EFFECT OF ENVIRONMENT AND GENETICS ON FLAVONOID LEVELS IN SORGHUM GRAINS

A Thesis

by

VICTOR MANUEL TALEON ALBAN SR.

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

May 2010

Major Subject: Food Science and Technology

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Approved by:

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ABSTRACT

Effect of Environment and Genetics on Flavonoid Levels in Sorghum Grains.

(May 2010)

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Chair of Advisory Committee: Dr. Lloyd W. Rooney

Sorghum flavonoids have antioxidant, anti-inflammatory and anti-cancer properties and potential as natural colorants in foods. Sorghums with pigmented pericarp have high levels of flavonoids, especially 3-deoxyanthocyanins, flavanones and flavones. The effect of environment on flavonoid levels in sixteen sorghum genotypes grown in four locations in Texas (Corpus Christi, College Station, Halfway, and Weslaco) was evaluated.

Sorghums from Halfway were grown at lower temperatures and the grains were more weathered than grains in the other three locations, which affected flavonoid levels, flavonoid profile, antioxidant activity, color and hardness of the grain. In general, 3-Deoxyanthocyanin levels were higher in black sorghums (151.6 - 1047.5 μg/g) while flavanones and flavones were higher in two yellow sorghums (308.8 - 1823.1 μg/g) and red sorghum 99LGWO50 (144.9 – 394.0 μg/g), respectively. Among locations, black sorghums at Halfway had lower levels of 3-deoxyanthocyanins (292.1 μg/g), but red and yellow sorghums had higher levels (100.4 and 37.2 μg/g, respectively). Flavanone levels in black and yellow sorghums at Halfway (77.9 and 525.7 μg/g, respectively) were lower than those from the other three locations. Weathered black sorghum from Halfway had

lower flavone levels than those grown in other locations (11.1 μg/g) while in the red 99LGWO50, the levels were higher in Halfway (246.8 μg/g). For all flavonoids, there was a genotype by environment interaction ($p < 0.001$), which suggested that environment had a different effect on flavonoid levels depending on the genotype.

Environment, especially weathering, affected flavonoid levels and profile of sorghums which had an impact in color; hardness of the grain also was affected by environmental conditions. Evaluation of the effects of these changes in processing of sorghum foods is necessary.

DEDICATION

To my father Manuel Taleon and my mother Gloria Otilde Alban

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INTRODUCTION

Sorghum is the fifth most important cereal crop in the world and third in the United States not only because of its total production (FAOSTAT 2009) but also because of its wide use and adaptation to diverse environmental conditions (Maunder 2000), especially drought, where other crops cannot produce as efficiently as sorghum does (Blum 2004). Sorghum is associated with health benefits; for example, sorghum bran has shown anti-inflammatory effects and anticancer activity which were correlated with their high content of phenolic compounds and high levels of flavonoids (Burdette et al 2007; Bralley et al 2008).

All the major groups of flavonoids have been identified in sorghum grains (Nip et al 1969, 1971; Waniska et al 1989; Dykes and Rooney 2006) from which three have been found in high amounts in a diverse range of sorghums: 3-deoxyanthocyanins, flavones and flavanones (Dykes 2008). The 3-deoxyanthocyanins which have shown anticancer cell proliferation (Shih et al 2007), and also potential as natural colorant in food systems at low pH (Awika et al 2004a) are found in high concentrations in black sorghum grains (Awika et al 2004b; Dykes 2008). Flavanones and flavones which have shown anticancer activity (Kuntz et al 1999) are found in high concentrations in yellow and red sorghums, respectively.

This thesis follows the style of Cereal Chemistry.

Most flavonoids in sorghum are located in the outer layers of the grain (Shirley et al 1998; Awika et al 2005); consequently, differences in pericarp, testa and aleurone characteristics of sorghums controlled by their genotype and environment influence the flavonoid concentration and profile. The flavonoid concentration and profile have great influence in the color of the pericarp. Sorghum grains are classified as black, red, yellow and white pericarp color, which genetically are determined by the R and Y genes (Rooney and Miller 1982).

The metabolism of sorghum flavonoids is regulated by its genotype, but also by environmental conditions (Stafford 1990; Boddu et al 2005; Shih et al 2006). Research in sorghum mesocotyls show that the synthesis of flavonoids is influenced by light (Orczyk et al; Weiergang et al 1996) or fungal infection (Nicholson et al 1992). Christie et al (1994) reported that temperature has an effect on the production of anthocyanins in maize. Dykes (2008) found that environment affected total concentration of anthocyanins in black, red and yellow sorghum grains, but the effect of environment on the concentration of major flavonoids in each type of sorghum is not described.

Change in flavonoids profile was found in each group of sorghum in different environments in preliminary results from Dykes (2008). With the information available, it is still difficult to screen sorghum varieties to identify unique sources of natural colorants or specific flavonoids. Considering this, a study with a wide range of environmental conditions representative of the production areas of sorghum and their effect on the flavonoid concentration in sorghum grains is necessary to understand the response of the sorghum genotype to external conditions.

Objectives

- Determine the effect of environment on major flavonoids, antioxidant activity levels color and hardness of the grain.
- Identify genotypes that produce more of each type of flavonoid in specific environmental conditions.
- Determine the effect of a change in flavonoid profile on the antioxidant capacity and color of the grain.

LITERATURE REVIEW

Sorghum Grain Use

In Asia and Africa sorghum grain is used mostly for human foods (FAOSTAT 2009). The most common types are flat breads from fermented or unfermented dough, thin or thick fermented or unfermented porridges, cous cous, and fried products (Rooney and Waniska 2009; INTSORMIL 2006; Leder 2004). The types of sorghums used for vary from white, yellow or red sorghums. In the United States, South America and Australia it is used principally for animal feed, and the sorghum used for human food is white sorghum, which is used as a substitute for corn and wheat. In recent years, the production of specialty colored sorghums free of tannins and high in flavonoids have become important (Waniska and Rooney 2000), because of their alternative uses as a source of natural colorants (Awika et al 2004a) and phytochemicals beneficial for human health (Burdette et al; Shih et al 2007; Bralley et al 2008).

Sorghum Processing

Most of the sorghum used for foods involves a milling process where the bran is removed and the endosperm is reduced to flour (Rooney and Waniska 2000; Taylor 2003). Traditionally in Africa, sorghum milling has been done manually using a mortar and pestle, limiting the production of homogeneous products because the batches are small and the sorghum characteristics are variable. In modern operations, the use of mechanized mills and sorghum with standard quality parameters makes the production of homogeneous products that can be used to make products such as prepared foods or

instant porridges. A considerable amount of bran can be obtained from these non-traditional operations and this can be used as well.

Sorghum Bran

The bran of sorghum grain is composed principally by the pericarp, testa, aleurone and germ; endosperm also is present and its quantity depends on the degree of decortication of the sorghum. The brans of colored sorghums have high levels of flavonoids because most of the flavonoids are located in the outer layers that are removed by decortication (Awika et al 2005; Dykes et al 2009). The bran, apart from its uses as source of fiber, also can be added to foods as natural colorants or as a nutraceutical ingredient (Burdette et al; Shih et al 2007; Bralley et al; Farrar et al 2008).

Sorghum Color

Genetic factors determine the pericarp color, pericarp thickness, presence of a pigmented testa and pigmentation of the glumes (Rooney and Miller, 1982). Pericarp color is the factor that most influences the color of the grain. Sorghum grains can be classified by the color of their pericarp as red, yellow and white. These properties are determined by the R and Y genes. The pericarp is white when the gene Y is homozygous recessive (rryy or R_yy). It is yellow when genes R and Y are homozygous recessive and homozygous dominant, respectively (rrYY). When both genes R and Y are dominant (R_Y_), the pericarp is red. Some sorghums with (R_Y_) genes in the presence of intense light turn black at maturation; these sorghums are known as black

sorghums (Dykes et al 2005). The three main groups of flavonoids: anthocyanins, flavones and flavanones, are important compounds that give color or function as copigmentation factors (Rein 2005).

Sorghum Flavonoids

Flavonoids are components of most plant seeds and grains. They are secondary metabolites derived from products of aromatic amino acid biosynthesis (phenylalanine) and the Krebs cycle (acetyl Co A). Flavonoids have a C_6-C_3-C_6 configuration and in nature the principal subgroups found are anthocyanins, flavones, flavonols, flavanones, and flavanols (Shirley 1998). All the major groups of flavonoids have been identified in sorghum (Nip et al 1969, 1971; Dykes 2006). Three have been found in high amounts in a diverse range of sorghums grains: 3-deoxyanthocyanins, flavones and flavanones (Dykes 2008). The 3-deoxyanthocyanidins are a special kind of anthocyanidins that have been found in only a limited number of species including sorghum and maize (Shirley 1998). The difference between the more stable 3-deoxyanthocyanidins compared to the common anthocyanidins is that they do not have the hydroxyl group in the C ring (Figure 1). In sorghum, four 3-deoxyanthocyanidins have been found in considerable amounts: Apigeninidin, luteolinidin, 5-methoxyluteolinidin and 7-methoxyapigeninidin. Luteolin and apigenin are commonly found flavones and eriodictyol and naringenin are the principal flavanones. Most of the flavonoids in sorghum grains are located in the outer layers of the grain (Shirley 1998; Awika et al 2005; Dykes et al 2009).

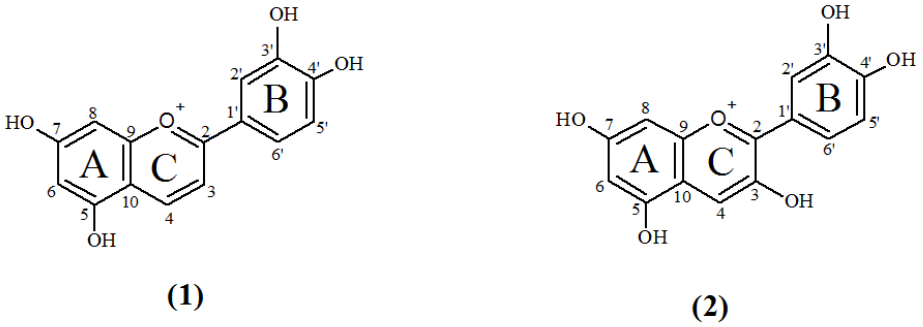

Fig. 1. Structure of the 3-deoxyanthocyanidins (1) and anthocyanidins (2).

Importance of Sorghum Flavonoids

The major class of flavonoids studied in sorghum are the 3-deoxyanthocyanins because of their special phytoalexins 3-deoxyanthocyanidins which have shown anticancer cell proliferation (Shih et al 2007), and also have a potential as natural colorants in food systems at low pH (Awika et al 2004a). They are found in high concentrations in black sorghum grains (Dykes et al 2009). Flavanones and flavones which also have shown anticancer activity (Kuntz et al 1999) are found in high concentrations in yellow and red sorghum (Dykes 2008), respectively. All these compounds contribute to the black, purple, red and yellow color in sorghum plants and grains.

Metabolism of Flavonoids

The metabolism of sorghum flavonoids is regulated by its genotype; for example, Dykes (2008) found that red pericarp sorghum grain contain high levels of 3-deoxyanthocyanidins while those with yellow pericarp contain low levels of 3-deoxyanthocyanidins. The metabolism of sorghum flavonoids is affected by environmental conditions (Stafford 1990). The synthesis of flavonoids is influenced by light (Orczyk et al; Weiergang et al 1996; Dykes et al 2009) or fungal infection (Snyder and Nicholson, 1990; Nicholson and Hammerschmidt, 1992; Lo and Nicholson 1998). Seitz (2004) found higher levels of 3-deoxyanthocyanidins in white sorghum grains affected by molds than in non molded grains. Christie (1994) reported that temperature affected the production of anthocyanin in maize. Dykes (2008) found that environmental conditions affected the total concentration of anthocyanins in black, red and yellow sorghum grains.

Biosynthesis of Flavonoids

Flavonoids have a C_6-C_3-C_6 configuration. For most of the sorghum flavonoids, the biosynthesis pathway is the same as in the well characterized flavonoid biosynthesis where phenylalanine and acetyl Co A are the precursors of the basic flavonoid molecules, the chalcones (Lo and Nicholson, 1998; Winkel, 2006; Vermerris and Nicholson, 2006) (Figure 2).

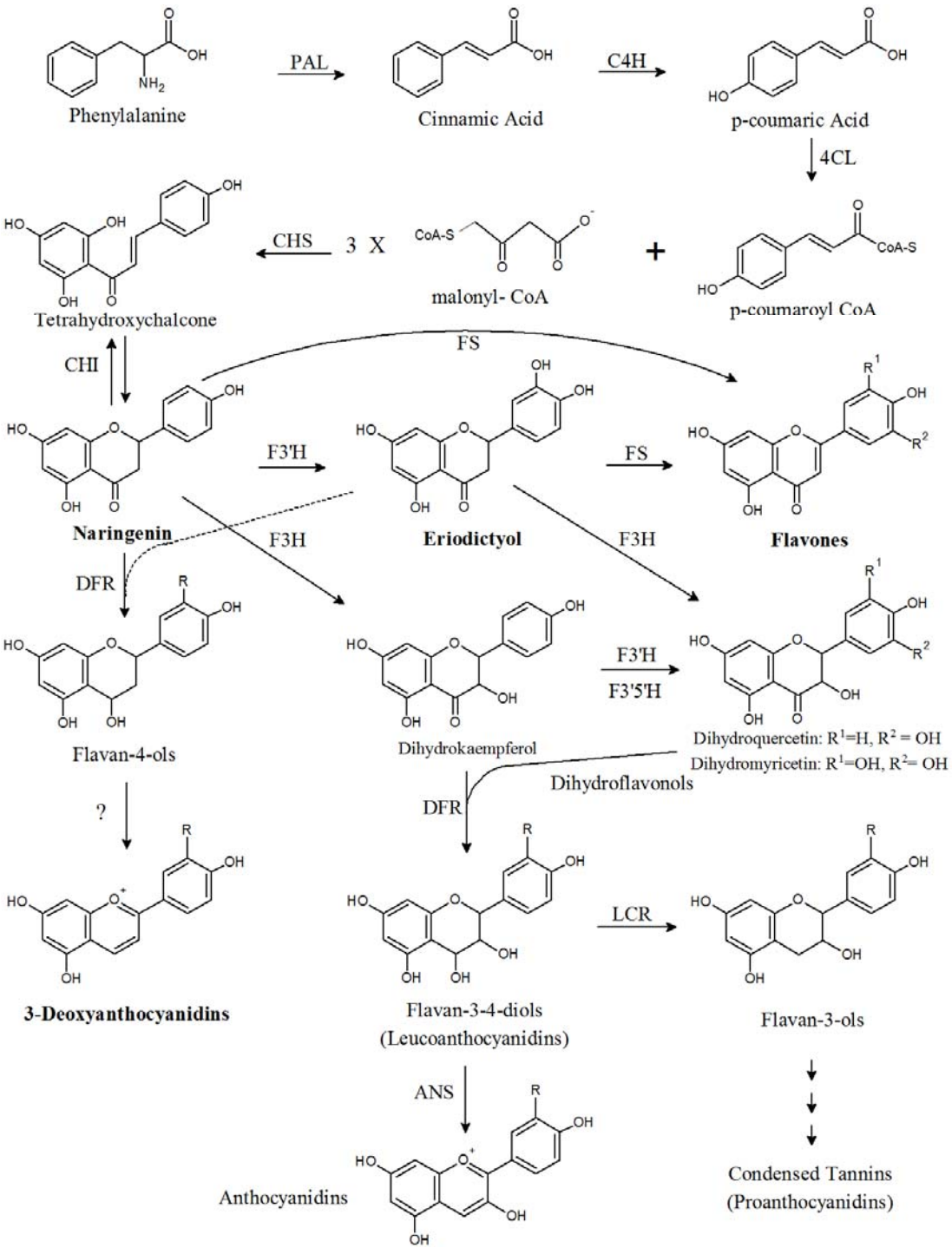

Fig. 2. Biosynthesis of sorghum flavonoids.

