# INHERITANCE OF BLACK PERICARP TRAIT IN SORGHUM

An Undergraduate Research Scholars Thesis

by

KATHLEEN MARIE HILL

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Research Advisor: Dr. William Rooney

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# ABSTRACT

Inheritance of Black Pericarp Trait in Sorghum. (May 2015)

Kathleen Hill
Department of Horticulture
Texas A&M University

Research Advisor: Dr. William Rooney
Department of Soil & Crop Science

Black sorghum is rich in health benefits and potential for the natural food markets. It has the highest levels of antioxidants of any cereal crop and contains other phenolic compounds of interest, such as tannin and 3-deoxyanthocyanidin. However, it is lower yielding than other commercial hybrids, with only 64% of the yield, and when crossing a black sorghum with other elite grain sorghums, the black pericarp trait is recovered on a very low percentage of the offspring. Therefore, there is a need for a stable commercial hybrid be created. Relatively little is known of the genetic effects and heritability of the black pericarp trait, so to better understand the inheritance pattern, a generation means analysis was performed on a cross between Tx3362, a black sorghum, and BTx623, a white elite hybrid. JMP Pro 11.0.0 and a joint scale test were used to analyze the material. The black pericarp trait was found to be recessive, controlled by many genes, highly epistatic, and heritable. Although more research into this trait will be necessary to fully comprehend the complexities of the genes controlling it, selecting for this trait to create high yielding black sorghum populations should be possible for breeders.

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# NOMENCLATURE

3-DOA.....3-Deoxyanthocyanidins

GMA .....Generation mean analysis

NIR..... Near-infrared spectroscopy

*L**.....Lightness value

*a**.....Red/green value

*b**.....Yellow/blue value

*m*..... Midparent

*a*..... Additive

*d*.....Dominance

*a x a*..... Additive x additive

*a x d*.....Additive x dominance

*d x d*..... Dominance x dominance

# CHAPTER I

# INTRODUCTION

With the worldwide rise in obesity (W.H.O., 2000), there has also been an increase in consumer demand for healthier food products. This global trend has also affected modern day plant breeding with the advent of crops like Golden Rice or biofortified sweet potatoes. Because of this trend, there is potential for other cereal crops like sorghum, which contains many beneficial health compounds (Dykes and Rooney, 2006), particularly black sorghum (Awika et al., 2005), to be bred to maximize these health compounds. This research expounds upon the advantages of creating a stable, commercially viable black sorghum and the challenges that face doing so.

Sorghum is high in many important phytochemicals, including, tannins, plant sterols, and anthocyanins. However, the presence of 3-deoxyanthocyanidins (3-DOA) is unique to black pericarp sorghum (Pfeiffer, 2014). Among anthocyanins, 3-DOA is different because it lacks a hydroxyl group at the C3 position (Awika and Rooney, 2004). Due to this structural difference, the molecule is stable at both high and low pH levels, making it a viable candidate for use as a natural food colorant and preservative (Pfeiffer, 2014). Sorghum is also naturally gluten free (Pfeiffer, 2014) making it an excellent wheat substitute for those who suffer from Celiac Disease.

Consuming products made of sorghum high in phenolic compounds like flavonoids, 3-DOA, and tannins, has also been connected to lower incidence of cardiovascular disease, cancer, and obesity (Awika and Rooney, 2004). These compounds act as antioxidants (Dlamini et al., 2007) and in the body, ward off free radicals that lead to disease (Rajendran, et. al., 2014). Sorghum,

compared to other cereals, has the highest level of antioxidants (Pfeiffer, 2014). In fact, black sorghum has levels equivalent to blueberries (Awika and Rooney, 2004) and 40 times greater than white sorghum grain hybrids (Pfeiffer, 2014).

Tannin rich sorghum, when consumed, binds to proteins and carbohydrates making them insoluble and indigestible leading to weight loss (Awika and Rooney, 2004). While this may be undesirable for livestock, it poses great possibilities for humans in a time when obesity is on the rise (Awika and Rooney, 2004).

