WIDE HYBRIDIZATION, GENOMIC, AND OVERWINTERING CHARACTERIZATION OF HIGH-BIOMASS *SORGHUM* SPP. FEEDSTOCKS

A Thesis

by

DAVID KYLE WHITMIRE

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

MASTER OF SCIENCE

August 2011

Major Subject: Plant Breeding

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Approved by:

Chair of Committee,	Russell W. Jessup
Committee Members,	Byron L. Burson
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ABSTRACT

Wide Hybridization, Genomic, and Overwintering Characterization of High-Biomass *Sorghum* spp. Feedstocks. (August 2011)

David Kyle Whitmire, B.S., Oklahoma State University

Chair of Advisory Committee: Dr. Russell W. Jessup

The federally mandated 36 billion gallons a year production goal for "advanced biofuels" by 2022 has created a demand for lignocellulosic feedstocks that are inexpensive to produce. The current lack of market development for lignocellulosic feedstocks incentivizes the development of versatile biomass products with greater end-use possibilities, as in either a forage or bioenergy system. High-biomass, perennial grasses offer dual-use potential in either forage or biofuel systems.

In 2009 and 2010 controlled pollinations were made to evaluate the efficiency of producing interspecific hybrids between homozygous recessive *iap/iap* and *Iap/-* *Sorghum bicolor* (L.) Moench, cultivated sorghum, and three *S. halepense* (L.) Pers., johnsongrass, genotypes. The *iap/iap* genotype removes reproductive barriers to alien pollen in *S. bicolor* and aids in wide hybridization. Total seed set, germinable seed set, and hybrid production were significantly higher using the *iap/iap* genotype. The *iap/iap* *S. bicolor* genotype is a valuable tool available to plant breeders for the creation of wide hybrids with *S. halepense*.

In a related study a bulked segregant analysis was conducted using bulked samples of *S. bicolor*, typical flowering *S. halepense*, non-flowering *S. halepense*, and putative triploid hybrids of the two species to identify unique markers for each bulk and to evaluate *S. bicolor* genetic material introgression into the non-flowering *S. halepense* genome. Thirty-nine and 23 markers were found to be unique to the *S. bicolor* and typical flowering *S. halepense* bulks, respectively. These unique markers could be used in a breeding program to identify interspecific hybrids. Alleles at fifteen markers were found in both the *S. bicolor* and non-flowering *S. halepense* bulks but not in typical flowering *S. halepense* and may help explain the non-flowering phenotype.

In 2010 and 2011 a study was conducted to investigate the rhizome composition of 11 genotypes of *Sorghum* species and its relationship to overwintering. Genotype, environment, and sampling date had significant effects on rhizome metabolite concentrations. Overwintering capacity was related to fructans and crude protein concentrations and NIRS (Near Infrared Spectroscopy) was effective at estimating these values. This information can be used to screen for stronger perennial parents to be used in future breeding programs.

DEDICATION

I would like to dedicate this work to my family. Firstly to my wife Bethany and my two sons, Sam and Corbin, I love you all very much. I also dedicate this work to both of my grandfathers, Bobby Chandler and Ernest Whitmire; you two are the reason I have made agriculture my career and I miss both of you very much.

ACKNOWLEDGEMENTS

I would like to respectfully thank my committee chair, Dr. Russell Jessup, and my committee members, Dr. Byron Burson, Dr. Seth Murray, and Dr. Tryon Wickersham, for their continued guidance and support throughout this research.

I would also like to thank Texas A&M University and the Department of Soil and Crop Sciences for the opportunity to attend an exceptional institution. I also extend my gratitude to Dr. George Hodnett and Dr. Jim Heitholt for their assistance and guidance.

Finally I would like to express my appreciation to my fellow graduate students, Jake Washburn, Chuck Dowling, and Mason Kearns, and the undergraduate student workers, Ethan Fortenberry, Jordan Haralson, Grant Brooks, and Zach Farrow, for without their assistance this research would not have been possible.

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CHAPTER I

INTRODUCTION

Sorghum has been utilized worldwide for the production of grain, forage, sugar, and more recently biofuels. *Sorghum bicolor* (L.) Moench is the 5th most important grain crop in the world in terms of grain production (ICRISAT, 2011). The grain is primarily used for human consumption throughout Asia, Africa, and Central America and for animal feed in the United States, Australia, and South America (ICRISAT, 2011). The subspecies *S. bicolor* ssp. *drummondii* (Nees ex. Steud.) de Wet & Harlan, sudangrass, and hybrids between *S. bicolor* and *S. bicolor* ssp. *drummondii* have been used as a source of high biomass forage and hay in the U.S. for decades (Armah-Agyeman et al., 2002). Recently *Sorghum* species have been evaluated as bioenergy feedstocks with ethanol being produced from grain starch, stem sugar, and lignocellulosic biomass (Rooney et al., 2007; Wu et al., 2008; Miller and Ottman, 2010; Sattler et al., 2010). These recent evaluations were fueled by rising transportation fuel prices and the federally mandated production goal of 36 billion gallons of "advanced biofuels" a year by 2022 with a cap of 15 billion gallons a year from starch-based ethanol derived from corn grain (Sissine, 2007). The production goal and enforced cap on corn grain creates a 21 billion gallon void that could be filled with suitable *Sorghum* species or interspecific hybrids.

This thesis follows the style of Crop Science.

The majority, if not all previous studies concerning *Sorghum* for bioenergy were conducted using the annual species, *S. bicolor*. *Sorghum bicolor* has an annual growth cycle in temperate climates and exhibits weak perenniality in tropical and subtropical climates. A perennial grass production system offers ecological and environmental benefits not present in annual row crop production such as increased soil organic carbon, reduced soil erosion, reduced inputs of fertilizer and herbicides, and a higher energy return because of the reduction of production inputs (Costanza et al., 1997; Lewandowski et al., 2003; Kort et al., 1998; McLaughlin and Walsh, 1998; Khanna et al., 2010). Taking into account the ecological and environmental benefits and agronomic sustainability of perennial production systems, a high-biomass, perennial grass would be a valuable biofuel feedstock. Unfortunately, a significant current issue with lignocellulosic ethanol production is the lack of a large-scale commercial market. In the U.S. most lignocellulosic ethanol production facilities are only at the pilot plant stage and the market is not expected to significantly develop for at least 5 years (Gnansounou and Dauriat, 2010). However, there are currently 588 million acres of rangeland and pastureland and 61.5 million acres of hay land in the U.S. (USDA/RMA, 2011). The current market status and availability of abundant forage and hay acreage provides the opportunity for a perennial, high-biomass forage feedstock that may have multiple end-uses and provide great value to producers.

The objectives of this research were to: 1) evaluate the efficiency of interspecific hybridization between *S. bicolor* and *S. halepense*, 2) investigate the genomic relationships between groups of *Sorghum* species and putative interspecific hybrids

using genetic markers, and 3) characterize rhizome metabolite composition and its relationship to overwintering capacity in a collection of *S. halepense* (L.) Pers. and *S. almum* Parodi genotypes.

CHAPTER II

LITERATURE REVIEW

Taxonomy of *Sorghum* Species

The genus *Sorghum* is divided into five sections or subgenera: *Eu-sorghum* or *Sorghum*, *Parasorghum*, *Stiposorghum*, *Heterosorghum*, and *Chaetosorghum* (de Wet, 1978). The annual, non-rhizomatous species *S. bicolor* (2n=2x=20) and perennial, rhizomatous species *S. halepense* (2n=2x=40) both belong to the *Eu-sorghum* section. *Sorghum propinquum* (Kunth) Hitchcock (2n=2x=20), also in the *Eu-sorghum* section, is perennial and rhizomatous and is native to Southeast Asia (de Wet, 1978). *Sorghum propinquum* was not included in this research because available germplasm is extremely limited with only one accession available in the USDA National Plant Germplasm System (NPGS); in comparison to *S. halepense* which is a noxious weed with naturalized populations found throughout the United States (USDA/GRIN, 1992). The natural distribution of *S. bicolor* and *S. halepense* spans throughout Africa, Southern Europe, and Asia (Price et al., 2005; de Wet 1978). Within the U.S., *S. bicolor* and *S. halepense* occur in every state except for Maine, Minnesota, and West Virginia (USDA Plants Database, 2011). It is a reasonable assumption that the distribution of both species also extends into Maine, Minnesota, and West Virginia as well, because all states that border these states have documented populations. An additional species in the *Eu-sorghum* section, *S. almum* (2n=2x=40), Columbusgrass, is considered to be a natural hybrid between *S. bicolor* and *S. halepense* (Parodi, 1943). This species was discovered

in Argentina and has been difficult to separate from *S. halepense* morphologically (Parodi 1943, Endrizzi 1957).

Natural Hybridization

There are opportunities for natural hybridization between *S. bicolor* and *S. halepense* because populations of *S. halepense* are extensive and overlap with *S. bicolor*. This possibility of natural hybridization is a serious concern because of the possible escape of engineered genes such as herbicide resistance from *S. bicolor* into "weedy" *Sorghum* species hybrid populations. Hybridization can occur at the diploid, 2x, and tetraploid, 4x, levels in *S. bicolor* but in order to attain a 4x ploidy level, chromosome doubling must take place. Arriola and Ellstrand (1996) reported that hybridization occurred when *S. halepense* plants were located as far as 100 m away from a *S. bicolor* field. They also determined that hybridization rates were inconsistent but generally increased to approximately 12% as the distance between the *S. bicolor* field and *S. halepense* stands decreased. This maximum frequency of natural hybridization was also determined to be near the average frequency, 11%, of hybridization in controlled pollinations in a greenhouse setting, where per cent germination and seedling vigor was similar to that of the *S. halepense* parents (Arriola and Ellstrand, 1997). Introgression of *S. bicolor* genetic material into *S. halepense* was documented by a survey of 77 *S. bicolor* specific alleles in multiple populations of *S. halepense* from the eastern and central U. S. (Morrell et al., 2005). This survey also revealed that individual *S. bicolor* specific allele frequency ranged from 0.134 in a population with long term exposure of at least 20 years to *S. bicolor* production to 0.075 in a population that had little to no

exposure to *S. bicolor* production. These results suggest that introgression is not only taking place but the hybrid progeny persist for long periods of time within populations.

2x Controlled Hybridization in *Sorghum* Species

Naturally occurring self-sterility has been observed in both *S. bicolor* and *S. halepense* and it can be utilized to create more efficient interspecific hybrid production systems. Cytoplasmic male sterility (CMS) systems, where A line parents are self-sterile but cross-fertile, have been used extensively within *S. bicolor* for the purpose of grain and hybrid production (Reddy et al., 2007). A self-sterile *S. halepense* strain was described by Casady (1961) and was cross-fertile with a tetraploid *S. bicolor* line; this was the case whether the *S. halepense* strain was used as the staminate or pistillate parent. CMS is a widely used and is a fairly common system in diploid grasses but self-sterility in *S. halepense* is very rare and would be extremely valuable in a breeding program.

Controlled hybridizations between *S. bicolor* and *S. halepense* have been successful but with varying results. Dweikat (2005) reported a 2n=2x=20 F_1 interspecific hybrid with 90% seed set when self-pollinated and morphological characteristics intermediate to the parents. In other studies, Endrizzi (1957) reported interspecific hybrids that were phenotypically indistinguishable from *S. halepense* based on characteristics such as seed production, number of panicles per plant, number of tillers per plant, and above- and belowground biomass production. Self-fertility of the F_1 hybrids varied across ploidy levels, with triploids (2n=3x=30) and tetraploids (2n=4x=40) having 1.1% and 66% seed set, respectively (Endrizzi 1957).

