IMPLEMENTATION OF GENOMIC AND PHENOMIC TOOLS FOR INTROGRESSION OF REINSTATED SORGHUM CONVERSION (RSC) GERMPLASM

A Dissertation

by

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ABSTRACT

Genotypic variance is necessary for trait improvement as limited diversity can reduce genetic gain in crop improvement. To maintain genetic diversity, a wealth of germplasm exists in the USDA-ARS sorghum [*Sorghum bicolor* (L.) Moench] collection, but most of the accessions are not adapted to temperate climates. Methodologies aimed at incorporating tropical germplasm have been evaluated extensively by public and private breeding programs due to their beneficial alleles for improved agronomic performance. However, concerns as to how and when material from this program should be tested for its agronomic value have been expressed. Three different methodologies were conducted to assess partially converted, early-generation lines from the Reinstated Sorghum Conversion (RSC) program. Our first methodology was to investigate the utility of using markers for the identification of high levels of tropical genome recovery, while elucidating the relationship between marker data and agronomic performance. The utilization of markers to predict hybrid performance was not observed, nonetheless, the ability to prescreen lines with high amounts of tropical genome recovery proved useful. Expanding upon these results, the second methodology focused on the phenotypic evaluation of partially converted, early-generation lines. From the lines evaluated, I was able to release lines that combined agronomic productivity with greater genetic diversity as confirmed via genotyping-by-sequencing. These eleven parental germplasms are being released to provide new genetic diversity for forage and grain hybrid improvement programs. Finally, noticing the value of phenotypic observations and its implications on selecting valuable germplasm, I further investigated plant height using high-throughput phenotyping via unmanned aerial systems (UAS). Within both advanced and early generation sorghum trials, genotypic variation estimates

were comparable to manual measurements with highly repeatable estimates of plant height, indicating the value of UAS in plant breeding programs.

DEDICATION

For my Mom, who made sure my siblings and I never did without. Her strong faith, diligence to work hard, and passion to care for others have helped me become the person I am today.

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NOMENCLATURE

DAP	Days after planting
DSM	Digital surface model
GBS	Genotyping-by-sequencing
GCV	Genotypic Coefficient of Variation
HPH	High Parent Heterosis
Max	Maximum percentile of structure-from-motion data
MPH	Mid-Parent Heterosis
LRT	Likelihood Ratio Test
P95	Ninety percentile of structure-from-motion data
R	Repeatability on an entry mean basis
RCBD	Randomized Complete Block Design
REML	Restricted maximum likelihood
RSC	Reinstated Sorghum Conversion
SC	Sorghum Conversion
SCA_T	Sugarcane Aphid Tolerance
SfM	Structure from motion
SNP	Single Nucleotide Polymorphism
TAMU	Texas A&M University
UAS	Unmanned aerial systems
USDA-ARS	United States Department of Agriculture-Agricultural Research Service

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1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) originated from the tropical to sub-tropical northeast quadrant of Africa where vast amounts of variability for wild types and cultivated forms are present (Doggett, 1970; Miller and Kebede, 1984). Since its domestication nearly 6000 years ago, it has experienced cultivation across numerous continents with differing climates and geographies (Klein et al., 2016). However, due to its center of origin, plants exhibited tall growth stature and were short-day in photoperiod response (Webster, 1976). Most of these cultivars did not initiate floral development until day lengths were less than 12 h 20 min (Klein et al., 2013). As such, most of the world collection of sorghum is photoperiod sensitive and unadapted to the U.S. (Shands et al., 1991). Therefore, it is difficult to utilize this germplasm for breeding purposes due to the flowering date differences with photoperiod insensitive material.

It was not until the 17th century that sorghum was introduced to the U.S., most likely with the slave trade (Kimber, 2001; Klein et al., 2016). Immediate selection for adaptation in the U.S. was centered on flowering time; photoperiod insensitive types were strongly selected for both grain and seed. Consequently, a majority of the sorghum varieties contributing to the United States grain production in the 1950's and 1960's contained very little representation of the world's genetic resources (Stephens et al., 1967). Following the discovery of cytoplasmic male-sterility (CMS) from the reciprocal crossing of two races, Durra (Milo) and Kafir (Stephens and Holland, 1954), hybrid sorghum breeding focused on the use of A1 cytoplasm. While grain yields improved, most of the hybrids produced were created from only these two races resulting in very little genotypic variation (Klein et al., 2008). To mitigate these issues of low variation among adapted lines and the utilization of only one male-sterile inducing system, the Texas Agricultural Experiment Station – United States Department of Agriculture (TAES-USDA)

Sorghum Conversion (SC) Program was launched in 1963. Utilizing four known maturity loci (Ma_1, Ma_2, Ma_3, and Ma_4) and four dwarfing genes (Dw_1, Dw_2, Dw_3, and Dw_4) identified by (Quinby, 1974),the goal of converting tall, photoperiod-sensitive alien sorghums to more temperate adaptation began (Stephens et al., 1967). Since the inception of the SC program, over 840 converted lines were publicly released to increase genetic diversity for sorghum improvement (Klein et al., 2008). They have been used as germplasm in parental line development (Rooney et al., 2011), as a source of disease resistance (D.T. Rosenow et al., 1997; Burrell et al., 2015), insect resistance (Schertz, 1977; Johnson et al., 1973) and for drought tolerance (Walulu et al., 1994). In addition, lines released from the SC program have contributed to new sources of male-sterility inducing systems (Schertz, 1977; Schertz and Ritchey, 1978).

Success generated from the SC program prompted researchers to continue their efforts in converting photoperiod-sensitive tropical accessions from the USDA-ARS germplasm collection (Stephens et al., 1967; Klein et al., 2013). In doing so, the Reinstated Sorghum Conversion (RSC) program was initiated in 2009 with the goal of reducing the time required for conversion to temperate adaptation using genomic background selection (Klein et al., 2013). To achieve a reduction time in development and release, early-generation, partially converted germplasm selected for a higher frequency of the tropical parent in the progeny, requiring fewer backcrosses than in the original SC program, was implemented (Klein et al., 2013). Most of the converted lines from the RSC originated from Sudan and Ethiopia and were predominately of race Durra or Caudatum (Klein et al., 2013, 2016). While the RSC conversion focused on elite yet unadapted sorghum lines and led to the release of over 155 new conversion lines (Klein et al., 2013, 2016), additional methods of release were warranted. Questions as to when material from this program

would be tested for value in a breeding program and how to identify high-combining ability of partially-converted sorghum lines utilizing genetic marker information needed to be evaluated.

Continual investigation of alternative methods for the introduction of tropical material have been proposed, while phenotypic selection of tropical lines followed by line evaluation may prove to be the method of choice. Although, an understanding that while increasing genetic diversity in elite sorghum germplasm is important, diversity associated with reduced performance is not desired. Unfortunately, this has limited the use of a vast majority of the exotic accessions in the USDA-ARS sorghum germplasm collection (Stephens et al., 1967; Gerloff and Smith, 1988). As such, these diverse sources must be adapted and tested, typically by public sector sorghum programs to introduce converted or partially-converted sorghum lines that can directly contribute to prebreeding programs of commercial grain hybrids (Jordan et al., 2011). Techniques utilizing early-generation genotyping-by-sequencing (GBS) of partially converted germplasm could be identified with a greater frequency of the tropical parental genome in the progeny thereby accelerating the conversion process permitting their release in earlier generations in field evaluations (Klein et al., 2013, 2016).

While genotyping technology has evolved, phenotyping is now the primary 'bottleneck' in crop genetic improvement programs (Furbank and Tester, 2011). Alternative methods aimed at identifying phenotypic traits are useful, yet evaluations in field settings for phenotyping under agricultural conditions are critical for exploiting genotype-by-environment-by-management complexities. Recently expressed interest in high-throughput techniques applied in the field may help researchers to further mitigate issues related to these interactions in a more efficient manner and at lower error rates than previous methodolgies (Furbank and Tester, 2011; Tester and Langridge, 2010; Araus and Cairns, 2014; Shi et al., 2016a). Specific technology via unmanned

aerial systems, or UAS, have been proposed as the new methodology of choice for phenotyping (Shi et al., 2016a). Despite this newly proposed method of phenotyping via UAS, the implications and utilization of UAS for plant breeding programs needs to be examined as interest for more expeditious methodologies become sought (Furbank and Tester, 2011; Shi et al, 2016a).

To determine the validity of including UAS for phenotypic measurements, plant height in grain sorghum may serve as a trait of exploration (Shi et al., 2016a; Watanabe et al., 2017). Plant height is highly correlated with grain yield in both sorghum and maize, especially in hot, dry, and stressful environments (Cassady, 1965). Traditional measurements of manual labor are tedious, exhausting, error prone, and are typically only captured at the terminal point of growth. Numerous researchers and programs have conducted experiments testing the application of UAS (Anthony et al., 2014; Chapman et al., 2014; Shi et al., 2016a; Watanabe et al., 2017), however, most anaylses have only been evaluated at a whole field level as opposed to the plot level. (Shi et al., 2016a; Watanabe et al., 2017). Challenges with the interpretation of results however prove to be difficult, as validation of UAS measurements relies on the assumption that manual measurements are accurate and repeatable. In comparison, it is also probable to assert that automated measurements are superior to manual measurements. To do so, evaluations through the consistency of measurements between replicates across different genotypes or treatments in a field setting are warranted.

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2. IDENTIFICATION OF THE RELATIONSHIP BETWEEN TROPICAL GENOME RECOVERY AND AGRONOMIC PERFORMANCE IN REINSTATED SORGHUM CONVERSION (RSC) LINES

2.1. Synopsis

Introduction of tropical sorghum [*Sorghum bicolor* (L.) Moench] into temperate breeding programs often proves to be a difficult task due to its origins of tropical adaptation where tall plant stature and late flowering tendencies are exhibited. The Reinstated Sorghum Conversion (RSC) program sought to convert Ethiopian and Sudanese germplasm to temperate-zone grain production utilizing genomic information. Utilizing high-throughput genotyping-by-sequencing (GBS) to identify genomic regions where the recovery of the tropical genome in an adapted background was the greatest, I investigated the relationship between recovery of the tropical genome and agronomic performance in partially-converted RSC progeny. A total of 23 selected $F_{3:4}$ lines across four RSC populations, three testers, and their respective line by tester combination (69 hybrids) were evaluated in four locations over three years for eight agronomic traits. Statistical analyses were structured with a focus on population, tropical genome recovery (level), tester, and tropical genome recovery (level) by tester to investigate how to best elucidate the predictive value of markers (SNPs). Results indicate that tropical genome recovery (based on SNP markers) showed no predictive ability to determine hybrid agronomic performance. However, it was observed that the lines with the lowest tropical genome recovery performed best for grain yield in inbred evaluations, whereas, the lines with the highest level of genome recovery performed better for grain yield in hybrid evaluations. Despite their lack of predictive ability of agronomic performance, markers proved helpful in pre-screening progeny to reduce the number of generations needed to convert tropical germplasm.

2.2. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) originated in tropical to sub-tropical Africa where plants exhibit tall growth stature and are short-day in photoperiod response (Webster, 1976). Most of these cultivars do not initiate floral development until day lengths are less than 12 h 20 min (Klein et al., 2013), with most of the world collection of sorghum being photoperiod sensitive and unadapted to the U.S. (Shands et al., 1991). As such, it is difficult to utilize this germplasm for breeding due to the flowering date differences with photoperiod insensitive material.

Sorghum was introduced in the U.S. in the 17th century, most likely with the slave trade. Immediate selection for adaptation was around flowering time; photoperiod-insensitive types were strongly selected for both grain and seed. Consequently, a majority of the sorghum varieties contributing to the United States grain production in the 1950's and 1960's contained very little representation of the worlds genetic resources (Stephens et al., 1967).

Harlan and de Wet (1972) formulated a simplified classification of cultivated sorghum into five races: Bicolor (B), Guinea (G), Caudatum (C), Kafir (K), and Durra (D). Of the five races, the Kafir and Durra (Milo) have contributed significant advances to the development of hybrid sorghum (Webster, 1976). These two races are responsible for the production of hybrid sorghum in the United States due to their usage in cytoplasmic male sterility (CMS) (Stephens and Holland, 1954). This method primarily focused on the use of A1 cytoplasm. Following the discovery of CMS and its wide spread use of creating sorghum hybrids, grain yields improved, however, most of the hybrids produced were created from the Durra (Milo) and Kafirs (Klein et al., 2008).

Limited variation among adapted lines and the availability of only one male-sterile inducing system lead to the development of the Texas Agricultural Experiment Station – United States Department of Agriculture (TAES-USDA) Sorghum Conversion (SC) Program in 1963. Utilizing four known maturity loci (Ma_1, Ma_2, Ma_3, and Ma_4) and four dwarfing genes (Dw_1, Dw_2, Dw_3, and Dw_4) identified by (Quinby, 1974),the goal of converting tall, photoperiod-sensitive alien sorghums to more temperate adaptation began (Stephens et al., 1967). Since the inception of the SC program, over 840 converted lines were publicly released to increase genetic diversity for sorghum improvement (Klein et al., 2008).

These materials have been an invaluable breeding and genomic resource to the sorghum research community. They have been used as germplasm in parental line development (Rooney et al., 2011), as a source of biotic stress tolerance to diseases such as downy mildew (*Sclerospora sorgi*) and anthracnose (*Collectotrichom graminicola*) (D.T. Rosenow et al., 1997; Burrell et al., 2015), and insects such as green bug (*Schizaphis graminum*) (Schertz, 1977), and sorghum midge (*Contarinia sorghicola*) (Johnson et al., 1973). They have also been used as a source of drought tolerance (Walulu et al., 1994). In addition to generating numerous sources of resistance to abiotic and biotic stresses, lines released from the SC program have contributed to additional sources of male-sterility inducing systems (Schertz, 1977; Schertz and Ritchey, 1978).

The success of the TAES-USDA SC program prompted researchers to continue converting photoperiod-sensitive tropical accessions from the USDA-ARS germplasm collection. The Reinstated Sorghum Conversion (RSC) program sought to reduce the time required for conversion to temperate adaption using genomic background information (Klein et al., 2013). By utilizing early-generation, partially converted germplasm selected for a higher

frequency of the tropical parent in the progeny, germplasm from this program requires fewer backcrosses and is released sooner than in the original SC program (Klein et al., 2013).

Previous experience with conversion material highlights that there are relevant questions regarding the use of this material. For example, there are questions as to when material from this program will be tested for value in a breeding program and how to identify high-combining ability of partially-converted sorghum lines utilizing genetic marker information. Thus, the goal of this research is to elucidate the appropriate use of RSC germplasm in a sorghum breeding program using marker-based criteria to serve breeding programs with the information needed to incorporate tropical germplasm into their program.

2.3. Materials and Methods

2.3.1. Germplasm and Experimental Design

Germplasm obtained for this project was provided by the USDA-ARS crop germplasm research group in College Station, TX and are outlined in Klein et al. (2013, 2016). Much like the goal of the SC program, the recovery of the tropical genome in an early flowering, short derivative was the primary objective of the RSC program. Thus, those lines in which the highest recovery of the unadapted (tropical) parental genome were recovered were considered for early generation evaluation of agronomic performance. To exploit the differences within each of those RSC lines within each population, varying levels (High, Medium, and Low) of tropical genome recovery were assigned. Utilizing restriction-site DNA sequencing technology developed by Morishige et al. (2013), I sought to examine the ability to predict agronomic performance in newly Reinstated Sorghum Conversion (RSC) lines.

In the summer of 2014, four selected RSC populations (RSC17, RSC37, RSC114, and RSC135) comprised of six $F_{3:4}$ derived lines per population were chosen (Table 1). Selected

progeny were planted into a crossing block and top-crossed with three elite testers; A3Tx436 (Miller et al., 1992), ATx645 (Rosenow et al., 2002), and ATx2928 (Rooney, 2003). These selected testers represent distinct diversity groups and are common testers within the Texas A&M sorghum breeding program.

Table 1. List of the four Reinstated Sorghum Conversion (RSC) populations with their respective male lines, PI Information, percentage of tropical genome recovery, and their designated level.

Population	Male Pedigree	PI Information	% Tropical Genome Recovery	Designated Level
RSC17	RSC17-3-F2-4-CS1	PI 665660, Mali, WG 50: D, Dry	0.39	High
	RSC17-3-F2-4-CS2	PI 665660, Mali, WG 50: D, Dry	0.39	High
	RSC17-3-F2-7-CS4	PI 665660, Mali, WG 50: D, Dry	0.40	High
	RSC17-3-F2-2-CS2	PI 665660, Mali, WG 50: D, Dry	0.35	Medium
	RSC17-3-F2-12-CS1	PI 665660, Mali, WG 50: D, Dry	0.32	Low
RSC37	RSC37-3-F2-12-CS3	PI 665668, Sudan, WG 31: C-Nigr, Dry	0.28	Medium
	RSC37-3-F2-12-CS1	PI 665668, Sudan, WG 31: C-Nigr, Dry	0.28	Medium
	RSC37-3-F2-12-CS4	PI 665668, Sudan, WG 31: C-Nigr, Dry	0.28	Medium
	RSC37-3-F2-2-CS3	PI 665668, Sudan, WG 31: C-Nigr, Dry	0.34	High
	RSC37-3-F2-6-CS3	PI 665668, Sudan, WG 31: C-Nigr, Dry	0.26	Low
	RSC37-3-F2-14-CS3	PI 665668, Sudan, WG 31: C-Nigr, Dry	0.28	Medium
RSC114	RSC114-3-F2-12-CS2	PI 665646, Ethiopia, WG 51: Nandyal, Dry	0.32	High
	RSC114-3-F2-12-CS4	PI 665646, Ethiopia, WG 51: Nandyal, Dry	0.32	High
	RSC114-3-F2-5-CS3	PI 665646, Ethiopia, WG 51: Nandyal, Dry	0.31	High
	RSC114-3-F2-6-CS2	PI 665646, Ethiopia, WG 51: Nandyal, Dry	0.22	Low
	RSC114-3-F2-9-CS2	PI 665646, Ethiopia, WG 51: Nandyal, Dry	0.28	Medium
	RSC114-3-F2-9-CS3	PI 665646, Ethiopia, WG 51: Nandyal, Dry	0.28	Medium
RSC135	RSC135-3-F2-11-CS1	PI 665655, Ethiopia, WG 51: Nandyal, Dry	0.28	Medium
	RSC135-3-F2-11-CS2	PI 665655, Ethiopia, WG 51: Nandyal, Dry	0.28	Medium
	RSC135-3-F2-12-CS1	PI 665655, Ethiopia, WG 51: Nandyal, Dry	0.26	Low
	RSC135-3-F2-8-CS1	PI 665655, Ethiopia, WG 51: Nandyal, Dry	0.32	Medium
	RSC135-3-F2-9-CS2	PI 665655, Ethiopia, WG 51: Nandyal, Dry	0.35	High
	RSC135-3-F2-9-CS4	PI 665655, Ethiopia, WG 51: Nandyal, Dry	0.35	High

Both parental lines and their line by tester combinations within each of the four RSC populations (RSC17, RSC37, RSC114, and RSC135) were evaluated across four locations (Corpus Christi, TX; College Station, TX; Halfway, TX; and Rio Farms, TX) over a period of three years between 2015-2017. A randomized complete block design (RCBD) with four replications per entry was used for all populations. Standard agronomic practices were implemented for each location.

2.3.2. Data Collection – Phenotypic Traits

Across all trials, the following phenotypic observations were recorded. Days-to-anthesis (DF) was the number of days from planting to when 50 percent of the plot was at 50 percent mid-anthesis. Plant height (PH, cm), flag-leaf height (FL, cm), and panicle exsertion (EX, cm), were measured as a representative mean of each plot. Average panicle length (Avg.PL, cm) and average panicle width (Avg.PW, cm) were calculated from a representative sample of five panicles within each plot. Test weight (TW, kg/hL) and grain yield (GY, Mt ha-1) were evaluated for each entry with grain yield adjusted to 14% moisture.

2.3.3. Statistical Analysis

Before evaluating our results using a mixed linear model, phenotypic estimates were checked for outliers and normality of the data in R (version 3.4.0; R Development Core Team, 2017). Due to limitations of experimental area and to more appropriately assess genotypic differences, the combination of year and location were considered as an environmental term. Thus, our analyses focused on the effects due to the respective RSC population and % tropical genome recovery level, instead of an overall pedigree performance for inbreds and hybrids.

To investigate the significance of each factor, a Likelihood Ratio Test (LRT) was performed for both inbreds (model 1) and hybrids (model 2);

$$Y_{ijlmno} = \mu + P_i + L_{j(i)} + E_l + (EL)_{l(j(i))} + Rep_{m(l)} + Ra_{n(l)} + Ro_{o(l)} + \varepsilon_{ijlmno}, \quad \text{Eq. [1]}$$

where Y_{ijlmno} are the respective indices for each factor, μ is the overall mean, P_i is the effect of the i^{th} RSC population, $L_{j(i)}$ is the j^{th} level of the %EGR within population, E_l is the l^{th} environment, $(EL)_{l(j(i))}$ is the interaction of the ith environment with the j^{th} level within population, $Rep_{m(l)}$ is the m^{th} replication within environment, $Ra_{n(l)}$ is the n^{th} range within environment, $Ro_{o(l)}$ is the o^{th} row within environment, and ε_{ijlmno} is the residual term associated with each Y_{ijlmno}. Model two was as follows;

$$Y_{ijklmno} = \mu + P_i + M_{j(i)} + F_k + (MF)_{jk} + E_l + (ME)_{jl} + Rep_{m(l)} + Ra_{n(l)} + Ro_{o(l)} + \varepsilon_{ijklmno}, \quad \text{Eq. [2]}$$

where $Y_{ijklmno}$ are the respective indices for each factor, μ is the overall mean, P_i is the effect of the i^{th} RSC population, $M_{j(i)}$ is the j^{th} male within population, F_k is the k^{th} tester, $(MF)_{jk}$ is the interaction of the j^{th} male with the k^{th} tester, E_l is the l^{th} environment, $(ME)_{jl}$ is the interaction of the j^{th} male with the l^{th} environment, $Rep_{m(l)}$ is the m^{th} replication within environment, $Ra_{n(l)}$ is the n^{th} range within environment, $Ro_{o(l)}$ is the o^{th} row within environment, and ε_{ijlmno} is the residual term associated with each $Y_{ijklmno}$.

To estimate LSmeans for population and level of inbreds and LSmeans of population in hybrids, model 3 was used;

$$Y_{ilmno} = \mu + G_i + E_l + Rep_{m(l)} + Ra_{n(l)} + Ro_{o(l)} + \varepsilon_{ilmno}, \quad \text{Eq. [3]}$$

where Y_{ilmno} are the respective indices for each factor, fixed effect μ is the overall mean, fixed effect G_i is the i^{th} population or level respective to either inbred or hybrids, and random effects; E_l is the l^{th} environment, $Rep_{m(l)}$ is the m^{th} replication within environment, $Ra_{n(l)}$ is the n^{th}

range within environment, $Ro_{o(l)}$ is the o^{th} row within environment, and ε_{ilmno} is the residual term associated with each Y_{ilmno}. To obtain LSmeans for the effect of level in hybrids, model four was used;

$$Y_{iklmno} = \mu + l_i + F_k + (Fl)_{ik} + E_l + (FE)_{kl} + Rep_{m(l)} + Ra_{n(l)} + Ro_{o(l)} + \varepsilon_{iklmno}, \quad \text{Eq. [4]}$$

where Y_{ilmno} are the respective indices for each factor, fixed effects; μ is the overall mean, L_i is the i^{th} level within population, F_k is the k^{th} tester, $(Fl)_{ki}$ is the interaction of the i^{th} level with the k^{th} tester, random effects; E_l is the l^{th} environment, $(FE)_{kl}$ is the interaction of the k^{th} tester with the l^{th} environment, $Rep_{m(l)}$ is the m^{th} replication within environment, $Ra_{n(l)}$ is the n^{th} range within environment, $Ro_{o(l)}$ is the o^{th} row within environment, and ε_{iklmno} is the residual term associated with each Y_{ijlmno}. Means separation test using Tukey', alpha .05 was performed for each factors LSmeans.

