GENETIC AND ENVIRONMENTAL EFFECTS ON PHENOLIC COMPOSITION AND AGRONOMIC PERFORMANCE IN BLACK SORGHUM (*Sorghum bicolor* L.) HYBRIDS

A Thesis

by

CHAD MATTHEW HAYES

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

MASTER OF SCIENCE

Approved by:

Chair of Committee, William L. Rooney
Committee Members, Joseph M. Awika
Stephen R. King
Head of Department, David D. Baltensperger

December 2012

Major Subject: Plant Breeding

ABSTRACT

A healthy diet requires a shift from the consumption of highly refined and processed foods with low nutritional value and towards whole grain food that have increased levels of antioxidants and phenolic compounds. Certain specialty sorghum genotypes have been identified as potential whole grain foods that have high levels of desired chemical compounds. Specifically, sorghums with a black pericarp are excellent sources of antioxidant compounds, including the unique compound 3-deoxyanthocyanin (3-DOA).

Although extensive work has been performed on the chemistry and food properties of black sorghum inbreds, little research has been done studying the grain phenolic composition and agronomic performance of black sorghum hybrids in production environments. The goal of this study was to evaluate black sorghum hybrids with differing genetic backgrounds for grain phenolic composition and agronomic performance across multiple environments and identify the best lines for producing these hybrids.

Results from this study confirm that sorghums with a black pericarp contain elevated levels of phenols, tannins and 3-DOA. Concentrations of these beneficial compounds were many times the concentration levels seen in traditional grain hybrids. Grain yields of black sorghum hybrids averaged 36% below commercial hybrids in this study. Lower grain yields could be due to reduced heterosis in black sorghum hybrids and other unknown factors associated with the black phenotype.

From this study, the Texas AgriLife Sorghum Breeding Program has developed two elite black sorghum lines for release as the first publicly available black pericarp seed parents. These newly developed seed parents, proposed for release as Tx3363 and Tx3364, allow for the creation of the first temperately adapted black seeded grain hybrid. These hybrids help address the growing demand for products that are healthy, and provide natural and stable antioxidant compounds for a growing health food industry.

DEDICATION

I dedicate this work to my wife and family for providing unwavering support.

“I don’t care how poor a man is; if he has a family, he’s rich”

ACKNOWLEDGEMENTS

I greatly appreciate and respect the guidance and patience that Dr. Bill Rooney has provided me during this process. I am looking forward to many years of collaboration and friendship. I would also like to recognize and thank my committee members Dr. Joseph Awika and Dr. Stephen R. King for providing excellent knowledge and support.

I must also thank Dr. John Burke and Mr. Charles Woodfin of USDA-ARS in Lubbock for believing in me and supporting my future as a plant breeder. Special thanks to Dr. Cleve Franks for providing fun times at ARS, reinforcing my plans to become a plant breeder.

Many thanks to the present and past members of the Texas A&M Sorghum breeding program. It was a pleasure working with you all, and I look forward to collaborating with you all in the future.

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CHAPTER I

INTRODUCTION

Sorghum (*Sorghum bicolor L.*) Moench) is the fifth most widely grown and utilized crop throughout the world; it is especially adapted in semi-arid regions where other cereal grains are not (Rooney, 2004). Sorghum improvement, in various forms, has occurred for thousands of years, resulting in a wide array of races, subraces and landrace varieties that are productive as grain, forage and industrial crops (Kimber, 2000). Early domestication began in Africa approximately 5000 years ago (Rooney, 2004). Agronomically important traits such as non-shattering grain, flowering time, and plant height were selected by ancestral people within distinct geographic regions. Over time, geographic isolation resulted in races and subraces within domesticated sorghum (Kimber, 2000). Sorghum is widely known for its drought tolerance abilities, but variation for a wide range of traits from disease resistance to grain quality exist and are available for breeding.

More recently, sorghum has been identified as a potential source of natural phytochemical compounds (Awika et al., 2009). Phenolic compounds are known to exert beneficial effects as free radical scavengers within the body and consumption of grains rich in phenolic compounds are reported to reduce health problems, especially certain types of cancers (Guajardo, 2008). Although many types of sorghum contain beneficial phenolic compounds, black sorghum has been identified as a potential source of anti-cancerous compounds

Black pericarp sorghum contains a rare anythocyanin compound known as 3-deoxyanthcyanidin (3-DOA), which has been identified as a potential natural food colorant (Dykes and Rooney, 2006). Awika (2004) found the black sorghum bran has higher anythocyanin concentrations than most sources; only elderberry was higher in total anthocyanin concentrations. This 3-DOA also has in vitro antioxidant activity (Awika and Rooney, 2004). Although many of the chemical characteristics are characterized for black sorghums, there has been little work in the breeding and agronomic production of the crop.

Although relatively rare, black pericarp sorghums are known. A black pericarp colored sorghum was identified and characterized in a set of sorghum germplasm obtained in 1978 by Texas A&M University researchers. The original accession designated as “Shawaya Black” was photoperiod sensitive and required hybridization and selection to produce photoperiod insensitive versions that could be grown in temperate environments.

From that breeding effort, Tx3362 was developed and released in 2012. This line is the first improved black sorghum genotype released in the USA (Rooney et al., 2012). Tx3362 is a 3-dwarf, early inbred line that produces a very black pericarp color when the developing grain is exposed to normal summer daylight and experimental versions of this line were used in many of the characterizations studies (Awika and Rooney, 2004). Interestingly, when the panicle is covered with a bag, the grain does not develop a black pericarp, but rather produces a red phenotype. While this genotype possesses desirable grain qualities, the grain yield is quite low (Rooney et al., 2012) and hybridization is not an option because crossing Tx3362 with nay standard seed parent results in a hybrid with red grain color (Rooney, personal communication). Thus, production of a black sorghum hybrid will require both parents to possess the trait.