4x Controlled Hybridization in *Sorghum* Species

To increase the possibility of producing fertile interspecific hybrids between *S. bicolor* and *S. halepense,* doubling the chromosome number of the *S. bicolor* parent was suggested by Piper and Kulakow (1994). Chromosome doubling would convert *S. bicolor* from a diploid (2n=2x=20) to an autotetraploid (2n=4x=40), the same ploidy level as *S. halepense*. Resulting hybrids between tetraploid *S. bicolor* and *S. halepense* should be tetraploids as well. Sangduen and Hanna (1984) reported that crossability was as high as 83% when *S. halepense* was used as the female parent and 33% when the tetraploid *S. bicolor* was used as the female parent. A possible explanation for the difference in crossability could be a species response to the plastic bag emasculation technique used to create male-sterile, female parents that were used in this study. Seed set from hybridizations between tetraploid *S. bicolor* ssp. *drummondii* and the hybrid *S. halepense* x tetraploid *S. bicolor* ssp. *drummondii* was similar to that from hybridizations between diploid *S. bicolor* ssp. *drummondii* and the hybrid *S. halepense* x tetraploid *S. bicolor* ssp. *drummondii*, but the seed produced using the diploid female parent did not have endosperm development and had a shriveled appearance (Casady and Anderson, 1952). Phenotypes of progeny produced from crosses between tetraploid *S. bicolor* and *S. halepense* varied greatly and ranged from resembling the *S. bicolor* parent to intermediate of the parents to resembling the *S. halepense* parent (Casady and Anderson, 1952; Sangduen and Hanna, 1984; Piper and Kulakow, 1994; Yim and Bayer, 1997).

Reproductive Barriers

Reproductive barriers that reduce the possibility of recovering interspecific hybrids between *S. bicolor* and *S. halepense* occur naturally. Hodnett et al. (2005) reported that reduced pollen germination and failure of the pollen tubes to grow through the pistils of the female parents were barriers to fertilization when a *S. bicolor* line was pollinated with pollen from 14 different alien *Sorghum* species. Of the species crossed, the most successful had only 0.6% of the pollen tubes growing into the ovary.

Laurie and Bennett (1989) discovered a sorghum accession in which maize (*Zea mays* L.) pollen germinated on its stigmas and the pollen tubes grew into the ovary. They determined that this only occurred when the recessive allele (*iap*) was present in the homozygous state in the female sorghum parent. The dominant allele *Iap,* known as 'Inhibitor of alien pollen', suppresses pollen tube growth of alien pollen in *S. bicolor* pistils. Price et al. (2006) pollinated two *S. bicolor* genotypes, one was *Iap,-* and the other was *iap/iap,* with pollen from three alien *Sorghum* species and pollen tube growth to the ovary dramatically increased in the homozygous recessive genotype versus the genotype with the dominant allele. Kuhlman and Rooney (2011) transferred the *iap* allele into an agronomically superior germplasm line that can be used to circumvent pollen-pistil incompatibilities and increase the frequency of interspecific hybridization in the genus.

Hybrid Progeny Ploidy

Hybridization between *S. bicolor* and *S. halepense* would be expected to produce triploid (2n=3x=30) progeny upon normal gamete formation by each species (n=x=10

and n=2x=20, respectively). Most triploid embryos, however, do not survive because of endosperm abortion and embryo rescue and culture are needed to obtain progeny. At metaphase I in pollen mother cells of triploid hybrids, varying numbers of univalents, bivalents, trivalents, and quadrivalents are produced and as meiosis proceeds there are lagging chromosomes and micronuclei that render the gametes non-functional and the plants sterile (Sengupta and Weibel 1971; Mariam et al., 1996; Kosmala et al,. 2006). Triploid progeny are of value because their sterility eliminates the possibility of outcrossing with either cultivated populations of *S. bicolor* or wild populations of *S. halepense*.

Marker Assisted Hybrid-Verification

Bulked segregant analysis (BSA) is a technique of quickly identifying markers linked to chromosome regions or genes of interest at much lower costs. It is carried out by grouping individuals within segregating populations or of varying phenotypes that share common traits into bulked or pooled DNA samples, with the resulting bulks being dissimilar for a particular trait or traits (Michelmore et al., 1991). This technique has been successfully used in a variety of species, including *S. bicolor*, and primarily for identifying chromosome regions and genes associated with disease resistance. Regions and/or genes relating to freezing tolerance in *Medicago sativa* L., alfalfa, and apomixis in *Pennisetum cilare* (L.) Link, bufflegrass, have been successfully identified using this technique (Rémus-Borel et al., 2010; Fondevilla et al., 2008; Singh *et al.*, 2006; Boora *et al.*, 1999; Dwivedi *et al.*, 2007).

Sorghum halepense is theorized to have originated from a hybridization between *S. propinquum* and *S. bicolor* ssp. *arundinaceum* (Desv.) de Wet & Harlan, common wild sorghum, (2n=2x=20) followed by chromosome doubling (de Wet, 1978). *S. halepense* and *S. bicolor* have a very similar subgenome in common as *S. bicolor* ssp. *arundinaceum* is believed to be the progenitor of modern *S. bicolor* (Hadley, 1953;; Celarier, 1958). *Sorghum halepense* has approximately two times as much DNA as *S. bicolor* and *S. propinquum,* and all three species have similar haploid chromosome complement sizes (Price *et al.*, 2005). The difference in DNA content is a result of *S. halepense* being a tetraploid and both *S. bicolor* and *S. propinquum* being diploids. This further supports the theory that *S. halepense* is a polyploid sharing one subgenome in common with *S. bicolor*. Paterson *et al.* (1995) reported that 117 out of 125 RFLP alleles found in *S. halepense* were accounted for by species-representative germplasm collections of *S. bicolor* and *S. propinquum* collectively. Markers unique to *S. halepense* and *S. bicolor* would be beneficial for identifying hybrids and determining the amount of introgression from each species as was determined by Dweikat (2005) for a diploid interspecific hybrid between these two species.

Rhizome Morphology

Rhizomes are underground structures present in many grasses that serve as nutrient storage organs and meristematic conduits for perenniality. More specifically, rhizomes are modified stems that are located beneath the soil surface and consist of nodes, internodes, leaves, and axillary buds. The highly modified leaves of rhizomes are short and scale-like in appearance compared to aboveground leaves. New shoots and

rhizomes can arise from the axillary buds and thus increase the vegetative biomass of the crop. Rhizomes further provide a means of vegetative propagation via harvesting them from the soil and replanting. Vegetative propagation is necessary when seed production is very low or non-existent, such as with a triploid plant. Finally, some authors hypothesize that rhizomes provide the critical factor in overwintering by existing at soil depths that do not freeze (Warwick et al., 1986).

Rhizome Composition

An additional facet to overwintering via rhizomes could be the metabolite composition of the structures themselves. There have been limited investigations into the composition of *Sorghum* rhizomes and how this relates to the ability to store energy and overwinter. Studies addressing *S. halepense* rhizomes have tended to focus on carbohydrate composition and metabolism. Glucose and sucrose concentrations were inversely correlated to rhizome length, with glucose concentrations increasing in shorter rhizomes and sucrose concentrations increasing in longer rhizomes (McWhorter 1974). This relationship to length may however be explained by the longer rhizomes being more mature storage structures, in which sucrose is less mobile than glucose.

Vegetation removal is known to effectively lower carbohydrate reserves in rhizomes through remobilization to actively initiate regrowth in aboveground vegetation and once adequate regrowth is achieved, typically within 30 days, carbohydrates begin to be partitioned to the rhizomes for storage (McWhorter 1974). Rhizome carbohydrate levels, including glucose, sucrose, starch, and dextrins, vary throughout the growing season in response to growth stage, with levels decreasing during sprouting and seed

formation. After seed production, sucrose concentrations in the rhizomes significantly increase for overwinter storage (Rapp 1948; McWhorter 1961). A reasonable prediction is that other metabolites associated with energy storage and/or winter survival would similarly increase, once reproductive maturity has occurred.

Although carbohydrates have been measured in *S. halepense* rhizomes, there are a number of other metabolites that plants use for both long and short term storage. Additional metabolites have been found to aid in the perenniality of species other than *S. halepense*. The rhizome nodes of cold-tolerant seashore paspalum (*Paspalum vaginatum* Sw.) have significant increases in the unsaturated fatty acid linolenic acid in response to cold treatment (Cyril et al., 2002). The stolons and rhizomes of Zoysiagrasses (*Zoysia* spp.) accumulate proteins associated with winter hardiness when exposed to cold stress (Patton et al., 2007). In contrast, belowground vegetative structures of the non-grass species *Gentiana triflora* Pall. accumulated proteins associated with cold and dehydrating stresses when plants were exposed to winter field conditions and when grown in more ideal greenhouse conditions. This consistent accumulation of stress related proteins implies that some stress genes are constitutive rather than inducible (Takahashi et al., 2006). Kavanová and Gloser (2004) determined that rhizomes of the warm season grass *Calamagrostis epigejos* (L.) Roth, wood small-reed, served as a transport system for, not a source of, free amino acids. Differential winter survival between rhizomes of *Agropyron repens* (L.) Beauv., quackgrass, and *S. halepense* was partially attributed to variation in lipid composition. In the genotypes and environments investigated, rhizomes of *A. repens* had a higher concentration of lipids and a higher

ratio of unsaturated fatty acids to saturated fatty acids than those of *S. halepense* (Stoller, 1977). This information suggests that predictive correlations between composition and overwintering ability might be present.

CHAPTER III

MATERIALS & METHODS

Sorghum bicolor x *S. halepense* Hybridization

Ten *S. bicolor* parental A-lines presumed to be *Iap/-*, and one A-line that was *iap/iap* were used as female parents in controlled hybridizations with three *S. halepense* genotypes (Table 1). Crosses were made in both a greenhouse and field setting in the fall of 2009 and 2010. The greenhouse crosses were made by taking one panicle of a female parent with exerted receptive stigmas and placing it inside a pollination bag with one panicle of a male parent with approximately half of its anthers exerted but before pollen dehiscence. The panicles were placed in direct contact with the male parent's panicle slightly above the female panicle to facilitate pollen shed upon receptive stigmas. The pollination bags were agitated each morning for at least 3 d. Crosses were also made in the field as described except agitation only took place for 1 d and was done by wind movement instead of physically tapping the bag. The agitation only took place for 1 d because female plants were potted and had to be physically taken to the male *S. halepense* plants that were located at the Texas A&M University Research Farm (Burleson County, TX).

Pollinated panicles were allowed to mature for at least 4 wk. Seed were checked for full maturity, by hardness and moisture content, before each panicle was harvested. The panicles were harvested by hand and placed within cold seed storage at a temperature of 50 °F and 30% relative humidity for 1 wk to dry. Once the panicles were

dried, their florets were counted and the seed were threshed. Threshing was done by hand using a ribbed rubber mat and a sanding block covered in the same ribbed rubber material. Seed set was determined by dividing the total number of seed by the total number of female florets. The seed were subjectively classified based on outward physical characteristics. Characteristics such as stigma/style fragments still attached, lobes present and severity, and pericarp appearance were used for classification (Fig. 1). Each seed was sized using a millimeter scaled ruler under a dissecting microscope; seed length, width, and depth measurement were taken to the nearest 0.1 mm. A weight was also taken for each seed using an analytical balance accurate to 0.0001 g.

Once the seed were measured and weighed, germination tests were conducted in a growth room. Two different regimes of light were used, 10 and 12 h, because the growth room was used for multiple flowering induction procedures with other plant material. Germination occurred using both periods of light duration. Temperature during light periods was approximately 29°C and approximately 21°C during the dark periods. Before germination, the seed were placed in a heated water bath of 70°C for 6 min to aid in breaking seed dormancy and rinsing pathogenic spores off the seed (Gritton and Atkins, 1963). This was done by wrapping seed in a Kimwipe® until forming a bundle and then placing the bundled seed into a test tube. The tube was then filled with water from a heated water bath and submerged within the bath. The seed was allowed to air dry and germination was conducted on sterile germination pads in Petri dishes. A 1% KNO_3 solution was used to keep the pads moistened and applied daily as needed to each pad (Shanmugavalli et al. 2007). The KNO_3 solution was used to help break seed

dormancy and facilitate an increased germination rate. Pads were kept moist for 2 wk and then allowed completely dry for 1 wk. After the 1 wk drying period, the pads were moistened daily for an additional 2 wk before terminating the germination test. Approximately 1 g Banrot® WP was added directly to the Petri dishes to combat fungal growth on the germination pads.