Variance components from models one and two were used to calculate genotypic coefficient of variation (GCV) and repeatability (R) on an entry mean basis. Estimates for GCV were calculated using,

$$GCV = \left(\frac{\sqrt{MS_G}}{\bar{x}_G}\right) \times 100$$

where GCV is the genotypic coefficient of variation, MS_G is the mean square error of genotype and $\bar{x}_G$ is the overall mean of genotype. Due to the absence of a familial structure, repeatability (R) estimates on an entry mean basis were calculated using,

$$\mathrm{R} = \frac{\sigma_{\mathrm{g}}^2}{\sigma_{\mathrm{g}}^2 + \frac{\sigma_{\mathrm{e}}^2}{\mathrm{r}}}$$

where R is the repeatability score, σ_g^2 is the genotypic variance, σ_e^2 is the error variance, and r is the number of replications (Nakagawa and Schielzeth, 2010).

Utilizing LSmeans, mid-parent (MPH) and high-parent heterosis (HPH) were estimated for each agronomic trait based on their desirable performance. The following calculation was used for MPH,

$$\text{MPH} = \left(\frac{TC_{ij} - \mu P_{ij}}{\mu P_{ij}}\right) * 100$$

where MPH is the mid-parent heterosis value, TC_{ij} is the LSmean of the interaction of the i^{th} line by the j^{th} tester in a line by tester analyses and μP_{ij} is the LSmean of the i^{th} line by the j^{th} tester. High parent heterosis was calculated using the following formula:

$$\text{HPH} = \left(\frac{TC_{ij} - HP_k}{HP_k}\right) * 100$$

where HPH is the high-parent heterosis value, TC_{ij} is the LSmean of the interaction of the i^{th} line by the j^{th} tester in a line by tester analyses and HP_k is the highest LSmean of the kth parent.

2.4. Results and Discussion

2.4.1. Inbred Performance

Because the classification of percent tropical genome recovery is a relative and subjective classification, the data is nested with each male line and differences between each male are present, our analyses focused on the effects of the RSC population and the percent tropical genome level. Statistical differences for each source of variation across the eight agronomic traits were observed from the LRT, highlighting the importance of conducting a more in-depth analysis of the differences between population and level (Table 2).

Table 2. Likelihood Ratio Test (LRT) for each source of variation across each sources eight agronomic traits for parental lines.

Source	Agronomic Traits							
	DF (d)	PH (cm)	EX (cm)	FL (cm)	Avg.PL (cm)	Avg.PW (cm)	TW (kg/hL)	GY (Mt ha^{-1})
Population	140.01***	140.94***	88.60***	107.88***	335.51***	36.10***	56.86***	45.92***
Level	227.27***	605.92***	145.60***	406.63***	240.29***	53.77***	147.84***	163.41***
Environment	1231.15***	535.61***	224.90***	308.11***	179.26***	80.97***	340.73***	352.36***
Level x Environment	97.22***	225.96***	45.00***	200.62***	64.47***	36.33***	49.54***	153.33***

Significance codes: 0***, 0.001***, .01**, 0.05*

Overall means for each of the four RSC populations varied within their respective trait across each of the eight agronomic traits (Table 3). Population RSC17 was the earliest population to flower at 62 days (d). Observations from grain yield components reveal that population RSC135 had the longest and widest panicles (Table 3). Population RSC37 was the shortest population. The RSC114 population did not appear at the top of any respective traits across each agronomic category, however, RSC114 was not statistically different than RSC37 for panicle exsertion (EX, cm) and was not statistically different from RSC135 for test weight (TW, kg/hL) (Table 3).

Comparisons made across the agronomic traits revealed specific combinations of RSC populations that performed better than others depending on the overall goal of the breeding program. For instance, RSC17 was the highest yielding, earliest to flower, had an acceptable plant height, and was not statistically different from RSC135 for average panicle width (Table 3). However, the exsertion in this population was greater than the other populations. Based on these data, population RSC17 has the most potential to produce hybrids with high yield, early flowering, wider panicles, and acceptable grain sorghum plant height. Alternatively, population RSC135 was the latest and tallest population (Table 3).

Table 3. LSmeans of each RSC population within their respective trait. LSmeans are ranked by their desirable agronomic performance for this study.

Trait	Population	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]		
DF (d)	RSC17	62.52	3.70	4.27	47.27	77.77	A		
	RSC37	65.78	3.65	4.08	50.30	81.27		B	
	RSC114	67.64	3.67	4.13	52.20	83.08		B	C
	RSC135	69.28	3.65	4.08	53.79	84.76			C
PH (cm)	RSC37	87.47	7.90	6.07	59.91	115.03	A		
	RSC17	105.01	8.18	6.90	77.72	132.31		B	
	RSC135	124.24	7.74	5.65	96.44	152.05			C

Table 3. Continued

Trait	Population	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]		
	RSC114	125.06	7.96	6.12	97.36	152.77			C
EX (cm)	RSC37	8.68	1.63	6.86	3.24	14.12	A		
	RSC114	9.04	1.65	6.94	3.55	14.54	A		
	RSC135	13.34	1.58	6.13	7.86	18.82		B	
	RSC17	17.14	1.72	8.34	11.70	22.57			C
FL (cm)	RSC37	63.71	5.41	7.72	46.26	81.17	A		
	RSC17	69.99	5.84	10.04	52.32	87.66	A		
	RSC135	85.67	5.18	6.63	68.18	103.17		B	
	RSC114	99.30	5.51	7.80	81.57	117.03			C
Avg.PL (cm)	RSC135	21.10	0.62	4.80	18.70	23.49	A		
	RSC37	19.01	0.68	6.59	16.71	21.32		B	
	RSC17	16.98	0.76	9.46	14.66	19.30			C
	RSC114	16.45	0.70	6.66	14.10	18.81			C
Avg.PW (cm)	RSC135	4.98	0.13	4.92	4.49	5.47	A		
	RSC17	4.74	0.17	12.24	4.25	5.23	A	B	
	RSC37	4.69	0.14	7.21	4.22	5.15		B	
	RSC114	4.61	0.15	7.08	4.11	5.10		B	
TW (kg/hL)	RSC135	67.21	2.56	5.30	57.78	76.64	A		
	RSC114	66.57	2.54	5.06	56.99	76.14	A		
	RSC37	66.54	2.48	4.71	56.87	76.21	A		
	RSC17	61.59	2.54	5.15	52.10	71.08		B	
YD (Mt ha^{-1})	RSC17	2.44	0.25	10.39	1.69	3.19	A		
	RSC37	2.06	0.23	7.47	1.33	2.80	A		
	RSC135	1.67	0.22	6.29	0.93	2.41		B	
	RSC114	1.59	0.23	7.29	0.82	2.35		B	

[†]SE = Standard Error
[‡]df = degrees of freedom
[§]lower.CL = lower Confidence Level
[¶]upper.CL = upper Confidence Level
[#]Group = Statistical rankings using connecting letters report; letters sharing the same character are not significantly different from one another

Since the level of tropical genome recovery was associated with a specific RSC population, mean separation test of the levels across all populations was possible, with comparisons being made within each population. Variation within each population for level was observed along with their relationship with agronomic performance (Table 4). Across the four populations, the general trend for yield related traits was that "Low" levels of tropical genome recovery performed the best (Table 4). For the other traits, "Medium" or "High" levels of tropical genome recovery performed better across the agronomic traits per se (Table 4).

Table 4. RSC parental test with their respective level of % tropical genome recovery ranked by their desirable agronomic performance for each trait within test.

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				DF (d)					
RSC17	High	64.12	5.63	1.00	-145.02	273.25	A		
	Low	64.51	5.68	1.03	-128.82	257.85	A		
	Medium	65.68	5.68	1.03	-126.53	257.90	A		
RSC37	Low	64.54	3.80	3.13	46.80	82.27	A		
	Medium	66.29	3.76	3.01	48.19	84.40		B	
	High	71.82	3.80	3.12	54.06	89.58			C
RSC114	Medium	70.33	5.64	2.02	28.22	112.43	A		
	High	71.43	5.63	2.01	29.07	113.78	A	B	
	Low	73.06	5.67	2.05	31.70	114.42		B	
RSC135	Medium	68.52	5.15	3.01	43.73	93.31	A		
	Low	72.10	5.19	3.10	47.69	96.51		B	
	High	72.91	5.17	3.05	48.29	97.53		B	
				PH (cm)					
RSC17	Low	92.92	9.21	2.35	36.26	149.58	A		
	Medium	103.06	9.29	2.40	47.28	158.83	A	B	
	High	107.46	8.86	2.04	42.16	172.76		B	
RSC37	Medium	90.49	4.37	5.21	75.38	105.59	A		
	Low	106.68	7.84	37.24	87.09	126.27	A	B	

Table 4. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				DF (d)					
RSC37	High	118.27	7.75	37.99	98.90	137.64		B	
	Medium	127.79	11.10	3.27	77.77	177.82	A		
RSC114	High	129.91	10.98	3.13	78.76	181.06	A		
	Low	134.39	11.54	3.76	87.18	181.59	A		
	High	96.39	8.84	5.31	66.09	126.70	A		
RSC135	Medium	135.66	8.53	4.68	104.69	166.62		B	
	Low	141.10	9.72	7.62	111.51	170.68		B	
				EX (cm)					
	High	14.37	2.93	2.14	-5.92	34.66	A		
RSC17	Low	19.05	3.29	3.28	4.26	33.84	A	B	
	Medium	23.85	3.37	3.45	9.27	38.44		B	
	Medium	7.79	2.27	3.22	-2.57	18.15	A		
RSC37	High	12.35	2.62	5.54	3.52	21.18		B	
	Low	17.27	2.63	5.54	8.41	26.14		B	
	High	9.75	1.69	3.81	2.90	16.59	A		
RSC114	Medium	10.29	1.78	4.71	3.86	16.72	A		
	Low	10.73	2.08	8.42	4.57	16.89	A		
	High	9.33	1.66	7.75	4.30	14.37	A		
RSC135	Low	13.21	1.99	15.32	7.88	18.54	A	B	
	Medium	14.91	1.54	5.85	9.82	20.00		B	
				FL (cm)					
	Low	53.98	7.31	2.41	10.29	97.66	A		
RSC17	Medium	63.27	7.38	2.46	20.17	106.38		B	
	High	77.90	6.99	2.05	26.62	129.19			C
	Medium	65.64	3.11	6.69	55.81	75.47	A		
RSC37	Low	67.95	6.16	53.23	52.76	83.13	A		
	High	88.74	6.10	55.04	73.73	103.76		B	
RSC114	Medium	100.33	9.33	3.46	60.03	140.63	A		

Table 4. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				FL (cm)					
RSC114	High	102.82	9.16	3.23	61.13	144.50	A		
	Low	106.19	9.92	4.34	68.81	143.56	A		
RSC135	High	61.72	6.69	7.90	41.57	81.88	A		
	Medium	95.00	6.19	5.92	74.63	115.37		B	
	Low	101.98	8.04	15.81	80.54	123.43		B	
				Avg.PL (cm)					
RSC17	Low	21.45	0.94	1.80	13.07	29.83	A		
	Medium	17.27	0.99	1.97	9.62	24.91		B	
	High	15.50	0.82	1.08	-8.28	39.28			C
RSC37	Low	21.27	1.32	3.45	15.55	26.99	A		
	High	19.23	1.31	3.43	13.51	24.94	A	B	
	Medium	19.04	1.16	2.13	11.00	27.08		B	
RSC114	Low	19.08	1.15	9.89	15.77	22.38	A		
	Medium	17.03	0.95	4.69	13.58	20.48	A	B	
	High	15.85	0.88	3.17	11.81	19.89		B	
RSC135	High	22.26	0.82	5.53	19.51	25.01	A		
	Medium	20.93	0.78	4.81	18.12	23.74		B	
	Low	19.16	0.91	8.27	16.45	21.86			C
				Avg.PW (cm)					
RSC17	High	4.97	0.12	1.57	3.57	6.37	A		
	Low	4.46	0.20	9.63	3.88	5.04		B	
	Medium	4.10	0.22	12.36	3.48	4.72		B	
RSC37	Low	4.75	0.19	7.21	4.15	5.34	A		
	Medium	4.53	0.14	2.38	3.66	5.40	A		
	High	4.52	0.19	7.21	3.93	5.11	A		
RSC114	Medium	4.84	0.31	2.31	2.89	6.79	A		
	Low	4.72	0.32	2.75	3.03	6.42	A	B	
	High	4.51	0.30	2.14	2.41	6.61		B	

Table 4. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				Avg.PW (cm)					
	Low	5.27	0.19	6.47	4.66	5.88	A		
RSC135	Medium	4.97	0.17	4.47	4.33	5.62		B	
	High	4.76	0.18	4.94	4.13	5.39		B	
				TW (kg/hL)					
	Low	65.32	4.93	2.28	33.83	96.81	A		
RSC17	High	59.22	4.80	2.06	24.35	94.09		B	
	Medium	58.86	4.96	2.32	27.89	89.83		B	
	High	69.32	4.21	3.34	50.65	87.98	A		
RSC37	Low	68.32	4.16	3.19	49.25	87.39	A		
	Medium	62.94	4.10	3.03	43.35	82.54		B	
	Low	71.13	2.34	4.98	62.89	79.37	A		
RSC114	High	66.73	2.07	3.30	57.48	75.98		B	
	Medium	59.24	2.29	4.67	50.92	67.56			C
	Medium	69.36	0.66	5.71	67.15	71.58	A		
RSC135	High	68.93	0.99	7.02	65.83	72.02	A		
	Low	67.64	0.85	14.96	65.35	69.93	A		
				YD (Mt ha^{-1})					
	Low	3.18	0.67	2.37	-0.90	7.26	A		
RSC17	Medium	2.39	0.68	2.44	-1.60	6.38		B	
	High	2.17	0.64	2.05	-2.56	6.89		B	
	Low	2.41	0.40	3.92	0.80	4.01	A		
RSC37	Medium	2.04	0.38	3.09	0.26	3.82	A		
	High	1.94	0.40	4.00	0.34	3.53	A		
	Low	2.15	0.29	6.68	1.24	3.05	A		
RSC114	High	1.65	0.24	3.62	0.63	2.68	A	B	
	Medium	1.52	0.26	4.61	0.57	2.47		B	
	Low	2.38	0.30	7.43	1.48	3.29	A		
RSC135	Medium	1.70	0.26	4.53	0.75	2.66		B	
	High	1.59	0.27	5.19	0.66	2.52		B	

[†]Level = Percent tropical genome recovery designation

Table 4. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]

[‡]SE = Standard Error
[§]df = degrees of freedom
[¶]lower.CL = lower Confidence Level
[#]upper.CL = upper Confidence Level
[††]Group = Statistical rankings using connecting letters report; letters sharing the same character are not significantly different from one another

Maintaining or increasing selectable genetic variation within a breeding program is essential to further genetic improvement. Two components commonly used to determine the amount of variation represented within a population are to analyze the percent coefficient of variation due to genotype (GCV) and the repeatability (R). The GCV is a measure of the variability in relation to the mean for that trait or population, the higher the value, the higher the variability of the data. Thus, a lower value for our study indicates a lower amount of variation was observed for those genotypes relative to that populations mean performance for a specific trait. Repeatability estimates which are calculated like heritability (H^2), was used herein due to the absence of a familial structure.

The GCV varied among populations for the eight agronomic traits of interest (Table 5). Three of the populations, RSC17, 37, and 114 had high amounts of GCV for panicle exsertion (EX, cm) with respective values of 27.61%, 54.50%, and 14.49% indicating the greatest amount of variation present within these populations for selection would come from selections made on panicle exsertion length. Population RSC135 had the largest amount of GCV for flag leaf height with a value of 31.28%. Days-to-anthesis (DF, d) had the lowest amount of GCV for populations RSC17 and RSC114 indicating low amounts of selection would be beneficial within these two populations (Table 5). Populations RSC37 and RSC135 had zero percent GCV for average panicle width and test weight, respectively.

Repeatability estimates within and across each of the populations varied with most of these values being high (Table 5). Repeatability estimates for population RSC17 varied from 0.68 to 0.96 with DF having the highest repeatability estimate. Populations RSC37 and RSC135 ranged from 0.00 to 0.97 for their repeatability estimates, with plant height (PH, cm) having the greatest repeatability estimate for both, an indication that plant height within these populations will serve as a true estimate of stature within subsequent cycles of selection. Average panicle width for RSC37 and test weight for RSC135 contained the lowest repeatability estimates indicating that no change in yield related traits can be observed with continued selection. RSC114 population had the highest repeatability estimates overall (Table 5). However, repeatability estimates for yield for the three other populations were higher than that of RSC114 with values ranging from 0.68 to 0.78 and GCV values ranging from 13.27 to 17.53.

Table 5. Genotypic coefficient of variation (GCV) and Repeatability (R) estimates on an entry mean basis for eight agronomic traits within their respective population for inbreds. Traits are ranked by their repeatability (R) estimate on an entry mean basis from high to low.

Population	Trait	GCV (%)	R
RSC17	DF (d)	2.98	0.96
	Avg.PW (cm)	10.23	0.94
	EX (cm)	27.61	0.89
	TW (kg/hL)	7.02	0.88
	FL (cm)	14.16	0.86
	PH (cm)	7.31	0.86
	Avg.PL (cm)	13.62	0.80
	YD (Mt ha^{-1})	14.02	0.68
RSC37	PH (cm)	30.13	0.97
	FL (cm)	34.59	0.96
	DF (d)	3.62	0.93
	EX (cm)	54.50	0.93
	TW (kg/hL)	4.67	0.85
	Avg.PL (cm)	6.90	0.78
	YD (Mt ha^{-1})	13.27	0.71

Table 5. Continued

Population	Trait	GCV (%)	R
RSC37	Avg.PW (cm)	0.00	0.00
RSC114	TW (kg/hL)	7.01	0.99
	FL (cm)	10.73	0.85
	PH (cm)	7.27	0.82
	DF (d)	1.74	0.81
	Avg.PL (cm)	9.69	0.73
	EX (cm)	14.49	0.63
	Avg.PW (cm)	3.31	0.40
	YD (Mt ha^{-1})	9.54	0.26
RSC135	PH (cm)	22.37	0.97
	FL (cm)	31.28	0.93
	Avg.PL (cm)	9.84	0.92
	DF (d)	2.70	0.89
	Avg.PW (cm)	4.48	0.82
	YD (Mt ha^{-1})	17.53	0.78
	EX (cm)	14.72	0.66
	TW (kg/hL)	0.00	0.00

2.4.2. Hybrid Performance

A likelihood ratio test (LRT) for each source of variation within the hybrid trial across each of the eight agronomic traits was conducted to determine if significant variation was observed. Significant differences for most of the sources of variation across the eight agronomic traits was observed, with strong significance (<0.001) observed across all traits for population, tester, and level (Table 6). While each of these individual sources of variation showed strong significance for variation, the interaction of Tester-by-Level (hybrid) was not significant for grain yield (Mt ha^{-1}) (Table 6). Despite its lack of significance, a means separation test was calculated to determine the effects of population, level, and tester in hybrid combinations.

Table 6. Likelihood Ratio Test (LRT) for each source of variation across eight agronomic traits with their significance for hybrids.

Source	Agronomic Traits							
	DF (d)	PH (cm)	EX (cm)	FL (cm)	Avg.PL (cm)	Avg.PW (cm)	TW (kg/hL)	GY (Mt ha^{-1})
Population	103.41^{***}	98.23^{***}	28.54^{***}	112.34^{***}	143.78^{***}	70.37^{***}	21.05^{***}	8.89^{**}
Tester	151.79^{***}	141.08^{***}	103.51^{***}	63.62^{***}	161.03^{***}	102.17^{***}	87.52^{***}	199.48^{***}
Level	437.86^{***}	1273.88^{***}	55.47^{***}	1118.75^{***}	415.53^{***}	63.73^{***}	86.48^{***}	36.05^{***}
Environment	2905.25^{***}	1164.32^{***}	305.59^{***}	799.18^{***}	432.86^{***}	269.41^{***}	2306.41^{***}	403.16^{***}
Tester x Level	78.06^{***}	60.41^{***}	9.54^{**}	56.89^{***}	15.83^{***}	4.13^{*}	28.65^{***}	1.75^{NS}
Level x Environment	243.33^{***}	145.04^{***}	9.79^{**}	131.46^{***}	25.25^{***}	38.38^{***}	17.12^{***}	32.84^{***}

Significance codes: 0***, 0.001***, .01**, 0.05*, Not Significant^{NS}

Population trends for hybrid testcrosses were similar to the inbred results (Table 7). As in the inbred, populations RSC17 and RSC135 were the earliest and latest flowering populations, respectively (Table 7). For plant height population RSC17 was the shortest and population RSC114 was the tallest (Table 7). Panicle exsertion was lowest in the RSC114 while the RSC17 population had the greatest exsertion (Table 7). Population RSC135 had the shortest flag leaf height, and the longest and widest panicles (Table 7). Population RSC114 had the tallest FL height and shortest panicle length, while RSC37 contained the shortest Avg.PW (cm) (Table 7). Both TW (kg/hL) and grain yield (Mt ha^{-1}) for the hybrids had population orders different than that of their inbreds, where Population RSC17 now had the highest TW and Population RSC37 was the highest yielding population (Table 7). Hybrids of Population RSC37 had the lowest TW and RSC135 hybrids had the lowest grain yields at 2.36 Mt ha^{-1}.

Table 7. Eight agronomic traits by RSC hybrid test ranked by their desirable agronomic performance respective to each trait.

Trait	Population	LSmean	SE†	df‡	lower.CL§	upper.CL¶	Group#		
DF (d)	RSC17	63.68	3.59	4.10	48.52	78.83	A		
	RSC37	64.83	3.57	4.03	49.57	80.09	A		
	RSC114	66.97	3.58	4.06	51.76	82.18		B	
	RSC135	67.65	3.57	4.02	52.38	82.93		B	
PH (cm)	RSC17	149.83	8.93	5.53	117.48	182.17	A		
	RSC135	152.93	8.79	5.21	120.23	185.63	A		
	RSC37	159.93	8.84	5.32	127.36	192.50		B	
	RSC114	177.56	8.88	5.39	145.03	210.09			C
EX (cm)	RSC114	13.91	1.55	5.73	8.37	19.46	A		
	RSC135	15.02	1.52	5.37	9.43	20.61	A		
	RSC37	16.63	1.54	5.62	11.08	22.18		B	
	RSC17	17.77	1.57	6.04	12.27	23.26		B	
FL (cm)	RSC135	109.99	6.88	5.40	84.81	135.17	A		
	RSC17	110.59	7.14	6.22	85.90	135.28	A		
	RSC37	122.55	6.99	5.73	97.60	147.49		B	

Table 7. Continued

Trait	Population	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]		
FL (cm)	RSC114	143.19	7.04	5.82	118.21	168.17			C
Avg.PL (cm)	RSC135	24.56	0.74	4.14	21.44	27.69	A		
	RSC37	22.16	0.76	4.50	19.12	25.20		B	
	RSC17	22.14	0.78	4.96	19.17	25.10		B	
	RSC114	20.73	0.77	4.58	17.69	23.77			C
Avg.PW (cm)	RSC135	5.69	0.16	4.20	5.02	6.36	A		
	RSC114	5.54	0.17	4.89	4.89	6.19	A	B	
	RSC17	5.33	0.18	5.96	4.71	5.95		B	C
	RSC37	5.11	0.17	4.82	4.46	5.75			C
TW (kg/hL)	RSC17	61.12	9.22	5.42	27.43	94.82	A	B	
	RSC135	59.73	9.04	5.01	25.49	93.96	A		
	RSC114	58.39	9.05	5.03	24.16	92.63	A	B	
	RSC37	56.79	9.05	5.03	22.58	90.99		B	
YD (Mt ha^{-1})	RSC37	3.08	0.29	7.13	2.12	4.03	A		
	RSC17	2.83	0.31	8.69	1.87	3.78	A	B	
	RSC114	2.76	0.29	7.31	1.80	3.72	A	B	
	RSC135	2.36	0.28	6.08	1.39	3.32		B	

[†]SE = Standard Error
[‡]df = degrees of freedom
[§]lower.CL = lower Confidence Level
[¶]upper.CL = upper Confidence Level
[#]Group = Statistical rankings using connecting letters report; letters sharing the same character are not significantly different from one another

The performance of testcross hybrids based on percent tropical genome were similar to that seen in inbreds for the traits DF, PH, EX, and FL. For these traits the category of "Medium" tropical genome recovery produced optimum results (Table 8). Testcross hybrid results differed from the inbred performance for yield related traits, indicating that dominant action is of importance for those traits (Table 8). A tropical genome recovery level of "Medium" was

observed for Avg.PL and TW, while a “High” level of tropical genome recovery was observed for both Avg.PW and YD in hybrids (Table 8).

