ASSESSMENT OF NOVEL SORGHUM KERNEL CHARACTERISTICS AND FUNCTIONALITY

A Thesis

by

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ABSTRACT

Sorghum (*Sorghum bicolor L. Moench*) is an important food grain in semi-arid regions. Recent food trends in the United States have made grain sorghum a food grain crop of interest to food processors. The Texas A&M Agrilife Research Sorghum Breeding Program has developed sorghum seed and pollinator parents that can produce specialty grain sorghum hybrids with distinct end uses: high digestibility protein; waxy endosperm; and high popping efficiency. In these types, the goal of this program is to provide inbred lines suitable for the production of specialty grain sorghum hybrids. The purpose of this study is to assess the yield potential, grain functionality and quality of waxy hybrids across multiple production environments. In multiple environments, the waxy sorghum hybrids had increased grain yields and acceptable agronomic traits when compared to the waxy check hybrid used. The waxy hybrids also had flour pasting properties distinctly different than the heterozygous waxy and homozygous non-waxy checks. The waxy hybrids had higher water solubility indexes and higher peak viscosities with large environmental variation. Additionally, the waxy sorghum hybrids had lower pasting temperatures that had little environmental variation compared to the checks. In the evaluation of sorghum genotypes for popping attributes, variation existed among the tested genotypes for popping efficacy, expansion ratio and flake size. Threshing method significantly impacted pop sorghum traits and the threshing methods that have metal to seed contact reduce popping yields the most.

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NOMENCLATURE

ANOVA	Analysis of Variance
DF	Degrees of Freedom
ER	Expansion Ratio
FE-SEM	Field Emission Scanning Electron Microscope
FS	Flake Size
HD	High Digestible
HI	Kernel Hardness Index
LSD	Least Significant Difference
MS	Means Squared
PE	Popping Efficacy
SG	Starch Granule
TEM	Transmission Electron Microscope
UPK	Un-popped Kernels
WAI	Water Absorption Index
WSI	Water Solubility Index
wx	Waxy Allele

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1. INTRODUCTION

Sorghum (*Sorghum bicolor L. Moench*) is the fifth most widely grown cereal grain in the world (FAO 2007). In many semi-arid regions of the world, it is a staple food crop for millions of people. In North America, sorghum is used predominantly as an animal feed in the form of grain, forage or silage. However, the demand for high quality food grade sorghums has increased exponentially in the past five years as it uniquely fills several specialty niche food markets, such as gluten free breads, natural sweeteners and confectionary snacks.

Several types of specialty grain sorghum exist and can be utilized for a variety of food products. White food-grade sorghums are milled to produce flour used in quick breads (ie, tortillas), leavened breads and various types of porridges. Other grain sorghum types can be either puffed or popped and consumed as a snack food (Rooney and Murty 1981). Selected dark red or even black colored grain sorghums possess high levels of polyphenols and 3-deoxyanthocyanidins that have high antioxidant activity and are perceived to be health foods (Awika 2017). Additionally, some specialty grain sorghums have high amylopectin content and are classified as waxy. These waxy sorghums can be used for malting alcoholic beverages or fermenting biofuels (Wu et al. 2013; Mezgebe et al. 2018).

Karper (1933) first reported that the waxy endosperm trait was controlled by a single gene in the homozygous recessive genotype. Since that time, sorghum breeders have maintained the waxy trait within the improvement programs, but it has rarely been commercialized. During World War II, there was interest in waxy sorghum production as a replacement for cassava which could not be imported because of the war. However, interest soon waned after the war because waxy endosperm lines (and eventually hybrids) were somewhat lower in yield than their non-waxy counterparts.

Whether this yield drag is due to limited breeding or pleiotropic effects remains debatable. Jones et al. (1952) reported that the genotypes with the waxy phenotype had a 10% reduction in yield compared to similar nonwaxy genotypes. Similarly, (Rooney et al. 2005) also found that the presence of the waxy phenotype conferred a reduction in hybrid yield and that factors such as pleiotropy, genetic linkage and lack of breeding are likely the cause of the yield reduction. Regardless, there have been waxy parental lines developed that have improved agronomics and increased yield potential that may help the development of waxy hybrids (Miller et al. 1992; Miller et al. 1996). The recent interest in waxy sorghum hybrids for human food, animal feed and ethanol production drive the demand to develop agronomically acceptable hybrids with high yield potential.

Although it is used as a staple crop in some regions of the world, the protein digestibility of sorghum grain is low relative to other cereal grains (Maclean et al. 1981). In the sorghum endosperm, the predominant protein structure (kafirins) are spherical bodies and the specific arrangement of these kafirins hinders enzymatic access and hence digestion of the protein when it is consumed (Oria et al. 1995). Variation in protein digestibility exists and is often caused by changes in the structure of the protein body (Weaver et al. 1998). One specific mutation that causes the kafirins to arrange abnormally, leading to misshapen protein bodies, results in increased protein digestion compared to the normal spherical shaped protein bodies (Oria et al. 2000). Incorporation of this misshapen, high digestible (HD), protein body trait into sorghum genotypes could reduce the amount of protein supplements needed in animal feeding, improve protein intake in protein deficient populations and help make sorghum economically viable as a grain crop.

Similar to the popular snack food popcorn, certain genotypes of sorghum grain will readily pop. Unlike popcorn, pop sorghum is much smaller in size, and possesses a neutral flavor profile. Due to these differences pop sorghum fills a specific niche markets including granola or health food bars.

While there is commercial interest in food-grade sorghums with these specialty traits, significant germplasm development and characterization is important. For example, genotypes with waxy endosperm are known in sorghum but have not been produced because they have inherently low yield potential and susceptibility to grain weathering caused by pathogens such as *Fusarium thapsinum* (Jones et al. 1952). In pop sorghum, variation for both popping efficacy and expansion ratio are known among genotypes (Murty et al. 1988) but little work has been completed to identify the best commercially grown food-grade sorghums for popping. Additionally, the effects of different harvesting and processing approaches on the popping quality of has not been studied for sorghum.

The Texas A&M Agrilife Research Sorghum Breeding Program has developed seed and pollinator parents that can produce hybrids with high digestibility and waxy endosperm traits. Additionally, screening of genotypes that have favorable popping characteristics has identified several hybrids and lines with these characteristics. The first purpose of this research is to assess the flour functionality and agronomic performance of developed waxy lines in testcross hybrid combinations being developed to meet the commercial needs for waxy endosperm and high digestibility sorghum hybrids. The second purpose is to identify elite popping type sorghums and how harvest and processing affect popping characteristics. The specific objectives and sub-objectives are as follows.

The first objective of this research aims to assess the agronomics and grain functionality of waxy endosperm and/or high digestible protein grain sorghum hybrids with superior agronomics relative to traditional grain sorghum hybrids. The sub-objectives are 1a) to identify waxy endosperm sorghum hybrids that also possess the HD protein mutation using a field emissions scanning electron microscope; 1b) to assess the yield potential of waxy and/or HD sorghum hybrids relative to a standard waxy check hybrid; and 1c) to evaluate the waxy endosperm trait for functionality and stability in quality over numerous environments.

The second objective of this research is to identify grain sorghum genotypes that possess superior popping ability and investigate whether harvest methods and harvest maturities cause kernel damage that affects popping quality. The sub-objectives are 2a) to evaluate the popping attributes of four sorghum hybrids and three inbred lines for popping attributes; 2b) to test five different methods of threshing for their effect on popping attributes; and 2c) to test the effects of maturity and moisture content at time of harvest has on popping attributes.

2. AGRONOMICS AND GRAIN FUNCTIONALITY OF WAXY AND/OR WAXY HIGH DIGESTIBLE SORGHUM HYBRIDS

2.1. Background

2.1.1. Waxy Endosperm

Sorghum endosperm is predominately comprised of starch. This starch is composed of both amylopectin and amylose and while the exact proportions are subject to some variation, amylopectin accounts for approximately 75% and amylose the remaining 25% (Rooney et al. 2005). These two carbohydrates are both composed of glucose; amylose is a linear molecule whereas amylopectin is highly branched (James et al. 2003). In terms of functionality, the highly branched nature of amylopectin allows more enzymatic access for amylases which increases the rate of digestibility compared to amylose. Consequently, waxy starch demonstrates faster rates of digestion at lower processing temperatures, which means that waxy starches require less energy to be converted to ethanol than non-waxy starches (Zhao et al. 2009).

Karper (1933) reported that the waxy starch phenotype is conditioned by a single gene that when in the homozygous recessive form (*wx wx*) cease granule-bound starch synthase (GBSS) avtivity (Pedersen et al. 2005). Since sorghum endosperm is triploid, the recessive *wx* allele must be present at all three loci for the visible waxy starch phenotype to be observed. Lichtenwalner et al. (1978) reported that some additivity exists in the trait as each recessive *wx* allele reduces amylose content, but significant amylose reduction or elimination occurs with three *wx* alleles. In recent years, the *wx* gene was mapped to chromosome 1 and has an approximate length of 4.5 kb nucleotides (McIntyre et al. 2008). To date, three *wx* alleles are known and are designated as wx^a, wx^b, wx^c. The wx^a allele lacks GBSS coding sequence whereas wx^b and wx^c both have inactive GBSS and only differ by a point mutation (Kawahigashi et al. 2013).

Waxy endosperm in cereal grains has long been known to improve quality for several applications. Akay et al. (2001) reported that dairy cows fed a waxy maize (*Zea mays*) silage hybrid produced more milk and fat compared to dairy cows fed conventional, non-waxy, maize silage hybrids. Similarly, waxy rice cultivars are preferred for particular applications due to the waxy starch's unique physiochemical properties (Bao et al. 2004).

Niche markets for food grade sorghum would also benefit from waxy starch sorghums. Sorghum with waxy starch and high protein digestibility have superior flour properties and may be better for dough-based products (Elhassan et al. 2015). Additionally, Mezgebe et al. (2018) reported higher grain malt yields of waxy starch sorghums, which may be due to the greater starch granule swelling property of amylopectin.

There are a few, if any, commercial grain sorghum hybrids with waxy endosperm. The reason for the scaricity of waxy sorghum hybrids is that limited commercial interest along with significant efforts required to improve the waxy germplasm base in order to develop competitive hybrids. Other cereal grains, such as wheat, rice, barley and maize, have already benefited from targeted breeding for waxy starch genotypes and have found their way into animal feed markets.

The waxy starch phenotype in grain sorghums generally results in lower yields compared to similar non-waxy starch sorghums. Jones et al. (1952) reported roughly a 10% yield drag for genotypes possessing the waxy phenotype compared to isogenic non-waxy genotypes. The exact reason for the observed yield drag in waxy starch sorghum is unknown with pleiotropy, undesirable genetic linkage and limited breeding being the best explanation (Rooney et al. 2005). However, waxy starch sorghum lines with comparable yields to non-waxy starch lines have been reported (Rooney et al. 2005). The effect of yield drag with the waxy starch phenotype has also been reported with other cereal grains, such as wheat and barley (Graybosch 1998; Oscarsson et al. 1998).

Additionally, HD sorghums have traditionally been believed to be lower yielding than non-waxy, non-HD sorghums. By using a recombinant inbred line population, (Jampala et al. 2012) showed that inbreds with either/both the waxy or HD phenotype yielded comparable to inbreds that were non-waxy and non-HD within the population. This is promising for the development of high yielding waxy, HD parents and demonstrates a need to test in hybrid combinations in replicated yield trials.

2.1.2. Protein Digestibility

Compared to other cereal grains, sorghum has relatively low protein digestibility. Cooked wheat, maize, and rice have protein digestibility of 81%, 73% and 66%, respectively, compared to sorghum at 46% (Maclean et al. 1981). With the large number of humans and livestock that rely on sorghum grain as a nutrition source, it is important to improve the protein digestibility. Sorghum protein digestibility is influenced by disulfide linkage between storage proteins that are known as kafirins (Aboubacar et al.

2003). These kafirins come in the form of β-kafirins, α-kafirins and ϒ-kafirins and are distinguished from one another based on molecular weight, solubility and structure (Shull et al. 1991). The three forms of kafirins form protein storage molecules called prolamins in the endosperm and exist in the form of spherical protein bodies. Normally, β- and ϒ- kafirins reside on the periphery of the protein body and α-kafirins in the inside of the protein body. The β-kafirins and ϒ-kafirins form disulfide linkages on the periphery of the protein body that hinders the digestion of the α-kafirins that are inside the protein body (Oria et al. 2000).

