

EVALUATION OF PHOTOPERIOD SENSITIVE GRAIN SORGHUM HYBRIDS
FOR HETEROSIS AND GRAIN QUALITY TRAITS

A Thesis

by

BETHANY JOANN ANDREWS

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Chair of Committee,	William L. Rooney
Committee Members,	Steve Hague
	Joseph Awika
Chair of Interdisciplinary	
Faculty,	Dirk Hays

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ABSTRACT

In many dry crop production regions of the world, food-insecure farmers rely on sorghum because of its drought tolerance and ability to yield with low inputs. In Central America and Sub-Saharan Africa, these farmers grow photoperiod sensitive (PS) sorghums, which match natural rainfall patterns with the varieties' growth and development so that they grow in the rainy season and mature into the dry season. Hybrids, despite their proven yield increases, have not been adopted in these regions. While there are several reasons for the absence of hybrids in these systems, an important factor is the complicated logistics of producing photoperiod-sensitive hybrids and the poor seed production systems in the region. To bypass these limitations, PS hybrids were created from photoperiod insensitive (PI) parents, allowing the seed to be produced anywhere in the world. The objectives of the study were to 1) assess PS hybrids for heterosis and grain quality and 2) review and assess the potential of this seed production system. Results indicate that grain yield heterosis is present in PS hybrids at high enough levels to be economically viable. In addition, variability for grain quality and for grain yield suggests that further breeding progress can be made. Five hybrids have been chosen for further testing based upon their high yields.

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NOMENCLATURE

PI	Photoperiod Insensitive
PS	Photoperiod Sensitive
QTL	Quantitative Trait Loci
NGO	Non-Governmental Organization
US AID	United States Agency for International Development
INTSORMIL	International Sorghum and Millet
TKW	Thousand Kernel Weight
LD	Long day
SD	Short Day

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Sorghum Background

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal in the world behind rice, wheat, maize and barley (FAO Food Security Department, 1999). Sorghum is an annual grass grown for food, feed, forage, syrup and biomass (House, 2000). Primarily an animal feed in the United States, food insecure farmers in Western and Central Africa, Asia and Central America grow sorghum as a food security crop. Originating under semi-arid and sub-tropical environments, sorghum reliably produces in hot, dry conditions (CGIAR, Taylor, 2003). Despite increases in land area where sorghum is being cultivated, yields have not increased in these areas, likely due to low inputs and failure to adopt hybrids (Axtell, et al., 1999, Okiyo, et al., 2010). Yields in the majority of the countries in this region are consistently lower than those in the United States, Brazil and South Africa (Figure 1) (FAO Statistics Division, 2015).

Photoperiod Sensitivity

Flowering in plants is regulated by four main pathways: photoperiod, gibberellic acid promotion, vernalization and autonomous (Bhosale, et al., 2012). The photoperiod pathway links floral initiation and the circadian clock to regulate the flowering based on day length; this response is strongly influenced by the latitude at which a species evolved (Taiz and Zeiger, 2010). Originating in Africa, sorghum is a short day plant, only flowering when day length reaches a critical threshold. Natural variability in

flowering time and degree of photoperiod sensitivity has been found in sorghum and photoperiod insensitive (PI) lines have been developed to allow for cultivation under all day length conditions (Stephens, et al., 1967).

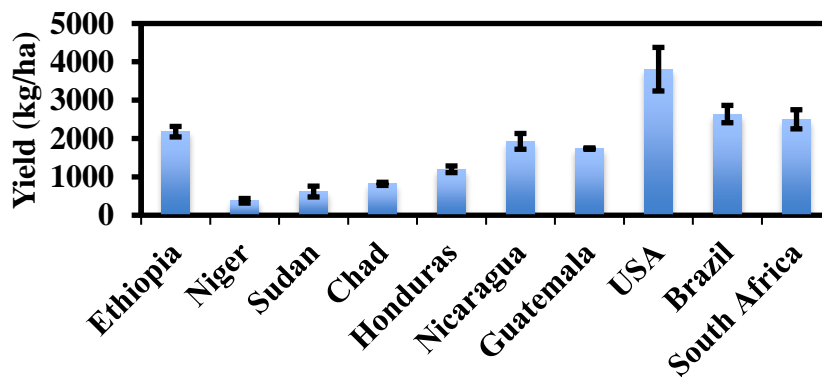


Figure 1: Average sorghum yields from 2010-2014. Data taken from FAO Statistics Division (2015)

The current model for flowering in sorghum involves six maturity genes (Ma_{1-6}), typically grouped as Ma_{1-4} and Ma_{5-6} . Within the first group, Ma_1 has the largest influence on flowering with Ma_2 , Ma_3 and Ma_4 only being expressed when Ma_1 is in the dominant condition (Quinby and Karper, 1945). Dominant Ma_1 alone or in any combination with dominant Ma_{2-4} results in a plant that flowers late and accumulates a large number of leaves due to an extended period of vegetative growth (Quinby, 1966). Ultimately, dominant Ma_{1-4} promote flowering under short days and inhibit flowering under long days (Childs, et al., 1997). Ma_5 and Ma_6 interact via complementary

dominant epistasis. Regardless of the state of Ma_{1-4} , if dominant alleles are present for both Ma_5 and Ma_6 , in either the heterozygous or homozygous condition, the sorghum will be photoperiod sensitive (PS) and flower only under short days. This allows for the creation of photoperiod sensitive hybrids from photoperiod insensitive parents that have the genotypes: $Ma_5Ma_5ma_6ma_6$ and $ma_5ma_5Ma_6Ma_6$, resulting in a hybrid that is heterozygous at both loci (Rooney and Aydin, 1999).

Ma_1 has been identified as pseudoresponse regulator protein 37 (PRR37), a central repressor in a regulatory pathway that controls flowering in sorghum, possibly through the repression of Flowering Locus T and other flowering genes in long day conditions (Murphy, et al., 2011). The observation that mutations in Ma_3 in sorghum and PhyB in *Arabidopsis thaliana* both resulted in insensitivity to inhibitory photoperiods followed by the molecular mapping of PhyA, PhyB, PhyC, and Ma_3 in sorghum led to the discovery that Ma_3 encodes a phytochrome B. Phytochrome B is responsible for shoot elongation, chlorophyll content and red light mediated deetiolation and it has been hypothesized that it interacts with the short day induced flowering pathway or responds directly to day length itself (Childs, et al., 1997). Ma_6 is a light dependent repressor of flowering in long days that is controlled by the circadian clock. When both are in the dominant condition, Ma_1 and Ma_6 act additively to repress flowering (Murphy, et al., 2014). Quantitative trait loci (QTL) studies suggest that Ma_5 is phytochrome C (PhyC). Ma_5 has epistatic interactions with both Ma_1 and Ma_6 (Yang, et al., 2014). This recent characterization of the photoperiod system has revealed that Ma_1 , Ma_5 and Ma_6 all interact to define photoperiod sensitivity and also the degree of photoperiod sensitivity.

Seed Systems

A seed system is defined as all the channels through which farmers acquire genetic materials, including all physical, organizational and institutional components and actions that have a role in determining seed supply and use (Mekbib, 2008, Nagarajan and Smale, 2005, Van Amstel, et al., 1996). Farmers in Africa and Central America acquire seeds through a combination of three systems: the formal seed system, the informal seed system, and more recently, the integrated seed supply system. The informal seed system involves seed kept by the farmer to plant the following year, seed traded or sold to neighbors and seed purchased from small grain markets or merchants. The formal seed system is a more defined process of seed production and distribution that is often backed by national legislation and internal quality controls. The integrated seed supply system, also known as farmer seed enterprises, has been endorsed by many non-governmental organizations (NGOs) as a way to get higher quality seeds to farmers and promotes the use of technology from the formal sector to locally produce seed and breed for improved varieties (Louwaars, 1996, Oyekale, et al., 2012).

The formal seed system is limited in many African and Central American countries and supplies only 20-30% of seed (Oyekale, et al., 2012, Sperling and Cooper, 2003). The systems that do exist operate without sufficient numbers of trained workers, inadequate funding to obtain and test new varieties and must contend with poor infrastructure (IFDC, 2001, Tahirou, et al., 2009). Because of this, the formal sector often releases varieties for crops of commercial importance rather than those of economic or social importance to small farmers (Oyekale, et al., 2012). For most

commercial companies, it is more profitable to produce modern varieties to sell to governments and nonprofits as relief seed than to develop varieties for direct purchase by small farmers (Tripp and Rohrbach, 2001). The formal supply of sorghum seed is often limited, not adapted to the agro-ecology or arrives too late for planting, leading farmers to use informal channels instead (David, 2004, Sperling and McGuire, 2010).

Farmer Preferences and Planting Practices for Sorghum

Farmer varieties in Africa are usually photoperiod sensitive land races that can be planted at the time of the early rains and then harvested 6-9 months later (McGuire, 2007b). These varieties offer the advantage of flowering at the end of the rainy season which allows developing grain to avoid mold, is prior to terminal drought pressure and maturity is in sync with other wild flora to minimize bird damage (Kouressy, et al., 2008). Farmers rely on planting a mixture of varieties to avoid a total loss of yield if abiotic or biotic stresses are present. Seeding rates are usually 3-6 times higher than the recommended plant populations due to poor germination, seedling disease loss, and deeper planting depth due to ox plow (McGuire, 2007b, Mekbib, 2008). Farmers will sow repeatedly at the beginning of the season to get a good stand. If this stand fails, farmers will sow photoperiod insensitive varieties. Typically, farmers have sufficient seed for the first planting and will only become seed insecure after multiple sowing attempts (McGuire, 2007a).

Small Central American farmers depend largely on maicillos criollos, which are uniquely Central American photoperiod sensitive landraces. These landraces have low, but stable productivity, even under erratic rainfall (Gómez, 1995, Peterson, 1999). Often

maize and sorghum are intercropped to safeguard against a total loss of yield in dry years. In some parts of Honduras, up to 90% of calories consumed come from sorghum during the dry season (Domínquez, 1998). Maicillos criollos are often susceptible to leaf diseases, lodging and do not show major yield increases in response to improved management practices (Domínquez, 1998, Gómez, 1995). The U.S. AID program INTSORMIL has produced improved maicillos by crossing the landrace with elite breeding lines and these lines have lower lodging, better stay green, good adaptation and tortilla quality (Domínquez, 1998, Peterson, 1999). Like African farmers, most Central American farmers rely on saved seed and informal seed systems (Barbentane, 2000, Haugen, 2000).