To increase grain yield of a black sorghum there is a need to develop the appropriate inbred lines to produce such hybrids. Thus, the objectives of this thesis are:

1. Identify and develop the release document for black-seeded A/B lines for the production of black-grain sorghum hybrids.
2. Evaluate the chemical composition of the black sorghum hybrids for total phenols, 3-deoxyanthocyanins and tannins; which are the important antioxidants present in these sorghums.
3. Evaluate the agronomic performance of black sorghum hybrids when compared with commercial grown sorghum hybrids for grain yield, height, and days to half-bloom.
4. Evaluate the relative importance of any GxE interactions for agronomic and composition traits so that environments best for production can be identified.

CHAPTER II

PHENOLIC COMPOSITION AND AGRONOMIC PERFORMANCE OF NOVEL BLACK SORGHUM HYBRIDS

Introduction

Various types of sorghum have grain with phytochemicals that can impart health benefits when consumed (Awika and Rooney, 2004). Specifically, flavonoids concentrated in the outer layers of the pericarp contain anti-cancer and anti-inflammatory characteristics when consumed. Three groups of flavonoids exist in varies different sorghum genotypes; 3-deoxyanthocyanins (3-DOA), flavones and flavanones. The concentrations of these beneficial compounds vary greatly between genotype and are influenced by environmental factors such as light, temperature, and fungal infection (Dykes et al., 2009).

Diets high in sorghum grain consumption can reduce incidence of esophageal and colon cancers in humans (Bralley et al., 2008). The anti-cancerous characteristics of some sorghum grain are associated with high flavonoid and tannin concentrations within the grain (Yang et al., 2009). Sorghum grain containing high levels of tannins have also shown the potential to reduce obesity in laboratory animals. Numerous reports have shown that animals fed high tannin sorghum diets had reduced nutritive values. This is primarily due to tannins binding proteins and carbohydrates into insoluble complexes that cannot be broken down in the digestive system (Muriu et al., 2002). Although there are no reports on weight reduction in humans to date, epidemiological data suggest that certain cultures in Africa that consume large amounts of high tannin grain are experiencing slowed digestion due to elevated tannins in their diet.

Although relatively rare, black pericarp sorghums have been present in sorghum breeding programs for several years. A black pericarp colored sorghum was identified and characterized in a set of sorghum germplasm obtained in 1978 by Texas A&M University researchers. The original accession designated as "Shawaya Black" was photoperiod sensitive and required hybridization and selection to produce photoperiod insensitive versions that could be utilized in temperate growing environments.

The black phenotype found in black sorghum has been described as a complex trait highly influenced by the environment but the exact inheritance is not known (Rooney, personal communication). When panicles of a black sorghum are covered with a bag at anthesis, the grain is red and does not develop the black color. Figure 1 provides images of the three genetic colors of sorghum and the genes associated with that color.

Even without a complete understanding of the genetic basis of the trait, Tx3362 was released in 2012 (Rooney et al., 2012). Tx3362 is a 3-dwarf, early inbred line that produces a very black pericarp color when the developing grain is exposed to normal summer daylight and experimental versions of this line were used in many of the characterization studies of sorghum with a black pericarp (Awika, 2003; Awika et al., 2004; Dykes and Rooney, 2006). Figure 2 provides images of Tx3362 and five breeding lines with varying pericarp color. While this genotype possesses desirable grain qualities, the grain yield is quite low (Rooney et al., 2012) and hybridization is not an option because crossing Tx3362 with any standard seed parent results in a hybrid with red grain color (Rooney, personal communication). Thus, production of a black sorghum hybrid will require that both parents possess the trait. Unfortunately, there are no known seed parents with a black pericarp.

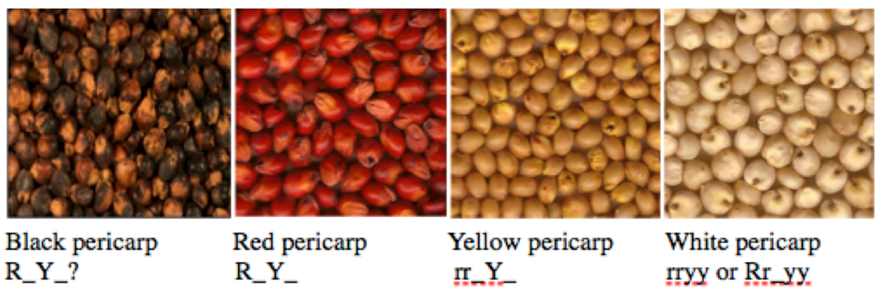

Figure 1. Examples of sorghum pericarp color and the genes associated

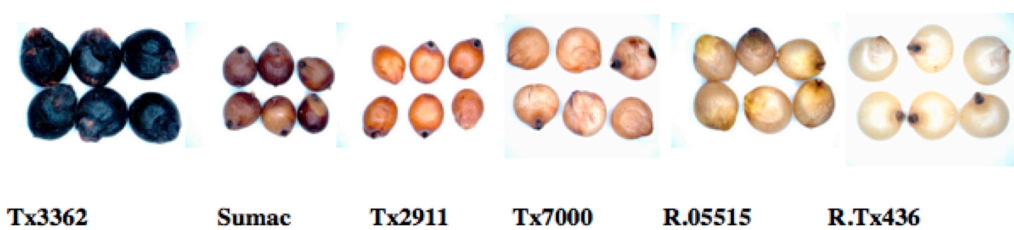

Figure 2. Photographs of the black grain pollinator Tx3362, and five breeding lines representing the diversity of pericarp color in sorghum.

Given this need for these types of seed parents, the Texas Agrilife sorghum breeding program has been developing black pericarp grain, grain sorghum seed parents. In theory, these lines should be used to produce black grain hybrids in combination with Tx3362. From this program, a series of experimental black seed parent lines (Figure 3) were developed and sterilized. Using these experimental seed parents, it was possible to produce experimental black grain hybrids (Figure 4). Figure 5 provides images of three hybrids differing in genetic pericarp color.

Although extensive food quality research has been done on sorghums with a black pericarp, little research has been done concerning the agronomic production of black pericarp sorghum lines or hybrids. Some of this is due to the fact that only limited inbred lines were available for evaluation. Using the now available sorghum germplasm, the objective of this study was to evaluate both experimental inbreds and hybrids for both agronomic and compositional traits with the goal of assessing the relative effects genotype, environment and genotype x environment interactions.

