

**GENETIC DIVERSITY AND COMBINING ABILITY AMONG SORGHUM
CONVERSION LINES**

A Dissertation

by

RAFAEL ARTURO MATEO MONCADA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2006

Major Subject: Plant Breeding

**GENETIC DIVERSITY AND COMBINING ABILITY AMONG SORGHUM
CONVERSION LINES**

A Dissertation

by

RAFAEL ARTURO MATEO MONCADA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee, William L. Rooney

Committee Members, Monica Menz

Stephen R. King

Kevin Crosby

Head of Department, C. Wayne Smith

December 2006

Major Subject: Plant Breeding

ABSTRACT

Genetic Diversity and Combining Ability Among Sorghum

Conversion Lines. (December 2006)

Rafael Arturo Mateo Moncada, B.S., Escuela Agricola Panamericana, Honduras;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. William Rooney

Sorghum (*Sorghum bicolor* [L] Moench) was first introduced to the United States in the 1800s. These introductions consisted of tropical varieties with a short day photoperiod response that limited their use in temperate hybrid breeding programs. Commercial exploitation of F1 hybrids in grain sorghum started by the mid 1950s with the use of cytoplasmic male sterility system CMS (A1). Even though other CMS are available, most sorghum hybrid seed production still relies on the A1 system. Genetic gain in most agronomic crop species is limited by several factors. In the specific case of sorghum, the uniform use of the CMS (A1) system and the recent introduction of sorghum to the United States have resulted in a reduction of its genetic base. In order to create enough genetic variability, plant breeders might utilize exotic non adapted material, exotic adapted material or existing elite material as a source of new alleles that will protect and improve genetic gain through selection. This study provides an estimate of the genetic diversity existing in a set of sorghum conversion lines. The objectives of this study were: (1) to estimate the genetic diversity present among a set of 16 sorghum conversion lines; (2) to classify this set of lines based on genetic similarities estimated

using AFLP markers and (3) to estimate heterosis, general and specific combining ability for grain yield among the set of conversion lines.

Genetic diversity was present in the set of conversion lines evaluated. For the lines included only in this study, Caudatum was the most homogenous race (average GS = 0.69), and this race was closely related to the Durra race (Average GS = 0.66). Two other homogenous races were Bicolor and Kafir with average GS of 0.67. Highest GCA effects were obtained from the Kafir and Caudatum races. Good heterotic responses were obtained from Durra-Kafir races and Caudatum-Kafir races. Estimation of SCA, MPH and BPH identified specific crosses that were numerically superior than those of the checks.

The use of AFLP markers allowed the identification of five strong clusters through estimates of genetic similarities. This classification did not group the lines by either their genetic background or their fertility reaction. This study provides information to identify specific combinations that would help to understand heterotic relationships in sorghum, and support the suggestions made by Menz and Gabriel that races in sorghum are not well defined.

DEDICATION

To my mother Juanita and my sister Marcela.

ACKNOWLEDGMENTS

My sincere gratitude to Dr. William Rooney for believing in me since I met him in 1997 in Honduras. Special thanks to Dr. Monica Menz for always taking the time to help and unselfishly sharing her knowledge with me. My gratitude to Dr. Stephen King and Dr. Kevin Crosby for serving on my committee and their valuable input. I am also grateful to Dr. John Yu for kindly substituting for Dr. Menz on my dissertation defense.

Thank you to my friends at the Sorghum lab: Karen Prihoda, Cindy King, Delroy Collins, Bill Lyles and Stephen Labar for all their help and support during my time at TAMU.

Thanks also to the doctors and future doctors: Jorge, Cleve, Gabriel, Hector, Les, Ryan, Dan, Leo and Joaquim for the good summer times. Thank you to my buddies who in one way or another made these six years a great experience in Aggieland: Sara Martha, Gerardo Reyes Mateo, Ndambe, Carlos, Paulo, Carmen, Gaby, Odete, MP, GG, Mole, Sergio and Arnaldo.

And last but not least, thank you to all the soccer gang “Strikers FC”, it is time for me to quit soccer guys. All the best!

TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
DEDICATION.....	v
ACKNOWLEDGMENTS.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES	ix
LIST OF TABLES.....	x
 CHAPTER	
I INTRODUCTION.....	1
II REVIEW OF LITERATURE	6
Sorghum origin.....	6
Sorghum importance.....	7
Sorghum production.....	8
TAES/USDA-ARS sorghum conversion program.....	9
Heterosis.....	10
Amplified fragment length polymorphism (AFLP)-technique.....	12
Molecular marker-based genetic diversity studies.....	15
III MATERIALS AND METHODS.....	18
Field evaluation.....	18
Plant material.....	18
Experimental design.....	21
Phenotypic evaluation.....	23
Statistical analyses.....	24
Heterosis and combining ability.....	27
Phenotypic correlations.....	30
Amplified fragment length polymorphism (AFLP)-analysis.....	31
DNA extractions	31
Pre-amplification and adapter ligation.....	32
Selective amplification.....	32

CHAPTER	Page
Gel analysis and AFLP images.....	33
Data analysis.....	33
IV RESULTS AND DISCUSSION.....	36
Field evaluation.....	36
Agronomic performance.....	36
Grain yield (GYL).....	36
Days to mid anthesis (DMA).....	45
Plant height (PHE).....	46
Panicle exertion (PEX).....	47
Panicle number (PAN).....	48
500 seed weight (SEW).....	48
Panicle length (PLE).....	49
General combining ability affects.....	50
Kafir race.....	51
Durra race.....	51
Caudatum race.....	52
Guinea race.....	52
Bicolor race.....	53
Specific combining ability (SCA), mid and better parent heterosis (MPH and BPH).....	62
Grain yield (GYL).....	62
Other traits.....	67
Amplified fragment length polymorphism (AFLP) analysis.....	71
Cluster analysis.....	71
Correlation between genetic similarity and hybrid performance.....	75
V CONCLUSIONS.....	76
REFERENCES.....	78
APPENDIX.....	90
VITA.....	108

LIST OF FIGURES

FIGURE	Page
1 Dendogram of 16 sorghum conversion lines and four testers lines revealed by cluster analysis of genetic similarity estimates generated by 1338 AFLP markers.....	72

LIST OF TABLES

TABLE		Page
1	List of 16 sorghum conversion lines and four testers for field and molecular evaluation	20
2	Agronomic, environmental and soil characteristics on the four environments in Texas in which lines and hybrids were evaluated.....	22
3	Expected mean squares for the individual environment analysis of variance	25
4	Expected mean squares for the combined analysis of variance across environments.....	26
5	Mean squares of grain yield and agronomic traits in sorghum testcross hybrids, parents and checks across four environments– College Station, Corpus Christi, and Weslaco in 2003 and College Station in 2004.....	38
6	Mean squares of grain yield and agronomic traits in sorghum hybrids, parents and checks at Weslaco in 2003.....	41
7	Mean squares of grain yield and agronomic traits in sorghum hybrids, parents and checks at College Station in 2003.....	42
8	Mean squares of grain yield and agronomic traits in sorghum hybrids and parents at Corpus Christi in 2003	43
9	Mean squares of grain yield and agronomic traits in sorghum hybrids, parents and checks at College Station in 2004.....	44
10	General Combining Ability (GCA) estimates for grain yield and other agronomic characters for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.....	54
11	General Combining Ability (GCA) estimates for grain yield $\text{MT}\cdot\text{ha}^{-1}$ for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.....	55

TABLE	Page
12	General Combining Ability (GCA) estimates for days to mid-anthesis for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis..... 56
13	General Combining Ability (GCA) estimates for plant height (cm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis..... 57
14	General Combining Ability (GCA) estimates for panicle exertion (cm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis..... 58
15	General Combining Ability (GCA) estimates for number of panicles/plot for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis..... 59
16	General Combining Ability (GCA) estimates for 500-seed weight (gm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis..... 60
17	General Combining Ability (GCA) estimates for panicle length (cm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis..... 61
18	Specific Combining Ability (SCA) values of line x tester hybrids for grain yield ($\text{MT}\cdot\text{ha}^{-1}$), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004..... 64
19	Mid Parent Heterosis (MPH) values of line x tester hybrids for grain yield ($\text{MT}\cdot\text{ha}^{-1}$), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004..... 65
20	Better Parent Heterosis (BPH) values of line x tester hybrids for grain yield ($\text{MT}\cdot\text{ha}^{-1}$), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004..... 66

TABLE		Page
21	Specific Combining Ability (SCA) values of line x tester hybrids for 500-seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.....	68
22	Mid Parent Heterosis (MPH) values of line x tester hybrids for 500-seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.....	69
23	Better Parent Heterosis (BPH) values of line x tester hybrids for seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.....	70
24	Average genetic similarities within and between races based on AFLP molecular markers.....	74

CHAPTER I

INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) evolved and was domesticated in arid areas of Africa. The crop is produced for its grain, fiber, and stalk in developing countries while in developed countries the crop is used primarily as animal feed. While grain sorghum can be successfully produced in a wide range of environments, its production has been limited to marginal subtropical and tropical regions of the world where water and heat stresses are present. Under optimal conditions, sorghum has a high yield potential comparable to other cereals such as rice, maize or wheat. In 2005, the total annual sorghum production was 58.6 million MT from approximately 44.7 million ha, with an average yield of 1.31 MT·ha⁻¹ (FAOSTAT data, 2006).

Sorghum was first introduced to the United States in the 1800s. These introductions consisted of tropical varieties with a short day photoperiod response. Selection of early maturing and short plants from these tropical strains appeared to be the method of adaptation of sorghum in the United States (Quinby, 1974). By 1916, the inheritance of many traits in sorghum were already determined (Schertz and Stephens, 1966), allowing selection of desirable parents with reasonable assurance that these parents will fulfill the objectives of breeding programs when they are crossed to create a new variety. Later on, after hybrid vigor was recognized in corn, and cytoplasmic male sterility discovered in onions, tremendous efforts were made to develop sorghum

hybrids. Commercial exploitation of F₁ hybrids in grain sorghum started by the mid 1950s with the use of the cytoplasmic male sterility system (CMS) A1 (Quinby, 1974). When the CMS system was implemented in sorghum, it allowed a cost effective way to produce F₁ sorghum hybrids. Once hybrid seed was produced, it was rapidly accepted by sorghum producers and replaced sorghum open pollinated cultivars in a period of less than ten years (Maunder, 1999). Currently, most sorghum hybrid seed production still relies on the A1 CMS system described by Stephens and Holland (1954). With the adoption and acceptance of sorghum hybrids along with improved agronomic inputs, yields in sorghum have doubled over a period of 60 years (Maunder, 1999). However, while efforts continue to increase genetic yield potential in sorghum and other crops, the rate of gain has reduced (Troyer, 1999). Genetic gain is limited by several factors such as harsh environments, disease and insect pressure, and water availability, which have a direct impact on the agronomic performance of genetic material. Along with these factors, the uniform use of the A1 CMS system and the recent introduction of sorghum to the United States have resulted in a relatively narrow genetic base of sorghum, which may be restricting genetic gain (Menz et al., 2004). However, early foresighted sorghum breeders were concerned about the implications of having a limited germplasm base, and of losing genetic diversity in the crop. For that reason, the TAES/USDA-ARS sorghum conversion program was created in 1963. The objective of the conversion program was to provide new sources of genetic variation in sorghum from accessions of different sorghum collections around the world that were of suitable height and maturity for use in sorghum improvement programs in the United States (Rosenow and Dahlberg, 2000).

As a result, the conversion of tall photoperiod sensitive sorghum genotypes to short photoperiod insensitive genotypes was made feasible, making a significant impact that has accounted for many of the improvements in sorghum hybrid production in the past 30 years (Rooney and Smith, 2001). Nowadays, there still exists a huge array of genetic diversity within *Sorghum bicolor*, with which the cultivated elite lines have the potential to exchange alleles for the improvement of the crop. This diversity for a breeding program directly affects the potential for genetic gain through selection. It also allows the plant breeder to make a classification of germplasm into heterotic groups to maximize heterosis (Menz et al., 2004). In absence of more information related to genetic diversity, and also for logistic reasons, sorghum germplasm has been separated into two groups: male lines (R-fertility-restorer) and female lines (A/B sterile-maintainer that lacks fertility restoration genes for the A1 CMS); these two groups have traditionally served as heterotic groups in sorghum. However, lines from different groups still share a lot of their genetic background that might be restricting the exploitation of heterosis in the crop. Moreover, it is well documented that hybrids of closely related sorghum parents show poorer vigor (Karper and Quinby, 1937; Nesbitt, 1994). That is why, with alternative methods, such as a better delineation of races, working groups and heterotic groups in sorghum, supported by the use of modern molecular tools, and appropriate interpretation of all the information generated by these methods, plant breeders will be able to better understand heterotic relationships in sorghum. Genetic diversity studies in sorghum have been previously conducted utilizing several types of markers (Menz et al, 2004; Ahnert et al., 1996), as well as field based

studies (Gabriel, 2005). For instance, Menz et al., (2004) described the use of AFLP markers in sorghum and their importance to calculate molecular-marker based genetic distance estimations (Vos et al., 1995, Mueller and Wolfenbarger, 1999; Barret and Kidwell, 1998; Zhu et al., 1998; Ajmone et al., (1998), allowing the identification of new heterotic groups that would assist breeders in the identification of new inbred lines for use as parents in a hybrid breeding program, one of the most costly and time consuming phases in sorghum hybrid development. Gabriel (2005) attempted to determine heterotic relationships existing between groups of genotypes observed by cluster analysis in the molecular marker-based diversity study by Menz et al., (2004), showing some accordance with Menz's findings. However, based on Menz's and Gabriel's findings, and also as previously documented by Gilbert (1994), heterotic groups in sorghum have not been clearly delineated.

The challenge for young plant breeders is to create enough genetic variability to keep improving genetic yield potential. Plant breeders might utilize exotic non adapted material, exotic adapted material or existing elite material as a source of new alleles that will protect and improve genetic gain through selection. In one more effort to understand heterotic relationships in sorghum, and to characterize a different portion of the sorghum germplasm not covered by Menz et al. (2004) and Gabriel (2005); this study attempts to provide an estimate of the genetic diversity existing in a set of sorghum conversion lines.

The objectives of this study were:

1. To estimate the genetic diversity present among a set of 16 sorghum conversion lines.
2. To classify this set of lines based on genetic similarities estimated using AFLP markers.
3. To estimate heterosis, general and specific combining ability for grain yield among the set of conversion lines.

CHAPTER II

REVIEW OF LITERATURE

Sorghum Origin

It is believed that cultivated races of sorghum (*Sorghum bicolor* [L.] Moench) were domesticated in north-eastern Africa, where the greatest variability of this species is still found. As the very early domesticated sorghum plants were selected and dispersed, genetic adaptation and intercrossing followed by selection and continued intercrossing in isolated ecosystems gave rise to new and stable sorghum biotypes. Reintroduction of native biotypes and introduction of biotypes evolved in other locations offered additional opportunity for intercrossing and development and selection of additional biotypes. This movement and evolution of biotypes gave rise to five sorghum races: bicolor, caudatum, guinea, kafir and durra (Rooney and Smith, 2000). The origin of this domestication probably started between 5,000 and 7,000 years ago (Murdock, 1959; Ehret, 1988; Harlan, 1989). Sorghum domestication has been associated with human migrations, trade, and shipping routes through Africa, and through the middle east to India, 3,000 years ago (Kimber, 2000). Some authors suggest the origin of sorghum in India (Meadow, 1996; Haaland, 1995), while others have proposed the origin and domestication of sorghum in southern China (Qiao and Zhenshan, 1970) and northern China (Kimber, 2000). Sorghum was first taken to the western hemisphere through the slave trade from West Africa. Later, it was re-introduced in the late 19th

century for commercial cultivation. All theories about the origin and domestication of sorghum are based on archaeological evidence. However the time and locations are not necessarily accurate (Kimber, 2000).

Nowadays, cultivated sorghum is found in a wide range of environments in Africa, Asia, Australia, North, South and Central America.

Sorghum Importance

While in developed countries sorghum is mainly used for livestock feed, in developing countries it is considered as a staple food crop that is utilized as an ingredient in malts, ready to cook breakfast, flour, beer, weaning foods, tortillas and noodles (Rooney et al., 1980; Murty and Kumar, 1995). Furthermore, sorghum is used to make syrup, ethanol, silage (sweet sorghum) and brooms from the branches of broom corn seed clusters. Besides all the uses previously mentioned, sorghum's strength derives from a great number of morphological and physiological characteristics that gives a great advantage over other cereals, being able to produce a harvestable crop where many other cereal crops would fail. These characteristics include an extensive root system and a waxy bloom on the leaves, which reduces water loss and the ability to stop growth during periods of drought and to resume growth when conditions become favorable - contributing to sorghum's adaptation to harsh environments, where water availability and soil fertility are marginal. These unique advantages of sorghum must be considered

for the future, especially in the presence of an increasing world population that demands more food with an efficient use of water and land resources.

Sorghum Production

Sorghum is broadly adapted and grown in a great range of environments. One of its strongest traits is its great adaptability to tropical and subtropical areas of the world where water availability and soil conditions are considered marginal for other grain crops. Under optimal conditions, sorghum has a high yield potential comparable to other cereals such as rice, maize or wheat. In 2005, the total annual sorghum production was 58.6 million MT from approximately 44.7 million ha, with an average yield of 1.31 $\text{MT}\cdot\text{ha}^{-1}$, making sorghum the fifth most important cereal in the world (FAOSTAT, 2006). Sorghum grain yield can be as high as 15 $\text{MT}\cdot\text{ha}^{-1}$, and a good yield is usually between 7 and 9 $\text{MT}\cdot\text{ha}^{-1}$ when rainfall is not a limiting factor. Under average conditions, sorghum yield can vary between 3 and 4 $\text{MT}\cdot\text{ha}^{-1}$, and can decrease to 0.3 to 1 $\text{MT}\cdot\text{ha}^{-1}$ under drought conditions (House, 1982). This drastic variation in grain sorghum yield among different sorghum production systems through out the world is mainly due to the use of hybrids and improved equipment and production techniques in developed countries, compared with the use of land races and subsistence agriculture that predominate in the developing world.

TAES/USDA-ARS Sorghum Conversion Program

With the replacement of open pollinated sorghum cultivars with hybrids, the successful acceptance of hybrid sorghum by the mid 1950's, and the ability of sorghum to withstand heat and water stresses, breeders knew the potential of sorghum as an important crop for the future. However, the genetic base of sorghum was reduced by the frequent use of the same tropical tall, photoperiod-sensitive accessions in the temperate US, and the extensive use of CMS A1 for hybrid seed production (Quinby, 1974). This concern led to the development of the TAES-USDA-ARS Sorghum Conversion Program, which was initiated in 1963. The objective of this program was to convert exotic tropical photoperiod-sensitive sorghum lines into temperate-adapted photoperiod insensitive lines suitable for breeding programs in the United States (Stephens et al., 1967). The conversion program has been successful and has had a great impact around the world. Most sorghum hybrids that are grown today have sorghum conversion lines in their pedigree (Rooney and Smith 2001). Another great impact created by this program is the identification of different CMS systems such as the A1 (Schertz, 1977; Schertz and Ritchey, 1978) and A3 systems (Quinby, 1980) along with the creation of lines possessing insect and disease resistance, and resistance to pre and post flowering stresses (Rosenow and Dahlberg, 2000).