Heterosis in Sorghum

Fu et al. (2015) defines heterosis, also known as hybrid vigor, as a natural phenomenon where hybrids created from diverse individuals outperform those individuals in terms of yield, adaptability, and resistance to abiotic and biotic stress. There are two traditional theories to explain heterosis in plants. The dominance theory, first suggested by Davenport (1908), states that the introduction of new desirable dominant alleles in the hybrid explain the superior phenotype. The overdominance theory postulates that heterosis is caused by complementary actions between both parental alleles and suggests that the heterozygous gene condition is more advantageous than the homozygous (Crow, 1948, Hull, 1945). It has also been reported that epistasis may also play a role in heterosis especially in complex traits such as yield (Blum, 2013, Fu, et al., 2015). Although there has been extensive study on heterosis in plants, there is

no consensus as to its genetic basis (Blum, 2013, Kaeppler, 2012)

Heterosis in sorghum is manifested in earlier flowering, more seeds per plant, heavier seeds, increased height, and increased forage and grain yield (Bartel, 1949, Quinby, 1963). The larger plant size results from greater cell number due to more rapid cell division in the apical meristem (Quinby, 1963, Quinby and Karper, 1945).

Midparent heterosis values are used to determine the percent increase or decrease in a trait for a hybrid relative to the average of its parents while high parent heterosis is used to compare the hybrid to the best performing parent. Haussmann et al. reported 13-127% increases in yield compared to the midparent with an average yield increase of 46% (1998).

End Use and Grain Quality

Sorghum has a wide variety of end uses in Central America and Africa and each of these requires specific grain quality characteristics (Rooney and Waniska, 2000). In general, endosperm texture, also known as grain hardness, and pericarp thickness have the largest effect on end use and milling quality (Bean, 2006, Munck, et al., 1982, Rooney, 1984). Rooney et. al (1986) suggested three distinct classes of sorghum based on endosperm textures and made recommendations as to which classes should be used for each end product. The first class includes sorghum with hard endosperm texture and is used for thick porridges, such as tô from Mali, and for couscous. The second class includes intermediate endosperms and is used for unfermented breads including tortillas and for brewing. Soft endosperms are ideal for fermented bread.

In African and Central American countries, many still rely on hand milling with

a mortar and pestle and thus have specific grain quality requirements (Rooney, 1986). A village milling trial in Mali found that sorghum with a thick pericarp and hard endosperm can be decorticated faster and with higher yield than sorghum with thin pericarps or floury endosperms. In addition, environmental conditions can lead to soft or discolored grain that is not acceptable for milling (Rooney, 1984). The Single Kernel Characterization System predicts weight, diameter, moisture and kernel hardness for 300 grains and gives an average and standard deviations. The kernel hardness values have a moderate correlation with hardness values determined by abrasive decortication and thus are useful for determining milling quality (Bean, 2006).

Seed Production of Photoperiod Sensitive Hybrids

Prior to the mid 1990s, the genetic basis of Ma₅ and Ma₆ were not understood and it would have been infeasible to create a photoperiod sensitive hybrid from photoperiod insensitive parents (Rooney and Aydin, 1999). A system for developing biomass sorghum hybrids from photoperiod insensitive parents was suggested in Rooney et al (2007) to maximize the amount of lignocellulose accumulated in a growing season. In addition to increased biomass accumulation, this system also allows for seed production under any day length and enables mechanical seed harvest (Packer and Rooney, 2014). Although this system has been deployed for forage, biomass and sweet sorghum hybrid production, to this author's knowledge no such programs have been developed for grain sorghum.

CHAPTER II

HETEROSIS AND GRAIN QUALITY

Introduction

Most farmers in Western and Central Africa and Central America prefer photoperiod sensitive sorghum varieties that only flower under short days (Gómez, 1995, McGuire, 2007b). Photoperiod sensitivity in these regions has the advantage of matching the crop cycle to natural growing seasons, which are affiliated with rainfall patterns. This environmental adaptation allows plants to avoid mold, insect and bird damage that are often problems for early maturing photoperiod insensitive varieties (Cavatassi, et al., 2011). Although Rattunde et al. (2013) demonstrated that photoperiod sensitive hybrids can be developed with maturity similar to local checks, such hybrids have not been adopted. It has been suggested that hybrids are not grown in these regions due to higher seed costs and/or the unavailability of sufficient seed (Feed the Future, 2012, Kumara Charyulu, et al., 2011). In addition, seed industries have a large number of uncontrollable factors making it difficult to produce quality seed under the substandard agronomic and meteorological conditions present in many developing countries (Banerjee, 1983). A yield increase of 20-30%, which is in the low range for heterosis in sorghum, has been suggested as sufficient to make the purchase of hybrid seed profitable (Gómez, 1995, Haussmann, et al., 1998).

Traditionally, photoperiod sensitive hybrids could only be produced at latitudes where short day flowering occurred, eliminating common areas of industrial sorghum

seed production. However, utilization of the *Ma1Ma5Ma6* gene interaction in sorghum allows photoperiod sensitive hybrids to be produced from photoperiod insensitive parents (Rooney and Aydin, 1999). By expanding the available production area to include countries with established seed systems, it is possible to minimize seed production constraints normally found in developing countries.

The system described above for creating a photoperiod sensitive hybrid from two photoperiod insensitive parents has been deployed for both forage and high biomass sorghum (Rooney, et al., 2007). By utilizing marker-assisted backcrossing, inbred lines possessing the desired alleles are efficiently selected and then evaluated for their potential as pollinator lines (Mullet, et al., 2010). Further evaluation and selection of these lines was based on biomass yield; grain yield was never considered in the selection and advancement process. Given the value of photoperiod sensitive sorghum in many production systems and the difficulty in producing such hybrids in those environments, there is value in evaluating this system for grain yield. Thus, the objectives of this study are: characterize photoperiod sensitive sorghum hybrids produced from photoperiod insensitive parents for 1) agronomic performance and heterosis and to 2) assess the quality of grain produced from these photoperiod sensitive sorghum hybrids.

Materials and Methods

Germplasm

A total of 17 pollinator lines (R-lines) were tested in combination with three male sterile seed parents (A-lines). The R-lines were developed using the pedigree method within the Texas A&M AgriLife Sorghum Improvement Lab. These lines were

developed specifically for use as biomass sorghum pollinators (Table 1). At the key maturity loci, these lines are homozygous $MA_1ma_5MA_6$ and are photoperiod insensitive regardless of environment. When combined with most seed parents, which are complementary at these maturity loci, $ma_1MA_5ma_6$, the hybrids are strongly photoperiod sensitive and do not initiate reproductive growth until daylengths drop below 12'15" (Rooney, et al., 2007).

The seed parent lines were Tx2928, Tx642 and Tx645, all of which are three dwarf ($dw1Dw2dw3dw4$) inbred lines, contain A1 cytoplasm and possess the following maturity alleles: $ma_1Ma_5ma_6$. These seed parent lines were selected because they represent three distinct phylogenetic clades of females in the publically available breeding lines (Klein, et al., 2004). Tx2928 has the pedigree (RS4906/BTx399)/RS4906 and has a white pericarp and tan plant color with moderate susceptibility to grain mold (caused by *Fusarium* spp. and *Alternaria* spp.), anthracnose (caused by *Colletotrichum graminicola* (Ces)) and extreme susceptibility to head smut (caused by *Sporisorium relianum* (Kuhn)) (Rooney, 2003). Tx642 was derived from the pedigree of (BTx406*IS12555(SC35)F3)*IS12555 and has a yellow pericarp and purple plant color and has been shown to have good specific combining ability. It possesses stay green in a dominant form which is present in hybrids produced with it. Tx642 is resistant to head smut but susceptible to anthracnose and most leaf diseases (Rosenow, et al., 2002a). Tx645 has the pedigree (BTx623*(BTx625*B35)) and has a red pericarp, red glumes, a purple plant color. It has been shown to have excellent general combining ability (Rosenow, et al., 2002b).

Table 1: Germplasm pedigrees and characteristics of the three seed parents and seventeen pollinator parents used in the evaluation of photoperiod sensitive hybrids for grain production. R.10733, R.10258, R.10788, and R.11367 are segregating for pericarp color

Seed Parent	Pedigree	Pericarp Color	Plant Color
B.Tx645	(BTx623*(BTx625*B35))-B37-BBK-BHBK-P3-L1-P2-L1-P1	Red	Purple
B.Tx642	[(BTx406*IS12555(SC35)F3)*IS12555]	Yellow	Purple
B.Tx2928	((RS4906/BTx399)/RS4906) -C4-T3-C1-C1	White	Tan
Pollinator Parent	Pedigree	Pericarp Color	Plant Color
R.11365	(SC599/R07007)-PRF2-CS7-WF1-CS2	Red	Tan
F.10805-3dw	((96CA5986/87BH606-6)-CS3/R07007)-WFF2-WF68-CS1-CS1-WF2	White	Purple
F.10810-3dw	(HEGARI/R07007)-PRF2-WF87-CS1-CS1-WF2	Red	Purple
F.10801-3dw	((96CA5986/87BH606-6)-CS3/R07007)-WFF2-WF68-CS1-CS1-WF1	White	Purple
F.10762-3dw	(RCV/R07007)-PRF2a-CS35-WF1-CS2-WF2-CS2	White	Tan
R.10712	(Macia/R07007)-PRF2-CS44-WF11-CS1-WF1	White	Tan
R.10702	(R07007/R07012) -PRF2-CS15-WF2-CS2	Yellow	Purple
R.10733	(R07018/R07007)-PRF2-CS9-WF2	Brown/Yellow	Tan
R.10258	(RIO/R07007) -WFF2-CS9-CA1-CS1-CS1	Yellow/White	Purple
R.10744	(R07018/R07007)-PRF2-CS1-WF1	White	Purple
R.10715	(R07007/R07012) -PRF2-CS19-WF2-CS1	Yellow	Purple
R.10704	(R07007/R07012) -PRF2-CS17-WF2-CS1	Yellow	Purple
R.11358	(R07007/R09015)-PRF1-PRF2-CS3-WF2-CS1	Yellow	Purple
R.11369	(SC748-5/R07007)-PRF2-CS6-WF2-CS2	Yellow	Purple
R.10779	(R07007/R07008)-PRF2-CS10-WF1	Yellow	Purple
R.10788	(R07007/R07020)-PRF2-CS1-WF2	Yellow/White	Purple
R.11367	(SC748-5/R07007)-PRF2-CS6-WF1-CS2	Yellow/White	Purple
R. Tx436	Standard grain pollinator (create check hybrids)	White	Tan
R. Tx437	Standard grain pollinator (create check hybrids)	White	Tan

Field Layout

Hybrid seed was produced from pollinations made in June 2014 in College Station, TX. Each R-line was crossed to the three A-lines to produce enough seed for multi-location testing. Seed was threshed on an Almaco Small Vogel Plot Thresher

(Almaco, Nevada, Iowa) and then stored at 10°C until planting. The hybrid trial was planted in a randomized complete block design in two environments with three replicates/environment. Planting dates and locations are outlined in Table 2. Day lengths for planting and harvest were calculated using the National Oceanic and Atmospheric Administration's Solar Day Calculator (Commerce, 2016). Fields were maintained as is standard for sorghum production in each location.