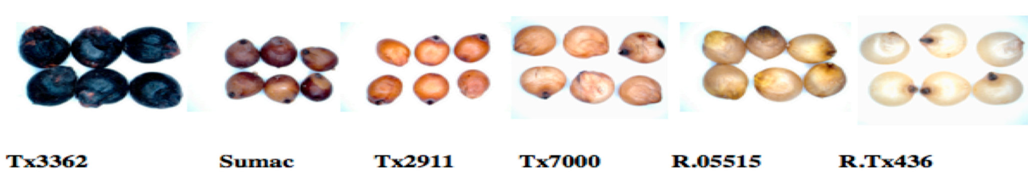

Figure 3. Photographs of the black grain pollinator Tx3362, and five breeding lines representing the diversity of pericarp color in sorghum.

Figure 4. Photographs of the six experimental black seed parent lines in hybrid combination with the pollinator Tx3362.

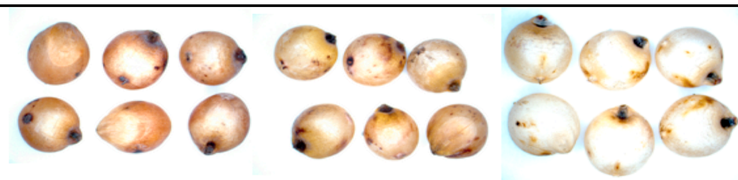

Figure 5. Photographs of three common grain hybrids with differing (red. yellow, white) pericarp color.

Materials and Methods

Line and Hybrid Development

The pollinator parent used in this study was Tx3362, which was described and released in Rooney et al., (2012). The seed parents were developed in the Texas Agrilife Sorghum Breeding Research Program and are all selections derived from the breeding cross of BTxARG-1ms3/Tx3362 (Table 1). B.TxARG-1 is a commonly used grain sorghum seed parent with temperate adaptation that was released by Texas Agrilife Research (Miller et al., 1992). BTxARG-1 is genetically a 3 dwarf ($dw_1Dw_2dw_3dw_4$) in height with a white seed, tan plant color and waxy endosperm that. Following selection of these lines, they were sterilized using ATxArg-1 as a donor parent for A1 cytoplasm. Seed for these six black sorghum hybrids were produced using an isolation block in Chilicothe, Texas in 2008. In addition to these six hybrids, eight standard grain sorghum hybrids representing the hybrids different grain quality and composition characteristics were included as references for the black sorghum (Table 2).

Table 1. Designation and pedigree information of seven experimental black sorghum lines developed by Texas Agrilife Research used in this study.

Inbred Line	Pedigree	Pericarp color
Tx3362	((R.Tx430)*ShawayaBlack)	Black
A/B05023	((BTxARG-1ms3*Tx3362)-F1*Tx3362)-CS15	Black
A/B05027	(BTxARG-1ms3*Tx3362)-WF55-CS1	Black
A/B05028	(BTxARG-1ms3*Tx3362)-WF55-CS1	Black
A/B05029	(BTxARG-1ms3*Tx3362)-WF70-CS0	Black
A/B05030	(BTxARG-1ms3*Tx3362)-WF70-CS1	Black
A/B05031	(BTxARG-1ms3*Tx3362)-WF70-CS2	Black

Table 2. Grain hybrids of experimental black and commercial grain checks used in this study.

Hybrid	Source	Pericarp color	Mesocarp thickness	Testa layer
84G62	Pioneer Hybrid International	Red	Thin	No
Tannin Hybrid	Sorghum Partners 8330	Red	Thick	Yes
ATx631/RTx436	Texas Agrilife Research	White	Thin	No
ATx2928/RTx437	Texas Agrilife Research	White	Thin	No
ATx642/Tx2953	Texas Agrilife Research	Yellow	Thin	No
ATx2752/RTx437	Texas Agrilife Research	Red	Thin	No
ATx623/RTx430	Texas Agrilife Research	Red	Thin	No
ATx2752/RTx430	Texas Agrilife Research	Red	Thin	No
A05023/Tx3362	Texas Agrilife Research	Black	Thick	Partial
A05027/Tx3362	Texas Agrilife Research	Black	Thick	Partial
A05028/Tx3362	Texas Agrilife Research	Black	Thick	Partial
A05029/Tx3362	Texas Agrilife Research	Black	Thick	Partial
A05030/Tx3362	Texas Agrilife Research	Black	Thick	Partial
A05031/Tx3362	Texas Agrilife Research	Black	Thick	Partial

Experimental Design

All experimental inbreds (Table 1) and hybrids (Table 2) were arranged in a single test using the randomized complete block design (RCBD) with two replications per location. This test was grown in three Texas locations: Weslaco, College Station and Halfway. These locations represent a range of different sorghum productions regions in Texas. Weslaco is in the Rio Grande Valley in a semi-arid but humid sub-tropical climate. College Station is located in the South Central region and is a subtropical environment. Halfway is in the Texas High Plains and has a dry, temperate climate. Within each environment, agronomic practices standard to the region were utilized for production. Supplemental irrigation was utilized in all three locations as needed. In both years, supplemental irrigation was applied at least once to each test in all environments. In 2008, a plot was one row six meters in length with row spacing of 0.76 meters in College Station and 1.00 meters in Weslaco and Halfway. In 2009, plot size changed in that a plot was two rows instead of one row to minimize differences in plot stands. Seed of all entries were treated with a seed treatment mixture of Captan, Gaucho and Concept to allow the application of Dual herbicide for weed control. Planting and harvest dates for each environment varied depending on climate and local agronomic practices (Table 3).

Table 3. Planting and harvest dates of each test location grown in 2008 and 2009

Location	Plant Date	Harvest Date
Weslaco		
2008	February 4	May 26
2009	February 7	May 25
College Station		
2008	April 2	July 31
2009	April 4	August 2
Halfway		
2008	May 21	September 30
2009	May 19	September 29

Data Collection

At anthesis, days-to-flowering was recorded as the date on which 50% of the panicles were at mid-anthesis. Just prior to harvest, plant height was measured as the height from the ground to the top of the panicle in meters and panicle exertion was measured as the distance between the flag leaf and peduncle in centimeters. If necessary, lodging was measured as a total percent of the plot that lodged. At grain maturity, all inbred and hybrid plots were hand harvested and grain was threshed using an ALMACO head-thresher. Total plot weights were recorded, as well as moisture percent and test weight. Plot yields were corrected to 13% moisture.

