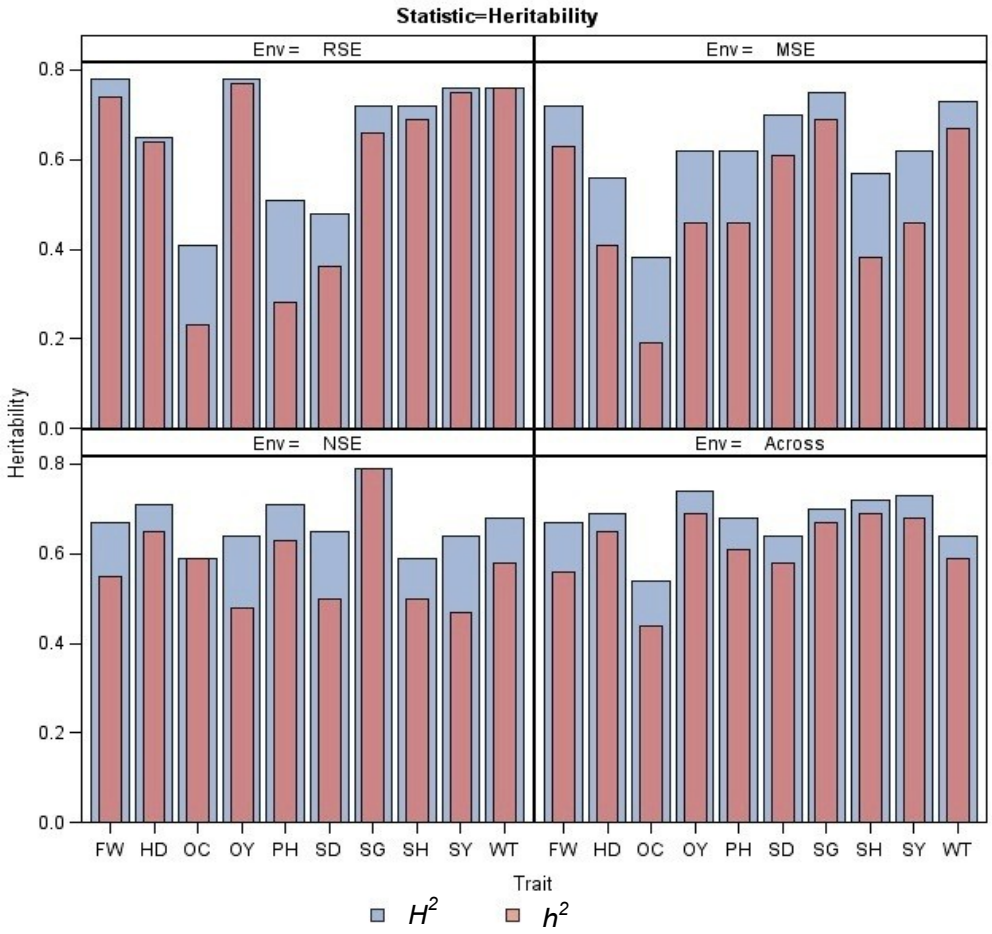

Figure 5.3 Broad sense (H^2) and narrow sense (h^2) heritability of the traits measured in three different moisture stress environments: RSE=random stress environments; MSE = managed drought stress environments; NSE = nonstress environments; and Across = mean across the three levels of environments. FW = days to 50% flowering; HD = head diameter (cm); OC = percent oil content; OY = oil yield (kg ha^{-1}); PH = plant height (cm); SD = stem diameter (cm); SG = stay green canopy (1-5 scale; 1 = 0 - 20% green leaves, 5 = 80 - 100% green leaves); SH = number of seeds per head; SY = seed yield (kg ha^{-1}); WT = 1000-seed mass (g).