Unfortunately, black sorghum is lower yielding than other grain sorghum hybrids, with only 64% of the yield of a commercial red or white sorghum hybrid (Pfeiffer, 2014). However, with more understanding about the genetic effects, heritability, and loci that control the black pericarp trait, breeding for a high yielding black sorghum would be possible. Past work has revealed that when crossing a black sorghum to a red sorghum, the black pericarp trait was determined to be recessive, controlled by many genes, and heritable (Pfeiffer, 2014). This research compares those results to a cross made between a white and black sorghum.

**Objectives**

The objectives of this research are to:

- Determine the genetic effects controlling the black pericarp trait using generation means analysis of a cross between a white sorghum and black sorghum,
- Determine the heritability of the black pericarp trait, and
- Estimate the number of genes controlling this trait

# CHAPTER II

# METHODOLOGY

Generation means analysis (GMA) is a widely used method of determining the inheritance of quantitative traits (Pfeiffer, 2014). Two parents, $P_1$ and $P_2$, are crossed to make the $F_1$ generation, which is then selfed to create the $F_2$ generation. A backcross is also made between the $F_1$ and each parent, creating $BC_1P_1$ and $BC_1P_2$, respectfully (Pfeiffer, 2014).

The parents of this material were Tx3362, a black sorghum, and BTx623, a white sorghum. The six generations ($P_1$, $P_2$, $F_1$, $F_2$, $BC_1P_1$, and $BC_1P_2$) were grown in three locations -College Station, Weslaco, and Halfway, Texas in 2013. They were visually evaluated in the field as well as tested using a colorimeter and a near-infrared scanner. A bleach test was performed to categorize grain as with or without a testa layer and then was evaluated visually to classify as with or without a spreader gene. Using the data that was retrieved (L*, a*, b*, phenols, tannins, fiber, etc.) a generation means analysis was performed using JMP statistical software and JNTScale software.

Using JMP Pro 11.0.0, an analysis of variance was performed to determine if environment, genotype, and environment x genotype interactions were significant. With the tabulate platform in JMP, standard descriptive statistics (mean, variance, standard error) for the traits of interest within each generation were calculated. Also, a joint scale test using a six parameter model was used to determine if additive, dominance, and midparent, as well as additive x additive, additive x dominance, and dominance x dominance interactions effects were significant. To estimate heritability, the equation $H^2 = \frac{F_2 - \frac{P_1+P_2+F_1}{3}}{F_2}$ was utilized in which $P_1$, $P_2$, $F_1$, and $F_2$ represent the

means of both parents and the $F_1$ and $F_2$ generations (Pfeiffer, 2014). The number of genes controlling the black pericarp trait was estimated using $N = \left(\frac{(P_1 - P_2)^2}{8}\right)\left(\hat{\sigma}^2_{F_2} - \hat{\sigma}^2_{P_1,P_2,F_1,\text{pooled}}\right)$. $P_1$ and $P_2$ are the means of BTx623 and Tx3362, $\hat{\sigma}^2_{F_2}$ is the variance of the $F_2$ generation, and $\hat{\sigma}^2_{P_1,P_2,F_1,\text{pooled}}$ is the pooled variance of BTx623, Tx3362 and the $F_1$ generation (Pfeiffer, 2014). With the data that was generated from the analysis and using the calculations above, the relative genetic effects (additive, dominance, and epistasis), number of genes, and the trait's heritability was estimated.

# CHAPTER III

# RESULTS

The material was grown in three locations (College Station, Halfway, and Weslaco, Texas), and it was determined through an analysis of variance that while differences were recorded, the generation x environment interaction was not considered significant in comparison to the generation x generation interactions. Therefore, the data was analyzed as a whole rather than by each location.

## Means

Table 1 shows the results of the means calculated for the color traits. High L* values signify a lighter appearance. BTx623, the white parent, had the highest value with a mean of 68.51 ± 0.46 indicating it was the lightest generation, while the black parent Tx3362 had the lowest value (30.46 ± 0.22) making it the darkest generation. The $F_1$ value fell in the middle as did the $F_2$, which was slightly darker than the $F_1$. Each backcross was closest to its respective parent in value. Overall, the results of L* were as expected. For b*, the degree of yellow, the results acted in a similar fashion to that of L*, with the white parent containing the most yellow and the black parent the least. For a*, which is the amount of redness, $F_1$ had the highest value and the parents the smallest value, with white having the least of all. This again is expected. When crossing the black and white parent, the $F_1$ generation consisted of all red progeny. The $F_2$, $BC_1P_1$, and $BC_1P_2$ were segregating for color and therefore had lower values than the $F_1$, but higher than both the $P_1$ and $P_2$.