Genetic variation for protein digestibility is known to exist is sorghum. A mutation that displaces the β-kafirins and ϒ-kafirins and causes misshapen protein bodies with exposed α-kafirins has been reported to increase the digestibility of protein bodies within sorghum endosperm. The source of the HD mutation was first described by (Weaver et al. 1998) in two genotypes known as P850029 and P851129. These lines were derived by crossing P721Q, a high lysine mutant, with a hard endosperm genotype. These genotypes were observed to have a quicker rate of α-kafirins digestion. In fact, (Weaver et al. 1998) reported ~90-95% α-kafirin digestion in 60 minutes in these genotypes compared to ~45-60% α-kafirin digestion with comparable, non-HD sorghum. The increased digestion rate of α-kafirin is significant in that kafirins make up roughly 80% of endosperm starch (Hamaker et al. 1986) and that α-kafirins are 80% of total kafirins (Shull et al. 1992). Both P850029 and P851129 have mostly soft, floury endosperm and are unacceptable agronomically due to yield and gain weathering. Combining this trait in hard endosperm, agronomically acceptable hybrids is needed and the process is underway.

There has been limited genetic studies and mapping of the HD trait. Originally, the HD trait was believed to be a simply inherited single gene. Winn et al. (2009) reported findings that contradict this and claimed that the HD trait is quantitatively inherited due to a wide range and distribution of protein digestibility ratings among the entries they evaluated. Winn et al. (2009) also found two antagonistic QTLs on chromosome 1 (i.e. one QTL significant with HD and one QTL significant for the normal digestibility). Given that several factors affect protein digestibility of sorghum grain, it is logical that a more accurate phenotyping method may be useful to characterize this trait.

A major problem in improving digestibility in sorghum is the difficulty in phenotyping the HD trait in a hard endosperm background. The presence of the HD trait is not visible to the naked eye and thus cannot be phenotyped quickly in the field. To date, three methods have been developed to identify the presence of the HD trait. An *in vitro* protein digestibility test using a pepsin assay was developed by (Mertz et al. 1984; Aboubacar et al. 2003). Another method, described by (Oria et al. 2000), uses a transmission electron microscope (TEM) to visually identify HD protein bodies. Unfortunately, both of these methods of identification have significant limitations. Teferra et al. (2018) reported that the pepsin assay for protein digestibility was at best inconsistent in phenotyping for the HD trait which was confounded further with genetic x environment interactions. Additionally, Teferra et al. (2019) proposed that the use of a field emission scanning electron microscope (FE-SEM) is an accurate method for phenotyping the HD trait. Currently, identification via FE-SEM is believed to be the most accurate method; however, this method is time consuming, laborious and expensive. Even with FE-SEM's limitations, it is believed to be the best available option.

Although there have been some genetic studies with the HD trait, there has been limited breeding with the trait, primarily due to the difficulty in phenotyping. The source of the HD trait isfrom genotypes that have soft endosperm (Weaver et al. 1998), which is agronomically undesirable and limits its application.

Over the past ten years, the Texas A&M Agrilife Research has been developing grain sorghum inbreds that possess both HD and waxy endosperm in combination with hard endosperm and agronomic desirability. The goal of this research is to assess the agronomics and flour functionality of waxy endosperm and/or high digestible protein grain sorghum hybrids with superior agronomics relative to traditional grain sorghum hybrids. The objectives are 1a) to identify of waxy endosperm sorghum hybrids that also possess the HD protein mutation using a field emissions scanning election microscope; 1b) to assess the yield potential of waxy and/or HD sorghum hybrids relative to a standard waxy check hybrid; and 1c) to evaluate the waxy endosperm trait for functionality and stability in quality over numerous environments.

2.2. Materials and Methods

2.2.1. Germplasm

Breeding crosses were made in the summer of 2015 with the intent to produce new and waxy pollinator parents for use in hybrid combination. F_1 crosses were selfpollinated in the winter nursery to create F_2 populations. Head to row selection was practiced in the summer seasons of 2016 and 2017. A total of 50 F_5 lines selected for combinations of waxy, and HD were testcrossed to seed parents to produce testcross hybrids. All of these testcross hybrids were evaluated in the summer of 2018; From this set, a final selection of the top 23 testcross hybrids were re-evaluated in 2019 for a second year. All twenty-three hybrids were homozygous for the waxy starch trait and varied for the HD protein trait (Table 1 and Table 2). The waxy hybrid that was used as an agronomic check was ATxARG-1/RTx2907 (Miller et al. 1992; Miller et al. 1996). It is also non-HD.

These 23 waxy grain sorghum hybrids were grown in two environments in 2018 and 2019 for a total of 4 environments. In 2018, tests were grown in College Station and Taft, Texas while in 2019, the tests were in Bishop and College Station, Texas. For the purpose of this study, College Station, Texas was designated as Central Texas whereas Bishop and Taft are both located near Corpus Christi, Texas and were designated as South Texas. All tests were rainfed and used standard agricultural practices that were appropriate for the environment. The experimental design for each test was a randomized complete block with two replications per environment. Each plot consisted of two rows 5.3 m in length with variable row spacing between 0.76 and 1.0 m. In these locations, agronomic practices standard to the region was used and all environments were rainfed.

Table 1. List of Waxy Sorghum Hybrids Tested from the Texas A&M AgriLife Research Sorghum Breeding Program and the Corresponding Pedigrees.

Hybrid Designation	Seed Parent Pedigree	Pollinator Parent Pedigree
A11029_wx/R17230_wx	(BTxARG-1/(RTx436*P850029)	RTx2907/P850029
A11029_wx/R17231_wx	(BTxARG-1/(RTx436*P850029)	RTx2907/P850029
A11030_wx/R17230_wx	(BTxARG-1/(RTx436*P850029)	RTx2907/P850029
ATxARG-1/R17010_wx	ATxARG-1	R11120/R08224
ATxARG-1/R17012_wx	ATxARG-1	R11120/R08224
ATxARG-1/R17013_wx	ATxARG-1	R11120/R08224
ATxARG-1/R17016_wx	ATxARG-1	R11120/R08224
ATxARG-1/R17021_wx	ATxARG-1	R11121/R05330
ATxARG-1/R17022_wx	ATxARG-1	R11121/R05330
ATxARG-1/R17023_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17034_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17035_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17036_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17037_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17038_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17039_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17040_wx	ATxARG-1	R11122/90EON343
ATxARG-1/R17041_wx	ATxARG-1	RTx2907//OB124/P851171
ATxARG-1/R17043_wx	ATxARG-1	RTx2907//OB124/P851171
ATxARG-1/R17044_wx	ATxARG-1	RTx2907//OB124/P851171
ATxARG-1/R17045_wx	ATxARG-1	RTx2907//OB124/P851171
ATxARG-1/R17049_wx	ATxARG-1	Sodamchal selection
ATxARG-1/RTx2907 (Waxy Hybid Check)	ATxARG-1	RTx2907

Table 2. List of Waxy Sorghum Hybrids Tested from The Texas A&M Agrilife Research Sorghum Breeding Program and Their Corresponding Phenotypes.

Hybrid/Trait	Grain Color	Plant Color
A11029_wx/R17230_wx	White	Purple
A11029_wx/R17231_wx	White	Purple
A11030_wx/R17230_wx	Red	Tan
ATxARG-1/R17010_wx	White	Tan
ATxARG-1/R17012_wx	White	Tan
ATxARG-1/R17013_wx	White	Tan
ATxARG-1/R17016_wx	White	Tan
ATxARG-1/R17021_wx	White	Tan
ATxARG-1/R17022_wx	White	Tan
ATxARG-1/R17023_wx	White	Tan
ATxARG-1/R17034_wx	Red	Tan
ATxARG-1/R17035_wx	Red	Tan
ATxARG-1/R17036_wx	White	Tan
ATxARG-1/R17037_wx	White	Tan
ATxARG-1/R17038_wx	Red	Tan
ATxARG-1/R17039_wx	White	Tan
ATxARG-1/R17040_wx	White	Tan
ATxARG-1/R17041_wx	White	Tan
ATxARG-1/R17043_wx	White	Tan
ATxARG-1/R17044_wx	White	Tan
ATxARG-1/R17045_wx	White	Tan
ATxARG-1/R17049_wx	Red	Purple
ATxARG-1/RTx2907 (Waxy Hybrid Check)	White	Tan

2.2.2. Kernel Characteristics, HD and Waxy Phenotyping

After harvest, grain samples from all locations were cleaned and prepared for secondary analysis using a Winterstieger LD180. Cleaned grain was sampled to measure kernel characteristics, high digestibility protein phenotyping and waxy characterization. The kernel characteristics of diameter, weight and hardness, were measured using a Kernel Hardness Test, Model 4100 (Perten Instruments). This machine takes the average of 300 individual kernels for each trait measured. Two samples of each entry are passed through the machine which records kernel hardness by crushing each seed. Kernel hardness is a relative measurement recorded as an index value. Seed weight and diameter are recorded for each kernel averaged as individual weight and diameter.

The endosperm tissue of a caryopses is triploid; a waxy phenotype requires homozygosity of the *wx* allele at all three loci. To confirm the presence of the waxy phenotype in kernel endosperm, a modified procedure developed by (Oria et al. 2000) was used (Pedersen et al. 2004). Two seed from each experimental hybrid was crushed and stained with potassium iodide. After one minute, the two seeds were scored either on the color of the endosperm: reddish brown (waxy) and bluish-black (non-waxy). In addition, endosperm was also visually scored: opaque (waxy) and translucent (non-waxy).

The HD mutation was phenotyped with a field emission-scanning electron microscope (FE-SEM) with a method that was described by Teferra (et al. 2019). For each hybrid, the pollinator parent was phenotyped for the HD trait because the seed parents were known to have normal digestibility and the hybrids will segregate for the trait if the respective pollinator parent was HD. Phenotyping the pollinator for the mutation because the line would be homozygous for the trait which will reduce any misclassification compared to phenotyping the hybrid, which would be heterozygous for the trait.

Each seed subjected to the FE-SEM was prepared by drying for twenty-four hours in a dryer at roughly 46^{o} Celsius and then submerged in liquid nitrogen for three minutes to reduce moisture content. Each seed was then split sagittal and mounted onto the microscope sample holder. To improve surface conductivity of the mounted samples and image quality, each sample was then sputter-coated with 80% platinum and 20% palladium.

2.2.3. Agronomic Traits

Data on several important agronomic traits were collected. Days to mid-anthesis was recorded as the days from planting to when 50% of a plot was at mid-flowering. Plant height and panicle exsertion were recorded at maturity just prior to harvest as the height in cm of a representative plant from the ground to the panicle tip and the distance from the flag leaf to the lowest rachis branch of the panicle, respectively. Each plot was harvested manually and threshed in the field using a Kincaid plot thresher in Central Texas. In South Texas, plots were combine harvested and grain samples were hand-harvested and brought to College Station for threshing. Grain yield was udjusted for 15% moisture. Percent moisture at harvest and test weight were measured at harvest using a Mini GAC 2500 (Dickie-john, Auburn, Illinois).

2.2.4. Waxy Flour Properties

Whole grain from the twenty-three hybrids was milled through a l mm screen. Flour pasting properties were recorded on the top seven agronomically performing hybrids using an RVA 4500 (Perten Instruments, Springfield, Illinois), which used a cup and stirrer. This provided peak viscosity, final viscosity, holding strength, breakdown value, setback value and pasting temperature. Water absorption index (WAI) and Water Solubility Index (WSI) were measured on flour samples from all twenty-three hybrids. Three checks, two homozygous non-waxy and one heterozygous waxy, that were grown in College Station, Texas in 2019 were included in the flour pasting, WAI and WSI profiles so that comparisons can be made between the starch types.