For all the traits measured, both H^2 and h^2 in the NSE and across the three stress environments were moderate to high. The h^2 ranged from 0.45 for OC to 0.78 for SG, while H^2 ranged from 0.50 for oil content to 0.78 for SG in the NSE (Figure 5.3). There were greater fluctuations in the heritability estimates among traits in both the RSE and MSE compared to the NSE and across

stress environments (Figure 5.3). In the RSE, h^2 for OC, PH and SD was low at less than 0.40 but high for the other traits at greater than 0.60, while H^2 was moderate to high ranging from 0.4 for OC to 0.78 for OY. In the MSE, h^2 for OY was very low at 0.19 and H^2 was moderate to low at 0.38. For the other traits h^2 in the MSE ranged from 0.40 for SH to 0.69 for SG and H^2 ranged from 0.56 for HD to 0.75 for SG (Figure 6.3). As most of the traits in this study had moderate to high heritability estimates except OC in all three stress environments, the magnitude of the estimates indicate that breeding progress in stress conditions is likely to be as effective as when conducting selection under favourable conditions (Atlin et al., 2004). Broad sense heritabilities for seed yield and oil yield obtained in this study are of similar magnitudes to those obtained by Alza and Fernandez-Martinez (1997) and Reif et al. (2013) but substantially lower for oil content. The low heritability for oil content is possibly due to the fact that, over the past decade, sunflower breeding in South Africa appeared to have focused more on seed yield than on oil content. A low selection pressure being applied for oil content in all likelihood lead to a general slowdown in genetic gain for the trait (Chigeza et al., 2012). Another factor which may have contributed to low heritability of oil content in the RSE and MSE is that drought during the flowering and grain filling stages causes a substantial decrease in seed oil content. Škorić (2009) recorded a 7-8% decrease and therefore less variability among the genotypes.

5.3.5 Genetic correlations and selection efficiency

5.3.5.1 Type A genotypic and additive genetic correlations and selection efficiency

Three traits, namely head diameter (HD), stem diameter (SD) and stay green canopy (SG) were used as secondary traits for selecting for oil yield. These traits were chosen on the basis that they are fast, cheap and easy to measure in a non-destructive fashion, and measurements can be taken before harvesting which may effectively allow assessment of two generations per year. The other traits SY, OC, SH, WT except FW and PH were considered direct components of oil yield (Gardner, 1995). According to Škorić (2009), selection for stay green canopy is the most practical, rapid and reliable approach to improving drought tolerance in sunflower though care should be taken in the choice of breeding material. Genotypes with a high level of self-fertility should be used when breeding for stay green canopy (Škorić et al. 2007). The stay green canopy also increases tolerance to stem canker (*Phomopsis helianthi)* and charcoal rot *(Macrophomina phaseolina)* (Škorić, 2009). The phenotypic (r_P), genotypic (r_G) and additive genetic (r_A) correlations of the three selected secondary traits with oil yield (Table 5.4), were all positive and significantly ($P<0.05$) different from zero in all the three stress environments.

Table 5.4 Type A genotypic and additive genetic correlations as well as phenotypic correlations between oil yield and secondary traits along with estimates of indirect selection efficiency in random stress, managed stress and non-stress environments

Environment	Trait	Heritability		Correlation with oil yield			Indirect selection efficiency (ISE)[1]	
		H^2	h^2	r_P	r_G	r_A	ISE_G	ISE_A
RSE	OY	0.78	0.77	-	-	-	-	-
	HD	0.65	0.64	0.34	0.65	0.77	0.59	0.70
	SD	0.48	0.36	0.44	0.62	0.83	0.49	0.57
	SG	0.72	0.66	0.44	0.82	0.72	0.79	0.67
MSE	OY	0.62	0.46	-	-	-	-	-
	HD	0.56	0.41	0.50	0.79	0.65	0.75	0.62
	SD	0.70	0.61	0.47	0.88	0.61	0.94	0.70
	SG	0.75	0.69	0.57	0.75	0.80	0.82	0.98
NSE	OY	0.64	0.48	-	-	-	-	-
	HD	0.71	0.65	0.47	0.66	0.66	0.69	0.77
	SD	0.65	0.50	0.48	0.74	0.73	0.74	0.75
	SG	0.79	0.79	0.36	0.71	0.73	0.78	0.93