Flavonoid Extraction

The extraction of sorghum flavonoids is difficult because these compounds are located in the cell wall matrix which reduce their availability to the extraction solvent. In whole grains, 75 to 85% of the phenols are present in the bound form (Hahn et al 1984; Waniska et al 1989) while in fruits and vegetables there are more free than bound phenolic compounds (Liu 2007). Different methods have been used in the extraction of phenolic compounds from plant materials, depending on the matrix of the material. In sorghum, Hahn (1984) and Gous (1989) found that 1% HCl in methanol was an efficient solvent; whereas 70% aqueous acetone was reported as a very efficient solvent for extracting phenols from fruits (Kallithraka et al 1995). Other solvents have been used for the extraction of phenolic compounds from fruits and vegetables as well as cereals with varying degrees of success. For example, aqueous acetone has been considered a good solvent for the extraction of procyanidins, anthocyanins and other phenolic compounds in fruits and vegetables (Garcia-Viguera et al 1998; Kallithraka et al 1995). Lu and Foo (2001) observed significant anthocyanin interaction when aqueous acetone was used as extraction solvent for fruits and vegetables. Modification of the HPLC-spectral characteristic of 3- deoxyanthocyanins associated with formation of pyranoluteolinidin and pyranoapigeninidin, which resulted in significantly lower levels of detectable anthocyanins was reported by Awika et al (2004b). Combinations of acetic, citric and tartaric acids in aqueous ethanol gave the same profiles of 3-deoxanthocyanins as the commonly used 1% HCl in methanol extractions with 50% reduction in extract (Njongmeta et al 2007).

Sorghum Emergence, Growth and Grain Development

Emergence of sorghum occurs between 5 to 10 days after planting. After emergence, three growth stages occur. This first growth stage is characterized by vegetative growth; the second growth stage is the period when reproductive structures of the panicle form and the maximum number of seed per plant are set; and the third stage is when the plant flowers and the grain is formed. The period between flowering to physiological maturity of sorghum grains varies among genotypes and usually takes around one third of the total crop cycle. The period can be predicted using the growing degree units (GDU) that each genotype needs during this stage (Gerik et al 2003). The GDU are directly related to the temperature of the environment; higher temperatures help the plant to reach maturity quicker.

The sorghum grain development can be classified in four stages: milk, soft dough, hard dough and physiological maturity. The first ten days after fertilization, is when a rapid accumulation of dry matter occurs in the grain, this period is called milk. From the day 15 to 25 after flowering, the grain accumulate around 50 % of its final dry weight, this stage is called soft dough. During the following fifteen days about three fourths of the dry matter is accumulated. The last stage takes around ten days and is when the grain reaches its maximum dry matter content; this point is considered as the physiological maturity of the grain (Warrick 2009). Physiological maturity of sorghum can be identified when a black layer is observed in the grain (Castor 1981). Sorghum generally is left in the field more days until harvest to reduce its moisture content; if this period is too long the grain can be exposed to weathering (Bandyopadhaya 2000).

Sorghum Grain Molds and Weathering

Grain mold is a disease that appears when sorghum is grown in conditions with high moisture and high temperatures during the flowering to the physiological maturity period (Bandyopadhyay et al 2000). Weathering is a result of different environmental factors that affect grain appearance including grain mold (damage by pathogens), late season weathering, sprouting and grain discoloration. (Munera 1996). The term weathering is used generally when the damage occurs after physiological maturity; during this period is when most of the damage by environmental factors is expressed on the surface of the grain.

Sorghum Grain Hardness

Sorghum grain hardness is an important quality attribute in processing and quality of final products (Bean et al 2006). Milling quality of sorghum grain has been related to grain hardness (Rooney and Waniska 2000). Cagampang and Kirleis (1984) reported that sorghum cooking quality parameters such as adhesion, cooked grain texture, alkali gel stiffness, and amylograph viscosities were related to grain vitrousness; while Rooney et al (1986) reported that sorghum grain hardness was the most important component related to porridge quality. Grain hardness also plays a role in plant defense against molds and insect attack (Chandrashekar and Mazhar 1999). Grain hardness has also been linked to mold and weathering resistance in sorghum (Jambunathan et al 1992). Lichtenwalner et al (1979) reported low hardness values with weathered sorghum grains.

MATERIALS AND METHODS

Samples

Sixteen sorghum genotypes were grown in four locations in 2008. Eight were black, five red, two yellow and one white sorghum (Table 1). Seeds were obtained from Texas Agri-Life sorghum breeding program at College Station.

Environments

The environments used were major ecological regions in the state of Texas where significant amounts of sorghum are produced (TASS 2009). The regions selected were in the High Plains (Halfway, TX), East Central Texas Plains (College Station, TX) and Western Gulf Coastal Plain (Corpus Christi, TX and Weslaco, TX) (Griffith et al 2004).

Standards and Reagents

Gallic acid, and 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and Folin-Ciocalteu reagent were obtained from Sigma (St. Louis, MO). Trolox was obtained from Aldrich (Milwaukee, WI). Naringenin was obtained from Sigma-Aldrich (St. Louis, MO). Apigenin and luteolin were obtained from Indofine Chemical Co., Inc. (Hillsborough, NJ). Eriodictyol, luteolinidin chloride, and apigeninidin chloride were obtained from ALSACHIM (Strasbourg, France) and 7-methoxyapigeninidin chloride was obtained from ChromaDex (Santa Ana, CA). all solvents were HPLC grade.

Table 1

Genotypes of Sorghums Evaluated

Sorghum lines or hybrids	Genotype			
	Colored testa	Pericarp color	Pericarp thickness	Plant color
BLACK Tx430	$b_1b_1B_2B_2$	RRYY	zz	PPQQ
Shawaya Black	$b_1b_1B_2B_2$	RRYY	zz	PPQQ
B05028 Black	$B_1B_1B_2B_2$	RRYY	zz	PPQQ
B05030 Black	$B_1B_1B_2B_2$	RRYY	zz	PPQQ
A05028/Black Tx430	$B_1b_1B_2B_2$	RRYY	zz	PPQQ
A05030/Black Tx430	$B_1b_1B_2B_2$	RRYY	zz	PPQQ
A05028/Shawaya	$B_1b_1B_2B_2$	RRYY	zz	PPQQ
A05030/Shawaya	$B_1b_1B_2B_2$	RRYY	zz	PPQQ
SC719-11E	$B_1B_1B_2B_2$	RRYY	zz	PPqq
TX2911	$b_1b_1B_2B_2$	RRYY	zz	PPqq
99LGWO50	$b_1b_1B_2B_2$	RRYY	zz	ppQQ
98CA4779	$b_1b_1B_2B_2$	RRYY	ZZ	PPQQ
B9904	$b_1b_1B_2B_2$	RRYY	ZZ	PPqq
RO7007	$b_1b_1B_2B_2$	rrYY	zz	PPqq
SC748-5	$b_1b_1B_2B_2$	rrYY	ZZ	PPQQ
ATx631/RTX436	$b_1b_1B_2B_2$	rrYY	ZZ	ppQQ

Sample Preparation

The grains were harvested 7 to 14 days after physiological maturity. The glumes were removed mechanically and the grains were fumigated and stored at 4ºC. The samples were cleaned prior to analysis.

Grain Weathering Score

The grains were evaluated subjectively by an experienced pathologist. The scores were in a scale from 1 to 9. Where 1 was for sorghum free of damage by weathering and 9 was for sorghum completely weathered (Isakeit 2007).

Grain Color Determination

Color of whole cleaned grains was obtained using a Minolta CR-310 Colorimeter (Osaka, Japan). Results were expressed as L^*, a^*, and b^* values (CIELAB) (Commission Internationale de l'Éclairage 1986). L^* values ranged from 0 – 100 where 0 is dark and 100 is light. Positive a^* values indicate red color and positive b^* values indicate yellow color; negative values or values close to 0 indicates green or blue color, respectively.

Physical Properties of the Grain

The hardness index was determined with a single kernel hardness tester (SKHT, model SKCS 4100, Perten Instruments, Reno, NV). Grain weight, diameter and moisture content was measured with the SKHT. Hardness also was measured with the tangential abrasive dehulling device (TADD) (model 4E-230, Venable Machine Works, Saskatoon, Canada) using 20 g of sample with 3.5 min abrasion time. The hardness index value with the SKHT was expressed in a scale with a range from -20 to 120, where higher values indicated harder grains. The value of hardness with TADD was expressed as a value between 0 and 100 which represents the percentage of the grains that was not

disintegrated by abrasive force. Higher hardness index and TADD values indicates harder grains more resistant to breakage.

Extraction for Total Phenols and Antioxidant Activity

All samples were ground through a cyclotec mill (UDY Corp., Fort Collins, CO) (0.5 mm mesh) prior to extraction. For all assays three replicates (0.1-0.5 g) were extracted in 25 mL 1% HCl/methanol (v/v) for two hours while shaking at low speed using an Eberbach shaker (Eberbach Corp., MI). All extracts were then centrifuged at 2790*g* for 10 min in a Sorvall SS-34 centrifuge (DuPont Instruments, Wilmington, DE) and were decanted. To avoid oxidation, extracts were stored in the dark at -20°C and analyses were performed within 24 hours.

Extraction for HPLC Analysis

The extraction of flavonoids was performed as described by Dykes (2008). Three replicates of ground samples (1 g) were extracted in 10 mL of 1% HCl/methanol (v/v) for two hours while shaking at low speed using an Eberbach shaker. The extracts were centrifuged at 2790*g* for 10 minutes and then decanted. A second set of extracts were prepared for flavanone analysis. Samples (1g) were extracted in 10 mL of 1% HCl/methanol (v/v) for two hours in an Eberbach shaker. Each supernatant was then transferred to glass tubes, sealed, and placed in a water bath for 90 min at 80ºC; after equilibration at room temperature all extracts were filtered using a 0.45 μm nylon membrane filter (Whatman Inc., Maidstone, UK) prior to HPLC analysis.

Total Phenols Analysis

Total phenols of the acidified methanol extracts were measured using the modified Folin-Ciocalteu method of Kaluza et al (1980). One aliquot of the extract (0.1 mL) was dissolved in 1.1 mL of water and reacted with 0.4 mL of Folin-Ciocalteu reagent and 0.9 mL of 0.5*M* ethanolamine. The reaction was allowed to stand for 20 min at room temperature and the absorbance was read at 600 nm.

Antioxidant Activity Analysis

Antioxidant activity of sorghum extracts were measured *in vitro* by the ABTS assay. The $ABTS^{\cdot+}$ was obtained by reacting 3 mM of $K_2S_2O_8$ with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The $ABTS^{\cdot+}$ solution was then diluted with a pH 7.4 phosphate buffer (50:42.5:9.5; water:0.2 M Na_2HPO_4:0.2 M NaH_2PO_4) solution containing 150 mM NaCl (PBS) to obtain an initial absorbance of 1.5 at 734 nm. Fresh $ABTS^{\cdot+}$ solution was prepared each day of analysis. Dilutions of Trolox in methanol were used to prepare the standard curve. Samples and standards (100 μm) were reacted with the $ABTS^{\cdot+}$ solution (2900 μm) for 15 min.

HPLC Analysis

Extracts were analyzed on an Alliance 2695 system (Waters Corp., Milford, MA) with a Waters 996 photodiode array detector (PDA). Sorghum flavonoids were separated using a Luna C18 column (150 mm x 4.6 mm i.d., 5 μm) from Phenomenex (Torrance, CA). Column temperature was conditionated at 35 ºC. Injection volume was 20 μL. The

mobile phase consisted of 4% formic acid in water (v/v) (Solvent A) and acetonitrile (Solvent B). The solvent flow rate was 1.0 mL/min. The 3-deoxyanthocyanins were separated using the following gradient: 0-20 min., 12-20% B; 20-40 min., 20-50% B; 40-50 min., 50% B. Flavones and flavanones were separated using the following gradient: 0-45 min., 15-41% B; 45-50 min., 41% B. The 3-deoxyanthocyanins, flavones, and flavanones were measured at 485 nm, 340 nm, and 280 nm respectively (Dykes 2008). Identification of sorghum flavonoids was based on commercial standards' retention times, UV-Vis spectra, and LC-MS data. Quantification of each compound was done by comparing peak areas with that of a standard curve of each authentic standard. Molecular weight correction factors were used to quantify 5-methoxyluteolinidin and 7-methoxyapigeninidin (Chandra et al 2001; Wu et al 2006). Data was collected and processed using the Empower software version 1.0 (Waters Corp., Milford, MA).