Phenotyping for Agronomic Traits

Plant height was recorded as the distance from the base of the plant to the top of the panicle at maturity. *Stem diameter* was measured in an internode at the middle of a representative plant; middle of the plant was determined by taking 50% of the height measurement. *Bird damage* was given a visual score from 0 to 100% based on the percentage of open glumes without seed.

At harvest, a defined area of each plot was hand harvested (Table 2). Scaled images of two representative panicles from each plot were made in Puerto Rico in 2015. Plots were threshed using the Almaco thresher described above and grain weight was recorded. Grain was cleaned of glumes and panicle particulates using the Wintersteiger LD180 (Wintersteiger, Ried im Innkreis, Austria). Panicle length was determined from photographs mentioned above using ImageJ (Schneider, et al., 2012).

Phenotyping for Grain Quality Traits

Grain quality was determined for the best five hybrids, their parental inbred lines and the photoperiod insensitive checks. Grain hardness was determined using the Perten SKCS 4100 which measures the hardness index, diameter, moisture and weight for 300

individual kernels and then reports an average with standard deviations (Perten Instruments, Hägersten Sweden). Classification levels for kernel hardness were: soft is less than or equal to 60; intermediate is between 60 and 75; hard is above 75 (Rooney and Waniska, 2000). Average individual kernel weight was used to calculate thousand kernel weight (TKW) for each sample.

Grain composition was determined using a FOSS XDS MasterLab Analyzer (FOSS NIR Systems, Eden Prairie, MN) and ISIScan routine analysis software (Infrasoft International LLC, State College, PA). Composition of ash, lipids, starch, protein and fiber was determined from intact grain using standards developed within the Texas A&M AgriLife Sorghum Improvement (Dykes et al., 2014).

Table 2: Planting and harvest specifications for evaluation of photoperiod sensitive hybrids produced from photoperiod insensitive parents. The row length harvested refers to grain. Data was collected in 2015 College Station for biomass and specifics for those harvests are denoted with an asterisk (*).

Environment	Latitude	Longitude	Planting Date	Planting Day Length	Harvest Date	Harvest Day Length	Row Length (m)	Row Spacing (m)	Rows per Plot	Row Length Harvested (m)
2015 Guayanilla, Puerto Rico (short day)	18°00'N	66°47'W	11/18/14	11hr 15min	3/6/15	11hr 53min	4.572	0.76	1	1.8288
2015 College Station, TX (long day)	30°32'N	96°26'W	4/9/15	13hr 5min	8/3/15 8/24/15*	13hr 34min 13hr*	5.49	0.76	2	1.524 5.49*
2016 Guayanilla, Puerto Rico (short day)	18°00'N	66°47'W	11/24/15	11hr 12min	2/23/16	11hr 41min	4.572	0.76	1	1.8288

Heterosis and Combining Ability Calculations:

High parent heterosis was calculated using the formula: $\frac{F_1 - HP}{HP} \times 100\%$ where F_1 is the performance of the F_1 hybrid and HP is the performance of the best parent.

General combining ability (GCA) was calculated using the formula $GCA_A = A^* - Y^{**}$ where A^* is the average performance for line A when in hybrid combination and Y^{**} is the average for all hybrids in the test.

Statistical Analysis

Data was analyzed using JMP Pro 11 (SAS Institute Inc., Cary, NC). Outliers in the data set were removed using the Jackknife Outlier Procedure with an alpha level of 0.05. Student's t test was used to detect differences between genotypes. The complete dataset from individual environments was analyzed using the model $\text{trait} = \text{genotype} + \text{rep}$ and hybrids were analyzed using $\text{trait} = \text{rep} + \text{male} + \text{female} + \text{male} * \text{female} + \text{error}$. Although heterogeneous error terms were detected, no methods of adjustments eliminated the heterogeneity, consequently, a combined analysis was performed for the complete dataset where $\text{trait} = \text{genotype} + \text{rep}(\text{environment}) + \text{environment} + \text{error}$ and for the hybrids where $\text{trait} = \text{environment} + \text{rep}(\text{environment}) + \text{male} + \text{female} + \text{male} * \text{female} + \text{male} * \text{environment} + \text{female} * \text{environment} + \text{male} * \text{female} * \text{environment} + \text{error}$. The square root of yield was used to normalize the residuals for the combined analysis. Rep and environment were run as random effects and the percent bird damage detailed above was run as a covariate for yield.

Results and Discussion

Individual Environment Analysis

In 2015 genotype had a significant effect for all traits (A-1). Rep was significant only for diameter. Bird damage was highly significant for yield despite the average bird damage score only being 1.5%. This is due to specific early maturing genotypes being damaged more heavily in all reps than later maturing genotypes. Ergot (*Claviceps africana*) was present only on certain genotypes, and infection tended to be present on all hybrids made with a specific pollinator. The presence of ergot is an indication of male sterility in the hybrid as the disease only infects unfertilized ovaries and is not a common problem in fully male fertile sorghum lines or hybrids (Bandyopadhyay, et al., 1998). The inability to fully restore fertility had not been previously reported for these pollinators because up to this point they have been exclusively used for biomass production and were never rated for fertility restoration.

Yields were lower and the hybrids were earlier in 2016 than in 2015. Genotype was again significant for all traits and rep was significant for all traits except yield (A-2). Bird damage was highly significant and had a much larger effect on yield than in 2015. On average, each plot had a bird damage rating of 28.1% and the plots with the highest incidences of bird damage were more distinguishable by maturity class than by genotype. Ergot pressure was higher in 2016 than in 2015 but the same patterns of failure to restore fertility were observed.

Combined Analysis

Genotype was significant for all measured traits in the combined analysis (Table

3). Environment had the largest relative effect on yield, height and exsertion but it was not significant for diameter (Table 3). A significant genotype by environment interaction is present for all traits and emphasizes the need for more years of testing and additional testing in the target environment.

Table 3: Combined environment analysis of variance for photoperiod sensitive hybrids and photoperiod insensitive inbred parental lines. Mean squares were determined after combining data from 2015 and 2016 Puerto Rico.

Variance Source	Yield [†]		Height		Exsertion		Stem Diameter	
	DF	MS	DF	MS	DF	MS	DF	MS
Genotype	76	405.2***	76	14912.7***	76	380.7***	76	17.6***
Rep(env)	4	71.6	4	1499.4***	4	241.6***	4	38.22***
Env	1	20070.0***	1	48433.0**	1	14137.1**	1	9.91
Genotype*Env	76	222.5***	76	276.3*	76	125.4***	76	5.3*
Bird Damage	1	1662.3***						
Error	291	75.4	304	196.1	303	50.5	304	3.7
R ²	0.86		0.95		0.78		0.63	

* significant at p <.05

** significant at p<.01

*** significant at p<.001

[†] residuals were normalized by using the square root of yield

Relative additive and dominance effects were determined using an ANOVA of the hybrids and looking at the interaction term between the male and female sources of variance. Dominance effects were assumed when the interaction term was significant while additive effects were assumed when the male or female term was significant either alone or in combination with a significant interaction term. Male, female, and the male*female interaction sources of variance were all significant for yield and diameter suggesting that these traits are controlled by both additive and dominance effects (Table 4). Several studies have looked at additive and dominance effects for yield and although

they agree that both are involved in grain yield, there is conflicting evidence as to which effect is largest (Kenga, et al., 2006, Liang and Walter, 1968, Plett, et al., 1991). Height and exertion are significant for male and female sources of variation and thus additive effects control those traits. This is in agreement with the model outlined by Quinby that specifies that for each recessive dwarfing gene, there is a specific height reduction (Quinby and Karper, 1954, Rooney, 2000)

Environment is the largest source of variation for yield, height and exertion (Table 4). Both male and female were significant for all traits but male effects accounted for a larger proportion of variation for height and stem diameter. This is likely due to the extremely diverse germplasm from the Sorghum Conversion Project represented in the bioenergy lines used as males in this study (Rosenow and Dahlberg, 2000).

Table 4: Analysis of variance for photoperiod sensitive hybrids created from photoperiod insensitive parents. Mean squares were determined after combining data from 2015 and 2016 Puerto Rico.