[1]Indirect selection for oil yield based on the secondary traits

RSE=random stress environments; MSE = managed drought stress environments; NSE = non-stress environments; OY = oil yield (kg ha^{-1}), HD = head diameter (cm); SD = stem diameter (cm); SG = stay green canopy on scale of 1 to 5; 1 = 0-20% green leaves, 5 = 80-100% green leaves; H^2 = broad sense heritability; h^2 = narrow sense heritability; r_P = phenotypic correlation based on means of the testcrosses; r_G = genotypic correlation; r_A = additive genetic correlation; ISE_G = indirect selection efficiency based on genotypic correlation; ISE_A = indirect selection efficiency based on additive genetic variance.

The phenotypic correlations, r_P, of the secondary traits HD, SD and SG with oil yield in the RSE ranged from 0.34 for HD to 0.44 for SG. In the MSE, r_P ranged from 0.47 for SD to 0.57 for SG and in the NSE, the range was from 0.36 for SG to 0.48 for SD. Overall, r_P for secondary traits with OY were moderate in magnitude and could be used for indirect selection for oil yield with some modest expected gains in genetic improvement. Razi and Assad (1999) obtained correlations of similar magnitude for SD but substantially lower for HD. Syed et al. (2004) found no significant correlation between OY and HD, while Hladni et al. (2003) found a moderate correlation between HD and seed yield of 0.62.

The genotypic correlation, r_G, in the RSE between the secondary traits and OY ranged from 0.62 for SD to 0.82 for SG. In the MSE, r_G between the secondary traits and OY ranged from 0.75 for SG to 0.88 for SD. In the NSE, the range was from 0.66 for HD to 0.74 for SD. The r_G

estimates were marginally higher in the MSE than in the RSE and NSE for both traits except SG in the RSE indicating that more gains will be achieved in drought managed trials than trials carried out under random stress or optimal conditions. The values obtained in this study were higher than those obtained by Razi and Assad (1999) for SD but of similar magnitude for HD. The additive genetic correlation, r_A, in the RSE ranged from 0.72 for SG to 0.83 for SD, while in the MSE the range was from 0.61 for SD to 0.80 for SG. In the NSE, r_A ranged from 0.66 for HD to 0.73 for SG. High r_G and r_A between SG and OY suggests that TCHs that have a high stay green canopy are the same TCHs that, on average across the environments, tend to have high OY both in MSE, RSE and NSE. In maize, Duvick (2005) reported a gradually significant increase in stay-green canopy during the past six decades of breeding under favourable conditions.

Overall, r_A was higher in the RSE than in the MSE and NSE indicating that selection using breeding values can be effectively carried out under natural rainfall stress environments. The greatest challenge in using RSE for selection is low heritabilities due to uneven plant stand (Bänzinger et al., 2004). In this study good and even plant establishment was achieved by either irrigating to field capacity before planting (Potchefstroom) or by planting in soils with adequate residual moisture for germination (Bothaville). Atlin et al. (2004) demonstrated that direct selection for yield under stress was effective if within-trial field heterogeneity is controlled.

Given that the baseline relative efficiency for direct selection for the trait of interest, oil yield in this case, is considered to be 1.0 (Mackay and Falconer, 1996), using secondary traits to select for oil yield in the RSE, MSE or NSE was considered as effective as direct selection when the ISE values were close to 1.0. For some secondary traits, such as SG, the efficiency based on ISE_A was close to 1.0 in the MSE and NSE (Table 5.4), indicating that using SG as a secondary trait for selecting oil yield was as effective as direct selection for oil yield under MSE and NSE conditions. The indirect selection efficiency of HD for OY based on ISE_G ranged from 0.59 in the RSE to 0.75 in the MSE while based on ISE_A it ranged from 0.62 under MSE to 0.77 in the NSE. For SD, the ISE_G ranged from 0.49 in the RSE to 0.75 in the MSE, and the ISE_A ranged from 0.57 in the RSE to 0.75 in the NSE. In both cases, indirect selection for oil yield using secondary traits was least in the RSE conditions except for SG (Table 5.4).