Upon closer examination of the tropical genome level from Table 8 with its corresponding population, differences between the levels within their respective population are observed. However, to make inferences about which population performed agronomically better for its desired trait in Table 7, focus will only be made on that populations associated level of tropical genome recovery from Table 8. Population RSC17 “High” was the earliest for days to flowering, yet not statistically different from a tropical genome recovery level of “Medium” (Table 8). This same level of tropical genome recovery was observed for the inbreds as well. While population RSC17 remained the population with the shortest plant height in both inbreds and hybrids, the level associated with that population changed from “Low” in the inbreds to “Medium” being the shortest plant height (PH, cm) (Table 8). Population RSC114, the population with the lowest exsertion exhibited in the hybrids, performed better with a level of “Medium” as opposed to that of “High” in inbreds, however not statistically different from the two remaining levels (Table 8). Population RSC135 maintained the same level of “High” tropical genome recovery for FL and Avg.PL while maintaining a tropical genome recovery level of “Low” for Avg.PW (Table 8). Test weight (TW, kg/hL) for population RSC17 in hybrid combination was greatest for a “Medium” level of tropical genome recovery as opposed to a “Low” level of tropical genome recovery when in the inbred trial. An evaluation of grain yield (Mt ha^{-1}) in Table 8 revealed population RSC37 contains the highest grain yield estimate when a tropical genome level of “High” is present as opposed to its “Low” level of tropical genome recovery when evaluated as an inbred. Results from Tables 4 and 8 highlighting the differences between the level of tropical genome recovery and its contribution to a desired agronomic traits

performance may indicate that selection of the "Lowest" amount of tropical genome recovery in inbreds may not result in the greatest grain yield and grain yield related traits in hybrid performance.

Table 8. Each RSC hybrid population with their respective level of percent tropical genome recovery ranked by their desirable agronomic performance for each trait.

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				DF (d)					
	High	65.28	4.86	1.00	-116.55	247.11	A		
RSC17	Medium	65.88	4.86	1.01	-112.03	243.78	A		
	Low	--	--	--	--	--	--		
	Medium	65.65	3.85	3.00	47.10	84.20	A		
RSC37	Low	66.60	3.85	3.01	48.09	85.10		B	
	High	67.38	3.85	3.01	48.87	85.88			C
	Medium	69.18	4.50	2.00	35.22	103.14	A		
RSC114	High	70.75	4.49	2.00	36.73	104.77		B	
	Low	71.88	4.50	2.01	38.03	105.73			C
	Medium	67.74	5.07	3.00	43.27	92.22	A		
RSC135	Low	67.80	5.07	3.01	43.35	92.25	A		
	High	69.84	5.07	3.00	45.37	94.30		B	
				PH (cm)					
	Medium	116.43	9.32	2.14	52.12	180.74	A		
RSC17	High	155.67	9.17	2.01	86.76	224.57		B	
	Low	--	--	--	--	--	--		
	Low	128.45	8.73	3.42	90.42	166.49	A		
RSC37	Medium	175.20	8.48	3.05	134.83	215.57		B	
	High	195.70	8.74	3.42	157.64	233.76			C
	Medium	177.11	16.45	3.02	98.23	256.00	A		
RSC114	High	186.45	16.44	3.02	107.47	265.44		B	
	Low	189.58	16.50	3.06	111.21	267.94		B	

Table 8. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				PH (cm)					
	High	118.28	9.87	4.35	81.11	155.44	A		
RSC135	Medium	163.14	9.75	4.12	125.39	200.89		B	
	Low	173.80	10.03	4.62	137.19	210.40			C
				EX (cm)					
	High	17.61	3.42	2.02	-7.95	43.17	A		
RSC17	Medium	20.93	3.52	2.25	-1.87	43.74		B	
	Low	--	--	--	--	--	--		
	High	17.85	1.72	4.08	11.13	24.57	A		
RSC37	Medium	18.40	1.61	3.11	10.88	25.92	A		
	Low	18.82	1.72	4.08	12.11	25.52	A		
	Medium	14.00	1.60	3.47	7.09	20.91	A		
RSC114	Low	15.13	1.70	4.36	8.75	21.51	A		
	High	15.15	1.59	3.47	8.28	22.02	A		
	High	11.62	1.15	6.65	7.98	15.27	A		
RSC135	Low	13.24	1.25	9.16	9.60	16.88	A	B	
	Medium	14.66	1.06	4.80	10.86	18.46		B	
				FL (cm)					
	Medium	74.68	8.48	2.22	18.67	130.68	A		
RSC17	High	119.37	8.28	2.02	57.49	181.26		B	
	Low	--	--	--	--	--	--		
	Low	83.35	6.70	3.84	56.28	110.41	A		
RSC37	Medium	134.72	6.34	3.09	104.88	164.56		B	
	High	155.86	6.71	3.84	128.77	182.96			C
	Medium	142.42	14.26	3.03	74.17	210.67	A		
RSC114	High	149.38	14.24	3.02	81.04	217.72		B	
	Low	152.22	14.31	3.08	84.55	219.89		B	
	High	76.20	7.94	4.73	47.54	104.86	A		
RSC135	Medium	121.16	7.73	4.25	91.70	150.62		B	
	Low	136.10	8.18	5.31	108.04	164.16			C
				Avg.PL (cm)					

Table 8. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]		
				Avg.PL (cm)					
	Medium	21.96	1.00	1.30	4.56	39.37	A		
RSC17	High	20.43	0.94	1.03	-11.94	52.79		B	
	Low	--	--	--	--	--	--		
	Low	24.97	1.16	2.30	17.63	32.30	A		
RSC37	Medium	22.47	1.13	2.03	14.12	30.82		B	
	High	21.12	1.17	2.32	13.84	28.41			C
	Medium	21.14	1.13	2.08	13.02	29.25	A		
RSC114	Low	21.04	1.15	2.20	13.39	28.68	A		
	High	20.58	1.13	2.07	12.42	28.74	A		
	High	26.62	0.87	4.40	23.38	29.86	A		
RSC135	Medium	24.18	0.85	4.14	20.89	27.48		B	
	Low	21.59	0.88	4.72	18.40	24.77			C
				Avg.PW (cm)					
	High	5.21	0.24	1.02	-3.25	13.66	A		
RSC17	Medium	4.90	0.25	1.21	-0.36	10.17		B	
	Low	--	--	--	--	--	--		
	High	5.12	0.16	2.68	4.24	6.00	A		
RSC37	Medium	5.04	0.15	2.07	3.93	6.15	A		
	Low	4.86	0.16	2.68	3.98	5.73		B	
	Medium	5.47	0.28	2.08	3.43	7.51	A		
RSC114	High	5.30	0.28	2.09	3.27	7.32	A	B	
	Low	5.23	0.29	2.21	3.31	7.14		B	
	Low	5.81	0.18	4.88	5.17	6.45	A		
RSC135	Medium	5.68	0.17	4.18	5.01	6.35	A	B	
	High	5.60	0.18	4.48	4.94	6.25		B	
				TW (kg/hL)					
	Medium	69.86	4.37	1.04	-75.92	215.64	A		
RSC17	High	68.79	4.33	1.00	-92.10	229.67	A		
	Low	--	--	--	--	--	--		

Table 8. Continued

Population	Level[†]	LSmean	SE[‡]	df[§]	lower.CL[¶]	upper.CL[#]	Group[††]	
				TW (kg/hL)				
	High	67.73	3.98	2.18	40.85	94.61	A	
RSC37	Low	65.24	3.98	2.19	38.51	91.96		B
	Medium	64.17	3.96	2.13	36.55	91.79		B
	Low	68.26	2.64	3.15	56.03	80.50	A	
RSC114	High	67.21	2.61	3.05	54.79	79.63	A	B
	Medium	66.01	2.63	3.12	53.70	78.32		B
	Medium	59.80	11.05	4.00	16.29	103.30	A	
RSC135	Low	59.54	11.05	4.01	16.05	103.04	A	B
	High	58.83	11.05	4.01	15.34	102.33		B
				YD (Mt ha^{-1})				
	High	2.59	0.41	2.03	-0.50	5.67	A	
RSC17	Medium	2.39	0.43	2.32	-0.29	5.08	A	
	Low	--	--	--	--	--	--	
	High	3.46	0.49	3.64	1.43	5.49	A	
RSC37	Medium	3.44	0.46	3.07	1.24	5.64	A	
	Low	2.61	0.48	3.61	0.57	4.64		B
	Low	3.12	0.47	3.46	1.08	5.15	A	
RSC114	High	3.01	0.46	3.15	0.89	5.14	A	
	Medium	2.59	0.46	3.17	0.47	4.71		B
	Low	2.58	0.48	4.37	0.78	4.37	A	
RSC135	High	2.28	0.47	4.21	0.47	4.09	A	B
	Medium	2.21	0.47	4.08	0.38	4.04		B

[†]Level = Percent tropical genome recovery designation
[‡]SE = Standard Error
[§]df = degrees of freedom
[¶]lower.CL = lower Confidence Level
[#]upper.CL = upper Confidence Level
[††]Group = Statistical rankings using connecting letters report; letters sharing the same character are not significantly different from one another

To identify which populations resulted in superior hybrid combinations for each of the eight agronomic traits, three commonly used testers within the Texas A&M sorghum breeding program were evaluated in a line-by-tester analysis. Significant differences across the three testers for each of the populations were minimal (Table 9), as was observed in the LRT analysis (Table 6). An important observation to make is that for traits like Avg.PW, TW, and YD, no statistically significant differences within each of the four populations for the three different testers was observed (Table 9). Thus, an indication that specific combinations of an RSC population and one of these three testers will not have significant differences for grain yield.

Grain yield (Mt ha^{-1}) across the four populations revealed that tester ATx2928 produced the highest yielding hybrids (Table 9). While each breeding program contains their own set of desirable agronomic traits with which it has specific selection criteria, the overall goal in a hybrid sorghum breeding program is grain yield. By understanding this pivotal point and based on the overall performance of these three testers with each of the four populations, utilization of specific populations with the tester ATx2928 may prove to result in generally acceptable yields. However, one must ensure that the level of genotypic variability and repeatability will allow for the continued advancement of superior genotypes within a population.

Table 9. RSC hybrid population with their respective testers ranked by their desirable agronomic performance for each trait within population.

Population	Tester	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]
			DF (d)				
	A3Tx436	65.77	4.87	1.01	-109.00	240.54	A
RSC17	ATx645	66.21	4.87	1.01	-108.63	241.06	A
	ATx2928	--	--	--	--	--	--
	ATx2928	65.87	3.86	3.04	47.47	84.27	A
RSC37	ATx645	66.76	3.86	3.04	48.37	85.16	A
	A3Tx436	66.99	3.86	3.04	48.59	85.38	A

Table 9. Continued

Population	Tester	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]	
				DF (d)				
	ATx2928	69.95	4.50	2.01	36.13	103.78	A	
RSC114	ATx645	70.57	4.50	2.01	36.73	104.41	A	
	A3Tx436	71.29	4.50	2.01	37.45	105.12	A	
	ATx645	67.58	5.08	3.02	43.19	91.96	A	
RSC135	ATx2928	67.96	5.08	3.02	43.59	92.34	A	
	A3Tx436	69.84	5.08	3.02	45.45	94.22		B
				PH (cm)				
RSC17	A3Tx436	128.74	9.44	2.25	67.60	189.87	A	
	ATx645	135.92	9.43	2.24	74.60	197.25	A	
	ATx2928	--	--	--	--	--	--	
	A3Tx436	164.16	9.07	3.95	128.18	200.14	A	
RSC37	ATx2928	165.23	9.06	3.95	129.24	201.22	A	
	ATx645	169.96	9.07	3.96	134.00	205.93	A	
	A3Tx436	175.42	16.59	3.12	97.99	252.86	A	
RSC114	ATx2928	185.33	16.59	3.13	107.92	262.73	A	B
	ATx645	192.39	16.58	3.12	114.92	269.87		B
	ATx2928	146.81	10.13	4.79	110.48	183.14	A	
RSC135	A3Tx436	149.93	10.03	4.61	113.30	186.57	A	
	ATx645	158.48	10.01	4.58	121.78	195.17	A	
				EX (cm)				
	ATx645	21.38	3.63	2.53	0.80	41.96	A	
RSC17	A3Tx436	21.66	3.63	2.53	1.10	42.23	A	
	ATx2928	--	--	--	--	--	--	
	ATx2928	15.62	1.69	3.79	8.74	22.50	A	
RSC37	ATx645	18.69	1.69	3.81	11.82	25.55		B
	A3Tx436	20.76	1.69	3.81	13.88	27.63		B
	ATx2928	12.12	1.68	4.18	5.68	18.56	A	
RSC114	A3Tx436	15.72	1.67	4.10	9.23	22.21		B
	ATx645	16.44	1.66	4.01	9.91	22.97		B

Table 9. Continued

Population	Tester	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]	
				EX (cm)				
	ATx2928	11.72	1.20	7.58	8.06	15.37	A	
RSC135	ATx645	13.18	1.14	6.14	9.50	16.87	A	B
	A3Tx436	14.63	1.14	6.26	10.94	18.31		B
				FL (cm)				
	A3Tx436	87.43	8.43	2.17	30.19	144.67	A	
RSC17	ATx645	92.07	8.42	2.16	34.57	149.56	A	
	ATx2928	--	--	--	--	--	--	
	A3Tx436	119.91	6.99	4.47	93.99	145.83	A	
RSC37	ATx645	126.54	6.99	4.48	100.64	152.45	A	
	ATx2928	127.48	6.98	4.46	101.55	153.41	A	
	A3Tx436	137.73	14.31	3.07	70.07	205.39	A	
RSC114	ATx2928	152.20	14.31	3.08	84.58	219.82		B
	ATx645	154.08	14.30	3.07	86.37	221.79		B
	A3Tx436	108.15	8.12	5.13	79.89	136.41	A	
RSC135	ATx2928	109.33	8.27	5.50	81.36	137.30	A	
	ATx645	115.98	8.10	5.07	87.67	144.30	A	
				Avg.PL (cm)				
	ATx645	23.33	1.01	1.34	7.25	39.42	A	
RSC17	A3Tx436	22.46	1.01	1.36	6.63	38.28	A	
	ATx2928	--	--	--	--	--	--	
	ATx645	24.03	1.15	2.22	16.45	31.61	A	
RSC37	A3Tx436	22.81	1.15	2.21	15.18	30.43		B
	ATx2928	21.72	1.15	2.21	14.09	29.36		B
	ATx645	22.24	1.13	2.10	14.20	30.27	A	
RSC114	A3Tx436	21.22	1.14	2.11	13.24	29.20	A	
	ATx2928	19.30	1.14	2.12	11.37	27.23		B
	ATx645	24.85	0.87	4.55	21.63	28.06	A	
RSC135	A3Tx436	24.30	0.88	4.57	21.09	27.51	A	B
	ATx2928	23.24	0.89	4.79	20.06	26.42		B

Table 9. Continued

Population	Tester	LSmean	SE†	df‡	lower.CL§	upper.CL¶	Group#
			Avg.PW (cm)				
	ATx645	5.02	0.25	1.22	-0.10	10.14	A
RSC17	A3Tx436	4.87	0.25	1.23	-0.23	9.96	A
	ATx2928	--	--	--	--	--	--
	ATx2928	5.17	0.19	4.35	4.45	5.88	A
RSC37	ATx645	5.14	0.19	4.36	4.42	5.86	A
	A3Tx436	4.71	0.19	4.36	3.99	5.43	A
	ATx2928	5.45	0.30	2.45	3.71	7.18	A
RSC114	ATx645	5.43	0.29	2.41	3.67	7.19	A
	A3Tx436	5.11	0.29	2.43	3.37	6.86	A
	ATx645	5.87	0.19	5.71	5.24	6.50	A
RSC135	ATx2928	5.73	0.19	6.04	5.10	6.35	A
	A3Tx436	5.49	0.19	5.75	4.86	6.12	A
			TW (kg/hL)				
	ATx645	67.07	4.01	2.29	41.56	92.57	A
RSC37	A3Tx436	65.51	3.97	2.15	38.19	92.83	A
	ATx2928	64.56	3.97	2.14	37.10	92.03	A
	ATx645	68.25	2.89	4.25	57.23	79.27	A
RSC114	A3Tx436	66.88	2.71	3.46	55.15	78.61	A
	ATx2928	66.35	2.68	3.35	54.50	78.21	A
	A3Tx436	60.43	11.05	4.02	16.98	103.89	A
RSC135	ATx645	60.22	11.10	4.08	16.98	103.46	A
	ATx2928	57.52	11.06	4.02	14.07	100.97	A
			YD (Mt ha^{-1})				
	A3Tx436	3.48	0.71	6.08	1.15	5.81	A
RSC17	ATx645	0.93	0.71	6.08	-1.40	3.26	A
	ATx2928	--	--	--	--	--	--
	ATx2928	3.95	0.78	9.15	1.67	6.23	A
RSC37	A3Tx436	3.52	0.78	9.17	1.24	5.80	A
	ATx645	2.04	0.78	9.17	-0.24	4.32	A

Table 9. Continued

Population	Tester	LSmean	SE[†]	df[‡]	lower.CL[§]	upper.CL[¶]	Group[#]
			YD (Mt ha^{-1})				
	ATx2928	3.68	0.78	9.07	1.42	5.94	A
RSC114	A3Tx436	2.92	0.77	9.04	0.66	5.18	A
	ATx645	2.13	0.77	9.01	-0.14	4.39	A
	ATx2928	3.31	0.74	11.89	1.27	5.36	A
RSC135	A3Tx436	2.33	0.74	11.75	0.29	4.38	A
	ATx645	1.42	0.74	11.75	-0.63	3.46	A

[†]SE = Standard Error
[‡]df = degrees of freedom
[§]lower.CL = lower Confidence Level
[¶]upper.CL = upper Confidence Level
[#]Group = Statistical rankings using connecting letters report; letters sharing the same character are not significantly different from one another

Like the observations within the inbred trial, both the percent genotypic coefficient of variation (GCV) and the repeatability (R) on an entry mean basis for each of the agronomic traits within each population were evaluated (Table 10). Population RSC17 GCV values ranged from 22.26 (FL, cm) to 2.30 (DF, d) across the eight traits, indicating that DF (d) contains the lowest amount genotypic variation to select and FL (cm) containing the largest amount of selectable genotypic variation within the population. An interesting point to highlight is that both the inbred trial and hybrid trial contained the lowest amount of genotypic variation present for DF in population RSC17, implying that there is little variation for this trait in that population. The highest repeatability estimate for RSC17 was 0.97 (FL, cm) with the lowest estimate being 0.33 for grain yield (YD, Mt ha^{-1}), almost half its repeatable estimate from the inbred trial. Population RSC37 GCV values ranged from 0.01 (YD, Mt ha^{-1}) to 19.55 (FL, cm), two completely different traits with low and high amounts of genotypic variation compared to their inbred performance. Thus, coupled with its low GCV value and repeatability score of zero, selection of grain yield within this population is limited for the continual improvement of hybrid performance (Table

10). The highest repeatability estimates from Table 10 revealed that plant height (PH, cm) for this population contained the highest overall genotypic variation present, different from that of panicle exsertion in the inbred trial. Hybrid performance of population RSC114 remained the population with the least amount of variation across all traits, with values for GCV ranging only from 1.60 (TW, kg/hL) to 11.56 (EX, cm). However, repeatability estimates for this population ranged from 0.93 (FL, cm) to 0.12 (YD, Mt ha^{-1}). Population RSC135 contained the largest range in variation across the eight agronomic traits for GCV, ranging from 1.96 (TW, kg/hL) to 26.99 (FL, cm). Population RSC135 also contained the highest amount of repeatability estimates on an entry mean basis for all eight agronomic traits across the four populations, indicating that genotypic variation within this population was the highest and most repeatable favoring the exploitation of genotypic differences within.

Table 10. Genotypic coefficient of variation (GCV) and Repeatability (R) estimates on an entry mean basis for eight agronomic traits within their respective population for hybrids. Traits are ranked by their repeatability (R) estimate on an entry mean basis from high to low.

Population	Trait	GCV (%)	R
RSC17	FL (cm)	22.26	0.97
	PH (cm)	14.51	0.95
	Avg.PL (cm)	9.93	0.93
	DF (d)	2.30	0.92
	EX (cm)	18.72	0.86
	TW (kg/hL)	2.30	0.75
	Avg.PW (cm)	3.78	0.63
	YD (Mt ha^{-1})	17.45	0.33
RSC37	PH (cm)	13.85	0.95
	FL (cm	19.55	0.94
	Avg.PL (cm)	7.78	0.92
	TW (kg/hL)	2.86	0.90
	DF (d)	1.59	0.86
	EX (cm)	9.78	0.67
	Avg.PW (cm)	3.87	0.55

Table 10. Continued

Population	Trait	GCV (%)	R
RSC37	YD (Mt ha^{-1})	0.01	0.00
RSC114	FL (cm)	6.56	0.93
	PH (cm)	5.72	0.92
	Avg.PL (cm)	7.18	0.90
	DF (d)	1.68	0.81
	EX (cm)	11.56	0.70
	TW (cm)	1.60	0.64
	Avg.PW (cm)	3.27	0.54
	YD (Mt ha^{-1})	7.53	0.12
RSC135	PH (cm)	17.55	0.97
	FL (cm)	26.99	0.96
	Avg.PL (cm)	8.80	0.94
	DF (d)	2.18	0.93
	EX (cm)	16.36	0.80
	Avg.PW (cm)	3.71	0.73
	TW (kg/hL)	1.96	0.68
	YD (Mt ha^{-1})	16.55	0.44

2.4.3. Heterosis

Both Mid-Parent (MP) and High Parent (HP) heterosis of this material were examined. Bernardo (2002) defined MP heterosis as the superiority of the hybrid over the mean of both parents and HP heterosis as the superiority of the hybrid over the best parent in the cross. Breeding programs have specific desirable agronomic characteristics specialized for their growing regions, yet they all require the need for high grain yield.

For grain yield (YD, Mt ha^{-1}), hybrids outperformed the parental lines (Table 11 & 12). However, as observed in numerous studies, hybrid performance was not well correlated with inbred performance (Jordan et al., 2003). For example, the highest yielding male parent crossed with the highest yielding tester (Table 11), produced a hybrid with one of the lowest grain yields

(Table 12). However, the highest yielding hybrid was produced from a combination of "Medium" by the second highest yielding tester (Table 11; Table 12). Overall, the best tester in hybrid combination was ATx2928 (Table 12) but the tester with the highest yield per se was BTx645 (Table 11).

Table 11. LSMeans of eight agronomic traits for each RSC male line and maintainer line sorted by grain yield (YD, Mt ha^{-1}) from highest to lowest. Numbers in bold within each trait correspond to their respective high and low values. Overall mean is presented as the mean quantile of the LSMeans for each trait.