Grain samples were collected for color and compositional analysis. Selected grain compositional traits were estimated using NIR (Near Infrared Spectroscopy). Composition traits measured by NIR were total phenols, 3-deooxyanthocyanins and tannins; as these compounds are associated with antioxidant and shelf stability properties

(Awika 2004). The NIR prediction model was developed in the Texas A&M sorghum breeding program and was used to estimate total phenol, 3-deoxyanthocyanin and tannin content. The model created was a multivariate-regression method that is capable of accurately predicting the characteristics and properties of unknown samples. The multivariate-regression methods used in this study and most frequently used in NIR spectroscopy were principal component regression (PCR) and partial least-squares (PLS) regression (Blanco and Villarroya, 2002).

Statistical Analysis

All effects were analyzed using SAS 9.2 and JMP 8.0. Homogeneous variances were found for all traits measured except for grain yield in this study. Heterogeneous variance components for grain yield could be explained by the highly variable production locations chosen for the study and differing agronomic practices. Although not all trait variances were homogenous in this study, a combined analysis of the data was performed to analyze the experimental black and check hybrids across locations and years. The model for the combined mixed model was *Dependent Variable = μ + Hybrid + Location + Year + Rep[Loc*Year] Hybrid*Location + Hybrid*Year + Location*Year + Hybrid*Location*Year + Error.* In the combined mixed model, hybrid and location effects were considered as fixed effects and the year effect was considered as a random effect. High-parent heterosis (HPH) was calculated using the equation $HPH= ((X_{hybrid}-x_j)/100)$, where X_{hybrid} is the hybrid mean and (x_j) is the mean of the highest yielding inbred used to create that hybrid.

Results and Discussion

Combined Analysis

Significant variation for at least one main effect was detected for every agronomic and composition trait measured. Trait data was relatively consistent indicated by acceptable C.V. values (Table 4). The effect due to hybrid was a highly significant for all traits measured in this test.. For most traits the hybrid effect accounted for the majority of the measured variation (Table 4). Black sorghum hybrids produced in this study had grain yields on average that were 64% of the mean of the commercial hybrid (Table 5) and the top performing black hybrid A05029/Tx3362 yielded 78% of the commercial hybrid mean (Table 5). Compared to the commercial check, test weights were lighter, possibly because the endosperm of the black hybrids is more floury than the control. All black hybrids were significantly earlier than the commercial check for maturity (Table 5). Significant variation for plant height was observed in this study but the differences had no agronomic impact as lodging was not observed.

Table 4. Combined phenolic and agronomic analysis of variance results for black hybrids and commercial check hybrids

		Total phenols	Tannins	3-DOA	Grain yield	Test weight	Days to anthesis	Plant height
Source of Variation	df	Mean Squares						
Year	1	31**	45	3903**	579	397**	1040**	1940**
Location	2	16*	265**	557	22129**	980**	1025**	168
Rep[Location* Year]	8	1	10	401	206	4	10	7
Location*Year	2	19**	178**	8215**	2526**	462**	1058**	4790**
Hybrid	6	368**	2015**	35070**	14919**	184**	28**	274**
Hybrid*Year	6	9**	49**	451	1995**	14	6	109
Hybrid*Location	12	9**	48**	714**	1037*	32**	4	118
Hybrid*Location*Year	12	10**	54**	452*	458	4	5	37
Error	90	2	14	214	343	12	5	81
C.V., %		14	19	14	16	5	4	7

Table 5. Phenolic and agronomic differences between black hybrids and a commercial check hybrid combined across locations and years using Fishers LSD.

Hybrid†	Phenols	Tannins	3-DOA	Grain yield	Test weight	Days to anthesis	Height
	mg GAE/g	mg CE/g	Abs/mL/g	MT ha^{-1}	kg hl^{-1}	d	cm
A.05023	16.9	30	168	2.4	60	59	119
A.05030	15.7	30.8	117.8	3.3	66	59	126
A.05028	15.2	29.1	109.6	3.9	65.1	59	125
A.05029	15.1	31.2	107.9	4	65.5	60	123
A.05031	11.7	21.6	114.6	3.5	65.8	61	134
A.05027	10.1	16.7	135.8	2.6	64.6	61	124
Check‡	2.1	0	5.4	5.1	70.3	68	132
LSD	2.7	8.1	25.8	0.3	5.8	1	6

*,** Denote significant at $p<.05$ and .01, respectively
† All black females hybridized with Tx3362
‡ ATx631/RTx436 grain hybrid

For composition, black sorghum hybrids in this test also contained elevated levels of antioxidant compounds when compared to commercial grain hybrids. The top performing hybrid for total phenol concentration was A05023/Tx3362 with a concentration of 16.9 mg GAE/g (Table 5) and the average black sorghum hybrid had 12.4 mg GAE/g of phenol concentration. Compared to the white grained commercial check hybrid ATx631/RTx436, the black hybrids produced over six times the phenol concentrations of the commercial check hybrid. A05023/Tx3362 also contained the highest levels of 3-DOA in this test with a concentration of 168.0 Abs mL/g and black sorghum hybrids averaged 125.7 Abs mL/g (Table 5). This average represents an increase in concentration over 23 times the commercial hybrid average concentration of 5.4 Abs mL/g. In essence the commercial hybrid did not possess measurable 3-DOA concentrations. The top performing hybrid for tannin concentration was A05030/Tx3362 with 30.8 mg CE/g and the average black hybrid produced 26.5 mg CE/g. The commercial hybrid did not have a measurable concentrations of tannins in this study and the tannin levels observed in these black hybrids were similar to the levels reported in hybrid sorghums with a pigmented testa. Reduced test weights in the black sorghums may result in increased concentrations of all composition compounds present in the pericarp, but the extreme differences between the black sorghum hybrids and the check hybrid confirm that black sorghum hybrids do contain elevated antioxidant compounds.