These results have practical implications in developing and modifying breeding strategies for yield testing as indirect selection using secondary traits is not necessarily practiced because it's

more efficient than direct selection for yield but because of limited resources including time (Bernardo, 2002). Scoring SG in sunflower is faster and less resource demanding than the primary trait, OY; hence for a given budget, early screening could depend on traits that are faster and cheaper to measure. In maize, use of secondary traits such as anthesis silking interval increased the selection efficiency in developing drought tolerant maize genotypes (Edmeades et al., 1997). However, Gaillais (1984) noted that there are only a few instances where indirect selection using one secondary trait clearly demonstrated superiority over direct selection, thus some form of a selection index for multiple secondary traits should be used when developing breeding strategies to develop drought tolerant sunflower cultivars.

5.3.5.2 Type B genotypic and additive genetic correlations

All correlations reported here were significantly ($p<0.05$) different from zero. Phenotypic correlations (r'_P) based on TCHs means between MSE and RSE, and between NSE and RSE were moderate for oil yield at 0.67 and 0.59, respectively (Table 5.5). The other trait which had a moderate r'_P of 0.60 was HD between NSE and RSE. For the other traits, r'_P, was low ranging between 0.26 for HD to 0.35 for SG between MSE and RSE and 0.25 for SD to 0.35 for SG between NSE and RSE (Table 5.5). The Pearson's correlation coefficient r''_P of female GCA effects was higher than r'_P for all traits (Table 5.5). The r''_P for oil yield between RSE and the other two environments, MSE and NSE were moderately high at 0.73 for both environments. The r''_P, between MSE and RSE and between NSE and RSE for SD and SG were low but that for HD was moderate at 0.66 between NSE and RSE (Table 5.5). The low to moderate r'_P between the selection environments and target environments indicates that re-ranking of genotypes will occur from one environment to another and the magnitude of re-ranking increases as the r'_P decreases. Similarly, low to moderate r''_P coefficients obtained for the other traits in this study except for OY (0.73) is an indication that rank changes of female CMS lines based on GCA effects for the other traits will be moderate to high, but for OY, rank changes will be moderate to low. These results supports earlier findings based on ranking of the best TCHs according to OY in the RSE (Table 5.2), in which 60% and 50% of the genotypes in the top 20 in RSE were also in the top 20 in MSE and NSE, respectively.

Table 5.5 Type B Genotypic and additive genetic correlations for oil yield along with selection efficiency in managed drought stress and non-stress in comparison with direct selection in random stress conditions

				Correlation with RSE				Indirect selection efficiency	
Environment	**Trait**	H^2	h^2	r'_P	r'_G	r''_P	r'_A	ISE'_G	ISE'_A
MSE	OY	0.62	0.46	0.67	0.97	0.73	1.23	0.86	0.96
	HD	0.56	0.41	0.26	0.44	0.44	0.86	0.40	0.69
	SD	0.70	0.61	0.33	0.57	0.35	0.74	0.69	0.97
	SG	0.75	0.69	0.35	0.48	0.39	0.58	0.49	0.59
NSE	OY	0.64	0.48	0.59	0.83	0.73	1.21	0.75	0.96
	HD	0.71	0.65	0.60	0.89	0.66	1.03	0.92	1.04
	SD	0.65	0.50	0.25	0.45	0.31	0.72	0.53	0.85
	SG	0.79	0.79	0.35	0.47	0.42	0.58	0.49	0.63
RSE	OY	0.78	0.77	-	-	-	-	-	-
	HD	0.65	0.64	-	-	-	-	-	-
	SD	0.48	0.36	-	-	-	-	-	-
	SG	0.72	0.66	-	-	-	-	-	-

[1]*ISE*-Indirect selection for oil yield based on type B genotypic (ISE'_G) and additive genetic correlations (ISE'_A); RSE = random stress conditions; MSE = managed drought stress conditions; NSE = non-stress/well watered conditions; OY = oil yield (kg ha^{-1}); HD = head diameter (cm); SD = stem diameter (cm); SG = stay green canopy on scale of 1 to 5, 1 = 0 - 20% green leaves, 5 = 80 - 100% green leaves; H^2 = broad sense heritability; h^2 = narrow sense heritability; r'_P = phenotypic correlation based on means of the testcrosses; r''_P = Pearson correlation of GCA effects; r'_G = genotypic correlation; r'_A = additive genetic correlation; ISE'_G = indirect selection efficiency based on genotypic correlation; ISE'_A = indirect selection efficiency based on additive genetic variance.