**Table 1.** Means and standard errors of the color traits.

| Generation | N | *L** | *a** | *b** |
|---|---|---|---|---|
| B.Tx623 | 110 | 68.51 ± 0.46 | 5.05 ± .06 | 22.66 ± 0.11 |
| $BC_1$B.Tx623 | 310 | 52.08 ± 0.51 | 11.02 ± .24 | 23.03 ± 0.16 |
| $F_1$ | 110 | 40.09 ± 0.35 | 15.88 ± .08 | 19.85 ± 0.28 |
| $F_2$ | 619 | 42.82 ± 0.34 | 12.84 ± 0.13 | 19.23 ± 0.20 |
| $BC_1$Tx3362 | 309 | 37.97 ± 0.24 | 13.12 ± 0.11 | 15.98 ± 0.26 |
| Tx3362 | 110 | 30.46 ± 0.22 | 6.74 ± 0.14 | 6.83 ± 0.28 |

Table 2 contains the results of the means of the phenolic traits. Like a*, tannins and phenols had the highest values in the $F_1$ generation and the lowest in the $P_1$ and $P_2$ generations. This can be explained by the $B_1$ and $B_2$ genes. Two genes control tannins, $B_1$ and $B_2$, and both must be dominant for tannins to be present in significant amounts (Dykes and Rooney, 2006). Each of the parents were dominant for only one gene and therefore they had little to no tannin. BTx623, which was sequenced in a previous study (Paterson et al. 2009), is genetically $b_1$_$B_2$_, whereas Tx3362 is $B_2$_$b_1$_ (Pfeiffer, 2014). When combined, the $F_1$ progeny was heterozygous for both genes and therefore had the highest levels of tannin overall. 3-DOA acted very differently than tannins and phenols, however. Levels of 3-DOA in Tx3362 (192.67 ± 5.11) were almost quadruple that of the black backcross (50.25 ± 2.32), the next highest generation, and ten times higher than BTx623 (19.08 ± 1.32), the white parent.

**Table 2.** Means and standard errors of the phenolic traits

| Generation | N | Phenols† | Tannins‡ | 3-DOA§ |
|---|---|---|---|---|
| B.Tx623 | 72 | 3.75 ± 0.15 | 6.18 ± 0.36 | 19.08 ± 1.32 |
| $BC_1$B.Tx623 | 202 | 7.28 ± 0.28 | 14.31 ± 0.76 | 15.80 ± 0.94 |
| $F_1$ | 73 | 13.23 ± 0.22 | 27.62 ± 0.61 | 22.25 ± 1.56 |
| $F_2$ | 435 | 8.21 ± 0.22 | 14.84 ± 0.56 | 16.24 ± 0.77 |
| $BC_1$Tx3362 | 210 | 8.82 ± 0.32 | 16.07 ± 0.91 | 50.25 ± 2.32 |
| Tx3362 | 75 | 8.33 ± 0.33 | 11.35 ± 0.74 | 192.67 ± 5.11 |

† Total phenols
‡ Condensed tannins
§ 3-deoxyanthocyanidins

## Effects of Tannin and a Spreader Gene

As both tannins and the presence of a spreader gene play a role in the phenotypic color of the sorghum, their relationship was tested in regards to levels of phenols and 3-DOA present (Table 3). The presence/absence of tannin (based off of NIR results) greatly influences both phenol and 3-DOA levels. The presence of a spreader (evaluated visually) had less of an effect. Phenols were significant, and 3-DOA was higher if a spreader was present, however, it wasn't considered significant. Overall, however, the presence of a tannin rich testa layer and spreader gene did in fact increase the amount of beneficial phenolic compounds found in the sorghum.