All seed that germinated were transferred to damp potting soil with Banrot® WP mixed in to further prevent fungal growth. Seed were not transferred until the coleoptile of each seedling came into contact with the lid of the Petri dish or if any fungal growth appeared to encroach upon the seedling. Once the seedlings produced sufficient mature leaf material, their ploidy levels were determined using a flow cytometer to identify the hybrids. To do so, approximately 1 cm^2 sections of mature and healthy leaf blades of both a diploid standard, as confirmed by root tip squashes or by using the registered germplasm Tx3361, and each putative hybrid were chopped using a standard razor blade. The leaf material was chopped in 0.25 mL of Galbraith's buffer in a Petri dish using a new razor blade. Once the material was properly macerated, 1.0 mL of Galbraith's buffer was added to the material and filtered through a 20 mμ filter into a 2.0 mL microtube. Leaf material was kept on ice before and after maceration. Fifty μL of propidium iodide was added to each microtube and allowed to set for 15 minutes in a covered ice chest. Each sample was then analyzed for DNA content using a Partec CyFlow Counter (Partec GmbH, Münster, Germany). At least 3,000 particles or nuclei of each sample were analyzed for each hybrid determination.

Data were analyzed using PROC TTEST and PROC GLM statements in SAS version 9.1 (SAS Institute, 2003). Differences between female *S. bicolor* genotypes were analyzed in terms of total seed set, germinable seed set, hybrid production, seed size, and seed weight. Seed class measurement means were separated using SNK means separation at a 0.05 significance level.

Sorghum Species Genomic Relationships

Plant materials were collected from different geographical areas and from within different *Sorghum* species to conduct a bulked segregant analysis (BSA) similar to the procedure described by Michelmore et al. (1991). Ten parental lines of diploid *S. bicolor* with varying genetic backgrounds were obtained from the USDA NPGS. Both A and B parental lines were used, but none of the 10 selected parental lines were from the same isogeneic A/B pair (Table 2). Nine genotypes of typical flowering tetraploid *S. halepense* were collected with various origins. Seven genotypes were collected within TX, one genotype was collected in NC, and one genotype was obtained from the USDA NPGS via Dr. Seth Murray (Table 3). Three non-flowering tetraploid *S. halepense* genotypes were collected within TX (Table 4). The non-flowering phenotype was identified by observing the genotypes in a greenhouse for over one year along with observing two of the genotypes in a separate field experiment. Four putative triploid hybrid genotypes were collected within TX (Table 5). The *S. halepense* and putative triploid hybrid genotypes that were collected were separated by space or morphology so that each genotype is distinctly different from the others. The ploidy level of the *S. bicolor* parental lines was assumed to be diploid with 20 chromosomes. This was not

confirmed as these are released parental lines. The ploidy levels of all other genotypes, both tetraploids and triploids, were confirmed by flow cytometry using a 2n=4x=40 *S. halepense* genotype as a standard as verified by conducting root tip squashes and metaphase chromosome counts. The procedure of this ploidy level verification was the same as that mentioned in the Methods section for the hybridization study except that instead of using a diploid standard, as was used in hybrid verification, a tetraploid standard was used.

The Simple Sequence Repeat (SSR) markers used in the BSA were developed by taking 50,000 base pair portions of sequence data, FASTA files, from across the sequenced *S. bicolor* genome, located online at http://www.phytozome.net, and mining the repeated sequences using the program SSRLocator that can be found at http://www.ufpel.edu.br/faem/fitotecnia/fitomelhoramento/faleconosco.html. Forward and reverse primers were then designed for the mined repeated sequences using the same program. The forward and reverse primer sequences were submitted to Sigma Life Science for oligonucleotide synthesis. The marker sequences ranged in size from 100-400 base pairs. Regions associated with "weedy" attributes such as rhizome production and length, shattering, tillering, and regrowth as described by Patterson et al. (1995) were saturated more so, by density, with markers than the rest of the genome. Markers outside of the "weedy" regions were located approximately every 2.5 million base pairs and within the regions the markers occur as frequently as every 200,000 base pairs.

Genomic DNA was extracted from one plant from each of the genotypes using a modified salt-extraction procedure as outlined by Aljanabi and Martinez (1997). The leaf

tissue used for DNA extraction was harvested from the youngest exposed leaf and was kept on ice until being stored in a -80°C freezer. At the time of extraction, the leaf material was removed from the -80°C freezer and 50-100 mg of leaf tissue was homogenized with 400 µL of homogenizing buffer in a 1.7 mL microtube. Immediately following homogenization, 40 µL of 20% SDS and 8 µL of 20 mg/mL proteinase K were added to the microtube and vortexed for 5 s. The mixture was then incubated at 65°C for at least 1 h in a heated water bath. After the 65°C incubation period, 300 µL of 6M NaCl was added to each microtube and vortexed for 30 s. The samples were spun at 12,000 rpm for 10 min; the resulting supernatant was then transferred to new microtubes. These new microtubes were spun at 12,000 rpm for 10 min and the resulting supernatant was transferred to another new microtube, taking care not to disturb the pellet of material in the bottom of the microtube. Then 800 µL of iopropanol, stored at -20°C, was added to the supernatant solution and the microtubes were gently inverted approximately 25 times until the cloudy interphase had passed. The samples were incubated at -20°C for at least 1 h. Following the -20°C incubation period, the samples were spun at 10,000 rpm for 3 min and the supernatant was discarded. Then 500 µL of EtOH, stored at -20°C, was added to the material remaining after the supernatant was discarded and spun at 10,000 rpm for 5 min. The resulting supernatant was discarded and the microtubes were opened, inverted, and placed overnight in a microtube rack to dry the pellet of material. This pellet was the extracted genomic DNA. The genomic DNA in each microtube was then re-suspended in 100 µL of autoclaved, de-ionized H_2O. The re-suspended genetic material was mixed vigorously to ensure the pellet was well dissolved in the H_2O.

The solution containing the extracted genetic material was quantified for DNA content using a spectrophotometer (Eppendorf, Hamburg, Germany). The DNA solution was diluted by placing 1 µL of the DNA solution into a cuvette with 49 µL of de-ionized and autoclaved H_2O. Once the DNA quantifications were completed, bulk samples were created to be used in the BSA. The bulks were made up as follows; the first bulk consisted of the *S. bicolor* parental lines; the second bulk was made up of the typical *S. halepense* genotypes; the third bulk consisted of the non-flowering *S. halepense*; and the fourth bulk was made up of the putative triploid hybrid genotypes. Each genotype was represented equally within its respective bulk and the final DNA concentration of each bulk was 50 ng μL^{-1}.

Once the bulks were made, 329 SSR primers were individually surveyed with each bulk upon PCR amplification. Each SSR PCR mixture consisted of 11.8 µL of de-ionized and autoclaved H_2O, 2 µL of 25 mM $MgCl_2$, 2 µL of 10X reaction buffer, 1 µL of 50 ng μL^{-1} bulk template DNA, 1 µL of 25mM deoxynucleoside triphosphates (dNTPs), 0.2 µL of 5 U μL^{-1} *Taq* DNA polymerase, 1 µL of the 40 mM forward primer, and 1 µL of the 40 mM reverse primer. The SSR PCR's were conducted in 96-well plates and temperature cycling was carried out using a PTC-220 Dyad Thermal Cycler (MJ Research Inc., Waltham, MA). The PCR method began with an initial denaturation of 95°C for 3 min; followed by 40 cycles of 95°C for 25 s, 55°C for 25 s, and 70°C for 45 s; and finished with a final extension of 72°C for 10 min.

Amplification products were separated by electrophoresis using a MEGA-GEL (C.B.S. Scientific, Del Mar, CA) high-throughput vertical unit and nondenaturing gels

with final concentrations of 6% acrylamide, 0.5X TBE (tris-borate-EDTA) Buffer, 0.07% ammonium persulfate, and 0.08% TEMED (Tetramethylethylenediamine) as described by Wang et al. (2003). The gels were stained with 50 µL of 10 mg mL^{-1} ethidium bromide. Before the amplification products were loaded into the gel wells, 2 µL of a solution containing 35 mL of 50% glycerol, 2.5 mL of 10X TBE, 2 mL of 0.5M EDTA, 0.5 mL of 20% SDS, 10 mL de-ionized H_2O, and 0.05 g of bromophenol blue powder was added into each well in the 96-well plate and spun at 340 rpm. The gels were then loaded with the amplification products and electrophoresis was carried out. Run times and voltages differed depending on the size of the amplicon but the wattage and amperage were held constant at 400 W and 400 mA. For sequences ranging from 100-199 base pairs, run time was 2 h at a voltage of 350 V. Sequences ranging from 200-299 base pairs had a run time of 3 h at a voltage of 350 V. The largest sequences, ranging in size from 300-400 base pairs, had a run time of 8 h at a voltage 175 V.

Gels that completed the electrophoresis process were then photographed using UV light to illuminate the allele bands. The contrast and white levels of the photographs were manipulated using Adobe Photoshop® and scored for the presence or absence of allele bands within each bulk according to the procedure set forth by Rodriguez et al. (2001).

Cluster and ordination analysis were performed using NTSYS-pc version 2.0 (Rohlf, 1997). Similarity coefficients were calculated using Jaccard's coefficient, SJ=a/(a+u), where a is the number of bands in which the two operational taxonomic units (OTUs) agree and u the number of bands present in one OTU but absent in the

other (Jaccard, 1908) with the SIMQUAL function. Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) algorithm within the SAHN function. Markers that were unable to be scored for the *S. bicolor* bulk were discarded on the assumption that PCR failure occurred.

Rhizome Composition and Overwintering

The plant material for the rhizome composition and overwintering study consisted of nine *S. halepense* genotypes that were collected from several areas in TX, one genotype collected in NC, and one genotype from the USDA NPGS provided by Dr. Seth Murray (Table 6). The genotypes were separated spatially, morphologically, or both so that each genotype is distinctly different from the others.

Trials consisted of plants transplanted directly into a cultivated field setting as well as plants being grown in PVC tubes. Trials were conducted at Commerce and College Station, TX and used randomized complete block design with three replications and two sampling dates, in the fall before frost and in the spring after greenup. One set of plants, consisting of one plant from each genotype, was sampled in the fall and a separate set was sampled in the spring. The sampling technique was destructive to the plants so that the same plant could not be sampled in both the fall and spring. The Commerce planting was located on Texas A&M University Commerce property (Hunt County, TX) and consisted of plants growing in 1.01 m sections of 15.2 cm diameter PVC tubes placed into 91.4 cm holes dug by a PTO-driven auger and tractor. The tube spacing at Commerce was 1.8 m to accommodate the movement of the tractor. Two plantings were located at the College Station Texas A&M University Research Farm

(Burleson County, TX). One planting was similar to the Commerce planting except that in College Station the holes into which the tubes were placed were 45.7 cm deep and on a 1 m spacing. The second College Station planting was conducted by transplanting plants directly into a field plot on 2 m spacings. The soil level in the tubes at the Commerce planting was even with the soil level of the field but the soil level in the tubes at College Station was 45.7 cm above the soil level of the field. The placement of the soil level in the College Station tubes in relation to the soil surface of the field exposed the upper half of the soil column in the tubes to more severe cold conditions. The upper half of the soil column was not expected to receive the insulation provided by the soil profile in the field that is beneficial to rhizome survival.