High r'_G for OY between the MSE and RSE of 0.97, and between NSE and RSE of 0.83 were obtained. Additive genetic correlations r'_A, for OY between the MSE and RSE, and between NSE and RSE were not constrained to 1.0 to avoid bias (Weber et al., 2013). The r'_A for oil yield was 1.23 between the MSE and RSE, and 1.21 between the NSE and RSE (Table 5.5). For the secondary traits, r'_A was moderate to high and in all cases greater than the r'_G (Table 5.5). A high r'_A is an indication that OY in the paired environments MSE and RSE, and NSE and RSE was under the control of the same set of additive genes (Lorenzana and Bernardo, 2008), hence cultivars developed for drought tolerance should also produce high oil yields in the presence or absence of drought stress.

The efficiency of selecting in the MSE and NSE for the RSE was also found to be trait dependent (Table 5.5); for OY, ISE'_G was higher in MSE at 0.86 than in the NSE at 0.75, while there was no difference for ISE'_A as both selection environments had a similar efficiency of 0.96.

These results show that the predictive power of ISE'_G for OY compared with direct selection in the RSE improved with increased stress levels from 0.75 for NSE to 0.86 for MSE, (Table 5.5). The predictive power of ISE'_A in this study was not affected by stress level as both the MSE and NSE had similar coefficients of 0.96 indicating that selection under either stress level can be effective for generating gains in the RSE. A similar magnitude of indirect selection efficiency based on r'_G was reported by Weber et al. (2012) in maize who obtained an indirect selection efficiency of 0.70 for genotypes adapted to random stress when selected under managed drought stress for grain yield.

Efficiency of indirect selection in the MSE or NSE in comparison with direct selection in the RSE based on ISE'_A was consistently high for all the traits compared to selection based on ISE'_G (Table 6.5). This finding should have major utility in developing drought tolerant co-hybrids/joint hybrids from elite female CMS lines exchanged between different breeding programmes in the same country or different countries without compromising the intellectual proprietary rights of the participating organisations, since the CMS lines cannot be reproduced without the maintainer B-lines. Drought tolerant CMS lines from one breeding programme could be crossed to tester lines from another breeding programme thereby creating joint TCHs which can then be evaluated in the stress managed environments to validate their performances. In maize, Kebede et al. (2013) concluded that genotypic correlations between performance in Mexico and eastern and southern Africa was high both for the line *per se* evaluations and testcrosses performance hence trial results and elite lines developed in either region could have a direct and immediate use in the other region.

5.4 Conclusion

It was evident from this exploratory study that oil yield decreased with moisture stress level in both the commercial hybrid checks and TCHs. As was expected, the reduction in oil yield was highest in MSE, followed by RSE. The best TCHs out-yielded the best commercial hybrids in the MSE and RSE indicating better performance of some TCHs under moisture stress conditions. In two of the three NSE trials there were no significant differences in performance between the best TCHs and commercial hybrid checks whereas differences were detected under MSE and RSE conditions indicating that significant differences in tolerance to the imposed moisture stress levels exist between the TCHs and the commercial hybrid checks.

Both broad and narrow-sense heritabilities for all traits in the MSE, RSE and NSE were moderate to high indicating that breeding progress for developing drought tolerant genotypes could be achieved with the current set of genotypes though further research with different genotypes and mating designs needs to be done to verify these early results. The SG trait had the highest estimate of r_G and r_A indicating its suitability as a secondary trait to use in breeding for oil yield in sunflower. In addition to high r_G and r_A, scoring of SG is less time consuming and according to Škorić (2009) the stay green characteristic also increases tolerance to stem canker and charcoal rot.