For agronomic traits, location was significant for all traits measured except for plant height in this study. In terms of grain yield, Halfway was the most productive environment (Table 6). Yield differences between Halfway and the other two locations could be due to several possible factors such as adaptation and/or grain mold and weathering. Both College Station and Weslaco are subtropical productions environments while Halfway is a temperate production environment. Sorghum hybrids are often adapted to one or the other, but rarely to both (Miller and Kebede, 1984). Furthermore, grain weather pressure is consistently worse in South and Central Texas and while grain weathering was not rated in these tests, the lower test weights in College Station and Weslaco are typical of grain weathering, whether it is visible or not (Williams and Rao, 1981). It should be stated that seed companies have been using the High Plains of Texas, which includes Halfway, as a seed production location for many decades due to the favorable conditions mentioned above.

For compositional traits, phenolic and tannin concentrations in the grain were influenced by location while 3-DOA concentrations were not. The Halfway location produced the highest concentrations of tannins with an average of 25.9 mg CE/g (Table 6) while College Station produced the highest concentrations of phenols and 3-DOA in this study with averages of 12.8 mg GAE/g and 114.1 Abs/mL/g, respectfully. Given that there was no difference in 3-DOA concentrations across the three locations, it appears that the Halfway locations is the most suitable for both composition and grain yield.

Table 6. Location differences for phenolic composition and agronomic performance traits of experimental black hybrids using Fishers LSD.

Location	Phenols†	Tannins‡	3-DOA§	Grain yield	Test weight	Days to flower	Height
	mg GAE/g	mg CE/g	Abs/mL/g	MT ha^{-1}	kg hl^{-1}	d	cm
Halfway	12.2	25.9	103.3	4.5	70.1	57	127
College Station	12.8	21.7	114.1	3	59.8	57	124
Weslaco	12.3	20.3	109.2	3.2	67	67	125
LSD	0.5	3.1	11.6	0.4	2.5	1	5

† GAE = gallic acid equivalents;
‡ CE = catechin equivalents
§ 3-DOA = 3-deoxyanthocyanin

Year was a significant effect for all traits measured except for tannins and grain yield in this study (Table 4). For agronomic traits, differences in rainfall and temperature among other things contributed to the differences between the 2008 and 2009 growing years. A significant year effect on compositional traits implies that end users must be prepared for yearly differences in 3-DOA and phenol concentrations due to random seasonal variation. For the plant breeder, it also implies that multiple years must be used in identifying the hybrids with the highest and most stable phenolic concentrations.

For agronomic traits, significant effects for the hybrid x environment interaction terms were detected for grain yield and test weight but not for plant height or days-to-anthesis (Table 4). These types of interactions are consistent with previous yield data in sorghum as yield is more affected by genotype x environment interactions that height or maturity. It indicates that multi-location testing is critical to identify the best locations to produce these hybrids. For composition traits, all three traits had significant genotype x environment interactions (Table 4). Producers must be able to accurately identify genotypes that perform best in the production environment selected.

Heterosis of Black Sorghum Hybrids

Grain yields in all black hybrids were significantly higher compared to the lines per se (Table 7). For the black hybrids, high parent heterosis for grain yield averaged 172%, indicating that performance is substantially improved relative to the lines per se. In comparison, the high parent heterosis for the commercial check averaged 148% (Table 7). Although the high parent heterosis value is lower in the check commercial compared to the black hybrids, the overall grain yields are significantly higher due to higher inbred grain yields.

The cause of the lower yield in the black inbreds is not specifically known but could be due to factors associated with the black pericarp phenotype and the floury endosperm, as well as grain mold susceptibility. Heterotic responses varied greatly for the black hybrids, implying differences in both general and specific combining ability (Table 7). Given that the pollinator parent Tx3362 is present in the pedigree of the black seed parents, it is likely that heterosis and overall grain yield can be increased over time with additional breeding to separate the lineages of the pollinator and seed parents. Ultimately, a premium will be required as an incentive to produce black sorghum hybrids. End-users will need to justify premium prices and lower yields for the unique compounds produced by black sorghum hybrids.

Table 7. Heterosis for six black hybrids and a commercial hybrid averaged across three locations and two years.

Trait	A.05023†	A.05027	A.05028	A.05029	A.05030	A.05031	Check§
Phenols (mg GAE/g)¶							
Female	16.3	9.6	14.6	15	17.8	8.9	1.8
Hybrid	16.9	10.1	15.2	15.1	15.7	11.7	2.1
Heterosis‡	104%	82%	104%	101%	88%	95%	116%
Tannins (mg CE/g)#							
Female	26.1	13.4	27.7	28.5	35.8	16	2.3
Hybrid	30	16.7	29.1	31.2	30.8	21.6	0
Heterosis	115%	78%	105%	109%	86%	100%	0.00%
3-DOA (Abs/mL/g)††							
Female	176.9	124.8	94.3	98.3	98	106.1	8.8
Hybrid	168	135.8	107.9	107.9	117.8	114.6	5.4
Heterosis	95%	83%	66%	66%	72%	70%	83%
Grain Yield (MT/ha^{-1})							
Female	1.9	2.2	2.4	2.5	1.9	1.2	3.5
Hybrid	2.4	2.6	3.9	4	3.3	3.5	5.1
Heterosis	126%	119%	163%	160%	174%	292%	148%

† All black females hybridized with Tx3362
‡ High-parent heterosis
§ ATx631/R.Tx436 grain hybrid
¶ GAE = gallic acid equivalents;
# CE = catechin equivalents
††3-DOA = 3-deoxyanthocyanin

Heterotic responses for composition traits also varied greatly in this test (Table 7). Percent heterosis for phenols and tannins averaged around 100%, implying little to no advantage for hybrids over inbreds in increasing phenol and tannin concentrations (Table 7). The full expression of phenol and tannin concentration levels in both the inbred and the hybrid is easily explained by dominant gene action. Percent heterosis for 3-DOA, however, averaged 75%, indicating that there is a decrease in 3-DOA concentration in the hybrid compared to the high parent inbred (Table 7). These results could indicate a more additive gene effect for 3-DOA concentrations in black sorghum hybrids or it could be due to other undefined factors. For all composition traits measured, little to no advantage was seen in the hybrid over the high parent inbred. The real advantage of the hybrid is in grain production.