Heterosis

Heterosis is defined as the superior performance of the offspring compared to the parents. Heterosis is also known as “hybrid vigor” which is usually maximized when we cross individuals that are not genetically related. The term heterosis was first used by Shull in 1952. Even though heterosis is seen in plant species, its level of expression is usually variable, depending on the crop and its natural mode of reproduction as well as its natural level of heterozygosity. Heterosis can be expressed as mid parent heterosis (MPH) and high parent heterosis (HPH). MPH is the performance of the offspring compared with the average performance of the parents. HPH is the performance of the offspring compared with the best parent in the cross. Out of the two methods of measuring heterosis, the HPH is the most important to breeders. A better performance of hybrids, such as yield increase or number of seeds, is only meaningful if it has increased value over the better parent.

There is extensive debate over how to explain heterosis (Crow 1948, 1952). Two hypotheses have been proposed: the dominance hypothesis (Davenport, 1908; Bruce, 1910; Jones, 1917 and Collins 1921) and the overdominance hypothesis (Shull 1908; East 1908). Furthermore, a review of recent researchers using molecular based markers studies point out the possibility of the presence of pseudo-overdominance (Stuber et al., 1992; Cockerham and Zeng 1996; Carr and Dudash, 2003).

Sorghum is commercially grown as a hybrid crop. Due to the nature of hybrid seed production that involves two different kinds of lines, inbred line identification and

evaluation comprise one of the most costly and time consuming phases in sorghum hybrid development. However, line performance *per se* does not predict the performance of sorghum hybrids for grain yield. For that reason, predictors of single cross hybrid value or heterosis between parental inbred lines are extremely important to increase the efficiency of hybrid improvement programs. Furthermore, in order to maximize heterotic potential, appropriate classification of germplasm is also extremely important. Crosses between unrelated, or genetically distant parental lines that belong to different heterotic groups have shown greater hybrid vigor than crosses between genetically related parental lines (Stuber, 1994). In the particular case of sorghum, the exploitation of hybrid vigor is well documented. Karper and Quinby (1937), reported high levels of hybrid vigor between milo and hegari with kafir, feterita, kaoling and sumac sorghum working groups. They also reported that relatedness between sorghum parents resulted in poorer vigor of the hybrid. In contrast, crosses between kafir and zerazera have shown good heterosis in hybrid combinations. Specific crosses from different sorghum groups have shown a very particular pattern, for example; crosses between zerazera by kafir and milo by kafir have shown poor heterosis in hybrid combination (Nesbitt, 1994).

Amplified Fragment Length Polymorphism (AFLP)-Technique

AFLP is considered a new polymerase-chain-reaction (PCR)-based technique. It is a relatively expensive (not on a data point basis), fast and reliable method that generates hundreds of informative genetic markers (Vos et al., 1995; Vos and Kuiper., 1997; Hillis et al., 1996). The key feature of AFLP-PCR is its capacity for the simultaneous screening of many different DNA regions distributed randomly throughout the genome. AFLP technique combines the strengths of two methods, the reproducibility of restriction fragment analysis and the power of the PCR (Mueller and Wolfenbarger, 1999). In essence, the AFLP technique involves the digestion of DNA template with specific restriction enzymes, followed by the ligation of a specially designed adapter onto the sticky ends of the sample DNA, using amplification of subsets of genomic restriction fragments (Vos et al., 1995, Vos and Kuiper., 1997). The results are highly informative fingerprints. Fingerprinting is the identification of individuals based on a pattern of DNA markers that can be detected in the genomic DNA of an individual (Fairbanks and Andersen, 1999). Fingerprints are an increasingly popular method for crop diversity studies through estimation of genetic distances. Ideally, a fingerprinting technique does not require any kind of investment on sequence analysis, primer synthesis or characterization of DNA probes.

Currently, AFLP markers are also used in biodiversity studies, population and conservation genetics, and QTL mapping (Mueller and Wolfenbarger, 1999). The polymorphism observed when AFLP markers are used is due to the differences in

restriction sites (similar to RFLP) - differences due to the selective nucleotide insertions and deletions in the DNA sequence (Menz, unpublished). The use of AFLP markers is based on their advantages: they are highly sensitive and show a high level of polymorphism, are highly reproducible, and widely applicable. Moreover, AFLPs just require the use of generic primers to produce a practically unlimited number of markers (Vuylsteke et al., 2000). One of the strongest advantages of these markers is their capacity to resolve extremely small genetic differences. For instance, AFLPs markers have been used to distinguish near-isogenic lines of soybeans that differ only in a single small region of the entire genome (Maughan, et al., 1996). Given this advantage, AFLP markers are suitable for analysis of relatedness, parentage, mating frequency and other genetic parameters (Vos et al., 1995). Among other utilities of the AFLP technique is its capacity of generating markers from any organism with DNA, without any prior knowledge about the genomic make up of the organism. For example, AFLPs markers have been efficiently used in bacteria (Jansen et al., 1997; Huys et al., 1996; Keim et al., 1997), fungi (Gonzalez et al., 1998; Majer et al., 1998; Rosendahl and Taylor 1997), nematodes (Semblat et al., 1998), vertebrates (Otsen et al., 1996; Ajmone et al., 1998; Liu et al., 1998), cultivated crops (Menz et al., 2004; Maughan et al., 1996; Jin et al., 1998; Mackill et al., 1996) and trees (Arens et al., 1998; Gaiotto et al., 1997; Paglia and Morgante, 1998). Another great advantage of AFLP markers is that their error levels are less than those of any other molecular markers. Moreover, AFLP amplifications are performed under high selection at high stringency conditions, eliminating any artifactual variation that is commonly seen in RAPD-PCR markers. In this sense, repeated AFLP

amplifications show near perfect reproducibility (Vos et al., 1995; Jones et al., 1997; Mueller and Wolfenbarger, 1999), and overall errors, including mis-priming and scoring, generally amount to less than 2% (Jansen et al., 1997; Tohme et al., 1996; Arens et al., 1998; Winfield et al., 1998). Another benefit of the AFLP technique is that it requires a minimal amount of DNA, and partially degraded samples can be used compared with other molecular markers that require high amounts of DNA (Vos et al., 1995). In addition, AFLP markers can be generated at great speed. Mackill et al. (1996) and Muller and Wolfenbarger (1999) estimated that a single researcher could assay thousands of loci per month, of which at least 30% are polymorphic. Finally, AFLP markers segregate in a Mendelian fashion (Vos et al., 1995; Maughan et al., 1996; Liu et al., 1998) and can be used for population genetic and QTL analyses.

For all the advantages previously mentioned, PCR-based markers such as AFLPs are likely to remain a key molecular tool for some time to come. The high reliability of AFLP markers could lead to the displacement of RAPD and RFLPs, and at least better address some problems such as QTL mapping and possibly population differentiation. However, because AFLPs are dominant markers, they are unlikely to compete against co-dominant markers such as micro-satellites or allozymes, which allow more powerful population-genetic analyses. Moreover, AFLP markers are technically demanding and some times they might show problems of interpretation. Also, there are still limitations to the use of the AFLP technique - high license fees are required to access this technique, limiting its public use. Nowadays researchers interested in genetic diversity, population structure, phylogeny or QTL mapping should carefully examine the relative strengths of

AFLP markers, and their limits in the context of a particular research question (Mueller and Wolfenbarger, 1999).

Molecular Marker -based Genetic Diversity Studies

Molecular marker-based diversity studies have become very popular among cereal crops. Data generated from molecular distance analysis aids in the correct classification of germplasm into heterotic groups, providing a better understanding of the phenomenon of heterosis between genetically distant or unrelated genotypes (Saghai-Maroo et al., 1997; Zhang et al., 1994, Menz 2004). Plant breeders have been trying to predict increased heterosis by measuring the genetic distance computed using data of parental lines. However, it is well documented that correlations between hybrid performance and molecular marker diversity of parental lines are not always of practical value (Dudley et al., 1991). In sorghum, numerous studies have been conducted with the objective of estimating genetic diversity of available germplasm, using a variety of molecular marker systems (Menz, 2004; Tao et al., 1993; Vierling et al., 1994; Taramino et al., 1997; Grenier et al., 2000; Dillon et al., 2005) but the germplasm in most of these studies have been limited, as were the number of molecular markers. One of the most complete studies was conducted by Menz et al., (2004), evaluating 50 sorghum genotypes that have traditionally been used in hybrid breeding programs in the United States. Using extensive marker coverage with 1814 AFLP and 100 SSR markers, B and

R lines did not show a consistent genetic dissimilarity characteristic of heterotic groups, and the groups that did appear to exist were somewhat in accordance with the working group system. Other studies involved the efficiency of molecular-based systems in identifying phylogenetic relationships among sorghum lines, as well as with different methods of analysis (Vierling et al., 1994; White et al., 1995). However, these studies evaluated the ability of various approaches to yield relationship estimates that corresponded with the assumed heterotic A/B and R groups. Molecular marker-based studies have been well documented in other cereals as well. Barret and Kidwell (1998), measured genetic diversity in 54 spring wheat cultivars from the Pacific Northwest. In this study, AFLP markers exhibited a high level of efficiency for detecting DNA polymorphisms among the 54 cultivars, providing AFLP-based genetic distances suggesting a hierarchical pattern of genetic diversity within the 54 Pacific Northwest adapted cultivars. Zhu et al. (1998) used AFLP as a DNA fingerprinting technique in rice germplasm analysis. Analyzing biodiversity of 57 rice germplasm accessions, three groups were clearly identified which corresponded to genotypes of Isozyme Groups I, II and VI. They also estimated the appropriate number of markers needed for robust classification of rice germplasm, and the establishment of the diversity between/within groups. Ajmone et al. (1998) surveyed genetic divergence among 13 inbred maize lines using RFLP and AFLP markers, and assessed the relationship between genetic distance and hybrid performance in a diallel set of crosses. Considerable variation among inbred lines was detected in both set of markers. This study also found that AFLP markers detected polymorphisms more efficiently in comparison to RFLPs due to the larger

number of loci assayed in a single PCR reaction. Moreover, they showed that genetic distances, calculated from both sets of marker data, were greater among lines belonging to different heterotic groups compared to those calculated from lines of the same heterotic group. Furthermore, the cluster analysis based on genetic distances revealed the association among lines which agreed with expectations based on pedigree information.

All the studies previously mentioned have pointed out the importance and the great impact of markers-based molecular studies, assessing genetic diversity among exotic non adapted materials, exotic adapted material or existing elite material that might expedite crop improvement.

CHAPTER III

MATERIALS AND METHODS

Field Evaluation

Plant Material

Sixteen sorghum conversion lines (SC), four testers, and six hybrid checks were included in this study (Table 1). The 16 SC lines were a representation of different races in sorghum and previously included in other diversity studies (Menz et al., 2004). Two female parental lines and two male parental lines were used as testers for this study. The two female lines in this research were A/BTx399 and A/BTx623 (Stephens and Karper, 1965; Miller, 1986). These female parental lines were originally developed and released in the A1 CMS system by Miller et al. (1999). The male parental lines used in this study were RTx430 and RTx436 (Miller 1984a, b; Miller et al., 1992). The Texas Agricultural Experiment Station released all these four parental lines. These four testers were chosen on the basis of their historical significance; they all have been or are currently used for the production of commercial hybrid sorghums. Also, the availability of A3 CMS in male lines played a key role in their selection, making crosses possible since these male lines were used as females. The six hybrid checks originated from crosses among the four testers previously described; these hybrid checks were: ATx623*RTx430, ATx623*RTx436, ATx399*RTx430, ATx399*RTx436, A3Tx436*RTx430 and ATx623*BTx378. The fertility reaction of all the genotypes in this study included A/B-

lines and R-lines. A-lines, commonly referred to as *females*, possess an A cytoplasm [A], lacking a dominant *Rf* (restoration of fertility) allele in the nuclear genome, resulting in a male sterile plant. B-lines, commonly referred to as *maintainers*, possess an N cytoplasm [N], and also lack a dominant *Rf* allele. The sole purpose of the B-line is to perpetuate or maintain the A-line. The A-line and the B-line are *isocytosplasmic*, meaning that these two lines are genetically identical, except that the A-line plants that are male sterile can be pollinated with pollen from B-line plants to produce the next generation of A-lines or male sterile plants. The R-lines, commonly referred to as *restorer lines*, carry the dominant *Rf* alleles, restoring fertility in the progeny of the A-line.

Using each of the sixteen SC lines crossed with each tester, 64 hybrid genotypes were created. During the summer of 2003, seed for all 64 hybrids were produced via hand pollination in a crossing block at the Texas Agricultural Experiment Station farm located at College Station, TX.

Table 1. List of 16 sorghum conversion lines and four testers for field and molecular evaluation.

Line	Release designation	Sorghum conversion designation	Pedigree†-working group§	Race††	Fertility ‡ Reaction
BTx399	Tx399		SA6697, Kafir-Milo	Kafir	B
BTx623	A/BTx623		(BTx3197*SC170-6-4-4), Kafir X Zera-Zera	Kafir-Caudatum	B
RTx436	RTx436		((SC120-6-sel*Tx7000)*Tx7000), Zera-zera and Kafir Milo	Caudatum	R
RTx430	RTx430		(Tx2536*SC170-6-5-1-E2), Feterita X Zera-zera	Caudatum	R
SC155	IS12646C-TAM	SC 155	45-50: Dur-Doc-Sub	Durra Bicolor	R
SC192	IS1105C	SC 192	49: Cernuum	Guinea-bicolor	R
SC214	IS1598C-TAM	SC 214	12: Dochna	Bicolor	R
SC250	IS5322C-TAM	SC 250	1: Roxburghii	Guinea	R
SC283	IS7173C-TAM	SC 283	3: Conspicuum	Guinea	B
SC303	IS3620C-TAM	SC 303	5: Margaritiferum	Guinea	B
SC311	IS2482C-TAM	SC 311	12: Dochna	Bicolor	P
SC326	SC326-6	SC326	BC derivative of IS3758C	Caudatum	R
SC333	IS3063C-TAM	SC 333	33: Caudatum	Caudatum	R
SC392	IS7229C-TAM	SC 392	34: Caud-Kaura	Caudatum	R
SC441	IS5142C-TAM	SC 441	41: Durra	Durra	R
SC625	IS8003C-TAM	SC 625	22: Caffrorum	Kafir-Caudatum	R
SC628	IS3169C-TAM	SC 628	22: Caffrorum	Kafir-Caudatum	R
SC680	IS8264C	SC 680	31(1): Dobbs	Guinea-Caudatum	R
SC748	IS3552C-TAM	SC 748	35: Caud-Guin	Durra-Caudatum	R
SC798	IS3541C-TAM	SC 798	39(1): Zerazera	Caudatum	R
SC303	IS3620C-TAM	SC 303	5: Margaritiferum	Guinea	B

† For complete pedigrees please review the germplasm release notice in Crop Sciences and TAES.

‡ R= restorer (progeny all male fertile); B=maintainer (progeny all male sterile); P=partial restorer.

†† Race is based on Harlan and De Wet (1972).

§ Working group numbers and names are based on a modified Snowden's Classification by Murty and Govil (1967)

Experimental Design

Using line by tester analysis described by Kempthorne (1957), lines and their hybrids were planted in a randomized complete block design with three replications. Since two R-line genotypes in A3 cytoplasm were used as female testers, as well as two A/B-lines (with reaction fertility that lack the A1 restorer gene) being used to create hybrids [A3] x SC [B] and [A] x SC [B], pollinator rows were interspersed throughout the test at regular intervals to ensure that pollen was available for pollination of the male-sterile hybrids. These pollinator rows were composed of male fertile hybrids of variable maturity to ensure that pollen was shed throughout the duration of anthesis (Lee et al., 1992). The test, composed of 16 SC lines, four testers and six hybrid checks, was evaluated in four different environments: College Station, TX in the summer of 2003 (CS03), Corpus Christi, TX in the summer of 2003 (CC03), Weslaco, TX in the summer of 2003 (WE03), and College Station, TX in the summer of 2004 (CS04) (table 2). In all four locations, plantings were made in March while harvests were made in July which is the typical production season. In order to minimize exogenous variability and maximize genetic expression of all genotypes; the tests were grown under the agronomic practices standard for each location, that included fertilization, weed and insect control as well as supplemental irrigation to avoid drought stress. Each experimental unit was composed of one row 5 m long with row spacing of 0.76 m.

Table 2. Agronomic, environmental and soil characteristics on the four environments in Texas in which lines and hybrids were evaluated.

	Soil Type	Altitude (m)	Latitude	Longitude	Plot length	Row spacing	Fertilizer regime	Irrigations	Rainfall*
College Station (2003)	Ships clay loam	96.0	30°40'N	96°21'W	5m	0.75m	60-40-40 lbs/ac preplant, sidedressing of 60 lbs N/ac 05/08	two-04/17, 06/03	
Weslaco (2003)	Raymondville clay loam	22.5	26°09'N	97°59'W	5m	0.75m	50 gal/ac of 4-10-10/ac and 2qt Zinc/ac; 100-0-0/ac	One-04/08	
Corpus Christi (2003)	Houston black clay	15.0	27°48'N	97°24'W	5m	0.75m	100 lbs N/ac; 0.71lbs Zinc/ac	none	21.10cm
College Station (2004)	Ships clay loam	96.0	30°40'N	96°21'W	5m	0.75m	60-40-40 lbs/ac preplant, sidedressing of 60 lbs N/ac 05/05	none	67.05cm

*Rainfall refers to the amount of moisture that fell during the growing season.

Phenotypic Evaluation

Seven agronomic traits were measured in all genotypes at all environments: 1) Days to mid-anthesis (DMA): DMA were recorded as the number of days from planting to the date that half the plants in the plot reach 50% anthesis. 2) Plant height (PHE): PHE is the average distance in centimeters from the ground to the tip of the panicle at maturity. 3) Panicle length (PLE): PLE was recorded as the distance in centimeters from the lowest panicle branch to the tip of the panicle at maturity. 4) Panicle exertion (PEX): PEX is the average distance in centimeters from the flag leaf's blade to the base of the lowest panicle branch at maturity. 5) Number of panicles per plot (PAN): PAN is the number of panicles in each row plot that were harvested. 6) 500-seed weight (SEW): SEW is the weight of 500 seeds in grams, from grain samples collecting during threshing. 7) Grain yield (GYL): GYL is the weight of the grain harvested per plot, expressed in metric tons per hectare ($\text{MT}\cdot\text{ha}^{-1}$) adjusting the weight to 13% moisture. Plant stand among plots did not show a problem (visual observations) in any locations. However, PAN and GYL variables were combined to create an extra variable, panicle weight (PEW). This variable was calculated by dividing the weight of the harvested grain per plot by the number of panicles counted in that specific plot. Measurements of PHE, PLE, PEX and PAN were collected one day prior to harvest. Panicles of all genotypes were hand harvested and threshed using an ALMACO Large Plot Thresher. Grain was weighed and moisture content was estimated to estimate grain yield.

Statistical Analyses

Once all genotypes' data from all environments were collected, data from a total of 90 entries were utilized to perform all statistical analyses. Individual environments' analyses of variance were performed with the GLM and PROC MIXED procedures included in SAS 9.1[®] (Table 3). Genotypes were considered fixed effects while replications were considered random effects. The linear model utilized for individual analyses was as follows:

$$Y_{ij} = \mu + g_i + r_j + \varepsilon_{ij}$$

Where

Y_{ij} is value of the ij^{th} plot;

μ is the population mean;

g_j is the effect due to the i -th genotype;

r_i is the effect due to the j -th replicate;

ε_{ij} is the environmental effect associated with the ij^{th} individual observation.

Table 3. Expected mean squares for the individual environment analysis of variance.

Source	df †	Mean Squares	Expected Mean Squares‡
Replications	r-1	MS _R	$\sigma^2_{\epsilon} + g'\sigma^2_r$
Genotypes	g-1	MS _G	$\sigma^2_{\epsilon} + r'\sigma^2_G$
Error	(r-1)(g-1)	MS _{ϵ}	σ^2_{ϵ}
Total	rg-1		

† varied depending upon the number of missing observations at each environment.