	Agronomic Traits							
Male Line[†]	**DF (d)**	**PH (cm)**	**EX (cm)**	**FL (cm)**	**Avg.PL (cm)**	**Avg.PW (cm)**	**TW (kg/hL)**	**YD (Mt ha^{-1})**
RSC17-3-F2-12-CS1-Low	62.94	94.54	18.78	53.36	21.80	4.57	67.48	**3.19**
RSC37-3-F2-6-CS3-Low	63.01	98.40	15.22	63.26	20.64	4.89	69.65	2.39
RSC37-3-F2-14-CS3-Medium	65.24	135.90	14.37	104.61	16.65	4.75	64.09	2.36
RSC17-3-F2-2-CS2-Medium	64.73	104.84	**23.85**	62.75	17.03	**4.22**	61.38	2.33
RSC17-3-F2-4-CS1-High	**60.76**	115.72	18.36	79.85	15.92	4.67	65.42	2.28
RSC17-3-F2-4-CS2-High	62.30	103.91	11.30	74.57	15.53	**5.50**	60.10	2.27
RSC135-3-F2-12-CS1-Low	70.65	**141.82**	14.21	102.51	19.07	5.24	65.49	2.19
RSC114-3-F2-6-CS2-Low	69.82	130.17	9.32	103.00	18.61	4.72	71.35	2.09
RSC37-3-F2-12-CS3-Medium	64.73	67.36	**2.99**	48.77	20.33	4.74	68.03	2.04
RSC17-3-F2-7-CS4-High	66.03	102.26	10.38	74.95	15.98	5.19	**53.91**	2.03
RSC37-3-F2-12-CS1-Medium	64.73	69.39	3.66	49.90	19.34	4.57	62.22	1.97
RSC37-3-F2-2-CS3-High	70.77	109.61	10.09	83.70	18.81	4.69	71.47	1.95
RSC114-3-F2-12-CS4-High	66.06	129.69	7.82	104.07	**13.61**	4.86	63.93	1.90
RSC114-3-F2-9-CS2-Medium	66.68	135.38	6.76	110.98	16.49	5.09	61.18	1.75
RSC135-3-F2-11-CS1-Medium	69.65	126.00	18.10	79.88	**23.93**	5.24	67.13	1.67
RSC135-3-F2-9-CS4-High	70.98	99.93	10.39	62.41	22.88	4.75	66.78	1.65
RSC37-3-F2-12-CS4-Medium	66.21	**55.38**	3.05	**40.35**	17.69	4.57	63.30	1.62
RSC114-3-F2-5-CS3-High	67.62	114.20	10.96	84.86	17.98	4.42	**72.42**	1.61
RSC135-3-F2-8-CS1-Medium	66.67	167.82	14.57	**132.62**	18.43	4.77	70.78	1.54
RSC135-3-F2-11-CS2-Medium	65.28	113.13	15.51	71.75	20.34	4.80	65.00	1.50
RSC114-3-F2-12-CS2-High	69.29	135.66	6.32	112.69	15.11	4.44	64.50	1.46
RSC114-3-F2-9-CS3-Medium	66.50	113.93	10.85	85.95	16.96	4.73	60.74	1.22
RSC135-3-F2-9-CS2-High	**73.84**	94.35	10.40	62.20	21.59	4.72	67.73	**1.12**
Overall mean[‡]	66.72	111.28	11.62	80.39	18.47	4.79	65.39	1.92
Maintainer Line								
BTx645	68.85	**116.81**	12.51	75.21	**28.26**	**5.40**	72.52	**3.29**

Table 11. Continued

	Agronomic Traits							
	DF (d)	**PH (cm)**	**EX (cm)**	**FL (cm)**	**Avg.PL (cm)**	**Avg.PW (cm)**	**TW (kg/hL)**	**YD (Mt ha^{-1})**
Maintainer Line								
BTx2928	**67.87**	**106.25**	**7.99**	**75.35**	23.54	5.33	**66.41**	2.35
BTx436	**71.17**	115.10	**15.51**	**74.29**	**22.85**	**4.75**	**73.71**	**1.87**
Overall mean[‡]	69.29	112.72	12.00	74.95	24.88	5.16	70.88	2.50

[†]RSC male line with its associated level of % tropical genome recovery.
[‡]Overall mean is the mean quantile of the LSmeans.

Table 12. LSMeans of each hybrid for eight agronomic traits sorted by grain yield (YD, Mt ha^{-1}) from high to low with their overall arithmetic mean for that trait. Numbers in bold within each trait correspond to the high and low value for that respective trait.

	Agronomic Traits							
Hybrid	**DF (d)**	**PH (cm)**	**EX (cm)**	**FL (cm)**	**Avg.PL (cm)**	**Avg.PW (cm)**	**TW (kg/hL)**	**YD (Mt ha^{-1})**
ATx2928/RSC37-3-F2-12-CS4-Medium	63.88	147.65	12.37	115.90	20.47	5.62	54.27	**4.72**
ATx2928/RSC114-3-F2-12-CS2-High	67.10	187.35	11.60	155.20	18.47	5.54	58.62	4.38
A3Tx436/RSC17-3-F2-12-CS1-Low	64.38	123.74	20.24	77.65	25.79	5.14	61.77	4.25
ATx2928/RSC37-3-F2-12-CS1-Medium	63.58	156.20	13.38	124.68	20.35	5.46	53.76	4.19
ATx2928/RSC114-3-F2-6-CS2-Low	66.22	186.29	10.90	155.26	19.23	5.53	58.62	4.10
ATx2928/RSC37-3-F2-2-CS3-High	65.71	184.16	13.08	152.44	19.24	5.42	59.12	4.04
ATx2928/RSC37-3-F2-14-CS3-Medium	64.61	186.20	19.06	150.61	21.27	5.31	**52.47**	4.04
ATx2928/RSC17-3-F2-7-CS4-High	64.96	155.63	8.87	129.52	19.55	5.84	54.78	4.00
ATx2928/RSC37-3-F2-12-CS3-Medium	63.88	160.11	13.99	127.51	21.35	5.13	57.20	3.99
A3Tx436/RSC17-3-F2-4-CS2-High	63.28	158.36	16.75	120.17	21.06	5.41	**64.62**	3.86
A3Tx436/RSC17-3-F2-4-CS1-High	62.97	153.68	20.01	113.44	21.47	5.09	63.41	3.79
ATx2928/RSC135-3-F2-12-CS1-Low	66.77	179.47	15.90	143.21	20.43	5.91	57.34	3.76
A3Tx436/RSC37-3-F2-12-CS1-Medium	66.45	153.35	17.94	116.91	21.51	4.94	56.62	3.68
A3Tx436/RSC37-3-F2-2-CS3-High	66.26	181.04	20.12	140.29	20.89	4.87	60.95	3.65
ATx2928/RSC17-3-F2-4-CS2-High	**61.28**	159.61	13.58	127.50	19.25	5.54	58.54	3.65
ATx2928/RSC17-3-F2-2-CS2-Medium	63.19	115.34	14.29	77.95	21.98	5.33	59.14	3.57
ATx2928/RSC17-3-F2-4-CS1-High	61.31	152.40	16.17	116.12	21.31	5.59	59.73	3.52
ATx2928/RSC135-3-F2-9-CS2-High	67.98	114.15	9.55	76.39	25.40	5.64	56.58	3.45
A3Tx436/RSC37-3-F2-12-CS3-Medium	65.37	150.52	16.20	112.93	23.47	4.94	56.20	3.43
ATx2928/RSC114-3-F2-5-CS3-High	65.83	169.52	**8.74**	138.90	19.08	5.22	61.22	3.39
A3Tx436/RSC17-3-F2-7-CS4-High	65.08	160.42	18.38	121.38	20.06	5.19	60.32	3.39
A3Tx436/RSC37-3-F2-14-CS3-Medium	65.04	185.66	17.71	147.61	20.95	4.85	55.64	3.36
A3Tx436/RSC17-3-F2-2-CS2-Medium	63.72	123.09	**23.51**	79.50	23.28	4.98	62.75	3.34
A3Tx436/RSC135-3-F2-11-CS2-Medium	67.29	158.32	16.10	116.40	23.60	5.73	59.31	3.29
A3Tx436/RSC37-3-F2-12-CS4-Medium	65.71	140.36	17.30	102.12	22.30	**4.83**	55.08	3.28

Table 12. Continued

Hybrid	Agronomic Traits							
	DF (d)	PH (cm)	EX (cm)	FL (cm)	Avg.PL (cm)	Avg.PW (cm)	TW (kg/hL)	YD (Mt ha^{-1})
ATx2928/RSC37-3-F2-6-CS3-Low	65.42	116.67	13.94	78.68	23.67	5.13	56.93	3.16
A3Tx436/RSC114-3-F2-12-CS2-High	68.21	178.71	17.36	137.66	21.84	5.30	57.42	3.14
A3Tx436/RSC114-3-F2-6-CS2-Low	69.06	170.25	15.06	131.66	21.65	5.12	59.21	3.14
A3Tx436/RSC37-3-F2-6-CS3-Low	66.42	121.65	19.75	77.75	24.14	4.85	56.55	3.10
ATx2928/RSC114-3-F2-9-CS3-Medium	64.13	166.85	13.51	132.90	20.44	5.74	56.52	3.07
A3Tx436/RSC114-3-F2-12-CS4-High	65.86	166.78	14.18	132.74	19.38	5.43	58.78	3.06
ATx2928/RSC135-3-F2-11-CS1-Medium	67.11	129.41	14.47	85.38	24.89	5.73	58.86	3.06
A3Tx436/RSC114-3-F2-9-CS2-Medium	65.39	169.90	12.87	137.89	20.14	5.22	57.10	3.03
ATx2928/RSC114-3-F2-9-CS2-Medium	65.39	175.02	11.08	143.50	**18.01**	5.87	53.79	2.93
ATx2928/RSC135-3-F2-11-CS2-Medium	64.72	171.23	11.05	136.38	22.32	5.74	58.44	2.87
A3Tx436/RSC135-3-F2-8-CS1-Medium	67.34	187.90	17.72	148.78	23.36	5.59	60.54	2.81
A3Tx436/RSC135-3-F2-9-CS4-High	70.41	129.36	16.97	83.10	27.17	5.54	59.47	2.70
A3Tx436/RSC114-3-F2-9-CS3-Medium	65.53	161.12	14.50	126.47	21.24	5.42	58.75	2.53
A3Tx436/RSC135-3-F2-11-CS1-Medium	68.87	147.94	18.37	100.40	25.75	5.52	60.05	2.49
ATx645/RSC37-3-F2-12-CS4-Medium	64.76	159.59	16.09	119.55	21.95	5.17	60.46	2.44
A3Tx436/RSC135-3-F2-12-CS1-Low	67.41	164.31	15.52	121.67	21.92	5.68	60.55	2.41
ATx645/RSC114-3-F2-12-CS2-High	67.09	196.68	16.19	**159.80**	22.19	5.86	59.96	2.41
ATx645/RSC37-3-F2-12-CS1-Medium	64.33	170.65	18.60	132.58	23.09	5.41	59.72	2.38
ATx645/RSC114-3-F2-6-CS2-Low	67.80	196.26	17.30	156.74	21.40	5.52	59.56	2.38
ATx645/RSC114-3-F2-9-CS2-Medium	65.13	181.23	13.20	148.96	21.48	5.76	57.93	2.20
ATx645/RSC37-3-F2-14-CS3-Medium	64.23	190.50	18.81	150.71	20.96	5.17	58.44	2.17
ATx645/RSC37-3-F2-2-CS3-High	67.36	190.47	14.94	154.04	21.99	5.57	60.29	2.16
ATx645/RSC37-3-F2-12-CS3-Medium	66.50	161.81	18.81	118.50	26.09	5.44	60.47	2.12
ATx645/RSC114-3-F2-5-CS3-High	66.62	180.01	16.07	142.30	22.42	5.21	61.29	1.98
ATx645/RSC114-3-F2-9-CS3-Medium	64.97	172.46	13.34	136.61	24.14	5.67	58.86	1.85

Table 12. Continued

Hybrid	Agronomic Traits							
	DF (d)	**PH (cm)**	**EX (cm)**	**FL (cm)**	**Avg.PL (cm)**	**Avg.PW (cm)**	**TW (kg/hL)**	**YD (Mt ha^{-1})**
ATx645/RSC114-3-F2-12-CS4-High	66.11	185.09	15.00	149.19	20.63	5.64	57.06	1.83
ATx645/RSC135-3-F2-12-CS1-Low	66.13	176.80	11.88	139.41	22.48	5.91	59.79	1.58
A3Tx436/RSC135-3-F2-9-CS2-High	**71.27**	117.17	12.46	75.15	26.24	5.00	59.58	1.50
ATx645/RSC135-3-F2-9-CS4-High	67.69	120.46	14.65	71.23	**28.49**	**6.13**	60.64	1.46
ATx645/RSC135-3-F2-8-CS1-Medium	66.44	**198.39**	16.18	155.35	23.54	5.79	60.69	1.46
ATx645/RSC135-3-F2-11-CS1-Medium	66.29	162.19	20.64	108.85	25.83	5.95	61.02	1.43
ATx645/RSC135-3-F2-9-CS2-High	67.75	116.93	14.55	**69.56**	27.35	5.76	60.95	1.40
ATx645/RSC17-3-F2-4-CS1-High	63.40	169.08	19.46	124.54	23.58	5.40	--	1.32
ATx645/RSC17-3-F2-7-CS4-High	66.09	172.40	13.57	139.88	20.59	5.48	--	1.31
ATx645/RSC17-3-F2-12-CS1-Low	63.92	123.61	21.23	76.85	26.93	5.14	--	1.27
ATx645/RSC135-3-F2-11-CS2-Medium	65.43	178.45	13.92	137.98	23.30	5.67	59.87	1.20
ATx645/RSC17-3-F2-4-CS2-High	63.95	177.13	18.64	134.63	22.57	5.48	--	1.18
ATx645/RSC37-3-F2-6-CS3-Low	65.49	**113.84**	18.11	70.88	25.68	5.01	58.77	1.08
ATx645/RSC17-3-F2-2-CS2-Medium	65.09	129.19	22.22	81.22	23.13	5.10	--	**1.06**
ATx2928/RSC114-3-F2-12-CS4-High	--	--	--	--	--	--	--	--
A3Tx436/RSC114-3-F2-5-CS3-High	--	--	--	--	--	--	--	--
ATx2928/RSC135-3-F2-8-CS1-Medium	--	--	--	--	--	--	--	--
ATx2928/RSC135-3-F2-9-CS4-High	--	--	--	--	--	--	--	--
ATx2928/RSC17-3-F2-12-CS1-Low	--	--	--	--	--	--	--	--
Overall mean[†]	65.73	160.00	15.75	121.39	22.40	5.42	58.75	2.79

[†]Overall mean is the mean quantile of the LSmeans.

While grain yield is the primary trait of selection for sorghum breeding programs, additional traits and their relationship with grain yield must be considered. Continual examination of the highest performing hybrid from Table 12 revealed additional pro's and con's associated with this hybrids high yielding capabilities, as seen in Table 13. In example, for MP heterosis, a reduction in flowering time (DF, d) occurred, while an increase in plant height (PH, cm), panicle exsertion (EX, cm), and flag-leaf (FL, cm) were observed with its combination of ATx2928. Despite this specific hybrid having the greatest HP heterosis for YD (Mt ha^{-1}), a reduction in both Avg.PL (cm) and TW (cm) were observed, while an increase in Avg.PW (cm) was obtained (Table 13). As observed from Table11, our highest yielding male parent and our highest yielding maintainer parent (BTx645) did not result in a specific hybrid combination with the highest HP heterosis; interestingly it was one of the worst combinations for HP heterosis in YD (Table 14). In addition, most of the RSC17 males performed poorly in specific combinations with tester ATx645, which was the overall worst performing tester in hybrid combinations for YD (Table 14). The same poor performance across the remaining seven agronomic traits for both MP and HP heterosis were observed with ATx645 (Table 14). Across all three testers, male lines within population RSC37 contained the highest HP heterosis for grain yield (YD, Mt ha^{-1}). Finally, Tx436, produced the greatest range in HP heterosis of grain yield (YD, Mt ha^{-1}) and had the greatest range in values for all other traits except for PH, EX, and FL (Table 15).

Table 13. Mid-parent (MP) and High-parent (HP) heterosis estimate for each male parent with ATx2928 in a line by tester analysis across each agronomic trait, ranked by YD (Mt ha^{-1}) from highest to lowest. Numbers in bold correspond to the range in variation across all males within each trait.

Male Parent	Mid-Parent				High-Parent			
	DF (d)	PH (cm)	EX (cm)	FL (cm)	Avg.PL (cm)	Avg.PW (cm)	TW (kg/hL)	YD (Mt ha^{-1})
RSC37-3-F2-12-CS4-Medium	-1.18	20.68	31.06	25.08	-13.02	5.33	-18.28	**100.66**
RSC114-3-F2-12-CS2-High	-0.54	13.72	15.56	16.27	-21.54	3.85	-11.74	86.47
RSC37-3-F2-12-CS1-Medium	-1.03	19.46	32.43	24.78	-13.54	2.38	-19.05	78.30
RSC114-3-F2-6-CS2-Low	-0.96	14.40	6.50	18.53	-18.30	3.80	-17.85	74.22
RSC37-3-F2-2-CS3-High	-1.30	17.66	11.18	22.92	-18.27	1.74	-17.28	71.93
RSC37-3-F2-14-CS3-Medium	-0.73	13.45	17.62	16.85	-9.64	-0.44	**-20.99**	71.18
RSC17-3-F2-7-CS4-High	-0.74	12.32	**-0.84**	18.09	-16.92	9.63	-17.52	70.28
RSC37-3-F2-12-CS3-Medium	-0.91	**21.11**	**38.71**	**26.37**	-9.28	**-3.80**	-15.92	69.52
RSC135-3-F2-12-CS1-Low	-0.90	11.17	10.82	15.26	-13.20	**10.94**	-13.66	59.92
RSC17-3-F2-4-CS2-High	**-1.46**	12.97	10.19	17.52	-18.21	0.78	-11.86	55.20
RSC17-3-F2-2-CS2-Medium	-1.17	**2.32**	-2.56	3.22	-6.60	-0.06	-10.94	51.78
RSC17-3-F2-4-CS1-High	-1.17	9.33	5.69	12.41	-9.46	4.85	**-10.06**	49.81
RSC135-3-F2-9-CS2-High	-1.01	3.45	0.98	2.77	**7.93**	5.74	-16.47	46.77
RSC114-3-F2-5-CS3-High	-0.71	13.45	-1.95	18.35	-18.94	-2.14	-15.48	44.34
RSC37-3-F2-6-CS3-Low	**-0.01**	3.50	5.04	3.38	0.58	-3.77	-18.26	32.24
RSC114-3-F2-9-CS3-Medium	-1.14	12.89	10.86	16.20	-13.15	7.74	-14.90	30.75
RSC135-3-F2-11-CS1-Medium	-0.60	2.86	2.74	**2.50**	4.03	7.46	-12.32	30.03
RSC114-3-F2-9-CS2-Medium	-0.70	11.22	12.56	13.51	**-23.47**	10.14	-19.01	24.67
RSC135-3-F2-11-CS2-Medium	-0.70	14.03	-1.48	21.36	-5.17	7.62	-12.00	**22.14**
RSC114-3-F2-12-CS4-High	--	--	--	--	--	--	--	--
RSC135-3-F2-8-CS1-Medium	--	--	--	--	--	--	--	--
RSC135-3-F2-9-CS4-High	--	--	--	--	--	--	--	--
RSC17-3-F2-12-CS1-Low	--	--	--	--	--	--	--	--

Table 14. Mid-parent (MP) and High-parent (HP) heterosis estimate for each male parent with ATx645 in a line by tester analysis across each agronomic trait, ranked by YD (Mt ha^{-1}) from highest to lowest. Numbers in bold correspond to the range in variation across all males within each trait.

Male Parent	Mid-Parent				High-Parent			
	DF (d)	PH (cm)	EX (cm)	FL (cm)	Avg.PL (cm)	Avg.PW (cm)	TW (kg/hL)	YD (Mt ha^{-1})
RSC37-3-F2-12-CS4-Medium	-1.03	**21.34**	26.72	26.73	-22.32	-4.27	-16.63	**-25.75**
RSC114-3-F2-12-CS2-High	-0.72	13.95	18.00	17.52	-21.48	8.64	-17.31	-26.68
RSC37-3-F2-12-CS1-Medium	-0.92	20.82	32.52	**27.99**	-18.29	0.26	-17.65	-27.51
RSC114-3-F2-6-CS2-Low	-0.55	14.73	14.65	18.98	-24.29	2.18	-17.87	-27.66
RSC114-3-F2-9-CS2-Medium	-0.97	10.93	9.25	15.00	-23.99	6.71	-20.12	-33.15
RSC37-3-F2-14-CS3-Medium	-1.05	12.69	9.99	16.91	-25.83	-4.17	-19.41	-33.91
RSC37-3-F2-2-CS3-High	-0.88	17.06	8.07	23.47	-22.20	3.24	-16.86	-34.32
RSC37-3-F2-12-CS3-Medium	**-0.11**	18.93	**35.69**	22.79	-7.70	0.72	-16.61	-35.44
RSC114-3-F2-5-CS3-High	-0.59	13.96	9.23	19.45	-20.68	-3.49	**-15.48**	-39.79
RSC114-3-F2-9-CS3-Medium	-1.00	12.37	3.57	17.38	-14.58	5.08	-18.83	-43.80
RSC114-3-F2-12-CS4-High	-0.50	12.54	11.91	16.61	-27.01	4.43	**-21.32**	-44.24
RSC135-3-F2-12-CS1-Low	**-1.30**	9.18	-2.77	14.22	-20.47	9.57	-17.55	-51.98
RSC135-3-F2-9-CS4-High	-0.80	2.79	6.98	0.88	**0.80**	**13.55**	-16.38	-55.44
RSC135-3-F2-8-CS1-Medium	-0.49	9.85	4.88	12.37	-16.70	7.35	-16.30	-55.46
RSC135-3-F2-11-CS1-Medium	-1.07	8.40	8.72	10.09	-8.59	10.33	-15.86	-56.58
RSC135-3-F2-9-CS2-High	-1.26	2.69	6.76	**0.31**	-3.24	6.70	-15.95	-57.55
RSC17-3-F2-4-CS1-High	-0.54	11.36	6.53	15.16	-16.58	0.02	--	-59.82
RSC17-3-F2-7-CS4-High	-0.50	14.35	4.64	21.58	**-27.13**	1.50	--	-60.15
RSC17-3-F2-12-CS1-Low	-0.75	4.24	8.92	4.89	-4.69	-4.70	--	-61.28
RSC135-3-F2-11-CS2-Medium	-0.61	13.80	**-0.15**	21.95	-17.55	5.02	-17.45	-63.48
RSC17-3-F2-4-CS2-High	-0.62	15.13	14.14	19.95	-20.14	-0.45	--	-64.04
RSC37-3-F2-6-CS3-Low	-0.16	**1.45**	7.66	0.60	-9.12	**-7.15**	-18.96	-67.14
RSC17-3-F2-2-CS2-Medium	-0.64	4.14	5.56	4.44	-18.16	-5.45	--	**-67.68**

Table 15. Mid-parent (MP) and High-parent (HP) heterosis estimates for each male parent with A3Tx436 in a line by tester analysis across each agronomic trait, ranked by YD (Mt ha-1) from highest to lowest. Numbers in bold correspond to the range in variation across all males within each trait.