2.2.5. Statistical Analysis

Analysis of data was conducted using JMP®, Version *14* (SAS Institute). Individual environments for agronomic traits were first analyzed using the model ($Y = \alpha_i + \beta_j + \varepsilon$), where α=genotype (i=1…23), β=field replication (j=1,2) and $\varepsilon = error$. Normality of data was tested using the Shapiro-Wilk test and the data was transformed in order to normalize if necessary. Once deemed appropriate by homogeneity of variances, combined ANOVA was conducted to analyze all agronomical traits using the model ($Y = \alpha_i + \theta_k + \alpha\theta_{ik} + \beta(\theta)_{jk} + \varepsilon$), where α=hybrid (i=1…23), β=replication (j=1,2), θ=environments (k=1,2,3,4) and $\varepsilon = error$. Similarly, grain quality data (flour pasting, WSI and WAI) was analyzed first by individual environments using the model ($Y = \alpha_i + \beta_j + \varepsilon$), where α=genotype (i=1…23), β=laboratory replication (j=1,2) and ε=error. Once deemed appropriate by homogeneity of variances, combined ANOVA was conducted to analyze all dependent flour variables using the model ($Y = \alpha_i + \theta_k + \alpha\theta_{ik} + \beta_j + \varepsilon$), where α=genotype (i=1…23), β=laboratory replication (j=1,2), θ=environments (k=1,2,3,4) and $\varepsilon = error$. All means separations performed were done using a Fischer’s LSD test. The coefficient of variation (CV%) was used to determine consistency of data for a measured trait.

2.3. Results and Discussion

2.3.1. Kernel Characteristics, HD and Waxy Phenotyping Results

Eight of the pollinator lines were confirmed to have the HD trait; the remaining lines were classified as having wild-type, normal digestibile protein bodies (Table 3). The method of phenotyping this mutation with the FE-SEM developed by Teferra et al. (2019) was successful in identifying pollinator lines with the HD mutation (Figure 1). However, there are a few major disadvantages to this method, with the most notable one being the time investment that it takes for sample preparation and image collection, which can take up to an hour and a half for six individual seeds (two genotypes). If this trait is truly quantitatively inherited, as Winn et al. (2009) reported, phenotypes from a sizeable population across several environments are needed to obtain a useful quantitative trait loci. Regardless of inheritance, molecular markers or a high throughput phenotyping method is needed in order to improve this trait and incorporate it into commercial hybrids.

Table 3. List of Texas A&M Waxy Sorghum Pollinator Lines and Their Corresponding Phenotypes for the High Digestible (HD) Protein Mutation.

Pollinator Line	HD Trait Present	Pollinator Line	HD Trait Present
R17010_wx	No	R17038_wx	Yes
R17012_wx	No	R17039_wx	No
R17013_wx	Yes	R17040_wx	No
R17016_wx	No	R17041_wx	Yes
R17021_wx	No	R17043_wx	No
R17022_wx	No	R17044_wx	Yes
R17023_wx	No	R17045_wx	No
R17034_wx	Yes	R17049_wx	No
R17035_wx	Yes	R17230_wx	No
R17036_wx	Yes	R17231_wx	No
R17037_wx	Yes	RTx2907	No

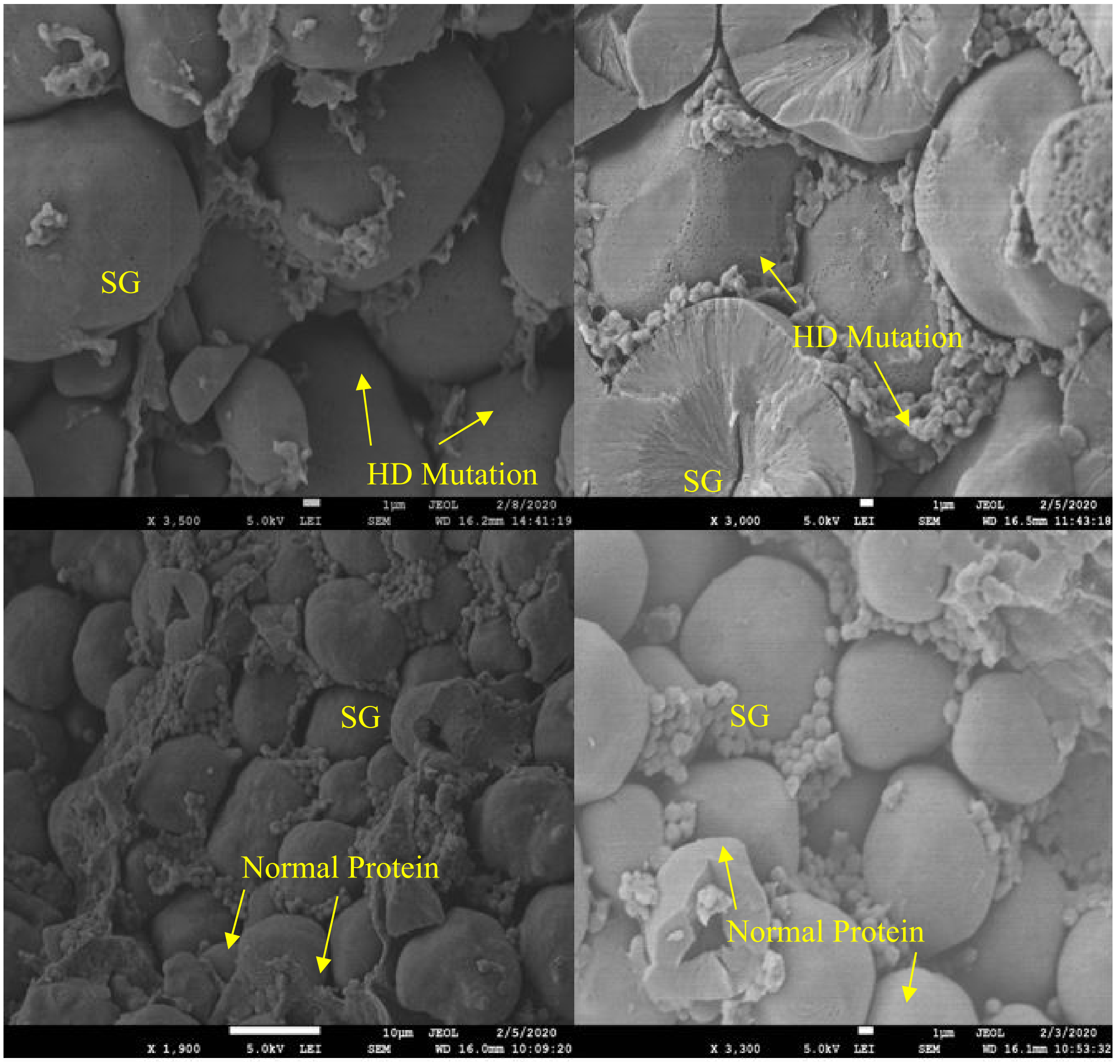

Figure 1. Field Emission-Scanning Electron Microscope (FE-SEM) images of Selected Texas A&M Waxy and Waxy HD Pollinator Lines. Top right: R17036_wx (HD) Top Left: R17034_wx (HD): Bottom Right: R17016_wx (Non-HD) Bottom Left: R17012_wx (Non-HD). SG: starch granule and HD: high digestible protein body mutation.

Genotype, environment and the interaction of genotype*environment were highly significant (P=<0.0001) for the three traits measured. Laboratory replications performed were not significant for any of the traits measured. The three R^2s are considerably high and the CV%s are within the acceptable range for the given traits (Table 4).

Table 4. Combined ANOVA and Summary Statistics for Kernel Hardness Index, Diameter and Weight Across Central and South Texas in 2018 and 2019 For the Twenty-Three Waxy Sorghum Hybrids.

		Hardness Index		Diameter (mm)		Weight (mg)	
Source of Variation	DF	MS		MS		MS	
Genotype	22	122.22	***	0.09	***	34.65	***
Genotype*Environment	66	19.71	***	0.01	***	7.44	***
Environment	3	1344.98	***	0.34	***	179.35	***
Laboratory Rep	1	0.23	ns	0.00	ns	0.02	ns
Error	91	1.34		0.00		0.75	
R^2		0.98		0.98		0.96	
Root Mean Square Error		1.16		0.03		0.86	
Mean of Response		80.85		2.61		27.12	
CV%		1.43		1.19		3.18	
Observations		184		184		184	

Note: *, ** and *** reflect significance at (*P*=<0.05), (P=<0.001) and (P<0.0001), respectively.

Significant variation for kernel hardness, diameter and weight was detected among the waxy hybrids and the check. It is a common perception that waxy starch hybrids have softer kernels and that softer sorghum kernels are more susceptible to grain weathering (Jambunathan et al. 1992). The average kernel hardness and diameter for non-waxy sorghum hybrids grown in the United States in 2015 were 67.1 and 2.61mm, respectively (Bard and Schroeder 2016) and the data in this test indicates that these hybrids are at or above the average for kernel hardness (Table 5). This implies waxy hybrids can have acceptable kernel hardness and diameter. Funnell-Harris et al. (2015), found that waxy sorghum inbreds were not more susceptible to grain weathering than non-waxy inbreds. Given past reports and the data herein, several of the tested waxy hybrids had improved agronomics compared to the waxy check and it should be possible to develop waxy sorghum hybrids with grain quality parameters that are competitive with non-waxy grain sorghum hybrids. Further testing to determine the true susceptibility of waxy sorghum hybrids to grain weathering is needed to make such claims but the observed data supports that waxy sorghum hybrids can be a viable option for sorghum grain production.

Multiple environments of testing indicated that the environment affects kernel characteristics. In fact, there was a 15% difference between environment with the heaviest seed and the lightest seed (Table 6). This variation has been observed for non-waxy sorghum hybrids (Maman et al. 2004) as well as for waxy sorghum hybrids. This will need to be taken into consideration when recommending waxy sorghum hybrids for commercial production in order to maximize yields.

Table 5. Combined Means Separation for Kernel Hardness Index, Diameter and Weight Across Central and South Texas in 2018 and 2019 for the Twenty-Three Waxy Sorghum Hybrids.

Hybrid/Trait	Hardness Index	Moisture (%)	Diameter (mm)	Weight (mg)
ATxARG-1/R17010_wx	85.92	11.85	2.41	23.67
ATxARG-1/R17012_wx	85.45	11.63	2.53	23.70
ATxARG-1/R17043_wx	85.18	11.73	2.62	26.31
ATxARG-1/R17013_wx	84.67	11.67	2.46	25.16
A11030_wx/R17230_wx	84.25	11.55	2.65	31.94
A11029_wx/R17230_wx	84.19	11.69	2.58	28.83
A11029_wx/R17231_wx	83.50	11.82	2.48	24.60
ATxARG-1/R17035_wx	83.06	11.54	2.70	27.79
ATxARG-1/R17021_wx	83.00	11.65	2.57	26.74
ATxARG-1/R17039_wx	82.57	11.39	2.74	28.62
ATxARG-1/R17044_wx	82.32	11.82	2.69	28.79
ATxARG-1/R17034_wx	81.85	11.85	2.70	28.47
ATxARG-1/R17045_wx	80.89	11.73	2.56	26.19
ATxARG-1/R17036_wx	79.34	11.77	2.71	26.56
ATxARG-1/R17038_wx	79.17	11.72	2.73	26.88
ATxARG-1/R17037_wx	78.87	11.74	2.65	27.99
ATxARG-1/RTx2907 (Waxy Hybrid Check)	78.58	11.79	2.65	27.85
ATxARG-1/R17040_wx	78.07	11.75	2.76	30.22
ATxARG-1/R17022_wx	77.94	11.84	2.53	26.50
ATxARG-1/R17016_wx	77.87	11.74	2.57	28.15
ATxARG-1/R17023_wx	77.46	11.73	2.73	29.00
ATxARG-1/R17041_wx	76.41	11.79	2.55	24.75
ATxARG-1/R17049_wx	69.02	11.90	2.43	25.02
LSD (α=0.05)	0.81	0.06	0.02	0.60

Table 6. Environmental Means Separation for Kernel Hardness Index, Diameter and Weight for Each Across Central and South Texas in 2018 and 2019 for the Twenty-Three Waxy Sorghum Hybrids.

Environment/Trait	Hardness Index		Diameter (mm)		Weight (mg)	
2018 Central Texas	76.6	D	2.64	B	27.38	B
2018 South Texas	88.33	A	2.52	D	25.26	D
2019 Central Texas	81.31	B	2.71	A	29.78	A
2019 South Texas	77.17	C	2.57	C	26.06	C

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

2.3.2. Waxy Agronomics

Of the main effects, hybrid, environment, pedigree*environment and field rep[environment] were all significant for yield, test weight and days to mid anthesis (Table 7). Hybrid, environment, and the interaction of the two were highly significant (P=<0.0001) for plant height and whereas pedigree was highly significant (P=<0.0001) for panicle exsertion. The R^2 for the models are relatively high and the CV%s are in the acceptable range for the given trait.