Statistical Analysis

Three replicates of each genotype from each location were analyzed for color, total phenols, antioxidant activity and flavonoids while for hardness using TADD and SKHT two replicates were analyzed. Effect of genotype, environment and genotype x environment interaction was analyzed using a General Linear Modeling procedure. The analysis was done using SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

Temperature and Rainfall of the Locations in 2008

Rainfall during grain maturation of sorghum in Corpus Christi (June-July), College Station (June-July), Weslaco (May-June) and Halfway (September-October) was 9.7, 1.8, 3.3 and 14.2 mm, respectively (Table 2). The high concentration of rainfall during the period between flowering and harvest in Halfway produced a high incidence of molds and weathering in the grains (Fig. A.1, A.2 and A.3). Grains from Weslaco, Corpus Christi and College Station were less affected by weathering because rain in those locations was moderate or low during the last stage of grain maturation. Average temperature of the locations during the maturation of the grains were 27.6 °C in Corpus Christi, 29.7 °C in College Station, 18.6 °C in Halfway and 28.3 °C in Weslaco (Table 2). Low temperatures in Halfway reduced the growing degree units at this location, prolonging the time to reach physiological maturation and exposing the grains to environmental conditions favoring mold growth, discoloration and potential modification of components.

Table 2

Average Monthly Temperature and Rainfall for the Locations Evaluated During Maturation of the Sorghum Grains

Location		April	May	June	July	Aug	Sept	Oct
Corpus Christi	Rainfall (mm)		3.2	1.8	4.7			
	Temperature (°C)		26.7	28.3	27.8			
College Station	Rainfall (mm)			0.7	1.0			
	Temperature (°C)			29.7	29.8			
Halfway	Rainfall (mm)					3.4	4.6	6.3
	Temperature (°C)					23.3	18.6	13.9
Weslaco	Rainfall (mm)	1.0	1.5	0.8				
	Temperature (°C)	25.0	29.4	30.6				

Source: Office of the Texas State Climatologist, 2009.

Effect of Environment on Hardness of the Grains

Hardness of Black Sorghums

Hardness values of black sorghum ranged from 52.6 – 78.3 and 44.4 – 73.8 for SKHT and TADD, respectively (Table 3). Differences in hardness of the grains with the two methods (SKHT and TADD) were found among locations. For hardness index measured with the SKHT, the average value was 68.9 for College Station and 65.7, 63.5, and 62.9 for Corpus Christi, Weslaco and Halfway, respectively (Table 4) (MSD = 5.0; α = .05). Environmental effect also was observed in hardness measured with TADD, where College Station had the highest average (63.8) while for Corpus Christi, Weslaco and Halfway the values were 54.4, 58.4 and 55.0, respectively (Table 4) (MSD = 4.9 α = .05). Differences among genotypes occurred in both methods (Table 5). Genotype x environment interaction also occurred; variability among genotypes was greater than among locations in both methods (Table B.1, B1.1, B.2 and B.2.1). Low relation (r^2 = 0.19) was found between weathering and hardness of black sorghum grains, contrary to the results from Lichtenwalner et al (1979) who found that weathering significantly reduced hardness of the grains. Thus, the samples in these studies had different levels of weathering while Lichtenwalner analyzed extensively weathered grains. The correlation between the hardness values of black sorghum grains by the SKHT and TADD was low (r = 0.70).

Table 3

Hardness of Black Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	Hardness[2]		Weathering Score
		(SKHT)[3]	(TADD)[4]	
CC	Tx430 Black	77.1	73.0	5
CS	Tx430 Black	70.2	73.8	4
WE	Tx430 Black	70.7	72.7	4
HW	Tx430 Black	58.1	59.8	8
CC	Shawaya Black	52.6	44.7	4
CS	Shawaya Black	78.3	68.8	7
WE	Shawaya Black	54.2	49.7	6
HW	Shawaya Black	61.8	51.1	7
CC	B05028 Black	82.7	65.2	3
CS	B05028 Black	77.0	67.2	2
WE	B05028 Black	78.0	64.5	2
HW	B05028 Black	69.7	47.9	6
CC	B05030 Black	60.4	44.4	3
CS	B05030 Black	62.1	42.8	2
WE	B05030 Black	58.0	52.3	3
HW	B05030 Black	65.0	47.2	7
CC	A05028/Black Tx430	67.2	51.1	3
CS	A05028/Black Tx430	67.6	69.5	2
WE	A05028/Black Tx430	68.1	59.3	2
HW	A05028/Black Tx430	67.0	62.9	6
CC	A05030/Black Tx430	56.6	47.4	3
CS	A05030/Black Tx430	58.2	57.1	4
WE	A05030/Black Tx430	61.5	54.1	3
HW	A05030/Black Tx430	57.6	52.0	6
CC	A05028/Shawaya	72.3	60.1	3
CS	A05028/Shawaya	73.9	71.3	5
WE	A05028/Shawaya	66.8	62.0	3
HW	A05028/Shawaya	63.6	62.9	6
CC	A05030/Shayawa	56.9	49.4	3
CS	A05030/Shayawa	63.5	59.7	5
WE	A05030/Shayawa	50.4	52.0	3
HW	A05030/Shayawa	60.4	56.1	7

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 1.4 for SKHT and 6.8 for TADD. $\alpha = .05$.

[3] SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester.

[4] TADD = % of kernel residues by Tangential Abrasive Dehulling Device.

Table 4

Effect of Location on Hardness (SKHT and TADD) of 8 Black Sorghum Grains

Location[1]	Hardness[2]		Weathering[2]
	(SKHT)[3]	(TADD)[4]	Score
CS	68.9[a]	63.8[a]	3.9[b]
CC	65.7[ab]	54.4[b]	3.4[b]
WE	63.5[b]	58.4[b]	3.3[b]
HW	62.9[b]	55.0[b]	6.6[a]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 5.0 for SKHT, 4.9 for TADD and 1.6 for Weathering Score values with different letter are statistically different for each column. $\alpha = .05$.

[3] SKHT = Hardness Index by Single Kernel Hardness Tester.

[4] TADD = Hardness by Tangential Abrassive Dehulling Device.

Table 5

Hardness (SKHT and TADD) of 8 Black Sorghum Grains Grown at Different Locations

Line or hybrid	Hardness[1,2]		Weathering
	(SKHT)[3]	(TADD)[4]	Score
Tx430 Black	69.0 [ab]	69.8 [a]	5.3
B05028 Black	76.8 [a]	61.2 [bc]	3.3
A05028/Shawaya	69.1 [ab]	64.1 [ab]	4.3
A05028/Black Tx430	67.5 [b]	60.7 [bcd]	3.3
Shawaya Black	61.7 [bc]	53.6 [cde]	6.0
A05030/Shayawa	57.8 [c]	54.3 [cde]	4.5
A05030/Black Tx430	58.5 [c]	52.7 [de]	4.0
B05030 Black	61.4 [bc]	46.7 [e]	3.8

[1] Average or 4 locations.

[2] Minimum Significant Difference = 8.3 for SKHT, 8.2 for TADD and 4.2 for Weathering Score values with different letter are statistically different for each column. $\alpha = .05$.

[3] SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester.

[4] TADD = % of kernel residues by Tangential Abrasive Dehulling Device.

Hardness of Red Sorghums

Hardness values of red sorghum ranged from 59.5 – 91.7 and 54.9 – 85.6 for SKHT and TADD, respectively (Table 6). For the hardness index measured with the SKHT, environmental effect was observed but in a lower response compared to the differences in genotypes; sorghums from halfway had the lowest hardness values (69.9) compared to 81.6, 80.1 and 79.1 from Corpus Christi, College Station and Weslaco, respectively (Table 7) (MSD = 3.7, $\alpha = .05$). Interaction of the genotypes with the environment was observed (Table B.3 and B.3.1). Environmental effect also was observed in hardness measured with TADD, where the lowest average value was found in Halfway (68.1) and 74.4, 73.4 and 72.1 for Corpus Christi, College Station and Weslaco, respectively (Table 7) (MSD = 3.3, $\alpha = .05$). The genotype had higher effect than the location effect. Interaction of genotype with locations also was observed for hardness measured with TADD (Table B.4 and B.4.1). Differences in hardness of the grains with the two methods (SKHT) and (TADD) were found in red sorghum genotypes (Table 8) (MSD = 4.3 and 3.8, respectively; $\alpha = .05$). Sorghum damaged by weathering had softer grains with both methods. This confirm the results of Lichtenwalner et al (1979) who observed that weathering significantly reduces the hardness of the grains. The correlation between the hardness values of black sorghum grains by the SKHT and TADD was high ($r = 0.91$).

Table 6

Hardness of Red Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	Hardness[2]		Weathering Score
		(SKHT)[3]	(TADD)[4]	
CC	99LGWO50	89.5	81.0	2
CS	99LGWO50	83.6	83.5	2
WE	99LGWO50	87.3	82.0	2
HW	99LGWO50	83.7	76.5	4
CC	Tx29911	70.3	64.7	3
CS	Tx29911	71.0	54.9	3
WE	Tx29911	64.9	56.6	2
HW	Tx29911	61.3	51.1	5
CC	SC719-11E	71.1	61.7	3
CS	SC719-11E	68.6	63.7	3
WE	SC719-11E	67.3	56.7	3
HW	SC719-11E	59.5	61.2	5
CC	98CA4779	85.5	79.4	3
CS	98CA4779	86.6	81.2	2
WE	98CA4779	87.4	79.6	3
HW	98CA4779	69.4	74.2	4
CC	B9904	91.7	85.1	3
CS	B9904	90.7	83.4	3
WE	B9904	91.3	85.6	4
HW	B9904	75.6	77.6	5

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.7 for SKHT and 3.4 for TADD. $\alpha = .05$.

[3] SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester.

[4] TADD = % of kernel residues by Tangential Abrasive Dehulling Device.

Table 7

Effect of Location on Hardness (SKHT and TADD) of 5 Red Sorghum Grains

Location[1]	Hardness[2]		Weathering[2]
	(SKHT)[3]	(TADD)[4]	Score
CC	81.6[a]	74.4[a]	2.8[b]
CS	80.1[a]	73.4[a]	2.6[b]
WE	79.1[a]	72.1[a]	2.8[b]
HW	69.9[b]	68.1[b]	4.6[a]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 3.7 for SKHT, 3.3 for TADD and 1.1 for Weathering Score values with different letter are statistically different for each column. $\alpha = .05$.

[3] SKHT = Hardness Index by Single Kernel Hardness Tester.

[4] TADD = Hardness by Tangential Abrassive Dehulling Device.

Table 8

Hardness (SKHT and TADD) of 5 Red Sorghum Grains Grown at Different Locations

Line or hybrid	Hardness[1,2]		Weathering
	(SKHT)[3]	(TADD)[4]	Score
B9904	87.3 [a]	82.9 [a]	3.8
99LGWO50	86.0 [ab]	80.8 [ab]	2.5
98CA4779	82.2 [b]	78.6 [b]	3.0
SC719-11E	66.6 [c]	60.8 [c]	3.5
Tx29911	66.9 [c]	56.8 [d]	3.3

[1] Average or 4 locations.

[2] Minimum Significant Difference = 4.4 for SKHT, 3.9 for TADD and 2.2 for Weathering Score values with different letter are statistically different for each column. $\alpha = .05$

[3] SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester.

[4] TADD = % of kernel residues by Tangential Abrasive Dehulling Device.

Hardness of Yellow Sorghums

Hardness values of yellow sorghum ranged from 67.1 – 101.0 and 61.4 – 88.5 for SKHT and TADD, respectively (Table 9). For the hardness index measured with the SKHT, environmental effect was observed. Sorghums from Halfway had the lowest values of hardness with the SKHT (59.9) while on Corpus Christi, College Station and Weslaco the values were 81.5, 86.4 and 86.1, respectively (Table 10) (MSD = 3.9; α = .05). Environmental effect also was observed in hardness with TADD where sorghums from Halfway had the lowest values (70.7) compared to 82.6, 83.3 and 81.7 from Corpus Christi, College Station and Weslaco, respectively (Table 10) (MSD = 5.3; α = .05). Differences in hardness of yellow sorghum genotypes were found with the two methods (SKHT) and (TADD) (Table 11). The genotype x environment interaction had higher effect than the location for SKHT and TADD hardness (Table B.5, B5.1 B.6 and B.6.1). The correlation between hardness values from SKHT and TADD was high (r = 0.97). As in red sorghums, yellow sorghums also had lower hardness values in Halfway, where a higher weathering damage was observed.

Table 9

Hardness of Two Yellow and One White Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	Hardness[2]		Weathering Score
		(SKHT)[3]	(TADD)[4]	
CC	ATx631/RTx436	89.0	81.1	2
CS	ATx631/RTx436	84.3	78.3	2
WE	ATx631/RTx436	88.4	82.9	2
HW	ATx631/RTx436	78.6	71.0	4
CC	RO7007	67.9	78.0	3
CS	RO7007	74.0	78.6	2
WE	RO7007	71.0	74.8	2
HW	RO7007	45.2	61.4	7
CC	SC748-5	95.2	87.1	3
CS	SC748-5	98.8	87.9	5
WE	SC748-5	101.0	88.5	4
HW	SC748-5	74.6	80.0	6

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.7 for SKHT and 3.4 for TADD. $\alpha = .05$.

[3] SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester.

[4] TADD = % of kernel residues by Tangential Abrasive Dehulling Device.

Table 10

Effect of Location on Hardness (SKHT and TADD) of 2 Yellow Sorghum Grains

Location[1]	Hardness[2]		Weathering[2]
	(SKHT)[3]	(TADD)[4]	Score
CS	86.4[a]	83.3[a]	3.5[a]
WE	86.1[a]	81.7[a]	3.0[a]
CC	81.5[b]	82.6[a]	3.0[a]
HW	59.9[c]	70.7[b]	6.5[a]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 3.9 for SKHT and 5.3 for TADD. $\alpha = .05$ values with different letter are statistically different for each column.

[3] SKHT = Hardness Index by Single Kernel Hardness Tester.