Variance Source	Yield [†]		Height		Exsertion		Stem Diameter	
	DF	MS	DF	MS	DF	MS	DF	MS
Female	2	1125.1***	2	7124.4***	2	2159.4***	2	24.5*
Male	15	1022.1***	15	41229.1***	15	435.4***	15	27.2***
Female*Male	30	178.0***	30	272.8	30	74.7	30	7.3**
Female*Env	2	643.9***	2	211.5	2	59.5	2	2.0
Male*Env	15	448.4***	15	145.4	15	155.7***	15	8.2*
Female*Male*Env	30	164.5***	30	221.6	30	79.18	30	1.8
Rep(Env)	4	114.3	4	822.9**	4	151.4*	4	27.0***
	1	15160.8***	1	28643.6**	1	10803.8**	1	5.25
Env								
Bird Damage	1	529.7**						
Error	177	74.9	188	206.6	187	56.6	188	3.9
R ²		0.86		0.95		0.74		0.56

* significant at p <.05

** significant at p<.01

*** significant at p<.001

[†] residuals were normalized by using the square root of yield

Orthogonal contrasts did not detect differences between all hybrids and the

pollen and seed parents across environments, possibly due to a large number of hybrids failing to restore fertility and bird damages (Table 5). In 2015 hybrid yields were not significantly different from pollinator parents but were higher than seed parents while the opposite was true in 2016. The higher levels of bird damage on early genotypes in 2016, specifically the seed parent ATx2928 may explain this inconsistency.

Table 5: Orthogonal contrasts for mean grain yield of PS hybrids and parental lines for individual and combined environments. Letters indicate differences between hybrids and parental lines at an alpha level of 0.05.

	All Hybrids	Seed Parents	Pollinator Parents	Photoperiod Insensitive Checks
	kg/ha			
Across Environments	1,669 ^B	1,338 ^B	1,544 ^B	2,567 ^A
2015 Puerto Rico	2,863 ^B	1,566 ^C	2994 ^B	3,829 ^A
2016 Puerto Rico	908 ^B	1,296 ^A	605 ^C	1,753 ^A

General and specific combining ability are used to select parents that will result in a hybrid with the highest level of heterosis. (Hariprasanna, et al., 2012, Kambal and Webster, 1965). Among the genotypes in this study, R10733 had the best general combining ability and was not significantly different from the photoperiod insensitive checks RTx437 and RTx436 (Table 6). Other pollinator lines, R10258, F10810-3dw and R10744, all had numerically high general combining ability but were not used in the statistical analysis due to seed unavailability for one hybrid combination each in 2016. F10805-3dw and F10801-3dw had the lowest general combining ability because they failed to completely restore fertility to the hybrid. Among the seed parents, ATx645 had

the highest level of general combining ability for the seed parents which was in agreement with Rosenow et. al (2002b). The negative GCA for ATx2928 is likely due to the earlier maturing of all hybrids in which is was the seed parent, leading to a higher incidence of bird damage.

Table 6: General combining ability for yield of seed and pollen parents of photoperiod sensitive hybrids. Parents connected by different letters are significant at $\alpha=.05$ using the Student's T Test.

Pollen Parents	GCA	Seed Parents	GCA
	kg/ha		kg/ha
R10733	1,130 ^A	ATx645	360 ^A
RTx437	965 ^A	ATx642	-81 ^B
RTx436	671 ^{AB}	ATx2928	-316 ^B
R10258	618 [†]		
F10810-3dw	483 [†]		
R10744	467 [†]		
R10702	2967 ^{BC}		
R11358	281 ^{BC}		
R10712	151 ^{BCD}		
R10788	54 ^{BCD}		
R11369	-2 ^{CD}		
F10762-3dw	-63 ^{CD}		
R10704	-220 ^{DE}		
R10715	-268 ^{DE}		
R11365	-585 ^{EF}		
R11367	653 ^{EFG}		
R10779	-879 ^{FG}		
F10805-3dw	-915 ^{FG}		
F10801-3dw	-1009 ^G		

[†] General combining ability calculated from 2015 Puerto Rico due to insufficient seed for one hybrid combination in 2016

Heterosis

R10733 had over 30% high parent heterosis for yield with all three female parents, further supporting the notion that it would make an excellent parent for PS grain hybrids (Table 7). In addition, ATx642 in combination with R10702, R11369, F10762-3dw, and ATx645 in combination with F10810-3dw and R10744 all had high parent heterosis greater than 20-30%, which is the level recommended for hybrids to be profitable (Gómez, 1995, Haussmann, et al., 1998). However, heterosis alone cannot determine economic feasibility. For instance, ATx642/R11369 has high levels of heterosis but only yields 1620.28kg/ha, which is below the average for all hybrids in the test (A-3). This suggests that the high heterosis is due to poorly performing parents rather than a high yielding hybrid. Other hybrids also had negative high parent heterosis, but some of this was due to bird damage. Specifically, ATx2928/RTx436 and ATx2928/RTx437 showed negative heterosis due to bird damage and these are highly heterotic hybrids under other conditions.

Although yield heterosis was demonstrated in this study, it should be noted that these plants were planted and harvested under short day conditions. In the target environment, these hybrids would be planted in the summer under long day conditions and then harvested in the winter under short day. Testing for heterosis in that environment will require two planting dates, one for the PS hybrids and another several months later for the PI checks and the PI inbred lines. Although it is likely that the heterosis observed in this study will be present in that system, further testing is necessary.

High parent heterosis for height ranged from -11.14% to 53.16% (Table 8). The negative height heterosis is found in hybrids with the male parents F10801-3dw or F10805-3dw, which are three dwarf and create a short photoperiod sensitive hybrid. In general, height heterosis in this study was higher than that found in photoperiod sensitive hybrids under long day conditions by Packer and Rooney (2014). The higher level of heterosis in this study is due to the use of photoperiod insensitive pollinator parents and the short day environment under which the hybrids were grown.

Table 7: High parent heterosis for yield across environments for photoperiod sensitive hybrids created from photoperiod insensitive parents. Percent yield heterosis calculated from Least Squares Means from Puerto Rico 2015 and 2016.

Male Parent	Female Parent		
	ATx2928	ATx642	ATx645
R10733	59.3	30.7	39.0
F10810-3dw	11.8 [†]	8.2	23.4
R10702	11.7	29.0	12.2
R10744	8.4 [†]	14.6	20.0
R11369	8.3	22.8	16.9
R10258	1.2	-25.3 [†]	15.5
R10712	0.5	-7.1	-8.0
F10762-3dw	-0.7	38.8	5.1
R10788	-3.8	-13.2	12.3
R11365	-5.6	-0.2	-9.8
R11367	-6.4	5.8	-18.0
R10779	-6.7	-35.4	-20.7
RTx437	-7.0	6.4	32.3
R11358	-22.6	0.8	-18.8
F10805-3dw	-26.8	-30.8	-19.0
R10715	-30.0	-3.3	-8.8
RTx436	-30.9	5.7	17.9
R10704	-38.8	-37.3	7.3
F10801-3dw	-41.3	-27.1	-22.2

[†] Heterosis calculated from 2015 Puerto Rico due to insufficient seed in 2016

Table 8: High parent heterosis for height across environments for photoperiod sensitive hybrids created from photoperiod insensitive parents. Percent height heterosis calculated from Least Squares Means from Puerto Rico 2015 and 2016.

Male Parent	Female Parent		
	ATx2928	ATx642	ATx645
F10762-3dw	15.1	13.2	8.6
F10801-3dw	-11.1	-5.8	-6.3
F10805-3dw	-9.8	3.4	-3.4
F10810-3dw	20.8 [†]	40.8	44.1
R10258	-2.1	6.2 [†]	7.0
0702	2.9	11.7	10.4
R10704	-3.2	10.5	4.6
R10712	44.0	53.2	42.5
R10715	-2.4	10.3	6.6
R10733	7.7	16.6	13.3
R10744	-2.8 [†]	5.1	3.3
R10779	4.0	11.9	12.1
R10788	0.6	5.5	8.6
R11358	1.7	2.2	7.1
R11365	9.1	25.0	24.4
R11367	2.1	9.6	13.8
R11369	0.6	5.0	2.2
RTx436	6.3	15.0	12.3
RTx437	12.7	19.3	22.6

[†] Heterosis calculated from 2015 Puerto Rico due to insufficient seed in 2016

Stem diameter heterosis was not as large in magnitude as expected, likely due to the method of phenotyping (Table 9). By taking diameter measurements without removing the leaves, the shorter genotypes, including the photoperiod insensitive parental lines and short hybrids, had diameters that were inflated due to the presence of overlapping leaves. For example, the highest heterosis were in the short hybrids ATx642/F10801-3dw, ATx642/F10805-3dw and the photoperiod insensitive check, ATx2928/RTx436.

Table 9: High parent heterosis for stem diameter across environments for photoperiod sensitive hybrids created from photoperiod insensitive parents. Percent stem diameter heterosis calculated from Least Squares Means from Puerto Rico 2015 and 2016.

Male Parent	Female Parent		
	ATx2928	ATx642	ATx645
F10762-3dw	-27.6	-26.2	7.8
F10801-3dw	18.2	27.5	4.3
F10805-3dw	6.3	21.0	5.6
F10810-3dw	-2.0 [†]	7.2	-9.8
R10258	-18.8	10.0 [†]	-8.4
R10702	3.8	13.2	-1.0
R10704	-12.6	1.8	-6.0
R10712	2.2	7.8	1.0
R10715	-1.7	10.3	-3.4
R10733	-9.5	6.6	-4.1
R10744	-12.1 [†]	3.7	-11.9
R10779	-4.4	-6.9	-8.9
R10788	-19.4	10.5	-16.2
R11358	-8.9	4.2	11.3
R11365	-10.1	-3.5	-16.5
R11367	-11.1	-8.5	-11.3
R11369	-4.1	4.5	-6.2
RTx436	44.2	11.5	9.1
RTx437	-8.5	10.4	16.8

[†] Heterosis calculated from 2015 Puerto Rico due to insufficient seed in 2016

Panicle length shows varying levels of heterosis depending on the genetic background of the hybrids (Premalatha, et al., 2006, Ringo, et al., 2015). Panicle length heterosis levels have been reported as varying from negative to as high as 48.4% (Premalatha, et al., 2006). In this study, panicle length heterosis was small or negative for all hybrids (Table 10). Because R07007, the donor of the recessive *ma5* allele, is a durra with low yield and small panicle, panicle length of the PI pollinators is lower than that of the seed parents for all combinations with the exception of ATx642 by R11365.

Although yield heterosis was present for the hybrids (Table 7), this increase in yield cannot be attributed to an increase panicle length.

Table 10: High parent heterosis for panicle length from Puerto Rico 2015 for photoperiod sensitive hybrids created from photoperiod insensitive parents. Percent height heterosis calculated from Least Squares Means from Puerto Rico 2015.