Conclusion

Black sorghum hybrids have the potential to provide health food companies with a novel grain source with increased health benefits. Compared to commercially available grain sorghum hybrids, black sorghum hybrids in this test contained elevated levels of three phenolic compounds known to be antioxidants. Grain yields were reduced overall in black hybrids compared to the commercial hybrid due to reduced hybrid vigor and unknown traits associated with the black phenotype. The parental lineage of the black hybrids also could of reduced grain yields due to a strong susceptibility to grain mold. High parent heterosis was much higher than the commercial hybrid, mostly due to very low black inbred yields. The creation of black sorghum hybrids seem justified due to increased hybrid yields over the black inbreds. These first generation black grain hybrids can and will be improved over time with additional breeding and selection for yield and combining ability. Of the experimental seed parents tested in this study, the line A.05029 produced the highest yielding hybrids with acceptable grain quality.

The black sorghum hybrids consistently produced more tannins, phenolics and 3-DOA content than the commercial grain hybrid. However, F_1 hybrids did not increase the concentrations of phenolic compounds in this study and for 3-DOA, the concentrations were reduced compared to the parental lines. The seed parent A.05023 produced hybrids with the highest phenol and 3-DOA concentrations. In addition, the A.05023 hybrid was quite high in tannins, ranking third with an average concentration of 30.0 mg CE/g. While A.05023 was the best performing black seed parent for antioxidant composition, very low grain yields make release and production of A.05023 unreasonable.

As expected, the different environments affected grain yield and the top yielding environment for grain yield was Halfway. Phenolic composition was significantly effected by the environment but the overall differences were minimal. Significant genotype x environment interactions were observed in this study indicating the importance of multiple test locations. Based on environmental results, Halfway is proposed as an ideal production environment due to higher observed grain yields and test weights. While Halfway did not produce the highest concentrations of phenolic compounds in some black hybrids, the GxE effect observed in this study merits Halfway as the ideal production location due to superior yield potentials.

CHAPTER III

DEVELOPMENT OF SORGHUM GERMPLASM Tx3363 AND Tx3364

Introduction

Sorghum ranks fifth in cereal crop grain production in the world (FAO, 2006). In the Western Hemisphere sorghum is primarily used as a food grain whereas in Africa and Asia it is used primarily as a food crop (Rooney, 2004). Production limitations for sorghum throughout the world include abiotic stresses like drought tolerance and biotic stresses like insect and disease pressure. The sorghum species presents significant amounts of variability for a wide array of different traits, ranging from stress tolerance to grain quality (Dykes and Rooney, 2006; Rooney, 2004).

Variation for specific compounds within sorghum grain that contribute to antioxidant activity and overall health benefits has been extensively documented (Awika et al., 2004); Dykes and Rooney, 2006). In particular, the 3-deoxyanthocyanins (3-DOA) are more pH stable than other common anthocyanins and could be used as a natural food colorant (Awika et al., 2004). The highest concentrations of 3-DOA have been reported in the pericarp of selected sorghum accessions with a black grain color; this is the color of Tx3362 sorghum germplasm, which was developed and released by the Texas AgriLife Sorghum Breeding Program (Rooney et al., 2012). The sorghum germplasm Tx3362 is the first temperately adapted photoperiod-insensitive line that produces high levels of 3-DOA. However, Tx3362 yields relatively poorly as a line per se and when used as a pollinator to produce hybrids with common seed parent, it produces only red hybrids with low 3-DOA (Rooney et al, 2012). The inability to produce a black hybrid with only a single black-seeded pollinator indicates that the black seeded trait is a recessive trait and the expression in a hybrid will require both parents possessing the trait.

Given this background, a goal of the Texas Agrilife Sorghum breeding program was to develop black grain-sorghum seed parents with high levels of 3-DOA so that these lines can be used to produce black grain hybrids in combination with Tx3362. Tx3363 and Tx3364 are proposed for release because they are seed parents that possess black grain color, are high in 3-DOA and when hybridized with Tx3362, produce hybrids with the same characteristics and improved grain yield. Hybrids produced using these parents are designed for specialty use in the food industry.

Methods

The lines proposed for release as Tx3363 and Tx3364 were selected, evaluated and increased in the Texas Agrilife Research sorghum breeding program at College Station, TX. Both Tx3363 and Tx3364 were selections derived from the breeding cross of BTxARG-1ms3/'Tx3362'. BTxARG-1 is a commonly used grain sorghum seed parent with temperate adaptation that was released by Texas Agrilife Research (Miller et al., 1992). B.TxARG-1 is genetically a 3-dwarf ($dw_1Dw_2ds_3dw_4$) in height with a white seed, tan plant color and waxy endosperm. Tx3362 was released by Texas Agrilife Research in 2012 (Rooney et al., 2012) Tx3362 is genetically a 3-dwarf ($dw_1Dw_2dw_3dw_4$) in height and photoperiod insensitive. Tx3362 is unique because the line is genetically red for seed color but turns a dark black when exposed to sunlight.

This line is a pollinator parent, but in this case it served as a source of the black pericarp as that trait was not available in a seed parent background.

The original breeding cross for Tx3363 and Tx3364 was made in College Station and the F_1 hybrid was grown and self-pollinated in a fall nursery in Weslaco Texas. The F_2 population was grown in College Station and selection was based primarily on acceptable height, maturity and grain color (dark red to black). Subsequent selections in the F_3 to the F_6 generation were made in one or more of the following locations: Corpus Christi Texas, College Station Texas, and Weslaco Texas. At the F_6 generation, multiple lines from this cross were evaluated across a range of environments (Weslaco, Corpus Christi, College Station, and Lubbock, Texas) for stability and consistency of a dark black pericarp; from this evaluation, a set of ten lines were selected for further development and evaluation.