‡ g' and r' denote harmonic means for genotypes and replications, respectively.

In order to combine data from individual environments, a Bartlett's test for heterogeneity of error variances (Steel and Torrie, 1980) was conducted. Results indicated that the error variances across environments were heterogeneous. Out of all locations, CS04 was the only one that showed a non normal distribution, indicating the lack of discrimination power among genotypes. However, elimination of CS04 for combined analyses did not affect the final conclusions of this study; there was no change in genotype ranking and the data for this location is just the reflection of the environmental conditions that predominated that summer season, which was characterized by high precipitation. For that reason, CS04 was included in the final combined analysis. Data transformation failed to normalize variances; thus, combined analyses of variance were calculated on untransformed data. As in the individual analyses, the combined analyses were performed using the GLM and PROC MIXED procedures included in SAS 9.1[®] for comparison purposes (Table 4). Genotypes and

environments were considered fixed effects while replications were considered random effects. The linear model utilized for the combined analyses was as follows:

$$Y_{ijk} = \mu + g_i + e_j + (ge)_{ij} + r(e)_{jk} + \varepsilon_{ijk}$$

Where,

Y_{ijk} is the value of the ijk^{th} plot,

μ is the population mean;

g_i is the effect due to the i -th genotype;

e_j is the effect due to the j -th environment;

$(ge)_j$ is the effect due to the interaction of i^{th} genotype with j^{th} environment;

$r(e)_{jk}$ is the effect due to the k -th replicate within the j^{th} environment;

ε_{ijl} is the error associated with the ijk^{th} individual observation.

Table 4. Expected mean squares for the combined analysis of variance across environments.

Source	df†	Expected Mean Squares‡
ENV	e-1	$\sigma^2_{\varepsilon} + g'\sigma^2_{R(E)} + r'\sigma^2_{GE} + r'g'\sigma^2_E$
REP(ENV)	e(r-1)	$\sigma^2_{\varepsilon} + g'\sigma^2_{R(E)}$
GENOTYPES	g-1	$\sigma^2_{\varepsilon} + r'\sigma^2_{GE} + r'e'\sigma^2_G$
GENOTYPE * ENV	(e-1)(g-1)	$\sigma^2_{\varepsilon} + r'\sigma^2_{GE}$
ERROR	e(g-1)(r-1)	σ^2_{ε}
Total	egr-1	

† varied depending upon the number of missing observations at each environment.

‡ g' and r' and e' denote harmonic means for genotypes and replications and environments, respectively.

Eight agronomic traits were measured in all genotypes in all environments, including panicle weight (PEW) that was created as an extra variable in order to detect plant stand problems. However, no changes in genotypes' performance in individual environments and across environments were detected using this variable, indicating that the same statistical inference was possible using panicle number (PAN) and grain yield (GYL) respectively. For that reason PEW was eliminated for the final analysis and not reported in this study.

For all final analyses, GLM means were used. GLM predicts values for missing observations that are not over or underestimated or sometimes not estimated, avoiding the problem of not having a true field observation. MIXED allows estimation of values for missing observations, that becomes a problem when an algorithm is used to compute a new estimate when observations are for such computation. For all the analyses reported in this study, GLM adjusted means were used.

Heterosis and Combining Ability

For each phenotypic trait, adjusted means were obtained using the lsmeans option in the MIXED procedure included in SAS 9.1[®]. To make subsequent calculations of heterosis, general combining ability (GCA) effects of parents, and specific combining ability (SCA) effects of hybrids, their corresponding standard errors and their mean squares were estimated using a line by tester analysis described by Kempthorne (1957).

Mid parent heterosis was calculated as:

$$\text{MPH} = \left(\frac{F_1 - \text{MP}}{\text{MP}} \right) * 100$$

Where, F_1 is the mean of the F_1 hybrid performance and $\text{MP} = (P_1 + P_2)/2$ in which P_1 and P_2 are the means of the inbred parents, respectively. High-parent heterosis was calculated as:

$$\text{HPH} = \left(\frac{F_1 - \text{HP}}{\text{HP}} \right) * 100$$

Where, F_1 is the mean of the F_1 hybrid performance and HP is the mean of the best parent. Estimations of general combining abilities (GCA) effects were calculated as:

For lines:

$$g_i = \left(\frac{x_{i..}}{tr} \right) - \left(\frac{x_{...}}{ltr} \right)$$

Where;

g_i is the estimation of GCA for the i -th line

$x_{i..}$ is the grand total value for the i -th line crossed with all testers.

$x_{...}$ is the grand total values for all crosses.

l is the number of lines.

t is the number of testers.

r is the number of replications.

For testers:

$$g_j = \left(\frac{x_{.j.}}{lr} \right) - \left(\frac{x_{...}}{ltr} \right)$$

Where;

g_j is the estimation of GCA for the j -th tester

$x_{.j.}$ is the grand total value for the j -th tester crossed with all lines.

$x_{...}$ is the grand total values for all crosses.

l is the number of lines.

t is the number of testers.

r is the number of replications.

Phenotypic Correlations

Phenotypic correlation coefficients (r) were estimated between genetic similarities (GS is further explained in the section dealing with molecular analysis) and single cross grain yield (F1), mid parent heterosis (MPH), high parent heterosis (HPH) and specific combining ability (SCA), from means per environment and across environments. The estimations of coefficients were done using the mixed procedure included in SAS 9.1[®]. The correlation r coefficient was calculated as:

$$r_{xy} = \frac{\text{Cov}(x,y)}{\sqrt{\sigma^2_x \sigma^2_y}}$$

Where:

r_{xy} is the correlation coefficient between x and y

$\text{Cov } x,y$ is the covariance between x and y

σ^2_x is the variance of x

σ^2_y is the variance of y

Amplified Fragment Length Polymorphism (AFLPs) Analysis

All the AFLPs markers in this study were generated using the protocol of Vos et al. (1995) with modifications by Klein (2000) and Menz (2002).

DNA Extractions

Genomic DNA was extracted for each of the sixteen sorghum inbred lines and the four testers to be analyzed (Table 1). DNA was extracted from a bulk of leaf tips from 20 different seedlings harvested seven days after germination using the procedure described in Williams and Ronald (1994) and modified by Klein (2000) and Menz (2002). Approximately 10-20 mg of lyophilized leaf tissue was cut into small pieces and transferred to a 1.5 ml microcentrifuge tube. Extraction buffer (800 μ l) containing 100 mM Tris pH 7.5, 10 mM EDTA pH 7.5, 700 mM NaCl, and 12.5 mM potassium ethyl xanthogenate (PEX) was added. Samples were incubated at 65° C for 1 h with occasional mixing. Following incubation, the supernatant was removed to a clean 1.5-ml microcentrifuge tube and centrifuged at 15000 g for 5 min. The supernatant (700 μ l) was transferred to a 1.5-ml microcentrifuge tube containing 700 μ l isopropanol and 70 μ l of 3 M sodium acetate pH 5.2, mixed and incubated for 15 min. The precipitated DNA was centrifuged at 15,000 g for 30 min, washed twice with 70% ethanol, air-dried and re-suspended in 100 μ l TE buffer. The genomic DNA was quantified using a DYNA Quant 200 fluorimeter (Hoefler Pharmacia Biotech, San Francisco, CA).

Pre-amplification and Adapter Ligation

Genomic DNA samples from all twenty genotypes were completely and simultaneously restricted with *EcoRI* and *MseI*. The incubation time was 2.5 h. Restricted genomic DNA fragments were ligated to *EcoRI* and *MseI* adapters overnight at 37° C and the reaction mixture diluted to 500 µl with TE buffer and stored at -20° C. Pre-amplification of the dilute template DNA was performed with AFLP primers having no E + 0; (GTAGACTGCGTACCAATTC) or one M+C; (GATGAGTCCTGAGTAA-C) selective nucleotide. Twenty µl PCR reactions were performed containing 5 µl dilute template DNA, 30 ng each E + 0 and M + C primers, 0.4 U *Taq* polymerase (Promega Corp., Madison, WI), 1× *Taq* buffer (10 mM Tris-HCl pH 9.0, 0.1% triton X-100, 50 mM KCl), 2.5 mM MgCl₂, and 200 µM dNTPs. Pre-amplification reactions were performed for 20 cycles of 30 sec at 94° C, 1 min at 56° C and 1 min at 72° C. Following pre-amplification, the reactions were diluted 10-fold with TE buffer and used as template for selective amplification.

Selective Amplification

Selective AFLP reactions were performed in a final volume of 10 µl containing 2 µl dilute preamplified template DNA, 15 ng *MseI* selective primer, 0.25-0.4 µl IRD-labeled *EcoRI* selective primer, 0.2 U *Taq* polymerase, 1× *Taq* buffer, 2.5 mM MgCl₂, and 200 µM dNTPs. Selective amplification reactions were performed as follows: 1 cycle of 2 min at 94° C followed by 36 cycles of 30 sec at 94° C, 30 sec annealing step (see below), and 1 min at 72° C. The annealing temperature in the first cycle was 65° C and was subsequently reduced 0.7°C for each of the next 12 cycles and was then

continued at 56° C for the remaining 23 cycles. Reactions were complete after a final extension of 5 min at 72° C.

Gel Analysis and AFLP Images

The AFLP amplification products were analyzed using a LI-COR model 4200L-2 dual-dye automated DNA sequencing system. Following amplification, an equal volume (5 µl) of PCR products labeled using the IRD-700 nm *EcoRI* primer (*EcoRI* + CAA) was pooled with the products labeled with the IRD-800 nm *EcoRI* primer (*EcoRI* + TGA). Basic fusion dye (2 µl) (LI-COR) was added to each pooled sample and the samples were denatured for 2.5 min at 95° C. Each sample (1 µl) was loaded on a 6.5% polyacrylamide gel containing 7 M urea. Gels were cast using LI-COR 25-cm plates with 0.25 mm-thick spacers and comb. Electrophoresis was performed at a constant power of 40 W and a constant temperature of 47.5°C for 3 h. The raw data from the LI-COR model 4200 sequencers is presented as an autoradiogram-like image that is stored in TIFF format.

Data Analysis

A total of 60 AFLP primer combinations were run to estimate genetic similarities between each pair of lines. These primer combinations generated around 1800 AFLP markers. However, based on the quality and clarity of the bands scored, only 1338 AFLP marker loci were included in the analysis. Dominant AFLP markers were scored visually as present (1) or absent (0). Samples of 18 lines were run in duplicate to provide an estimate of the reproducibility of band size determination. All data was

transformed to binary code producing a matrix of presence (1) versus absence (0) of each allele. The resulting matrix was used to estimate genetic similarity among all pairs of lines by Dice coefficient of similarity (Nei and Li 1979). Genetic similarity was estimated as follows:

$$GS_{ij} = \frac{2N_{ij}}{(N_i + N_j)}$$

Where:

GS_{ij} is the genetic similarity between the i-th and j-th line.

N_{ij} is the number of alleles (scored bands) shared by the i-th and j-th line.

N_i is the total number of scored bands in the i-th line.

N_j is the total number of scored bands in the j-th line.

When scoring the bands, negative matches (0-0) were not included. Values of genetic similarity may range from 1 (identical profile for all markers) to 0 (no bands in common). A similarity matrix was generated for pair comparisons among the 20 inbred lines. A dendrogram was created from the similarity matrix by the unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973).

To determine how accurately the dendrograms would represent the estimates of genetic similarities, 1000 permutations were run by comparing the observed values with the critical values obtained from a consensus tree. All procedures were performed by appropriate routines in NTSYSpc version 2.11a (Exeter Software, Setauket, NY). An estimate of the confidence limits for the grouping produced by each dendrogram was obtained by performing 1000 bootstrap resampling in WinBoot (Yap and Nelson, 1996).

CHAPTER IV

RESULTS AND DISCUSSION

Field Evaluation

Agronomic Performance

Grain Yield (GYL). Significant differences ($P < 0.05$) were detected among genotypes across environments except for the tester lines (referred as “testers” in the ANOVA table), showing a consistent performance of tester lines across environments ((Table 4). As expected, average GYL for hybrid checks (referred as “checks” in the ANOVA table) across environments was significantly higher ($P < 0.05$) than the rest of the genotypes with $2.61 \text{ MT}\cdot\text{ha}^{-1}$ compared to 2.01, 1.22 and $1.94 \text{ MT}\cdot\text{ha}^{-1}$ from testcross hybrids (referred as “hybrids” in the ANOVA table), parental SC lines (referred as “lines” in the ANOVA table) and tester lines respectively (Table 4). Although statistical comparisons should not be made between lines and hybrids, these high yields from hybrid checks are the result of capturing dominance gene action, along with additive gene action and their epistatic relationship that exploits heterosis to achieve higher yields. Furthermore, the tester lines crossed to create these hybrid checks are the reflection of many years of breeding, indicating why these tester lines have a historical significance in sorghum hybrid development, and why they are still used in sorghum hybrid production. Not surprisingly, the contrasts “checks vs parents and hybrids”, and “parents vs hybrids” also showed significant differences ($P < 0.05$).

While statistical comparisons can be made between tester lines and parental lines, this comparison is not fair, since, as mentioned previously, tester lines have gone through breeding selection and have been selected for a purpose such as high yield potential, disease resistance and combining ability, while the parental lines (conversion SC lines) are lines that have just been conversion to short, photoperiod insensitive plants, and not selected for improvement of their agronomic traits. For reasons previously mentioned, GYL from tester lines compared to parental line yield, was significantly ($P < 0.05$) higher with an average yield of $1.94 \text{ MT}\cdot\text{ha}^{-1}$ compared to $1.22 \text{ MT}\cdot\text{ha}^{-1}$ from the SC lines. The same rationale can be used to explain the significant difference between GYL of check hybrids and testcross hybrids with average GYL of 2.61 and $2.01 \text{ MT}\cdot\text{ha}^{-1}$, respectively (Table 4). Check hybrids originated from crosses between elite TAES-lines, assuring the exploitation of heterosis from two lines that have been selected to improve yield when tested in hybrid combinations. The testcross hybrids are derived from an elite line x exotic line cross, and the exotic line in the cross carries both desirable and undesirable alleles, the expression of which might result in lower yields.

Table 5. Mean squares of grain yield and agronomic traits in sorghum testcross hybrids, parents and checks across four environments– College Station, Corpus Christi, and Weslaco in 2003 and College Station in 2004.

Source of variation	df	Mean squares						
		†GYL	DMA	PHE	PEX	PAN	SEW	PLE
Environment	3	133.11*	3361.69*	3981.57*	2413.67*	37324.79*	177.47*	3568.82*
Rep(environment)	8	5.49*	28.65*	1337.68*	206.19*	4482.55*	3.63	28.33
Genotypes	89	4.51*	38.39*	3785.54*	107.07*	3002.34*	38.55*	113.72*
Checks	5	1.15*	22.88*	958.21*	22.84*	779.08*	16.10*	69.25
Parents	19	2.86*	44.56*	1491.80*	146.69*	3398.94*	72.88*	103.76*
Lines	15	1.96*	38.70*	1469.30*	180.80*	4084.14*	72.77*	122.07*
Testers	3	0.57	52.72*	1814.06*	21.99	1043.02*	49.13*	30.38*
Hybrids	63	3.46*	32.65*	3218.86*	98.20*	3106.67*	21.91*	85.12*
Checks vs (parents and hybrids) *	1	17.17*	60.63*	95.54*	661.09*	2041.79*	6.89	794.16*
Parents vs Hybrids	1	13.51*	173.31*	3654.28*	887.02*	75619.74*	255.98*	2154.12*
Genotypes * Environment	267	1.23*	9.74*	317.70*	18.05	634.77*	4.67*	32.02*
Checks * Environment	15	0.82*	6.25*	95.41*	13.13	333.03	1.44*	64.29
Parents * Environment	57	0.87*	7.58	205.36	22.99*	728.76*	5.91*	17.51*
Lines * Environment	45	0.68*	8.78	183.80	23.21	820.56	6.36*	19.25*
Testers * Environment	9	0.62	1.72	352.47*	27.97	327.45	3.18	9.77*
Hybrids * Environment	189	1.18*	10.94*	337.22*	17.17	618.26*	4.06*	31.07*
Checks vs (parents and hybrids) * Environment	3	6.93*	0.66	267.34	15.59	161.79	6.31	103.24
Parents vs Hybrids * Environment	3	8.35*	5.15	2487.00*	6.701	1993.55	28.95*	151.02*
Error	701	0.417	6.84	212.71	17.79	403.30	2.54	22.95
Mean of Checks ††		2.61	73.19	130.33	13.01	60.86	14.60	27.55
Mean of Hybrids		2.01	72.44	143.92	12.26	56.99	14.29	27.51
Mean of Lines		1.22	73.77	121.80	10.78	60.86	12.26	23.71
Mean of Testers		1.94	75.28	116.94	11.16	60.70	14.11	24.78
LSD (0.05)		0.18	0.75	4.11	1.17	8.33	0.50	1.35
CV (%)		33.97	3.59	10.57	35.12	34.54	11.46	17.93

†GYL = Grain yield, DMA = days to mid-anthesis, PHE = plant height, PEX = panicle exertion, PAN = number of panicles per plot, SEW = 500-seed weight, PLE = panicle length

*Significant at the p = 0.05 level

†† Mean of Checks = hybrid checks, Mean of Hybrids = testcross hybrids, Mean of Lines = parental SC lines, Mean of Testers = tester lines.

A significant ($P < 0.05$) genotype by environment interaction was detected for all genotypes except for tester lines (Table 4), demonstrating once more the wide adaptation and stable performance of these tester lines across environments in Texas. For SC lines and test cross hybrids, this significant genotype by environment interaction was somewhat expected and highly probable for a polygenic trait such as GYL. Also, the SC lines utilized in this study were selected as a representation of different sorghum races providing a range of genotypic variation; this was also the criteria based on which these lines were included in previous diversity studies. A meaningful genotype by environment interaction would represent a shift of ranks of genotypes where evaluated in different environments.

For individual environments, similar trends were observed as those of the combined analyses, where average GYL for check hybrids was significantly different ($P < 0.05$) from the other genotypes. The most productive environment was WE03 (Table 6), where the average GYL for hybrid checks was $4.092 \text{ MT}\cdot\text{ha}^{-1}$ compared to $2.762 \text{ MT}\cdot\text{ha}^{-1}$, $2.286 \text{ MT}\cdot\text{ha}^{-1}$ and $1.319 \text{ MT}\cdot\text{ha}^{-1}$ from CS03 (Table 7), CC03 (Table 8) and CS04 (Table 9) respectively.

The average GYL for test cross hybrids was also higher in WE03 with a mean of 2.964 $\text{MT}\cdot\text{ha}^{-1}$, followed by that of CC03 with 2.254 $\text{MT}\cdot\text{ha}^{-1}$, and CS03 and CS04 with values of 1.655 $\text{MT}\cdot\text{ha}^{-1}$ and 1.208 $\text{MT}\cdot\text{ha}^{-1}$ respectively. GYL for the SC lines was consistently lower in all environments compared to the rest of the genotypes with average values of 1.579, 1.139, 1.129 and 1.038 $\text{MT}\cdot\text{ha}^{-1}$ in WE03, CC03, CS04 and CS03 respectively. The means of the tester lines for GYL were also higher in WE03 with an average value of 3.327 $\text{MT}\cdot\text{ha}^{-1}$ yielding significantly ($P < 0.05$) more than the testcross hybrids in this environment. Also, in CS03 the average mean for GYL of tester lines (1.708 $\text{MT}\cdot\text{ha}^{-1}$) was numerically higher than that of the testcross hybrids but not significantly different. However, in CC03 the tester lines performed significantly ($P < 0.05$) lower than the testcross hybrids with an average value of 1.649 $\text{MT}\cdot\text{ha}^{-1}$. Furthermore, the tester lines performed numerically lower than the testcross hybrids but not significantly different.