As expected grain yield of these hybrids varied among genotypes and environments. Compared to the existing waxy check hybrid, ATxArg-1/RTx2907, several of these hybrids (ATxARG-1/R17041_wx, ATxARG-1/R17013_wx and ATxARG-1/R17040_wx) are significantly improved for grain yield with little to no change in basic agronomic traits (Table 8). This implies that these hybrids have commercial potential if a waxy grain sorghum market develops.

Table 7. Combined ANOVA and Summary Statistics for Yield, Test Weight, Days to Anthesis (DY), Plant Height (PH) and Panicle Exsertion (EX) Across Central and South Texas in 2018 and 2019 for the Twenty-Three Waxy Sorghum Hybrids.

		Yield ($kg\ ha^{-1}$)		Test Weight ($kg\ hL^{-1}$)		Days to Mid Anthesis (d)		Plant Height (cm)		Panicle Exsertion (cm)	
Source of Variation	DF	MS		MS		MS		MS		MS	
Hybrid	22	870520	***	4.68	***	28.59	***	1120.79	***	78.92	***
Environment	3	3333296	***	790.70	***	1230.21	***	1666.04	***	37.12	ns
Environment*Hybrid	66	692329	***	3.26	**	4.47	**	50.04	ns	21.26	ns
REP [Environment]	4	1444216	**	7.86	**	11.65	**	23.32	ns	20.55	ns
Error	88	288216		1.51		2.62		36.15		22.75	
R^2		0.76		0.95		0.95		0.91		0.62	
Root Mean Square Error		536.86		1.10		1.62		6.01		4.77	
Mean of Response		4149.34		72.34		77.06		120.91		11.10	
CV%		12.94		1.90		2.10		4.97		42.97	
Observations		184		184		184		184		184	

Note: *, ** and *** reflect significance at (*P*=<0.05), (P=<0.001) and (P<0.0001), respectively.

Table 8. Combined Means Separation for Yield, Test Weight, Days to Anthesis (DY), Plant Height (PH) and Panicle Exsertion (EX) Across Central and South Texas in 2018 and 2019 for the Twenty-Three Waxy Sorghum Hybrids.

Hybrid/Trait	Yield (kg ha^{-1})	Test Weight (kg hL^{-1})	Days to Mid Anthesis (d)	Plant Height (cm)	Panicle Exsertion (cm)
A11030_wx/R17230_wx	4,932	73	76	153	11
ATxARG-1/R17041_wx	4,717	72	71	118	17
A11029_wx/R17231_wx	4,548	72	77	111	6
ATxARG-1/R17013_wx	4,477	72	76	110	9
ATxARG-1/R17040_wx	4,470	73	78	118	11
A11029_wx/R17230_wx	4,454	73	78	154	10
ATxARG-1/R17034_wx	4,256	73	77	121	13
ATxARG-1/R17035_wx	4,230	72	76	120	11
ATxARG-1/R17038_wx	4,172	72	78	123	14
ATxARG-1/R17043_wx	4,168	73	74	117	13
ATxARG-1/R17039_wx	4,114	72	76	120	15
ATxARG-1/R17023_wx	4,102	71	79	115	6
ATxARG-1/R17036_wx	4,092	74	77	119	12
ATxARG-1/R17045_wx	4,078	71	74	121	16
ATxARG-1/R17044_wx	4,048	72	75	110	9
ATxARG-1/R17037_wx	4,032	72	77	125	15
ATxARG-1/R17012_wx	3,901	73	78	111	12
ATxARG-1/R17016_wx	3,839	74	77	133	10
ATxARG-1/R17049_wx	3,838	72	76	122	11
ATxARG-1/RTx2907 (Waxy Hybrid Check)	3,828	70	77	111	8
ATxARG-1/R17010_wx	3,751	73	78	117	10
ATxARG-1/R17022_wx	3,749	72	79	112	6
ATxARG-1/R17021_wx	3,636	73	79	111	10
LSD (α=0.05)	533	1	2	6	5

Variation across environments were also observed for the agronomic traits measured (Table 9). For example, yield varied 15% (650 kg ha^{-1}) between the highest and the lowest yielding environment. Additionally, the best and poorest yielding environments came from the same physical location (College Station, Texas) in different years. There are important environmental factors within each year, such as rainfall and temperature that affect performance of any genotype. This complex genotype by environment interaction observed here is not fully understood for waxy sorghum hybrids and may merit further investigation.

Table 9. Environmental Means Separation for Yield, Test Weight, Days to Anthesis (DY), Plant Height (PH) And Panicle Exsertion (EX) Across Central and South Texas in 2018 and 2019 for the Twenty-Three Waxy Sorghum Hybrids.

Environment/Trait	Yield ($kg\ ha^{-1}$)		Test Weight ($kg\ hL^{-1}$)		Days to Mid Anthesis (d)		Plant Height (cm)		Panicle Exsertion (cm)	
2018 Central Texas	4,483	A	74	B	81	A	123	B	10	B
2018 South Texas	4,194	B	66	D	69	D	114	D	12	A
2019 Central Texas	3,833	C	72	C	10	B	128	A	11	AB
2019 South Texas	4,087	B	77	A	78	C	119	C	11	AB

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

The top three yielding waxy and waxy HD hybrids in each environment were selected and compared in order to make further claims on how these waxy and waxy HD hybrids perform across environments (Table 10). There was crossover interaction for both of the hybrid classifications (waxy and waxy HD) for the top three waxy hybrids in each location, so the same three hybrids were not necessarily the same in each environment. However, the best performing waxy and waxy HD hybrids tended to consistently be in the top of the trial. It is also important to note that some hybrids, such as ATxARG-1/RTx17041_wx, were both waxy and HD and had high yields.

The waxy hybrids and the waxy HD hybrids outperformed the check hybrid, which was waxy non-HD, in every environment with the exception that the check outperformed the top three waxy HD hybrids in 2019 South Texas by 7.5% (Table 10). The largest difference between the check and the waxy hybrids was in 2019 Central Texas, where a 33.7% difference was observed. In every environment, the waxy non-HD outperformed the waxy HD, with the largest difference being 18.3%. Overall, the average yield for the top three waxy and waxy-HD hybrids were 10% and 24% higher than the waxy hybrid check.

Average yields for select waxy and waxy HD hybrids were 5,000 kg ha^{-1} and 4,250 kg ha^{-1}, respectively (Table 10). Additional yield tests that include waxy, non-waxy, waxy HD and non-waxy non-HD hybrids are still needed to make direct comparisons between the two types of starch and how they impact yield. The data herein supports observation by Rooney et al. (2005) that improved parental waxy lines can be produced making commercially viable waxy sorghum hybrids feasible. The current study also supports that claims by Jampala (et al. 2012) that combining the waxy and HD traits does not inherently reduce grain yield potential.

Table 10. Environment Means Separation for Top Three Yielding Waxy and Waxy High Digestible Protein (HD) Hybrids in Each of the Tested Texas Environments.

Environment	Waxy Hybrid Yield (kg ha^{-1})	Waxy HD Hybrid Yield (kg ha^{-1})	Waxy Check Hybrid Yield (kg ha^{-1})
2018 Central Texas	5,293 A	4,692 A	4,396 A
2018 South Texas	5,025 A	4,106 B	3,145 B
2019 Central Texas	5,056 A	4,131 B	3,354 AB
2019 South Texas	4,812 A	4,081 B	4,417 A

Notes: Values connected by a common letter in a column are not significantly different at α=0.05.The top three hybrids were not necessarily the same hybrids in each location.

2.3.3. Waxy Flour Functionality

The main effects of hybrid, environment and the interaction of hybrid*environment were highly significant (P=<0.0001) for all flour quality traits measured (Table 11).

Of the main effects, hybrid had the largest effect on flour pasting traits with the waxy hybrids consistently higher for peak viscosity and breakdown value (Table 12). The non-waxy and heterozygous waxy check hybrids had higher setback, pasting temperature and final viscosity values than waxy hybrids (Table 13). Graybosch (1998) observed similar results between the waxy and non-waxy pasting properties in wheat flour. It should be noted that quality of checks was evaluated only in 2019 and 2019 generally had higher values. Overall, variation exists among the experimental waxy genotypes for all the flour pasting traits measured.

Table 11. Combined ANOVA and Summary Statistics for Peak Viscosity, Holding Strength, Breakdown Value, Final Viscosity, Setback Value and Pasting Temperature Across Central and South Texas in 2018 and 2019 for the Top Seven Waxy Sorghum Hybrids.

		Peak Viscosity		Holding Strength		Breakdown Value (mPa.s)		Final Viscosity		Setback Value		Pasting Temperature (^{o}C)	
Source of Variation	DF	MS		MS		MS		MS		MS		MS	
Hybrid	6	122216	***	7178.8	***	113371	***	308376.4	***	291716.5	***	2.909642	***
Lab Replication	1	418	ns	44.6	ns	311	ns	185.8	ns	236.2	ns	0.008502	ns
Environment	3	5221326	***	339668.3	***	3002668	***	812147.5	***	108455.1	***	0.792645	***
Hybrid*Environment	18	114251	***	12528.2	***	59539	***	53646.3	***	17016.6	***	0.712554	***
Error	27	5963		659.8		3105		2902		1281.2		0.071	
R^2		0.99		0.99		0.99		0.99		0.99		0.94	
Root Mean Square Error		77.22		25.69		55.72		53.87		35.79		0.27	
Mean of Response		1939.98		950.04		989.43		1953.00		1002.30		79.08	
CV%		3.98		2.70		5.63		2.76		3.57		0.34	

Note: *, ** and *** reflect significance at (*P*=<0.05), (P=<0.001) and (P<0.0001), respectively.

Table 12. Combined Means Separation for Peak Viscosity, Holding Strength and Breakdown Value Across Central and South Texas in 2018 and 2019 for the Top Seven Waxy Sorghum Hybrids.

Hybrid/Trait	Peak Viscosity		Holding Strength mPa.s		Breakdown Value	
ATxARG-1/R17013_wx	2102	A	967	A	1135	A
ATxARG-1/R17049_wx	2066	A	915	A	1151	A
ATxARG-1/R17034_wx	2041	A	992	A	1046	AB
ATxARG-1/R17041_wx	1870	AB	979	A	891	ABC
ATxARG-1/R17040_wx	1851	AB	918	A	933	AB
ATxARG-1/R17035_wx	1837	AB	941	A	896	ABC
ATxARG-1/R17038_wx	1813	AB	939	A	874	ABC
ATx642/RTx436 (Non-Waxy Check Hybrid)	1672	AB	1043	A	629	ABC
AH14/RTx436 (Non-Waxy Check Hybrid)	1343	AB	917	A	427	BC
ATxArg-1/RTx436 (Heterozygous Waxy Check Hybrid)	1043	B	836	A	207	C

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

Table 13. Combined Means Separation for final viscosity, setback value and pasting temperature Across Central and South Texas in 2018 and 2019 for the Top Seven Waxy Sorghum Hybrids.