**Table 3**. Effects of the presence of tannins and a spreader on the level of phenols and 3-DOA.

| Tannins Present | Mean (Phenol) | Std. Error (Phenol) | Mean (3-DOA) | Std. Error (3-DOA) | N |
|---|---|---|---|---|---|
| yes | 11.360 | ± 0.187 | 19.962 | ± 1.073 | 259 |
| no | 3.563 | ± 0.126 | 10.761 | ± 0.922 | 176 |

| Spreader Presence | Mean (Phenol) | Std. Error (Phenol) | Mean (3-DOA) | Std. Error (3-DOA) | N |
|---|---|---|---|---|---|
| yes | 12.345 | ± 0.211 | 20.894 | ± 1.397 | 162 |
| no | 10.635 | ± 0.316 | 19.266 | ± 1.796 | 71 |

## Genetic Effects

A six parameter model was used estimate the genetic effects. Color traits (L*, a*, and b*) as well as the phenolic compounds (total phenols, condensed tannins, and 3-deoxyanthocyanidins) were tested. For every trait examined, midparent and additive were considered significant.

For the color traits (L*, a*, and b*), the more negative the number, the darker the pericarp color. For L*, (lightness) all six parameters were significant. Midparent, additive, dominance, and additive x additive were all significantly positive, while additive x dominance and dominance x dominance were significantly negative. The redness variable (a*) was high in both midparent and dominance. The yellowness variable (b*) was significantly positive for midparent, additive and dominance, and like L*, is significantly negative for both additive x dominance and dominance x dominance.

For the phenolic traits, phenols and tannins behaved very similarly. However, phenols are significant for midparent, additive, and *d* x *d* while tannins were significant only in midparent and additive. Tannins were also much higher in dominance and *d* x *d* than phenols. 3-DOA had very different effects with all six parameter acting significantly. Additive and dominance were both highly negative, whereas midparent, *a* x *a*, *d* x *d*, and *a* x *d* were highly positive. In summary, the traits of interest are controlled by many effects, and for most of the traits, by multiple genes, especially in the case of L* and 3-DOA in which they are highly epistatic.

**Table 5**. Estimation of the genetic effects using the six parameter model including midparent (*m*), additive (*a*), and dominance (*d*), as well as additive x additive (*a* x *a*), dominance x dominance (*d* x *d*), and additive x dominance (*a* x *d*).

| **Trait** | ***m*** | ***a*** | ***D*** | ***a* × *a*** | ***a* × *d*** | ***d* × *d*** |
|---|---|---|---|---|---|---|
| *L** | 40.64 ± 1.81** | 19.02 ± 0.25** | 9.26 ± 4.47** | 8.85 ± 1.79** | -9.83 ± 1.24** | -9.81 ± 2.80** |
| *a** | 8.96 ± 0.75** | -0.85 ± 0.07** | 8.58 ± 1.91** | -3.06 ± 0.74** | -2.49 ± 0.54** | -1.66 ± 1.19 |
| *b** | 13.67 ± 1.03** | 7.92 ± 0.15** | 16.07 ± 2.50** | 1.07 ± 1.02 | -1.73 ± 0.68** | -9.89 ± 1.60** |
| Phenols[†] | 6.67 ± 1.25** | -2.29 ± 0.18** | -0.41 ± 3.18 | -0.63 ± 1.23 | 1.49 ± 0.93 | 6.97 ± 2.01** |
| Tannins[‡] | 7.34 ± 3.28** | -2.58 ± 0.41** | 9.69 ± 8.49 | 1.42 ± 3.26 | 1.65 ± 2.50 | 10.59 ± 5.43 |
| 3-DOA[§] | 38.74 ± 6.45** | -86.80 ± 2.64** | -73.50 ± 18.14** | 67.14 ± 5.88** | 104.67 ± 7.28** | 57.02 ± 12.15** |

† Total phenols
‡ Condensed tannins
§ 3-deoxyanthocyanidins

## Heritability

Table 6 shows the results of the calculation of heritability for the traits of interest. All of the color traits are highly heritable, with a* (0.90) being the most heritable, followed by L* (0.81), and b* (0.75). Of the phenolic traits, both tannins (0.81) and phenols (0.79) are highly heritable and 3-DOA (0.41) is moderately so. Overall, both color and the compositional traits of interest in this study are heritable.