The tubes were constructed by cutting 3 m lengths of 15.2 cm diameter PVC pipe into two equal halves longitudinally with a reciprocating saw. The halves were then de-burred using a rotary file and cordless drill. The de-burred halves were stacked one on top of the other and cut into 1.01 m sections with a chop saw. A 2.5 cm hole was drilled through the top of the arch formed by each half-circumference of pipe and was centered 6.4 cm from the end of the pipe. On the opposite of the half-circumference of pipe, opposite the 2.5 cm hole, three evenly spaced 0.3 cm holes were drilled approximately 2.5 cm from the end of the pipe. The 2.5 cm holes were used to move the tubes around once they had been filled with soil by placing a steel bar through the holes and producing a handle. The 0.3 cm in holes had galvanized wire threaded through them and crossing through the center of the pipe to form a web-like design. The threading took place after the two halves were joined together and secured with several rounds of duct

tape at locations along the length of the tube. The top of the tube was easily determined by the presence of the 2.5 cm holes and the bottom of the tube had the threaded wire. A 30.5x61 cm piece of burlap was folded several times and placed at the bottom of each tube. The threaded wire prevented the burlap from falling out of the bottom of the tube. The purpose of the burlap was to keep the soil in the tube while still allowing water to flow out.

The tubes were filled with soil taken from the top 15.2 cm at the Texas A&M University Research Farm. The soil was a Weswood Silty Clay Loam and the reason for using this soil was because it more closely simulated field conditions within the tubes. The tubes were filled to the 2.5 cm hole located at the top of the tube; the tubes were then tamped on the ground three times so that compaction was even among the tubes. The space between the soil surface and the bottom of the 2.5 cm hole that was created from the tamping was filled with soil but no additional tamping took place. This filling process created a 91.4 cm column of soil in each tube due to soil only being present in the space between the burlap and the bottom of the 2.5 cm hole, which were 2.5 cm and 7.6 cm from each end of the tube respectively. A soaker-hose irrigation system was set up to water the tubes slowly overnight and any drop in soil level was refilled with additional soil to the base of the 2.5 cm hole.

Plants were transplanted into the College Station field plot on July, 8th 2010 and a minimum of 2 gallons of H_2O was poured within a 30.5 cm radius of the plant center. One wk after transplanting the plants were fertilized at 34 Kg N acre^{-1} as ammonium sulfate and watered in with sprinkler irrigation. Plants were transplanted into the tubes

for both locations on August, 16th 2010 and stored outside in a rack system. Plants were watered in with the same soaker-hose irrigation system. The plants in the tubes were fertilized at 34 Kg N $acre^{-1}$ as ammonium sulfate and watered in with the soaker-hose irrigation system on August, 30th 2010. The Commerce tubes designated for the fall and spring sampling were placed in the ground on September 17th 2010. The tubes designated for the fall sampling did not have the holes backfilled and the tubes designated for the spring sampling did have the holes backfilled. A soaker-hose irrigation system was set up to slowly water the tubes. The College Station tubes designated for the spring sampling were placed into the holes on November, 12th 2010, 2 d after the fall sampling was completed, and the holes were backfilled. Open-ended, plastic sleeves were fitted over each tube before it was placed in its respective hole. This sleeve allowed the tubes to be easily removed from the holes for sampling.

The fall sampling in Commerce took place on October, 22nd 2010 and the first reported low temperature below freezing was November, 25th 2010. The tubes designated for the fall were removed from the ground using the steel bar placed through the holes at the top of the tube. The plastic sleeve stayed in the hole because the soil adhered to the plastic sleeve instead of the tube. The duct tape on the tubes was cut and the tubes were opened while lying on the ground. The soil column stayed intact once the tubes were opened. The soil column was divided laterally into two equal halves of 45.7 cm. A large tub of water was used to wash large amounts of soil away so the rhizome samples could be collected. Rhizome samples were taken from the upper and lower halves and kept separately from one another, so that a 0-45.7 cm and 45.7-91.4 cm

sample was collected for every plant. Only the 0-45.7 cm samples were used in analyses because very few plants had rhizomes below 45.7 cm at both sampling dates. 20.3 cm lengths of rhizome were ideally taken with the tips included. Many plants did not have rhizomes 20.3 cm long; therefore rhizomes of different lengths were taken. Regardless of their overall length, every rhizome collected had the tip attached. The rhizome samples were placed into plastic bags and stored on ice until returning to Texas A&M University campus at College Station. Cold storage was necessary to minimize any degradation of metabolites that may occur. Upon arrival in College Station the samples were washed individually in a bucket with a high-pressure stream of water to remove all soil from the rhizomes. Following the final washing, a diameter measurement was taken from the middle of the first whole internode out from the cut site or growth point from nine rhizomes. The samples were then drained, placed into plastic bags, and stored in a -80°C freezer. The College Station tubes were sampled on November, 10th 2010 using a similar procedure that was used for the Commerce sampling. The sampling in College Station differed because a tub of water was not used to initially remove the soil and the tubes did not have to be removed from holes in the ground.

The fall sampling of the second College Station environment, where the plants were grown in a field setting and not in a tube, took place on November, 17th 2010 and November, 19th 2010. The first reported temperature below freezing occurred on November, 27th 010. The first replication was sampled on the earlier date and the remaining replications were sampled on the later date. The sampling procedure for the plants in the field differed from those in tubes. For ease of access to the rhizomes, each

plant had its above ground vegetation removed immediately before sampling. This was done so near the time of sampling so that metabolites in the rhizomes would not be mobilized for vegetative regrowth. Following vegetation removal, drain spades or sharp shooter spades were used to dig around the perimeter of the primary bunch of tillers. Once the main bunch of tillers was encircled, the plant and root/rhizome mass was inverted. This created a hole that was approximately 25.4-30.5 cm deep and placed the root/rhizome mass into the air. The hole's depth was increased to 45.7 cm and rhizomes were collected from both the inverted rhizome mass and the hole down to 45.7 cm. No rhizomes were found to be below 45.7 cm; therefore no 45.7-91.4 cm samples were collected. This may have been caused by a hard pan located at approximately 40.6 cm below the soil surface. Rhizomes were collected throughout the 0-45.7 cm space and ideally at 20.3 cm in length with the tips attached. As in the earlier samplings, rhizomes shorter than 20.3 cm were collected but the tips were always attached. After sampling, the rhizomes were placed into plastic bags and stored on ice until returning to Texas A&M University campus. From this point the procedure did not differ from that used for the tubes.

Prior to the spring sampling, several measurements of overwintering and spring regrowth were taken. Green shoot number within a 1 m^2 quadrat centered on the primary bunch of tillers for each plant as well as the highest leaf number among the shoots for each plant in the field planting at College Station were collected on March, 2nd 2011. Spring greenup measurements were taken the day of sampling, April, 1st 2011 in College

Station and April, 7th 2011 in Commerce, for each of the tubes at both locations. Greenup measurements consisted of spring regrowth being present or absent in the tube.

The spring sampling, at all the locations, was carried out in much the same manner as the fall sampling. Deviations from the fall sampling procedure included no tub of water being used at Commerce and the standing vegetation in the field planting at College Station was mechanically removed several wks before sampling. Only live rhizome tissue was collected in the spring samplings as some of the rhizomes had succumbed to freeze damage. This was not an issue in the fall sampling. Live rhizome tissue was determined by the rhizomes having a rigid and stiff structure and when bent there was resistance. Additionally a score of 1-5, 1 being the healthiest and 5 being the poorest, was assigned to each rhizome sample from the tube plantings. Many of the plants did not greenup in the spring or had rhizomes with an unhealthy, dark appearance.

To prepare the samples for analysis, the rhizomes were placed into mesh bags before being placed in a lyophilizer (Labconco, Kansas City, MO) to dry completely. The mesh bags allowed airflow through each sample and better facilitated drying. Samples were kept in the lyophilizer for at least 3 d at -40°C. Once the rhizome samples had completely dried, they were immediately ground or stored in a -80°C freezer until grinding could take place. Before grinding, all root material was removed the rhizomes. Samples were ground to a final 1mm particle size for analysis using first a coarse grinder and then a UDY Cyclone mill (Udy Corp, Fort Collins, CO). Once the material was ground to the final particle size of 1 mm, it was placed into glass vials and stored in cold storage at 50°F. The spring samples for the College Station and Commerce tubes

differed from the protocol described in that these samples were first placed in a -20°C freezer for 12 h before being transferred to a -80°C freezer. This was done to partially cool the material and not stress the -80°C freezer.

The analyses performed on the samples included determinations of crude protein (CP), crude fat (CF), starch, water soluble carbohydrates (WSC), and ethanol soluble carbohydrates (ESC). These measurements were outsourced to Dairy One (Utica, NY) for wet chemical analysis. WSC consists of primarily glucose, sucrose, fructose, maltose, lactose, and fructans. ESC primarily consists of glucose, sucrose, fructose, maltose, and lactose. An estimate of fructans concentration was obtained by taking the concentration difference between WSC and ESC for each sample. The samples were scanned using a Thermo Scientific Antaris II FT-NIR Analyzer (Thermo Scientific Inc., Waltham, MA) before the wet chemical analyses were conducted. To insure that the samples were packed uniformly in the vials prior to scanning, the vials were packaged together in a small box and tamped on a lab bench 15 times to pack that material in the bottom of the vials. The results of the wet chemical analyses for the fall samples were used with the predicted NIR values to create prediction curves using the OMNIC 8 software suite (Thermo Scientific Inc., Waltham, MA). To develop predictions in TQ Analyst spectral data was first smoothed using a Norris derivative filter by analyzing segments of the spectra at lengths of 5 units and gaps between segments were 5 units as well. Only spectra within the range of 4,000-8,000 wave numbers cm^{-1} were used in developing prediction curves. The first derivative of the spectral data was used in the calibration for the predictive curves of CP, starch, WSC, and ESC. The second

derivative of the spectral data was used to develop the curve for CF. The second derivative aids in separating peaks of interest, furthermore than the first derivative, in the spectral data. The number of PLS factors used was optimized by the software package and were 10, 7, 7, 7, and 2 for CP, starch, WSC, ESC, and CF, respectively. The number of factors used in CF was increased manually to 5. A mean centering data normalization technique was used to develop the predictive curves for CP, starch, WSC, and ESC. To create the predictive curve for CF a variance scaling data normalization technique was used. Variance scaling is useful when a value is predicted for a group of compounds that exist at varying levels within the sample. The prediction curve for CF required different parameters to increase the coefficient of correlation from 0.44 to 0.90. Finally, the greatest outlier was removed from each dataset for each metabolite.

The data was analyzed for genotype, environmental, and harvest date effects as well as an interaction between genotype and environment using a PROC GLM statement in SAS version 9.1 (SAS Institute, 2003). Because of differences in experimental design the three experiments at the two locations were treated as three separate environments. Correlations between CP, CF, starch, WSC, ESC, rhizome diameter, and overwintering capability were also determined using a PROC CORR statement. Means were separated using SNK and Tukey's at a 0.05 level of significance.

CHAPTER IV

RESULTS & DISCUSSION

Sorghum bicolor x *S. halepense* Hybridization

Total seed production differed significantly between the female *S. bicolor* genotypes regardless of which *S. halepense* was used as a pollinator (Table 7). Total seed production encompassed all seed classes (Table 8). Total seed production was greater for the *iap/iap* genotype with crosses made using the *S. halepense* genotypes 09TX04 and 09TX07. These results are similar to those reported by Price et al. (2006) where the *iap/iap* genotype increased pollen tube growth into the ovary over *Iap/-* for interspecific hybrids with three other *Sorghum* species, *S. halepense* not being one of the three tested. Crosses using the third *S. halepense* genotype 09TX09 as the male parent had significantly higher total seed production when the *Iap/-* genotype of *S. bicolor* was used as the female as compared to the *iap/iap* genotype. This is contrary to the results of the other crosses made in this study as well as the results reported by Price et al. (2006). The number of female florets pollinated with 09TX09 pollen was the lowest for all of the males used and this may have contributed to the unexpected results. Another possibility for the unexpected results could be a day effect, as all of the pollinations with the *iap/iap S. bicolor* occurred on the same day and conditions may not have been conducive for pollination on that day. Using more female florets would help overcome any possible confounding effects due to unequal numbers of attempted pollinations or less-than ideal pollinating procedures.