[4] TADD = Hardness by Tangential Abrassive Dehulling Device.

Table 11

Hardness (SKHT and TADD) of 2 Yellow Sorghum Grains Grown at Different Locations

Line or hybrid	Hardness[1,2]		Weathering Score
	(SKHT)[3]	(TADD)[4]	
SC748-5	92.4 [a]	85.9 [a]	4.5[a]
RO7007	64.5 [b]	73.2 [b]	3.5[a]

[1] Average or 4 locations.

[2] Minimum Significant Difference = 2.0 for SKHT, 2.7 for TADD and 3.3 for Weathering Score values with different letter are statistically different for each column. $\alpha = .05$.

[3] SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester.

[4] TADD = % of kernel residues by Tangential Abrasive Dehulling Device.

Effect of Environment on Grain *L**, *a** and *b** Values

Color of Black Sorghums

*L** values of black sorghum ranged from 32.8 – 38.6 (Table 12). The grains of black sorghums that were less affected by weathering had two distinct colored areas, one with a black color and one with a yellow/red color (Fig. 3). Areas with yellow/red color were covered by the glumes. Presumably the glumes protected these areas from the sunlight and low synthesis of 3-deoxyanthocyanins occurred, as indicated by Dykes (2009). Samples affected by weathering had more uniform black pericarp; in this case, the areas covered by glumes were not yellow/red, presumably because weathering conditions stimulated the production of 3-deoxyanthocyanins, producing the black color instead of the yellow/red. In addition, the rain solubilized part of the anthocyanins which moved them from the black areas to the areas covered by glumes.

All black sorghum samples from Halfway were darker in color than the other three locations as indicated by lower *L** values, except for the sorghum Shawaya Black from College Station which had a lower *L** value and also was highly affected by weathering (Fig. A.1). Average *L** value for black sorghums at Halfway was 33.7 while Corpus Christi, College Station and Weslaco was 36.2, 34.8 and 36.8, respectively (Table 13) (MSD = 0.5, $\alpha = .05$), showing that the genotypes had a color change (*L** value) in response to environment conditions. Differences in *L** values also occurred among genotypes (Table 14) .This change was not similar in all genotypes, as expressed by the environment by genotype interaction (Table B.7 and B.7.1).

Table 12

***L*, a*, b** Color Values of Black Sorghum Grains Grown at Different Locations[a]**

Location[a]	Line or hybrid	Color[b]		
		L*	***a****	***b****
CC	Black Tx430	34.4	4.9	4.0
CS	Black Tx430	34.0	5.6	3.2
WE	Black Tx430	35.4	5.4	4.9
HW	Black Tx430	32.7	3.5	1.5
CC	Shawaya Black	34.1	6.7	4.3
CS	Shawaya Black	32.1	5.6	2.1
WE	Shawaya Black	34.1	7.7	3.3
HW	Shawaya Black	32.8	5.8	2.7
CC	B05028	37.8	8.8	8.9
CS	B05028	36.0	7.4	6.6
WE	B05028	38.5	9.1	9.2
HW	B05028	34.4	7.2	4.7
CC	B05030	36.3	7.2	6.5
CS	B05030	36.7	8.7	7.5
WE	B05030	37.1	9.6	6.8
HW	B05030	34.5	7.1	4.8
CC	A05028/Black Tx430	37.2	7.1	7.5
CS	A05028/Black Tx430	35.0	7.0	5.5
WE	A05028/Black Tx430	38.6	7.2	9.4
HW	A05028/Black Tx430	34.3	6.4	4.5
CC	A05030/Black Tx430	36.7	7.5	7.1
CS	A05030/Black Tx430	35.6	8.0	6.2
WE	A05030/Black Tx430	37.4	7.1	7.9
HW	A05030/Black Tx430	33.9	6.0	3.7
CC	A05028/Shawaya	36.8	8.6	7.5
CS	A05028/Shawaya	34.3	7.6	4.8
WE	A05028/Shawaya	36.6	11.0	6.3
HW	A05028/Shawaya	33.8	7.4	3.8
CC	A05030/Shawaya	36.1	8.0	6.5
CS	A05030/Shawaya	34.4	8.6	5.0
WE	A05030/Shawaya	36.4	11.4	6.3
HW	A05030/Shawaya	33.1	6.3	3.1

[a] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[b] Minimum Significant Difference = 1.3 for *L**, 0.9 for *a** and 1.4 for *b**. $\alpha = .05$.

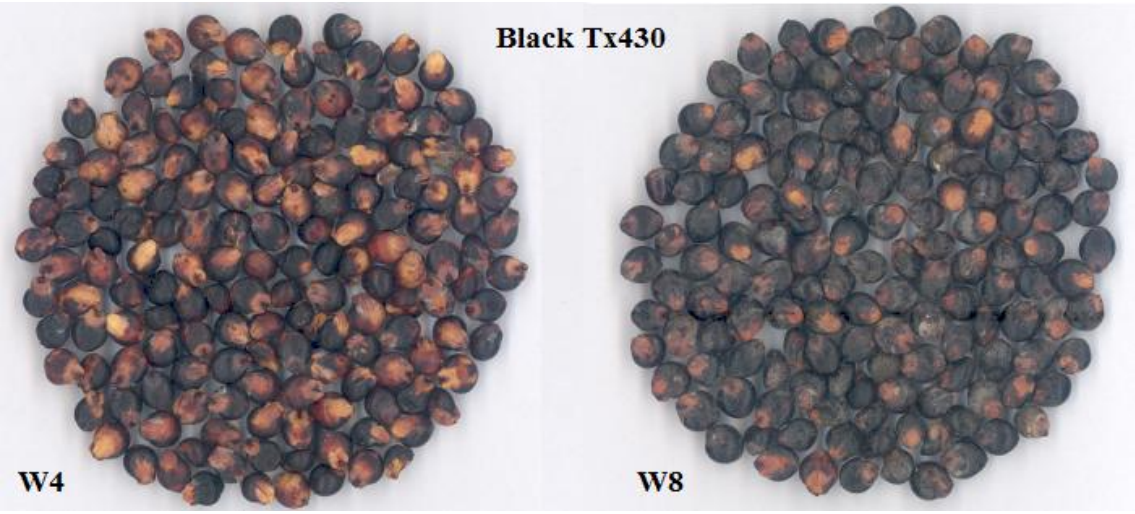

Fig. 3. Comparison of appearance between a low weathered grain from College Station (W4) and a highly weathered grain from Halfway (W8) of Black Tx430 grains. Low weathered sorghums (W4) had a defined red/yellow region in contrast to highly weathered grains (W8) which had a more uniform black pigmentation. (W4 = weathering score of 4 and W8 = weathering score of 8).

Table 13

Effect of Location on Average *L, *a**, and *b** Values of 8 Black Sorghum Grains**

Location[1]	Color[2]			Weathering
	*L**	a*	b*	Score
WE	36.76[a]	8.56[a]	6.75[a]	3.3[b]
CC	36.17[b]	7.35[b]	6.54[a]	3.4[b]
CS	34.75[c]	7.32[b]	5.12[b]	3.9[b]
HW	33.68[d]	6.20[c]	3.59[c]	6.6[a]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.5 for *L**, 0.6 for *a**, 0.6 for *b** and 1.6 for weathering values with different letter are statisticaly different for each column. $\alpha = .05$.

Table 14

Average *L, *a**, and *b** Values of 8 Black Sorghum Grains Grown at Different Locations**

Line or Hybrid	Color[1,2]			Weathering Score
	*L**	*a**	*b**	
B05028	36.7 [a]	8.1 [a]	7.3 [a]	3.3 [a]
A05028/Black Tx430	36.3 [ab]	6.9 [c]	6.7 [ab]	3.3 [a]
B05030	36.2 [abc]	8.1 [ab]	6.4 [abc]	3.8 [a]
A05030/Black Tx430	35.9 [bc]	7.2 [bc]	6.2 [bc]	4.0 [a]
A05028/Shawaya	35.4 [d]	8.6 [a]	5.6 [cd]	4.3 [a]
A05030/Shawaya	35.0 [d]	8.6 [a]	5.2 [d]	4.5 [a]
Black Tx430	34.1 [e]	4.8 [d]	3.4 [e]	5.3 [a]
Shawaya Black	33.3 [b]	6.5 [c]	3.1 [e]	6.0 [a]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 0.8 for *L**, 1.0 for *a**, 1.0 for *b** and 4.2 for weathering values with different letter are statistically different for each column. $\alpha = .05$.

The a^* values of black sorghums ranged from 3.5 – 11.4 (Table 12). The a^* values which indicates redness of the pericarp were lower in sorghums from Halfway, with an average of 6.2 compared to 7.4, 7.3 and 8.6 from Corpus Christi, College Station and Weslaco, respectively; Shawaya Black from College Station was the exception to this trend (Table 13) (MSD = 0.6, $\alpha = .05$). Significant differences in genotypes (Table 14) and also an interaction between the environment and genotypes were observed for a^* values (Table B.8 and B.8.1). The general response of low a^* values in Halfway and Shawaya Black from College Station can be explained because the surface of the grains was affected by molds and weathering.

The b^* values of black sorghums ranged from 1.5 – 9.4 (Table 12). The b^* values which indicates yellow color were lower in the samples from Halfway, showing significant effect of environment; the average b^* value for Halfway was 3.6 compared to 6.5, 5.2, and 6.8 from Corpus Christi, College Station and Weslaco, respectively (Table 13) (MSD = 0.6, $\alpha = .05$). Genotype effect also occurred (Table14) for b^* value. Like L^* and a^* values, the highly weathered sorghum Shawaya Black from College Station was the exception to this trend, indicating an interaction between the environment and genotypes (Table B.9 and B.9.1). The homogeneous black surface in sorghums from Halfway, which were affected by weathering, caused lower b^* values in this location.

Color of Red Sorghum Grains

*L** values of red sorghum ranged from 38.1 – 45.3 (Table 15). Differences in color were observed in the grains of red sorghums grown at different locations; these differences were due to different levels of weathering in the grains (Fig. 4). In general, sorghums from Weslaco had the highest *L** values (41.9) while College Station had the lowest values (40.3); the other two locations, Corpus Christi and Halfway had 41.1 and 41.0, respectively (Table 16) (MSD = 0.8, α = .05). Differences were found between genotypes for *L**, *a** and *b** values (Table 17). Interaction between environment and genotypes also was observed for *L**, *a** and *b** values (Tables B.10, B.10.1, B.11, B.11.1, B.12 and B.12.1). for example, the sample 99LGWO50 from Halfway had a higher *L** value than the sample from Weslaco.

Red color, expressed as *a** values of red sorghum ranged from 9.8 – 22.1 (Table 15). On average, *a** value was 14.4 for Halfway, indicating reduced red color in the pericarp. *a** values were higher in the locations with less weathered grains, the values in these locations were 17.3, 17.7 and 19.7 for Corpus Christi, College Station and Weslaco, respectively (Table 16) (MSD = 0.8, α = .05).

*b** values ranged from 10.7 – 19.7 (Table 15). The *b** values were lower in the samples from Halfway with an average value of 13.0, while Corpus Christi, College Station and Weslaco had 15.6, 14.5 and 15.3, respectively (Table 16) (MSD = 1.0, α = .05).

Table 15

***L**, *a**and *b** Color Values of Red Sorghum Grains Grown at Different Locations**

Location[a]	Line or hybrid	Color[b]		
		L*	***a****	***b****
CC	SC719-11E	41.8	19.4	17.9
CS	SC719-11E	40.1	19.9	15.5
WE	SC719-11E	42.8	20.4	17.2
HW	SC719-11E	41.2	17.0	14.1
CC	Tx2911	39.7	18.9	14.6
CS	Tx2911	39.7	19.9	14.4
WE	Tx2911	41.8	20.8	15.7
HW	Tx2911	38.7	15.7	11.1
CC	99LGWO50	38.3	18.0	12.5
CS	99LGWO50	38.1	17.0	11.4
WE	99LGWO50	38.3	22.1	11.4
HW	99LGWO50	40.4	9.8	13.0
CC	98CA4779	44.2	15.9	18.6
CS	98CA4779	44.7	17.9	19.7
WE	98CA4779	45.3	19.1	19.3
HW	98CA4779	44.4	12.8	16.0
CC	B9904	41.6	14.1	14.6
CS	B9904	38.9	13.8	11.6
WE	B9904	41.1	16.1	12.9
HW	B9904	40.0	12.3	10.7

[a] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[b] Minimum Significant Difference = 0.7 for *L**, 0.7 for *a** and 0.8 for *b**. $\alpha = .05$.

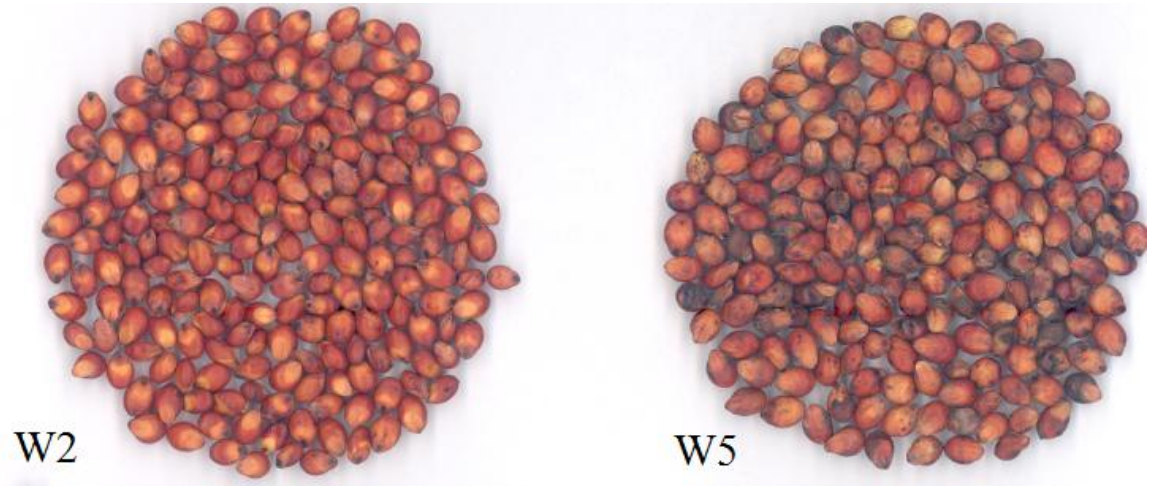

Fig. 4. Comparison of appearance of a low weathered red sorghum TX2911 from Weslaco (W2) and a highly weathered red sorghum TX2911from Halfway (W5). (W2 = weathering score of 2 and W5 = weathering score of 5).