For type B genetic correlations, indirect selection using r'_A in the MSE was equally effective as direct selection under RSE indicating that additive genetic correlation using GCA effects is effective for estimating correlated response to selection especially if the breeding populations are from factorial mating designs. Thus results from this exploratory study on developing drought tolerant sunflower cultivars using type A and type B genotypic and additive genetic correlations provide an initial basis from which to develop more advanced breeding strategies for drought tolerance in sunflower in South Africa.

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CHAPTER 6

General discussion and overview of results

6.1 Introduction

Adequate knowledge on phenotypic variance, heritability, combining ability, correlated response to selection and reliability of selection environments in a breeding program are cornerstones for future genetic gains in the target traits. This study focused on four major objectives:

(i) Quantifying genetic gain for seed yield and associated traits of sunflower populations in South Africa;
(ii) Estimating genetic variability, expected genetic advance and heterosis using recycled inbred lines from advanced cycle pedigree breeding (Lu and Bernardo, 2001);
(iii) Quantifying the importance of GCA and SCA of CMS inbred lines in advanced cycle pedigree breeding; and
(iv) Estimating indirect versus direct selection efficiency using secondary traits and test selection environments based on type A and type B genetic correlations.

6.2 Seed yield and associated trait improvements in South African sunflower cultivars

Empirical evidence from this study clearly demonstrates that the South African sunflower breeding programmes score more successes than failures. The results showed that:

(i) The absolute genetic gain for seed yield in side-by-side trials ranged from 18 to 32 kg ha^{-1} $year^{-1}$ with a mean of 24 kg ha^{-1} $year^{-1}$ resulting in a relative genetic gain of 1.5% $year^{-1}$.
(ii) The absolute seed yield increase under commercial production was 12 kg ha^{-1} $year^{-1}$, translating to a 1.9% $year^{-1}$ relative genetic gain.
(iii) The contribution of new cultivars to the total gain in commercial seed yields in sunflower was 56.3% for the period 1970 to 1989; 23.9% from 1990 to 2009; and the mean over the four decades under consideration from 1970 to 2009 was 41.6%.

Positive increases in genetic gains for oil yield, oil content and number of seeds per head were also obtained, although the gain in oil content was relatively low. From 1970 to 2009, the

contribution of new cultivars to commercial seed yields decreased by more than half, dropping from 56.3% for the period 1970 to 1989 to 23.9% from 1990 to 2009. The large contribution of cultivars to commercial yields from 1970 to 1989 was primarily due to the exploitation of heterosis when F_1 hybrids replaced open pollinated cultivars (OPC) (Chigeza et al., 2012). The reduction in yield gain from 1990 to 2009 following the introduction of F_1 hybrids is an indication as well as a warning that obtaining yield increases through conventional hybrid breeding is becoming more difficult (Fischer et al., 2009). New selection strategies should be developed to speed up the rate of genetic gain. Besides yield potential and yield stability, new traits such as high oleic acid and natural herbicide resistance should be pursued to add quality and diversity to the market. In South Africa breeding for these traits is still at its infancy stages.

6.3 Genetic variability and heterosis during advanced cycle pedigree breeding in sunflower

6.3.1 Genetic variability

Selection methods and procedures to maximise genetic gains and/or prevent loss of realised gains is of paramount importance for any breeding programme. Advanced cycle pedigree breeding is one method designed to maximize genetic progress while at the same time prevent loss of the already achieved gains by crossing good by good inbred lines of cultivars already in use or of obsolete cultivars (Bernardo, 2008). In crops like barley (*Hordeum vulgare* L.), industry guidelines and market demands necessitates use of specific narrow core germplasm with minimum introgression (Condón et al., 2008).