Male Parent	Female Parent		
	ATx2928	ATx642	ATx645
F10762-3dw	6.1	16.7	-11.6
F10801-3dw	-15.4	2.4	-22.0
F10805-3dw	-5.8	-3.3	-25.2
F10810-3dw	-15.7	-1.6	-24.4
R10258	-29.1	-15.4	-31.7
R10702	-28.6	-21.2	-35.3
R10704	-8.7	1.2	-16.1
R10712	-23.7	-5.0	-33.6
R10715	-14.2	-5.5	-28.4
R10733	-12.4	-4.3	-23.0
R10744	-9.4	5.9	-19.8
R10779	-12.1	-7.5	-31.2
R10788	-29.9	-19.5	-38.2
R11358	-22.9	-7.5	-26.3
R11365	-4.4	6.6	-23.1
R11367	-21.8	-12.8	-31.4
R11369	-38.8	-17.0	-38.9
RTx436	4.1	-7.8	-8.2
RTx437	8.5	-1.1	-17.5

Grain Quality

Grain quality traits were evaluated for the five best performing hybrids in order

to assess milling and end use quality. The best hybrids had thousand kernel weights that were higher than the PI checks, except for ATx642/R11358, which was not different from ATx642/RTx437 (Table 11). The same trend was present for seed diameter. R11369 had a similar TKW and diameter to the best hybrids. Increased seed weight and diameter can be desirable but kernel hardness is generally more important, as larger seeds tend to be soft and crack more easily (Munck, et al., 1982).

Kernel hardness for ATx645/R11369, ATx645/R10258, ATx645/R10733 and ATx2928/R10733 are all lower than the PI checks, indicating a softer kernel. Soft kernels fall into the third class as described by Rooney et. al (1986) and are ideal for fermented breads. Additional research on these hybrids will be necessary as decortication is more difficult and yield loss due to broken kernels is common on softer grain. It is possible that these hybrids would be desirable in dry areas, such as Sudan, where decortication is not necessary (Rooney and Waniska, 2000). ATx642/R11358 had an intermediate kernel hardness and is in the second class, recommended for unfermented breads such as tortillas or for brewing (Rooney, 1986). None of the best PS hybrids fell into the category of hard kernels, which are ideal for tradition milling and hand decortication. This should be an area of emphasis in future breeding efforts.

Table 11: Single kernel hardness tester results for best photoperiod sensitive hybrids, parental lines and photoperiod insensitive checks. Least square means for grain quality parameters are presented for the best hybrids, their parents and the PI checks. Data is from 15PR and 16PR. Genotypes connected by different letters are significantly at $\alpha=.05$ using the Student's T test.

Genotype	TKW	Diameter	Kernel Hardness
	g	mm	
ATx645/R11369	35.2 ^A	2.7 ^{ABC}	64.8 ^{IJ}
ATx645/R10258	33.6 ^{AB}	2.7 ^{ABC}	59.5 ^K
ATx645/R10733	33.1 ^{ABC}	2.7 ^{AB}	59.8 ^K
ATx2928/R10733	32.5 ^{BC}	2.7 ^A	60.8 ^{JK}
R11369	32.3 ^{BC}	2.8 ^A	57.5 ^K
ATx642/R11358	31.1 ^{CD}	2.6 ^{BC}	71.4 ^{FG}
ATx642/RTx437	29.3 ^{DE}	2.6 ^{CD}	79.4 ^{CD}
R10733	28.8 ^{DEF}	2.7 ^{AB}	67.2 ^{HI}
R10258	27.3 ^{EFG}	2.5 ^{DE}	59.7 ^K
ATx642/RTx436	27.3 ^{EFG}	2.5 ^{EF}	79.8 ^{CD}
R11358	27.1 ^{EFGH}	2.5 ^{DE}	69.8 ^{GH}
RTx437	26.5 ^{FGHI}	2.5 ^{DE}	85.7 ^{AB}
ATx2928/RTx436	25.8 ^{GHI}	2.5 ^{DE}	75.0 ^{EF}
ATx2928/RTx437	25.8 ^{GHI}	2.5 ^{DE}	83.4 ^{BC}
ATx645/RTx436	25.8 ^{GHI}	2.4 ^{GH}	77.7 ^{DE}
ATx645/RTx437	24.8 ^{HI}	2.4 ^{FGH}	82.0 ^{BC}
BTx642	24.2 ^{IJ}	2.5 ^{EF}	72.9 ^{FG}
RTx436	22.2 ^J	2.3 ^H	87.8 ^A
BTx2928	22.1 ^J	2.5 ^{EFG}	75.6 ^{DEF}
BTx645	21.7 ^J	2.1 ^I	83.7 ^{ABC}

Environment had the largest effect on grain composition followed by genotype and then genotype*env (Table 12). The large environment effect is likely due to variation in weather and management between Puerto Rico in 2015 and in 2016. This supports the conclusion by Griess et al. (2010) that environment has a larger effect on quality traits on food-grade sorghum than genotype or genotype by environment

interactions. This, again, suggests that these hybrids need to be grown and evaluated under farmer field conditions in the target environment.

Table 12: Analysis of variance for grain composition for photoperiod sensitive hybrids, inbred parental lines and photoperiod insensitive checks. Values were obtained via combined analysis from 15PR and 16PR.

Variance Source	Protein		Moisture		Fat	
	DF	MS	DF	MS	DF	MS
Genotype	63	6.53***	63	2.23***	63	0.98***
Genotype*Env	63	2.46***	63	0.74***	63	0.44***
Env	4	276.22**	4	132.10***	4	35.70**
Rep (Env)	1	4.06***	1	0.79**	1	0.55***
Error	212	0.27	215	0.21	216	0.03
R ²		0.94		0.89		0.95
Variance Source	Fiber		Ash		Starch	
	DF	MS	DF	MS	DF	MS
Genotype	63	0.05***	63	0.01***	63	3.96***
Genotype*Env	63	0.01***	63	0.01***	63	2.53***
Env	4	0.48**	4	0.61***	4	148.30***
Rep (env)	1	0.01***	1	0.00**	1	2.15***
Error	215	0	215	0	213	0.23
R ²		0.94		0.89		0.93

* significant at $p < .05$

** significant at $p < .01$

*** significant at $p < .001$

The best hybrids had the highest level of protein, varying from 9.8 to 12.1% as compared to the parents, which had protein levels from 8.6 to 9.6% (Table 13). The highest levels of fat were found in ATx645/R10258 and ATx645/R10733 but were not statistically different from ATx2829/RTx436, RTx436 and RTx437. The best PS hybrids

had lower levels of starch than the PI checks with the exception of ATx2928/RTx436, which was not statistically different than ATx645/R10258. ATx645/R11369 could not be included in the statistical analysis for composition traits due to insufficient seed in all three replications in 2016.

Table 13: Grain composition for the best photoperiod sensitive hybrids, photoperiod insensitive checks and parental lines. Genotypes not connected by the same letter are significantly different by the Student's t test at an alpha level of 0.05.

Genotype	Protein	Moisture	Fat	Fiber	Ash	Starch
	%					
ATx645/R11369	12.1 [†]	11.4 [†]	2.6 [†]	1.7 [†]	1.4 [†]	65.8 [†]
ATx645/R10258	11.1 ^A	11.6 ^C	2.4 ^{ABCD}	1.8 ^{EF}	1.4 ^{AB}	66.8 ^{FG}
ATx645/R10733	10.8 ^A	10.8 ^E	2.6 ^A	1.7 ^G	1.3 ^{CDE}	66.5 ^{GH}
ATx642/R11358	9.9 ^B	12.3 ^{AB}	2.3 ^{CD}	1.8 ^{DE}	1.4 ^A	66.4 ^{GH}
ATx2928/R10733	9.8 ^B	10.8 ^{EF}	2.0 ^{EFG}	1.7 ^G	1.3 ^{CDE}	66.0 ^H
ATx2928/RTx436	9.8 ^{BC}	11.9 ^{BC}	2.5 ^{ABC}	1.6 ^I	1.4 ^{AB}	67.2 ^{EF}
RTx437	9.6 ^{BCD}	11.94 ^{BC}	2.5 ^{AB}	1.8 ^{CDE}	1.4 ^{BCDE}	66.9 ^{FG}
BTx645	9.6 ^{BCD}	11.75 ^C	2.3 ^{BCD}	1.9 ^B	1.3 ^{DEF}	67.8 ^{CDE}
R11358	9.5 ^{BCD}	11.51 ^{CD}	1.8 ^{GH}	1.7 ^G	1.3 ^{EF}	67.3 ^{EF}
BTx2928	9.0 ^{CDE}	10.20 ^F	1.9 ^{GH}	1.6 ^I	1.3 ^{FG}	68.0 ^{BCD}
ATx642/RTx437	9.0 ^{CDE}	12.51 ^A	2.3 ^{BCD}	1.8 ^{CD}	1.4 ^{AB}	68.2 ^{BC}
RTx436	8.9 ^{DEF}	11.7 ^C	2.6 ^A	1.7 ^H	1.4 ^{ABC}	67.5 ^{DE}
ATx645/RTx436	8.8 ^{EF}	12.48 ^A	2.2 ^{DEF}	1.7 ^{FG}	1.4 ^A	68.3 ^{ABC}
R10733	8.8 ^{CDE}	10.46 ^{EF}	1.4 ^J	1.8 ^{EFG}	1.3 ^G	66.7 ^{FG}
R11369	8.7 ^{DEF}	10.51 ^{EF}	1.0 ^K	1.7 ^{GH}	1.3 ^G	67.1 ^{EFG}
ATx642/RTx436	8.7 ^{FG}	12.51 ^A	2.2 ^{DE}	1.7 ^{FG}	1.4 ^A	68.3 ^{ABC}
R10258	8.7 ^{FG}	11.52 ^{CD}	1.6 ^{IJ}	1.7 ^G	1.3 ^{DEF}	66.8 ^{FG}
BTx642	8.6 ^{FG}	11.05 ^{DE}	1.7 ^{HI}	1.9 ^A	1.3 ^{FG}	67.3 ^{EF}
ATx2928/RTx437	8.4 ^{FG}	11.76 ^{BC}	2.0 ^{FGH}	1.7 ^G	1.3 ^{DEF}	68.8 ^{AB}
ATx645/RTx437	8.0 ^G	12.57 ^A	1.9 ^{GH}	1.8 ^{BC}	1.4 ^{BCD}	68.7 ^A

[†] Composition values from 2015 Puerto Rico due to insufficient grain for analysis in 2016

Long and Short Day Comparison

Because the pollinator lines in this study were bred for use as biomass hybrid parents, PS hybrids were grown under LD conditions in College Station, TX, in the summer of 2015. Dry biomass yield rankings for the hybrids were compared to grain yield rankings from 2015 and 2016 (Figure 2). ATx645/R10733 was the second best hybrid yielding under SD and the best yielding hybrid under LD suggesting that it would be ideal for a dual-purpose system where sorghum grain is used for consumption and the stems are used for building materials or as forage. ATx2928/R10733, however, was much more representative of the study in that it was the best yielding hybrid under SD and the 40th under LD. There is a weak significant correlation of 0.31 between LD and SD yield ranks but with such a low correlation, selection for biomass related traits cannot be used in lieu of selecting for grain yield. This emphasizes the need for targeted breeding for grain yield in PS hybrids in the future.