Male Parent	Mid-Parent				High-Parent			
	DF (d)	PH (cm)	EX (cm)	FL (cm)	Avg.PL (cm)	Avg.PW (cm)	TW (kg/hL)	YD (Mt ha^{-1})
RSC37-3-F2-2-CS3-High	**-1.66**	15.28	14.30	19.40	-8.56	2.60	-17.32	**87.20**
RSC37-3-F2-12-CS1-Medium	-0.55	**16.56**	**21.80**	**22.07**	-5.88	3.99	-23.19	86.84
RSC135-3-F2-11-CS2-Medium	-0.34	9.68	0.95	14.85	3.28	**19.23**	-19.54	75.60
RSC37-3-F2-12-CS4-Medium	-1.08	16.17	21.62	19.54	-2.39	1.74	**-25.28**	75.02
RSC17-3-F2-4-CS2-High	-1.29	11.15	6.24	15.36	-7.84	**-1.65**	**-12.34**	70.19
RSC37-3-F2-12-CS3-Medium	-0.95	16.25	18.79	20.88	2.71	4.03	-23.76	67.89
RSC114-3-F2-12-CS2-High	-0.72	10.63	14.77	11.81	-4.44	11.64	-22.10	67.56
RSC17-3-F2-7-CS4-High	-1.28	11.90	10.52	15.66	-12.21	0.00	-18.17	66.57
RSC17-3-F2-4-CS1-High	-1.13	8.29	4.55	11.80	-6.03	7.19	-13.97	66.10
RSC114-3-F2-9-CS2-Medium	-1.28	8.92	3.91	12.21	-11.86	2.47	-22.53	61.72
RSC114-3-F2-12-CS4-High	-1.00	9.07	5.39	12.21	**-15.19**	11.74	-20.26	61.40
RSC135-3-F2-8-CS1-Medium	-0.57	8.21	4.46	10.95	2.25	17.39	-17.87	50.10
RSC114-3-F2-6-CS2-Low	-0.51	9.71	5.34	12.13	-5.24	7.76	-19.67	49.84
RSC135-3-F2-9-CS4-High	**-0.23**	5.08	7.75	5.39	**18.75**	16.52	-19.32	43.99
RSC17-3-F2-2-CS2-Medium	-1.56	2.98	4.87	4.01	1.89	4.95	-14.87	43.50
RSC37-3-F2-14-CS3-Medium	-1.16	11.98	4.64	16.25	-8.31	2.02	-24.52	42.65
RSC114-3-F2-9-CS3-Medium	-1.20	10.18	2.51	14.46	-7.03	14.20	-20.29	34.90
RSC17-3-F2-12-CS1-Low	-0.99	4.51	4.52	5.42	12.87	8.16	-16.21	33.43
RSC135-3-F2-11-CS1-Medium	-0.55	5.68	2.33	7.56	7.62	5.53	-18.53	33.22
RSC37-3-F2-6-CS3-Low	-0.25	3.49	7.14	3.26	5.63	-0.89	-23.29	29.61
RSC135-3-F2-12-CS1-Low	-1.23	6.98	1.11	9.41	-4.05	8.41	-17.85	9.95
RSC135-3-F2-9-CS2-High	-0.43	**2.97**	**-0.95**	**2.53**	14.84	5.35	-19.18	**-19.90**
RSC114-3-F2-5-CS3-High	--	--	--	--	--	--	--	--

2.5. Conclusions

Incorporation of RSC germplasm into a sorghum breeding program is a notable strategy for breeding programs that are trying to increase diversity within their program while maintaining high agronomic mean. However, based on the results presented herein, the relative level of tropical germplasm in the RSC lines and populations (as measured by SNP assays) has no predictive value related to the performance of hybrids created from those lines. For example, a "Low" level of tropical genome recovery yielded the best overall in inbred performance across all four populations, however, in hybrid evaluation a level of "High" contained an overall greater grain yield (YD, Mt ha^{-1}). Regarding performance of the testers, ATx2928 showed higher HP heterosis compared to that of the remaining testers in question. Based on pedigree information of the male lines with BTx406 as their donor parent, such results with the three testers were unexpected. ATx2928 has a closer genotypic relationship with BTx406 as opposed to the remaining two testers (William L. Rooney, personal communication); yet maintained the highest HP heterosis for YD (Mt ha^{-1}), which contradicts the norm of how to obtain maximum heterosis. Similar results were reported by Menz et al (2004) where the utilization of AFLP and SSR markers revealed that R- and B-lines are not good representations for heterotic pools in sorghum. This indicates that further investigation is needed on how to most appropriately combine parents for the maximization of heterosis. Thus, our study hypothesizes that breeding programs will need to utilize early generation tropical material with testers optimal for their program to achieve their desired agronomic performance.

With the primary goal of the RSC program aimed at recovering high amounts of tropical genome in early flowering, short plants, its emphasis was paralleled to that of the SC program for "conversion," rather than agronomic performance. In doing so, selection for lines having high

amounts of tropical genome were considered of high breeding value and thus, resulted in the amount of variation present between the "levels" presented herein to be minimal within each population. While both the SC and the RSC programs were focused on conversion of tropical material, their utilization of markers for obtaining the highest amount of tropical recovery appeared to be an effective method. However, to accurately address whether differing levels of tropical genome were a good predictor of agronomic performance, one must assess not only those lines with the highest tropical recovery, but also examine those lines where the lowest amount of tropical recovery are observed to tease out true differences of "level". To appropriately utilize the material presented herein, the evaluation of line performance per se should be conducted, followed by hybrid testing evaluations with various testers from that program. Nonetheless, when using this germplasm in a breeding program the interactions of population, level, their interaction, and the tester in hybrid combinations need to be evaluated with caution when making claims about predictive performance. Based on the several agronomic traits assessed herein, an index selection methodology may better serve to aid in the discovery of hybrids with agronomically desirable traits.

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3. REGISTRATION OF 11 DIVERSE SORGHUM GERMPLASM LINES FOR GRAIN AND SILAGE HYBRID PRODUCTION

3.1. Synopsis

Genotypic variance is necessary for trait improvement as limited diversity can reduce genetic gain in crop improvement. To maintain genetic diversity, a wealth of germplasm exists in the USDA-ARS sorghum [*Sorghum bicolor* (L.) Moench] collection, but most of the accessions are not adapted to temperate climates. To address this issue, the Reinstated Sorghum Conversion program (RSC, circa 2009-2014) converted 155 tropical accessions to combine height, early maturing F_3 and BC_1F_3 families. Herein the identification of 11 germplasm lines (Reg. No. GS-###, Tx3429 to Reg. No. GS-###, Tx3439) released by Texas A&M AgriLife Research in 2019 and derived from the RSC program are described. This germplasm was derived from F_3, BC_1F_3, and BC_1F_4 RSC families that was selected based on testcross hybrid performance for either grain or silage production. Six lines are grain sorghum pollinators, one line is a seed parent, and four lines are silage pollinators. These lines combined agronomic productivity with greater genetic diversity as confirmed via genotyping-by-sequencing. These eleven parental germplasms are being released to provide new genetic diversity for forage and grain hybrid improvement programs.

3.2. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] originated in the northeast quadrant of Africa where the greatest amount of variability is contained for both cultivated and wild types (Doggett, 1970; Miller and Kebede, 1984). Since domestication nearly 6000 years ago, sorghum has been cultivated across numerous continents with differing climates and geographies (Klein et al., 2016). As it moved to the United States by means of the slave trade from West Africa (Kimber, 2001; Klein et al., 2016), only a small fraction of the world's sorghum germplasm collection was grown due to photoperiod sensitivity restrictions that limited flowering and seed production in long-day environments (Rosenow and Dahlberg, 2000; Duncan et al., 1991).

While increasing the genetic diversity in elite sorghum germplasm is important, diversity associated with reduced performance is not acceptable. This fact has limited the use of a vast majority of the tropical accessions in the USDA-ARS sorghum germplasm collection (Stephens et al., 1967; Gerloff and Smith, 1988). As such, these diverse sources must be adapted and tested, typically by public sector sorghum programs, to introduce converted or partially-converted sorghum lines that can directly contribute to prebreeding programs of commercial grain hybrids (Jordan et al., 2011).

To mitigate these issues, both the Sorghum Conversion program and the Reinstated Sorghum Conversion (RSC) program converted unadapted sorghum accessions from the USDA-ARS germplasm collection (Stephens et al., 1967; Klein et al., 2013). In the RSC, conversion focused on elite yet unadapted sorghum lines and led to the release of 155 new conversion lines (Klein et al., 2013, 2016). Most of the converted lines from the RSC originated from Sudan and Ethiopia and are predominately of race Durra or Caudatum (Klein et al., 2013, 2016). By utilizing early-generation genotyping-by-sequencing (GBS), partially converted germplasm

could be identified with a greater frequency of the tropical parental genome in the progeny to thereby accelerate the conversion process permitting their release in earlier generations (Klein et al., 2013, 2016).

From the RSC program, eleven parental germplasm lines (Reg. No. GP-###, TX3429 to Reg. No. GP-###, TX3439) were released by Texas A&M AgriLife Research in 2019 based on agronomic desirability, unique combinations of traits including biotic stress tolerance (anthracnose, sugarcane aphids), and yield in hybrid combinations. The germplasm described herein represents further refinement of this material to identify lines that demonstrate acceptable agronomic characteristics and high heterosis in hybrid combination. Further, they maintain unique genetic diversity relative to existing seed and pollinator breeding groups.

3.3. Materials and Methods

3.3.1. Breeding and Selection Method

The RSC lines utilized in the present study were bred from intentional crosses using the pedigree method of plant breeding as detailed previously (Klein et al., 2013, 2016). A total of 375 RSC (F_3, BC_1F_3, and BC_1F_4) lines derived from 70 unique tropical accessions were evaluated in 5.48 meter two-row plots in College Station, Texas (TX) during the summer of 2016 using standard agronomic practices. The 70 tropical accessions from which the RSC partially converted lines were derived represented a subset of the unadapted accessions converted by the RSC program; this subset was selected based on prior knowledge of RSC material related to their agronomic fitness and desirability by the principal investigators of the RSC program (F.R. Miller, R.R. Klein, unpublished observations). These lines were evaluated for standard phenotypic descriptors including (but not limited to) plant and seed color, plant height, foliar health, awns, seed size, tillering, stalk stiffness, and plot uniformity. Based on these and other

observations, a total of 30 $F_{3:4}$, 10 $BC_1F_{3:4}$, and 26 $BC_1F_{4:5}$ lines were advanced for further testing.

3.3.2. Experimental Design

The 66 lines (from the F3:4, BC1F3:4, and BC1F4:5 generations) were planted in 5.48 meter single row plots in College Station, TX in 2017 and top-crossed to two female testers, ATxARG-1 (Miller et al., 1992b) and ATx2928 (Rooney, 2003a). Lines were evaluated for standard agronomic traits such as flowering date, plant height, and phenotypic uniformity.

Testcross hybrids were planted in three locations in 2017 and 2018. In August 2017, a testcross hybrid observation test was planted to assess sugarcane aphid tolerance (SCAT) along with plant height and uniformity. Hybrids that had phenotypic uniformity and plant heights between 102 and 140 cm were classified as grain types. Uniform hybrids taller than 140 cm with desirable forage phenotypes were classified as silage types.

Grain-type experimental lines were planted in 2018 for agronomic evaluation in four locations (College Station, TX; College Station-Anthracnose nursery, TX; Lubbock, TX; and Weslaco, TX). Included with these lines were three restorer (R) and three maintainer (B) lines (BTx406, BTx2928 (Rooney, 2003a), BTxArg-1 (Miller et al., 1992b), RTx436 (Miller et al., 1992c), RTx437 (Rooney et al., 2003), and RTx2783 (Peterson et al., 1984)). Hybrids from the grain-type experimental lines were planted in an RCBD with three replications in College Station, TX in 2018. Two public check hybrids (ATxArg-1/RTx437 and ATx2928/ RTx437) were included for comparison.

Forage-type experimental lines and their hybrids were planted in an RCBD in College Station, TX during the summer of 2018. Two medium to medium-early commercial silage hybrids were included within the study for comparison (Super Sile 30 (DynaGro Seed), NK300

(Sorghum Partners), and one grain type commercial hybrid (NK5418 (Sorghum Partners)). Standard agronomic practices for sorghum were implemented in both grain and silage trials in all environments.

3.3.3. Phenotypic Evaluation

For all trials, days-to-anthesis (d) was recorded as the date at which 50 percent of the plot was at 50 percent mid-anthesis. Plant height (cm) was measured from the ground to the tip of the panicle at the end of the growing season as a representative mean for each plot. Head length (cm) and panicle exsertion (cm) were measured as a representative mean of the plot. Parental lines were assessed for their response to anthracnose (*Collectotrichum sublineolum* P. Henn., Kabát and Bubák), with fungal inoculations being administered 45 days after planting. Anthracnose disease ratings for the parental grain ideotypes across 11 different dates throughout the growing season were scored on a scale of one to nine; one being little disease and nine being severe disease (Burrell et al., 2015). Six classes of ratings were constructed by fitting an area under the disease progress curve (AUDPC) representing a quantitative summary of disease intensity over time relative to known resistant and susceptible checks. Observational data on hybrids for $SCA_{\mathbf{T}}$ in Weslaco, TX 2017 was scored using a one to nine scale, with one being tolerant and nine being susceptible, a protocol similar to the one implemented by Mbulwe et al. (2016). For grain yield determinations, grain hybrids were combine-harvested and adjusted to 14% moisture, with yield values recorded as Metric tonnes per hectare (Mt ha^{-1}). Forage yield was machine harvested, adjusted to 65% moisture and recorded as fresh weight yield in Metric tonnes per hectare (Mt ha^{-1}). Quality analysis of parental and hybrid silage types were conducted using Near-Infrared Spectroscopy (NIR) as dry weight percent for Crude Protein (%CP), Acid

Detergent Fiber (%ADF), Neutral Detergent Fiber (%NDF), and Total Digestible Nutrients (%TDN) using standard NIR calibration curves for forage quality.

3.3.4. Statistical Analysis

Statistical comparisons were analyzed using JMP 14 (SAS Institute, 2014). Tukey's honest significant difference (HSD) test was used for means separation of agronomic traits, alpha set at $p \leq 0.05$.

3.3.5. Genotypic Analysis

To assess the genotypic diversity of the released lines, genotyping-by-sequencing (GBS) was conducted on Tx3429 to Tx3439 and 25 open-pedigree inbreds. The 12 restorer (R) inbreds were RTx2536 (Duncan et al., 1991), RTx2737 (Johnson et al., 1982), RTx2783 (Peterson et al., 1984), RTx2903 (Miller and Prihoda, 1996), RTx2907 (Miller et al., 1996), RTx2917 (Rooney, 2003b) , RTx430 (Miller, 1984), RTx433 (Miller and Rosenow, 1984a), RTx434 (Miller and Rosenow, 1984b), RTx435 (Miller, 1986a), RTx436 (Miller et al., 1992c), and RTx437 (Rooney et al., 2003). The 13 maintainer (B) inbreds included BTx2752 (Johnson et al., 1982), BTx2928 (Rooney, 2003a), BTx3197 (Stephens and Karper, 1965), BTx378, BTx399, BTx406, BTx623, BTx626 (Miller, 1986b), BTx631 (Miller, 1986b), BTx635 (Miller et al., 1992a), BTx642 (Rosenow et al., 2002), BTx645 (Rosenow et al., 2002), and BTxARG-1 (Miller et al., 1992b).

This germplasm was genotyped using the high-throughput GBS method as described by Morishige et al. (2013). Genomic DNA, extracted using the Quick-DNA™ Plant/Seed Miniprep Kit (Zymo Research), was digested with a methylation-sensitive enzyme *Ngo*MIV, and Illumina template library was prepared as described by Burrell et al. (2015) with single-end sequencing performed on the Illumina HiSeq2500 (Texas A&M AgriLife Genomic and Bioinformatics Services). The 150 bp reads obtained by sequencing were sorted and trimmed to 125 bp using

custom perl and python scripts. The trimmed reads were uploaded to the CLC Genomics Workbench 11.0.1, mapped to the reference BTx623 genome (Sbicolor 3.1), and variants (SNPs) were detected as described by Patil et al. (2017). Markers were excluded based on any of the following criteria; physically close to one another (<10,000 bp), had >75% missing data across the genotypes, or contained SNPs that were unique to the reference genome (BTx623). Missing data were imputed using FastPhase (Scheet and Stephens, 2006) and only SNPs with a minor allele frequency of 5% or more were retained for further downstream analysis. Of the remaining SNPs, 88.14 % mapped to the reference genome (Sbicolor 3.1) resulting in a total of 12,342 genetic markers for evaluation of genotypic diversity between the RSC parental inbreds and elite public inbreds. The 12,342 SNPs were formatted as two alphabetized alleles and concatenated to create a sequence for each genotype. The sequences were imported in MEGA X (Kumar et al., 2018) to construct a Neighbor-Joining tree (Saitou and Nei, 1987) and to calculate pairwise genetic distances using the Tajima-Nei model (Tajima and Nei, 1984) with 1,000 bootstraps. Principal Coordinate Analysis (PCoA) was performed in RStudio (v1.1.463) using the prcomp and cmdscale functions (Gower, 1966).

3.4. Results and Discussion

From the analysis, a total of seven grain types were released as Tx3429 to Tx3435 and four silage types were released as Tx3436 to Tx3439 (Table 16). Two of these lines (Tx3429 and Tx3438) were derived from the USDA-ARS Sudanese collection; the remainder of the lines have an origin from the Ethiopian collection.

3.4.1. Parental Lines

Tx3429 is a maintainer (B line) of sterility in the A_1 cytoplasmic-genetic male-sterility (CMS) system, and the remaining 10 release lines are restorers of fertility (R lines) in the A_1

CMS system. Currently, a male-sterile version of Tx3429 is in development but not yet available. Ten of the 11 lines have a pigmented plant color while Tx3429 segregates for plant color (i.e., tan or purple plant types). While the 11 released parental inbreds differ for pericarp color (red, yellow, or white), none possessed a pigmented testa or produced a hybrid with a pigmented testa with the testers used in this study (Table 16). Seven of the lines are awnless and all lines contain pigmented glumes (Table 16). Phenotypic variation for panicle architecture ranged from compact, to semi-open for grain type lines Tx3429 to Tx3435; based on their intended use, panicle architecture was not recorded for silage types Tx3436 to Tx3439 (Table 16).

Grain types (Tx3429-Tx3435) are 3-dwarf lines (*dw*$_1$*Dw*$_2$*dw*$_3$*dw*$_4$) with similar agronomic characteristics to common elite checks with a few exceptions (Table 17). Tx3431 is shorter in plant height (cm) and Tx3430 exhibits greater panicle exsertion (cm) compared to Tx2783 (Table 17). Head length (cm) for the release lines and checks were not different (Table 17). Tx3429 was highly resistant to anthracnose while Tx3434 and Tx3435 were resistant (Table 17).

Table 16. Designation, experimental pedigree, idiotype, race, and phenotypic observations of Tx3429 to Tx3439 parental lines.

Designation	Experimental Pedigree	Idiotype	Race†	Working Group†	Plant Color‡	Grain Color§	Awns	Panicle Arch.¶
Tx3429	(BTx406/RSC67)-F2-9-1-CS1	Grain	C	WG 30: C	TP	W	No	SO
Tx3430	(BTx406/RSC107)-F2-15-1-CS1	Grain	D	WG 51: Nandyal	P	R	No	SC
Tx3431	(BTx406/RSC109)-F2-2-2-CS2	Grain	D	WG 50: D	P	R	No	SC
Tx3432	(BTx406/RSC118)-F2-17-1-CS1	Grain	D	WG 51: Nandyal	P	R	Yes	SC
Tx3433	(BTx406/RSC118)-F2-2-1-CS1	Grain	D	WG 51: Nandyal	P	R	Yes	C
Tx3434	(BTx406/RSC148-F2-2//RSC148)-F2-12-2-CS2	Grain	DK	WG 150: D-K	P	Y	Yes	SO
Tx3435	(BTx406/RSC148-F2-2//RSC148)-F2-4-1-CS1	Grain	DK	WG 150: D-K	P	Y	No	SC
Tx3436	(BTx406/RSC103)-F2-3-2-CS2	Silage	DB	WG 91: D-Dochna	P	R	Yes	--
Tx3437	(BTx406/RSC145)-F2-9-1-CS1	Silage	D	WG 51: Nandyal	P	R	No	--
Tx3438	(BTx406/RSC76-F2-4//RSC76)-F2-16-3-1-CS1	Silage	CD	WG 140: C-D	P	W	No	--
Tx3439	(BTx406/RSC111-F2-11//RSC111)-F2-10-13-1-CS1	Silage	DK	WG 150: D-K	P	R	No	--

†Race is as described by Harlan and de Wet (1972), where C = Caudatum; D = Durra; CD = Caudatum Durra; DB = Durra Bicolor; DK = Durra Kafir. Working group numbers and names are based on a Modified Snowden's Classification (Dahlberg, 2001). Single letter working group abbreviations (C, D, K) are the same as for race.

‡P = Pigmented; TP = Tan Pigmented.

§R = Red; W = White; Y = Yellow.

¶C = Compact; SC = Slightly Compact; SO = Slightly Open

Table 17. General agronomic characteristics of grain lines Tx3429 to Tx3435 and standard seed parents across Texas in 2018 (College Station, College Station Anthracnose, Lubbock, and Weslaco).

Designation	Days to Anthesis (d)[†]	Plant Height (cm) [‡]	Head Length (cm) [‡§]	Panicle Exsertion (cm) [‡]	Anthracnose Rating (AUDPC) [¶]	Anthracnose Class (AUDPC) [#]
Tx3429	69	99	22	11	36.5	VR
Tx3430	73	94	17	23	120.0	MR
Tx3431	71	80	19	14	85.0	R
Tx3432	76	102	19	18	59.0	R
Tx3433	71	104	25	10	88.0	MR
Tx3434	75	102	23	8	44.0	R
Tx3435	72	93	21	7	44.0	R
BTx406 (Check)	69	78	18	18	120.5	MS
BTx2928 (Check)	70	99	21	4	89.5	MR
BTxARG-1 (Check)	76	106	24	10	89.5	MR
RTx436 (Check)	72	107	22	15	84.5	MR
RTx437 (Check)	69	107	23	9	193.5	S
RTx2783 (Check)	76	108	19	3	330.5	S
Test Mean[††]	74	101	20	11	102.8	MR
$HSD_{(0.05)}$	10	27	NS	19	--	--

[†]LSMeans from across three environments (College Station, College Station Anthracnose, and Weslaco).
[‡]LSMeans from across three environments (College Station, Lubbock, and Weslaco).
[§]Head Length was not significantly different across all three environments.
[¶]Anthracnose rating is the Area Under the Disease Progress Curve (AUDPC) from one replication in a separate trial from College Station, TX in 2018 inoculated with isolates of *C. sublineola*.
[#]Anthracnose Class is calculated from the AUDPC Rating and is relative to known resistant and susceptible checks from the trial. R = Resistant; S = Susceptible; MR = Moderately Resistant; MS = Moderately Susceptible; VR = Very Resistant; VS = Very Susceptible.
[††]Test Mean refers to the average of all lines in the trial. A total of 40 lines contributed to this test mean.

Of the silage releases, Tx3436 to Tx3439 are 2-dwarf lines ($dw_1Dw_2Dw_3dw_4$), photoperiod-insensitive, and have minimal variation for days to anthesis (Table 18). Plant height (cm) ranged from 119 to 144. Head Length (cm) and panicle exsertion (cm) for the released lines were similar (Table 18).

Table 18. LSMeans for general agronomic characteristics of silage lines Tx3436 to Tx3439 and three check hybrids (Super Sile 30, NK300, and NK5418) in College Station, TX in 2018.

Designation	Days to Anthesis (d)	Plant Height (cm)	Head Length (cm)	Panicle Exsertion (cm)	Harvest moisture (%)	Fresh Weight Yield (Mt ha^{-1})[†]
Tx3436	65	144	15	18	69.37	33.13
Tx3437	75	119	21	0	72.20	31.77
Tx3438	76	133	20	0	72.83	27.93
Tx3439	70	153	27	1	69.77	32.50
Super Sile 30 (Hybrid Check)	PS[‡]	217	--	--	81.70	53.67
NK300 (Hybrid Check)	PS[‡]	148	--	--	80.13	46.76
NK5418 (Hybrid Check)	62	108	30	3	66.10	28.00
Test Mean[§]	66	129	19	5	69.67	30.80
$HSD_{(0.05)}$	3	39	9	6	8.72	11.12

[†]Silage Fresh Weight Yield (Mt ha^{-1}) is adjusted to 65% moisture.
[‡]PS = Photoperiod-Sensitive.
[§]Test Mean refers to the average of all lines in the trial. A total of 27 lines contributed to this test mean.

3.4.2. Genotypic Diversity of Released Parental Germplasm and Elite Public Inbreds

Using 12,342 SNP markers, pairwise distances were calculated based on the nucleotide diversity between the panel of elite public inbreds and RSC parental inbreds (A-1). PCoA was used to visualize the clustering of the RSC parental inbreds in relation to elite public inbreds (Figure 1). Three principal components explained 33.1%, 20.8%, and 10.3% of the total variation. In general, inbreds clustered into three distinct groups; one group represents elite public restorer (R) lines, a second group is comprised of elite public maintainer lines (B), and a third group comprising the released RSC parental inbreds. Exceptions to these groupings included BTx642 that clustered in the area encompassing Tx3430 to Tx3439 while Tx3429 clustered with standard public B lines (Figure 1). The remaining newly released pollinator grain and silage lines did not cluster with common public inbreds used in this analysis, highlighting the genetic diversity and potential uniqueness of these released parental germplasm lines (Figure 1).