Total seed production represented all seed, even seed with dramatically diminished endosperm and reduced probability of germination. A more informative form of seed production was germinable seed production, or seed that had evident endosperm development and are more likely to germinate. Germinable seed are able to produce progeny and shriveled seed confirm that fertilization occurred but seed development was lacking. Casady and Anderson (1952) and Dweikat (2005) made distinctions between shriveled and well-developed seed. In both of these cases only the well-developed seed were able to germinate. Germinable seed consisted of seed classes A, B, and C (Fig. 1). Germinable seed production was significantly different only for the crosses made using the *S. halepense* genotype 09TX07 as the male parent (Table 9). No germinable seed was produced in the crosses using the *S. halepense* male parent 09TX09. More germinable seed may have been produced if more florets had been pollinated or if pollinations had been conducted on multiple days. In this study only the germinable seed germinated and only classes A and B produced hybrids.

Hybrids were recovered only from crosses using the *S. halepense* male parent 09TX07 and hybrid production was significantly higher when the *S. bicolor iap/iap* genotype was used as the female. Examples of flow cytometry hybrid determination are given in Fig. 2. This method of confirmation was efficient due to the fact that all hybrids produced were polyploids and when compared to a diploid standard, the DNA content of their nuclei was greater and easily detectable. Hybrid production is an indicator, in addition to total seed and germinable seed production, that the *iap/iap* genotype results in greater interspecific hybridization. Morphologically the hybrids more closely

resembled the *S. halepense* parent and their seed set ranged from 56.4 to 69.8% when self-pollinated. All hybrids were tetraploid, which differs from the expected triploid ploidy level. Tetraploid and triploid hybrid production has been previously reported in varying ratios by others (Karper and Chisholm, 1936; Hadley, 1953; Endrizzi, 1957; Bennett and Merwine, 1966; Merwine and Bennett, 1966; Sengupta and Weibel, 1971). The development of a triploid hybrid occurs by the fertilization of normal haploid egg cell (gamete) from the diploid *S. bicolor* with a haploid sperm nucleus (gamete) from tetraploid *S. halepense.* The formation of tetraploid hybrids has multiple possible explanations. Syncyte formation during megasporogenesis was proposed by Merwine and Bennett (1966) to create these tetraploid interspecific hybrids and Kidd (1952) suggested that syncyte formation was responsible for triploid *S. bicolor* plants in a monoculture of *S. bicolor*. Genotype effects when *S. bicolor* is the female are other possible explanations for tetraploid hybrids. Bennett and Merwine (1966) recovered only tetraploid with one *S. bicolor* genotype and Sengupta and Weibel (1971) reported triploid and tetraploid hybrids from four *S. bicolor* cultivars and only tetraploid hybrids from one cultivar. Hadley (1958) reported a higher frequency of tetraploid hybrids when cytoplasmic or genetic male sterile *S. bicolor* genotypes were used as females in lieu of hot water emasculation. Parental genotype interaction could play a role in obtaining hybrids with differing ploidy levels. Endrizzi (1957) sites fertilization of diploid egg as the explanation for tetraploid hybrids. Interspecific hybridizations between *S. bicolor* and *S. macrospermum* Garber (2n=4x=40) yielded only the expected triploid hybrids (Price et al., 2005; Kuhlman et al., 2008; Kuhlman et al., 2010). This suggests that some

mechanism may be affecting ploidy level in hybrids between *S. bicolor* and *S. halepense* that is not seen with other male parents; e.g. *S. macrospermum*.

Sorghum Species Genomic Relationships

Genetic similarities between the bulks are presented in Fig. 3 and Table 10. The genetic similarities between the bulks are graphically represented using a dendrogram constructed from the data generated from the presence or absence of alleles and shows the separation of the bulks from one another (Fig. 3). The highest degree of similarity was observed between the typical and non-flowering *S. halepense* bulks. This high degree of similarity follows logic as the only observable difference between the bulks was a differential flowering time phenotype, typical vs. non. The triploid/putative hybrid bulk had a higher degree of genetic similarity with the *S. halepense* bulks than did the *S. bicolor* bulk. This similarity scheme was also very logical. Considering the triploid/putative hybrid bulk consisted of true interspecific hybrids between *S. bicolor* and *S. halepense* then 66.67% (two sets of chromosomes ~2x) of the hybrids' genetic would be expected to come from the *S. halepense* parent and 33.33% (one set of chromosomes ~1x) would be expected from the *S. bicolor* parent. When the gametes fuse to form the hybrid embryo the resulting genome configuration would be triploid, 3x. The percentages would remain the same with other proposed genomic formulas, *S. bicolor* being a tetraploid and *S. halepense* being an octaploid (Tang and Liang, 1988).

The pairwise genetic similarity coefficients are presented in Table 10 and show that the typical *S. halepense* and the non-flowering *S. halepense* bulks had similarity values of 0.5265 and 0.4983, respectively with the *S. bicolor* bulk. These values are near

the 0.5 value that would be expected if *S. halepense* is an allotetraploid with the *S. bicolor* genome representing half of its genetic material (Hadley, 1953; Celarier, 1958; de Wet, 1978). The putative triploid hybrid bulk had a pairwise similarity value of 0.6724 with the typical *S. halepense* bulk and this value is near the expected value of 0.6667 if the putative hybrids actually were hybrids between *S. bicolor* and *S. halepense*. The reason for the expected value has been explained in the above text. The pairwise similarity values for the triploid/putative hybrid bulk with the *S. bicolor* and non-flowering *S. halepense* were 0.5979 and 0.5987, respectively. The *S. bicolor* value should be closer to 0.3333 because one set of chromosomes in the putative triploid hybrid would come from the diploid *S. bicolor* parent. The pairwise value of 0.5987 should be closer to 0.6667 as with the typical *S. halepense* bulk, because the only morphological distinction between the typical and non-flowering bulks was flowering time. The typical and non-flowering *S. halepense* bulks had a similarity value of 0.6296, and if the bulks were very similar because they consist of genotypes within the same species, the value should be closer to 1.0. The departures from the expected values in the last three pairwise genetic similarity values discussed may be explained by bulks consisting of fewer genotypes as compared to previous BSA's, where in some cases more than 40 genotypes were used to create a single bulked sample (Michelmore et al., 1991; Singh et al., 2006; Fondevilla et al., 2008; Remus-Borel et al., 2010). A larger quantity of heterogenic genotypes would increase the possibility of identifying a higher percentage of alleles unique to that bulk and similarities or differences with other bulks.

Markers unique to the *S. bicolor* and *S. halepense* bulks (Table 11) could be utilized for future marker assisted hybrid verification in lieu of flow cytometry, as was used in the hybridization study. Dweikat (2005) used *S. bicolor* specific markers along with morphological characteristics to verify interspecific hybrids with *S. halepense*. The 15 markers found in both the *S. bicolor* and non-flowering *S. halepense* bulks, but absent from the other bulks may help to explain the *S. halepense* non-flowering phenotype by introgression of *S. bicolor* maturity genes into the *S. halepense* genome. Maturity genes have quantitative effects on flowering time in *S. bicolor* with *Ma5* and *Ma6* loci controlling photoperiod sensitivity (Rooney and Aydin, 1999).The unique markers for each bulk were associated with loci from across the genome.

Rhizome Composition and Overwintering

Genotypic effects on rhizome metabolite concentrations varied with levels of significance and both between and among environments (Table 12). Genotype by environment interactions were significant for multiple metabolites for the spring and fall sampling dates as well as the concentration level difference between the dates (Table 13). These interactions explain why no single genotype consistently produced the largest concentration of a single metabolite across environments. Environmental effects were significant on rhizome composition and characteristics at both sampling dates. Measures of spring regrowth were significantly affected by genotype at each environment; shoot counts in the field planting at College Station and presence of green shoots in both tube plantings.

Sampling date also had significant effects, at the 0.01 level, on the concentration of every metabolite except ethanol soluble carbohydrates (ESC). Seasonal effects on varying rhizome composition have been reported for *S. halepense* and *Phalaris arundinacea* L. (Rapp, 1947; McWhorter, 1961; McWhorter, 1974; Xiong et al., 2009). From these results it is apparent that many factors affect rhizome composition. Separated means for measured components of each genotype for each environment and sampling date are shown in Tables 14-19. Separation was not efficient for most concentration means in at the Commerce environment. This could be because the samples had to remain on ice for the minimum of 4 h during the return trip to College Station before they could be cooled to -80°C and some degradation may have occurred. Differences in metabolite concentrations between sampling dates are given in Tables 20-22. The differences were calculated by subtracting the spring concentrations from those from the fall; a negative value means a decrease from the fall and a positive valued means an increase from the fall. Genotypic effects were significant for differences in concentration between sampling dates for some metabolites as well. Genotype effects on rhizome health scores were only significant for the Commerce tube environment. The genotypes with the best and healthiest rhizome score means at the College station tube planting, 09TX05 and 09TX06, were the only genotypes that had green shoots across all replications in the spring, even though genotypes effects were not significantly different.

Plant material was subjected to the most severe winter conditions at the College Station tube environment and this environment also had the most obvious differences in spring green up and rhizome scores. This environment was used to demonstrate

overwintering capacity in the most extreme sense. The Pearson correlation coefficient for green up and rhizome score at this environment is -0.61 at a significance of 0.01 and illustrates that as rhizome scores are healthier then green up is more likely to occur. The coefficient is negative because the lower scored rhizomes had a healthier appearance. I hypothesize that green up and rhizome score can both be used as a measure of overwintering capacity. Spring fructans levels had Pearson correlation coefficients with rhizome score and green up of -0.49 and 0.56 respectively and these values were significant at a 0.01 level. Similar coefficients were observed with the difference in fructans concentration between the fall and spring sampling dates. Regression coefficients of determination for spring fructans levels and differences in frutans levels from the fall when regressed upon rhizome scores and green up were not greater than 0.31 but were among the highest values for all the metabolites. Fructans have primarily are present in cool-season grasses and at low concentrations only in warm season grasses (Pollock and Cairns, 1991). The results from this study disagree with what Pollock and Cairns (1991) reported this and suggest that genotypes that can accumulate fructans have a higher potential for overwintering. Water soluble carbohydrates (WSC) had slightly lower correlation coefficients than fructans because fructans are included in WSC. Hoffman et al. (2010) determined that crowns of *Lolium perenne* L. accumulated WSC in response to temperatures of 2°C. Total carbohydrate accumulations have also been reported to increase freeze tolerance in the crowns of barley (*Hordeum vulgare* L.) (Livingston et al., 1989). Shahba et al. (2003) determined that rhizomes of *Distichlis spicata* (L.) Greene, saltgrass, accumulated non-structural carbohydrates to increase

freeze tolerance. In contrast, the accumulation of crude protein (CP) appears to decrease the possibility of spring green up. Patton et al. (2007) determined that specific proteins, not necessarily crude protein, increased freeze tolerance in zoysiagrasses. Pearson correlation coefficients for CP spring levels with rhizome score and green up were 0.53 and -0.38 respectively and were significant at the 0.10 level. Pearson coefficients for CP concentration differences between fall and spring sampling dates with rhizome score and green up were near those reported for spring concentrations of CP. The spring sampling means and difference between fall and spring sampling means of CP and fructans for the genotypes 09TX05 and 09TX06, the only genotypes to green up across all replications, had some of the lowest CP levels and highest fructans levels among the genotypes evaluated. These genotypes also had some of the greatest means for spring shoot number in the College Station field environment. CF was did not appear to be an indicator of overwintering capacity in this study. It has been found in *Paspalum vaginatum* and *Lolium perenne* that total lipids may not be as influential as the ratio of different fatty acids (Cyril et al., 2002; Hoffman et al., 2010).