Table 6. Mean squares of grain yield and agronomic traits in sorghum hybrids, parents and checks at Weslaco in 2003.

Source of variation	Df	†GYL MT·ha ⁻¹	DMA	PHE Cm	PEX Cm	PAN	SEW gm	PLE cm
Reps	2	2.27*	17.00*	502.77*	3.14	1948.73	4.92*	2.8393
Genotypes	89	3.47*	21.43*	1165.76*	42.61*	2734.68*	17.99*	41.1742*
Checks	5	0.36	12.69*	235.41*	8.69	384.72	5.04*	14.7670
Parents	19	2.74*	25.13*	517.29*	64.93*	1523.93	32.68*	37.2721*
Lines	15	4.62*	36.81*	2831.61*	64.15*	4858.19	26.93*	76.4140*
Testers	3	21.70*	128.46*	5363.52*	216.71*	4443.77	44.11*	240.3721*
Hybrids	63	2.77*	19.16*	994.71*	37.46*	3352.46*	9.66*	36.4738*
Checks vs. (Parents, Hybrids)	1	32.07*	0.98	1610.87*	26.46	60.20	39.24*	6.5611
Parents vs. hybrids	1	48.31*	158.76*	28469.81*	128.63*	427.87	305.70*	577.6189*
Error	177	0.31	1.43*	20.63*	8.43	1661.60	0.74	9.6635
Mean of checks ††		4.092	77.22	126.72	16.02	72.94	15.71	23.57
Mean of hybrids		2.964	76.37	142.52	15.15	73.54	14.75	23.77
Mean of lines		1.579	77.90	118.00	13.34	76.11	11.73	19.79
Mean of testers		3.327	79.58	115.46	14.08	81.50	14.19	22.01
LSD (0.05)		0.316	0.68	2.58	1.62	22.92	0.67	1.77
CV (%)		19.99	1.56	3.34	19.56	54.62	6.00	13.53

†GYL = Grain yield, DMA = days to mid-anthesis, PHE = plant height, PEX = panicle exertion, PAN = number of panicles per plot, SEW = 500-seed weight, PLE = panicle length

*Significant at the p = 0.05 level

†† Mean of Checks = hybrid checks, Mean of Hybrids = testcross hybrids, Mean of Lines = parental SC lines, Mean of Testers = tester lines.

Table 7. Mean squares of grain yield and agronomic traits in sorghum hybrids, parents and checks at College Station in 2003.

Source of variation	Df	†GYL MT·ha ⁻¹	DMA	PHE Cm	PEX Cm	PAN	SEW gm	PLE cm
Reps	2	16.13*	71.47*	2128.66*	22.69	16617.84*	3.64*	19.85
Genotypes	89	2.67*	21.91*	1757.40*	43.42*	1668.89*	14.06*	83.46*
Checks	5	2.00*	8.11	356.86	39.71*	906.32	8.66*	9.46
Parents	19	1.07*	19.47*	644.67*	53.28*	2438.32*	22.00*	53.79
Lines	15	4.00*	46.66*	3624.75*	48.30*	1113.56	23.17*	136.10*
Testers	3	17.95*	59.94*	9797.83*	276.57*	10105.55*	22.51*	127.39
Hybrids	63	2.73*	23.35*	1594.37*	40.19*	1462.44*	10.12*	75.26*
Checks vs. (Parents, Hybrids)	1	25.26*	4.46	1832.90*	76.75*	367.85*	4.67*	4.01
Parents vs. hybrids	1	10.40*	65.53*	40098.25*	42.63	5330.66*	120.92*	1609.54*
Error	174	0.46	8.13	190.07	11.80	517.87	0.71	37.13
Mean of checks ††		2.762	69.94	125.66	10.30	48.06	15.63	29.63
Mean of hybrids		1.655	68.86	143.32	8.40	41.02	15.51	31.56
Mean of lines		1.038	69.54	115.33	7.57	53.81	12.89	25.19
Mean of testers		1.708	72.55	105.94	6.88	42.58	14.98	27.31
LSD (0.05)		0.383	1.69	7.83	1.95	12.91	0.53	3.46
CV (%)		41.63	4.12	10.18	41.34	51.84	5.58	20.25

†GYL = Grain yield, DMA = days to mid-anthesis, PHE = plant height, PEX = panicle exertion, PAN = number of panicles per plot, SEW = 500-seed weight, PLE = panicle length

*Significant at the p = 0.05 level

†† Mean of Checks = hybrid checks, Mean of Hybrids = testcross hybrids, Mean of Lines = parental SC lines, Mean of Testers = tester lines.

Table 8. Mean squares of grain yield and agronomic traits in sorghum hybrids and parents at Corpus Christi in 2003.

Source of variation	Df	†GYL MT·ha ⁻¹	DMA	PHE Cm	PEX Cm	PAN	SEW gm	PLE cm
Reps	2	2.95**	21.28**	2451.01**	741.33**	1389.58**	1.34	31.93*
Genotypes	89	1.50**	7.62**	1164.46**	42.35**	1005.80**	10.34**	41.77**
Checks	5	0.82*	1.73	235.41**	8.69	52.22	3.76	12.35
Parents	19	0.90**	9.57**	516.09**	63.77**	1163.51**	18.10**	44.86**
Lines	15	1.54**	6.14*	2831.61**	63.94**	822.97*	17.52**	46.45**
Testers	3	5.55**	42.31**	5363.52**	214.37**	12909.67**	23.78**	365.56**
Hybrids	63	1.04**	5.36**	994.71**	37.35**	1063.83**	7.28**	35.90**
Checks vs. (Parents, Hybrids)	1	1.22	0.03	1614.19**	27.31	266.90	0.37	12.76
Parents vs. hybrids	1	45.08**	149.32**	28373.30**	133.14**	88.94	89.82**	536.02**
Error	172	0.35	1.89	20.94	8.79	218.44	3.84	9.73
Mean of checks ††		2.286	70.17	127.56	15.17	64.44	13.00	23.35
Mean of hybrids		2.254	69.53	143.30	14.30	60.82	13.18	25.05
Mean of lines		1.139	71.19	118.96	12.49	58.28	11.36	21.65
Mean of testers		1.649	72.00	116.10	13.02	64.08	13.22	21.38
LSD (0.05)		0.337	0.78	2.59	1.68	8.40	1.12	1.77
CV (%)		29.12	1.96	3.35	21.21	24.32	15.23	12.90

†GYL = Grain yield, DMA = days to mid-anthesis, PHE = plant height, PEX = panicle exertion, PAN = number of panicles per plot, SEW = 500-seed weight, PLE = panicle length

*Significant at the p = 0.05 level

†† Mean of Checks = hybrid checks, Mean of Hybrids = testcross hybrids, Mean of Lines = parental SC lines, Mean of Testers = tester lines.

Table 9. Mean squares of grain yield and agronomic traits in sorghum hybrids, parents and checks at College Station in 2004.

Source of variation	Df	†GYL MT·ha ⁻¹	DMA	PHE Cm	PEX Cm	PAN	SEW gm	PLE cm
Reps	2	0.75	4.6104	358.29	50.44	362.33	6.52	55.41
Genotypes	88	0.46	17.4964	644.39	36.22	1026.98	4.68	43.26
Checks	5	0.44	16.1139	416.77	5.16	434.93	1.10	229.88**
Parents	18	0.61	15.5086	469.55	42.19	587.35	6.75	20.77
Lines	15	0.52	13.8283	1138.20**	36.69	774.41	6.39*	47.36*
Testers	3	1.29*	57.4741*	702.37	81.14	937.61	3.90	52.88
Hybrids	63	0.42	17.8204	619.90	37.14	1224.49	4.08	30.35
Checks vs. (Parents, Hybrids)	1	0.28	0.5721	71.38	0.58	12.25	1.77	305.22**
Parents vs. hybrids	1	0.18	64.8659*	7220.45**	55.92	990.70	25.05*	102.52
Error	154	0.61	17.0120	622.68	41.70	1158.81	5.23	35.70
Mean of checks ††		1.319	75.44	141.39	10.58	58.00	14.12	33.94
Mean of hybrids		1.208	74.73	146.32	10.96	59.41	13.87	29.61
Mean of lines		1.129	75.68	134.78	9.74	55.48	13.08	28.15
Mean of testers		1.104	76.91	130.28	10.69	54.67	13.88	28.42
LSD (0.05)		0.452	2.42	14.11	3.62	19.01	1.32	3.39
CV (%)		65.11	5.50	17.41	60.03	57.91	16.56	20.16

†GYL = Grain yield, DMA = days to mid-anthesis, PHE = plant height, PEX = panicle exertion, PAN = number of panicles per plot, SEW = 500-seed weight, PLE = panicle length

*Significant at the p = 0.05 level

†† Mean of Checks = hybrid checks, Mean of Hybrids = testcross hybrids, Mean of Lines = parental SC lines, Mean of Testers = tester lines.

Days to Mid Anthesis (DMA). Differences in maturity among environments and genotypes were significant ($P < 0.05$) (Table 4). Test cross hybrids were significantly earlier across environments than the other genotypes with an average DMA of 72.44 days compared to that of hybrid checks with 73.19 days, SC lines with 73.77 days and the tester lines with an average value of 75.28 days. Comparisons of hybrid checks vs parents and test cross hybrids were highly significant ($P < 0.05$) as was the comparison between SC lines and tester lines vs testcross hybrids (Table 4). A significant genotype by environment interaction ($P < 0.05$) was only detected for hybrid checks (Table 4) indicating that these genotypes shift their ranks across environment and did not perform in a stable way. A close look at the hybrid checks in individual environments showed that their days to mid-anthesis range from 69.94 days in CS03 (Table 6) to 77.22 days in WE03 (Table 5). For the other genotypes - testcross hybrids range from 68.86 days in CS03 to 76.37 days in WE03; SC lines ranged from 69.54 days in CS03 to 77.90 days in WE03; while tester lines ranged from 72.00 and 72.55 days in CC03 and CS03 respectively to 79.58 days in WE03. It is noticeable that all genotypes were earlier in CS03, and a similar trend was observed in CS04. While CC03 is an intermediate environment for DMA, lateness in all genotypes was observed in WE03. Even though evaluation of all genotypes was conducted during typical production seasons in all environments, climatic conditions proper of each environment had an impact on DMA. In theory, DMA should be less affected by environmental conditions, since it is an oligogenic trait. It is most likely that climatic conditions such as high temperature during vegetative growth can lead to earliness, while cooler temperatures

during early phases of development can reduce growth rate resulting in late maturity of genotypes. Although a significant genotype by environment interaction was detected in the combined analyses (Table 4), genotypes only showed a difference no larger than 2.5 DMA across environments, reflecting a uniform performance in maturity.

Plant Height (PHE). Significant variation was detected in PHE among genotypes ($P < 0.05$) as well as among environments (Table 4). Testcross hybrids were significantly taller with an average height across environments of 143.92 cm compared to check hybrids (130.33 cm), SC lines (121.80 cm) and tester lines (116.94 cm). Testcross hybrids are, surprisingly, not taller than hybrid checks; most of the SC lines they are derived from are taller than average sorghum cultivars. This can also be seen when comparing tester lines to SC lines; the tester lines are significantly ($P < 0.05$) shorter (by 5 cm) than the SC lines. As expected, the contrast comparison between hybrid checks vs SC lines and tester lines, and SC lines and tester lines vs testcross hybrids are significant ($P < 0.05$) (Table 4).

Significant ($P < 0.05$) genotype by environment interactions were detected in hybrid checks, testcross hybrids and tester lines, indicating shift of ranks in these genotypes across environments. On the other hand, non-significant differences were observed for SC lines, indicating that these lines performed very stably across environments for this specific trait. Greater heights were observed in CS04 (Table 8), followed by CC03 (Table 7), WE03 (Table 5) and CS03 (Table 6). Plant height is a polygenic trait which is affected by environmental conditions. Furthermore, the existence of genetic loci that modify the four main genes that control height in sorghum,

might be the reasons for variation in performance among and within hybrid checks, testcross hybrids and tester lines.

Panicle Exsertion (PEX). Differences in PEX among genotypes were significant ($P < 0.05$). Hybrid checks showed higher exsertion values with an average mean across environments of 13.01 cm, significantly different from SC lines with 10.78 cm and tester lines with 11.16 cm, but not significantly different from testcross hybrids with 12.26 cm (Table 4). These kinds of exsertion values for hybrids checks is not a surprise; since they are the result from a cross between elite lines, superior agronomic characteristics are expected from these hybrids, one of them panicle exsertion. Normally, values between 12 and 15 cm are desirable for cultivated varieties, in this range we also have the testcross hybrids, a positive trait to be account of, since they come from a cross between an exotic and an elite line. Although, SC lines and tester lines do not fit in this range of desirable exsertion, it is extremely important to notice that information on line *per se* performance is not sufficient for making final decision of selecting a genotype - lines must be evaluated in hybrid combination. As expected, contrast comparisons between hybrid checks vs SC lines and tester lines, and SC lines and tester lines vs testcross hybrids were significant ($P < 0.05$) (Table 4).

Significant ($P < 0.05$) genotype by environment interaction was only detected for SC lines (Table 4), indicating a shift of ranks in lines across different environments. The rest of the genotypes showed a very stable performance across environments in accordance with the ANOVA (Table 4). For individual environments the same trend as the combined analyses was observed. The environment with highest values of exsertion

was WE03 (Table 5) followed by CC03 (Table 7), CS04 (Table 8) and CS03 (Table 6); higher exertion values in WE03 and CC03 have been previously observed through the years in the TAES sorghum breeding program. Genotypes also showed a consistent pattern in individual environments, with higher exertion values for check hybrids, followed by testcross hybrids, and both kind of lines.

Panicle Number (PAN). According to the ANOVA (Table 4), significant ($P < 0.05$) differences among genotypes were detected, indicating different number of panicles within each group of genotypes. PAN means across environments were 60.86, 56.99, 60.86 and 60.70 panicles for hybrid checks, testcross hybrids, SC lines and tester lines respectively. PAN can be a very useful variable when estimating GYL when experimental observations have problems with plant stand; in such cases, PAN can play an important role, making a difference in ranking genotypes. However, since plant stand was not a problem in all environments (visual observations) and the statistical analysis just proves it, the results from PAN in this experiment shown that adjustment for yield was not necessary, confirming that the use of panicle weight was not of any statistical use.

500 Seed Weight (SEW). Significant differences ($P < 0.05$) were expected in SEW within genotypes and among environments (Table 4). Not surprisingly, hybrid checks showed higher values of SEW, but their average mean across environments of 14.60 gm was only significantly different from that of the SC lines with a SEW value of 12.26 gm. SEW mean across environments for testcross hybrids and tester lines is 14.29 gm. When a contrast was created to detect differences between hybrid checks vs SC

lines and tester lines and checks, significant ($P < 0.05$) differences were not detected. However, when comparing SC lines and tester lines vs hybrids checks, significant differences were detected. The same rationale used to explain higher values in GYL in hybrids can be used to explain these differences in SEW. Hybrids capture dominant gene effects along with additive gene effects and their epistatic relationship, while lines just possess additive effects, making the hybrid exploit heterosis.

A significant genotype by environment interaction was detected for all genotypes except tester lines. As previously discussed, tester lines are elite lines in the TAES breeding program, and their wide adaptability across environments has been proven many times, thus these results are not unexpected. For the rest of the genotypes, a shift in ranks for SEW was detected across environments, in accordance with the ANOVA (Table 4). For individual environments the same pattern was observed; check hybrids showed higher values of SEW in all locations except in CC03 where testcrosses were superior, followed by tester lines and SC lines. Higher values for SEW were observed in CS03 (Table 6), followed by WE03 (Table 5), CS04 (Table 8) and CC03 (Table 7).

Panicle Length (PLE). Classification of cultivated sorghum races (Doggett, 1988) is based on panicle shape, length, glumes and grain shape characteristics. All genotypes used in this study represent different sorghum races. Thus, differences in panicle length among genotypes were highly expected (Table 4). Hybrid checks and testcross hybrids were not significantly ($P < 0.05$) different from each other - however, they were significantly different from SC lines and tester lines. PLE means across environments were 27.55, 27.51, 23.71 and 24.78 cm for hybrid checks, testcross

hybrids, SC lines and tester lines respectively. Furthermore, contrast comparisons between hybrid checks vs SC lines and tester lines, and SC lines and tester lines vs testcross hybrids were significant ($P < 0.05$) (Table 4).

A significant genotype by environment interaction was detected for all genotypes except hybrid checks, indicating the shift of ranks in panicle length of genotypes across environments except for the checks. In individual environments, the same trend was observed as that across environments. Higher numerical values of panicle length were observed for hybrid checks and testcross hybrids followed by lower values of SC lines and tester lines. Higher values of panicle length were observed in CS04 (Table 8), followed by CS03 (Table 6), CC03 (Table 7) and WE 03 (Table 5).

General Combining Ability (GCA) Effects

According to pedigree information, SC lines were classified into five different sorghum races. In this study, the race Bicolor was represented by SC214, SC211, SC155 (Durra-Bicolor) and SC192 (Guinea-Bicolor). The race Guinea was represented by SC250, SC283 and SC303. The race Caudatum was represented by SC326, SC333, SC392, SC798, RTx430, RTx436 and BTx623 (Kafir-Caudatum). The race Durra was represented by SC441 and SC748 (Durra-Caudatum) while the race Kafir was represented by SC625, SC628, SC680 which are Kafir-Caudatum and BTx399 is a Kafir-Milo (Table 1).

Kafir Race. The highest average GCA effects for GYL across environments were observed in the Kafir race with a value of $0.30 \text{ MT}\cdot\text{ha}^{-1}$ (Table 9). Furthermore, the Kafir race showed a desirable low GCA effects for DMA indicating early maturity; low GCA effects for PEX indicating that genotypes within this race might produce hybrids with shorter exertion; and negative GCA effect for PHE indicating shorter plants. This GCA effect for GYL from the Kafir race was numerically higher than that of the tester lines (Table 9). Within the Kafir group, the line SC625 consistently had the highest GCA effect across environments (Table 10). Moreover, SC625 was the line that showed the highest GCA effects among all the lines evaluated in this study. Also, this line showed a constant average positive GCA effect across environments (except in CS03) for DMA with a value of 49 days (Table 11); a negative GCA value across environments for PHE (-14.62 cm) (Table 12) and an average positive effect across environments for panicle exertion (0.91 cm) (Table 13). The other two SC lines representing this race (SC628 and SC680), also showed positive and constant GCA effects across environments increasing the average on GCA estimates in the Kafir group. Detailed GCA values for each lines and traits can be observed in Tables 10-16.

Durra Race. Lines belonging to the Durra race showed the second highest average GCA across environments for GYL with a value of $0.27 \text{ MT}\cdot\text{ha}^{-1}$ (Table 9). The two lines in the Durra race, SC441 and SC748 showed their highest GCA effect for grain yield in CS03 and the lowest in CC03 and CS04. SC441 showed desirable negative SCA values for DMA (Table 11) which indicates that an early maturing hybrid can be obtained when this line is used; a similar trend can be observed with negative favorable

GCA values for PHE (Table 12). It is important to observe the high GCA effects for SEW across environments shown by SC748. This is an indication of the potential of this line to increase seed weight as a component of GYL.