Because the breeding cross involved a seed parent and pollinator parent both maintainers and restorers of pollen fertility were expected in the derived lines. Therefore all ten lines were crossed to ATxARG-1. Any lines that produced partially or fully fertile hybrids were discarded and any lines that produced male-sterile hybrids were backcrossed to introgress the A1 cytoplasmic sterility system. Sterile versions of Tx3363 and Tx3364 were backcrossed to their fertile counterparts until isocytoplasmic lines of Tx3363 and Tx3364 were developed. Male-sterile versions of Tx3363 and Tx3364 were top crossed to Tx3362 for evaluation of hybrid combination. From preliminary agronomic evaluation, these two lines produced the best combination of agronomic desirability and grain quality (i.e. darkest color and highest 3-DOA) (data not shown). Tx3363 and Tx3364 were tested under the designation of A/B.05029 and A/B.05030, respectively.

For comparison purposes, agronomic data for Tx3363 and Tx3364 and their hybrids is presented from field trials grown in College Station and Halfway, Texas in 2009. In addition to standard agronomic data (plant height, day-to-anthesis, grain yield, test weight and moisture content), grain samples were collected for chemical composition. Chemical composition of lines and hybrids were estimated using NIR analysis and calibration curves developed within the Texas AgriLife Sorghum Breeding Program. Composition traits estimated using NIR were total phenols, tannins, and 3-deoxyanthcyanin (3-DOA).

Characteristics

Inbred Lines

Tx3363 and Tx3364 are maintainers of sterility in the A1 cytoplasmic male sterility system. While their reactions in cytoplasmic genetic male sterility systems (A2 and A3) have not been tested, based on pedigree, both lines are likely maintainers of sterility in both of these systems. Both lines are genetically three-dwarf ($dw_1Dw_2ds_3dw_4$) and are photoperiod insensitive, flowering earlier than most standard checks and very similar to Tx3362 (Table 7). Agronomically, the line per se grain yields of Tx3363 and Tx3364 are similar to Tx3362 (Table 8).

Table 8. Agronomic trait means of Tx3363 and Tx3364 compared with Tx3362 and two standard breeding lines (BTx642, Tx2953) grown in College Station and Halfway Texas in 2009.

Trait	Tx3363	Tx3364	Tx3362	BTx642	Tx2953	L.S.D (P<.05)
Days to anthesis (d)						
College Station	66	70	66	76	82	1
Halfway	69	67	72	73	74	1
Combined	67	68	69	75	77	1
Panicle exertion (cm)						
College Station	7.6	2.5	2.5	0	0	1.6
Halfway	5.1	0	10.2	12.7	10.2	3.2
Combined	6.5	1.5	4.6	5.2	5.1	2.4
Moisture content (%)						
College Station	13.8	13.7	11.5	12	11.4	0.5
Halfway	15.2	14.3	16.4	12.9	15.3	0.5
Combined	14.3	13.9	14.8	12.4	13.6	0.5
Test weight (kg hl^{-1})						
College Station	55	53	58.4	63	63.3	1.4
Halfway	67.3	67	60.6	74.9	72.9	1.8
Combined	61.1	59.9	59.7	67.6	68.5	1.7
Plant height (cm)						
College Station	91.4	99.1	109.2	99.1	109.2	3
Halfway	109.2	114.3	119.4	109.2	139.7	3.5
Combined	101.4	106.3	112.8	105.5	119.3	3.2
Grain yield (kg ha^{-1})						
College Station	1425	2377	521	780	1187	340
Halfway	1594	2140	1550	643	2238	450
Combined	1509	2291	1036	702	1712	390

Total phenols, tannins, and 3-DOA concentrations were higher for Tx3363 and Tx3364 than other common sorghum seed parents (Table 9). Both Tx3363 and 3364 have higher 3-DOA levels than sumac, but they are lower than the concentrations found in Tx3362 (Table 9). Tannin concentrations were highest in 'sumac', but the moderately high concentrations observed in both Tx3363 and Tx3364 were somewhat surprising as neither Tx3363 nor Tx3364 have an obvious pigmented testa layer (Table 9) (Dykes et al., 2006). This was also reported in Tx3362 and it indicates that all of these lines contain a partial testa, which is often difficult to detect phenotypically (Table 9).

Table 9. Composition trait means relative to antioxidant activity of Tx3363 and Tx3364 compared with Tx3362 and three standard breeding lines (B.Tx642, Tx2953, and Sumac) grown in College Station and Halfway Texas in 2009.

Trait	Tx3363	Tx3364	Tx3362	BTx642	Tx2953	Sumac	L.S.D (P<.05)
College Station							
Phenols (mg GAE/g)†	14.1	15.8	14.2	5.3	3.9	20.4	1.8
Tannins (mg CE/g)‡	26.2	31.5	25.1	3.4	2.8	43.4	1.2
3-DOA (Abs/mL/g)§	116.1	142.1	158.9	58.6	29.5	35.3	3.1
Halfway							
Phenols (mg GAE/g)†	15.8	21.3	15.2	2.6	4.1	20	1.8
Tannins (mg CE/g)‡	35.7	50.8	33.8	3.2	5.5	45.1	3.1
3-DOA (Abs/mL/g)§	99.1	132.1	171.9	29.9	17.9	46	4.5

† GAE = gallic acid equivalents
‡ CE = catechin equivalents
§ 3-DOA = 3-deoxyanthocyanin

Hybrid Performance and Heterosis

Compared to the lines per se, grain yield in the black sorghum hybrids (ATx3363/RTx3362 and ATx3364/RTx3362) was significantly higher than any of the lines per se (Tables 8 and 10). Across all environments and hybrids, high parent heterosis for grain yield averaged 180% and indicates that performance can be substantially improved over line per se. A large high parent heterosis value in these hybrids can also be explained by the relatively poor performance of the black inbreds. This performance could be due to unknown genetic factors associated with the black pericarp, as well as grain mold susceptibility caused by a floury endosperm. Heterosis was substantially different between the two lines; Tx3363 has higher heterosis than Tx3364, implying that it combines better with Tx3362. Given that the pollinator parent (Tx3362) is in the pedigree of the seed parent, it is likely that heterosis can be improved with additional breeding to separate the lineages of the seed and pollinator parents. The differences in heterosis between Tx3363 and Tx3364 may be due to these factors as was observed in Tx623 and Tx430 in previous research (Menz et al., 2004).