Wet chemical analysis can be very expensive and a more affordable alternative is NIRS analysis. NIRS can also deliver results for multiple analyses quickly. A number of NIRS models were fit for calibration. Ultimately a model using the whole spectrum with Norris smoothing of 5 unit segments and 5 unit gaps NIRS predictions for CP, CF, WSC, ESC, and starch for the fall sampling date had correlation coefficients of 0.99, 0.44, 0.93, 0.85, and 0.98 respectively when predicted concentrations were regressed onto the concentrations determined by wet chemical analysis. The efficiency for each of

these predictions was 89.6, 35.3, 76.4, 59.0, and 80.7 for CP, CF, WSC, ESC, and starch, respectively. Adjusting the parameters for the CF prediction increased the correlation coefficient to 0.90 and increased the efficiency to 37.8. Mitchell et al. (1998) reported an NIRS correlation of 0.96 for total non-structural carbohydrates in peppermint (*Mentha piperita* L.) rhizomes which is similar to values reported herein.

Predictions for the spring concentrations were made using the predictive curve developed with the fall results. When the spring predicted concentrations were regressed onto the concentrations determined by wet chemical analysis the correlation coefficients were 0.94, 0.05, 0.81, 0.76, and 0.90 for CP, CF, WSC, ESC, and starch, respectively. When the adjusted parameters were used for the CF predictions, the correlation coefficient decreased to 0.01. From these values, it appears that NIRS is a reliable tool to estimate concentrations of CP and starch and to a lesser extent WSC and ESC. NIRS, however, is not useful for predicting CF concentrations of *Sorghum* species rhizomes.

Of the metabolite measured, those that appear to be significant indicators of overwintering, based on the College Station tube environment data, are fructans and CP. While fructans was not measured directly using wet chemistry or predicted using the NIR, WSC and ESC were. The difference between WSC and WSC is a measure of fructans concentration. The NIR was very effective at predicting CP concentrations. NIR could be useful for selecting stronger perennial parents in a breeding program.

CHAPTER V

SUMMARY

Sorghum bicolor x *S. halepense* Hybridization

The *iap/iap S. bicolor* genotype was more effective at increasing total seed production, germinable seed production, and hybrid seed production than *Iap/- S. bicolor* in controlled hybridizations with at least one *S. halepense* male. The hybrids produced were morphologically more similar to the *S. halepense* parent than the *S. bicolor* parent and had at least 56.4% seed set when self-pollinated. All hybrids were surprisingly tetraploids. Several possible explanations are reported in the literature. Overall the *iap/iap* genotype is a valuable tool for breeders to introgress wild genetic material into *S. bicolor* and create interspecific hybrids between *S. bicolor* and *S. halepense*.

Sorghum Species Genomic Relationships

The BSA provided additional evidence for the theory that *S. halepense* is an allopolyploid that includes the *S. bicolor* genome, and it aided in explaining the non-flowering phenotype present in some of the *S. halepense* genotypes, as can be seen in the genetic similarity coefficients. There were departures from expected pairwise similarity coefficients with some of the genetic similarity coefficients but this may be alleviated by using bulked samples that contain more genotypes and more genetic diversity. This method of analysis was also effective at identifying unique markers for future interspecific hybrid determination and could possibly be used to explain the non-flowering phenotype present in the non-flowering *S. halepense* bulk. The unique markers

identified would need to be surveyed with both the *S. bicolor* parent and the *S. halepense* parent to determine if the particular parents used shared alleles that were determined to be unique. Marker assisted hybrid verification is now possible and this method was more economically feasible than surveying many individuals within populations.

Rhizome Composition and Overwintering

Genotype, environment, genotype by environment interactions, and sampling date had significant effects on rhizome. Many factors affect rhizome composition but it is likely possible to select *S. halepense* genotypes with greater overwintering capacity that have lower CP concentrations and greater fructans concentrations within their rhizomes in the spring. NIR was effective at determining certain metabolite concentrations within rhizome samples and in particular CP and WSC, which includes fructans. NIR could be used in selecting stronger perennial parents while minimizing the need for costly wet chemical analyses.

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APPENDIX A

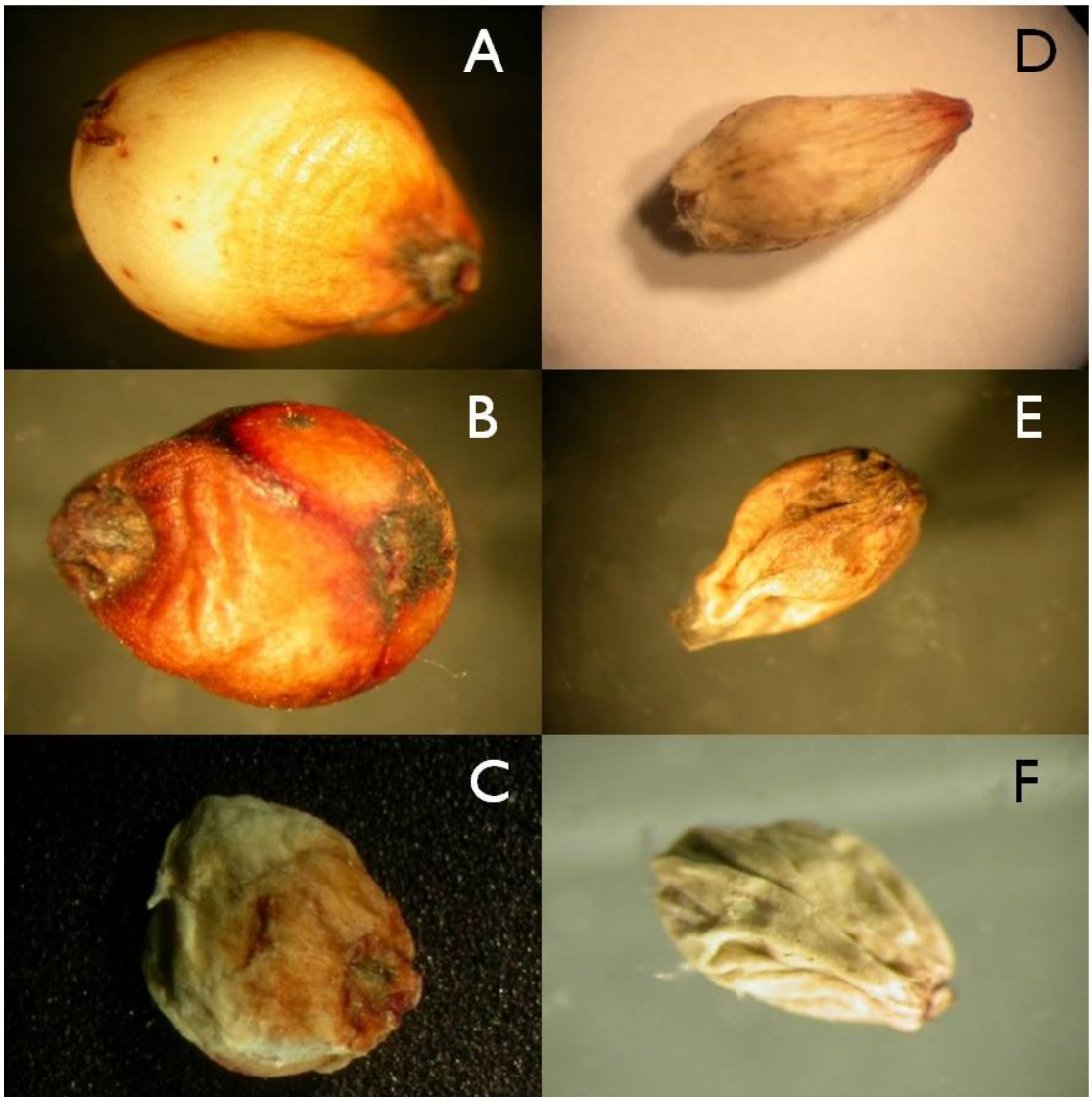

Fig. 1. Seed classes produced from hybridizations between *S. bicolor* and *S. halepense*. (A) Large, full endosperm and slightly lobed; (B) Large, full endosperm and heavily lobed; (C) Moderate endoperm with wrinkled pericarp; (D) Diminished and irregular endosperm with minimal pericarp folds; (E) Diminished endosperm with moderate pericarp folds; (F) Diminished endosperm, pericarp with many folds, and a stigma/style remnant.

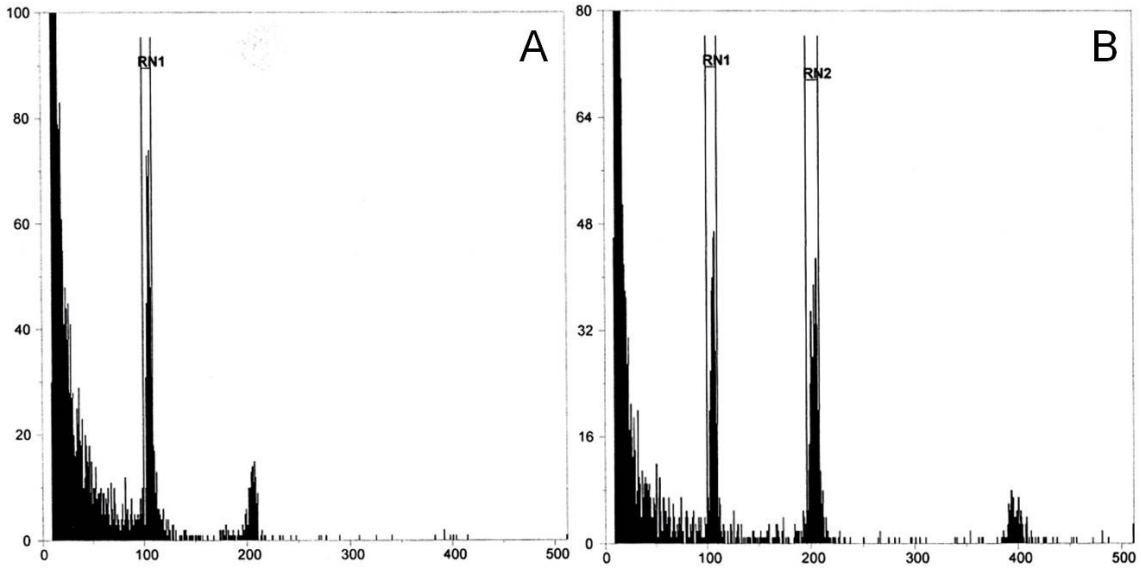

Fig. 2. Verification of hybrids using flow cytometry. X axis is the photon intensity associated with DNA content. Y axis is the particle or nuclei count. (A) Diploid parent and standard with 2C peak aligned at 100 on the X axis. (B) Diploid standard and a tetraploid hybrid with 2C peaks aligned at 100 and 200 respectively on the X axis.