**Table 6.** Estimate of broad sense heritability of both color traits and phenolic compounds.

| **Trait** | Heritability (Broad) |
|---|---|
| *L** | 0.81 |
| *a** | 0.90 |
| *b** | 0.75 |
| Phenols† | 0.79 |
| Tannins‡ | 0.81 |
| 3-DOA§ | 0.41 |

† Total phenols
‡ Condensed tannins
§ 3-deoxyanthocyanidins

## Estimation of Genes Involved

The number of genes affecting each of the traits in question were estimated using Lande's Method (Table 7). Based on this conservative model, 2 to 3 genes control the lightness of the grain (L*), 1 gene affects redness (a*) and 1 to 2 genes play a role in the yellowness (b*) present.

Both tannins and phenols are affected by 1 gene, whereas 3-DOA is controlled by as many as four.

**Table 7.** Estimation of the number of genes involved

| Trait | Number of Genes |
|---|---|
| *L** | 2.12 |
| *a** | 0.02 |
| *b** | 1.63 |
| Phenols† | 0.08 |
| Tannins‡ | 0.01 |
| 3-DOA§ | 3.75 |

† Total phenols
‡ Condensed tannins
§ 3-deoxyanthocyanidins

# CHAPTER IV

# DISCUSSION

As hypothesized, the inheritance of black pericarp trait and its associated beneficial compounds is complex. All of the traits were controlled by multiple genetic effects (Table 5) and most are highly epistatic which confirms that these traits are controlled by multiple genes (Table 7). All of the traits however, are moderately to highly heritable (Table 6).

The black pericarp trait is recessive; this was confirmed by the low number of progeny that were recovered from the $F_1$, $F_2$, and backcrosses (Table 1). Of the phenolic compounds, 3-DOA is strongly correlated to black pericarp color as indicated by the means calculated by the analysis of variance (Table 1). No other generation had 3-DOA levels close to that of the black parent, therefore, this trait is also highly recessive.

Condensed tannins and total phenols are significantly increased when a spreader gene and tannin rich testa layer are present (Table 3) and, as established previously, tannins are only present in those which are dominant in both the $B_1$ and $B_2$ loci. Therefore, sorghums which are dominant at both tannin loci and are dominant for the spreader gene contain higher levels of beneficial compounds than those that are not. Unlike 3-DOA, the progeny contained higher levels of tannins and phenols than either of the parents. Therefore, while tannins and the presence of a spreader do actually darken the appearance of the grain and consequently are found in higher quantities in darker generations (i.e. the white backcross versus the black backcross –Table 1), there is not a strong correlation between a true black pericarp and levels of condensed tannins

and total phenols, i.e. not all black pericarp sorghums have tannin and tannin is not exclusively found in black sorghum.

One of the problems that arose with using a generation means analysis with this material was the GMA model assumes that one of the parents contributes all of the beneficial genes and the other parent provides none and this was not the situation for all traits. Tannins, as we have already stated, require that both genes be dominant for the trait to be expressed. This explains the estimation of genetic effects for tannins in which, although a high value was given, the model did not consider tannins to be significant in either dominance or *d* x *d*.

The method used to calculate the number of genes contributing to a trait poses another issue. It assumes no linkage or epistasis between genes. However, after determining genetic effects, it has been established that epistasis does in fact occur. This may explain the relatively low number of estimated genes that control the traits of interest.

To fully comprehend the complexities of the black pericarp trait and associated phenolic traits more research should be conducted. While estimating the number of genes that are involved with these traits is a step forward, more work should be done to locate chromosomal regions and possibly even individual genes that control the traits of interest using a bulked segregant analysis.

As stated above, the black pericarp trait is complex, being recessive, epistatic, and controlled by multiple genes. This accounts for the lack of having established a stable, commercially viable

black sorghum. However, although it is complex, these traits are heritable. Black sorghum has the possibility to be a widely used commodity in the constantly growing health food market, therefore, the benefit of creating a black sorghum containing the beneficial compounds outlined in this research would be a wise investment and would be possible through standard breeding practices.

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