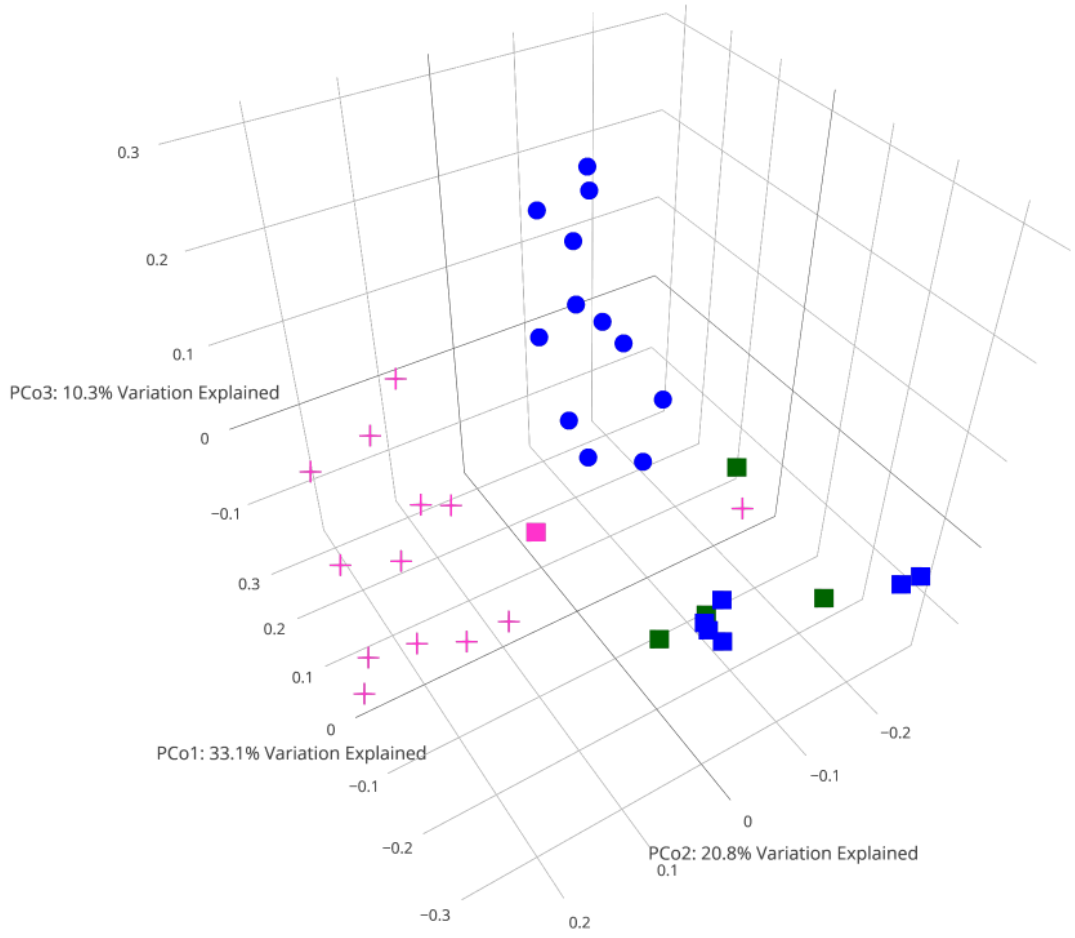

Figure 1. Principal Coordinate Analysis (PCoA) of elite public maintainer (B) and restorer (R) lines compared to released RSC parental germplasm Tx3429 to Tx3439. Percentage of variation explained by each coordinate are displayed. Pink crosses, 13 elite public B-lines; blue circles, 12 elite pubic R-lines; blue squares, RSC grain type R-lines (Tx3430-Tx3435); pink square, RSC grain type B-line Tx3429; and green squares, silage type RSC R-lines (Tx3436-Tx3439).

3.4.3. Agronomic Performance of Hybrids

Testcross grain hybrids of Tx3429 to Tx3435 were similar to the hybrid checks for most agronomic traits with a few exceptions (Table 19). For example, testcross hybrids of Tx3430 and Tx3432 were later than the check (Table 19). There was no difference in grain yield (Mt ha^{-1}) between the testcross hybrids and the check hybrids (Table 4). Testcross hybrids of Tx3429,

Tx3431, Tx343, and Tx3435 had grain test weights (kg hL^{-1}) greater than the hybrid check (Table 19). Sugarcane aphid tolerance, which was evaluated in the fall of 2017 revealed that testcross hybrids of Tx3432 were highly tolerant with several other hybrids possessing moderate tolerance (Table 19). Given the variation of SCA_T response across environments, SCA_T tolerance reported herein should be subject to further evaluation for confirmation.

Table 19. LSMeans of general agronomic performance for grain hybrids from Tx3429-Tx3435 with testers A1TxARG-1 and A1Tx2928 in College Station, TX, 2018 and their Sugarcane Aphid tolerance rating from 2017 Weslaco, TX.

Designation	Days to Anthesis (d)	Plant Height (cm)	Head Length (cm)†	Panicle Exsertion (cm)†	Grain Yield (Mt ha^{-1})‡	Test Weight (kg hL^{-1})	SCA_T (1-9)§
Tx3429	77	131	22	9	5.78	72.7	3
Tx3430	78	116	19	14	6.08	72.2	6
Tx3431	76	112	23	13	5.39	72.9	7
Tx3432	78	125	24	15	5.48	75.2	1
Tx3433	76	120	24	13	5.48	72.1	6
Tx3434	76	124	25	10	5.60	71.2	4
Tx3435	76	128	27	10	5.82	73.7	6
Tx437 (Check)	75	119	24	11	6.12	70.3	--
Test Mean¶	78	125	24	10	5.34	71.5	--
$HSD_{(0.05)}$	2	17	NS	NS	1.48	2.1	--

†Designated lines across both testers for Head Length (cm) and Panicle Exsertion (cm) were not statistically different from one another.
‡Grain Yield (Mt ha^{-1}) is adjusted to 14% moisture.
§Sugarcane Aphid Tolerance is from observational data of hybrid trials presented as a mean across the two testers from one rep in one location in Weslaco fall, 2017. Rating is from 1 (tolerant) to 9 (susceptible).
¶Test mean refers to the average of all hybrids within the trial. A total of 25 hybrids contributed to this test mean.

Silage-type testcross hybrids (Tx3436-Tx3439) flowered between 64 and 71 days after planting (Table 20). All testcross hybrids, except for TxArg-1/Tx3437, were taller than the checks and exhibited no lodging (Table 20). Head length (cm) and panicle exsertion (cm) among the testcross hybrids (Tx3436-Tx3439) are similar but could not be compared because Super Sile 30 and NK300 have delayed maturity and did not flower by harvest (Table 20). Moisture (%) at harvest for the testcross hybrid combinations were significantly lower than both hybrid checks, which is likely due to maturity at harvest (Table 20). Fresh weight yields (Mt ha^{-1} adjusted to

65% moisture) of these testcross hybrids were similar to both photoperiod-sensitive (nonflowering) commercial checks (Table 20).

Forage quality of these testcross hybrids were similar for protein content and TDN and generally lower for ADF and NDF than the check hybrids (Table 20). Some of these differences may be due to maturity differences between the testcross and check hybrids. Like the grain hybrids, observational ratings for sugarcane aphid tolerance indicate moderate tolerance in Tx3437, Tx3438 and Tx3439, depending on the specific hybrid evaluated (Table 20). Much like the grain trials, these same lines demonstrate a greater range of genetic diversity with comparable agronomic performance. Further, these hybrids are early and represent new silage hybrids for the early season maturity market.

Across both grain and silage hybrids, the testcross hybrid performance was not superior to check hybrids (Tables 19 and 20). However, these sources provide comparable yield with significant genetic diversity (Tables 19 and 20; Figure 1). As such, these sources have application as parents in hybrids and as germplasm for breeding programs to diversify the breeding populations. Finally, they demonstrate that useful germplasm can be derived from the RSC program.

Table 20. Agronomic performance of silage hybrids from Tx3436-Tx3439 parental lines with testers ATx2928 and ATxArg-1 in one environment in College Station, Texas, 2018 and their Sugarcane Aphid Tolerance rating from 2017 Weslaco, TX.

							NIR Quality Analysis‡				
Designation	**Days to Anthesis (d)**	**Plant Height (cm)**	**Head Length (cm)**	**Panicle Exsertion (cm)**	**Moisture (%)**	**Fresh Weight Yield (Mt ha^{-1})†**	**CP (%)**	**ADF (%)**	**NDF (%)**	**TDN§**	**SCA$_T$¶ (1-9)**
Tx2928/Tx3436	64	218	20	10	68.08	47.69	12	32	56	58	7
TxARG-1/Tx3436	65	228	22	15	71.07	46.66	12	35	63	56	5
Tx2928/Tx3437	70	225	24	4	68.74	51.96	12	33	61	57	5
TxARG-1/Tx3437	71	208	26	2	69.82	45.67	11	35	64	56	3
Tx2928/Tx3438	67	241	25	9	68.24	51.75	11	27	53	60	3
TxARG-1/Tx3438	69	253	23	10	69.92	48.50	11	34	60	57	3
Tx2928/Tx3439	66	237	26	10	67.59	46.58	10	33	63	57	5
TxARG-1/Tx3439	68	235	30	14	68.91	44.60	10	37	66	56	3
Super Sile 30 (Check)	PS	217	PS	PS	79.76	53.68	13	43	71	54	--
NK300 (Check)	PS	148	PS	PS	80.13	46.75	13	42	71	54	--
NK5418 (Check)	62	108	30	3	66.11	27.99	12	33	61	57	--
Test Mean#	66	207	24	9	69.55	44.20	11	34	62	57	--
$HSD_{(0.05)}$	3	39	9	17	6.90	14.64	2	9	14	NS	--

†Silage Fresh Weight Yield (Mt ha^{-1}) is adjusted to 65% moisture.

‡NIR Quality Analysis refers to CP = Crude Protein; ADF = Acid Detergent Fiber; NDF = Neutral Detergent Fiber; TDN = Total Digestible Nutrients.

§TDN was not significantly different across all hybrid types and checks.

¶ Sugarcane Aphid Tolerance is from observational data of hybrid trials presented as a mean across the two testers from one rep in one location in Weslaco fall, 2017. Rating is from 1 (tolerant) to 9 (susceptible).

#Test mean refers to the average of all hybrids within the trial. A total of 49 hybrids contributed to this test mean.

3.5. Availability

Seed of Tx3429 to Tx3439 will be maintained by personnel in the Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474. Request for germplasm can be directed to W.L. Rooney, AgriLife Research Sorghum Breeding or to Texas A&M Technology Commercialization, Texas A&M University, College Station, TX 77843-3369. Seed of these germplasms has been deposited in the National Plant Germplasm System, where they have been classified as parental germplasm and will be available 20 years after publication.

3.6. Conclusions

Tx3429 to Tx3439 were released to provide the sorghum breeding industry with sources of germplasm for both grain and silage that are diverse and have agronomic value. These release lines can be used to produce both breeding lines and hybrids that represent readily useable, early-generation sorghum germplasm developed from the RSC program.

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4. TEMPORAL ESTIMATES OF CROP GROWTH IN SORGHUM AND MAIZE BREEDING ENABLED BY UNMANNED AERIAL SYSTEMS*

4.1. Synopsis

To meet future world food and fiber demands, plant breeders must increase the rate of genetic improvement of important agricultural crops. One of the biggest obstacles now facing crop scientists is a phenotyping bottleneck. To ease this burden, the emerging technology of unmanned aerial systems (UAS) presents an exciting opportunity. To assess the utility of UAS, it is important to investigate their application across multiple crop species. Terminal plant height is of great importance to maize [*Zea mays*] and sorghum [*Sorghum bicolor* L. Moench] breeders and temporal plant height has been hypothesized to be a useful measurement but has been logistically impractical to measure in the field. In this study, I present an in-depth statistical analysis of the ability for UAS to estimate height in sorghum (Advanced and Early Generation material) and maize (Optimal and Late material) and the applications of these estimates in breeding programs. I found that UAS explains genotypic variation similarly to ground-truth methods and that the repeatability of the methodology is high (R = 0.61 – 0.99), indicating effective differentiation of genotypes. Additionally, correlations between ground-truth (GT) and UAS measurements were moderate to high for all materials (r = 0.4 – 0.9). Finally, I present a novel application for the technology in the form of high-resolution temporal growth curves. Using these UAS-generated growth curves, new physiological insights can be obtained, and new avenues of scientific investigation are possible.

*Adapted with permission from Pugh, N.A., D.W. Horne, S.C. Murray, G. Carvalho, L. Malambo, J. Jung, A. Chang, M. Maeda, S. Popescu, T. Chu, M.J. Starek, M.J. Brewer, G. Richardson, and W.L. Rooney. 2017. Temporal Estimates of Crop Growth in Sorghum and Maize Breeding Enabled by Unmanned Aerial Systems. The Plant Phenome Journal 1(1). doi: 10.2135/TPPJ2017.08.0006.

4.2. Introduction

Numerous reports have identified that crop yields need to increase at a rate of at least 2.4% per year to secure food for growing populations under a changing climate (Godfray et al., 2010; Cairns et al., 2013; Gleadow et al., 2013; Wheeler and Von Braun, 2013). As genotyping technology has evolved, phenotyping is now a primary 'bottleneck' in crop genetic improvement programs, caused by monetary, accessibility, and time-oriented limitations (Furbank and Tester, 2011). While greenhouse and growth chamber studies are useful to dissecting phenotypic traits where environmental effects are minimized, field phenotyping under agricultural conditions is critical to achieve gains that transcend the complex genetic by environment by management interactions that farmers routinely experience. High-throughput techniques applied in the field, especially those that utilize remote sensing platforms, are promising new tools to help close this gap and could account for other shortcomings in crop improvement pipelines (Furbank and Tester, 2011; Tester and Langridge, 2010; Araus and Cairns, 2014; Shi et al., 2016a). Unmanned aerial systems, or UAS, are the most promising of these emerging technologies that could serve as affordable, efficient high-throughput phenotyping platforms (Shi et al., 2016a). The capability of these systems to cover vast areas in a short period, as well as their ability to carry various payloads consisting of different sensors, makes them appealing to crop scientists (Shi et al., 2016a).

Currently, there is unlimited potential in UAS, but researchers must first objectively evaluate these technologies for their utility before implementation can occur. As a proof-of-concept, plant height is an excellent trait to explore the usefulness of UAS technology in agricultural research and plant breeding programs (Shi et al., 2016a; Watanabe et al., 2017). Plant height is a well-characterized quantitative trait in sorghum [*Sorghum bicolor* L. Moench],

maize [*Zea mays* L.], and other grasses (Fernandez et al., 2009), and is genetically simpler than complex traits such as grain yield (Quinby and Karper, 1953; Lin et al., 1995; Pereira and Lee, 1995). In practical terms, plant height (height) is highly correlated with grain yield in both sorghum and maize, especially in hot, dry, and stressful environments (Cassady, 1965). Measuring height in the field by traditional manual measurements is arduous, and is typically obtained only at the terminal point of growth. Interest for more expeditious methodologies is high (Furbank and Tester, 2011; Shi et al, 2016a). Previous studies have sought to estimate the height of sorghum and maize in a field environment using various UAS platforms (Anthony et al., 2014; Chapman et al., 2014; Shi et al., 2016a; Watanabe et al., 2017). While the correlations were moderate to high for these previous studies, they did not investigate UAS-derived data on a level beyond whole-field correlative analysis (Shi et al., 2016a; Watanabe et al., 2017). Such correlative analysis can be challenging, because it assumes that the manual measurements are correct and that automated measurements must therefore reproduce them; however, it is also conceivable that the automated measurements are superior to manual measurements and this can only be evaluated through the consistency of measurements between replicates across different genotypes or treatments.

In this study, our first objective was to understand UAS-derived height estimates within and among different ideotypes of material and to investigate their accuracy and usefulness in plant breeding programs. For UAS technology to be useful in crop genetic improvement, data generated must be statistically repeatable and useful to extract biologically meaningful differences between genotypes in the field (Fehr et al., 1987; Nakagawa and Schielzeth, 2010). To this end, it is important that the relative rankings of genotypes be compared between the

conventional ground-truth and UAS methodologies. Ultimately, UAS technologies need to be able to correctly select the genotypes to exclude from advancement in a breeding program.

In addition to using UAS to replace conventional field measurements, a more exciting use is making measurements that were not previously possible. New insights for both basic biology and applied breeding for yield could be obtained by measuring height temporally throughout crop growth and would require substantial amounts of time and money (Cooper et al., 2016; Chang et al., 2017). The second objective of our study was to apply UAS technology over the entire growth cycle to develop growth curves for sorghum and maize (Cooper et al., 2016; Chang et al., 2017). If two or more cultivars had different growth trajectories, they could theoretically be crossed to pyramid a new cultivar with a desirable growth phenotype that could not be detected through traditional terminal measurements alone. Furthermore, higher temporal resolution should permit the development of better physiological models to predict yield. Development and future refinement of these highly descriptive phenotypes could replace simple terminal height measurements in breeding programs. Detailed growth curves could be a useful tool for plant breeders to assess overall plant vigor and tolerance of various stressors throughout its growth (Pauli et al., 2016).

4.3. Materials and Methods

4.3.1. Germplasm and Experimental Design

A complete list of entries for both sorghum and maize tests appears in the appendix materials. Two sets of sorghum ideotypes (Advanced and Early Generation) were divided amongst two experimental tests composed of hybrids as well as inbred lines in varying assortments. The first test, Advanced, was comprised of elite material that included 36 experimental hybrids and four commercial, open-pedigree hybrids. The second test, Early

Generation, was comprised of exotic material that included nine inbred lines (six experimental, three commercial), four commercial hybrids, and 17 experimental hybrids. Both tests were planted in a randomized complete block designs (RCBD) with four replications. Replicates in each test consisted of 6.71m plots with 1.22m alleys and were planted in College Station, TX on March 23rd, 2016 and Corpus Christi, TX on March 29th, 2016. Standard agronomic practices for grain sorghum were used in this study.

The maize test (Maize) was comprised of seven commercial hybrid checks, two inbred lines, one segregating open-pollinated population, and 26 experimental hybrids made from elite Texas A&M University (TAMU) lines with expired plant variety protection (ex-PVP) lines, elite commercial testers, or other TAMU lines (see appendix materials). This was planted in two plantings in College Station, TX: Optimal was an early and typical planting date (March 13th, 2016), while the Late planting occurred at a later date (April 4th, 2016) when temperatures were higher. In addition, there was one Optimal planting in Corpus Christi, TX on April 1st, 2016. In each of the three trials an RCBD was used with four replicates: two of the replicates were two-row plots, one replicate was a four-row plot, and one replicate was a one-row plot. The one-row plot replicate in Corpus Christi did not have complete entries or notes and has been excluded. Plot length in College Station, TX was 7.62m including a 1.22m alley, in Corpus Christi, TX this was 6.10m including a 1.52m alley. A summary table for the experimental design for maize and sorghum can be found in the appendix materials (A-2).

4.3.2. Field Measurements for Ground-Truth Validation of Height

For sorghum, measurements of height were recorded differently depending upon the stage of growth of each plot. Sorghum plants that had not emerged from the whorl were measured from the ground to the apex (highest point) of the overall plant. Plants that had

emerged from the whorl (i.e. the stem had elongated) were measured from the ground to the tip of the panicle. The panicle tip measurement was analogous to the apex measurement of un-emerged plants. The maximum height (apex and panicle tip) was used as the "ground-truth" value in the subsequent statistical analyses. Measurements were taken as an estimated mean across the entire plot (i.e. one measurement per plot) between the dates of May 11th and July 8th (A-2). Of these weekly measurements, seven ground-truth measurements corresponded with flights by the UAS teams.

For maize, plants were measured eight times in College Station throughout growth, from May 7th to June 27th, but the earlier Optimal planting was not measured on the last two dates, which occurred well after flowering because it was believed that there should be no change in growth. In Corpus Christi, plants were measured five times from May 13th to July 1st (A-2). One representative plant from each experimental plot was measured from the ground to the top visible ligule, which tended to correspond to the highest flat leaf surface, which was easier to consistently measure in a windy field than other methods tested.

4.3.3. UAS Aerial Survey and Data Processing

Each UAS team (one in College Station, another in Corpus Christi) used standard but separate workflows. Flights were conducted at standard altitudes and high image overlap was obtained (Malambo et al., 2017). The College Station team used a DJI ® Phantom 3 Professional UAS to conduct flights, while the Corpus Christi group used a DJI ® Phantom 4 Professional. In addition to the Phantom 3, the maize in Corpus Christi, TX was also flown with a 3DR ® Solo and fixed-wing eBee mounted with RGB and near-infrared (NIR) cameras, respectively. Each flight team used portable or stationary (respectively) ground control points, or GCPs, that were uniformly placed within the fields. These GCPs were measured with survey-grade differential

GPS prior to image acquisition. A summary of flight details for each location is available in the appendix materials (A-2).

While there are other methods for estimating height using remote sensing, including light detection and ranging (LiDAR), the UAS teams used RGB imagery to produce 3D reconstructions for this study (Malambo et al., 2018). Each flight team generated 3D reconstructions, or point clouds, of the imaged crop surfaces using either Pix4Dmapper (Pix4D SA; Lausanne, Switzerland) in College Station or Agisoft Photoscan Pro (http://www.agisoft.com) in Corpus Christi. Pix4D mapper was also used for some of the flights in Corpus Christi. From the point cloud data, a digital surface model (DSM) was derived and subsequently used by the software to orthorectify the imagery mosaicking to create an orthomosaic. After generating DSMs, both groups subtracted the ground elevation from the DSMs using a bare-earth digital elevation model captured during pre-growth and exposed ground conditions to derive canopy height models for each flight. For a more detailed description of the SfM processing workflow applied to derive a DSM from UAS acquired imagery, as well as pertinent information regarding GCPs and flight parameters, please refer to the appendix materials (A-3).

4.3.4. Data analysis and statistics

Sorghum and maize ground measurements and UAV estimates were checked for normality and outliers in JMP Pro 12.2.0 software (SAS Institute Inc., 1989 - 2017). Restricted maximum likelihood analysis (REML) was conducted within environments using Fit Model (all random) in JMP. The statistical model used for this analysis was

$$Y = \alpha_i + \beta_j + \gamma_t + \delta_k + \varepsilon$$

, where α = genotype (i), β = replication (j), γ = row (ι), δ = range (k), and ε = error, where the row and range are spatial adjustments of what are sometimes called the column and row, respectively (D'Agostino et al., 2006). Effects with negative variance components were removed from the model. The percentage of total variation that could be explained by genotype was calculated using this model as was the repeatability. Repeatability (R) estimates were calculated using the equation:

$$R = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

, where R = the repeatability score, σ_g^2 = the genotypic variance, σ_e^2 = the error variance, and r = the number of replications (Nakagawa and Schielzeth, 2010). Repeatability uses the variance of each component to give researchers an indication about the consistency of their techniques. It is calculated similarly to heritability (H^2), but is distinct from it since the sorghum and maize populations used in this study did not have a familial structure. Pearson's correlation coefficients (r) were calculated using R software (R Development Core Team, 2008). Correlations were conducted within all five tests in both environments. Least squares means (LSMeans) for each entry within test and environment were calculated using the same statistical REML model noted previously, except setting genotype as a fixed effect to calculate best linear unbiased estimators, and leaving all other effects as random terms. The consistency of genotypes was evaluated by the percent of variation explained by genotype in the statistical model and by the repeatability of that measurement. These approaches were useful for not only comparing UAS based measurements to conventional ground truth (GT) measurements, but also for refining and improving approaches in UAS methods. Least squares means were used to determine the relative rankings of the

genotypes for height in both the ground truth and SfM datasets in sorghum (See appendix, A-4) and all quantitative data used in this study is available in the appendix materials.

4.4. Results and Discussion

Two different, and mostly independent, methods were used to evaluate the accuracy and precision of height estimates derived from UAS data using structure-from-motion (SfM) algorithms across sorghum and maize. First, the consistency of genotypes across each replicate, which does not require a manual measurement, and secondly, correlations between UAS and the traditionally obtained manual measurements, which have been investigated elsewhere (Shi et al., 2016[a]; Watanabe et al., 2017). For example, multiple UAS SfM point cloud percentiles were evaluated but only P95 (the 95% highest point in the point cloud for a plot) and Max (the highest point in the point cloud for a plot) are presented herein as they were the most predictive.

In early stages of plant development, any differences among plots or genotypes that existed were smaller than manual measurement tools could accurately capture. At this stage, I expected limited genotypic variation or field spatial variation, and most differences could only be attributed to error variation. As plants grew larger and the differences between genotypes became more prominent, genetic variation increased.

4.4.1. Genotypic Variation Explained by Test and Repeatability

For sorghum and maize, the trends observed for genotypic variation and repeatability were consistent in both College Station and Corpus Christi. In the early stages of growth, the variation explained by genotype was relatively low. This was particularly evident for the early ground-truth data in sorghum where differences between genotypes were smaller than ground researchers could accurately measure (Figure 2). The same trends were also seen in Corpus Christi, and thus College Station is presented as representative of the two locations (Figure 2).