Table 16

Effect of Location on Average *L, *a** and *b** Values of 5 Red Sorghum Grains**

Location[1]	Color[2]			Weathering
	*L**	*a**	*b**	Score
WE	41.9^{a}	19.7^{a}	15.3^{ab}	2.8^{b}
CC	41.1^{ab}	17.3^{b}	15.6^{a}	2.6^{b}
HW	40.9^{bc}	14.4^{c}	13.0^{c}	2.8^{b}
CS	40.3^{c}	17.7^{b}	14.5^{b}	4.6^{a}

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.8 for *L**, 0.8 for *a**, 1.0 for *b** and 1.1 for weathering values with different letter are statisticaly different for each column. $\alpha = .05$.

Table 17

Average *L, *a** and *b** Values of 5 Red Sorghum Grains Grown at Different Locations**

Line or hybrid	Color[1,2]			Weathering
	*L**	*a**	*b**	Score
98CA4779	44.6 [a]	16.4 [c]	18.4 [a]	3.0 [a]
SC719-11E	41.5 [b]	19.1 [a]	16.2 [b]	3.5 [a]
Tx2911	40.0 [c]	18.8 [ab]	14.0 [c]	3.3 [a]
B9904	40.4 [c]	14.1 [d]	12.4 [d]	3.8 [a]
99LGWO50	38.8 [d]	16.7 [b]	12.1 [d]	2.5 [a]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 0.9 for *L**, 1.0 for *a**, 1.2 for *b** and 2.2 for weathering values with different letter are statistically different for each column. $\alpha = .05$.

Color of Yellow Sorghums

*L** values of yellow sorghum ranged from 44.9 – 57.0 (Table 18). Differences in color were observed in all locations, but principally in grains from Halfway which were more affected by molds and weathering (Fig. 5). In yellow sorghums significant effect of environment was identified (Table 19) (MSD = 3.2, α = .05). Samples from Halfway had lower *L** values (46.0), while the other locations had 52.1, 49.6 and 53.4 for Corpus Christi, College Station and Weslaco, respectively. Interaction between environment and genotypes was observed (Table B.13 and 13.1). The presence of molds was the factor that impacted *L** values of the yellow sorghums.

*a** values of yellow sorghum ranged from 6.9 – 7.8 (Table 18). In general, there ware no differences between locations in *a** values of yellow sorghums (Table 19) (MSD = 0.8, α = .05). No differences were found among genotypes (Table 20), but there was an interaction between the environment and genotypes (Table B.14 and 14.1). Presence of molds did not affect *a** values, because of the relative low amount of red color present in yellow sorghums compared to other pigments.

*b** values of yellow sorghum ranged from 13.8 – 26.5 (Table 18). The *b** values were reduced by weathering conditions (HW), with average values of 15.2 while the other three locations were between 21.0 and 23.8 (Table 19) (MSD = 3.8, α = .05). An interaction between environment and genotype occurred (Table B.15 and 15.1).

Table 18

***L*, a*, b** Color Values of Yellow Sorghum Grains Grown at Different Locations**

Location[a]	Line or hybrid	Color[b]		
		*L**	*a**	*b**
CC	RO7007	55.4	6.9	26.5
CS	RO7007	54.3	7.0	25.6
WE	RO7007	57.0	7.3	25.6
HW	RO7007	45.7	7.6	13.8
CC	SC748-5	48.9	7.6	21.1
CS	SC748-5	44.9	7.7	16.3
WE	SC748-5	49.9	7.8	20.2
HW	SC748-5	46.2	7.0	16.7

[a] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[b] Minimum Significant Difference = 0.6 for *L**, 0.7 for *a** and 0.8 for *b**. $\alpha = .05$.

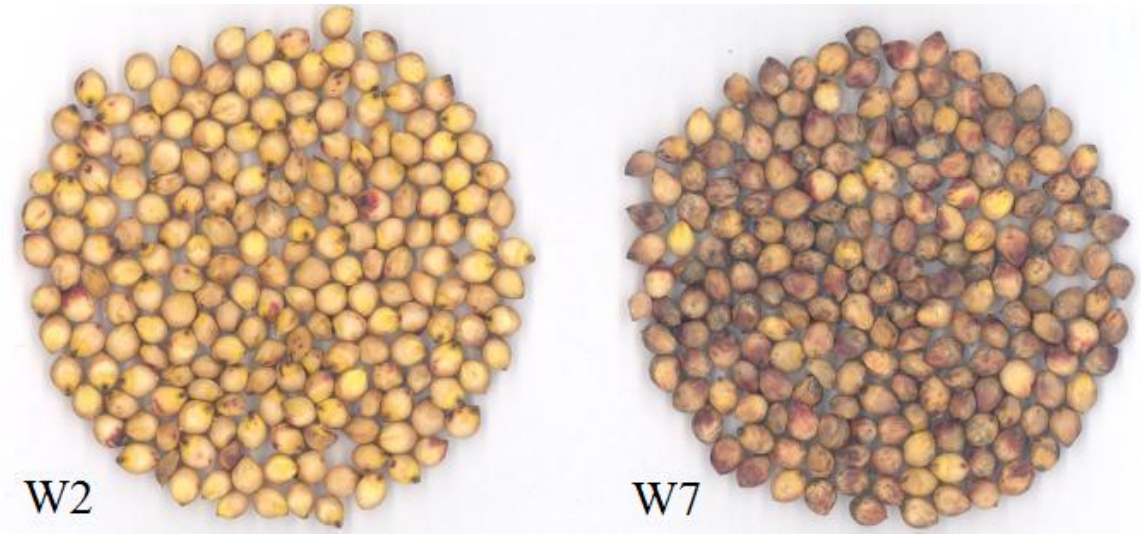

Fig. 5. Comparison of appearance of a low weathered yellow sorghum RO7007 from Weslaco (W2) and a highly weathered yellow sorghum RO7007 from Halfway (W7). (W2 = weathering score of 2 and W7 = weathering score of 7).

Table 19

Effect of Location on Average *L, *a** and *b** Values of 2 Yellow Sorghum Grains**

Location[1]	Color[2]			Weathering
	*L**	*a**	*b**	Score
WE	53.4[a]	7.6[a]	22.9[a]	3.0[a]
CC	52.1[ab]	7.2[a]	23.3[a]	3.0[a]
CS	49.6[b]	7.3[a]	21.0[a]	3.5[a]
HW	46.0[c]	7.3[a]	15.2[b]	6.5[a]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 3.2 for *L**, 0.8 for *a**, 3.8 for *b** and 5.3 for weathering values with different letter are statisticaly different for each column. α = .05.

Table 20

Average *L, *a** and *b** Values of 2 Yellow Sorghum Grains Grown at Different Locations**

Line or hybrid	Color[1,2]			Weathering
	*L**	*a**	*b**	Score
RO7007	53.1 [a]	7.2 [a]	22.9 [a]	3.5[a]
SC748-5	47.5 [b]	7.5 [a]	18.6 [b]	4.5[a]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 1.8 for *L**, .3 for *a** and 2.2 for *b**. α = .05

values with different letter are statistically different for each column.

Effect of Environment on Total Phenols and Antioxidant Activity of Sorghum Grain

Total Phenols of Black Sorghum Grains

Total phenols of black sorghum ranged from 4.8 – 22.0 mg GAE/g (Table 21). Total phenols of black sorghums grown at Halfway (14.5 mg GAE/g) were lower compared to Corpus Christi, College Station and Weslaco (16.3, 15.8 and 15.5 mg GAE/g), respectively, showing the effect of environment on the concentration of total phenols (Table 22) (MSD = 0.9 mg GAE/g, $\alpha = .05$). The total phenols of black sorghums were higher in samples with a pigmented testa (13.9 to 22.0 mg GAE/g) compared to Tx430 Black which does not have a pigmented testa (4.8 to 7.1 mg GAE/g) (Table 23) (MSD = 1.4 mg GAE/g, $\alpha = .05$). Environment by genotype interactions occurred, as in the case of the sorghum B05028 from Weslaco which had lower levels of total phenols than the sample from Halfway (Table B.16 and B.16.1). In general, weathered sorghums (HW) had the greatest reduction of phenol levels in black sorghums.

Antioxidant Activity of Black Sorghum Grains

Antioxidant activity of black sorghum ranged from 61.9 – 272.1 µmol TE/g (Table 21). In general, sorghums from Halfway had the lowest antioxidant activity (190.0 µmol TE/g) compared to Corpus Christi, College Station and Weslaco with 211.4, 208.9 and 204.5 µmol TE/g, respectively (Table 22) (MSD = 9.4 µmol TE/g, α = .05). The antioxidant activity in black sorghums was higher in samples with a pigmented testa (182.1 to 272.1 µmol TE/g) while the Tx430 Black had values in the range of 61.9 and 83.3 µmol TE/g (Table 23) (MSD = 9.4 µmol TE/g, α = .05). Interaction between the environment and genotypes occurred (Table B.17 and B.17.1). Weathered grains had the greatest reduction of antioxidant activity in black sorghums except for the sample B05028 from Weslaco. Antioxidant activity of black sorghums with testa was highly positively correlated with their total phenols content in all locations (r = 0.92).

Table 21

Phenol and Antioxidant Activity in Black Sorghum Grown at Different Locations

Location[1]	Line or hybrid	Total Phenols (mg GAE/g)[2,3]	ABTS (µmol TE/g)[4,5]
CC	Black Tx430	7.1	83.2
CS	Black Tx430	4.8	61.9
WE	Black Tx430	5.6	69.2
HW	Black Tx430	5.3	62.7
CC	Shawaya Black	18.1	242.3
CS	Shawaya Black	16.9	237.8
WE	Shawaya Black	18.0	242.9
HW	Shawaya Black	15.2	204.3
CC	B05028	15.6	188.1
CS	B05028	16.8	210.1
WE	B05028	14.6	182.1
HW	B05028	16.3	194.6
CC	B05030	20.5	263.2
CS	B05030	17.3	234.2
WE	B05030	17.2	233.1
HW	B05030	17.3	231.2
CC	A05028/Black Tx430	15.4	207.8
CS	A05028/Black Tx430	16.4	223.0
WE	A05028/Black Tx430	15.1	201.1
HW	A05028/Black Tx430	14.8	190.3
CC	A05030/Black Tx430	20.3	251.5
CS	A05030/Black Tx430	22.0	272.1
WE	A05030/Black Tx430	19.8	255.3
HW	A05030/Black Tx430	17.0	219.1
CC	A05028/Shawaya	15.2	209.7
CS	A05028/Shawaya	16.4	212.7
WE	A05028/Shawaya	15.3	205.8
HW	A05028/Shawaya	13.9	196.4
CC	A05030/Shawaya	18.0	245.4
CS	A05030/Shawaya	15.8	219.9
WE	A05030/Shawaya	18.3	246.4
HW	A05030/Shawaya	15.8	221.1

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 2.1 mg GAE/g for Total Phenols. $\alpha = .05$.

[3] GAE = Gallic acid equivalents.

[4] Minimum Significant Difference = 25.7 µmol TE/g for Antioxidant Activity. $\alpha = .05$.

[5] TE = Trolox equivalents.

Table 22

Effect of Location on Average Total Phenols and Antioxidant Activity of 8 Black Sorghum Grains

Location[1]	Total Phenols[2] (mg GAE/g)[3]	Antioxidant Activity[2] (µmol TE/g)[4]
CC	16.3[a]	211.4[a]
CS	15.8[a]	208.9[a]
WE	15.5[a]	204.5[a]
HW	14.5[b]	190.0[b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.9 mg GAE/g for Total Phenols and 9.4 µmol TE/g for Antioxidand Activity. $\alpha = .05$ values with different letter are statisticaly different for each column.

[3] GAE = Gallic Acid Equivalents.

[4] TE = Trolox Equivalents.

Table 23

Average Total Phenols and Antioxidant Activity of 8 Black Sorghum Grains Grown at Different Locations

Line or hybrid	Total Phenols (mg GAE/g)[2,3]	ABTS (µmol TE/g)[4,5]
A05030/Black Tx430	19.8 [a]	249.5 [a]
B05030	18.1 [b]	240.4 [ab]
A05030/Shawaya	17.0 [bc]	233.2 [b]
Shawaya Black	17.1 [bc]	231.8 [b]
A05028/Shawaya	15.2 [d]	206.1 [c]
A05028/Black Tx430	15.4 [d]	205.5 [c]
B05028	15.8 [cd]	193.7 [c]
Black Tx430	5.7 [e]	69.3 [d]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 1.4 mg GAE/g for Total Phenols. $\alpha = .05$.

3 GAE = Gallic acid equivalents.

[4] Minimum Significant Difference = 15.8 µmol TE/g for Antioxidant Activity. $\alpha = .05$.

[5] TE = Trolox equivalents.

Total Phenols of Red Sorghum Grains

Total phenols were detected in all samples; the values ranged between 1.9 - 5.1 mg GAE/g (Table 24). The average total phenols in red sorghums were lower in Halfway (2.9 mg GAE/g), while higher values were observed in Corpus Christi, College Station and Weslaco with 3.1, 3.55 and 3.2 mg GAE/g, respectively (Table 25) (MSD = 0.3, $\alpha = .05$). Sorghum 99LGWO50 had the highest values of total phenols (Table 26) (MSD = 0.3, $\alpha = .05$). Genotype by environment interaction occurred (Table B.18 and B.18.1). In general, weathered sorghums (HW) had the greatest reduction of phenol levels in red sorghums.