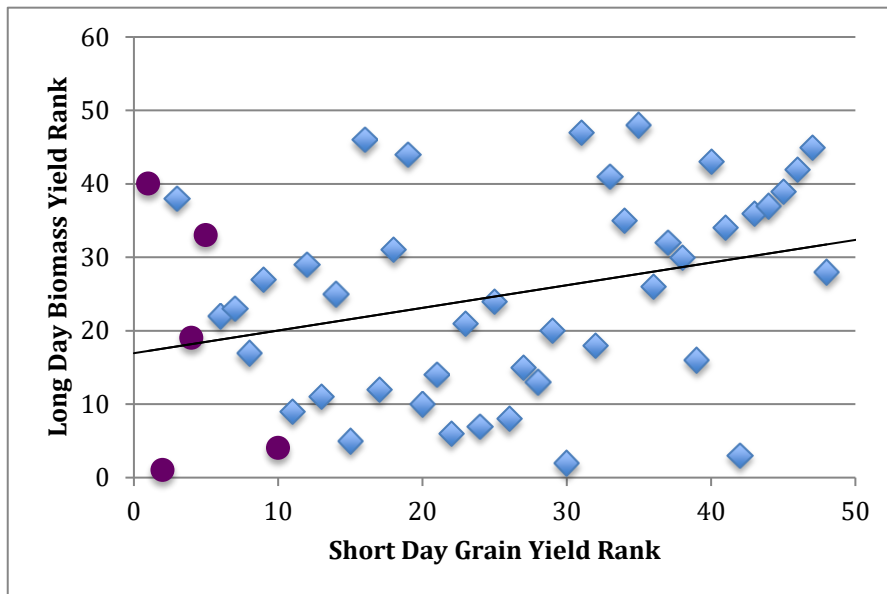


Figure 2: Long day biomass yield ranks versus short day yield ranks for photoperiod sensitive hybrids created from photoperiod insensitive hybrids. Rankings were given with the highest yield hybrid receiving a rank of one and the lowest yielding receiving a rank of 49. Long day yield rankings are from College Station 2015 and were measured as dry biomass yield. Short day rankings were determined from 15 and 16 Puerto Rico grain yield LS Means. Purple circles represent the selected five best hybrids.

Conclusions

The pollinator lines used in this study were bred for biomass hybrid production and this is the first time they have been studied for grain. As such, it is not surprising that varying levels of yield and heterosis were observed. Despite the fact that these lines had never been selected for grain yield, several high yielding hybrids were found. On average the photoperiod sensitive hybrids were equal to that of the parents and significantly lower than the checks.

In the future, PS hybrids should be tested for heterosis in the target environments,

planting under LD conditions and then harvesting under SD. Improvements to grain yield, heterosis, and grain quality can be made with targeted breeding efforts.

Ultimately, elite varieties from the target environments can be converted to have the desired maturity alleles using marker assisted backcrossing as demonstrated by Klein et. al (2016). Then, hybrid seed can be produced in locations with established seed infrastructure. These hybrid plants will have the agronomic and grain quality traits found in the elite varieties from Central America or Sub-Saharan Africa but with increased yield due to heterosis and fewer seed production limitations.

CHAPTER III
PHOTOPERIOD SENSITIVE GRAIN SORGHUM HYBRIDS FOR SUB-SAHARAN
AFRICA AND CENTRAL AMERICA

Introduction

Sorghum ranks second in cereal grain production in Africa and Central America (FAO Food Security Department, 2015). Sorghum is an important food security crop, as it reliably produces under harsh conditions with minimal inputs (Taylor, 2003). Many of these food insecure people live in Sub-Saharan Africa and Central America where subsistence farmers produce much of the sorghum crop. Most of these farmers grow a combination of different landrace varieties that are tall and photoperiod sensitive (McGuire, 2007b). Yields remain low for a variety of reasons including poor soil health, few or no inputs and failure to adopt hybrids (agra.org, 2015, Feed the Future, 2012).

Seed systems in Central America and Sub-Saharan Africa suffer due to poor infrastructure, non-availability of high quality seed, and limited trained workers (IFDC, 2001, Tahirou, et al., 2009). Most local seed companies find it more profitable to focus on crops for commercial production such as hybrid maize or to produce seed for relief organizations rather than target smallholder farmers (Tripp and Rohrbach, 2001). Traditionally, photoperiod sensitive sorghum could only be bred in these target zones often leading to its neglect, as it is not a high value crop (Gómez, 1997).

The identification of the maturity genes Ma_5 and Ma_6 have made breeding for photoperiod sensitive hybrids possible at any latitude (Rooney and Aydin, 1999). This

system has been utilized for the production of high biomass photoperiod sensitive sorghums for use as a bioenergy feedstock (Rooney, et al., 2007). However, this method has not previously been deployed for the production of grain hybrids. The objective of this paper is to review and assess the potential of PS grain hybrids created from PI parents.

Background

Importance of Hybrids

Heterosis in sorghum was first reported in 1937 (Karper and Quinby, 1937). It is manifested in taller plants, heavier seeds, higher yields, and improved performance under abiotic stress (Axtell, et al., 1999, Quinby, 1963). Mainly due to higher yield potentials and the success of hybrid maize, hybrid sorghum was deployed in the United States in 1956 (Smith and Frederiksen, 2000). Since that time, sorghum hybrids have produced yields that are 20-60% higher than improved inbreds and landrace varieties with high parent heterosis values commonly between 20 and 30% (Axtell, et al., 1999). Despite the success of hybrids in countries such as South Africa and Brazil, they have not been largely adopted in Sub-Saharan Africa and Central America. For this, and several other reasons, yields in these areas between 2010 and 2014 averaged 14 to 87 percent of the yields in countries that have successfully adopted hybrids (FAO Statistics Division, 2015).

Sorghum in Africa and Central America

Smallholder and subsistence farmers in Africa grow photoperiod sensitive sorghum landraces. Seed is generally sown at the beginning of the rainy season and then

grain is harvested 6-9 months later, at the beginning of or during the dry season (McGuire, 2007b). This system avoids grain mold by putting the grain fill period after the rains but before terminal drought stress occurs. In addition, this maturity is synchronized with the maturity of natural flora which minimizes bird damage (Kouressy, et al., 2008). In these regions, 70-80% of the seed is procured through the informal seed system, consisting of trading among farmers and small village markets (Oyekale, et al., 2012, Sperling and Cooper, 2003). Farmers sow a mixture of seeds in hopes that if one landrace fails due to disease or abiotic stress, another will survive. Planting rates are much higher than recommended and planting depth is much deeper as most farmers use animal or hand-planted systems (McGuire, 2007b, Mekbib, 2008). Farmers will sow seed multiple times to get a good stand and will only grow photoperiod insensitive varieties after several such attempts have failed (McGuire, 2007a). End-uses for sorghum grain in Sub-Saharan Africa range from porridge, fermented and unfermented breads to beer (Rooney and Waniska, 2000). In addition, sorghum stalks are often used as building materials and occasionally as a forage for livestock (House, 2000, Mohanraj, et al., 2011).

Maicillos criollos are tall, photoperiod sensitive landraces that were introduced to Central America from Africa during the colonial period and have evolved to fit the specific agro-climactic zone (Peterson, 1999). These landraces are still grown extensively by smallholder farmers because of their stable, albeit low, yields (Rooney, 2011). As land is a limiting factor, most farmers increase yields per unit area by intercropping maize and sorghum (Gómez, 1995). Like African farmers, the vast

majority of seed is acquired through the informal seed system (Barbentane, 2000, Haugen, 2000).

Past Attempts at Hybrid Deployment

In the 1980s, Sudan invested a large amount of money and resources into the deployment of hybrids in collaboration with INTSORMIL and ICRISAT. The reasoning was simple; hybrids had produced consistently higher yields in all areas they had been introduced. In those areas, initial yield advantages were 15-25% over the highest yielding parent and gave returns on investment of 4-5 times. Disadvantages included higher seed production costs, lower cold tolerance and higher susceptibility to midge (Maunder, 1983).

In Sudan, steps were taken to establish seed policy and seed certification systems based upon the advice gathered at a workshop at Gezira Research Station in Wad Medani, Sudan in 1983. The Plant Propagation and Variety Release Committee released Hageen Dura-1 as the first commercial hybrid in 1983. It is early maturing and yields were 150% of locally grown varieties under both rainfed and irrigated conditions (House, 2000). Hageen Dura-1 was initially very popular and large harvests resulted in 1985 and 1986. However, the markets were not well established and grain prices dropped drastically due to overproduction. In addition, inability to keep up with increased seed demand has led to lower levels of adoption than previously hoped (Ejeta, 1993). At last report in 1997, Hageen Dura-1 was produced on 12% of irrigated sorghum land (1997). Given the conflict in the region, it is unlikely that the hybrid is produced currently.