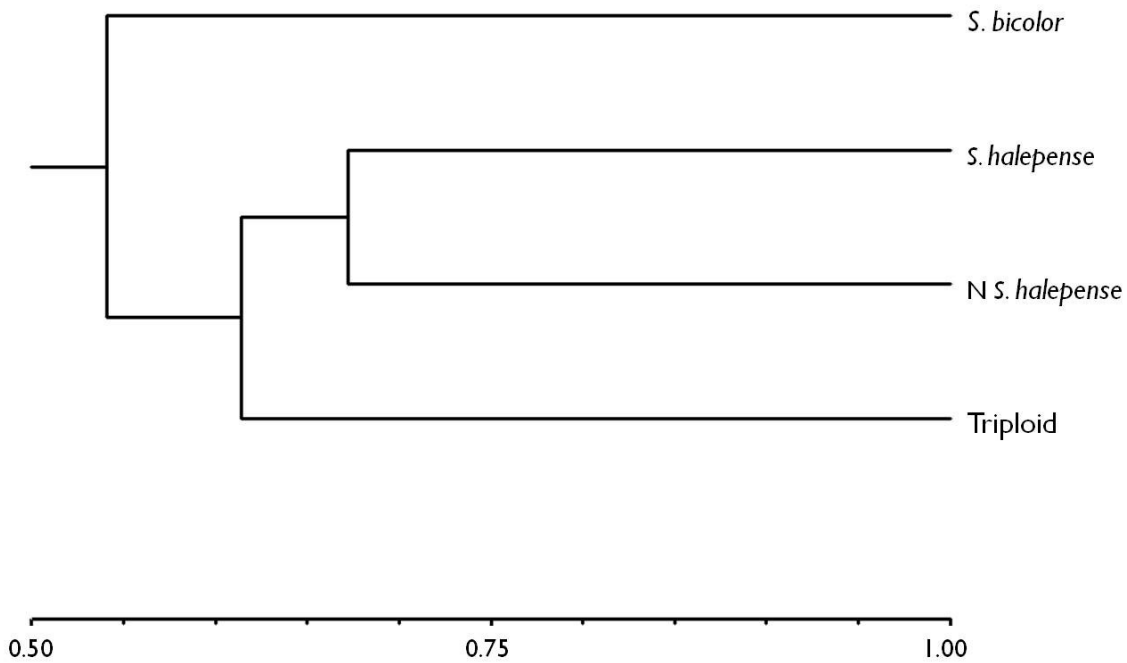

Fig. 3. Genetic similarity tree showing relatedness between bulks. *S. bicolor* is the diploid *S. bicolor* bulk, *S. halepense* is the typical flowering *S. halepense* bulk, N *S. halepense* is the non-flowering *S. halepense* bulk, and Triploid is the putative triploid hybrid bulk. The x axis is the proportional similarity value.

APPENDIX B

Table 1. Parent material used in hybridization comparison.

S. halepense Genotypes	*Iap/- S. bicolor* A-lines	*iap/iap S .bicolor* A-line
09TX04	PI 598118	Tx3361
09TX07	PI 598084, 598086, 598090, 598094, 598096, 598112, 598114, 598116, 598118	Tx3361
09TX09	PI 598118	Tx3361

Table 2. *S. bicolor* genotypes used in bulked segregant analysis and their genetic background.

PI #	Genetic Background
598091	60-Day Milo
598093	58-Day Milo
598094	Ryer Milo
598097	38-Day Milo
598105	Sooner Milo
598106	Sooner Milo
598109	Sooner Milo
598113	Texas Blackhull Kafir
598116	Acme Broomcorn
598118	Japanese Dwarf Broomcorn

Table 3. Typical *S. halepense* genotypes used in bulked segregant analysis and their origin.

Genotype	Origin
09NC01	Collected off of Interstate 77 at mile marker 90 near Union City, NC
09TX01	Collected off of FM 455 near Pilot Point, TX
09TX03	Collected at Texas A&M Research Farm S of FM 60, W of College Station, TX
09TX04	Collected at Texas A&M Research Farm S of FM 60, W of College Station, TX
09TX06	Collected off of Hwy 47 between Hwy 21 and FM 60, W of College Station, TX
09TX07	Collected off of Hwy 21 0.5 mi W of Hwy 290 intersection, NE of Bastrop, TX
09TX08	Collected off of Hwy 304 S of Bastrop, TX
09TX09	Collected off of Charles Blvd in Bastrop, TX city park
09TX20	PI#271615 (Country of origin : India)

Table 4. Non-flowering *S. halepense* genotypes used in bulked segregant analysis and their origin.

Genotype	Origin
09TX13	Collected off Hwy 71 S of La Grange, TX
09TX14	Collected off Hwy 71 S of La Grange, TX
09TX15	Collected off Hwy 71 S of La Grange, TX

Table 5. Putative triploid hybrid genotypes used in bulked segregant analysis and their origin.

Genotype	Origin
09TX02	Collected at Texas A&M Research Farm S of FM 60
10TX01	Collected at Texas A&M Research Farm S of FM 60
10TX02	Collected at Texas A&M Research Farm S of FM 60
10TX03	Collected at Texas A&M Research Farm N of FM 60

Table 6. *S. halepense* and *S. almum* genotypes and used in the rhizome composition study and their origin.

Genotype	Origin
09NC01	Collected off of Interstate 77 at mile marker 90 near Union City, NC
09TX05	Collected off of Hwy 47 between Hwy 21 and FM 60, W of College Station, TX
09TX06	Collected off of Hwy 47 between Hwy 21 and FM 60, W of College Station, TX
09TX07	Collected off of Hwy 21 0.5 mi W of Hwy 290 intersection, NE of Bastrop, TX
09TX08	Collected off of Hwy 304 S of Bastrop, TX
09TX10	Collected off of Hwy 47 between Hwy 21 and FM 60, W of College Station, TX
09TX11	Collected off of Hwy 95 near Bastrop, TX
09TX13	Collected off Hwy 71 S of La Grange, TX
09TX14	Collected off Hwy 71 S of La Grange, TX
09TX15	Collected off Hwy 71 S of La Grange, TX
09TX20	PI#271615 (Country of origin : India)

Table 7. Total seed production, size, and weight for the male *S. halepense* parent 09TX04.

S. bicolor Genotype	Florets	Seed	Seed Set	Mean Seed Length	Mean Seed Width	Mean Seed Depth	Mean Seed Weight
	no.	no.	%	mm	mm	mm	mg
				09TX04			
Iap/-	469	3***	0.64***	3.6	2.6	1.7**	9.5**
iap/iap	724	110***	15.19***	3.2	2.1	1.3**	2.7**
				09TX07			
Iap/-	2385	22***	0.92***	2.7**	1.7***	1.2	3.0
iap/iap	1977	276***	13.96***	3.4**	2.3***	1.3	3.9
				09TX09			
Iap/-	398	13***	3.27***	2.4	1.1	1.0	0.9
iap/iap	394	0***	0.00***	-	-	-	-

** Significantly different at 0.05 level
*** Significantly different at 0.01 level

Table 8. Seed class average length, width, depth, and weight values.

Seed Class	Seed Parameter							
	Mean Length		Mean Width		Mean Depth		Mean Weight	
	mm		mm		mm		mg	
A	4.0	a	3.5	a	2.2	a	22.3	a
B	4.0	a	3.3	ab	2.0	ab	17.0	b
C	3.6	a	3.1	b	1.8	b	9.2	c
D	3.8	a	2.2	c	1.8	b	4.9	d
E	2.5	c	1.2	e	1.0	c	1.0	e
F	3.1	b	1.9	d	1.1	c	1.4	e

Table 9. Germinable seed (classes A, B, and C) production, size, and weight.

S. bicolor Genotype	Florets	Seed	Seed Set	Mean Seed Length	Mean Seed Width	Mean Seed Depth	Mean Seed Weight	Hybrid Retrieved
	no.	no.	%	mm	mm	mm	mg	no.
				09TX04				
Iap/-	469	3	0.64	3.6*	2.6**	1.7*	9.5***	0
iap/iap	724	8	1.10	4.4*	3.5**	2.1*	18.6***	0
				09TX07				
Iap/-	2385	2***	0.08***	3.9	3.4	2.9*	0.0213**	0**
iap/iap	1977	35***	1.77***	3.6	3.2	1.8*	0.0110**	4**

* Significantly different at 0.10 level
** Significantly different at 0.05 level
*** Significantly different at 0.01 level

Table 10. Genetic similarity coefficients for groups of *Sorghum* species. *S. bicolor* is the diploid *S. bicolor* bulk, *S. halepense* is the typical flowering *S. halepense* bulk, N *S. halepense* is the non-flowering *S. halepense* bulk, and Triploid is the putative triploid hybrid bulk.

	S. bicolor	*S. halepense*	N *S. halepense*	Triploid
S. bicolor	1.0000			
S. halepense	0.5265	1.0000		
N *S. halepense*	0.4983	0.6296	1.0000	
Triploid	0.5979	0.6724	0.5987	1.0000

Table 11. Allele bands present and/or absent in individual bulks or bulk combinations. “+” indicates presence in the bulk and “-“ indicates absence from the bulk.

No. of Allele Bands	*S. bicolor*	*S. halepense*	N *S. halepense*	Triploid
39	+	-	-	-
23	-	+	-	-
26	-	-	+	-
22	-	-	-	+
15	+	-	+	+/-
12	+	-	+	-
22	+	-	-	+
20	-	+	+	-
22	-	+	-	+
14	-	-	+	+
10	+	+	+	-
21	+	+	-	+
21	+	-	+	+
33	-	+	+	+
59	+	+	+	+

Table 12. Genotype effects on rhizome metabolites and characteristics and spring regrowth.

Environment	CP	CF	Starch	WSC	ESC	Fructans	Diameter Mean	Rhizome Score	Shoots m^{-2}	Green Up
					Fall					
College Station Field	***		***	***	*	***	***	-	-	-
College Station Tubes	***	**	***	**	**		***	-	-	-
Commerce Tubes	*				***		***	-	-	-
					Spring					
College Station Field	***	***	***	***	***	**	***	-	***	-
College Station Tubes	**			**		***			-	***
Commerce Tubes		**	**	*	**		***	**	-	**
					Difference					
College Station Field		**	**	***	*	***		-	-	-
College Station Tubes				**		***		-	-	-
Commerce Tubes	*		*		*			-	-	-

* Significant effects at 0.10 level

** Significant effects at 0.05 level

*** Significant effects at 0.01 level

Table 13. Significance of effects of environment (env), genotype (geno), and genotype by environment interaction (geno*env) on metabolites and rhizome characteristics.

Effect	CP	CF	Starch	WSC	ESC	Fructans	Diameter Mean	Rhizome Score
				Fall				
env	***	**	***		**		***	-
geno	***	**	***	***	***		***	-
geno*env	***		**	**		***	*	-
				Spring				
env	***		***	***	***	***	***	***
geno	***	***	***	***	***	***	***	**
geno*env	**	**	**	***	*	***		
				Difference				
env	***		***	***	***	***	***	-
geno	*	***	***	***	**			-
geno*env	**			***		***		-

* Significantly different at 0.10 level
** Significantly different at 0.05 level
*** Significantly different at 0.01 level

Table 14. Rhizome composition and diameter means for plants harvested from the College Station, TX field planting, fall 2010.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm	
09TX05	77.0	abc	7.0	a	161.0	b	288.0	ab	172.0	a	116.0	ab	6.2	bc
09TX06	79.0	abc	7.0	a	164.0	b	309.0	a	177.0	a	132.0	a	6.7	bc
09TX07	82.0	abc	5.0	a	190.0	b	300.0	a	187.0	a	113.0	ab	7.6	ab
09TX08	94.0	abc	10.0	a	204.0	b	266.0	abc	153.0	a	113.0	ab	6.7	bc
09TX10	71.0	bc	8.0	a	157.0	b	256.0	abc	156.0	a	99.0	abc	6.5	bc
09TX11	102.0	a	10.0	a	189.0	b	223.0	bcd	152.0	a	71.0	bcd	6.0	bc
09TX13	67.0	c	7.0	a	148.0	b	269.0	abc	165.0	a	104.0	abc	7.3	abc
09TX14	78.0	abc	8.0	a	171.0	b	212.0	cde	177.0	a	35.0	d	9.1	a
09TX15	69.0	c	8.0	a	200.0	b	208.0	cde	153.0	a	56.0	cd	7.7	ab
09TX20	84.0	abc	8.0	a	305.0	a	154.0	e	118.0	a	36.0	d	6.3	bc
09NC01	97.0	ab	9.0	a	200.0	b	175.0	de	126.0	a	49.0	cd	5.7	c
Tukey's $MSD_{0.05}$	27.0		6.0		93.0		67.0		73.0		56.0		1.9	