Antioxidant Activity of Red Sorghum Grains

The values of antioxidant activity in red sorghum ranged from 28.1 - 92.7 µmol TE/g (Table 24). The average antioxidant activity of red sorghums from Weslaco were lower (51.88 µmol TE/g) compared to 53.9, 58.8 and 54.2 µmol TE/g from Corpus Christi, College Station and Halfway, respectively (Table 25) (HSD = 2.9, $\alpha = .05$). Sorghum SC719-1E had the highest antioxidant activity value (Table 26) (MSD = 3.3, $\alpha = .05$). Interaction between genotypes and environment was observed (Table B.19 and B.19.1). Antioxidant activity of red sorghums had a high positive correlation with their total phenols content ($r = 0.96$) in all locations.

Table 24

Total Phenols and Antioxidant Activity Levels in Red Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	Total Phenols (mg GAE/g)[2,3]	ABTS (µmol TE/g)[4,5]
CC	SC719-11E	3.6	87.7
CS	SC719-11E	3.9	92.7
WE	SC719-11E	3.5	84.0
HW	SC719-11E	3.2	82.7
CC	Tx2911	3.1	52.3
CS	Tx2911	4.1	61.1
WE	Tx2911	3.7	54.2
HW	Tx2911	3.0	52.8
CC	99LGWO50	4.2	67.2
CS	99LGWO50	5.1	75.6
WE	99LGWO50	4.5	61.8
HW	99LGWO50	4.1	74.5
CC	98CA4779	2.0	28.1
CS	98CA4779	2.4	31.6
WE	98CA4779	1.9	28.0
HW	98CA4779	2.1	30.4
CC	B9904	2.5	34.2
CS	B9904	2.3	32.8
WE	B9904	2.3	31.3
HW	B9904	2.3	30.6

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.5 mg GAE/g for Total Phenols. $\alpha = .05$.

[3] GAE = Gallic acid equivalents.

[4] Minimum Significant Difference = 3.5 µmol TE/g for Antioxidant Activity. $\alpha = .05$.

[5] TE = Trolox equivalents.

Table 25

Effect of Location on Average Total Phenols and Antioxidant Activity of 5 Red Sorghums

Location[1]	Total Phenols[2] (mg GAE/g)[3]	Antioxidant Activity[2] (µmol TE/g)[3]
CS	3.6[a]	58.8[a]
WE	3.2[b]	54.2[b]
CC	3.1[b]	53.9[b]
HW	2.9[b]	51.9[b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.3 mg GAE/g for Total Phenols and 2.9 µmol TE/g for Antioxidand Activity $\alpha = .05$ values with different letter are statisticaly different for each column.

[3] GAE = Gallic Acid Equivalents.

[4] TE = Trolox Equivalents.

Table 26

Average Total Phenols and Antioxidant Activity of 5 Red Sorghums Grown at Different Locations

Line or hybrid	Total Phenols (mg GAE/g)[2,3]	ABTS (µmol TE/g)[4,5]
99LGWO50	4.5 [a]	69.8 [b]
SC719-11E	3.5 [b]	86.8 [a]
Tx2911	3.4 [b]	55.1 [c]
B9904	2.3 [c]	32.2 [d]
98CA4779	2.1 [c]	29.6 [d]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 0.3 mg GAE/g for Total Phenols. $\alpha = .05$.

3 GAE = Gallic acid equivalents.

[4] Minimum Significant Difference = 3.3 µmol TE/g for Antioxidant Activity. $\alpha = .05$.

[5] TE = Trolox equivalents.

Total Phenols of Yellow Sorghum Grains

Total phenols values were in a range of 1.4 to 2.7 mg GAE/g (Table 27). The total phenols in yellow sorghums where lower in Halfway (1.7 mg GAE/g), while in Corpus Christi, College Station and Weslaco were 2.4, 2.0 and 1.9 mg GAE/g, respectively (Table 28) (MSD = 0.3, $\alpha = .05$) . No difference was observed among genotypes (Table 29). Interaction between genotypes and environment was observed (Table B.20 and B.20.1).

Antioxidant Activity of Yellow Sorghum Grains

The antioxidant activity of yellow sorghums was in the range of 42.4 and 72.3 μmol TE/g (Table 27). The antioxidant activity of yellow sorghums was lower in samples from Halfway (48.9 μmol TE/g) while in Corpus Christi, College Station and Weslaco was 68.9, 55.7 and 55.4 μmol TE/g, respectively (Table 28) (MSD = 7.7, $\alpha = .05$). No difference was observed among genotypes (Table 29). Interaction between environment and genotypes was observed (Table B.21 and B.21.1). The antioxidant activity of yellow sorghums had a high positive correlation with total phenol content ($r = 0.98$) in all locations.

Table 27

Total Phenols and Antioxidant Activity Levels in 2 Yellow Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	Total Phenols (mg GAE/g)[2,3]	ABTS (µmol TE/g)[4,5]
CC	RO7007	2.7	72.3
CS	RO7007	2.0	58.0
WE	RO7007	2.0	56.2
HW	RO7007	1.4	42.4
CC	SC748-5	2.2	65.6
CS	SC748-5	1.9	53.5
WE	SC748-5	1.8	54.5
HW	SC748-5	1.9	55.4

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.2 mg GAE/g for Total Phenols. $\alpha = .05$.

[3] GAE = Gallic acid equivalents.

[4] Minimum Significant Difference = 6.1 µmol TE/g for Antioxidant Activity. $\alpha = .05$.

[5] TE = Trolox equivalents.

Table 28

Effect of Location on Average Total Phenols and Antioxidant Activity of 2 Yellow Sorghum Grains

Location[1]	Total Phenols[2] (mg GAE/g)[3]	Antioxidant Activity[2] (µmol TE/g)[3]
CC	2.4[a]	68.9[a]
CS	2.0[b]	55.8[b]
WE	1.9[b]	55.4[b]
HW	1.7[b]	48.9[b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.3 mg GAE/g for Total Phenols and 7.2 µmol TE/g for Antioxidand Activity $\alpha = .05$ values with different letter are statisticaly different for each column.

[3] GAE = Gallic Acid Equivalents.

[4] TE = Trolox Equivalents.

Table 29

Average Total Phenols and Antioxidant Activity of 2 Yellow Sorghum Grains Grown at Different Locations

Line or hybrid	Total Phenols (mg GAE/g)[2,3]	ABTS (µmol TE/g)[4,5]
RO7007	2.0 [a]	57.2 [a]
SC748-5	2.0 [a]	57.2 [a]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 0.2 mg GAE/g for Total Phenols. $\alpha = .05$.

[3] GAE = Gallic acid equivalents.

[4] Minimum Significant Difference = 4.1 µmol TE/g for Antioxidant Activity. $\alpha = .05$.

[5] TE = Trolox equivalents.

Effect of Environment on 3-Deoxyanthocyanidins of the Sorghum Grains

3-Deoxyanthocyanidins of Black Sorghum Grains

Two methoxylated (5-methoxyluteolinidin and 7-methoxyapigeninidin) and two non- methoxylated (luteolinidin and apigeninidin) 3-deoxyanthocyanidins were identified and quantified in all black sorghum grains (Table 30). The average amount of total 3-deoxyanthocyanidins for Corpus Christi, College Station, Weslaco and Halfway was 498.2, 398.2, 305.4 and 292.1 μg/g, respectively (Table 31) (MSD = 58.9, α = .05). Among genotypes, Black Tx430 had the highest amount of each 3-deoxyanthocyanidin (Table 32) (Fig. 6). The average amount of luteolinidin in Halfway was (107.1 μg/g), while for the other three locations it was between 143.6 and 205.5 μg/g (Fig. 7) (Table 33) (MSD = 22.9, α = .05), where a significant difference was observed in all locations. The general effect of the environment was greater than the effect of genotype (Table B.22 and B.22.1). Samples from Halfway, which were affected by weathering, had the lowest luteolinidin levels, except for the samples B05030 and A05030/Shawaya from College Station, which had a low and high damage by weathering, respectively. This indicated a genotype by environment interaction (Table B.22.1). Apigeninidin concentrations were lower in all samples from Halfway with an average of 45.1 μg/g, and between 81.3 and 140.5 μg/g for the other locations (Fig. 7) (Table 33) (MSD = 20.9, α = .05). Location effect was greater than genotype effect (Table B.23). For apigeninidin also there was an interaction between genotypes and environment (Table B.23.1), which was low compared to the genotype and environment effects.

The two methoxylated 3-deoxyanthocyanidins identified had a different response to the environment compared to the non-methoxylated. The 5-methoxyluteolinidin had values between 54.3 and 107.6 μg/g (Fig. 7) (Table 33) (MSD = 16.6, α = .05); environmental effect occurred (Table B.24); in this case, sorghums from Halfway which were affected by weathering did not have the lowest values. The lowest levels of 5-methoxyluteolinidin were found in Weslaco and College Station where the damage by weathering was minor compared to the other locations (Fig. A.1). The rate of change in 5-methoxyluteolinidin was not the same for all the genotypes in the different environments which is explained in the environment by genotype interaction (Table B.24.1). The 7-methoxyapigeninidin had values between 26.3 and 57.1 μg/g (Fig. 7) (Table 33) (MSD = 8.5, α = .05), an environmental effect also was observed with this component (Table B.25). The lower 7-methoxyapigeninidin values were observed in Weslaco, with the exception of samples B05030 and A05030/Shawaya from College Station which had a low and high damage by weathering, respectively; this is shown in the environment by genotype interaction (Table B.25.1).

The trends were different for each type of 3-deoxyanthocyanins. The methoxylated 3-deoxyanthocyanins were not reduced in grains damaged by weathering stress to the same extent as in other locations were other factors induced lower levels. However, the non-methoxylated 3-deoxyanthocyanins levels were lower in sorghum grains damaged by weathering.

Table 30

3-Deoxyanthocyanidins in Black Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]				
		Luteolinidin	Apigenidin	5-Methoxy-luteolinidin	7-Methoxy apigeninidin	Total
CC	Tx430 Black	352.7	233.6	308.0	153.2	1047.5
CS	Tx430 Black	272.7	214.9	244.2	147.9	879.7
WE	Tx430 Black	226.4	144.3	147.2	77.6	595.5
HW	Tx430 Black	215.1	84.4	281.8	112.3	693.7
CC	Shawaya Black	230.2	101.0	90.1	31.1	452.4
CS	Shawaya Black	152.7	134.1	38.7	24.4	349.8
WE	Shawaya Black	184.2	89.5	65.3	21.3	360.3
HW	Shawaya Black	97.6	38.1	84.5	25.9	246.1
CC	B05028 Black	123.9	175.3	39.7	57.4	396.3
CS	B05028 Black	171.4	229.4	43.7	54.5	499.0
WE	B05028 Black	92.5	96.3	28.1	29.1	246.0
HW	B05028 Black	100.3	63.0	69.2	47.5	280.1
CC	B05030 Black	234.9	134.0	89.1	51.5	509.4
CS	B05030 Black	75.1	48.0	17.2	11.3	151.6
WE	B05030 Black	141.6	72.3	23.8	15.1	252.7
HW	B05030 Black	117.6	40.9	85.9	32.0	276.3
CC	A05028/Black Tx430	211.9	152.7	135.4	81.7	581.7
CS	A05028/Black Tx430	178.0	213.0	59.6	52.8	503.5
WE	A05028/Black Tx430	124.7	82.5	49.1	25.9	282.2
HW	A05028/Black Tx430	75.9	45.2	73.9	42.9	237.9
CC	A05030/Black Tx430	149.5	90.1	84.5	43.4	367.6
CS	A05030/Black Tx430	103.4	98.8	38.3	25.6	266.1
WE	A05030/Black Tx430	111.9	55.8	52.4	19.3	239.3
HW	A05030/Black Tx430	61.6	28.3	59.8	28.3	177.9
CC	A05028/Shawaya	134.7	86.8	38.0	22.2	281.7
CS	A05028/Shawaya	167.1	141.0	37.0	24.9	370.1
WE	A05028/Shawaya	107.9	60.9	24.7	11.7	205.2
HW	A05028/Shawaya	86.0	30.9	60.3	20.0	197.3
CC	A05030/Shayawa	206.1	50.6	75.9	16.5	349.0
CS	A05030/Shayawa	91.0	45.0	21.7	7.5	165.2
WE	A05030/Shayawa	159.3	48.6	43.7	10.7	262.3
HW	A05030/Shayawa	102.9	30.0	73.9	20.9	227.7

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 7.4 , 5.1, 5.1 and 3.1 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. $\alpha = .05$ values with different letter are statisticaly different for each column.

Table 31

Effect of Location on Average Total 3-Deoxyanthocyanidins of 8 Black Sorghum Grains

Location[1]	Total 3-Deoxyanthocyanidins[2] (µg/g)
CC	498.2[a]
CS	398.2[b]
WE	305.4[c]
HW	292.1[c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 58.9 µg/g. α = .05 values with different letter are statisticaly different.

Table 32

Average 3-Deoxyanthocyanidins in Black Sorghum Grains Grown at Different Locations

Line or hybrid[1]	µg/g[2]				
	Luteolinidin	Apigenidin	5-Methoxy-luteolinidin	7-Methoxy apigeninidin	Total
Black Tx430	266.7 [a]	169.3 [a]	245.3 [a]	122.8 [a]	804.1 [a]
A05028/Black Tx430	147.6 [bc]	123.3 [bc]	79.5 [bc]	50.9 [b]	401.3 [b]
B05028 Black	122.0 [cd]	141.0 [ab]	45.2 [ab]	47.1 [b]	355.4 [bc]
Shawaya Black	166.2 [b]	90.7 [cd]	69.6 [bc]	25.7 [cd]	352.1 [bc]
B05030 Black	142.3 [bcd]	73.8 [de]	54.0 [de]	27.5 [cd]	297.5 [dc]
A050028/Shawaya	124.0 [cd]	79.9 [d]	40.0 [d]	19.7 [cd]	263.6 [dc]
A05030/Black Tx430	106.6 [d]	68.2 [de]	58.7 [de]	29.2 [c]	262.7 [dc]
A05030/Shawaya	139.9 [bcd]	43.5 [e]	53.8 [e]	13.9 [d]	251.1 [d]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 38.2, 35.0, 27.8, 14.3 and 98.8µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. α = .05 values with different letter are statisticaly different for each column.