The results from these studies indicate that despite the breeding populations having gone through advanced cycle pedigree breeding, genetic variation for seed yield and oil yield were high, and that of oil content was low to moderate as indicated by their corresponding heritabilities. In maize, Yu and Bernardo (2004) found that the genetic variances of other traits were drastically reduced during advanced cycle breeding except that of grain yield indicating that genetic variance in the elite lines has not been exhausted and breeders are likely to continue practicing advanced cycle breeding until it is obvious that genetic variance has been completely depleted (Lu and Bernardo, 2004). Seed yield in sunflower is a quantitative trait and is an expression of the combined effects of large numbers of genes. Recurrent selection methods with the assistance of molecular markers provide breeders with the opportunity to

increase favourable alleles in source populations prior to inbred line extraction. These methods in conjunction with introgression with alternate germplasm such as wild species which are important sources of tolerance to both abiotic and biotic stresses could enhance the stability of hybrids produced by both public and private sector breeding programmes in the South Africa.

Although Yu and Bernardo (2004) concluded that it might be premature to predict exhaustion of genetic variance for any crop species, long-term genetic gains require broadening the genetic base beyond the current levels (Hoisington et al., 1999). One strategy which would fit broadening the genetic base as well as maintaining favourable gene combinations through the advanced cycle pedigree breeding is the use of elite exotic germplasm lines with high yielding potential and resistance to abiotic and biotic stresses. The Germplasm Enhancement in Maize (GEM) programme which has been in existence for more than 20 years and first started enhancement with 51 elite tropical maize and temperate accessions, is a good example of broadening genetic diversity using elite inbred lines (Pollak, 2003). Similar initiatives are therefore needed not only in the sunflower breeding programmes in South Africa but also in other crops of commercial importance in which the advanced cycle pedigree breeding strategy is commonly employed.

6.3.2 Heterosis in advanced cycle pedigree breeding populations

The word heterosis was coined by Shull in 1908 and in his own words "*it was chosen in the same spirit as Johannsen's word "gene," namely that it should be free from every hypothesis. It represented a group of observable phenomena for which any subsequent student was free to propose his own explanation without thereby being obliged to abandon the word 'heterosis'"* (Shull, 1948). Ever since then, vocabulary and terminology associated with the term heterosis has been applied in different experiments in plant breeding. Heterosis based on mid-parent (MPH) or better parent (BPH) have been extensively used in cross-pollinated crops (Duvick, 1999), but the cost to determine these parameters in cross-pollinated crops has been the subject of much scrutiny (Smith, 1997). According to Troyer and Wellim (2009), too much information on heterosis based on MPH or BPH in maize was "like too much diversification in financial investment: it becomes redundant". In a plant breeding programme with the goal to release hybrid cultivars, retaining the experimental testcross hybrids for further evaluation in multi-environment trials is based on performance relative to a standard or genetic check. Patnaik et al. (1990) and Makanda et al. (2009) used the term standard heterosis (SH) to define

the superiority or yield advantage of experimental F_1 hybrids or TCHs relative to the performance of commercial checks in rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L.), respectively. Thus SH measures the commercial breeding potential of experimental lines and is sometimes referred to as commercial heterosis (Meredith and Bridge, 1972). In this study another measure of heterosis is proposed if the genetic background of inbred lines is to be interpreted, termed founder parent heterosis (FPH). The FPH measures the genetic progress and also provides information on traits that may have been already improved in founder parents or have reached a plateau especially when inbred parents are recycled (Chigeza et al., 2013). On the basis of the results from this study FPH, in combination with SH, was found to be more relevant in cross pollinated crops such as sunflower if the principal aim is to develop inbred lines that have a high probability of outperforming both their founder parents in testcross combinations and commercial hybrid checks.