In Central America, hybrids were introduced when the seed industry was privatized in the late 1980s allowing seed companies to produce seed for import into Central America (Gómez, 1994). Large commercial farmers chose photoperiod insensitive, combine type hybrid seeds based upon on farm hybrid trials run by INTSORMIL (Gómez, 1994, 1997). However, small to medium scale farmers continued to rely largely on improved varieties and subsistence farmers continued to grow maicillos criollos (Peterson, 1999). Economic and practical production considerations limited the adoption of hybrids into these production situations. As food insecure farmers often intercrop sorghum and maize in order to harvest the maize first and then the sorghum later in the year as food supplies become limited, a fast maturing photoperiod insensitive hybrid cannot be adapted into this production system (Peterson, 1999). In addition, the increased cost associated with hybrid seed limited adoption in some cases. In 2011, 67% of grain sorghum grown was maicillos, despite the continued release of photoperiod insensitive hybrids and improved varieties (Rooney, 2011).

Approach

A method for creating photoperiod sensitive hybrids from photoperiod insensitive parents has been established in bioenergy and sweet sorghums (Rooney, et al., 2007). This method uses $MA_1ma_5MA_6$ photoperiod insensitive pollinator parents and $ma_1MA_5ma_6$ photoperiod insensitive seed parents, which results in a photoperiod sensitive F1 hybrid with the genotype $MA_1ma_1MA_5ma_5MA_6ma_6$ (Rooney and Aydin, 1999) (Figure 3). This approach for creating photoperiod sensitive hybrids has been effective in allowing production of biomass hybrid seed even in environments where

photoperiod sensitive varieties will not flower. This study looks into the feasibility of



Figure 3: Pictures of heterosis in PI parental lines and PS hybrids. A. Height and yield heterosis for photoperiod sensitive hybrid: ATx645/R11369 (center) compared to female inbred line: ATx645 and male inbred line: R11369 (right). B. Root mass heterosis for photoperiod sensitive hybrid: ATx2928/R09106 (center) compared to female inbred line ATx2928 (left) and male inbred line R09106 (right) and C. Panicle length heterosis for photoperiod sensitive hybrid ATx2928/R09106 (center) compared to female inbred line ATx2928 (left) and male inbred line R09106 (right).

using this system to create PS grain hybrids for Central America and Sub-Saharan Africa in order to bypass the complicated logistics and underdeveloped seed production systems

often found in these regions.

Three seed parents, ATx645, ATx642, and ATx2928 were crossed with 17 biomass pollinator lines developed by the Texas A&M AgriLife Sorghum Improvement Lab (Rosenow, et al., 2002a, Rosenow, et al., 2002b). F1 hybrids were grown in under short day conditions in Guayanilla, Puerto Rico in 2015 and 2016. The five best hybrids were selected based upon yield and agronomic traits and data for those hybrids is presented below.

Supporting Data

The five best hybrid yields were not statistically different from the PI checks by orthogonal contrasts (Table 14). The PI checks used in this experiment are frequently used to evaluate hybrid performance in the Texas A&M AgriLife Sorghum Improvement Lab suggesting that the best photoperiod sensitive hybrids may be able to compete with commercially available hybrids. The hybrids out yielded the seed and pollen parents by more than 1000 kg/ha (Table 15).

Similar patterns were observed for 2015 with the exception of pollen parents having a statistically higher yield than the seed parents. Yields on average were much lower in 2016 as compared to 2015. The best hybrids, seed parents, and PI checks all had statistically similar yields. Bird damage was much more extensive in 2016 and the earliest maturing plants suffered the largest yield losses.

Table 14: Orthogonal contrasts for yield between best photoperiod sensitive hybrids, seed and pollen parent inbred lines and photoperiod insensitive checks. Contrasts were performed for individual environments and in a combined analysis. Yields connected with a different letter within a row are significantly different at an alpha level of 0.05.

	Best Hybrids	Seed Parents	Pollen Parents	PI Checks
Across Environments	2,852.2 ^A	1,337.9 ^B	1,544.2 ^B	2,567.4 ^A
2015	4,489.5 ^A	1,566.1 ^B	2,994.5 ^C	3,828.8 ^A
2016	1,669.5 ^A	1,296.0 ^A	604.7 ^B	1752.8 ^A

Table 15: Yield LSMeans for the best photoperiod sensitive hybrids created from photoperiod insensitive parents and photoperiod insensitive checks. LSMeans were calculated for individual and combined environments. LSMeans not connected by the same letter are significant at an alpha level of 0.05 by the Student's t test.

Genotype	Yield		
	kg/ha		
	Across Environments	2015	2016
ATx645/RTx437	3,875 ^A	4,978 ^A	3,054 ^{AB}
ATx645/RTx436	3,452 ^{AB}	3,664 ^A	3,289 ^A
ATx2928/R10733	3,345 ^{AB}	4,158 ^A	2,693 ^{ABC}
ATx645/R10733	3,266 ^{AB}	5,123 ^A	2,044 ^{ABCD}
ATx642/R11358	2,802 ^{ABC}	4,278 ^A	1,659 ^{CDE}
ATx642/RTx436	2,800 ^{ABC}	3,137 ^A	2,625 ^{ABC}
ATx645/R10258	2,758 ^{ABC}	4,114 ^A	1,768 ^{BCDE}
ATx642/RTx437	2,527 ^{BC}	3,796 ^A	1,612 ^{BCDE}
ATx645/R11369	2,269 ^{ABC}	4,774 ^A	941 ^{BCDE}
ATx2928/RTx437	1,904 ^{BC}	4,235 ^A	744 ^{DE}
ATx2928/RTx436	1,173 ^C	3,164 ^A	232 ^E

Significant yield differences were not found between the best hybrids and the highest yielding PI check (Table 16). The pollinator line R10733 had high yields with both ATx645 and ATx2928 suggesting that R10733 possess good general combining

ability. Pedigree was not significant for the best hybrid and PI checks in 2015, as they are all similarly high yielding. ATx645/R11369 had the second highest yield of the PS hybrids for 2015 but the lowest yield in 2016. Bird damage is the primary cause for yield reduction; this hybrid received a bird damage score of 90-100% in all three replicates.

High parent heterosis levels for yield ranged from 0.84 to 59.30% for the best hybrids (Table 16). Only two of the five selected hybrids have heterosis levels above the recommended level of 20-30% heterosis for economic feasibility (Gómez, 1995, Haussmann, et al., 1998). High yielding hybrids do not always show high levels of heterosis. For example, all of the hybrids presented in this study were selected based upon high yields, however ATx645/R11369, ATx645/R10258 and ATx642/R11358 do not show adequate heterosis to justify purchasing hybrid seed. Negative heterosis was present in the PI checks, ATx2928/RTx437 and ATx2928/RTx436, due to high levels of bird damage. Further breeding efforts should focus on finding hybrids that have both high yields and high levels of yield heterosis.

PS hybrids and PI checks show positive high parent heterosis for height. This is positive, as tall plants are desirable in Central America and Sub-Saharan Africa where the stems are often used for building materials (House, 2000). Positive heterosis for thousand kernel weight was found in all of the PS hybrids but not in two of the PI checks.

Table 16: High parent heterosis for best photoperiod sensitive hybrids and photoperiod insensitive checks. These values were determined from LSM means of the best hybrids and their parental lines.

Genotype	High Parent Heterosis		
	Yield	Height	TKW
	%		
ATx2928/R10733	59.30	7.72	13.08
ATx645/R10733	38.99	13.32	15.00
ATx645/RTx437	32.25	22.60	-6.45
ATx645/RTx436	17.88	12.33	16.41
ATx645/R11369	16.86	2.20	8.85
ATx645/R10258	15.46	7.03	22.72
ATx642/RTx437	6.43	19.30	10.67
ATx642/RTx436	5.71	15.02	13.16
ATx642/R11358	0.84	2.23	14.68
ATx2928/RTx437	-6.97	12.68	-2.59
ATx2928/RTx436	-30.93	6.33	16.65

The best hybrids on average have higher TKW with ATx645/R11369 having the highest kernel weight (Table 17). Seed diameter for the best hybrids was higher than the inbred parental lines with the exception of R11369, which had the largest seed. The photoperiod sensitive hybrids have softer kernels than the photoperiod insensitive checks. Increased seed weight and diameter can be desirable grain quality traits but have been associated with softer kernels with a higher tendency of cracking during milling (Munck, et al., 1982).

Table 17: Single kernel hardness tester results for best photoperiod sensitive hybrids, parental lines and photoperiod insensitive checks. Least square means for grain quality parameters are presented for the best hybrids, their parents and the PI checks. Data is from 15PR and 16PR. Genotypes connected by different letters are significantly at alpha=.05 using the Student's T test.

Genotype	TKW	Diameter	Kernel Hardness
	g	mm	
ATx645/R11369	35.2 ^A	2.7 ^{ABC}	64.8 ^{IJ}
ATx645/R10258	33.6 ^{AB}	2.7 ^{ABC}	59.5 ^K
ATx645/R10733	33.1 ^{ABC}	2.7 ^{AB}	59.8 ^K
ATx2928/R10733	32.5 ^{BC}	2.7 ^A	60.8 ^{JK}
R11369	32.3 ^{BC}	2.8 ^A	57.5 ^K
ATx642/R11358	31.1 ^{CD}	2.6 ^{BC}	71.4 ^{FG}
ATx642/RTx437	29.3 ^{DE}	2.6 ^{CD}	79.4 ^{CD}
R10733	28.8 ^{DEF}	2.7 ^{AB}	67.2 ^{HI}
R10258	27.3 ^{EFG}	2.5 ^{DE}	59.7 ^K
ATx642/RTx436	27.3 ^{EFG}	2.5 ^{EF}	79.8 ^{CD}
R11358	27.1 ^{EFGH}	2.5 ^{DE}	69.8 ^{GH}
RTx437	26.5 ^{FGHI}	2.5 ^{DE}	85.7 ^{AB}
ATx2928/RTx436	25.8 ^{GHI}	2.5 ^{DE}	75.0 ^{EF}
ATx2928/RTx437	25.8 ^{GHI}	2.5 ^{DE}	83.4 ^{BC}
ATx645/RTx436	25.8 ^{GHI}	2.4 ^{GH}	77.7 ^{DE}
ATx645/RTx437	24.8 ^{HI}	2.4 ^{FGH}	82.0 ^{BC}
BTx642	24.2 ^{IJ}	2.5 ^{EF}	72.9 ^{FG}
RTx436	22.2 ^J	2.3 ^H	87.8 ^A
BTx2928	22.1 ^J	2.5 ^{EFG}	75.6 ^{DEF}
BTx645	21.7 ^J	2.1 ^I	83.7 ^{ABC}

The best hybrids had the highest level of protein, varying from 9.8 to 12.1% as compared to the parents, which had protein levels from 8.6 to 9.6% (Table 18). The highest levels of fat were found in ATx645/R10258 and ATx645/R10733 but were not statistically different from ATx2829/RTx436, RTx436 and RTx437. The best PS hybrids had lower levels of starch than the PI checks with the exception of ATx2928/RTx436,

which was not statistically different than ATx645/R10258. ATx645/R11369 could not be included in the statistical analysis for composition traits due to insufficient seed in all three replications in 2016.