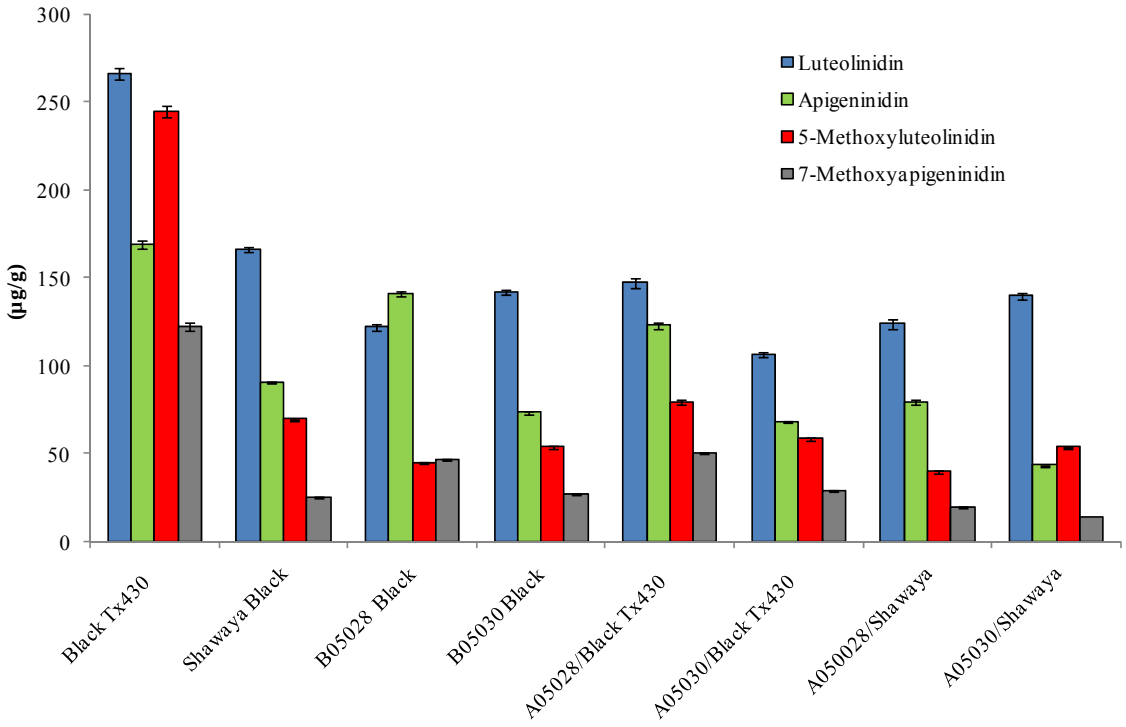

Fig. 6. Effect of genotype on average 3-deoxyanthocyanidins concentration of black sorghums grown in 4 locations.

Table 33

Effect of Location on Average 3-Deoxyanthocyanidins of 8 Black Sorghum Grains

Location[1]	ug/g[2]				
	Luteolinidin	Apigeninidin	5-Methoxy Luteolinidin	7-Methoxy Apigeninidin	Total
CC	205.5[a]	128.0[a]	107.6[a]	57.1[a]	498.2 [a]
CS	151.4[b]	140.5[a]	62.6[b]	43.6[b]	398.1 [b]
WE	143.6[b]	81.3[b]	54.3[b]	26.3[c]	305.5 [c]
HW	107.1[c]	45.1[b]	98.7[a]	41.2[b]	292.1 [c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 22.9, 20.9, 16.6, 8.5 and 58.9 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. $\alpha = .05$ values with different letter are statisticaly different for each column.

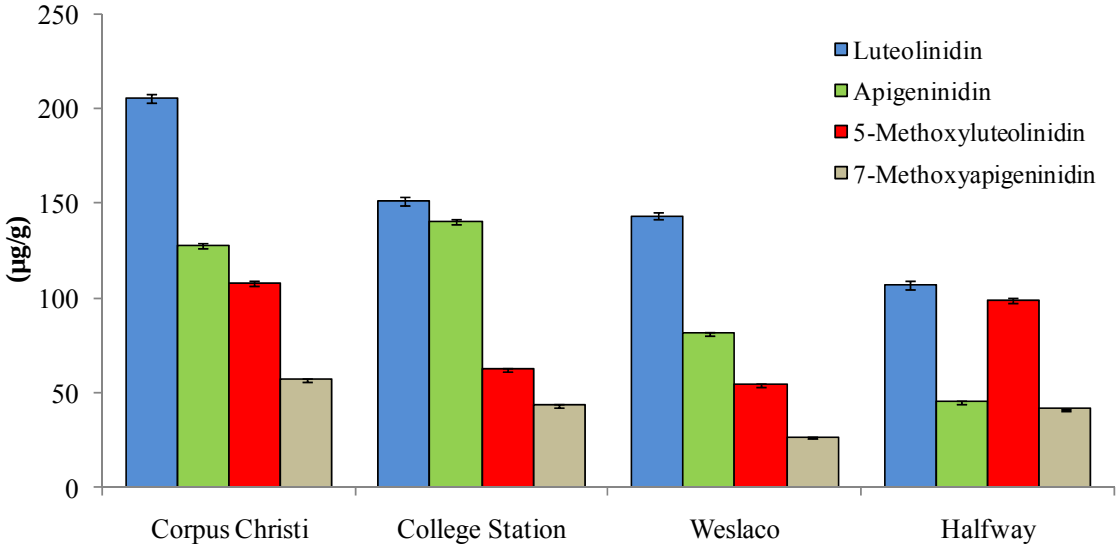

Fig. 7. Effect of environment on average methoxy and non-methoxy 3-deoxyanthocyanidin concentration in 8 black sorghums grown in different locations.

3-Deoxyanthocyanins of Red Sorghum Grains

Two non-methoxylated (luteolinidin and apigeninidin) and two methoxylated (5-methoxylyteolinidin and 7-methoxyapigeninidin) 3-deoxyanthocyanins were identified and quantified in all red sorghum grains (Table 34). The mean 3-deoxyanthocyanins for Corpus Christi, College Station, Weslaco and Halfway was 50.5, 38.9, 36.7 and 100.4 μg/g, respectively (Table 35) (MSD = 27.9, α = .05).The data showed that there was a general effect of the environment on the samples from Halfway which were affected by weathering. Among genotypes, Tx2911 had the highest amount of 3-deoxyanthocyanidins as average of all locations (Fig 8.) (Table 36). Luteolinidin levels were higher in Halfway (18.61 μg/g) while in the other locations were between 5.60 and 9.46 μg/g (Fig. 9) (Table 37) (MSD = 5.5, α = .05). For luteolinidin levels there was an interaction of the genotypes with the locations (Table B.26 and 26.1), which indicates that the environment had an effect on luteolidin concentration, but not all the genotypes had the same level of response to it. Apigeninidin concentrations were also higher in Halfway with an average of 37.14 μg/g, and between 15.72 and 25.8 μg/g for the other locations (Fig. 9) (Table 37) (MSD = 5.5, α = .05); with this component there was an interaction between genotypes and environment (Table B.27 and B.27.1).

The methoxylated 3-deoxyanthocyanins also had higher values in Halfway with an average value 5-methoxyluteolinidin of 16.8 μg/g, while in the other locations the levels were between 4.9 and 6.1 μg/g (Fig. 9) (Table 37) (MSD = 3.0, α = .05). Environmental effects occurred (Table B.28), but in this case, sorghums from Halfway which were affected by weathering did not have the lowest values. Interaction had an important impact (Table B.28.1). The 7-methoxyapigeninidin values from Halfway were higher (27.8 μg/g) compared to the other locations (8.1 and 10.3 μg/g) (Fig. 9) (Table 37) (MSD = 10.4, α = .05); an environmental effect also was observed with this component (Table B.29), also having interaction between environment and genotypes (Table B.29.1). Contrary to black sorghums, the levels of methoxy and non-methoxy 3-deoxyanthocyanins were higher in sorghums affected by weathering. The 3-deoxyanthocyanins are known as phytoalexins, being synthesized when plants are stressed by molds. Thus, stressed sorghums produce higher levels of 3-deoxyanthocyanins which was the case of red sorghums.

Table 34

3-Deoxyanthocyanidin in Red Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]				
		Luteolinidin	Apigenidin	5-Methoxy luteolinidin	7-Methoxy apigeninidin	Total
CC	SC719-11E	7.0	44.2	3.0	11.7	65.9
CS	SC719-11E	3.7	22.6	2.6	7.7	36.7
WE	SC719-11E	4.3	30.8	3.4	12.8	51.3
HW	SC719-11E	10.9	30.8	16.0	25.9	83.6
CC	Tx2911	11.9	46.7	11.4	30.5	100.5
CS	Tx2911	7.7	40.1	9.4	22.8	80.0
WE	Tx2911	6.9	23.7	10.0	21.9	62.5
HW	Tx2911	31.0	97.5	32.6	89.2	250.4
CC	99LGWO50	1.2	4.3	0.6	1.2	7.2
CS	99LGWO50	0.7	14.7	0.9	1.9	18.2
WE	99LGWO50	0.7	4.9	0.8	1.5	7.9
HW	99LGWO50	1.7	12.9	1.8	3.9	20.3
CC	98CA4779	25.5	19.1	7.6	3.3	55.6
CS	98CA4779	13.9	15.1	9.5	3.4	41.9
WE	98CA4779	16.4	10.6	14.4	4.2	45.7
HW	98CA4779	47.4	29.8	31.0	11.4	119.6
CC	B9904	1.7	14.9	1.9	4.9	23.4
CS	B9904	2.0	8.2	3.1	4.4	17.8
WE	B9904	1.6	8.5	1.9	4.0	16.0
HW	B9904	2.1	14.6	2.7	8.8	28.3

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 1.5 3.7, 0.9 and 2.0 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. $\alpha = .05$ values with different letter are statisticaly different for each column.

Table 35

Effect of Environment on Average Total 3-Deoxyanthocyanidins in Red Sorghum Grains

Location[1]	Total 3-Deoxyanthocyanidins[2]
HW	100.4[a]
CC	50.5[b]
CS	38.9[b]
WE	36.7[b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 27.9 µg/g. α = .05 values with different letter are statisticaly different.

Table 36

Average 3-Deoxyanthocyanidins in Red Sorghum Grains Grown at Different Locations

Line or hybrid[1]	µg/g[2]				
	Luteolinidin	Apigenidin	5-Methoxy-luteolinidin	7-Methoxy apigeninidin	Total
Tx2911	14.4 [b]	52.0 [a]	15.8 [a]	41.1 [a]	123.3 [a]
98CA4779	25.8 [a]	18.7 [c]	15.6 [a]	5.6 [b]	65.7 [b]
SC719-11E	6.5 [c]	32.1 [b]	6.3 [b]	14.5 [b]	59.4 [b]
B9904	1.9 [c]	11.6 [c]	2.4 [bc]	5.5 [b]	21.3 [c]
99LGWO50	1.1 [c]	9.2 [c]	1.0 [c]	2.1 [b]	13.4 [c]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 6.1, 13.4, 5.2, 12.6 and 33.2 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. α = .05 values with different letter are statisticaly different for each column.

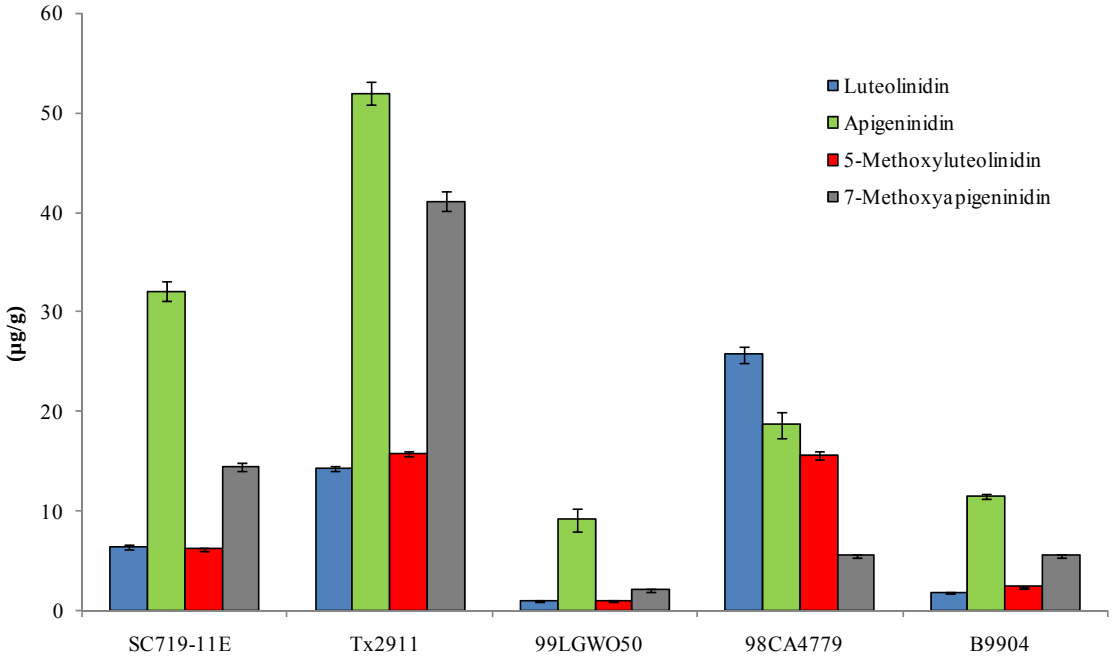

Fig. 8. Effect of genotype on average 3-deoxyanthocyanidins concentration of red sorghums grown in 4 locations.

Table 37

Effect of Location on Average 3-Deoxyanthocyanidins in Red Sorghum Grains Grown at Different Locations

Location[1]	Luteolinidin[2] (µg /g)	Apigeninidin[2] (µg/g)	5-Methoxy Luteolinidin[2] (µg /g)	7-Methoxy Apigeninidin[2] (µg/g)	Total
HW	18.6[a]	37.1[a]	16.8[a]	27.8[a]	100.3 [a]
CC	9.4[b]	25.9[b]	4.9[b]	10.3[b]	50.5 [b]
WE	5.9[b]	15.7[b]	6.1[b]	8.9[b]	36.6 [c]
CS	5.6[b]	20.1[b]	5.1[b]	8.1[c]	38.9 [c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 5.5, 11.3, 4.4, 10.6 and 27.9 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. $\alpha = .05$ values with different letter are statisticaly different for each column.