Hybrid/Trait	Final Viscosity		Setback Value		Pasting Temperature (°C)	
	mPa.s					
AH14/RTx436 (Non-Waxy Check Hybrid)	3335	A	2419	A	85.5	B
ATx642/RTx436 (Non-Waxy Check Hybrid)	3048	A	2005	B	81.4	C
ATxARG-1/R17013_wx	2035	B	1069	DE	78.1	E
ATxARG-1/R17034_wx	2068	B	1071	DE	79.6	D
ATxARG-1/R17035_wx	2151	B	1210	C	79.5	D
ATxARG-1/R17038_wx	2098	B	1159	CD	79.6	D
ATxARG-1/R17040_wx	1948	BC	1030	E	79.3	D
ATxARG-1/R17041_wx	1749	CD	770	F	79.2	D
ATxARG-1/R17049_wx	1622	D	707	F	78.4	E
ATxArg-1/RTx436 (Heterozygous Waxy Check Hybrid)	3118	A	2282	A	87.5	A

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

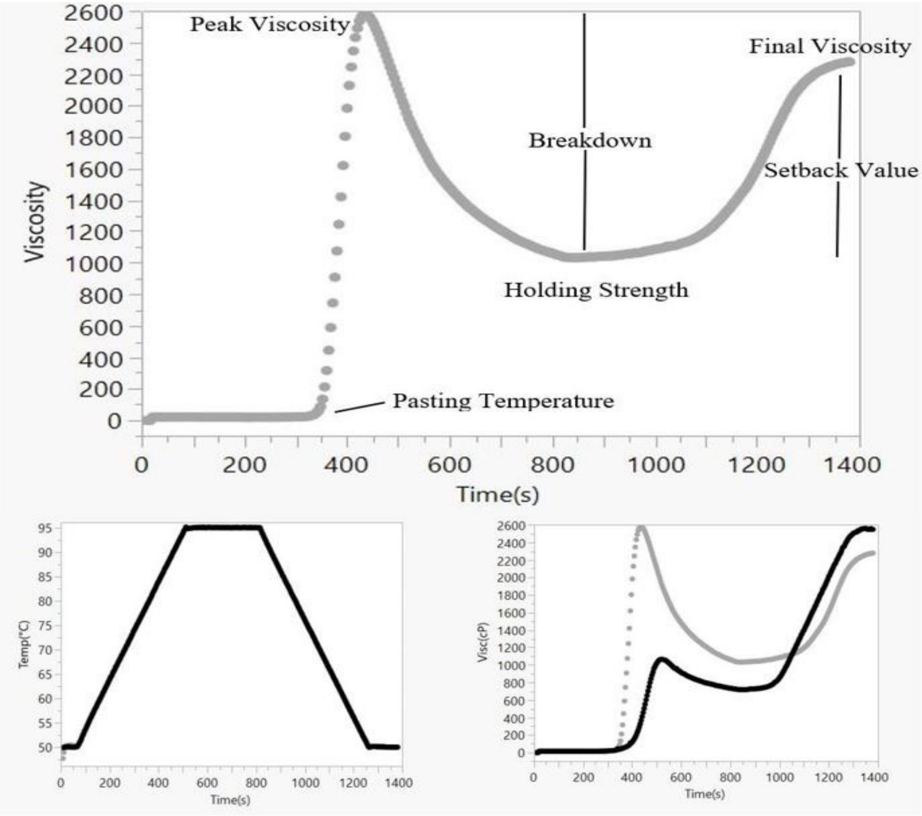

Figure 2. Top: Representation of Measurements Recorded from RVA 4500 (Perten Instruments). Bottom Left: Depiction of how the Temperature Changes Throughout Time Bottom Right: Depiction of How the Viscosity of A Sample Changes Throughout Time/ Heat. Flour Pasting Graphs Of Waxy (Gray Line) And Non-Waxy (Black Line).

A depiction of flour quality measuremntes is displayed in figure 2. The environment also affected all flour pasting traits except for pasting temperature (Table 14 and 15). As examples, the overall peak viscosity mean in 2019 South Texas is nearly twice the mean of 2019 Central Texas, and the breakdown mean in 2019 South Texas is

2.5x that of the mean in 2019 Central Texas (Table). The 2019 South Texas environment was consistently in the top group for all measured traits, whereas 2019 Central Texas was in the lowest group for all measured traits. This consistency in the ranking of the measured traits can be beneficial for determining the production region that is best suited for the end use purpose.

Table 14. Environmental Means Separation for Peak Viscosity, Holding Strength and Breakdown Value Across Central and South Texas in 2018 and 2019 for the Top Seven Tested Waxy Sorghum Hybrids.

Environment/Trait	Peak Viscosity		Holding Strength		Breakdown Value	
			(mPa.s)			
2018 Central Texas	1861	B	892	C	676	C
2018 South Texas	1602	C	926	B	966	B
2019 Central Texas	1474	D	1172	A	1652	A
2019 South Texas	2824	A	810	D	664	C

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

Table 15. Environmental Means Separation for Final Viscosity, Setback Value and Pasting Temperature Across Central and South Texas in 2018 and 2019 for the Top Seven Waxy Sorghum Hybrids.

Environment/Trait	Final Viscosity		Setback Value		Pasting Temperature	
	(mPa.s)				(ºC)	
2018 Central Texas	1895	B	969	C	79.1	A
2018 South Texas	1905	B	1010	B	79.1	A
2019 Central Texas	2291	A	1119	A	79.3	A
2019 South Texas	1721	C	911	D	78.8	B

Values connected by a common letter in a column are not significantly different at α=0.05.

The effects of genotype, environment, and the interaction of genotype by environment were all highly significant (P=<0.0001) for water solubility index (WSI) and water absorption index (Table 16).

Table 16. Combined ANOVA and Summary Statistics for Water Solubility Index (WSI) and Water Absorption Index (WAI) Across Central and South Texas in 2018 and 2019 for the Twenty-Three Tested Waxy Sorghum Hybrids.

Source of Variation	DF	Water Solubility Index (g/g) MS		Water Absorption Index (g/g) MS	
Genotype	22	4.64	***	0.07	***
Lab Replication	1	0.23	ns	0.01	ns
Environment	3	10.94	***	0.14	***
Genotype*Environment	66	2.32	***	0.03	***
Error	91	0.13		0	
R^2		0.96		0.91	
Root Mean Square Error		0.36		0.07	
Mean of Response		8.53		2.43	
CV%		4.17		2.76	
Observations		184		184	

Note: *, ** and *** reflect significance at (*P*=<0.05), (P=<0.001) and (P<0.0001), respectively.

Considerable variation for WSI among the waxy hybrids - a 30% difference existed between the waxy hybrids with the highest and lowest WSI (Table 17). All of the tested waxy hybrids had significantly higher WSI compared to the non-waxy and heterozygous waxy checks. Additionally, several of the tested waxy hybrids had higher WSI than the agronomic waxy hybrid check, ATxArg-1/RTx2907. Interestingly, the heterozygous waxy check had the lowest WSI out of all tested hybrids and may be due to unique interactions between the amylose and amylopectin starch molecules or other kernel characteristics that were not studied herein (Vamadevan and Bertoft 2020).

There was not a distinct separation between waxy and non-waxy hybrids for WAI as there was for WSI. The hybrid with the highest WAI was a non-waxy check and the hybrid with the second highest WAI was the heterozygous waxy check. One of the non-waxy check and the heterozygous waxy check had a significantly higher WAI than all of the waxy hybrids tested. However, several of the experimental waxy hybrids had significantly higher WAI than the other non-waxy check. Additionally, there was observed variation among the tested waxy hybrids for WAI. There was a 14% difference between the experimental waxy hybrid with the highest WAI and the experimental waxy hybrid with the lowest WAI.

The environment affected both of these traits with a 12% difference in WSI between 2019 South Texas and 2019 Central Texas (Table 18). The range of means was less for WAI, but they remained statistically different. While there were differences in both traits due to environment, there was no trend as to the specific source for that variation. For example, both WAI and WSI differed due to both location and year.

Table 17. Combined Mean Separation for Water Solubility Index (WSI) and Water Absorption Index (WAI) Across Central and South Texas in 2018 and 2019 for the Twenty-Three Tested Waxy Sorghum Hybrids.

Hybrid/Trait	Water Solubility Index (g/g)	Water Absorption Index (g/g)
ATxARG-1/R17013_wx	9.52	2.53
ATxARG-1/R17041_wx	9.44	2.49
ATxARG-1/R17012_wx	9.36	2.35
ATxARG-1/R17022_wx	9.34	2.35
ATxARG-1/RTx2907 (Waxy Hybrid Check)	9.18	2.34
ATxARG-1/R17040_wx	9.17	2.33
ATxARG-1/R17016_wx	9.09	2.32
ATxARG-1/R17021_wx	8.93	2.39
ATxARG-1/R17035_wx	8.87	2.5
ATxARG-1/R17038_wx	8.8	2.42
ATxARG-1/R17034_wx	8.78	2.4
ATxARG-1/R17010_wx	8.69	2.33
ATxARG-1/R17023_wx	8.63	2.39
ATxARG-1/R17039_wx	8.52	2.48
ATxARG-1/R17043_wx	8.51	2.7
ATxARG-1/R17037_wx	8.42	2.42
A11029_wx/R17230_wx	8.05	2.39
ATxARG-1/R17044_wx	8	2.46
A11029_wx/R17231_wx	7.87	2.33
ATxARG-1/R17045_wx	7.51	2.48
A11030_wx/R17230_wx	7.41	2.5
ATxARG-1/R17036_wx	7.32	2.47
ATxARG-1/R17049_wx	6.71	2.53
ATx635/RTx436 (Non-Waxy Hybrid Check)	6.11	3.01
AH14/RTx436 (Non-Waxy Hybrid	6.07	2.44
ATxArg-1/RTx436 (Heterozygous Waxy Hybrid Check)	4.38	2.86
LSD (α=0.05)	1.08	0.14

Table 18. Environmental Means Separation for Water Solubility Index (WSI) and Water Absorption Index (WAI) for Across Central and South Texas in 2018 and 2019 for the Tested Twenty-Three Waxy Sorghum Hybrids.

Location/Trait	Water Solubility Index (g/g)		Water Absorption Index (g/g)	
2018 Central Texas	8.42	B	2.46	A
2018 South Texas	8.31	B	2.43	B
2019 Central Texas	9.24	A	2.48	A
2019 South Texas	8.14	C	2.35	C

Values in a column connected by a common letter are not significantly different at α=0.05.

One of the primary interests in waxy sorghum hybrids is for ethanol production. The results observed herein document the importanc of both genotypic and environmental affect the utility of specific hybrid and environment combinations. For example, variation for ethanol fermentation efficiency is known to exist for different types of sorghums. Zhao et al. (2008) reported a strong negative correlation between ethanol yield and final viscosity, (ie, ethanol yield increased as final viscosity decreased). In this study, waxy hybrids had lower final viscosity than the non-waxy checks (39% lower on average) and the heterozygous waxy check (37% lower on average) (Table 13). Additionally, all of the waxy hybrids had a lower pasting temperature than the heterozygous waxy and non-waxy checks (Table 13). The lower pasting temperatures of the waxy hybrids are important in that it means it requires less heat (energy) to convert the waxt starch to ethonal. Wu et al. (2007) reported a 22% variation in ethanol yield and a 9% variation in fermentation efficiency for different types of sorghums with waxy sorghum hybrids across different environments. Given the variation observed with in the waxy hybrids herein, it is likely a same range among them would exist as well, but all would be better than non-waxy grain sorghum.

It is important to note that the heterozygous waxy hybrid check, which produces 75% non-waxy and 25% waxy grain, had pasting temperatures that were higher than both the non-waxy checks and the waxy hybrids. Since lower pasting temperatures are desired, especially for ethanol production, the heterozygous waxy hybrid would not be undesirable. There could be a unique interaction between the amylose and amylopectin molecules or other kernel factors affecting this. Regardless, additional studies would need to be done in order to make broad claims about heterozygous waxy hybrids' usefulness.

In addition to ethanol production, food processors are interested in waxy grain sorghum hybrids because it performs better under freeze/thaw conditions than non-waxy starches (Mohd Azemi and Wootton 1984). Wheat flour is the standard for performance because wheat flour based products have better dough functionality due to its water holding ability (Dexter et al. 1994). One way to improve sorghum-based food products is to improve its water holding ability. Evaluation of WAI and WSI among the tested hybrids indicates that waxy sorghum hybrids are not significantly different than normal non waxy hybrids for WAI but they have consistently higher WSI values. Elhassan et al. (2015) reported a similar result for WSI and that the waxy trait increase the solubility of the starch, which was also observed here. As both of these variables influence water holding capacity the increases in WSI and while maintaining WAI implies that waxy grain sorghum is an adequate candidate for use in batter-based food processing. Further, the variation among the tested waxy hybrids, indicates that improvement should be possible.

The environmental variation observed here is of no surprise. Variation among and within environments for flour quality is known and has been reported in other crops. For example, significant genotype by environment affects have been reported for wheat flour functionality (Bassett et al. 1989). Likewise, Akingbale and Rooney (1987) reported a significant year effect on sorghum flour pasting properties. Environmental factors that affect grain quality and functionality cannot always be controlled, however, these affects can be mitigated by marketing this grain to the optimal environments.

2.4. Chapter Summary

Identification of the HD trait using a FE-SEM was successful. Eight of the twenty-three pollinator lines were confirmed to be homozygous HD. Although, the FE-SEM was successful in phenotyping the HD trait, it was determined to be too laborious, expensive, time consuming and cumbersome to be used as a breeding tool.