Table 18: Grain composition of the best photoperiod sensitive hybrids, photoperiod insensitive checks, pollen and seed parent lines. Genotypes not connected by the same letter are significantly different by the Student's t test at an alpha level of 0.05.

Genotype	Protein	Moisture	Fat	Fiber	Ash	Starch
	%					
ATx645/R11369	12.1 [†]	11.4 [†]	2.6 [†]	1.7 [†]	1.4 [†]	65.8 [†]
ATx645/R10258	11.1 ^A	11.6 ^C	2.4 ^{ABCD}	1.8 ^{EF}	1.4 ^{AB}	66.8 ^{FG}
ATx645/R10733	10.8 ^A	10.8 ^E	2.6 ^A	1.7 ^G	1.3 ^{CDE}	66.5 ^{GH}
ATx642/R11358	9.9 ^B	12.3 ^{AB}	2.3 ^{CD}	1.8 ^{DE}	1.4 ^A	66.4 ^{GH}
ATx2928/R10733	9.8 ^B	10.8 ^{EF}	2.0 ^{EFG}	1.7 ^G	1.3 ^{CDE}	66.0 ^H
ATx2928/RTx436	9.8 ^{BC}	11.9 ^{BC}	2.5 ^{ABC}	1.6 ^I	1.4 ^{AB}	67.2 ^{EF}
RTx437	9.6 ^{BCD}	11.94 ^{BC}	2.5 ^{AB}	1.8 ^{CDE}	1.4 ^{BCDE}	66.9 ^{FG}
BTx645	9.6 ^{BCD}	11.75 ^C	2.3 ^{BCD}	1.9 ^B	1.3 ^{DEF}	67.8 ^{CDE}
R11358	9.5 ^{BCD}	11.51 ^{CD}	1.8 ^{GH}	1.7 ^G	1.3 ^{EF}	67.3 ^{EF}
BTx2928	9.0 ^{CDE}	10.20 ^F	1.9 ^{GH}	1.6 ^I	1.3 ^{FG}	68.0 ^{BCD}
ATx642/RTx437	9.0 ^{CDE}	12.51 ^A	2.3 ^{BCD}	1.8 ^{CD}	1.4 ^{AB}	68.2 ^{BC}
RTx436	8.9 ^{DEF}	11.7 ^C	2.6 ^A	1.7 ^H	1.4 ^{ABC}	67.5 ^{DE}
ATx645/RTx436	8.8 ^{EF}	12.48 ^A	2.2 ^{DEF}	1.7 ^{FG}	1.4 ^A	68.3 ^{ABC}
R10733	8.8 ^{CDE}	10.46 ^{EF}	1.4 ^J	1.8 ^{EFG}	1.3 ^G	66.7 ^{FG}
R11369	8.7 ^{DEF}	10.51 ^{EF}	1.0 ^K	1.7 ^{GH}	1.3 ^G	67.1 ^{EFG}
ATx642/RTx436	8.7 ^{FG}	12.51 ^A	2.2 ^{DE}	1.7 ^{FG}	1.4 ^A	68.3 ^{ABC}
R10258	8.7 ^{FG}	11.52 ^{CD}	1.6 ^{IJ}	1.7 ^G	1.3 ^{DEF}	66.8 ^{FG}
BTx642	8.6 ^{FG}	11.05 ^{DE}	1.7 ^{HI}	1.9 ^A	1.3 ^{FG}	67.3 ^{EF}
ATx2928/RTx437	8.4 ^{FG}	11.76 ^{BC}	2.0 ^{FGH}	1.7 ^G	1.3 ^{DEF}	68.8 ^{AB}
ATx645/RTx437	8.0 ^G	12.57 ^A	1.9 ^{GH}	1.8 ^{BC}	1.4 ^{BCD}	68.7 ^A

[†] Composition values from 2015 Puerto Rico due to insufficient grain for analysis in 2016

Future Research

Data from short day environments in Puerto Rico have shown promising yields and agronomic traits but further testing is required in the target environments. Hybrids should be sown in long days and grown into short days under farmer's field conditions in Africa and Central America to test for suitability to the established agronomic practices in the region. Comparisons should be made between not only the hybrid and its parents but also between hybrids and the landraces commonly grown in that region. The end users should make final decisions in determining the suitability of the grain based on local preferences.

The hybrids tested in this study have been bred for biomass potential and thus had varying yields and grain quality traits. As such, targeted breeding for grain yield and quality in this system needs to be undertaken. Ultimately, the best varieties from Sub-Saharan Africa and Central America can be converted to this system using marker assisted backcrossing so that the agronomics and grain quality match varieties that are currently grown but with increased yields due to heterosis.

Conclusion

Deployment and adoption of hybrids is essential for increased yields in Sub-Saharan Africa and Central America. The preliminary data presented above show promising levels of heterosis for grain yield in PS hybrids created from PI parents. In addition, yields are equal to that of the photoperiod sensitive checks. Targeted breeding for grain yield and heterosis will allow for future improvement and should be undertaken along with multi-location testing in the target environments. This system avoids seed

production problems often encountered in developing countries and will allow for high yielding hybrids to be deployed that meet farmer preferences for photoperiod sensitivity.

CHAPTER IV

CONCLUSIONS

The method for producing PS hybrids described above is well established for biomass sorghum but has not been deployed for grain (Rooney, et al., 2007). This method simplifies breeding and seed production logistics by producing seeds anywhere, especially in areas where seed system infrastructure is already in place. In addition, it has the potential to meet farmer preferences for photosensitivity and increase sorghum yields in food insecure regions of Central America and Sub-Saharan Africa. This study evaluated this method by looking at yields of the best hybrids, heterosis and grain quality.

Five PS hybrids, ATx2928/R10733, ATx642/R11358, ATx645/R10733, ATx645/R11369 and ATx645/R10258, were selected based on high yields and acceptable agronomics. They yielded equal to PI checks and should be tested in the target environments against varieties that are currently being grown. Only ATx2928/R10733 and ATx645/R10733 had adequate levels of heterosis, above the 20-30% threshold recommended for economic feasibility (Gómez, 1995, Haussmann, et al., 1998). This suggests that both high yields and high levels of heterosis should be bred for in the future.

Genotypic variation was present for grain yield heterosis suggesting that further breeding progress could be made. Seven hybrids showed acceptable levels of grain yield high parent heterosis. Negative heterosis was seen in several hybrids that failed to

restore fertility as evidenced by a high incidence of ergot infection in those genotypes. Environment had the largest effect on all traits except for diameter, further emphasizing that these hybrids need to undergo multi-location testing in the target environment, under farmer's field conditions.

Variation was present for all grain quality and composition traits. In general, hybrids had larger and softer seed than their parents. Factors that determine grain quality vary widely depending on the desired end use and grain quality in this study was largely affected by genotype by environment interactions. The women or men who will ultimately be preparing this grain should determine specific grain quality parameters to assist in the breeding process.

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APPENDIX

A-1: ANOVA for Puerto Rico 2015 for photoperiod sensitive hybrids, parental lines and photoperiod insensitive checks

Variance Source	Yield		Height		Exsertion		Diameter		Panicle Length	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Genotype	79	3467664***	79	7678.54***	79	396.07***	79	7.10***	79	51.20***
Rep	2	200860	2	134.34	2	106.73	2	28.45***	2	3.05
Bird	1	6584745**								
Error	152	929879	156	131.08	155	71.52	154	3.59	152	2.56

* significant at $p < .05$

** significant at $p < .01$

*** significant at $p < .001$

A-2: ANOVA for Puerto Rico 2016 for photoperiod sensitive hybrids, parental lines and photoperiod sensitive checks

Variance Source	Yield [†]		Height		Exsertion		Diameter	
	DF	MS	DF	MS	DF	MS	DF	MS
Genotype	72	252.62***	76	7559.39***	76	119.67***	76	14.80***
Rep	2	81.00	2	2856.57***	2	364.05***	2	48.33***
Bird	1	1158.47***						
Error	144	55.87	154	256.14	154	29.04	156	3.90

* significant at $p < .05$

** significant at $p < .01$

*** significant at $p < .001$

[†] residuals were normalized by using the square root of yield

A-3: Least square means for Puerto Rico 2015 and 2016 for photoperiod sensitive hybrids created from photoperiod insensitive parents and photoperiod insensitive checks

	ATx2928	ATx642	ATx645
	kg/ha		
F10762-3dw	1288.8	2054.65	1877.84
F10801-3dw	530.91	802.00	1051.68
F10805-3dw	795.76	731.53	1137.81
F10810-3dw	3573.15 [†]	1970.84	2603.10
R10258	2085.53	2443.25 [†]	2757.50
R10702	1753.43	2403.11	2145.40
R10704	927.12	993.15	2830.93
R10712	2157.74	1872.90	1833.01
R10715	972.03	1925.68	1707.90
R10733	3345.11	2190.74	3266.22
R10744	3353.88 [†]	2080.26	2460.55
R10779	1165.93	513.19	1093.51
R10788	1732.53	1444.47	2397.07
R11358	1619.39	2801.97	1833.03
R11365	1191.38	1075.47	1389.23
R11367	1127.89	1194.78	1128.31
R11369	1516.52	1620.28	2268.80
RTx436	1173.14	2799.59	3452.39
RTx437	1903.64	2526.61	3874.92

[†] Least square mean from 2015 Puerto Rico due to insufficient seed in 2016