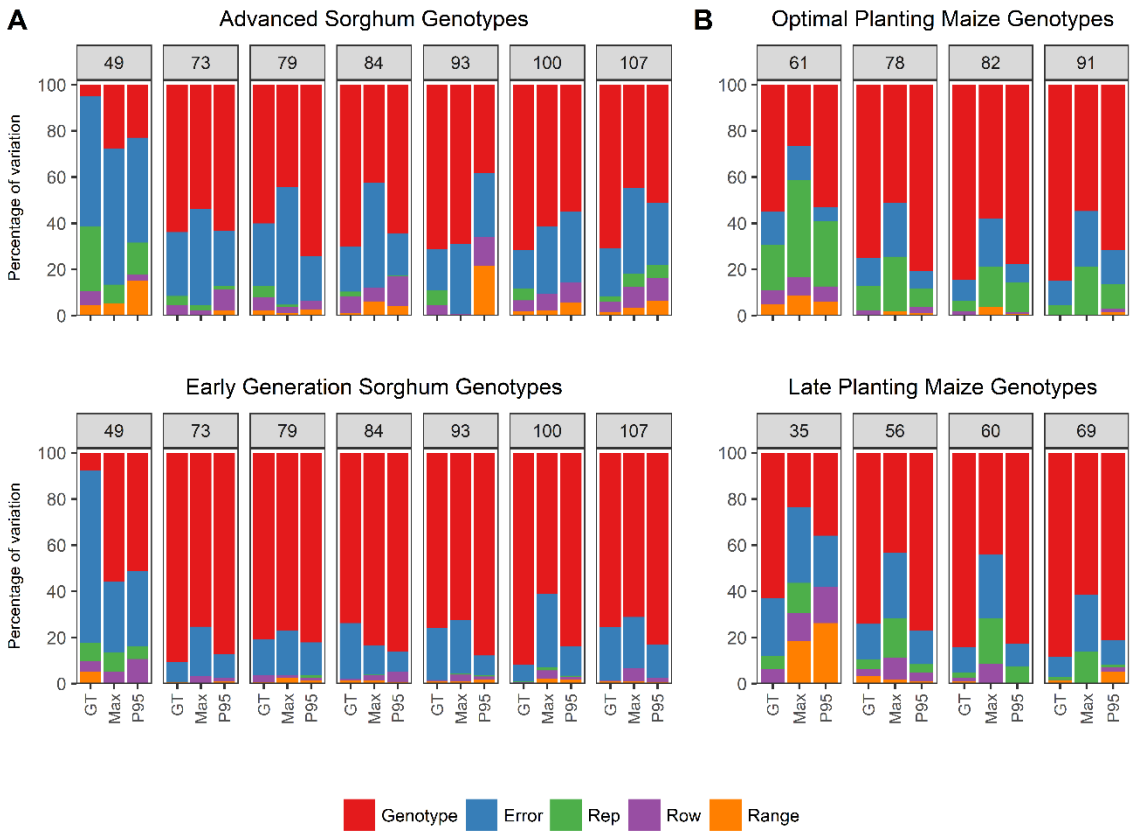

Figure 2. Percentage of Variation Explained in Sorghum and Maize. The percentage of variation explained for each test and flight in sorghum and maize (shown as the number of days after planting, or DAP, that the flight took place). Measurements include ground-truth (GT), the 95th percentile (P95), and maximum percentile (Max) of the UAS data. Two experimental sorghum tests, Advanced and Early Generation, were mapped for College Station, TX in 2016 (A). Two experimental maize tests, Optimal (early planting) and Late (late planting), were also mapped for College Station, TX in 2016 (B).

However, the genotypic variance of the Max percentile in maize rose sharply as the height and genetic differentiation increased, remaining moderate to high (Figure 2). In sorghum, the variation explained due to genotype was consistently high throughout the growing season when using UAS based measurements, but the ground-truth measurements were unable to capture the same levels of variation on the first date (Figure 2A). The maize data showed the

opposite trend, wherein the UAS based measurements were matched or even outperformed by researchers on the ground in capturing genotypic variation early on (Figure 2B). The reason for this is unknown; however, the data on the first flight date shows that, on average, plant heights for sorghum were much lower than they were in maize (see appendix materials). This could have made it more difficult to capture genotypic variation on that date.

In both crops, the genotypic variance was not perfectly linear with time and demonstrated multiple upward and downward variations. For ground truth measurements, these differences could have been due to day-to-day human error, exacerbated by wind movement of plants, different plant choices, and heat and fatigue stress. For UAS measurements, wind during flights, changing canopy height, image overlap, and potential errors in the photogrammetric 3D reconstruction process of the SfM appeared to be potential causes. However, further studies will be required to quantify exactly what effect, if any, these factors have upon UAS-based height estimates.

Repeatability calculations, shown herein on and entry-mean basis use variance estimates to assess the consistency of the measurements (Table 21).

Table 21. Repeatability Estimates for Sorghum and Maize. Repeatability (*R*) estimates for measurements of sorghum and maize height taken on the ground as well as with structure from motion (SfM) for each flight (P95 and Max) and the closest corresponding ground-truth (GT) measurement on specific number of days after planting, or DAP. Estimates were calculated using the equation, $R = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$ **. Percentiles for the SfM data included both the 95th percentile (P95) and the maximum percentile (Max). Estimates are for Advanced and Early Generation sorghum tests and Optimal (early planting) and Late (late planting) of the maize test Maize, each located in College Station and Corpus Christi, TX.**

College Station, TX				
DAP	Test	GT	P95	Max
49	Advanced	0.27	0.67	0.65
	Early Generation	0.29	0.88	0.86
61	Optimal Planting	0.94	0.97	0.88
35	Late Planting	0.91	0.87	0.74
73	Advanced	0.90	0.91	0.84
	Early Generation	0.98	0.97	0.93
78	Optimal Planting	0.96	0.98	0.89
56	Late Planting	0.95	0.96	0.86
79	Advanced	0.89	0.94	0.78
	Early Generation	0.95	0.96	0.94
82	Optimal Planting	0.97	0.98	0.92
60	Late Planting	0.97	0.97	0.87
84	Advanced	0.94	0.93	0.79
	Early Generation	0.92	0.97	0.96
91	Optimal Planting	0.97	0.95	0.90
69	Late Planting	0.98	0.97	0.91
93	Advanced	0.94	0.85	0.90
	Early Generation	0.93	0.98	0.93
100	Advanced	0.94	0.88	0.89
	Early Generation	0.98	0.96	0.89
107	Advanced	0.93	0.88	0.83
	Early Generation	0.93	0.96	0.93

Corpus Christi, TX				
DAP	Test	GT	P95	Max
29	Advanced	0.37	0.64	0.61
	Early Generation	0.78	0.88	0.88
26	Optimal Planting	0.85	0.91	0.91
44	Advanced	0.28	0.86	0.88
	Early Generation	0.96	0.98	0.97

Table 21. Continued

Corpus Christi, TX				
DAP	Test	GT	P95	Max
46	Optimal Planting	0.96	0.98	0.97
58	Advanced	0.80	0.87	0.86
	Early Generation	0.95	0.99	0.98
60	Optimal Planting	0.97	0.97	0.97
72	Advanced	0.79	0.86	0.93
	Early Generation	0.96	0.85	0.89
67	Optimal Planting	0.97	0.92	0.92
86	Advanced	0.94	0.81	0.92
	Early Generation	0.97	0.82	0.85
91	Optimal Planting	0.97	0.93	0.94

In sorghum, the repeatability of the UAS-derived height estimates was consistently high across the growing season (Table 21). Except for the first flight date (49 Days after planting, or DAP), the repeatability of the ground-truth measurements was consistently high as well (Table 21). The repeatability was high for all methodologies in both the Optimal and Late trials of maize (Table 21).

The low repeatability values observed early in sorghum growth was the result of physical limitations of the ground-truth methodology for short plants, where differences between genotypes were smaller than the measurement precision of the ground recorders (Nakagawa and Schielzeth, 2010). This tendency was most apparent in the Advanced sorghum test, but was also observed, to a lesser extent, in the Optimal trial of maize in College Station as well (Table 21). The ability for the UAS to retain high repeatability values in sorghum, especially early in the growing season, is an advantage over traditional ground-truth methodologies. However, in contrast, more genotypic variation could be captured early in maize than was captured by the UAS (Figure 2B). This is an important difference, as accurate height estimates during early vegetative growth is a key component when constructing growth curves in both crops. The lower

repeatability observed in some ground-truth situations, particularly in sorghum, are attributed to having too little variation between genotypes for ground-truth data to discern between them. However, the high repeatability exhibited in the UAS data across all four tests suggested that there was sufficient variation. Indeed, based on the comparison between earlier and later flight dates in sorghum, the threshold of genotypic variation necessary to be discernable by ground researchers was much higher than that which is necessary for the UAS (Figure 2). Interestingly, the Max percentile appeared less capable of extracting genetic variation than P95 in both sorghum and maize (Figure 2). This is likely due to several factors including variable error between percentiles calculated from the UAS data and the structural properties of the plants each of the percentiles is capturing.

4.4.2. Correlations Between Ground-Truth and SfM Heights

For sorghum, Pearson correlations (*r*) between ground-truth measurements and UAS-derived height estimates were moderate to high across the growing season in College Station and Corpus Christi, TX (Figure 3).

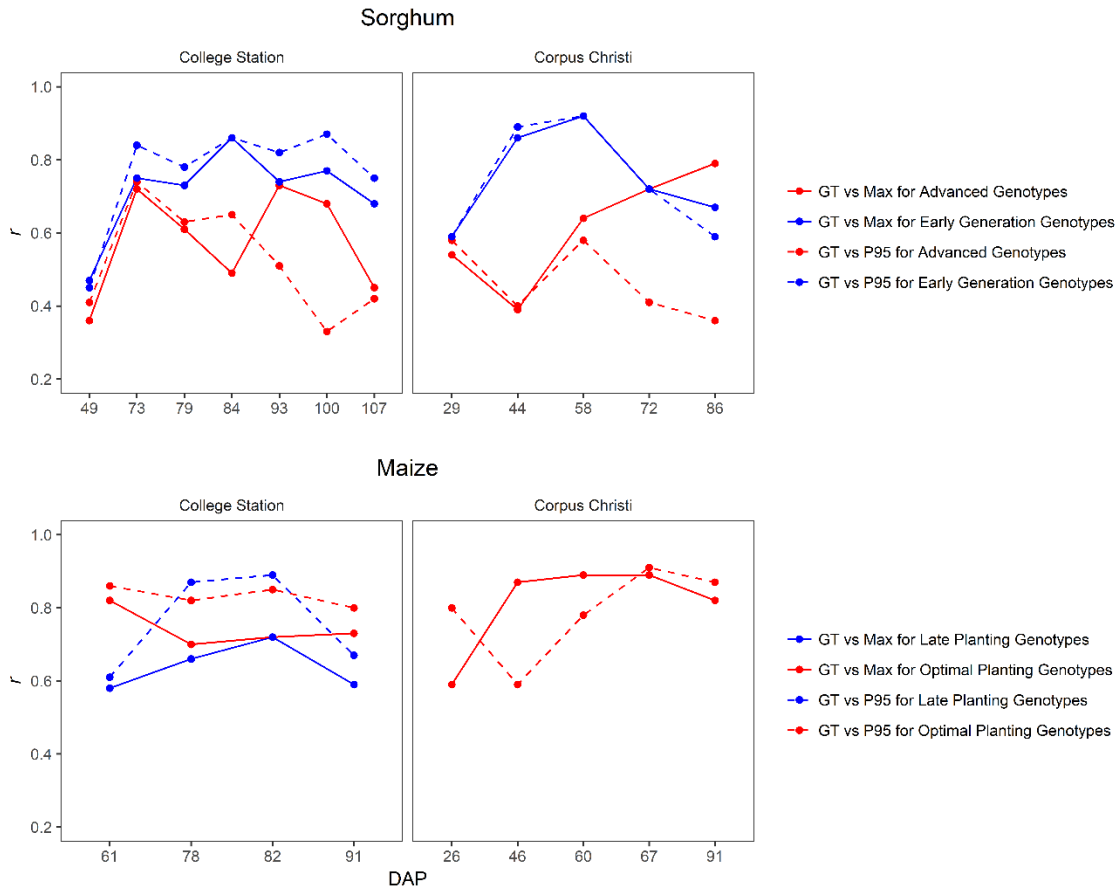

Figure 3. Pearson's Correlation Coefficients for Sorghum and Maize. Graphical representation of Pearson's correlation coefficients (*r*) over the course of the growing season in sorghum in College Station and Corpus Christi, TX in 2016. Four different groups of material are represented: Advanced and Early Generation tests for sorghum, and Optimal and Late tests for maize. Correlations were conducted between ground-truth (GT) data and two different percentiles of structure-from-motion (SfM) data. Percentiles for the SfM data include the 95th percentile (P95) as well as the maximum percentile (Max).

The Advanced test remained within a range of 0.4 to 0.8 *r* while the Early Generation test tended to spike rapidly up to a maximum of ~0.95 *r* before leveling off for the remainder of the flights. As observed in genotypic variance and repeatability metrics, correlations are expected to be stronger in the test with higher variation between genotypes (Early Generation). The maize

trials showed strong correlations in both environments. For Optimal maize in Corpus Christi, correlations were highest when using the Max percentile. In contrast, for maize in College Station, correlations were highest (range of 0.7 – 0.9 *r*) when using P95. However, when comparing College Station maize in the Optimal and Late trials, similar trends were observed between the plantings wherein a high correlation stayed relatively consistent but dropped on the final flight date (91 DAP and 69 DAP, respectively). This phenomenon was particularly evident in the Late planting of the trial (Figure 3).

Interestingly, Max was marginally superior for the Advanced sorghum test and the Optimal maize trial planted in Corpus Christi, while the P95 was preferable for estimating the Early Generation test as well as both plantings of the maize trial in College Station (Figure 3). The presence of two groups of material at different stages in the sorghum breeding pipeline meant that further differences could be spotted between these ideotypes based upon the percentile used. In other words, different percentiles could be utilizing different threshold values from the point clouds generated by SfM; Max could correspond slightly better to elite, less variable sorghum hybrids (Advanced) and the P95 could correspond better to material with more variation within plots (Early Generation) (Figure 3). More experiments are needed to determine if this observation is consistent over time.

In sorghum and maize, Shi et al. (2016) found field correlation between UAS-derived height estimates and those obtained with ground-truth methods to be low to moderate. It is worth noting that for sorghum those estimates used a fixed-wing platform on large plots. For maize, there was a substantial time difference between when the ground truth and UAS measurements were conducted (Shi et al., 2016[a]). This variation makes differences in resolution and accuracy logical. In Watanabe et al. (2017), the capability to predict height in small sorghum plots using a

UAS ranged in Pearson's coefficient from 0.5 to 0.7 *r*, depending on the percentile of point cloud data being used (50^{th}, 75^{th}, 90^{th}, or 99^{th}) as well as whether or not height estimates were corrected for the presence of adjacent plots, which can influence the ability for the researchers that are using UAS to estimate height. Though the correlations in that study were lower, the design of their experiment was different from the current study. Although diverse experimental material was used, the plots were very small (0.72 m x 1.80 m) and estimates of height were performed on two singular plants sampled from those plots (Watanabe et al., 2017). Additionally, correlations were not separated by the ideotypes of the material being investigated, which could explain why that study did not find large differences between the various percentiles of 3D point cloud data used. In this study, apparent differences between the capabilities of the different percentiles of SfM data were observed. Further studies could also investigate how well UAS technology can capture intra-plot variation, thus teasing out differences within genotypes. However, more experimentation and environments are needed to determine if this holds true in all situations or if it is necessarily the correct explanation for the discrepancy that I observed.

4.4.3. High-Resolution Temporal Growth Curve Analysis for Sorghum and Maize using SfM Data

A novel phenotype that UASs can enable in a field breeding program is temporal growth curves, which could allow a better separation of genetic differences at various stages of plant development; these also could parameterize physiological models for specific genotypes (Cooper et al., 2016). After constructing high-resolution temporal growth curves for both the Advanced and Early Generation sorghum tests in Corpus Christi, clear differences between the materials were observed (Figure 4).

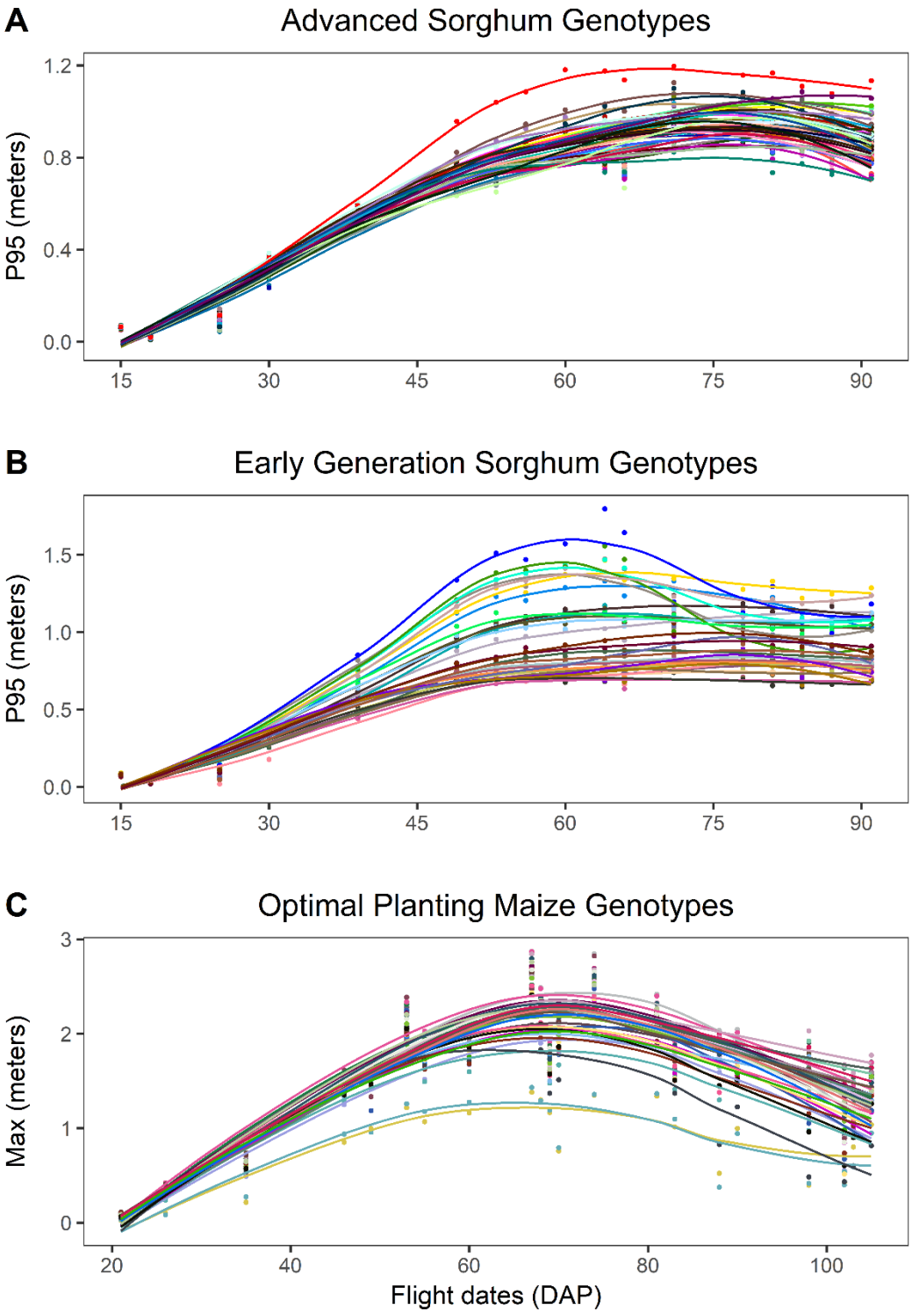

Figure 4. Multitemporal Growth Curves for Sorghum and Maize. Graphical representation of UAS-derived growth curves fit over the course of the growing season for two sorghum trials, Advanced (A) and Early Generation (B), and the Optimal maize trial (C) in Corpus Christi, TX in 2016. Growth over 16 flight dates

(Shown as days after planting, or DAP) for sorghum and 21 flight dates for maize is shown using the least squared mean (LSMean) height for each plot. Each color on the growth curve denotes the structure-from-motion (SfM) data (95th percentile or P95 for sorghum, maximum percentile or Max for maize) for a different genotype. The included points on the graph represent LSMean values for each entry on each flight date. While the sorghum material was flown using a DJI ® Phantom unmanned aerial system, the Optimal trial was flown using a combination of the DJI ® Phantom and senseFly ® eBee and Solo systems. The Advanced material flowered from 53 – 63 DAP, while the Early Generation material flowered from 53 – 61 DAP.

The smaller spread in various genotypes heights at any time in the Advanced trials (compared with Early Generation trials) demonstrated why genetic variance was lower; all remained relatively similar to one another throughout the growth period, as expected from uniform, elite hybrid material (Figure 4). The exception was genotype 50, which was considerably taller than the rest of the material throughout the growth period (Figure 4). As in the Advanced trial, the Early Generation genotypes reached their zenith at ~60 - 64 DAP and then slightly decreased over the rest of the growth period (Figure 4). However, several of the taller genotypes showed a more rapid decline immediately following maximum growth (Figure 4). To our knowledge this is the first report of decreased height in crops between flowering and harvest, however it is also the first experiment to our knowledge measuring crops temporally throughout this period. The decrease in height could be attributed to several possible factors including, lodging of genotypes due to wind and other environmental factors (though no significant lodging was noted at the time) or possibly a reduced accuracy of SfM measurements on materials that are too tall. Upon investigation of raw imagery for those plots, lodging was observed but since it did not affect all plants in a plot, it seems unlikely it would have affected P95 or Max metrics which gives credence to this proposed explanation.

In the Optimal maize test in Corpus Christi, a uniquely detailed growth curve was generated by combining SfM data from three different UAS platforms that were being investigated (Figure 4). These platforms included the DJI ® Phantom 4 Professional used in the rest of the study, as well as the addition of 3DR ® Solo and eBee UAS systems. By combining data from all three platforms, a curve with higher temporal resolution was generated that could be used to investigate plant development on a finer scale (Figure 4). Interestingly, the overall height of the maize plants gradually decreased for all three platforms after the crop had flowered, and to a greater extent than that observed in sorghum (Figure 4). This same phenomenon was also captured in College Station data with both UAS and manual measurements.

There are several possible explanations as to the cause of the decrease in the plant height estimates. Maize plants senesce (in sequence from the bottom leaves to the top leaves in Texas), and the height decrease could be due to the senescence and subsequent drooping of the tassels and canopy surface. Another possible explanation is the curvature (i.e. "melting" plants) of the stalk, which is similar to but not the same as stalk lodging. It is also possible that root lodging or stalk breakage within the research plots could have been ignored or overlooked by field researchers, and heights may still have been recorded only on plants that remained standing which might have been shorter; lodged or shrunken plants may have been picked up by the UAS sensors but it would take the majority of the plot to affect P95 and Max and this was not visually observed. It is also possible that the digital surface model or the altitude of the UAV was in error that day, but I could not determine this as a cause. Thus, while it is an outside possibility that plants shrink, other explanations are more likely in this case. To understand this process and elucidate what is causing the decrease in height later in the season, further studies will need to be conducted.

As demonstrated herein, growth in sorghum and maize is known to be logarithmic up to or slightly past flowering. This biological characteristic has been studied and described previously (Bartel and Martin, 1938; Arkin et al., 1976; Shi et al., 2016b). However, while researchers could previously study sorghum and maize growth in a few genotypes, it has not been efficient or applicable at scale in a field research program. These results demonstrate that it is possible to obtain growth information using UAS platforms and circumvent the limiting issues of labor and time of manual measurement.