6.4 Combining ability during advanced cycle pedigree breeding in sunflower

The proven performance of a line is assessed by its performance in hybrid combination/s based on general combining ability (GCA) or specific combining ability (SCA). Townsend et al. (2013) successfully identified parents for use in hybrid seed production based on GCA effects in the medicinal plant, *Artemisia annua* L. and use of the GCA effects was consistent with the QTL breeding approach. In this study, the probability of selecting the top 30 TCHs based on GCA and SCA effects was 0.80 and 0.47, respectively. Reif et al. (2013) found that prediction of hybrid performance based on GCA effects was high and additional approaches using genomic based selection could not increase the prediction of intergroup hybrid performance. In this study, inbred lines P4_1CMS, P2_3CMS, P3_20CMS, P1_19CMS, P1_26CHS and P1_19CMS and P3_8CMS were identified as consistently having high GCA effects and these lines could be used as parents for the next cycle of pedigree breeding or some form of recurrent selection.

The results also indicated that despite breeding populations being in advanced cycle pedigree breeding, significant GCA and SCA variance components and effects were present in the four breeding populations studied. Variance component for GCA were predominant for seed yield and oil yield thus early generation testing could be applied in these breeding populations. For oil content, non-additive gene action was found to be more important than additive. Early generation testing will be of limited value as the performance of TCHs in early generation testing will not be guaranteed in the subsequent later generations of more highly fixed lines.

6.5 Selection strategies for improving drought tolerance

The South African sunflower breeding programmes have recorded more successes than failures based on the modest seed yield increases evident from side-by-side evaluations and under commercial farming production. Some shortfalls are also evident regarding breeding objectives and strategies. Major emphasis has been placed on breeding for seed yield rather than a more holistic approach which should include breeding for improved oil content and other traits positively correlated with seed yield. The fluctuations of mean seed yield based on commercial production (Figure 2.1) is an indication of several factors causing yield instability, and drought was identified as one of the major causes of yield instability in crop production in the tropics (Fisher et al., 2009; Jordan et al., 2012). Tolerance to abiotic stresses such as drought tolerance is one breeding objective that has also not been given adequate attention in the past in the South African sunflower breeding programme. The reasons for not improving sunflower for abiotic stresses are not clear but it may be due to the perception that sunflower is generally regarded as a 'drought tolerant crop' suitable for harsher environments. In sorghum, a crop equally regarded as drought tolerant, selection for drought tolerance based on secondary traits, including stay green canopy, increased grain yield and reduced lodging for the natural rainfall production environments (Jordan et al., 2012). Based on the results obtained in this exploratory research study, the stay green canopy was found to be a suitable secondary trait for selecting drought tolerance. A number of morpho-physiological traits have been associated with drought tolerance in sunflower and therefore drought tolerant genotypes should possess more than one mechanism for drought tolerance. By logical extension, further research to identify a suite of secondary traits contributing to drought tolerance rather than from a single secondary trait would further improve the effectiveness of breeding drought tolerant sunflower. A selection index incorporating primary and secondary traits will assist breeders in selecting for such broad based drought tolerance. Indirect selection for oil yield in the managed stress environments was found to be as good as direct selection in the random stress environments although the biggest challenge in conducting selection in random stress environments is in ensuring good plant establishment each season.

6.6 Conclusion

The success of a breeding programme is measured by how well new cultivars perform and are adopted by farmers in the target production environments while retaining genetic options that future breeders and geneticists can exploit (Bernardo, 2008) to avoid reaching a yield plateau. As pointed out by Baenziger et al. (2011), understanding how to increase grain yield in crop species is a journey not a destination, therefore a better understanding of the basis for past genetic increases (or lack therefore) helps in developing or modifying breeding strategies that ensure sustained genetic gain in crop yields. The results from this study demonstrate clear progress in obtaining genetic gain for seed yield and oil yield but as with any other investment all putative gains should be accurately quantified and verified in order to properly evaluate accomplishments and failures. Parents and their hybrid progeny developed and released in one breeding era are used as parents for the next breeding era. Therefore advanced cycle pedigree breeding will continue to be used in most sunflower breeding programmes. Limited introgression of elite exotic accessions with the application of molecular markers is recommended to sustain future gains in sunflower yields in South Africa. Results from these exploratory studies indicate the use of secondary traits and drought managed stress environments will increase the selection efficiency in breeding for drought tolerance in sunflower in South African and consequently there is a clear need to develop managed drought selection environments for sunflower breeding programmes in South Africa.

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