Nine-teen of the twenty-three experimental waxy pollinators, when in test cross hybrid combinations, agronomically outperformed the standard Texas A&M Sorghum Breeding Program's waxy hybrid check. Additionally, the top three yielding waxy hybrids and waxy HD hybrids outperformed the waxy check hybrid in all environments, with the exception that the waxy check hybrid narrowly outperformed the waxy HD hybrids in one environment. The data from this study shows that the test experimental waxy pollinator lines could be used to produce agronomically favorable waxy sorghum hybrids.

The functionality tests performed herein show that the tested waxy sorghum hybrids have distinct performance compared to the non-waxy and heterozygous waxy checks. It was observed that the tested waxy hybrids produced flour that had higher peak viscosities, higher breakdown values, and higher WSIs than the check hybrids. Additionally, these hybrids also had lower WAIs, setback values and pasting temperature than the check hybrids. It was also observed that the environment drastically affected the flour properties of the test waxy hybrids.

This research and data observed herein may be used as a foundation for further research regarding waxy and waxy-HD sorghum. Further investigations into how the waxy and HD traits affect grain yield in hybrid combinations would be merited. Additionally, investigations into the environmental influence on the flour functionality of waxy hybrids would be merited.

3. ASSESSING THE EFFECT OF THRESHING METHOD AND HARVEST MATURITY ON POPPING EFFICIENCY IN SORGHUM

3.1. Background

Sorghum is one of several cereal grains such as maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum*) and barley (*Hordeum vulgare*) that can be popped (Huang et al. 2018). Like popcorn, sorghum produces a generally spherical puffed kernel but the popped kernels are significantly smaller than popped corn and they do not convey the flavor associated with corn. The smaller size of the popped sorghum and different flavor profile provides opportunities to fill niche markets that are not accessible to popcorn. In some situations, popped sorghum is superior to popcorn due to its tenderness and reduction of hulls (Rao and Murty 1982). Conversely, minimal breeding and research on popped sorghum hinders the ability of sorghum to compete with popcorn or fill niche markets.

Popped sorghum is a popular snack in India and Africa (Parker et al. 1999). In addition, popped sorghum possesses unique nutritional values compared to grain sorghum – popped sorghum has higher protein and starch digestibility compared to non-popped sorghum (Saravanabavan et al. 2013). This phenomenon could lead to an increase of pop sorghum flour-based products and animal feed. One disadvantage, however, is that dietary fiber drop in the popped grain (Llopart and Drago 2016).

Two methods, popping and puffing, are commonly used when processing expanding kernel sizes of corn and sorghum. Popping applies high temperatures at rapid speed to the kernel which causes high internal moisture pressure within the kernel. Once this internal pressure surpasses the strength of the endosperm and pericarp, it forcefully ruptures the pericarp, resulting in a popped kernel (Mishra et al. 2014). Grain puffing, in contrast, uses heat and high pressure to cause an instantaneous release of water vapor in a pre-gelatinized kernel to create a puffed kernel.

Popcorn quality is primarily measured by the expansion ratio and popping efficiency (Song et al. 1991). Expansion ratio is defined by dividing the volume after popping by the volume before popping of the popcorn kernels. Popping efficiency is defined as the percent of kernels that pop after being subjected to the popping method. With a long history in breeding for improved popcorn quality, popcorn now has roughly 95% popping efficiency and can have expansion ratios upwards of 35:1 (Lyerly 1942; Pordesimo et al. 1990).

Several grain quality attributes influence the quality of the popcorn including moisture content, kernel size, endosperm type and physical endosperm structure (Pordesimo et al. 1990; Pordesimo et al. 1991). These same factors likely influence pop sorghum quality but have yet to be tested. The inverse relationship between vitreous (hard) and floury (soft) endosperm influences popping quality. In all popped kernels, the light, white airy component is composed of denatured starch that is instantly gelatinized upon popping due the release of pressure and drop in temperature (Parker et al. 1999).

There are two shapes popcorn kernels pop into: butterfly and mushroom. The butterfly shape is an elongated, irregular shape whereas the mushroom shape is a generally spherical. Both shapes have unique qualities that make them desirable for either commercial or residential popping applications (Eldredge and Thomas 1959).

While that these two shapes may also exist in popped sorghum, they have not been identified or described. In addition, grain size, pericarp and endosperm to germ ratio are known to affect the popping efficacy in sorghum (Rooney and Murty 1981; Murty et al. 1988). Because these factors are all under genetic control, higher popping capacity is specific to genotypes possessing specific kernel characteristics. These superior popping genotypes possess a hard endosperm and high endosperm to germ ratio that results into high popping efficiency and expansion ratio, spherical popped shape, tender texture and favorable taste (Hoseney et al. 1983; Llopart and Drago 2016).

Even with the right genetics, extraneous factors influence popping capacity. In popcorn, mechanical damage during harvest (which appears as chipped and cracked kernels) reduces popping efficiency (Singh et al. 1997). These cracks cause structural weaknesses in the pericarp and endosperm of the kernel and this reduces popping efficiency by allowing avenues for the escape of water vapor. Using a razor blade to induce pericarp damage, (Singh et al. 1997) reported a 9.1%-47.5% reduction in expansion ratio of damaged popcorn kernels. Similarly, (Goneli et al. 2007) reported a reduced on expansion ratio with mechanically damaged popcorn. For these reasons, popcorn is often harvested at higher moisture or by the ear to minimize damage. Similar mechanical damage is possible to occur in sorghum, but this issue has not been studied to date.

Compared to popcorn, pop sorghum has had notably less breeding and research. (Murty et al. 1988) reported that sorghum popping was a quantitatively inherited trait. (Rooney and Rooney 2013) reported that variation among sorghum genotypes exist for popping ability. Pugh et al., (2017) used a recombinant inbred lines population and determined popping efficacy is influenced by environmental, genotypic and genotypic x environmental factors and is moderately to highly heritable. These studies indicate that it is possible to select for high-yielding sorghum genotypes with superior popping qualities. To further the development of pop sorghum, it is important to identify sources of popping variation, determine if threshing methods affect popping characteristics and to assess moisture content at harvest affects kernel quality.

The objective of this research is to identify grain sorghum genotypes that possess superior popping ability and investigate whether harvest methods and harvest maturities cause kernel damage that affects popping quality. The subobjectives are 2a) to evaluate the popping attributes of four sorghum hybrids and three inbred lines for popping attributes; 2b) to test five different methods of threshing methods for their effect on popping attributes; and 2c) to test the effects of maturity and moisture content at time of harvest has on popping attributes.

3.2. Materials and Methods

3.2.1. Germplasm

Seven sorghum genotypes tested previously and known to have relatively high popping efficiency (PE), expansion ratio (ER) and flake size (FS) were used in this study. Four of the seven genotypes are potential commercial pop sorghum F_1 hybrids and the other three genotypes are inbred lines that are known to have popping and food qualities (Rooney and Rooney, 2013; Pugh et al., 2017) (Table 19). ATx642, ATx2928 and AHF14 (Tx3447) are seed parents and RTx436 is a pollinator parent released from

Texas A&M AgriLife (Rooney 2003). R06321 and R11171 are experimental Texas A&M pollinator lines. Sureno is a inbred line with food quality attributes (Meckenstock et al. 1993). Macia is a cultivar of ICRISAT origin that has been released in numerous Southern African countries (Setimela et al. 1997). Sumac is an old forage/grain sorghum originally from Southern Africa and grown widely throughout Texas (Vinall et al. 1936). Numerous reselections of Sumac were made by producers over the years, eventually resulting an early sumac version officially released by Kansas State in 1922 (Vinall et al. 1936). Some of these versions of sumac are still used as pollinator parents for forage sorghum hybrids. The lines included in this study provide a base popping standard for the hybrids (Rooney and Rooney 2013).

All of these entries were grown in College Station, Texas, in the 2019 growing season and was grown under normal, rainfed, agronomic practices. The test was planted in a randomized complete block design with two field replications. Each treatment in the test had plot size of two 5.5 m rows.

Table 19. List of Texas A&M Sorghum Genotypes with Popping Attributes That Were Grown in 2019 College Station, Texas.

Genotype Designation	Type	Grain Color	Plant Color	1000 Kernel Weight (g)	Kenrel Hardness Index (x:1)
ATx642/R06321	F1 Hybrid	Yellow	Pigmented	30.0	80.5
ATx642/R11171	F1 Hybrid	Yellow	Pigmented	31.6	81.0
AHF14/RTx436	F1 Hybrid	White	Tan	26.0	79.0
ATx2928/RTx436	F1 Hybrid	White	Tan	25.2	80.6
Macia	Inbred Line	White	Tan	28.6	83.8
Sureno	Inbred Line	White	Tan	24.3	96.3
Sumac	Inbred Line	Brown (Red3)	Pigmented	16.0	64.4

3.2.2. Agronomic Traits

Data was collected on several agronomic traits that are important for production and/or influence grain production and popping. Days to mid-anthesis was the number of days from planting to when 50% of a plot is flowering. Plant height and panicle exsertion was recorded (cm) just prior to harvest as the height of representative plants from the ground to the panicle tip and the distance from the flag leaf to the lowest rachis branch of the panicle, respectively. For each treatment, whole plots were hand harvested to estimate yield potential and for samples for processing.

For each entry, grain was harvested at both a high and low moisture content levels (harvest maturity) which corresponds to an early and late harvest. The target range for the high moisture was 17-21% moisture and the target range for the low moisture treatment was 10-15% moisture. Immediately after hand harvest, the panicles of each genotype were divided and threshed using five different methods. These were a: i) John Deere 3300 combine modified for plot harvest; ii) an Almaco belt thresher; iii) a Kincaid plot thresher, iv) a Wintersteiger LD180 thresher and v) hand threshed. Because these threshing methods use different approaches to separate the grain from the panicle, they are likely to cause varying levels of kernel damage (Table 21). After threshing, each sample was dried to a storage moisture content (<12%).

Table 20. Threshing Methods That the Texas A&M sorghum Genotypes with Popping Attributes Were Subjected to and their Expected Damage.

Threshing Method	Action	Expected Physical Damage
JD3300 Combine	Metal concave beaters, high impact	High
Kincaid Plot Thresher	Metal concave beaters, high impact	High
Wintersteiger Thresher	High speed brushes, moderate friction	High
Almaco Belt Thresher	Rubber belts, moderate friction	Medium
Hand Threshed	Light hand pressure and friction	Low

3.2.3. Popping Methodology

Samples were stored in cold storage for a minimum for three months inorder to bring all samples to a stable storing moisture. Samples were then prepared for popping tests by adjusting grain moisture content of all samples to 14% as this was reported as an optimum popping moisture content (Gaul and Rayas-Duarte 2008). All popping replications contained 500 counted seed and prior to popping, all samples were verified to be free of glumes and any apparent broken kernels.

Samples were popped in a residential air popcorn popper (Presto Orville Redenbacher's® Hot Air Popper) for 2 minutes and 15 seconds. Two air poppers were used, and each sample was popped twice in each popper for a total of four popping assays. After popping, the total sample volume was measured using a graduated cylinder and was than sieved through a 4.8 mm screen – kernels that did not pass through the sieve were considered popped and those that passed through are classed as un-popped. The un-popped kernels (UPK) were counted and the volume of the popped kernels were measured using a graduated cylinder

The method to measure sorghum popping efficacy (PE) and expansion ratio (ER) developed by (Rooney & Rooney 2013) was used. Flake size (size of a single popped kernel) calculations were done following the methods of (Sharma et al. 2014). Equations used to calculate popping measurements are presented below:

PE=[(500-UPK)/500]x100%

ER=(Volume After Popping)/(Volume Before Popping)

FS=(Volume of Popped Kernels)/(Number of Popped Kernels)

3.3. Statistical Analysis

Analysis of data used JMP®, Version *14* (SAS Institute). Normality of the data was tested using the Shapiro-Wilk test. Data for PE, ER and FS was not normal, but transformation did not normalize the data nor improve the models. As such the data were analyzed without any transformation. The statistical model for analysis of each dependent variable (popping efficacy, expansion ratio and flake size) ($Y = \mu + \delta_i + \theta_m + \pi(\theta)_{mn} + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon$), where δ=field replication (l=1,2), θ=poppers (m=1,2), π= popper repetition (n=1,2), α=genotype (i=1,2,3,4,5,6,7), β=harvest Time (j=1,2), and γ=harvest method (k=1,2,3,4,5). All independent variables except for replications were considered as fixed effects. The coefficient of variation (CV%) was used to determine consistency of data for a measured trait. All Means separation test were conducted using a Fischer's LSD test.