While the black sorghum hybrids exhibited moderate to good heterosis, black sorghum hybrids still yielded significantly less than typical commercial hybrids (Table 10). Across all environments and hybrids, the black sorghum hybrids yielded on averaged 69% if the elite grain sorghum hybrids (Table 10). Composition trait means corresponding to antioxidant concentrations were also many times higher in black hybrids compared to traditional grain hybrids (Table 11). In the Halfway environment, the best black hybrid (Tx3363/RTx3364) yielded 71% of the best commercial grain sorghum hybrid (84G62). In addition, compared to the commercial checks, test weights were lighter, likely due to an endosperm that was more floury (Table 10). The lower yields will require a premium to induce a grower to produce the crop. Ultimately, end-users will determine if the value of the compounds produced justify the premium required for production.

Table 10. Agronomic trait means of hybrids of Tx3363 and Tx3364 compared with three commercial check hybrids grown in College Station and Halfway Texas in 2009.

Trait	ATx3363/ RTx3362	ATx3364/ RTx3362	Pioneer 84G62	ATx2752/ RTx437	ATx642/ Tx2953	LSD (P<.05)
Days to anthesis (d)						
College Station	63	64	69	64	71	1
Halfway	60	61	65	63	62	1
Combined	62	62	67	64	67	1
Panicle exertion (cm)						
College Station	7.6	1.3	1.3	10.2	6.4	2.6
Halfway	11.5	8.4	4.6	9.8	9.4	3.1
Combined	9.3	4.3	3.7	10	8.1	2.9
Moisture content (%)						
College Station	12.3	12.6	14.3	12.3	13.5	0.5
Halfway	14.4	14.1	15.3	14.3	13.6	0.5
Combined	12.9	13.3	14.9	12.9	13.5	0.5
Test weight (kg hl-1)						
College Station	55.5	53.1	60.9	70	67.3	1.8
Halfway	67.7	68.4	73	72.7	77.5	1.5
Combined	61.4	61.5	68.2	71.1	72.2	1.4
Plant height (cm)						
College Station	116.8	99.1	124.5	134.6	119.4	4
Halfway	139.7	129.5	127	139.7	147.3	3.5
Combined	131.6	130	127	135.1	129.4	3.5
Grain yield (kg ha-1)						
College Station	3044	2377	4762	4893	3891	480
Halfway	4843	3415	6346	5398	4651	410
Combined	3944	2896	5554	5145	4271	420

Table 11. Composition trait means relative to antioxidant activity of hybrids of Tx3363 and Tx3364 compared with three commercial check hybrids grown in College Station and Halfway Texas in 2009.

Trait	ATx3363/ RTx3362	ATx3364/ RTx3362	Pioneer 84G62	ATx2752/ RTx437	ATx642/ Tx2953	LSD (P<.05)
College Station						
Phenols (mg GAE/g)†	15.4	16	2.1	1.8	1.9	1.2
Tannins (mg CE/g)‡	27.2	26.5	0	0	0	0.9
3-DOA (Abs/mL/g)§	87.6	110.1	7.4	5.3	9.4	2.2
Halfway						
Phenols (mg GAE/g)†	15.3	16.2	1.9	2.2	2.3	1.2
Tannins (mg CE/g)‡	34.8	37.8	0	0	0	0.8
3-DOA (Abs/mL/g)§	114.5	138.9	7.9	7.8	11.1	2.7

† GAE = gallic acid equivalents;
‡ CE = catechin equivalents
§ 3-DOA = 3-deoxyanthocyanin

Conclusions

These seed parents provide the sorghum industry the first opportunity to produce a grain sorghum hybrid with a black pericarp color. Associated with this color is an increase in 3-DOA, tannins and phenols. Concentrations of all three compounds have multiple potential benefits including shelf stability and antioxidant activity (Awika et al., 2004; Dykes and Rooney, 2006). Tx3363 is being released because of its higher hybrid yield potential relative to other black pericarp seed parents (Table 10). Tx3364 is proposed for release because of its elevated (3-DOA) levels compared to Tx3363 (Table 11). Figure 6 provides images of Tx3363 and Tx3364 being grown in the Halfway environment in 2009. While the grain yield of these hybrids is not competitive with commercial grain sorghum hybrids, they represent a substantial improvement over the lines per se. Thus, production of these hybrids will likely be on a contract basis and any grain produced will be identity preserved for end use.

Figure 6. Rows of Tx3363 and Tx3364 being grown in Halfway, Texas in 2009.

CHAPTER VI

CONCLUSION

Sorghum with a black pericarp is a novel source of phytochemicals known for antioxidant potential and overall health. Flour from black sorghums can be sold into health food markets as a unique cereal grain with beneficial compounds not currently in the marketplace. The bran from black sorghums can also be removed and processed into an antioxidant rich concentrate. The willingness of consumers to pay premium prices for healthy food additives has cause health food companies to search for novel sources of healthy food inputs to meet growing demand. Although many plant based products with health benefits are known and on the market today, specific novel benefits within black sorghum which include shelf stability and high 3-DOA concentrations make black sorghum an ideal health food crop.

The black seed parents A.05029 and A.05030 are proposed for release in this study with the designation Tx3363 and Tx3364 due to high grain yield and elevated phenolic grain concentration. Tx3363 contains higher yield potential while Tx3364 contains higher phenols. Both provide the end user with novel health food compounds desired by the marketplace today. Halfway is proposed as an ideal production environment due to much higher grain yields compared to College Station and Weslaco. Halfway being a temperate growing environment helped facilitate higher grain yields in these hybrids. In addition, Halfway also produced similar concentrations of phenolic compounds in Tx3363 and Tx3364 when compared with the other locations. These results designate Halfway, or similar locales, as the ideal production environment for these black sorghum hybrids.

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