Caudatum Race. The Caudatum race showed negative unfavorable GCA effects for GYL. However, positive average GCA effects across environments were observed for SEW with a value of 1.37 gm. Furthermore, the line SC798, showed the second highest (among all genotypes in the test) stable GCA effects for GYL across environments with $0.44 \text{ MT}\cdot\text{ha}^{-1}$ (Table 9 and 10) but with unfavorable unstable positive GCA effect for DMA (Table 11) as well as a positive non favorable GCA value for PHE and positive favorable value for PEX, indicating that late maturity, tall, large exertion hybrids could be obtained when this line is used in hybrid combinations. For the rest of the lines that represented this race, SC333 showed a positive GCA effect for GYL while SC326 and SC392 showed a negative one decreasing the mean of GCA effects for GYL for the whole group of lines in the race.

Guinea Race. The lowest average GCA effects for GYL across environments were observed in the Guinea race with a value of $-0.30 \text{ MT}\cdot\text{ha}^{-1}$ (Table 9). Moreover, the tallest hybrids could be obtained from lines that represent this race, suggested by positive undesirable GCA effects for PHE, the highest values in the whole test. However, average GCA across environments effects for PAN were desirably positive, possibly indicating that number of tillers could be increased in hybrids when these lines are used in hybrid combinations, specially SC283 (Table 14).

Bicolor Race. Lines from the race bicolor showed the second lowest average GCA effects across environments for GYL with a value of $-0.243 \text{ MT}\cdot\text{ha}^{-1}$ (Table 9). However, this race showed a positive GCA effect for PEX, indicating that improvement for panicle exertion could be obtained from these lines. Furthermore, the line SC155 showed a positive, desirable and stable GCA effect for panicle length across environments, actually the highest in the whole test (Table 16).

The information obtained from estimation of GCA effects helps to facilitate the identification of lines with favorable traits, when combined in hybrids combination might help to identify superior genotypes. For instance, an increase in grain yield might possibly be achieved by combining the high GYL potential of SC625 with SEW potential of SC333 and SC748. Similarly, a reduction in DMA and PHE may be effected, using SC441 and SC628 respectively. In general, every sorghum race represented in this research showed at least one desirable trait that has a potential use in the future in sorghum breeding programs.

Table 10. General Combining Ability (GCA) estimates for grain yield and other agronomic characters for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	†GYL	DMA	PHE	PEX	PAN	SEW	PLE
Bicolor	Durra-bicolor	SC155	-0.243	-0.185	-2.788	0.505	0.645	-0.275	-0.065
		SC214							
	SC311								
	Guinea-bicolor	SC192							
Guinea		SC250	-0.307	-0.053	16.580	-1.213	12.283	-1.320	1.953
		SC283							
		SC303							
Caudatum		SC326	-0.015	0.993	0.945	0.520	-6.258	1.375	0.888
		SC333							
		SC392							
		SC798							
Durra (Durra-Caudatum)		SC441	0.275	-0.305	3.560	-1.155	-7.105	1.035	-2.500
		SC748							
Kafir (Kafir-Caudatum)		SC625	0.300	0.037	-15.910	0.010	-0.667	-0.813	-1.193
		SC628							
		SC680							
Kafir-Milo		Testers B.TX399	0.005	-0.013	0.077	0.005	0.040	0.000	-0.013
	Caudatum (Kafir-Caudatum)	B.TX623 R.TX436 R.TX430							
S.E. (GCA for line)			0.190	0.58	3.09	0.72	5.75	0.37	0.91
S.E. (GCA for tester)			0.140	0.44	2.33	0.55	4.35	0.28	0.69
S.E. (g _i - g _j) line			0.260	0.81	4.37	1.02	8.14	0.53	1.29
S.E. (g _i - g _j) tester			0.200	0.62	3.3	0.77	6.15	0.4	0.97

†GYL = Grain yield, DMA = days to mid-anthesis, PHE = plant height, PEX = panicle exsertion, PAN = number of panicles per plot, SEW = 500-seed weight, PLE = panicle length

Table 11. General Combining Ability (GCA) estimates for grain yield (MT·ha⁻¹) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations
		Lines					
Bicolor	Durra-bicolor	SC155	-0.34	-0.21	0.16	0.17	-0.07
		SC214	-0.61	-0.60	-0.86	-0.11	-0.55
		SC311	-0.34	0.19	0.15	0.02	0.08
	Guinea-bicolor	SC192	-0.51	-0.28	-0.76	-0.18	-0.43
Guinea		SC250	-0.21	-0.24	-0.32	-0.02	-0.18
		SC283	-0.02	-0.08	0.33	-0.25	0.00
		SC303	-1.01	-0.63	-1.07	-0.22	-0.74
Caudatum		SC326	0.39	0.38	0.76	-0.28	-0.25
		SC333	-0.41	0.10	0.86	-0.19	0.10
		SC392	-0.58	-0.23	-0.70	0.12	-0.35
		SC798	0.38	0.68	0.60	-0.06	0.44
Durra (Durra-Caudatum)		SC441	1.20	-0.17	-0.04	0.07	0.27
		SC748	0.83	0.21	0.26	-0.02	0.28
Kafir (Kafir-Caudatum)		SC625	0.73	0.32	0.85	0.39	0.58
		SC628	0.50	0.21	-0.36	0.21	0.11
		SC680	0.16	0.28	0.12	0.38	0.21
Kafir-Milo	Testers						
		B.TX399	0.16	0.06	-0.33	0.07	-0.01
Caudatum (Kafir-Caudatum)		B.TX623	0.51	-0.37	0.69	0.23	0.28
		R.TX436	0.15	0.44	0.42	-0.14	0.24
		R.TX430	-0.90	-0.14	-0.77	-0.17	-0.49
S.E. (GCA for line)			0.20	0.17	0.16	0.23	0.19
S.E. (GCA for tester)			0.15	0.13	0.12	0.17	0.14
S.E. (g _i - g _j) line			0.28	0.24	0.23	0.32	0.26
S.E. (g _i - g _j) tester			0.21	0.18	0.17	0.24	0.20

Table 12. General Combining Ability (GCA) estimates for days to mid-anthesis for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations
		Lines					
Bicolor	Durra-bicolor	SC155	1.64	1.31	1.71	-0.49	0.93
		SC214	0.31	-1.03	0.13	1.01	-0.01
		SC311	-1.19	-0.53	-2.20	0.90	-0.89
	Guinea-bicolor	SC192	-1.58	0.14	0.63	-1.99	-0.77
Guinea		SC250	-0.69	0.22	-0.04	0.90	0.03
		SC283	0.14	0.56	1.80	0.44	0.58
		SC303	-0.97	0.06	-2.29	0.62	-0.77
Caudatum		SC326	0.56	0.06	0.71	-1.29	2.34
		SC333	7.48	0.81	3.88	0.36	2.83
		SC392	-3.11	-1.20	-1.87	-1.20	-1.96
		SC798	3.71	-0.03	-1.45	1.81	0.76
Durra (Durra-Caudatum)		SC441	-1.52	-0.03	-1.29	-0.56	-0.96
		SC748	2.14	0.14	-1.95	0.44	0.35
Kafir (Kafir-Caudatum)		SC625	0.39	0.72	1.55	-0.24	0.49
		SC628	-2.58	-1.28	0.13	-1.57	-1.35
		SC680	2.92	0.06	0.55	1.09	0.97
Kafir-Milo	Testers						
		B.TX399	-1.28	-1.28	-1.83	-1.02	-1.35
Caudatum (Kafir-Caudatum)		B.TX623	0.49	0.33	0.32	-0.30	0.14
		R.TX436	-0.28	-0.01	-0.56	-0.35	-0.38
		R.TX430	1.31	0.95	2.07	1.65	1.54
S.E. (GCA for line)			0.82	0.40	0.35	1.19	0.58
S.E. (GCA for tester)			0.62	0.30	0.26	0.90	0.44
S.E. (g _i - g _j) line			1.16	0.56	0.49	1.68	0.81
S.E. (g _i - g _j) tester			0.88	0.42	0.37	1.27	0.62

Table 13. General Combining Ability (GCA) estimates for plant height (cm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

	Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations	
	Lines						
Bicolor	Durra-bicolor	SC155	-12.09	-9.74	-9.74	7.22	-5.94
		SC214	-13.78	-6.35	-6.35	-5.27	-7.80
		SC311	-3.62	-0.64	-0.64	6.38	0.01
	Guinea-bicolor	SC192	-2.35	0.21	0.21	11.67	2.58
Guinea		SC250	24.75	18.63	18.63	0.87	15.86
		SC283	3.37	3.60	3.60	-4.42	1.62
		SC303	32.79	35.35	35.35	25.00	32.26
Caudatum		SC326	-10.39	-8.26	-8.26	-1.46	-6.30
		SC333	9.08	6.14	6.14	-0.82	5.40
		SC392	-10.18	-8.89	-8.89	0.24	-7.29
		SC798	22.94	11.85	11.85	-0.61	11.97
Durra (Durra-Caudatum)		SC441	-6.16	-9.95	-9.95	-16.48	-10.49
		SC748	27.88	19.90	19.90	3.20	17.61
Kafir (Kafir-Caudatum)		SC625	-18.44	-15.03	-15.03	-10.56	-14.62
		SC628	-29.48	-24.34	-24.34	-5.05	-20.76
		SC680	-12.26	-12.49	-12.49	-9.92	-12.35
Kafir-Milo	Testers						
	B.TX399	-9.65	-10.58	-10.58	-0.13	-7.72	
Caudatum (Kafir-Caudatum)	B.TX623	21.20	14.45	14.45	4.05	13.95	
	R.TX436	-4.47	-3.81	-3.81	-5.11	-4.16	
	R.TX430	-8.09	-0.05	-0.05	1.19	-1.76	
S.E. (GCA for line)		3.98	1.32	1.31	7.20	3.09	
S.E. (GCA for tester)		3.01	1.00	0.99	5.45	2.33	
S.E. (g _i - g _j) line		5.63	1.87	1.85	10.19	4.37	
S.E. (g _i - g _j) tester		4.26	1.41	1.40	7.70	3.30	

Table 14. General Combining Ability (GCA) estimates for panicle exertion (cm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations
		Lines					
Bicolor	Durra-bicolor	SC155	-2.47	-1.60	-1.61	-3.41	-2.27
		SC214	2.18	4.33	4.31	0.19	2.76
		SC311	0.07	0.30	0.29	0.40	0.46
	Guinea-bicolor	SC192	1.97	-0.54	-0.56	3.36	1.07
Guinea		SC250	-0.99	-3.51	-3.73	-2.99	-2.80
		SC283	0.70	1.36	1.35	-0.03	0.85
		SC303	-1.84	-2.24	-2.04	-0.66	-1.69
Caudatum		SC326	3.24	4.11	4.10	1.88	1.38
		SC333	-1.20	-0.97	-0.77	1.03	-0.47
		SC392	-1.20	-1.18	-1.19	0.19	-0.84
		SC798	2.22	3.06	3.04	-0.03	2.01
Durra (Durra-Caudatum)		SC441	0.91	0.94	0.93	-1.51	0.33
		SC748	-4.38	-2.87	-2.88	-0.45	-2.64
Kafir (Kafir-Caudatum)		SC625	1.55	0.09	0.08	1.88	0.91
		SC628	0.84	-0.12	-0.13	1.24	0.38
		SC680	-1.47	-1.18	-1.19	-1.09	-1.26
Kafir-Milo	Testers						
		B.TX399	1.44	1.15	1.14	1.14	1.27
Caudatum (Kafir-Caudatum)		B.TX623	0.91	0.62	0.61	1.03	0.81
		R.TX436	1.13	1.36	1.40	-0.66	0.82
		R.TX430	-3.72	-3.14	-3.15	-1.51	-2.88
S.E. (GCA for line)			0.99	0.86	0.84	1.86	0.72
S.E. (GCA for tester)			0.75	0.65	0.63	1.41	0.55
S.E. (g _i - g _j) line			1.40	1.21	1.19	2.64	1.02
S.E. (g _i - g _j) tester			1.06	0.92	0.90	1.99	0.77

Table 15. General Combining Ability (GCA) estimates for number of panicles/plot for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations
		Lines					
Bicolor	Durra-bicolor	SC155	-16.52	-10.37	-14.06	-6.20	-9.44
		SC214	3.35	11.45	2.44	8.13	6.88
		SC311	-9.27	2.55	0.44	-2.37	-1.70
	Guinea-bicolor	SC192	12.48	4.10	1.86	7.46	6.84
Guinea		SC250	2.90	-0.40	-4.34	13.88	3.59
		SC283	18.98	11.76	65.19	1.96	24.90
		SC303	2.23	9.93	27.27	6.05	8.36
Caudatum		SC326	0.07	3.10	-4.40	-8.70	-3.77
		SC333	-7.56	-7.07	-9.98	0.23	-5.96
		SC392	-5.77	-6.73	-11.62	-9.45	-7.48
		SC798	-2.20	-3.82	-12.68	-7.54	-7.82
Durra (Durra-Caudatum)		SC441	3.73	-15.82	-4.68	-3.70	-4.43
		SC748	-7.02	-10.57	-13.90	-6.37	-9.78
Kafir (Kafir-Caudatum)		SC625	-2.52	-0.57	-4.65	-1.37	-1.91
		SC628	14.26	5.01	-11.80	15.30	4.45
		SC680	-7.74	7.18	-10.31	-7.29	-4.54
Kafir-Milo	Testers						
		B.TX399	13.53	10.98	4.62	4.90	8.81
Caudatum (Kafir-Caudatum)		B.TX623	4.50	6.10	4.71	2.32	4.94
		R.TX436	-0.41	8.20	5.51	-2.87	1.97
		R.TX430	-19.55	-24.47	-15.36	-4.25	-15.56
S.E. (GCA for line)			6.57	4.27	11.77	9.83	5.75
S.E. (GCA for tester)			4.97	3.23	8.90	7.43	4.35
S.E. (g _i - g _j) line			9.29	6.03	16.64	13.90	8.14
S.E. (g _i - g _j) tester			7.02	4.56	12.58	10.51	6.15

Table 16. General Combining Ability (GCA) estimates for 500-seed weight (gm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations
		Lines					
Bicolor	Durra-bicolor	SC155	-0.63	0.00	-0.24	-0.11	-0.25
		SC214	-2.39	-0.46	-1.72	0.07	-0.96
		SC311	-0.20	-0.42	-0.08	0.07	-0.12
	Guinea-bicolor	SC192	0.41	0.63	0.18	-0.24	0.23
Guinea		SC250	-0.94	-1.88	-1.22	-1.97	-1.53
		SC283	-0.82	-0.93	-0.73	-0.85	-0.85
		SC303	-2.38	-1.82	-1.92	0.25	-1.58
Caudatum		SC326	-0.37	-0.10	-0.25	0.23	-0.09
		SC333	3.48	1.86	3.40	0.34	2.21
		SC392	2.65	1.43	1.89	1.43	1.82
		SC798	1.63	2.11	1.58	-0.10	1.56
Durra (Durra-Caudatum)		SC441	1.85	-0.02	1.40	-0.16	0.69
		SC748	1.07	1.57	1.49	0.92	1.38
Kafir (Kafir-Caudatum)		SC625	-3.01	-1.07	-2.08	-0.07	-1.49
		SC628	-2.17	-0.31	-0.94	0.33	-0.61
		SC680	-0.81	-0.46	-0.18	-0.16	-0.34
Kafir-Milo	Testers						
		B.TX399	0.60	-0.20	0.97	-0.33	0.20
Caudatum (Kafir-Caudatum)		B.TX623	-0.62	-0.59	-0.59	-0.10	-0.43
		R.TX436	-1.14	-0.26	-1.04	0.08	-0.56
		R.TX430	1.21	1.03	0.64	0.34	0.79
S.E. (GCA for line)			0.24	0.57	0.25	0.66	0.37
S.E. (GCA for tester)			0.18	0.43	0.19	0.50	0.28
S.E. (g _i - g _j) line			0.34	0.80	0.35	0.93	0.53
S.E. (g _i - g _j) tester			0.26	0.60	0.27	0.71	0.40

Table 17. General Combining Ability (GCA) estimates for panicle length (cm) for 16 lines and 4 testers in a line x tester design evaluated in four environments, and the combined analysis.

		Lines/ Testers	College Station (2003)	Corpus Christi (2003)	Weslaco (2003)	College Station (2004)	Combined locations
		Lines					
Bicolor	Durra-bicolor	SC155	2.09	4.28	3.51	0.89	2.63
		SC214	-0.66	0.81	-1.36	2.36	0.15
		SC311	2.09	1.04	-0.30	-1.13	0.39
	Guinea-bicolor	SC192	-4.89	-3.04	-2.63	-3.04	-3.43
Guinea		SC250	6.33	3.32	3.22	2.68	3.90
		SC283	1.67	0.56	0.55	-0.82	0.46
		SC303	0.40	-1.34	2.87	4.16	1.50
Caudatum		SC326	0.19	0.78	2.24	-1.03	1.40
		SC333	2.94	0.99	0.33	0.56	1.30
		SC392	1.25	2.43	1.39	1.83	1.60
		SC798	-0.62	-1.55	-1.57	0.56	-0.75
Durra (Durra-Caudatum)		SC441	-0.66	-1.13	-0.01	-1.98	-0.86
		SC748	-6.85	-1.98	-6.23	-1.45	-4.14
Kafir (Kafir-Caudatum)		SC625	-2.56	-1.34	1.39	0.03	-0.59
		SC628	-5.47	-2.19	-1.15	-1.66	-2.61
		SC680	4.00	-0.92	-1.99	-1.88	-0.38
Kafir-Milo	Testers						
		B.TX399	-2.09	-2.42	-1.68	-1.04	-1.79
Caudatum (Kafir-Caudatum)		B.TX623	0.19	-2.14	-1.47	0.72	-0.70
		R.TX436	1.88	0.78	0.04	-0.79	0.52
		R.TX430	0.02	3.58	3.17	1.09	1.92
S.E. (GCA for line)			1.76	0.90	0.90	1.73	0.91
S.E. (GCA for tester)			1.33	0.68	0.68	1.30	0.69
S.E. ($g_i - g_j$) line			2.49	1.27	1.27	2.44	1.29
S.E. ($g_i - g_j$) tester			1.88	0.96	0.96	1.84	0.97

Specific Combining Ability (SCA), Mid and Better Parent Heterosis (MPH and BPH)

Grain Yield (GYL). The top six performing crosses having numerically high positive SCA effects (Table 18) and numerically high GYL with their respective values of MPH (Table 19) and BPH (Table 20) were:

1. BTx623 * SC798: SCA = 0.74, 3.48 MT·ha⁻¹, MPH = 107.37 and BPH = 76.95;
2. A3Tx436 * SC628: SCA = 0.51, 2.87 MT·ha⁻¹, MPH = 79.95 and BPH = 50.69;
3. A3Tx430 * SC214: SCA = 0.50, 1.48 MT·ha⁻¹, MPH = 1.17 and BPH = -13.48;
4. BTx623 * SC748: SCA = 0.49, 3.06 MT·ha⁻¹, MPH = 85.15 and BPH = 56.14;
5. BTx623 * SC441: SCA = 0.40, 2.96 MT·ha⁻¹, MPH = 79.95 and BPH = 50.69;
6. A3Tx430 * SC283: SCA = 0.40, 1.93 MT·ha⁻¹, MPH = 12.57 and BPH = 12.57;