3.4. Results and Discussion

3.4.1. Agronomics

Of the seven genotypes evaluated, a wide range of grain yields were observed (Table 21). As expected, Sumac was the lowest yielding; this is a tall forage parent that was included in the study because it has been shown to possess popping characters. Both Macia and Sureno had grain yields that were numerically lower but not different than the highest yielding entries even though they are pure line cultivars. However, other traits (ie, plant height, days to mid anthesis) make their commercial use logistically challenging at best, especially Sureno and Sumac. The hybrids ATx642/R06321 and ATx642/R11171 had the highest yields and best combinations of favorable agronomic traits compared to others in this test. For these two hybrids, these trends had been observed in previous studies as well (W.L. Rooney, personal communication).

Table 21. Means Separation for Grain Yield, Plant Height (PH), Panicle Exsertion (EX), Days to Mid Anthesis (DY) and Test Weight for Four Hybrids and Three Cultivars Grown in College Station, Texas in 2019.

Hybrid/Trait	Yield (kg ha^{-1})		PH (cm)		EX (cm)		DY (d)		Test Weight (kg hL^{-1})	
AHF14/RTx436	4991	A	132	F	20	C	73	D	71.6	A
ATx2928/RTx436	4859	A	130	G	13	D	71	E	71.3	A
ATx642/R06321	5757	A	147	C	25	A	71	E	67.5	BC
ATx642/R11171	5811	A	135	E	23	B	71	E	69.5	ABC
Macia	5045	A	142	D	10	E	78	B	70.3	AB
Sureno	4674	A	185	B	13	D	89	A	69.3	ABC
Sumac	2092	B	208	A	0	F	75	C	66.6	C

Note: Values connected by a common letter in a column are not significantly different at α=0.05

3.4.2. Analysis of Variance and Summary Statistics

Analysis of variance models were significant for all three measured popping quality traits (Table 22). The main effects of genotype and threshing method were significant for PE, ER and FS whereas harvest maturity was significant only for FS. Further, the two and three-way interactions involving genotype, threshing method and harvest maturity were significant for all traits.

Table 22. ANOVA And Summary Statistics for the Texas A&M Sorghum Genotypes with Popping Attributes for Popping Efficacy (PE), Expansion Ratio (ER) and Flake Size (FS) Grown In 2019 College Station, Texas.

Source of Varition	DF	Popping Efficacy (%) MS		Expansion Ratio (x:1) MS		Flake Size (cm^3) MS	
Field Rep	1	0.00	ns	0.14	ns	0.00	ns
Popper	1	0.21	**	20.17	**	0.00	ns
Popper Rep[Popper]	2	0.01	ns	2.78	ns	0.00	ns
Genotype	6	0.40	***	208.49	***	0.61	***
Threshing Method	4	0.47	***	182.29	***	0.27	***
Genotype*Threshing Method	24	0.05	***	18.07	***	0.03	***
Harvest Maturity	1	0.00	ns	0.25	ns	0.04	***
Genotype*Harvest Maturity	6	0.10	***	20.18	***	0.02	***
Threshing Method*Harvest Maturity	4	0.05	***	17.46	***	0.02	***
Genotype*Threshing Method*Harvest Maturity	24	0.01	***	2.90	***	0.00	***
Error	486	0.00		0.48		0.00	
R^2		0.83		0.92		0.94	
Root Mean Square Error		0.05		0.69		0.03	
Mean of Response		0.70		7.30		0.32	
CV%		7.75		9.46		8.46	

Note: *, ** and *** reflect significance at (*P*=<0.05), (P=<0.001) and (P<0.0001), respectively.

3.4.3. Harvest Maturity

Of the three main effects, harvest maturities had no effect on PE or ER, but it did influence FS - earlier harvest maturity had a slightly higher FS (Table 23). Inherent in testing, this factor was common practices in popcorn and rice, where the crop is often harvested earlier when the crop has higher moisture content. Presumably, the possibility of structural kernel damage is reduced which concomitantly will increase popping and milling quality of corn and rice, respectively.

However, in sorghum, the statistical and practical differences between the two harvest maturities are at best, minimal. Consequently, it is more important in sorghum to use threshing methods with reduced impact and suitable genotypes regardless of when the crop is harvested.

Table 23. Means Separation for Harvest Maturities of Seven Texas A&M Genotypes with Popping Attributes for Popping Efficacy (PE), Expansion Ratio (ER) and Flake Size (FS) Grown in 2019 College Station, Texas.

Harvest Maturity	Popping Efficacy (%)		Expansion Ratio (x:1)		Flake size (cm^3)	
Late	69.88	A	7.28	A	0.32	B
Early	69.64	A	7.32	A	0.33	A

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

3.4.4. Genotype Effect

Among the entries, the PE ranged from 60 to 78%. These PE numbers are similar to other reports in sorghum (Rooney and Rooney 2013; Aruna et al. 2020), but they are notably below those considered acceptable for popcorn. For PE, ATx642/R11171 and ATx642/R06321 were statistically better for PE than any other genotype (Table 24).

A wide range in ER, from 4.0 to 10.0, was observed among the genotypes (Table 24). These ER values reflect a similar ranges have been observed by (Rooney and Rooney 2013; Pugh et al. 2017). Of the genotypes, Sureno was the best by a significant margin but ATx642/R06321 and ATx642/R11171 did have acceptable ER values (ER value of 8+ could be acceptable but standards have not been established).

Flake size ranged from .43 to .21 cm^3 with the same three genotypes with the highest PE and ER having the highest FS (Table 24). From a practical standpoint, while differences do exist between the three genotypes with the highest FS, these differences may be nearly impossible to detect in a real-world application. Larger differences in FS were observed between the best popping entries and the poorest. For example, differences between Sureno and ATx2928/RTx436 were not only statistical but visually obvious. One possible reason for this wide range in FS may be kernel size - Gökmen (2004) reported that larger popcorn kernels yielded higher flake sizes. Additionally, flake size for the best pop sorghum hybrid observed here is roughly a tenth of the flake size observed in popcorn by (Pordesimo et al. 1990). Logically, it would be assumed that larger kernels would have higher FS, although a correlation of 0.47 was observed between kernel diameter and FS, the relationship is not necessarily always true. The genotype that had the largest kernel diameter (AHF14/RTx436) ranked last among the genotypes for FS. This demonstrates that factors other than kernel diameter affect FS.

Table 24. Genotype Means Separation of the Texas A&M Genotypes with Popping Attributes for Popping Efficacy (PE), Expansion Ratio (ER) and Flake Size (FS) Grown in 2019 College Station, Texas.

Genotype	PE (%)		ER (x:1)		FS (cm^3)	
ATx642/R11171	77.8	A	8.2	B	0.41	B
ATx642/R06321	77.6	A	7.9	C	0.37	C
Sureno	74.8	B	10.0	A	0.43	A
AHF14/RTx436	71.3	C	7.2	D	0.29	E
ATx2928/RTx436	64.3	D	5.0	F	0.21	F
Macia	63.2	D	6.4	E	0.35	D
Sumac	60.3	E	6.4	E	0.22	F

Note: Values connected by a common letter in a column are not significantly different at α=0.05.

The effect of the type of genotypes tested (hybrids vs inbreds) was significant for PE, ER and FS (Table 25). Hybrids, on average, outperformed the inbreds but Sureno did outperform all other genotypes for ER and FS (Table 23). The results seem to indicate that grain quality is more important than heterosis in conditioning popping quality. Hybrids which provide greater agronomic desirability and grain yield potential, can also have good popping characteristics.

Table 25. Contrast Between the Tested Hybrids and Inbreds From Texas A&M with Popping Attributes for Popping Efficacy (PE), Expansion Ratio (ER) and Flake Size (FS) Grown in 2019 College Station, Texas.

	PE (%)	ER (x:1)	FS (cm3)
hybrid vs. inbred Contrast	**	**	**

Note: *, ** reflect significance at (*P*=<0.05) and (P<0.0001), respectively.

Correlation between the popping quality traits was obvious – entries that performed well for PE also had the highest values for ER and FS (Table 24). Although the rank order and statistical differences varied among them, ATx642/R11171, ATx642/R06321 and Sureno were consistently the top performers for all three traits (Table 24). Considering the agronomics of these three genotypes, ATx642/R11171 and ATx642/R06321 are the best candidates for commercial production in the USA.

3.4.5. Threshing Methods

Variation among the five threshing methods was observed and the rank orders of the different methods indicate that kernel damage does influence popping characteristics. Across the different threshing methods, popping efficiency ranged from 61 to 77 and higher values were associated with the lighter impact threshing methods (Table 26). The hand threshing was expected to inflict the least amount of kernel damage; this method had the highest values for PE, ER and FS. Alternatively, the highest impact threshing methods consistently had lower PE values.

As was observed for PE, higher impact threshing methods also reduced ER and FS although the trend for FS was not as strong as for PE. In popcorn, Singh et al. (1997) and Goneli et al. (2007) also reported inflicted kernel damage reduced popping quality. Additionally, the reduction in popping quality in sorghum with more abrasive threshing methods was also observed in popcorn by Lien et al. (1975) where harvesting popcorn at different combine cylinder threshing speeds influenced popping quality.

The control, hand threshing, inflicted the least amount of pericarp damage followed by the belt threshing method. These two methods of threshing do not involve any contact with metal or other hard surfaces during the threshing process. The other three threshing methods do have metal to seed contact and always ranked lower than two threshing methods that did not have seed to metal contact. While it does not prove, it certainly implies that mechanical harvesting can have a significant impact on PE, ER and FS.

The threshed grain used for popping test was composed of whole, intact kernels with no obvious cracks or chips. Therefore, the damage is likely small microfractures of the pericarp or endosperm. Further work would be needed to confirm their presence, but the trends clearly imply that these small fractures or chips are occurring. Ultimately, if the grain is to be used for popping, the threshing method will influence the PE, ER and FS of the resultant popped grain.

Table 26. Threshing Method Means Separation of the Texas A&M Genotypes with Popping Attributes for Popping Efficacy (PE), Expansion Ration (ER) and Flake Size (FS) Grown in 2019 College Station, Texas.

Thresher	Action	Popping Efficacy (%)		Expansion Ration (x:1)		Flake Size (cm^2)	
Hand	Light hand pressure and friction	77	A	8.9	A	0.39	A
Belt	Rubber belts, moderate friction	74	B	8.5	B	0.37	B
Wintersteiger	High speed brushes, moderate friction	71	C	6.7	C	0.28	D
Pull Behind	Metal concave beaters, high impact	66	D	6.3	D	0.29	C
Combine	Metal concave beaters, high impact	61	E	6.1	D	0.30	C

Note: Values connected by a common letter in a column are not significantly different at $\alpha=0.05$.

3.4.6. Interaction Effects

In order to access the significant interactions noted in 3.5.2., the top three performing genotypes (ATx642/R11171, ATx642/R06321 and Sureno) were evaluated for their consistency of response for popping qualities. Among this group of three, the two- and three-way interactions between harvest maturity, genotype and threshing method were still significant ($P=<0.05$).

The interaction of threshing method and genotype on PE, ER and FS (Figure 3) follow similar trends. For PE, it appears that ATx642/R06321 and ATx642/R11171 suffer from grain kernel damage during threshing than Sureno. There was a significant reduction in PE when ATx642/R06321 and ATx642/R11171 when subjected to metal to seed contact threshing. Alternatively, Sureno was consistently more stable, albeit at a lower PE across most threshing methods, and somewhat surprisingly, the PE of Sureno actually increased when threshed using the Wintersteiger LD 180. There is no obvious explanation for this result.

For ER, Sureno had the highest values consistently and there were only minor interactions between ATx642/R06321 and ATx642/R11171 for the different threshing methods. For FS, Sureno and ATx642/R11171 consistently performed best, with minor interactions throughout the different threshing methods.

The exact reason for the interactions between threshing method and genotype is not clear. However, Sureno produces grain that is harder than the other two genotypes (Table 19); this hardness may be reduce kernel damage due to threshing. Baptestini et al. (2014) reported that popcorn genotypes have varying levels of susceptibility to kernel damage. It appears a similar difference is observed here with sorghum. Realistically, there is more likely a complex interaction of kernel hardness, diameter, weight, endosperm ratios, endosperm shapes and pericarp thickness causing these interactions that merits additional study.