Table 15. Rhizome composition and diameter means for plants harvested from the College Station, TX tube planting, fall 2010.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm	
09TX05	59.0	abc	6.0	ab	126.0	b	277.0	a	204.0	a	73.0	a	5.3	b
09TX06	44.0	cd	6.0	b	162.0	ab	257.0	ab	176.0	ab	81.0	a	4.6	b
09TX07	42.0	cd	8.0	ab	188.0	a	278.0	a	187.0	ab	92.0	a	7.0	a
09TX08	68.0	ab	9.0	ab	191.0	a	217.0	ab	135.0	ab	82.0	a	5.2	b
09TX10	52.0	bcd	7.0	ab	202.0	a	223.0	ab	125.0	b	98.0	a	5.5	b
09TX11	44.0	cd	7.0	ab	186.0	a	224.0	ab	145.0	ab	79.0	a	5.3	b
09TX13	47.0	cd	9.0	ab	198.0	a	232.0	ab	143.0	ab	89.0	a	5.9	ab
09TX14	56.0	abcd	8.0	ab	153.0	ab	248.0	ab	139.0	ab	109.0	a	7.1	a
09TX15	36.0	d	10.0	ab	212.0	a	192.0	ab	124.0	b	69.0	a	5.6	b
09TX20	72.0	a	14.0	a	152.0	ab	206.0	ab	130.0	ab	58.0	a	5.0	b
09NC01	56.0	abcd	13.0	a	197.0	a	180.0	b	115.0	b	65.0	a	5.6	b

Table 16. Rhizome composition and diameter means for plants harvested from the Commerce, TX tube planting, fall 2010.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm	
09TX05	46	a	9	a	115	a	252	a	163	ab	89	a	4.2	b
09TX06	41	a	11	a	138	a	217	a	147	abc	70	a	4.4	b
09TX07	31	a	11	a	153	a	212	a	160	abc	53	a	6.9	a
09TX08	48	a	12	a	128	a	241	a	183	a	57	a	5	ab
09TX10	41	a	10	a	132	a	206	a	142	abc	63	a	4.9	ab
09TX11	47	a	6	a	160	a	216	a	135	abc	81	a	4.8	ab
09TX13	53	a	6	a	151	a	199	a	115	bc	84	a	5.4	ab
09TX14	55	a	7	a	129	a	241	a	124	abc	117	a	5.7	ab
09TX15	49	a	9	a	88	a	223	a	126	abc	96	a	6.7	a
09TX20	50	a	18	a	122	a	264	a	133	abc	132	a	4.1	b
09NC01	45	a	8	a	133	a	187	a	98	c	75	a	4.9	ab

Table 17. Rhizome composition, diameter, and sprout number means for plants harvested from the College Station, TX field planting, spring 2011.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean		Shoots m^{-2}	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm		no.	
09TX05	67.0	c	16.0	c	112.0	bc	283.0	abcd	211.0	ab	73.0	ab	6.1	a	116.0	ab
09TX06	64.0	c	18.0	c	125.0	bc	276.0	abcd	208.0	ab	68.0	ab	6.5	a	144.7	a
09TX07	80.0	bc	18.0	bc	99.0	bc	319.0	ab	193.0	abc	126.0	a	8.2	a	31.0	a
09TX08	95.0	ab	17.0	c	228.0	a	180.0	e	124.0	c	56.0	b	6.6	a	50.7	bc
09TX10	73.0	bc	20.0	bc	112.0	bc	238.0	cde	183.0	abc	55.0	b	7.4	a	75.0	abc
09TX11	106.0	a	25.0	abc	122.0	bc	280.0	abcd	224.0	a	56.0	b	7.4	a	34.7	c
09TX13	75.0	bc	25.0	abc	76.0	c	347.0	a	250.0	a	97.0	ab	7.9	a	41.0	c
09TX14	84.0	abc	21.0	abc	86.0	c	287.0	abcd	201.0	ab	86.0	ab	7.8	a	52.7	bc
09TX15	69.0	c	26.0	abc	145.0	abc	292.0	abc	211.0	ab	81.0	ab	8.1	a	49.0	bc
09TX20	82.0	bc	32.0	a	180.0	ab	213.0	de	141.0	bc	72.0	ab	5.7	a	129.3	a
09NC01	95.0	ab	30.0	ab	100.0	bc	262.0	bcd	181.0	abc	81.0	ab	5.8	a	30.0	c
Tukey's $MSD_{0.05}$	22.0		12.0		83.0		76.0		73.0		69.0		2.5		72.2	

Table 18. Rhizome composition, diameter, and rhizome score means for plants harvested from the College Station, TX tube planting, spring 2011.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean		Rhizome Score	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm		1-5	
09TX05	51.0	ab	19.0	a	61.0	a	243.0	ab	151.0	a	92.0	ab	4.6	a	2.0	a
09TX06	61.0	ab	13.0	a	64.0	a	285.0	a	174.0	a	111.0	a	4.0	a	2.3	a
09TX07	66.0	ab	27.0	a	51.0	a	206.0	ab	138.0	a	68.0	abc	5.6	a	3.3	a
09TX08	83.0	a	29.0	a	89.0	a	106.0	b	89.0	a	17.0	c	5.0	a	4.0	a
09TX10	64.0	ab	25.0	a	62.0	a	154.0	ab	98.0	a	47.0	bc	4.0	a	4.0	a
09TX11	64.0	ab	17.0	a	51.0	a	128.0	ab	118.0	a	16.0	c	5.5	a	3.7	a
09TX13	74.0	ab	22.0	a	54.0	a	125.0	ab	103.0	a	23.0	c	5.8	a	4.5	a
09TX14	49.0	b	17.0	a	63.0	a	132.0	ab	116.0	a	16.0	c	5.6	a	2.7	a
09TX15	44.0	b	20.0	a	91.0	a	157.0	ab	149.0	a	12.0	c	5.2	a	2.7	a
09TX20	-		-		-		199.0	ab	128.0	a	-		5.0	a	4.5	a
09NC01	-		-		66.0	a	98.0	b	82.0	a	16.0	c	5.6	a	4.0	a

Table 19. Rhizome composition, diameter, and rhizome score means for plants harvested from the Commerce, TX tube planting, spring 2011.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean		Rhizome Score	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm		1-5	
09TX05	42.0	a	23.0	b	24.0	ab	179.0	ab	144.0	a	35.0	a	4.1	abc	1.0	b
09TX06	4.2	a	15.0	b	32.0	ab	157.0	ab	127.0	a	31.0	a	3.7	bc	1.3	b
09TX07	5.4	a	22.0	b	22.0	ab	184.0	ab	153.0	a	31.0	a	4.8	ab	1.0	b
09TX08	6.4	a	23.0	b	73.0	a	154.0	ab	117.0	a	37.0	a	4.6	abc	1.5	b
09TX10	5.0	a	14.0	b	8.0	ab	97.0	ab	81.0	a	16.0	a	3.6	c	2.0	ab
09TX11	6.2	a	22.0	b	24.0	ab	135.0	ab	100.0	a	35.0	a	4.4	abc	1.0	b
09TX13	7.2	a	29.0	b	11.0	ab	155.0	ab	106.0	a	49.0	a	4.6	abc	1.7	b
09TX14	4.9	a	28.0	b	67.0	ab	189.0	ab	155.0	a	33.0	a	5.2	a	1.3	b
09TX15	5.0	a	23.0	b	28.0	ab	212.0	a	144.0	a	69.0	a	5.2	a	2.0	ab
09TX20	7.8	a	56.0	a	8.0	ab	125.0	ab	116.0	a	9.0	a	3.6	c	1.3	b
09NC01	9.1	a	-		5.0	b	74.0	b	71.0	a	3.0	a	4.4	abc	3.0	a

Table 20. Rhizome composition and diameter means for plants harvested from the College Station, TX field planting, difference between fall 2010 and spring 2011.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm	
09TX05	-1.0	a	0.9	b	-4.9	ab	-0.5	bc	3.8	ab	-4.3	ab	-0.2	a
09TX06	-1.5	a	1.1	ab	-3.9	ab	-3.3	bc	3.1	ab	-6.4	b	-0.2	a
09TX07	-0.1	a	1.3	ab	-9.1	ab	1.9	abc	0.6	ab	1.3	ab	0.5	a
09TX08	0.0	a	0.7	b	2.3	a	-8.7	c	-2.9	b	-5.7	b	0.0	a
09TX10	0.2	a	1.2	ab	-4.5	ab	-1.8	bc	2.7	ab	-4.5	ab	1.0	a
09TX11	0.4	a	1.4	ab	-6.7	ab	5.7	ab	7.2	ab	-1.6	ab	1.4	a
09TX13	0.8	a	1.7	ab	-7.2	ab	13.5	a	8.5	a	0.5	ab	0.6	a
09TX14	0.6	a	1.3	ab	-8.6	ab	7.5	ab	2.4	ab	5.1	a	-1.3	a
09TX15	-0.1	a	1.9	ab	-5.5	ab	8.4	ab	5.9	ab	2.5	ab	0.3	a
09TX20	-0.1	a	2.4	a	-12.5	b	5.9	ab	2.4	ab	3.5	ab	-0.7	a
09NC01	-0.2	a	2.1	ab	-10.0	b	8.7	ab	5.5	ab	3.2	ab	0.0	a

Table 21. Rhizome composition and diameter means for plants harvested from the College Station, TX tube planting, difference between fall 2010 and spring 2011.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm	
09TX05	-0.7	a	1.3	a	-6.5	a	-3.4	a	-5.4	a	1.9	a	-0.7	a
09TX06	1.7	a	0.8	a	-9.8	a	2.9	a	-0.2	a	3.0	a	-0.7	a
09TX07	2.0	a	2.0	a	-13.7	a	-7.2	a	-4.8	a	-2.4	ab	-1.3	a
09TX08	1.3	a	1.9	a	-9.1	a	-12.2	a	-6.1	a	-6.1	ab	-0.6	a
09TX10	1.6	a	2.0	a	-12.5	a	-8.9	a	-2.7	a	-9.5	b	-1.6	a
09TX11	2.0	a	1.0	a	-13.4	a	-9.6	a	-2.7	a	-6.6	ab	0.2	a
09TX13	2.8	a	1.3	a	-14.7	a	-12.2	a	-5.1	a	-7.1	ab	-0.1	a
09TX14	-0.7	a	0.8	a	-9.1	a	-11.6	a	-2.3	a	-9.2	b	-1.5	a
09TX15	0.7	a	1.0	a	-12.1	a	-3.6	a	2.6	a	-5.3	ab	-0.4	a
09TX20	-		-		-		3.3	a	-3.3	a	-		0.4	a
09NC01	-		-		-11.7	a	-8.3	a	-3.5	a	-4.8	ab	0.4	a

Table 22. Rhizome composition and diameter means for plants harvested from the Commerce, TX tube planting difference between fall 2010 and spring 2011.

Genotype	CP		CF		Starch		WSC		ESC		Fructans		Diameter Mean	
	g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		g Kg^{-1}		mm	
09TX05	2.5	ab	1.3	a	-9.1	a	-7.3	a	-1.9	a	-5.4	a	-0.1	a
09TX06	0.1	b	0.4	a	-10.6	a	-6.0	a	-2.0	a	-3.9	a	-0.6	a
09TX07	2.2	ab	1.1	a	-13.1	a	-2.8	a	-0.6	a	-2.2	a	-2.1	a
09TX08	1.4	ab	1.2	a	-5.6	a	-9.8	a	-6.5	a	-3.3	a	-0.8	a
09TX10	0.9	ab	0.3	a	-12.4	a	-11.8	a	-7.7	a	-4.1	a	-1.5	a
09TX11	1.7	ab	1.6	a	-13.6	a	-8.2	a	-3.5	a	-4.6	a	-0.4	a
09TX13	1.4	ab	2.1	a	-13.9	a	-4.4	a	-0.9	a	-3.5	a	-0.8	a
09TX14	-1.3	b	2.1	a	-6.3	a	-3.8	a	3.1	a	-7.9	a	-0.5	a
09TX15	-0.2	b	1.4	a	-4.3	a	-1.0	a	1.7	a	-2.8	a	-1.5	a
09TX20	-		3.6	a	-11.3	a	-12.7	a	-0.6	a	-12.2	a	-0.5	a
09NC01	4.4	a	-		-		-16.0	a	-6.0	a	-10.0	a	-	

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