The Durra line SC441 and the Durra-Caudatum line SC748 showed high positive values for SCA effects as well as positive high values of MPH and BPH when crossed with the Kafir-Caudatum tester BTx623, suggesting that these lines might belong to different heterotic groups. However, heterotic relationships in sorghum have been explained for the past fifty years as a separation of genotypes according to their fertility reaction as R-lines and A/B-lines. In this specific case, the two Durra lines have an R fertility reaction type, while the tester BTx623 is a B fertility reaction type, resulting in the explanation of the high GYL shown by these two crosses. It is important to notice that BTx623 is an elite line which has been tested over the years in TAES breeding programs, while SC441 and SC748 are just conversion lines and improvement in their

traits has not been done. Thus, these high values of GYL, MPH and BPH from these two specific crosses might be the result of the outstanding combiner capacity of BTx623. Moreover, lines from the Caudatum race, SC326, SC333, SC392 and SC798 clearly showed positive values of SCA, MPH and BPH when crossed with BTx623; however, these four lines possess an R fertility reaction, possibly the reason for these positive high values. This is also in accordance with the logistical separation of A/B-lines and R-lines in sorghum, which has been proven to maximize heterotic responses (Gabriel, 2005). It is also noticeable that BTx623 is considered a Kafir-caudatum line; perhaps BTx623 has more of Kafir in its genetic background than Caudatum. This would also explain the heterotic response between SC441 a Durra-Caudatum line when crossed with BTx623, suggesting that the heterotic response in the BTx623 * SC441 cross comes from the Kafir-Durra response. Furthermore, the Caudatum lines except for SC326 also showed high values of SCA, positive values of MPH and intermediate values of BPH when crossed with the Kafir-Milo tester BTx399, indicating a good heterotic response between these two races, and also verifying more Kafir genetic background in BTx623. For this specific research, the data suggests that good heterotic responses can be obtained from the crosses derived from Durra-Kafir races and Caudatum-Kafir races. However, these data and results should be interpreted with caution, since this data would also seem to support and confirm that exploitation of heterosis in sorghum can be achieved by keeping lines separate by their fertility reaction type, by their genetic background or both. For that reason, crosses between lines from different races do not necessarily result in a good heterotic response. Similarly, crosses between lines with different fertility

Table 18. Specific Combining Ability (SCA) values of line x tester hybrids for grain yield ($\text{MT}\cdot\text{ha}^{-1}$), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo	Caudatum (Kafir-Caudatum)		
			B.TX399	B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-0.06	0.00	0.35	-0.30
		SC214	-0.26	-0.25	-0.01	0.50
		SC311	0.27	-0.19	0.16	-0.26
	Guinea-bicolor	SC192	0.18	-0.41	0.09	0.13
Guinea		SC250	-0.21	-0.34	0.24	0.30
		SC283	-0.08	-0.33	-0.02	0.40
		SC303	0.03	-0.26	-0.10	0.30
Caudatum		SC326	-0.11	0.29	-0.02	0.08
		SC333	0.15	0.23	-0.34	-0.06
		SC392	0.10	0.18	-0.39	0.10
		SC798	0.27	0.74	-0.71	-0.07
Durra (Durra-Caudatum)		SC441	-0.12	0.40	-0.22	-0.08
		SC748	-0.23	0.49	0.39	-0.68
Kafir (Kafir-Caudatum)		SC625	0.08	-0.16	0.09	-0.03
		SC628	-0.25	-0.16	0.51	-0.11
		SC680	0.20	0.06	-0.03	-0.25

S.E. (SCA for effects) = 0.37

S.E. ($s_{ij} - s_{kl}$) = 0.52

Contribution of lines = 43.95%

Contribution of testers = 32.15%

Contribution of (line x tester) = 25.13%

Table 19. Mid Parent Heterosis (MPH) values of line x tester hybrids for grain yield ($\text{MT}\cdot\text{ha}^{-1}$), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo	Caudatum (Kafir-Caudatum)		
			B.TX399	B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	25.51	63.38	92.66	-5.92
		SC214	-30.90	-5.97	9.94	1.17
		SC311	59.53	62.95	92.31	11.49
	Guinea-bicolor	SC192	10.37	-0.57	35.17	-7.68
Guinea		SC250	-0.52	18.97	59.89	20.78
		SC283	-2.60	6.78	24.50	12.57
		SC303	-2.41	9.15	23.88	2.93
Caudatum		SC326	14.29	62.52	46.48	15.58
		SC333	25.48	57.67	24.67	2.19
		SC392	24.86	67.24	23.33	11.95
		SC798	50.03	107.37	21.55	22.84
Durra (Durra-Caudatum)		SC441	20.76	79.95	43.89	13.34
		SC748	14.91	85.15	81.60	-26.05
Kafir (Kafir-Caudatum)		SC625	43.07	57.10	73.43	29.97
		SC628	-7.97	18.68	55.89	-13.29
		SC680	29.58	48.16	44.65	-6.71

Table 20. Better Parent Heterosis (BPH) values of line x tester hybrids for grain yield ($\text{MT}\cdot\text{ha}^{-1}$), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo	Caudatum (Kafir-Caudatum)		
			B.TX399	B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-16.26	12.73	34.70	-32.42
		SC214	-46.65	-23.92	-9.41	-13.48
		SC311	5.42	11.26	32.99	-20.84
	Guinea-bicolor	SC192	-21.56	-26.48	1.51	-28.44
Guinea		SC250	-27.88	-10.10	22.79	-4.12
		SC283	-14.01	-0.15	19.02	12.57
		SC303	-42.04	-33.78	-24.21	-35.91
Caudatum		SC326	-3.88	44.25	32.74	9.27
		SC333	0.75	33.12	7.32	-8.48
		SC392	-21.68	7.81	-19.63	-25.42
		SC798	21.65	76.95	5.79	11.30
Durra (Durra-Caudatum)		SC441	-3.77	50.69	22.83	0.61
		SC748	-7.83	56.14	56.15	-33.85
Kafir (Kafir-Caudatum)		SC625	19.06	37.85	55.33	21.37
		SC628	-16.81	13.82	52.95	-15.56
		SC680	8.06	30.31	29.86	-12.67

reaction types do not ensure a good heterotic response either. For example: Lines from the race Bicolor would be expected to have a highly superior SCA with all four testers, since the testers do not have any Bicolor in their genetic background. Furthermore, all the lines with R fertility reaction would have been expected to have higher values of SCA effects when crossed with BTx399 and BTx623.

From all the races represented in this study at least one of their lines made it to the top six crosses. Also, it is noticeable that certain lines also have above average performance within a same race, e.g. the crosses RTx430 * SC214 from the Bicolor race, RTx430 * SC283 from the Guinea race, and RTx436 * SC628 from the Kafir race. It is also notable that out of the six top crosses, BTx623 was present in three of them, verifying the great tester this line is.

Other Traits. A close look at SEW was made as a very important component of grain yield. However, the same trend was observed as previously described for GYL (Table 21 and 22). For the other six traits, the data is self explanatory and is reported in tables A1 to A17 in the appendix.

Table 21. Specific Combining Ability (SCA) values of line x tester hybrids for 500-seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo	Caudatum (Kafir-Caudatum)		
			B.TX399	B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-0.64	-0.09	0.09	0.65
		SC214	0.09	-0.21	0.88	-0.68
		SC311	0.54	0.51	-0.17	-0.88
	Guinea-bicolor	SC192	-0.74	0.13	0.48	0.13
Guinea		SC250	-0.62	-0.10	0.31	0.41
		SC283	0.54	0.42	-0.31	-0.64
		SC303	0.64	-0.18	-0.16	-0.30
Caudatum		SC326	0.30	-1.02	-0.01	0.72
		SC333	0.06	0.61	-1.29	0.62
		SC392	0.85	0.14	-0.25	-0.74
		SC798	-0.10	0.58	-0.12	-0.32
Durra (Durra-Caudatum)		SC441	-0.02	0.28	-0.33	0.06
		SC748	-0.78	-0.13	0.47	0.44
Kafir (Kafir-Caudatum)		SC625	-0.22	0.13	0.34	-0.25
		SC628	-0.15	-0.31	0.10	0.36
		SC680	-0.02	-0.27	-0.08	0.37

S.E. (SCA for effects) = 0.75

S.E. ($s_{ij} - s_{kl}$) = 1.06

Contribution of lines = 72.55%

Contribution of testers = 15.29%

Contribution of (line x tester) = 11.79%

Table 22. Mid Parent Heterosis (MPH) values of line x tester hybrids for 500-seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo	Caudatum (Kafir-Caudatum)		
			B.TX399	B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	1.88	9.26	21.53	17.00
		SC214	3.60	4.23	24.54	3.22
		SC311	8.37	11.40	16.10	3.30
	Guinea-bicolor	SC192	2.75	12.44	26.41	14.48
Guinea		SC250	-2.94	4.11	18.82	10.85
		SC283	6.51	8.83	13.05	3.11
		SC303	4.37	0.73	11.11	2.66
Caudatum		SC326	8.74	1.64	20.31	17.29
		SC333	-4.04	1.13	-4.04	3.27
		SC392	7.53	5.54	11.08	2.07
		SC798	10.97	19.31	24.49	14.54
Durra (Durra-Caudatum)		SC441	11.21	17.15	23.17	17.23
		SC748	5.02	12.79	27.85	18.65
Kafir (Kafir-Caudatum)		SC625	0.72	6.62	19.68	6.16
		SC628	3.29	5.07	19.03	12.64
		SC680	0.92	1.67	12.72	8.92

Table 23. Better Parent Heterosis (BPH) values of line x tester hybrids for seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo	Caudatum (Kafir-Caudatum)		
			B.TX399	B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-13.42	-1.74	19.73	0.11
		SC214	-13.31	-7.82	20.43	-13.06
		SC311	-5.09	3.49	13.73	-8.88
	Guinea-bicolor	SC192	-10.99	3.24	25.41	-0.13
Guinea		SC250	-21.39	-11.12	10.47	-9.66
		SC283	-9.77	-2.46	10.95	-12.06
		SC303	-13.69	-12.04	5.94	-14.54
Caudatum		SC326	-6.58	-7.51	20.08	1.47
		SC333	-12.77	-13.19	-23.72	-6.81
		SC392	5.89	-2.41	-5.63	-0.29
		SC798	1.49	16.12	16.37	5.55
Durra (Durra-Caudatum)		SC441	-3.49	7.77	21.94	2.45
		SC748	-3.96	9.76	19.53	9.32
Kafir (Kafir-Caudatum)		SC625	-18.60	-9.18	11.00	-13.65
		SC628	-12.62	-5.97	16.63	-4.06
		SC680	-10.04	-3.73	8.26	-2.19

Amplified Fragment Length Polymorphism (AFLP) Analysis

Cluster Analysis

A total of 1338 AFLP markers were identified and scored in the total set composed of four testers, seventeen SC lines (Table 1), and an extra observation of SC303 (IS3620). Values from GS ranged from 0.80 to 0.49 with an average of 0.64. The maximum GS value was obtained between SC680 (Guinea-Caudatum) and SC798 (Caudatum). Association among the lines evaluated in this study based on GS between pair of lines indices is presented in Fig. 1. The strongest groups identified by the cluster analysis are: Group 1, composed of Caudatum lines SC333, SC798 and a Guinea-Caudatum SC680; Group 2, composed of SC192 (Guinea-Bicolor), SC214 (Bicolor) and SC441 (Caudatum); Group 3, composed of the two Caudatum lines RTx430 and RTx436; Group 4, composed of two Guinea lines SC250 and SC283; Group 5, composed of BTx399 (Kafir), SC628 and SC625 that are Kafir-Caudatum. It seems that the identification of these five groups is based neither on sorghum races nor the lines' fertility reaction. There are discrepancies in affirming one or the other reason that justify the clustering. For example, lines from group 4 were not clustered with SC303 even though they belong to the same Guinea race. Moreover, clustering did not result in grouping of these lines by their fertility reaction either, since SC250 is an R- line; it should have been in a different cluster from SC283 and SC303 that have A/B fertility reaction type.

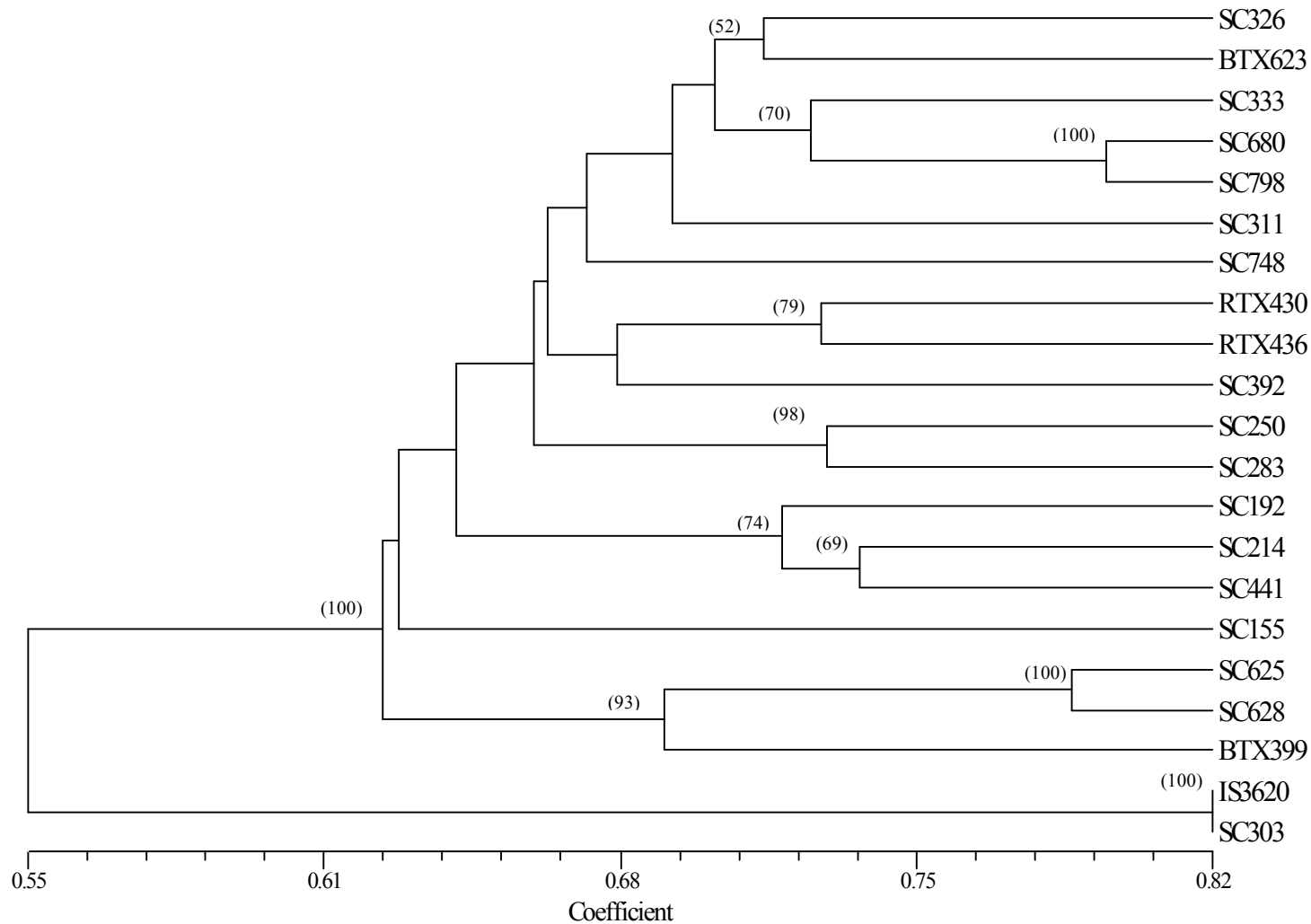


Figure 1. Dendrogram of 16 sorghum conversion lines and four testers lines revealed by cluster analysis of genetic similarity estimates generated by 1338 AFLP markers. Bootstrap confidence intervals are included in parentheses at the junction of each cluster

Another example is the clustering of lines in group 2; their clustering could be interpreted based on their fertility reaction since all three lines are restorer R-lines. However, a close look at their genetic background would show that these lines belong to a combination of races: Guinea-Bicolor, Bicolor and Durra respectively. Group 1 shows some accordance with race information as well as fertility reaction type, since all the lines from the group belong to the Caudatum race and possess R- fertility reaction type. However, these groups then should have been clustered with lines from group 3 that are also Caudatum and restorers of fertility.

There are lines that have not been clustered in any group. This could be the result of discrepancies or misclassification of these lines that have more than one race in their genetic background. For example, BTx623 possesses Kafir and Caudatum in its genetic background and was grouped closer to the Caudatum groups. Furthermore, it becomes more complex when lines from the Caudatum origin are distributed across several different clusters, suggesting that a lot of the lines utilized in this study have a substantial part of Caudatum in their genetic backgrounds. The clustering presented in this study should be interpreted with caution; another reason for not finding a clear clustering pattern among races represented here could be the lack of representation (in number of lines) of each race. There is an uncertain probability to pull individual lines within the same race that are completely apart (less related), indicating the high level of variation that exists within the races. A representative number of individuals by race might provide more detailed information about the existing variation in each race - if enough variation is found, identification of sub-races would be feasible. Based on Menz

et al., (2004) and Gabriel (2005), it is clear and evident that working groups and races in sorghum are not well defined. Studies including more lines per race or working group should provide a better interpretation of genetic diversity in sorghum.

Overall, Caudatum is the most homogenous race (average GS = 0.69), and this race on average is closely related to the Durra race (average GS = 0.66). The other two very homogenous races are Bicolor and Kafir with average GS of 0.67. It is also noticeable that race Bicolor is closely related to Durra with GS average of 0.67, the same level of similarity that can actually be seen within the Bicolor race.

Table 24. Average genetic similarities within and between races based on AFLP molecular markers.

Race	Bicolor	Guinea	Caudatum	Durra	Kafir
Bicolor	0.67				
Guinea	0.61	0.60			
Caudatum	0.65	0.63	0.69		
Durra	0.67	0.61	0.66	0.66	
Kafir	0.62	0.61	0.65	0.64	0.67

Correlation between Genetic Similarity and Hybrid Performance

Combined average values of GS were negatively correlated with GYL of testcross hybrids, SCA, MPH and HPH. Negative correlation ($P < 0.01$) between GS and SCA, GS and SCA, GS and MPH, and GS and BPH, combined across environments, was detected with r values of -0.46, -0.08, -0.35 and -0.50 respectively. Even though these correlations are significant, they are too low to be considered as predictors of hybrids' performance. Studies with genetic distances have demonstrated that genetic distances calculated from different heterotic groups and correlated to F1 hybrid grain yield, result in low correlation coefficients (Charcosset and Essioux, 1994). Although the r coefficients are self explanatory, according to trends observed from Menz et al., (2004) and Gabriel (2005), heterosis can decrease when diversity is excessively high. This suggests that, in general, the predictability of hybrid performance seems to be better when genetic similarities are high up to a certain threshold, depending on the germplasm under consideration (Betran et al., 2003; Moll et al., 1965)

CHAPTER V

CONCLUSIONS

Genetic diversity is present in the set of sixteen sorghum conversion lines evaluated in this study. AFLP technique allowed the identification of five strong clusters through the estimates of genetic similarities. However, these five groups do not agree with the classification proposed by Menz et al., (2005) where the lines evaluated in her study responded to sorghum race-groups. Contradictory to that study, the classification revealed by the cluster analysis for this study does not group the lines as sorghum germplasm has traditionally been done (either R- or B- lines). Different examples were mentioned in the discussion part regarding why these results should be interpreted with caution, especially with the lines that were not clustered under any race. It is proposed that this could be due to the result of misclassification of these lines that have more than one race in their genetic background. This interpretation becomes more complex, since the race caudatum is distributed across several different clusters, suggesting the strong presence of caudatum race in the lines evaluated in this study. Another reason for not finding a clear clustering pattern among the races in this study could be the lack of representation of the races in question. There is an uncertain probability to pull individual lines within the same race that are less related, indicating the high level of variation that exists within races. It is clear and evident and supported by Gabriel (2005) that working groups and races in sorghum are not well defined. Overall, Caudatum is the most homogenous race (average GS = 0.69), and this race on average is closely related to the Durra race (average GS = 0.66). The other two very

homogenous races are Bicolor and Kafir with average GS of 0.67. It is also noticeable that race Bicolor is closely related to Durra with GS average of 0.67.