To the authors' knowledge this is one of the first instances of growth curves being described for maize and sorghum using purely UAS-derived data; however, previous studies have used other high-throughput phenotyping systems to study various aspects of growth in other plant species (Pauli et al., 2016; Apelt et al., 2015; Grieder et al., 2015). In Grieder et al. (2015), wheat [*Triticum aestivum*] canopy cover was recorded temporally instead of plant height, though both are measures of different aspects of plant growth. In that study, sensors estimated the relative canopy cover over time showing the variability of canopy cover from week to week and differences between genotypes being detected (Grieder et al., 2015). Apelt et al. (2015) investigated various aspects of growth in Arabidopsis [*Arabidopsis thaliana*] using a specialized imaging system. However, these studies were conducted under controlled conditions where it is difficult to scale to the population sizes needed for breeding and genetics research or to observe the environmental interactions occuring in a research field. As shown in this study, the enhanced ability to discover and utilize previously unknown physiological attributes of the plants presents an exciting opportunity for researchers. An indication of novel value to these measurements was directly observed through correlations between terminal height and earlier season heights (A-5), which are low, combined with knowledge of the high genetic variance and repeatability (Table

21, Figure 2). This suggests that there are reliable and repeatable genetic determinants to early season and mid-season growth that cannot be captured by conventional terminal growth measurements. If incorporated with weather and physiological growth model data, new insights into how to breed crops may be gained (Cooper et al., 2016).

4.4.4. Time and Equipment Considerations

Getting the most accurate and repeatable data is important in plant science research and breeding; however, resources are often limited, so a fair appraisal of this new technology must be made. I estimated that the College Station maize tests (36 genotypes x 4 replicates x 2 tests x 2 measurements) took four to five hours with two people per ground truth time point (0.8 to 1 min per plot), the Corpus Christi maize tests (36 genotypes x 3 replicates x 3 plant measurements) required about 1.5 hours (approximately 0.8 minutes per plot). In both cases, a measuring stick and a Samsung Galaxy Tab 4 (~$170 USD) were used. For College Station and Corpus Christi sorghum tests (80 genotypes x 4 replicates x 2 tests x 2 measurements) required about one-hour (~10 seconds for per plot), but at a greater monetary cost up-front as a barcoded measuring stick and Zebra MT2090 (~$850 USD) barcode scanners were used to record heights. In contrast, to get the entirety of the sorghum and maize data by UAS (464 plots) in College Station and Corpus Christi I estimate it took 45 - 60 minutes to fly and 10 – 14 hours to transfer the imagery, process the data using SfM software, derive digital surface models, and extract values for each measurement date. However, UAS required a much greater fixed cost including the UAS itself (~$1200 USD), a powerful workstation (~$4000 - $17,000 USD) and proprietary software (~$500 – $2,000 USD per month). However, these same UAS images provide an important archive from which other traits can also be extracted. Decisions must be based on a cost-benefit

analysis by each individual scientist and should depend upon factors such as the number and types of traits desired, the amount of necessary funds, and the number of personnel available.

4.5. Conclusions

This study has been the first in-depth temporal and statistical evaluation of UAS for measuring plant height in sorghum and maize breeding and genetics programs and has provided a proof of concept and multiple key insights. It is important to reiterate that terminal plant height is strongly positively correlated to yield in Texas in both sorghum and maize, especially in dry and marginal environments where it seems to serve as a proxy to vigor under stress (Cassady, 1965; Farfan et al., 2013). The technology utilized in this study has shown to be highly repeatable and generally capable of dissecting genetic variation between research plots, dependent upon the growth stage and the material being investigated. This could enable new ways of understanding plant growth, as demonstrated by the highly detailed temporal growth curves presented herein. The three most important new biological findings for field research programs are; 1) that there is genotypic variation at each growth stage, 2) that early and late stage plant height are uncorrelated and likely independent, and 3) that plant heights appear lower post-anthesis. These discoveries demonstrate that frequent UAS measurements can provide a practical advantage over traditional measurement techniques. However, although the UAS technology works and is generally strongly correlated with ground-truth measurements of the trait, there are still limitations that must be considered. Specifically, the effectiveness of remote sensing technology to estimate height in maize and sorghum is largely contingent upon the material being measured; while the technology is effective at estimating height in exotic sorghum (Early generation) and hybrid maize, it is less able to differentiate more uniform elite sorghum hybrids (Advanced) and plants at early growth stages. Nevertheless, the use of high-throughput

phenotyping platforms, especially UAS, have potential to positively shift the phenotyping paradigm for modern research programs and alleviate the critical phenotyping bottleneck.

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5. CONCLUSIONS

With the primary goal of the RSC program aimed at recovering the tropical genome in early flowering, short plants, its emphasis was like that of the SC program for "conversion," rather than agronomic performance. Various methodologies presented within this dissertation, however, may offer new insight into how to best appropriately evaluate and utilize partially converted, early generation sorghum germplasm from the Reinstated Sorghum Conversion program. Incorporation of RSC germplasm into a sorghum breeding program is a notable strategy for breeding programs that are trying to increase diversity within their program while maintaining high agronomic mean. While our inability to elucidate hybrid performance using markers based on varying levels of tropical genome recovery, the importance of using markers to identify novel tropical material in early testing should be implemented. In doing so, our results show that to appropriately utilize the material presented herein, the evaluation of line performance per se should be prioritized (rather than marker-based selection), followed by hybrid testing evaluations with various testers from that program. Following such recommendations, it was possible to provide the sorghum breeding industry with sources of germplasm for both grain and silage that are diverse and have agronomic value from RSC germplasm. The released lines detailed in this dissertation can be used to produce both breeding lines and hybrids that represent readily useable, early-generation sorghum germplasm developed from the RSC program. For the continual selection of phenotypic measurements of agronomically desirable genotypes, this study showed that the utilization of high-throughput phenotyping via unmanned aerial systems (UAS) in an appropriate method for explaining genotypic variation and high repeatability estimates. This could provide new insight into understanding plant growth, as demonstrated by the highly detailed temporal growth curves

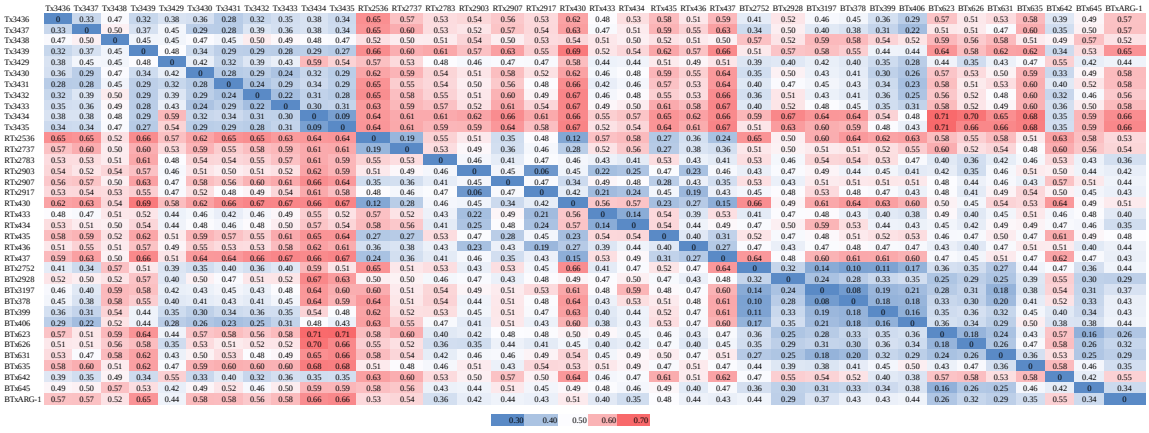
presented herein. In summary, these results show that utilization of newly reinstated sorghum conversion lines have their place within sorghum breeding programs and can deliver agronomically desirable genotypes with novel genetic diversity, and UAS is an appropriate tool for assisting breeders in evaluating the genetic diversity within their breeding programs.

APPENDIX A

Appendix Results and Discussion

Genotypic diversity of released parental germplasm and elite public inbreds

Using 12,342 SNP markers, pairwise distances were calculated based on the nucleotide diversity between the panel of elite public inbreds and RSC parental inbreds (A-1).



A-1: Correlations of genetic pairwise distances of Tx3429 to Tx3439 and common restorer (R) and maintainer (B) lines. Lines more closely related are in shades of blue, while lines with greater genetic distance are in shades of red.

APPENDIX B

Appendix Materials and Methods

Entry Information and Experimental Design

Information related to the number of entries, methods, and number of flights for both tests and both locations can be found in A-2.

A-2: Summary of Sorghum and Maize Experimental Design Lists all the experimental factors for the sorghum and maize trials in this study. Included are the locations (College Station and Corpus Christi, TX), the number of tests for each crop, the number of entries, the plot size, the number of replicates, and the number of flight dates.

College Station, TX		
	Sorghum	Maize
Tests	2	2
Entries	80	36
Plot Size	2 & 1 row (.76 m spacing), 6.7 m length	1, 2, & 4 row (.76 m spacing), 7.6 m length
Replicates	4	4
UAS	Rotary Wing	Rotary Wing
Flight Dates	7	4
Corpus Christi, TX		
	Sorghum	Maize
Tests	2	1
Entries	80	36
Plot Size	2 & 1 row (.76 m spacing), 6.7 m length	1, 2, & 4 row (.76 m spacing), 6.10 m length
Replicates	4	4
UAS	Rotary Wing	Rotary and Fixed Wing
Flight Date	5	5

UAS Aerial Survey and Data Processing

In College Station, weekly flights were conducted from April until July in 2016 using a DJI ® Phantom 3 Professional UAS. The maize and sorghum tests were planted adjacent to each other in College Station so that a single flight captured both crops at the same time. A 12-megapixel camera mounted on the UAS recorded 8-bit images in the visible spectrum (red, green, and blue, or RGB) in JPEG format. For each flight, a flight altitude of 20 m was maintained, and an image overlap of at least 90% was obtained as permitted by the weather. Using Pix4Dcapture, parallel flight lines were designed for autopilot to capture the images. For ground reference, each flight campaign utilized six to twelve portable ground control points (GCP) that were uniformly placed throughout the field. These GCPs were measured with GPS prior to image acquisition and were composed of a 47.5 cm by 61.0 cm wooden frame that was covered with canvas fabric. For a summary of flight details, refer to A-3.

A-3: Flight and Ground Control Point Details Summary of flight information from unmanned aerial system flights in both College Station and Corpus Christi, TX in 2016. The date each flight took place (SfM Date) is given as the Julian date. Dates that matched ground-truth measurements in sorghum or maize are given as the days after planting (DAP) of those respective crops. Ground sample distance (GSD) is given in cm/pixel for each flight. Information on the location and error of the ground control points (GCPs) for each flight is also given.

College Station, TX, 2016									
SfM date	DAP GT sorghum (days after SfM)	DAP GT maize (days after SfM)	Number images	GSD (cm/pixel)	GCP geolocation details				
					No. GCP	*Error(X) (cm)*	*Error(Y) (cm)*	*Error(Z) (cm)*	*Total RMS error (cm)*
94	.	.	1061	0.82	8	3.5	4.2	2.0	3.2
98	.	.	1215	0.86	8	2.0	2.6	4.1	2.8
118	.	.	1061	0.84	12	3.0	3.5	3.3	3.2
134	49 (-2)	61/39 (+4)	963	0.81	10	3.4	6.1	2.7	4.0
155	73 (+1)	78/56 (+0)	906	0.81	8	5.0	5.0	1.9	4.0
159	79 (+3)	82/60 (+0)	981	0.83	6	3.3	1.5	2.0	2.2
168	84 (-1)	91/69 (-3)	953	0.88	6	2.2	1.8	2.3	2.2
176	93 (+0)	.	1064	0.86	6	2.1	3.3	1.3	2.2
183	100 (+0)	.	906	0.88	6	5.0	5.1	1.9	4.0
190	107 (+0)	.	985	0.88	6	1.6	2.0	1.0	1.5

A-3. Continued

Corpus Christi, TX, 2016									
					GCP geolocation details				
SfM date	DAP GT sorghum (days after SfM)	DAP GT maize (days after SfM)	Number images	GSD (cm/pixel)	*No. GCP*	*Error(X) (cm)*	*Error(Y) (cm)*	*Error(Z) (cm)*	*Total RMS error (cm)*
98	.	.	241	1.05	20	6.67	4.92	3.53	9.01
103	.	.	245	1.08	20	6.92	5.95	7.41	11.75
106	.	.	452	1.28	20	4.07	2.44	9.11	10.27
113	.	.	143	1.32	13	2.36	1.59	4.70	5.49
118	29 (+0)	26 (+4)	280	1.30	18	3.18	3.86	4.32	6.61
127	.	.	641	1.34	20	3.58	6.80	4.04	8.68
137	44 (-1)	46 (-3)	419	1.38	20	4.76	7.02	4.33	8.48
141	.	.	428	1.34	20	5.34	6.72	4.05	9.49
144	.	.	430	1.42	20	7.42	6.46	4.06	10.64
148	58 (+2)	56 (+0)	440	1.42	20	4.93	7.76	5.39	10.66
152	.	.	518	1.46	20	4.73	7.90	4.93	10.44
154	.	.	529	1.34	8	3.86	5.28	1.54	6.72
159	72 (+0)	67 (-1)	518	1.2	7	2.69	2.68	2.38	4.48
166	.	.	682	0.90	9	3.94	4.81	3.83	7.36
169	.	.	715	0.92	9	2.63	1.78	3.02	4.38
172	.	.	695	0.75	10	2.86	2.16	2.38	4.30
175	86 (+0)	.	745	0.88	17	4.40	3.67	5.09	7.66
179	.	.	734	0.80	17	3.93	3.58	4.86	7.20
182	.	91	710	0.71	17	3.89	3.21	4.45	6.73
190	.	.	948	0.98	9	2.97	3.38	3.24	5.55
195	.	.	791	0.90	16	4.56	3.95	5.70	8.30
198	.	.	982	0.84	16	5.09	4.18	5.49	8.58
201	.	.	717	1.23	16	5.00	4.25	5.84	8.78
203	.	.	682	0.84	16	4.62	3.96	5.22	8.02
207	.	.	623	0.81	16	4.47	3.86	4.93	7.69
210	.	.	791	0.77	13	5.17	3.81	6.00	8.79

Using the Pix4Dmapper software, 3D point clouds and ortho-mosaics were generated. A standard structure from motion (SfM) workflow was followed, beginning with tie-point extraction and matching, triangulation, and bundle adjustment, followed by point cloud densification and ending with digital surface model (DSM) and orthomosaic generation.

Subsequent plant height estimates were generated from the 3D point cloud for each field plot. This procedure involved normalizing a 3D point cloud generated to the ground level and then using the resulting point clouds as the basis for the height estimation. The normalization of the point clouds was accomplished using the equation:

$$P_{tAGL}(x,y,z) = P_t(x,y,z) - g_t(x,y,z)$$

, where $P_t(x,y,z)$ is the original point, $g_t(x,y,z)$ is the ground surface, and $P_{tAGL}(x,y,z)$ is the resulting above-ground level (AGL) point cloud at date *t*. The ground surface was obtained by filtering the point cloud using linear prediction as implemented in the FUSION software and interpolating it into a 2m grid (Kraus and Pfeifer, 1998; McGaughey, 2009). The AGL point cloud was then split into multiple plot-level point clouds that were based on plot boundaries. To minimize edge effects, plot boundaries were buffered inwards by 0.2 m. Finally, each plot-level AGL point cloud was used to estimate the 95th and 100th (Max) percentiles of the AGL heights.

In Corpus Christi, a DJI Phantom 4 (DJI Technology, Shenzhen, China) was utilized as a platform to collect aerial images over the entire test field. A 12.4 mega pixel RGB camera sensor with a 94-degree field of view was attached. Aerial data acquisition was conducted five times during April, May, June, and July (Table S1). A flight on April 7th, 2016 conducted prior to crop emergence was used to develop a DSM. The UAS was pre-programmed to perform data collection at 20 or 30 m altitude above ground with 85% side- and forward-image overlap using the Pix4D capture mobile application. The approximate flight duration for each date was 20 minutes.

Although the approximate geographical location (longitude, latitude, and altitude) of the raw images were also recorded by the UAS platform's onboard GPS sensor for initial processing,

it does not provide enough accuracy for high quality, direct image geo-referencing. To address this limitation, a set of eight GCPs were installed around the sorghum field and their precise location was surveyed using a GPS device for accurate image geo-referencing across different dates. The surveyed GCPs were utilized to improve geo-referencing accuracy of the final geospatial products. The raw images for each Corpus Christi flight were processed using Agisoft Photoscan Pro (http://www.agisoft.com) SfM software with GCPs' geographic coordinate to generate geospatial data products such as orthomosaic images, 3D point clouds, and DSM. The spatial resolution of the resulting ortho-mosaic image and the DSM was less than 1.5 cm and 3cm, respectively (A-3). The RMSE of geo-referencing for each flight was less than 10 cm. After generating ortho-mosaics and DSMs, ground elevation was removed from the DSM to extract accurate crop height of the AGL. The 95^{th} and Max percentiles of the pixel values in each row were extracted to estimate plant height.

Appendix Results and Discussion

Comparison of Rankings by Genotype for Ground-Truth and SfM in Sorghum

In breeding and cultivar selection, a primary approach used is to rank genotypes. Ranks are more useful than absolute phenotypic values because the later are based on the limited number and specific environments, management and their interactions with the genetics evaluated, and cannot be repeated. Rankings of genotypes tends to be more stable across similar target environments. The effectiveness of SfM for ranking the final, pre-harvest heights of the various genotypes depended largely upon the environment and the stage of material that was being investigated (A-4).

A-4: Rankings by Genotype in Sorghum. Rankings of each genotype (GENO) using LSMeans for ground-truth estimates (GT) as well as SfM-derived estimates of height (P95) in the Advanced and Early Generation sorghum tests for College Station and Corpus Christi, TX. Genotypes that were deemed too tall for cutoffs of 1.40m (College Station) and 1.24m (Corpus Christi) are denoted by colored cells (Blue = Advanced, Red = Early Generation).

College Station, TX						Corpus Christi, TX					
Advanced			Early Generation			Advanced			Early Generation		
GENO	GT	P95	GENO	GT	P95	GENO	GT	P95	GENO	GT	P95
1	1.32	1.19	1	1.06	1.08	1	1.19	0.98	1	1.04	0.81
2	1.33	1.21	2	1.13	0.95	2	1.15	0.96	2	1.16	0.79
3	1.29	1.10	3	1.21	1.04	3	1.14	0.92	3	1.14	0.81
4	1.21	1.13	4	1.32	1.08	4	1.07	0.89	4	1.26	0.68
5	1.31	1.03	5	1.19	1.06	5	1.17	0.78	5	1.10	0.75
6	1.32	1.09	6	1.49	1.35	6	1.18	0.88	6	1.39	0.88
7	1.33	0.91	7	1.92	1.79	7	1.20	0.80	7	1.85	1.07
8	1.37	1.03	8	0.89	0.96	8	1.19	0.89	8	1.03	0.73
9	1.39	1.44	9	1.23	0.92	9	1.23	1.04	9	1.00	0.68
10	1.30	1.29	10	1.56	1.63	10	1.24	1.06	10	1.58	1.14
11	1.28	1.08	11	1.79	1.60	11	1.18	0.96	11	1.59	1.14
12	1.28	1.27	12	1.79	1.65	12	1.21	1.00	12	1.54	1.11
13	1.37	1.00	13	1.81	1.75	13	1.24	0.78	13	1.88	1.25
14	1.24	0.95	14	1.21	1.03	14	1.14	0.80	14	1.15	0.83
15	1.32	1.16	15	1.26	1.59	15	1.24	0.93	15	1.23	0.79
16	1.31	1.11	16	1.73	1.45	16	1.20	0.86	16	1.71	1.08
17	1.32	1.25	17	1.15	1.09	17	1.22	0.98	17	1.27	0.96
18	1.26	0.99	18	1.89	1.68	18	1.17	0.87	18	1.68	1.01
19	1.42	1.05	19	1.38	1.14	19	1.20	0.86	19	1.26	0.86
20	1.43	1.09	20	1.16	1.04	20	1.19	0.93	20	1.07	0.78
21	1.33	1.07	21	1.22	1.14	21	1.17	0.90	21	1.16	1.04
22	1.31	1.09	22	1.93	1.61	22	1.19	0.88	22	1.89	0.93
23	1.25	0.97	23	1.49	1.66	23	1.11	0.83	23	1.34	1.00
24	1.09	0.90	24	2.03	1.69	24	1.01	0.79	24	1.80	1.15
25	1.21	0.89	25	2.25	1.68	25	1.11	0.79	25	2.03	0.97
26	1.19	0.92	26	1.22	1.00	26	1.05	0.85	26	1.17	0.83
27	1.32	1.28	27	1.23	0.93	27	1.17	0.90	27	1.18	0.74
28	1.34	1.29	28	1.30	0.96	28	1.20	1.03	28	1.21	0.90
29	1.33	1.04	29	1.30	1.22	29	1.21	0.84	29	1.19	0.91
30	1.33	1.02	30	1.21	1.04	30	1.19	0.83	30	1.13	0.89
31	1.33	1.24				31	1.23	1.00			
32	1.32	1.10				32	1.18	0.91			
33	1.46	1.27				33	1.28	0.85			
34	1.44	1.15				34	1.24	0.88			
35	1.42	1.10				35	1.19	0.88			
36	1.38	1.18				36	1.24	0.77			

A-4. Continued

College Station, TX					
Advanced			Early Generation		
GENO	GT	P95	GENO	GT	P95
37	1.38	0.97			
38	1.44	1.02			
39	1.48	1.12			
40	1.39	1.13			
41	1.34	1.13			
42	1.34	1.21			
43	1.38	1.14			
44	1.40	1.12			
45	1.46	1.01			
46	1.43	1.12			
47	1.46	1.18			
48	1.38	1.11			
49	1.66	1.32			
50	1.75	1.54			

Corpus Christi, TX					
Advanced			Early Generation		
GENO	GT	P95	GENO	GT	P95
37	1.18	0.78			
38	1.23	0.82			
39	1.23	0.91			
40	1.31	0.97			
41	1.21	0.86			
42	1.19	0.83			
43	1.21	0.98			
44	1.22	0.92			
45	1.28	0.74			
46	1.19	0.78			
47	1.28	0.92			
48	1.20	0.85			
49	1.40	0.93			
50	1.47	1.08			

In College Station, the SfM-derived height estimates at either P95 or Max were ranked dis-similarly to ground-truth in the Advanced material, likely because of the narrow range of variation. However, in the Early Generation test, the relative rankings and proportion of genotypes to be trimmed were quite similar between ground-truth and SfM (A-4).

Maximum acceptable heights of sorghum have been determined based on grower preferences in the environment that the genotypes were being ranked. The cutoff value for sorghum was determined to be 1.40m in College Station, TX, and 1.24m in Corpus Christi, TX (since genotypes are expected to be shorter, on average, in that environment). For sorghum, two Advanced genotypes, 9 and 50 were estimated to be 1.44m and 1.54m, respectively, with genotype 50 being the tallest genotype estimated using both ground-truth and P95 in College Station (A-4). One caveat was that the number of genotypes above the cutoff plant height was much lower for the Early Generation test in that environment; only one genotype (Entry 13)

would have been removed based on the 95th percentile of UAS data, compared to 15 genotypes identified through ground data. Additionally, genotype 45 was 1.28m when estimated using the ground-truth methodology but was then estimated as the shortest genotype when using SfM data (A-4). This is an example of potential inaccuracies when attempting to use this technology to rank elite, highly uniform material. Furthermore, rankings conducted by SfM could be inaccurate due to segregation of the material or anomalies within plots.

Correlations between terminal height and earlier season heights

Information related to the correlation for end of the season plant height and initial plant heights for both tests in sorghum and maize are found in A-5.

A-5: Pearson's Correlation Coefficients Between Initial and Final Heights for Sorghum and Maize Pearson correlation coefficients (*r*) between heights of the first measurement date and the last measurement date in sorghum and maize using both ground-truth and unmanned aerial system, or UAS, data. Heights were measured as either ground-truth (GT), the 95th percentile of the UAS data (P95), and the maximum percentile of the UAS data (Max). Sorghum material includes the Advanced and Early Generation trials, and maize material includes the Optimal and Stress titles. Correlations were calculated for both College Station, TX and Corpus Christi, TX.

	College Station, TX			
	Advanced	Early Generation	Optimal	Stress
GT	-0.142	0.175	0.429	0.722
P95	-0.127	0.283	0.687	0.433
Max	-0.097	0.362	0.511	0.326

	Corpus Christi, TX		
	Advanced	Early Generation	Optimal
GT	0.069	0.334	0.485
P95	0.075	0.139	0.741
Max	0.056	0.263	0.765