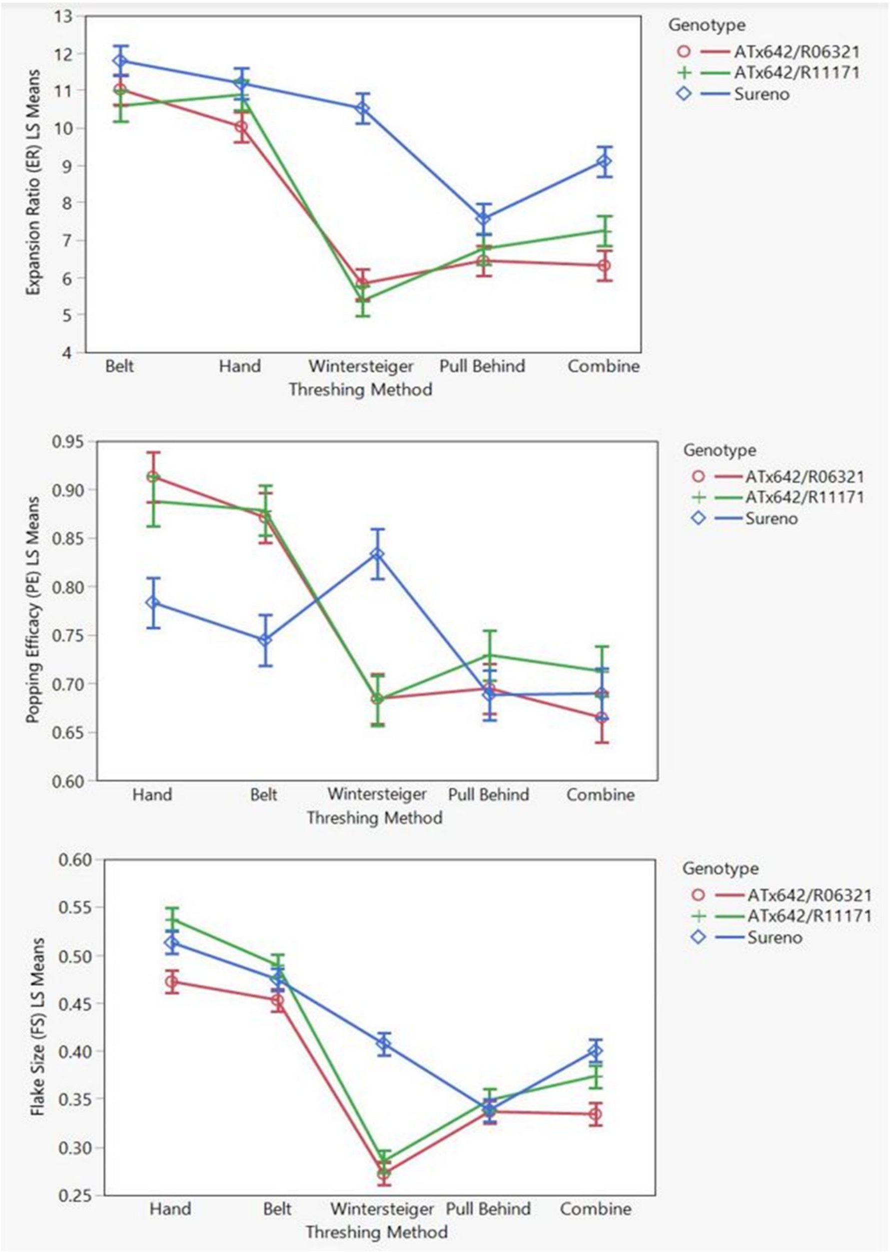

Figure 3. Interaction Plot of Threshing Method and Genotype for Popping Efficacy (PE) (Top), Expansion Ratio (ER) (Middle) and Flake Size (FS) (Bottom) of the Top Three Performing Texas A&M Genotypes with Popping Attributes Grown in 2019 College Station, Texas. The Threshing Methods are Placed From Left to Right in Order of Average PE Across All Entries in the Test.

The interaction of harvest maturity and genotype on PE, ER and FS (Figure 4) reveal interesting trends. Popping efficiency increases for all three genotypes with the late harvest maturity although the levels of increase vary. For ER both FS, ATx642/R06321 and ATx642/R11171 both decrease from the early to late harvest maturity whereas Sureno increased significantly from the early to late harvest maturity. The cause for this interaction between harvest maturity and genotype is unknown and minimal literature exists on this interaction. White et al. (1980) reported a decrease in expansion volume of popcorn as the harvest moisture increase. Ultimately, these interactions will require further study in order to understand their base cause.

Similar to harvest maturity and threshing method, there were no trends in the three-way interaction of harvest maturity, threshing method and genotype. The significance of the interactions and the interpretations from them herein do show that there are important interactions among the three factors. The best way to address these factors is for a producer of pop sorghum to try several genotypes, threshing methods and harvest maturities and decides which one works best for their operation.

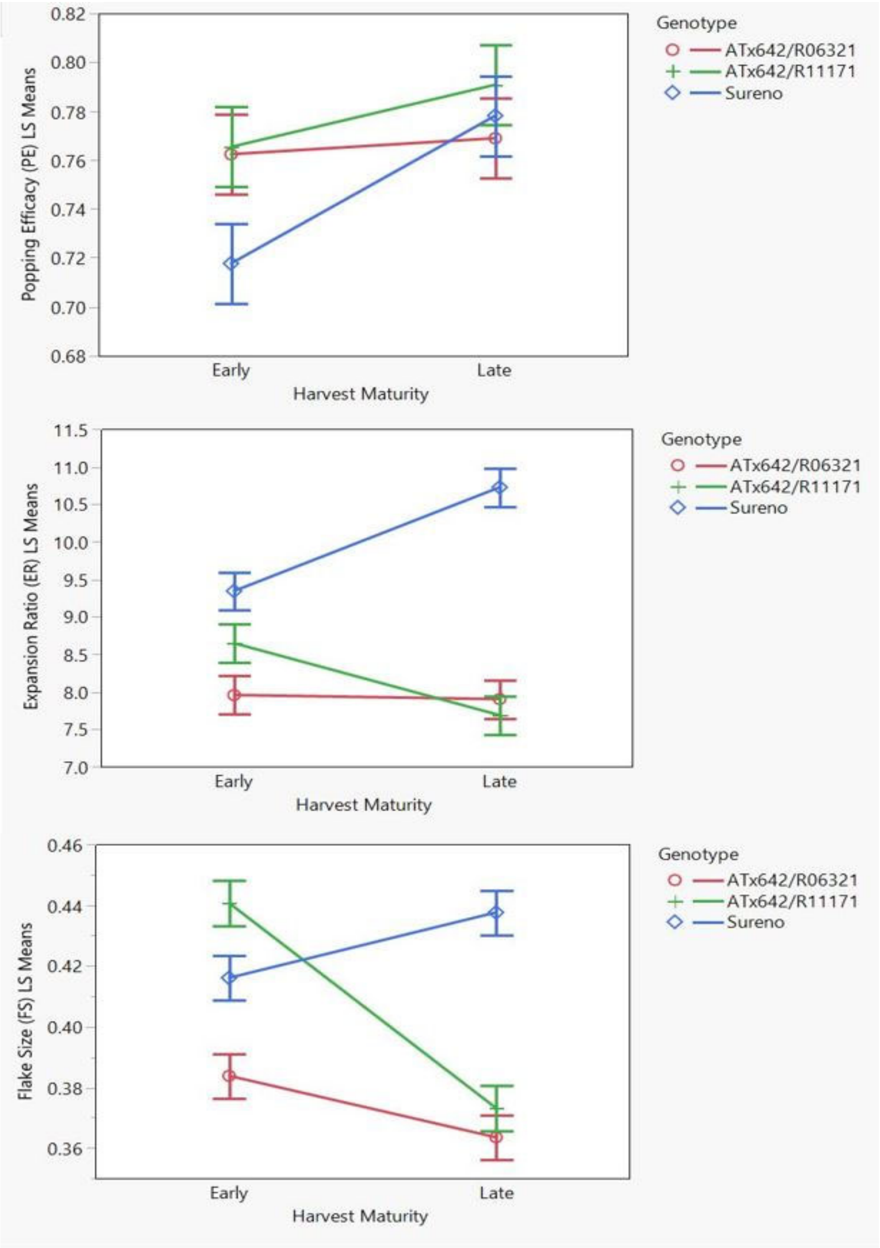

Figure 4. Interaction Plot of Harvest Maturity and Genotype for Popping efficacy (PE) (Top), Expansion Ratio (ER) (Bottom) and Flake Size (FS) (Bottom) for the Top Three Performing Texas A&M Genotypes with Popping Attributes Grown in 2019 College Station, Texas.

3.5. Chapter Summary

There was considerable variation among the tested genotypes for PE, ER and FS. There was not a clear distinction between the performance of hybrids and inbreds for popping quality. This indicates that grain quality is more important than heterosis in conditioning popping quality. From the tested genotypes, two hybrids (ATx642/R06321 and ATx642/11171) possess favorable agronomics and popping quality, which could make them a viable option for pop sorghum production.

The threshing methods tested caused varying amounts of kernel damage that was manifested in loss of popping quality. It was found that hand threshing (control) caused the least amount of kernel damage and that combine threshing caused the greatest amount of kernel damage. A clear distinction was observed between the threshing methods that had metal to seed contact and the threshing methods that did not have metal to seed contact. It is concluded here that metal to seed contact significantly reduces popping quality.

Harvest maturity did not affect popping quality nearly as much as the genotype and threshing method did. There was not a statistical difference between the early and late harvest for PE and ER. There was a statistical difference between the two harvest maturities for FS, although, this difference may be negligible in a real-world application.

This research and data observed herein may and should be used as a foundation for further research regarding pop sorghum. Such future research could be a further investigation of how kernel characteristics (i.e. hardness, size, weight, endosperm structure and pericarp thickeness) and their significance on popping traits in sorghum.

Additionally, an investigation of kernel characteristics and their role in kernel damage caused by threshing/processing would be merited.

4. CONCLUSIONS

The first goal of this research was to assess the agronomics and grain functionality of waxy endosperm and/or high digestible protein grain sorghum hybrids with superior agronomics relative to traditional grain sorghum hybrids. Within the goal of this study was three sub-objectives. The first sub-objective was to identify of waxy endosperm sorghum hybrids that also possess the HD protein mutation using a field emissions scanning election microscope. The method of identifying the HD mutants using a FE-SEM was successful, with eight genotypes being identified to be HD. The second sub-objective was to assess the yield potential of waxy and/or HD sorghum hybrids relative to a standard waxy check hybrid. It was found that several of the tested waxy and waxy HD hybrids outperformed the waxy check hybrid. The final sub-objective of this study was to evaluate the waxy endosperm trait for stability in quality over numerous environments. It was found that the tested waxy hybrids had distinct performance when compared to the non-waxy and heterozygous-waxy check hybrids. Additionally, it was observed that the environment in which the grain was grown in significantly affected the functionality of the flour.

The second goal of this research was to identify grain sorghum genotypes that possess superior popping ability and investigate whether harvest methods and harvest maturities cause kernel damage that affects popping quality. Within the goal of this study was three sub-objectives. The first sub-objective was to evaluate the popping attributes of four sorghum hybrids and three inbred lines for popping attributes. It was found that there was not a clear distinction between the performance of hybrids and inbreds for popping quality. This indicates that grain quality is more important than heterosis in conditioning popping quality. The second sub-objective was to test five different methods of threshing methods for their effect on popping attributes. From the observations found in this study, the five different threshing methods inflicted varying amounts of kernel damage, which was manifested in the decrease in popping quality. Additionally, it was observed that the threshing methods that had metal to seed contact significantly reduced the popping quality compared to threshing methods that do not have such contact. The final sub-objective was to test the effects of maturity and moisture content at time of harvest has on popping attributes. It was found that the harvest maturities did not affect PE and ER but did significantly affect FS. The significant difference in FS for the two different maturities was small and may be negligible in a real-world application.

From this research, it is concluded that the tested waxy sorghum hybrids are agronomically acceptable and many have increased grain yield when compared to the waxy hybrid check. Additionally, large environmental factors impact waxy sorghum flour functionality and specific environments should be targeted for particular end uses. It is also concluded from this research that factors of genotype, threshing method and harvest maturity can affect popping quality in pop sorghum and, depending on the desired end use, should be taken into consideration for commercial pop sorghum production.

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