GCA effects were estimated from the set of conversion lines. The highest GCA values were obtained for the Kafir and Caudatum races. The information obtained from GCA effects in this study might help in the identification of lines with favorable traits, since every sorghum race represented in this research showed at least one desirable trait that demonstrate their potential use in sorghum breeding programs. Estimations of SCA, MPH and BPH indicated specific crosses that were numerically superior to those of the checks. For this specific research, good heterotic responses were obtained from the crosses derived from Durra-Kafir races and Caudatum-Kafir races. However, these results should be interpreted with caution, since according to the field evaluation and supported by the AFLP analysis, exploitation of heterosis in sorghum can be obtained by keeping lines separate by either their fertility reaction type or by their genetic background or both. For that reason, crosses between lines from different races do not necessarily produce a good heterotic response. Moreover, crosses between lines with different fertility reaction type do not ensure a good heterotic response either. However, this kind of study provides information to identify specific combinations that would help to interpret heterotic relationships in sorghum.

REFERENCES

- Ahnert, D., M. Lee, D. F. Austin, C. Livini, S.J. Openshaw, J.S.C. Smith, K. Porter, and G. Dalton. 1996. Genetic diversity among elite sorghum inbred lines assessed with DNA markers and pedigree information. *Crop Sci.* 36:1385-1392.
- Ajmone M. P., P. Castiglioni, F. Fusari, M. Kuiper and M. Motto. 1998. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. *Theor. Appl. Genet.* 96:219-227.
- Arens, P., H. Coops, J. Jansen and B. Vosman. 1998. Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. *Mol. Ecol.* 7: 11-18.
- Barret, B.A. and K.K. Kidwell. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop Sci.* 38:1261-1271.
- Betrán, F. J., J. M. Ribaut, D. Beck, and D. Gonzalez de León. 2003. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. *Crop Sci* 43: 797-806.
- Bruce, A.B. 1910. The Mendelian theory of heredity and the augmentation of vigor. *Science.* 32:627-628.
- Carr, D.E. and M.R. Dudash. 2003. Recent approaches into the genetic basis of inbreeding depression in plants. *Phil. Trans. R. Soc. Lond. B* 358:1071-1084.
- Charcosset, A., and L. Essioux, 1994. The effect of population structure on the relation between heterosis and heterozygosity at marker loci. *Theor. Appl. Genet.* 89, 336-343.

- Cockerham, C.C. and Z.B. Zeng. 1996. Design III with marker loci. *Genetics* 143:1437-1456.
- Collins, G.N. 1921. Dominance and the vigor of first generation hybrids. *Amer. Nat.* 55:116-133.
- Crow, J.F. 1948. Alternative hypothesis of hybrid vigor. *Genetics* 33:477-487.
- Crow, J.F. 1952. Dominance and overdominance. *In* Gowen W (ed) *Heterosis*. Iowa State College Press. Ames, IA. pp. 282-297.
- Davenport, C.B. 1908. Degeneration, albinism and inbreeding. *Science* 28:454-455.
- Dillon, S.L., P.K. Lawrence and R.J. Henry. 2005. The new use of *Sorghum bicolor* derived SSR markers to evaluate the genetic diversity in 17 Australian Sorghum species. *Plant Genetic Resources: Characterization and Utilization*. 3: 19-28.
- Dudley, J.W., M.A. Saghai-Marooof and G.K. Rufener. 1991. Molecular markers and grouping of parents in maize breeding programs. *Crop. Sci.* 31: 718-723.
- East, E.M. 1908. Inbreeding in corn. *Rep. Conn.Agric. Exp. Stn.* 1907:419-428.
- Ehret, C. 1988. Language change and the material correlates of language and shift. *Antiquity*. 62:564-573.
- Fairbanks D., and W.R. Andersen. 1999. *Genetics, the continuity of life*. Pacific Grove, Brooks/Cole Pub, CA: Wadsworth Pub.
- Food and Agriculture Organization of the United Nations (FAOSTAT). 2006. Database of agricultural production. *FAO Statistical Databases*.
<http://faostat.fao.org/default.aspx>

- Gabriel, K. 2005. A study of heterotic relationships in sorghum. Ph.D. dissertation. Texas A&M University, College Station, TX.
- Gaiotto, F.A., M. Bramucci and D. Grattapaglia. 1997. Estimation of outcrossing rate in a breeding population of *Eucalyptus urophylla* with dominant RAPD and AFLP markers. *Theor. Appl. Genet.* 95: 842-849.
- Gilbert, M.L. 1994. Identification and search for heterotic patterns in sorghum. pp. 117-126. *In* D. Wikinson (ed.) *Proc. Annu. Corn and Sorghum Ind. Res. Conf.*, 49th, Chicago, IL. 9-10 Dec. 1994. Am. Seed Trade Assoc, Washington, DC.
- González, M., R. Rodríguez, M. E. Zavala, J. L. Jacobo, F. Hernández, J. Acosta, O. Martínez and J. Simpson. 1998. Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. *Phytopathology.* 88: 292-299.
- Grenier, C., M. Deu, S. Kresovich, P.J. Bramel-Cox and P. Hamon. 2000. Assessment of genetic diversity in three subsets constituted from the ICRISAT sorghum collection using random vs non-random sampling procedures B. using molecular markers. *Theor. Appl. Genet.* 101: 197-202.
- Haaland, R. 1995. Sedentism, cultivation and plant domestication in the Holocene middle Nile region. *J. of Field Archeol.* 22:157-174.
- Harlan J. R. and J. M. J. de Wet. 1972. A simplified classification of cultivated sorghum. *Crop. Sci* 12: 172-176.

- Harlan, J.R. 1989. The tropical African cereals. pp. 335-343. *In* D.R. Harris and G.C. Hillman (eds.), *Foraging and Farming: The Evolution of Plant Exploitation*. Unwin Hyman, London.
- Hillis, D., C. Moritz, and B. Mable. 1996. *Molecular systematics*. Sinauer Associates, Sinauer, Sutherland, MA.
- House, L.R. 1982. *El Sorgo: La Genetica del Sorgo*. Grupo Editorial Gaceta. Mexico City, Mexico.
- Huys, G., R. Coopman, P. Janssen and K. Kersters. 1996. High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. *Inst. J. Syst. Bacteriol.* 46: 572-580.
- Jin, H., L. Domier, F. L. Kolb, and C. M. Brown. 1998. Identification of quantitative loci for tolerance to barley yellow virus in oat. *Plant. Mol. Biol.* 35: 155-165.
- Jones C.J., K. J., Edwards, S. Castiglione, M. O. Winfield, F. Sala, C. van de Wiel, G. Bredemeijer, B. Vosman, M. Matthes, A. Daly, R. Brettschneider, P. Bettini, M. Buiatti, E. Maestri, N. Malcevski, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vasquez, and A. Karp. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol. Breed* 3:381-390.
- Jones, D.F. 1917. Dominance of linked factors as a means of accounting for heterosis. *Proc. Nat. Acad. Sci. Wash.* 3:310-312.
- Karper, R.E. and J.R. Quinby. 1937. Hybrid vigor in sorghum. *J. Hered.* 28:83-91.

- Keim, P., A. Kalif, J. Schupp, K. Hill, S.E. Travis, K. Richmond, D.M. Adair, M. Hugh-Jones, C.R. Kuske and P. Jackson. 1997. Molecular evolution and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. *J. Bacteriol.* 179: 818-824.
- Kemphorne, O., 1957. An introduction to genetic statistics. John Wiley and Sons, Inc; New York; Chapman & Hall, Ltd., London.
- Kimber, C.T. 2000. Origins of domesticated sorghum and its early diffusion to India and China. pp. 3-98. *In* Smith et al. (ed.). Sorghum: Origin, history, technology, and production. John Wiley & Sons, Inc, New York.
- Klein, P.E., R.R. Klein, S.W. Cartinhour, P.E. Ulanich, J. Dong, J.A. Obert, D.T. Morishige, S.D. Schlueter, K.L. Childs, M. Ale and J.E. Mullet. 2000. A high-throughput AFLP based method for constructing integrated genetic and physical maps: Progress toward a sorghum genome map. *Genome Res.* 10:789-807.
- Lee, R.D., B.E. Johnson, K.M. Eskridge, and J.F. Pedersen. 1992. Selection of superior female parents in sorghum utilizing A3 cytoplasm. *Crop Sci.* 32:918-921.
- Liu, Z., A. Nichols, P. Li, R.A. Dunham. 1998. Inheritance and usefulness of AFLP markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), and their F1, F2 and backcross hybrids. *Mol. Gen. Gent.* 258: 260-268.
- Mackill, D.J., Z. Zhang, E.D. Redona and P.M. Colowit. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome.* 39: 969-977.
- Majer, D., B.G. Lewis and R. Mithen. 1998. Genetic variation among field isolates of *Pyrenopeziza brassicae*. *Plant Pathol.* 47: 22-28.

- Maughan, P.J., M.A. Saghai Maroof, G.R. Buss and G.M. Huestis. 1996. Amplified fragment length polymorphism (AFLP) in soybean: Species diversity, inheritance, and near-isogenic line analysis. *Theor. App. Genet.* 93, 392-401.
- Maunder, A.B. 1999. History of cultivar development in the United States: From “Memoirs of A.B. Maunder-sorghum breeder”. pp. 191-223. *In* Smith et al. (ed.) *Sorghum: Origin, history, technology and production*. John Wiley & Sons, Inc., New York.
- Meadow, R.H. 1996. The origin and spread of agriculture and pastoralism in Northwestern South Asia. pp. 390-412. *In* D.R. Harris (ed.) *The origins and spread of agriculture and pastoralism in Eurasia*. UCL Press, London.
- Menz, M.A., R.R. Klein, J.E. Mullet, J.A. Obert, N.C. Unruh, and P.E. Klein. 2002. A high-density genetic map of *sorghum bicolor* (L.) Moench based on 2926 AFLP[®], RFLP and SSR markers. *Plant Mol. Biol.* 48:483-499.
- Menz, M.A., R.R. Klein, N.C. Unruh, W.L. Rooney, P.E. Klein, and J.E. Mullet. 2004. Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. *Crop Sci.* 44:1236-1244.
- Miller, F.R. 1984a. Registration of RTx430 sorghum. *Crop Sci.* 24:1224.
- Miller, F.R. 1984b. Registration of RTx432 sorghum. *Crop Sci.* 24:392.
- Miller, F.R. 1986. Registration of seven sorghum A- and B-line inbreds. *Crop Sci.* 26:216–217.

- Miller, F.R., J.A. Dahlberg, and P.W. Morgan. 1999. Registration of A3/B3 cytoplasmic–genetic male-sterile sorghum maturity and height parental lines. *Crop Sci.* 39:306–307.
- Miller, F.R., T.F. Dusek, K.L. Prihoda, and L.W. Rooney. 1992. Registration of RTx436 sorghum parental line. *Crop Sci.* 32:1518.
- Moll, R.H., J.H. Lonquist, J.V. Fortuna, and E.C. Johnson. 1965. The relationship of heterosis and genetic divergence in maize. *Genetics* 52:139–144.
- Mueller, U.G. and L. Wolfenbarger. 1999. AFLP genotyping and fingerprinting. *Trends Ecol. Evol.* 10: 389–394.
- Murdock, G.F. 1959. *Africa: Its people and their cultural history.* McGraw-Hill, London.
- Murty B. R. and J. N. Govil. 1967. Description of 70 groups in genus sorghum based on a modified Snowden's classification. *Indian J. Genet.* 27: 75-91.
- Murty, D.S. and K.A. Kumar. 1995. Traditional uses of sorghum and millets. pp. 185-221. *In* D.A.V. Dendy (ed.), *Sorghum and Millets: Chemistry and Technology.* American Association of Cereal Chemists, St. Paul, MN.
- Nei, M., and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76:5269-5273.
- Nesbitt, T.C. 1994. Evaluation of heterotic patterns in diverse sorghum hybrids. M.S. thesis, Texas A&M University, College Station, TX.

- Otsen, M., M. den Bieman, M.T. Kuiper, M. Pravenec, V. Kren, T.W. Kurtz, H.J. Jacob, A. Lankhorst, B.F. van Zutphen. 1996. Use of AFLP markers for gene mapping and QTL in the rat. *Genomics* 37: 289-294.
- Paglia, G. and M. Morgante. 1998. PCR-based multiplex DNA fingerprinting techniques for the analysis of conifer genomes. *Mol. Breed.* 4: 173-177.
- Qiao, K. and W. Zhenshan (ed.). 1970. Varieties of sorghum in China. Agricultural Publishing, Beijing.
- Quinby, J.R. 1974. Sorghum improvement and the genetics of growth. Texas A&M University Press, College Station, TX.
- Quinby, J.R. 1980. Interaction of genes and cytoplasm in male sterility in sorghum. pp. 175-184. *In* H.D. Loden and D. Wilkinson (eds.) Proc. 35th Ann. Corn and sorghum Ind. Res. Conf., Chicago, IL. 9-11 Dec. 1980, Am. Seed Trade Assoc., Washington, DC.
- Rooney, L.W., M.E. Blakely, F.R. Miller and D.T. Rosenow. 1980. Factors affecting the polyphenols of sorghum and their development and location in the sorghum kernel. pp. 25-35. *In* J.H. Hulse (ed.), Polyphenols in cereals and legumes. International Development Research Center, Ottawa, Ontario, Canada.
- Rooney, W.L. 2000. Techniques for developing new cultivars. pp. 329-347. *In* C.W. Smith and R.A. Frederiksen (eds.) Sorghum: Origin, history, technology and production. John Wiley & Sons, Inc., New York.

- Rosendhal, S. and J.W. Taylor. 1997. Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP. *Mol. Econ.* 6: 621-829.
- Rosenow, D.T. and J.A. Dahlberg. 2000. Collection, conversion, and utilization of sorghum. pp. 309-328. *In* C. W. Smith and R.A. Frederiksen (eds.) *Sorghum: Origin, history, technology and production*. John Wiley & Sons, Inc., New York.
- Saghai-Marouf, M.A., G.P. Yang, Q. Zhang and K.A. Gravois. 1997. Correlation between molecular marker distance and hybrid performance in U.S. Southern ling grain rice. *Crop. Sci.* 37: 145-150.
- Schertz, K.F. 1977. Registration of A₂Tx2753 and BTx2753 sorghum germplasm (Reg. no. GP30 and 31). *Crop Sci.* 17:983-983.
- Schertz, K.F. and J.C. Stephens. 1966. Compilation of gene symbols, recommended revisions and summary of linkage for inherited characters of *Sorghum vulgare* Pers. Texas Agricultural Experimental Station. Tech. Mono. 3, Texas A&M University, College Station, TX.
- Schertz, K.F. and J.M. Ritchey. 1978. Cytolasmic-genetic male-sterility systems in *Sorghum*. *Crop Sci.* 18:890-893.
- Semblat, J.P., E.Wajnerb, A. Dalmaso, P. Abad and P. Castagnone-Sereno. 1998. High-resolution DNA fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis. *Mol. Econ.* 7: 119-125.
- Shull, G.H. 1908. The composition of a field of maize. *Rep. Am. Breed. Assoc.* 4:296-301.

- Shull, G.H. 1952. Beginnings of the heterosis concept. pp. 14-48. *In* J.W. Gowen (ed.) Heterosis. Iowa State College Press. Ames, IA.
- Sneath, P.H.A. and R.R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco.
- Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York.
- Stephens, J.C. and R.F. Holland. 1954. Cytoplasmic male sterility for hybrid sorghum seed production. *Agron. J.* 46:20-23.
- Stephens, J.C., and R.E. Karper. 1965. Release of breeding stocks of male sterilized grain sorghum lines. Texas Agric. Exp. Stn. Miscellaneous Publication MP-758. Texas A&M University, College Station, TX.
- Stephens, J.C., F.R. Miller, and D.T. Rosenow. 1967. Conversion of alien sorghums to early combine genotypes. *Crop Sci.* 7:396.
- Stuber, C.W., S.E. Lincoln, D.W. Wolff, T. Helentjaris and E.S. Lander. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132:823-839.
- Tao, Y., J.M. Manners, M.M. Ludlow and R.G. Henzell. 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). *Theor. Appl. Genet.* 86: 679-688.
- Taramino, G., R. Tarchini, S. Ferrario, M. Lee and M.E. Pe. 1997. Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. *Theor. Appl. Genet.* 95: 66-72.
- Tohme, J., D. Orlando Gonzalez, S. Beebe and M.C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection. *Crop. Sci.* 36: 1375-1384.

- Troyer, A.F. 1999. Background of U.S. hybrid corn. *Crop Sci.* 39:601-626.
- Vierling, R.A., Z. Xiang, C.P. Joshi, M.L. Gilbert and H.T. Nguyen. 1994. Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theor. Appl. Genet.* 87: 816-820.
- Vos, P. and M. Kuiper. 1997. AFLP analysis, in DNA markers: protocols, applications and overviews. pp. 115-31. *In* Caetano-Anolles, G. and P. M. Gresshoff (eds.) Wiley-VCH, New York.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Pelman, M. Kuiper and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acid Research.* 23:4407-4414.
- Vuylsteke, M., R. Mank, B. Brugmans, P. Stam, and M. Kuiper. 2000. Further characterization of AFLP data as a tool in genetic diversity assessments among maize inbred lines. *Mol. Breed.* 6:265-276.
- White, P.S., M.L. Gilbert, H.T. Nguyen and R.A. Vierling. 1995. Maximum parsimony accurately reconstructs relationships of elite sorghum lines. *Crop. Sci.* 35: 1560-1565.
- Williams, C.E. and P.C. Ronald. 1994. PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization. *Nucl. Acids Res.* 22: 1917-1918.

- Winfield, M.O., G.M. Arnold, F. Cooper, M. Le Ray, J. White, A. Karp and K.J. Edwards. 1998. A study of genetic diversity on *Populus nigra* subsp. *betulifolia* in the Upper Severn area of the UK using AFLP markers. *Mol. Ecol.* 7:3-10.
- Yap, I.V. and R.J. Nelson. 1996. WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendograms. International Rice Research Institute. Manila, Philippines.
- Zhang, Q., Y. J. Gao, S. H. Yang, R. Ragab, M.A. Shagai Maroof, Z. B. Li. 1994. A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. *Theor. Appl. Genet* 89:185-192.
- Zhu, J., M. D. Gale, S. Quarrie, M. T. Jackson and G. J. Bryan. 1998. AFLP markers for the study of rice biodiversity. *Theor. Appl. Genet.* 96:602-611

APPENDIX

Table A1. Specific Combining Ability (SCA) values of line x tester hybrids for days to mid-anthesis, based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	0.43	-0.47	0.63	-0.54
		SC214	0.04	0.88	0.07	-0.93
		SC311	0.66	-0.66	0.02	0.03
	Guinea-bicolor	SC192	-1.04	0.56	-0.42	0.96
Guinea		SC250	-1.09	1.17	0.10	-0.14
		SC283	-1.56	0.66	-0.28	1.23
		SC303	2.38	0.43	-0.59	-2.17
Caudatum		SC326	0.45	-0.79	-0.02	-0.68
		SC333	2.44	-0.96	-0.94	-0.58
		SC392	0.40	-1.01	0.72	-0.07
		SC798	0.02	0.06	0.63	-0.61
Durra (Durra-Caudatum)		SC441	-1.27	-0.09	0.72	0.68
		SC748	-1.40	0.11	-0.46	1.80
Kafir (Kafir-Caudatum)		SC625	-0.80	2.05	-0.60	-0.60
		SC628	0.21	-1.61	-0.09	1.54
		SC680	-0.28	-0.52	0.59	.026

S.E. (SCA for effects) = 1.15

S.E. ($s_{ij} - s_{kl}$) = 1.63

Contribution of lines = 33.35%

Contribution of testers = 35.35%

Contribution of (line x tester) = 30.00%

Table A2. Specific Combining Ability (SCA) values of line x tester hybrids for plant height (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	3.28	0.24	-2.41	-1.42
		SC214	-7.99	7.59	-1.61	1.71
		SC311	4.10	-4.66	2.01	-1.75
	Guinea-bicolor	SC192	3.01	-2.15	1.99	-3.16
Guinea		SC250	-2.02	-0.82	-1.77	4.30
		SC283	-10.67	2.84	5.49	2.04
		SC303	1.69	10.50	-12.46	-0.04
Caudatum		SC326	-2.78	4.56	-2.11	-0.27
		SC333	5.94	-5.14	2.34	-3.44
		SC392	-4.36	-6.46	2.75	7.77
		SC798	1.88	7.87	3.61	-8.21
Durra (Durra-Caudatum)		SC441	4.44	-2.62	2.57	-4.70
		SC748	0.05	3.36	-7.75	4.04
Kafir (Kafir-Caudatum)		SC625	-1.00	-4.63	5.85	-0.57
		SC628	0.95	-8.87	5.85	1.76
		SC680	2.27	2.19	-5.53	0.76

S.E. (SCA for effects) = 6.17

S.E. ($s_{ij} - s_{kl}$) = 8.73

Contribution of lines = 66.99%

Contribution of testers = 25.81%

Contribution of (line x tester) = 8.07%

Table A3. Specific Combining Ability (SCA) values of line x tester hybrids for panicle exertion, based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	1.69	-0.81	0.24	-1.14
		SC214	-1.01	0.09	-1.19	2.09
		SC311	-0.29	-2.70	1.54	1.43
	Guinea-bicolor	SC192	1.32	-3.30	2.41	-0.45
Guinea		SC250	-0.11	0.78	1.20	-1.88
		SC283	-1.64	0.08	1.14	0.40
		SC303	-0.16	0.09	-1.19	1.24
Caudatum		SC326	-2.43	2.05	-0.70	1.94
		SC333	2.22	2.26	-1.35	-3.15
		SC392	-0.37	1.14	-2.46	1.67
		SC798	0.17	1.61	0.20	-1.40
Durra (Durra-Caudatum)		SC441	0.58	0.62	0.40	-1.62
		SC748	1.85	-0.87	-1.50	0.50
Kafir (Kafir-Caudatum)		SC625	0.63	-0.18	2.15	-2.62
		SC628	-1.17	-1.56	1.41	1.30
		SC680	-1.22	1.68	-2.25	1.77

S.E. (SCA for effects) = 1.45

S.E. ($s_{ij} - s_{kl}$) = 2.04

Contribution of lines = 38.64%

Contribution of testers = 34.42%

Contribution of (line x tester) = 27.60%

Table A4. Specific Combining Ability (SCA) values of line x tester hybrids for number of panicles/plot, based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-4.40	6.09	2.15	-3.99
		SC214	-12.93	-4.57	6.66	10.69
		SC311	4.49	-1.24	0.07	-3.48
	Guinea-bicolor	SC192	27.86	-11.78	0.36	-16.61
Guinea		SC250	-9.47	-14.86	0.45	23.73
		SC283	-15.29	-12.84	-9.03	37.00
		SC303	-6.74	-2.22	16.18	-7.38
Caudatum		SC326	-10.58	14.70	-5.41	1.87
		SC333	11.87	-5.56	-2.00	-4.47
		SC392	7.85	9.84	-11.23	-6.62
		SC798	4.94	9.63	-8.98	-2.11
Durra (Durra-Caudatum)		SC441	-1.24	-0.09	-8.03	9.21
		SC748	2.81	8.84	-2.52	-9.28
Kafir (Kafir-Caudatum)		SC625	0.11	-3.94	5.36	-1.69
		SC628	-3.16	1.87	18.26	-17.13
		SC680	4.24	6.93	-1.93	-9.40

S.E. (SCA for effects) = 11.51

S.E. ($s_{ij} - s_{kl}$) = 16.27

Contribution of lines = 27.40%

Contribution of testers = 32.13%

Contribution of (line x tester) = 40.25%

Table A5. Specific Combining Ability (SCA) values of line x tester hybrids for 500-seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-0.64	-0.09	0.09	0.65
		SC214	0.09	-0.21	0.88	-0.68
		SC311	0.54	0.51	-0.17	-0.88
	Guinea-bicolor	SC192	-0.74	0.13	0.48	0.13
Guinea		SC250	-0.62	-0.10	0.31	0.41
		SC283	0.54	0.42	-0.31	-0.64
		SC303	0.64	-0.18	-0.16	-0.30
Caudatum		SC326	0.30	-1.02	-0.01	0.72
		SC333	0.06	0.61	-1.29	0.62
		SC392	0.85	0.14	-0.25	-0.74
		SC798	-0.10	0.58	-0.12	-0.32
Durra (Durra-Caudatum)		SC441	-0.02	0.28	-0.33	0.06
		SC748	-0.78	-0.13	0.47	0.44
Kafir (Kafir-Caudatum)		SC625	-0.22	0.13	0.34	-0.25
		SC628	-0.15	-0.31	0.10	0.36
		SC680	-0.02	-0.27	-0.08	0.37

S.E. (SCA for effects) = 0.75

S.E. ($s_{ij} - s_{kl}$) = 1.06

Contribution of lines = 72.55%

Contribution of testers = 15.29%

Contribution of (line x tester) = 11.79%

Table A6. Specific Combining Ability (SCA) values of line x tester hybrids for panicle length (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-0.25	0.25	-0.23	0.27
		SC214	-1.67	1.99	0.14	-0.41
		SC311	0.52	1.33	-0.74	-1.08
	Guinea-bicolor	SC192	1.26	1.23	-1.48	-0.97
Guinea		SC250	0.81	0.14	0.93	-1.85
		SC283	-0.62	-0.65	0.25	1.07
		SC303	1.32	0.54	-1.21	-0.60
Caudatum		SC326	-0.24	0.36	-0.55	0.14
		SC333	0.34	-0.64	-0.52	0.86
		SC392	0.05	-0.62	0.80	-0.18
		SC798	-1.84	0.41	1.13	0.26
Durra (Durra-Caudatum)		SC441	0.29	-1.45	-0.13	1.33
		SC748	-0.67	-0.39	-0.23	1.33
Kafir (Kafir-Caudatum)		SC625	1.81	-0.45	-1.00	-0.32
		SC628	0.13	0.09	-0.60	0.43
		SC680	-1.15	-2.13	3.53	-0.21

S.E. (SCA for effects) = 1.82

S.E. ($s_{ij} - s_{kl}$) = 2.58

Contribution of lines = 58.55%

Contribution of testers = 26.75%

Contribution of (line x tester) = 14.62%

Table A7. Mid Parent Heterosis (MPH) values of line x tester hybrids for days to mid-anthesis, based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-2.50	-3.98	-3.82	-2.71
		SC214	-1.89	-1.07	-3.48	-2.12
		SC311	-0.70	-2.85	-3.25	-0.51
	Guinea-bicolor	SC192	-5.62	-3.71	-6.30	-1.82
Guinea		SC250	-3.05	-0.28	-3.05	-0.67
		SC283	-5.02	-2.31	-4.85	-0.22
		SC303	-1.08	-3.98	-6.63	-6.06
Caudatum		SC326	-2.12	-4.07	-4.35	-2.56
		SC333	4.32	-0.67	-1.96	1.20
		SC392	-2.11	-4.35	-3.32	-1.65
		SC798	0.29	-0.02	-0.59	0.45
Durra (Durra-Caudatum)		SC441	-5.21	-3.86	-4.09	-1.45
		SC748	-3.67	-1.90	-3.97	1.72
Kafir (Kafir-Caudatum)		SC625	-4.28	-0.77	-5.55	-2.90
		SC628	-2.38	-5.16	-4.42	0.51
		SC680	-2.96	-3.56	-3.40	-1.19

Table A8. Mid Parent Heterosis (MPH) values of line x tester hybrids for plant height (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	33.54	32.35	20.91	24.21
		SC214	11.76	28.50	11.97	17.04
		SC311	25.86	21.16	17.99	17.01
	Guinea-bicolor	SC192	29.34	27.04	21.99	19.82
Guinea		SC250	29.14	31.83	23.21	30.16
		SC283	8.21	22.55	16.41	15.71
		SC303	48.37	54.74	29.33	41.53
Caudatum		SC326	19.18	28.40	13.85	17.71
		SC333	24.37	18.35	15.86	13.33
		SC392	13.59	15.65	14.29	20.72
		SC798	32.65	38.74	27.53	19.95
Durra (Durra-Caudatum)		SC441	9.89	8.50	3.71	0.00
		SC748	33.15	37.01	20.43	32.02
Kafir (Kafir-Caudatum)		SC625	7.71	9.18	8.56	5.36
		SC628	5.22	2.11	4.66	3.38
		SC680	25.20	27.65	11.38	19.49

Table A9. Mid Parent Heterosis (MPH) values of line x tester hybrids for panicle exertion (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	63.76	19.75	26.07	-17.07
		SC214	13.82	14.94	2.61	12.14
		SC311	27.20	-3.31	29.69	12.19
	Guinea-bicolor	SC192	49.25	-2.40	43.78	0.52
Guinea		SC250	8.09	7.77	7.46	-48.56
		SC283	-5.14	1.13	5.58	-16.33
		SC303	53.87	43.05	20.74	30.23
Caudatum		SC326	9.70	36.33	12.18	18.08
		SC333	37.81	29.04	-5.77	-44.62
		SC392	18.98	24.13	-12.79	6.07
		SC798	40.30	43.67	26.90	-4.58
Durra (Durra-Caudatum)		SC441	21.97	14.29	8.79	-27.28
		SC748	21.80	-12.20	-21.13	-25.68
Kafir (Kafir-Caudatum)		SC625	20.84	6.98	21.12	-34.85
		SC628	-3.61	-12.85	5.67	-11.49
		SC680	54.11	77.70	19.23	53.73

Table A10. Mid Parent Heterosis (MPH) values of line x tester hybrids for panicle number, based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-9.57	14.96	6.95	-39.52
		SC214	-5.70	13.18	33.18	10.65
		SC311	-18.92	-23.96	-23.61	-50.38
	Guinea-bicolor	SC192	41.70	-10.11	8.18	-46.15
Guinea		SC250	-8.49	-13.40	11.76	23.57
		SC283	-8.20	-1.84	2.63	42.58
		SC303	8.02	22.22	56.23	-17.88
Caudatum		SC326	-2.29	55.24	13.97	-6.38
		SC333	38.94	13.35	21.35	-23.26
		SC392	23.18	36.88	-8.22	-35.32
		SC798	5.45	20.33	-15.46	-34.97
Durra (Durra-Caudatum)		SC441	-13.34	-8.29	-22.18	-21.54
		SC748	-11.86	1.49	-18.39	-58.87
Kafir (Kafir-Caudatum)		SC625	-7.44	-9.87	4.17	-34.91
		SC628	-4.24	7.71	33.65	-50.61
		SC680	-7.95	-0.13	-14.77	-53.53

Table A11. Mid Parent Heterosis (MPH) values of line x tester hybrids for 500-seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	1.88	9.26	21.53	17.00
		SC214	3.60	4.23	24.54	3.22
		SC311	8.37	11.40	16.10	3.30
	Guinea-bicolor	SC192	2.75	12.44	26.41	14.48
Guinea		SC250	-2.94	4.11	18.82	10.85
		SC283	6.51	8.83	13.05	3.11
		SC303	4.37	0.73	11.11	2.66
Caudatum		SC326	8.74	1.64	20.31	17.29
		SC333	-4.04	1.13	-4.04	3.27
		SC392	7.53	5.54	11.08	2.07
		SC798	10.97	19.31	24.49	14.54
Durra (Durra-Caudatum)		SC441	11.21	17.15	23.17	17.23
		SC748	5.02	12.79	27.85	18.65
Kafir (Kafir-Caudatum)		SC625	0.72	6.62	19.68	6.16
		SC628	3.29	5.07	19.03	12.64
		SC680	0.92	1.67	12.72	8.92

Table A12. Mid Parent Heterosis (MPH) values of line x tester hybrids for panicle length (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	14.79	15.41	21.14	22.77
		SC214	-4.09	9.38	9.50	7.72
		SC311	9.69	11.81	11.13	10.01
	Guinea-bicolor	SC192	9.44	8.12	4.41	7.12
Guinea		SC250	30.47	25.60	37.16	25.07
		SC283	9.12	7.92	19.43	22.46
		SC303	17.78	13.28	13.92	16.32
Caudatum		SC326	12.60	13.79	18.01	20.61
		SC333	13.47	8.34	16.43	21.66
		SC392	4.26	1.08	13.18	9.79
		SC798	-1.69	6.88	17.59	14.04
Durra (Durra-Caudatum)		SC441	9.05	0.86	14.15	20.03
		SC748	1.41	1.93	11.31	18.45
Kafir (Kafir-Caudatum)		SC625	25.46	13.50	19.65	22.31
		SC628	6.40	5.26	10.45	15.05
		SC680	16.39	10.32	45.50	27.41

Table A13. Better Parent Heterosis (BPH) values of line x tester hybrids for plant height (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	32.21	16.18	11.01	14.18
		SC214	5.19	20.39	10.11	15.26
		SC311	15.04	16.99	16.26	15.14
	Guinea-bicolor	SC192	19.89	20.87	21.93	19.71
Guinea		SC250	14.19	31.61	17.10	23.55
		SC283	-5.11	21.18	9.63	8.84
		SC303	32.52	53.24	24.27	35.82
Caudatum		SC326	13.64	18.77	10.47	14.36
		SC333	8.27	16.05	8.24	5.75
		SC392	5.30	10.03	14.23	20.61
		SC798	19.80	35.71	24.03	16.50
Durra (Durra-Caudatum)		SC441	-4.21	6.55	-2.96	-6.55
		SC748	18.24	36.57	14.99	25.90
Kafir (Kafir-Caudatum)		SC625	-1.90	5.83	6.55	3.28
		SC628	-3.18	-2.10	3.88	2.47
		SC680	24.87	12.78	2.95	10.59

Table A14. Better Parent Heterosis (BPH) values of line x tester hybrids for panicle exertion (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	18.46	-15.30	-12.59	-37.10
		SC214	-4.02	0.00	-8.00	-10.66
		SC311	25.34	-8.11	19.34	6.02
	Guinea-bicolor	SC192	45.64	-8.11	31.11	-4.10
Guinea		SC250	-2.91	-6.30	-9.23	-50.58
		SC283	-20.00	-12.02	-5.34	-33.34
		SC303	6.81	-2.70	-19.34	-5.63
Caudatum		SC326	-5.18	21.70	3.32	-3.77
		SC333	35.86	26.13	-10.92	-49.05
		SC392	12.64	13.51	-22.67	4.34
		SC798	37.05	41.73	21.04	-12.97
Durra (Durra-Caudatum)		SC441	13.33	10.00	8.33	-36.67
		SC748	16.49	-18.92	-29.40	-27.66
Kafir (Kafir-Caudatum)		SC625	7.55	-1.54	15.14	-45.46
		SC628	-17.81	-23.30	-4.11	-28.77
		SC680	0.99	14.45	-24.36	4.49

Table A15. Better Parent Heterosis (BPH) values of line x tester hybrids for panicle number, based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-27.65	-0.15	-3.47	-44.61
		SC214	-17.12	9.26	32.09	8.02
		SC311	-29.18	-38.86	-40.54	-61.82
	Guinea-bicolor	SC192	37.95	-16.41	-3.33	-52.56
Guinea		SC250	-16.89	-13.52	6.90	16.41
		SC283	-17.87	-19.38	-18.49	11.91
		SC303	-6.76	15.62	54.23	-18.14
Caudatum		SC326	-26.35	26.00	-4.22	-20.28
		SC333	-0.96	-13.69	-4.68	-39.00
		SC392	-8.45	9.33	-24.17	-45.86
		SC798	-12.84	8.43	-20.69	-38.06
Durra (Durra-Caudatum)		SC441	-16.61	-13.76	-29.72	-30.14
		SC748	-18.36	-0.79	-23.52	-62.02
Kafir (Kafir-Caudatum)		SC625	-11.37	-14.83	-5.48	-41.78
		SC628	-7.21	0.61	19.93	-56.30
		SC680	-9.35	-8.13	-24.62	-59.47

Table A16. Better Parent Heterosis (BPH) values of line x tester hybrids for seed weight (gm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	-13.42	-1.74	19.73	0.11
		SC214	-13.31	-7.82	20.43	-13.06
		SC311	-5.09	3.49	13.73	-8.88
	Guinea-bicolor	SC192	-10.99	3.24	25.41	-0.13
Guinea		SC250	-21.39	-11.12	10.47	-9.66
		SC283	-9.77	-2.46	10.95	-12.06
		SC303	-13.69	-12.04	5.94	-14.54
Caudatum		SC326	-6.58	-7.51	20.08	1.47
		SC333	-12.77	-13.19	-23.72	-6.81
		SC392	5.89	-2.41	-5.63	-0.29
		SC798	1.49	16.12	16.37	5.55
Durra (Durra-Caudatum)		SC441	-3.49	7.77	21.94	2.45
		SC748	-3.96	9.76	19.53	9.32
Kafir (Kafir-Caudatum)		SC625	-18.60	-9.18	11.00	-13.65
		SC628	-12.62	-5.97	16.63	-4.06
		SC680	-10.04	-3.73	8.26	-2.19

Table A17. Better Parent Heterosis (BPH) values of line x tester hybrids for panicle length (cm), based on combined data from four environments – College Station, Corpus Christi and Weslaco in 2003, and College Station in 2004.

		Lines/ Testers	Kafir- Milo B.TX399	Caudatum (Kafir-Caudatum)		
				B.TX623	R.TX436	R.TX430
Bicolor	Durra-bicolor	SC155	7.70	13.77	16.60	21.67
		SC214	-12.26	4.98	2.68	5.76
		SC311	3.70	11.12	7.82	8.15
	Guinea-bicolor	SC192	3.00	-2.91	-4.16	-5.77
Guinea		SC250	28.00	21.67	36.11	18.48
		SC283	6.60	5.00	19.04	16.49
		SC303	11.58	12.82	10.75	14.12
Caudatum		SC326	10.72	10.00	16.85	14.01
		SC333	7.91	8.34	13.64	18.88
		SC392	-7.62	-6.19	2.71	4.14
		SC798	-4.37	4.44	17.45	8.94
Durra (Durra-Caudatum)		SC441	8.18	-3.34	12.03	12.52
		SC748	-8.54	-12.09	-1.96	0.20
Kafir (Kafir-Caudatum)		SC625	17.79	1.67	9.57	7.35
		SC628	1.61	-4.16	2.84	2.58
		SC680	5.77	-4.16	29.10	8.54

VITA

Rafael Arturo Mateo Moncada was born on January 29, 1975 in Tegucigalpa, Honduras. He graduated from Escuela Agricola Panamericana, Zamorano, Honduras with a B.S. in agronomy in 1996. In December 2003, he received a M.S. in plant breeding from Texas A&M University. In December 2006, he received his Ph.D. in plant breeding from Texas A&M University.

Permanent mailing address:
Rafael Arturo Mateo Moncada
Colonia Hato de Enmedio, Sector # 4,
Bloque # 49, Casa 4907.
Tegucigalpa, Honduras
Telephone: (504) 2551982