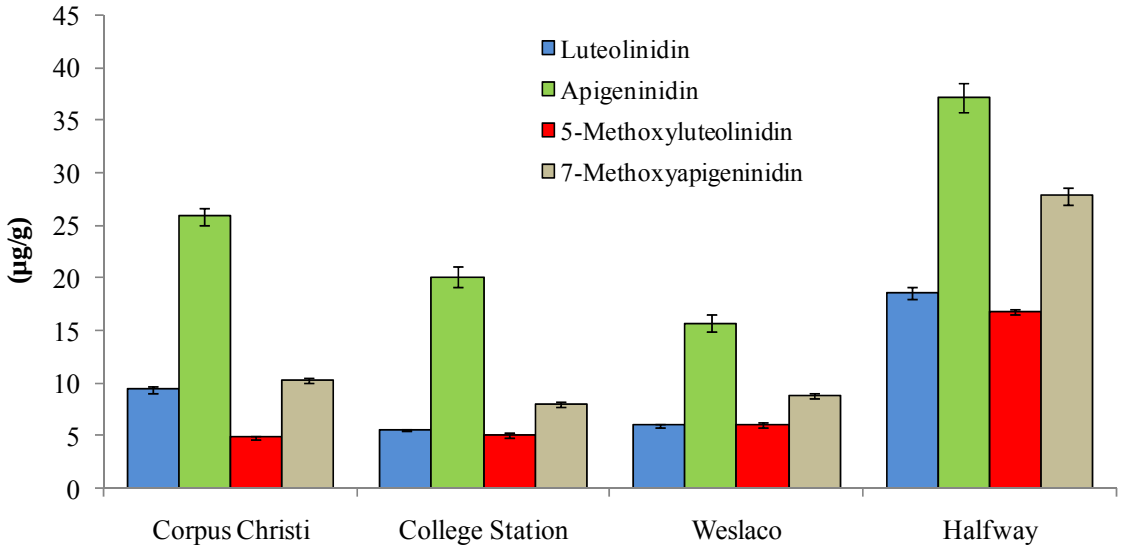

Fig. 9. Effect of environment on average methoxylated and non-methoxylated 3-deoxyanthocyanin concentration in 5 red sorghums grown in different locations.

3-Deoxyanthocyanins of Yellow Sorghum Grains

Two non-methoxylated (luteolinidin and apigeninidin) and two methoxylated (5-methoxyluteolinidin and 7-methoxyapigeninidin) 3-deoxyanthocyanins were identified and quantified in all yellow sorghum grains (Table 38). The average amount of total 3-deoxyanthocyanins for Corpus Christi, College Station, Weslaco and Halfway was 20.5, 13.9, 15.2 and 37.2 μg/g, respectively (Table 39) (MSD = 13.9, α = .05). This data indicates that there was a general effect of the environment on the samples from Halfway which were affected by weathering. Among genotypes, RO7007 had the highest amount of 3-deoxyanthocyanidins as average of all locations (Fig 10) (Table 40). Levels of luteolinidin were higher in Halfway (7.2 μg/g) while in the other locations were between 2.1 and 3.9 μg/g (Figure 11) (Table 41) (MSD = .8 α = .05). For luteolinidin, there was an interaction of the genotypes with the locations, which indicates that the environment had an effect on luteolidin concentration, but not all the genotypes had the same level of response to it (Table B.30 and B.30.1). Apigeninidin concentrations were also higher in Halfway with an average of 17.4 μg/g, and between 7.7 and 14.3 μg/g for the other locations (Fig. 11) (Table 41) (MSD = 5.5 α = .05), with this component also there was an interaction between genotypes and environment (Table B.31 and B.31.1).

The methoxylated 3-deoxyanthocyanins also had the highest levels in Halfway. 5-methoxyluteolinidin had an average value of 5.3 μg/g, while in the other three locations the levels were between 1.2 and 2.6 μg/g (Fig. 11) (MSD = 1.1 α = .05) (Table 41). Environment by genotype interaction occurred (Table B.32 and B.32.1). The 7-methoxyapigeninidin values from Halfway also were higher (7.3 μg/g) compared to the

other locations with values between 1.1 and 2.0 μg/g (Fig. 11) (Table 41) (MSD = 2.7 α = .05) indicating an environmental effect (Table B.33). Interaction between environment conditions and genotypes was also observed (Table B.33.1). Contrary to black sorghums, the levels of methoxy and non-methoxy 3-deoxyanthocyanins were higher in yellow sorghums affected by molds.

Table 38

3-Deoxyanthocyanidins in Yellow Sorghum Grains Grown at Different Locations

Location[1]	Line or hybrid	μg/g[2]				
		Luteolinidin	Apigenidin	5-Methoxy-luteolinidin	7-Methoxy apigeninidin	Total
CC	RO7007	2.4	16.5	ND[c]	1.0	20.0
CS	RO7007	1.4	14.2	0.4	1.5	17.5
WE	RO7007	1.4	9.9	0.6	2.0	13.9
HW	RO7007	5.5	27.5	2.6	10.9	46.4
CC	SC748-5	5.4	12.0	2.4	1.2	21.0
CS	SC748-5	2.8	2.9	3.2	1.4	10.3
WE	SC748-5	4.4	5.5	4.5	2.1	16.5
HW	SC748-5	8.9	7.3	8.1	3.7	28.0

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 1.1, 1.5, .8 and 0.6 μg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. α = .05 values with different letter are statisticaly different for each column.

[c] ND = non detectable.

Table 39

Effect of Environment on Average Total 3-Deoxyanthocyanidins in Yellow Sorghum Grains

Location[1]	Total 3-Deoxyanthocyanidins[2] (µg/g)
HW	37.2[a]
CC	20.5[b]
WE	15.2[c]
CS	13.9[c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 13.9 µg/g. $\alpha = .05$ values with different letter are statisticaly different.

Table 40

Average 3-Deoxyanthocyanidins in Yellow Sorghum Grains Grown at Different Locations

Line or hybrid[a]	µg/g[b]				
	Luteolinidin	Apigenidin	5-Methoxy-luteolinidin	7-Methoxy apigeninidin	Total
RO7007	2.7 [b]	17.0 [a]	0.9 [b]	3.9 [a]	24.5 [a]
SC748-5	5.4 [a]	6.9 [b]	4.5 [a]	2.1 [b]	19.0 [b]

[a] Average of 4 locations.

[b] Minimum Significant Difference = 0.5, 3.1, .6, 1.5 and 4.1 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. $\alpha = .05$ values with different letter are statisticaly different for each column.

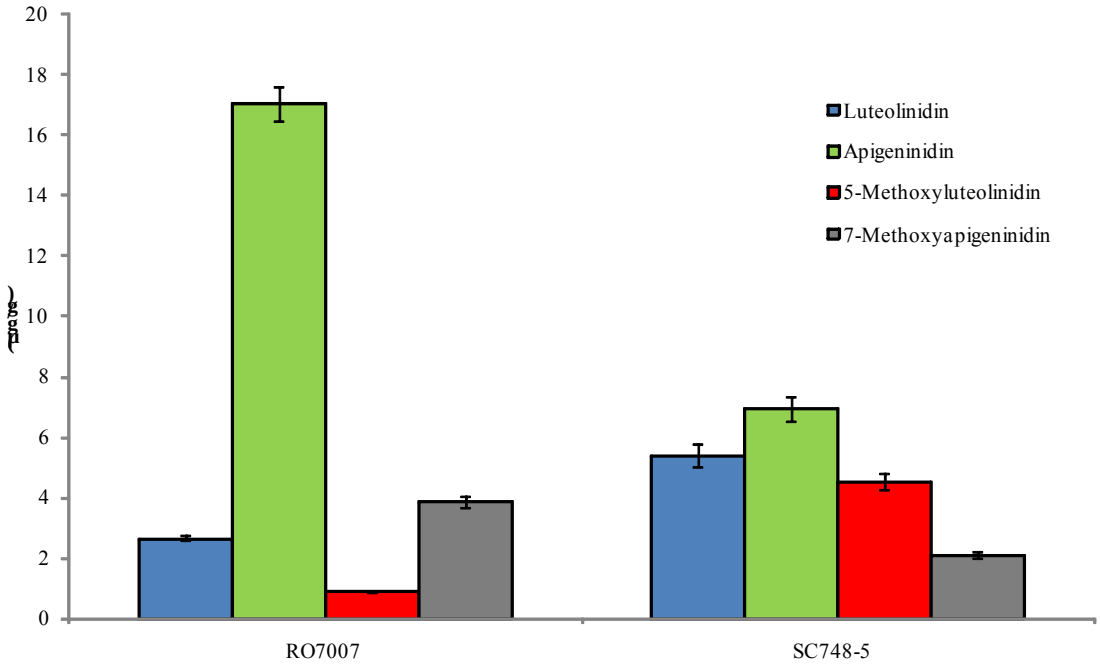

Fig. 10. Effect of genotype on average 3-deoxyanthocyanidins concentration of red sorghums grown in 4 locations.

Table 41

Effect of Location on Average 3-Deoxyanthocyanidins in Two Yellow Sorghum Grains Grown at Different Locations

Location[1]	Luteolinidin[2] (µg /g)	Apigeninidin[2] (µg/g)	5-Methoxy Luteolinidin[2] (µg /g)	7-Methoxy Apigeninidin[2] (µg/g)	Total
HW	7.2[a]	17.4[a]	5.3[a]	7.3[a]	37.2 [a]
CC	3.9[b]	14.3[ab]	1.2[c]	1.1[b]	20.5 [b]
WE	2.9[c]	7.7[c]	2.6[b]	2.0[b]	15.2 [c]
CS	2.1[c]	8.5[bc]	1.8[bc]	1.5[b]	13.9 [c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 0.9, 5.9, 1.2, 2.9 and 7.8 µg/g for luteolinidin, apigenidin, 5-methoxyluteolinidin, 7-methoxyapigeninidin and total, respectively, among genotypes. $\alpha = .05$ values with different letter are statisticaly different for each column.

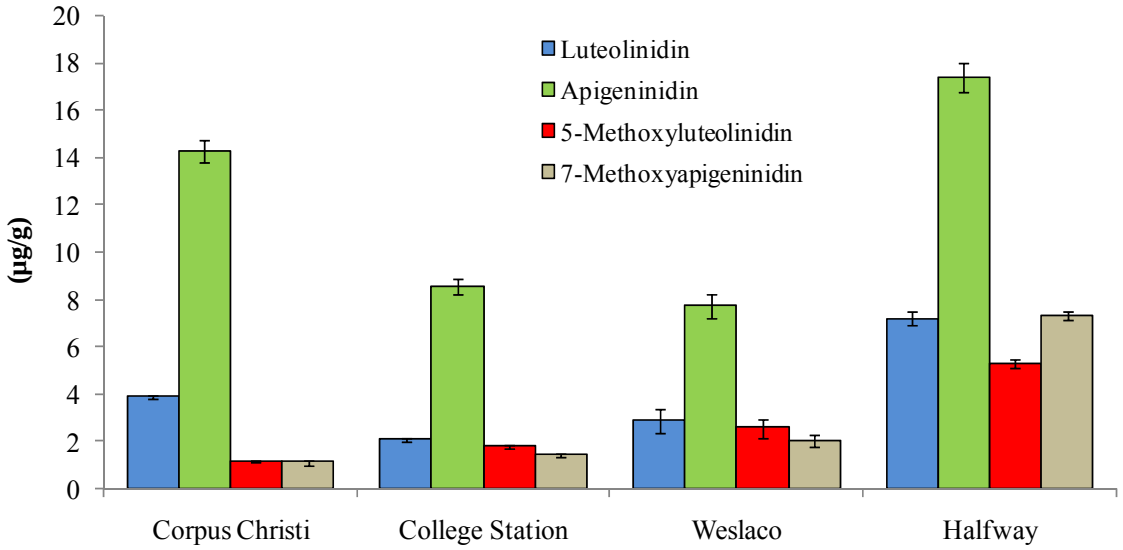

Fig. 11. Effect of environment on average methoxylated and non-methoxylated 3-deoxyanthocyanin concentration in 2 yellow sorghums grown in different locations.

Effect of Environment on Flavones of Sorghum Grains

Flavones in Black Sorghum Grains

Luteolin and apigenin were identified and quantified in all black sorghum from Corpus Christi, College Station and Weslaco except the sorghum B05030 grown at College Station (Table 42). Environmental effect was observed in luteolin levels, where grains from Halfway had the lowest levels (10.6 μg/g) while the other three locations had levels between 20.2 and 23.8 μg/g (Fig. 12) (Table 43) (MSD = 5.0, α = .05). Genotype effect was observed (Table 44). Environment by genotype interaction was observed but the interaction effect was low compared to the effect of location or genotype (Table B.34 and B.34.1). Apigenin levels were low in all samples; sorghums from Halfway and the B05030 from College Station had no detectable levels and in the other three locations the levels were between 3.7 and 4.4 μg/g (Fig. 12) (Table 43) (MSD = 4.7, α = .05), showing an environment affect. Environment by genotype interaction also was observed for apigenin (Table B.35 and B.35.1).

Table 42

Flavones in Black Sorghums Grown at Different Locations

Location[1]	Line or Hybrid	µg/g[2]		
		Luteolin	Apigenin	Total
CC	Tx430 Black	39.9	6.7	46.6
CS	Tx430 Black	54.3	11.3	65.7
WE	Tx430 Black	47.3	7.9	55.2
HW	Tx430 Black	21.3	3.9	25.1
CC	Shawaya Black	20.3	3.2	23.5
CS	Shawaya Black	14.9	3.1	18.0
WE	Shawaya Black	18.9	3.0	21.9
HW	Shawaya Black	9.6	ND[c]	9.6
CC	B05028 Black	14.7	2.6	17.2
CS	B05028 Black	15.2	2.3	17.5
WE	B05028 Black	10.5	1.6	12.1
HW	B05028 Black	9.7	ND	9.7
CC	B05030 Black	28.3	3.9	32.2
CS	B05030 Black	8.5	ND	8.5
WE	B05030 Black	46.9	7.7	54.7
HW	B05030 Black	12.3	ND	12.3
CC	A05028/Black Tx430	29.7	10.4	40.1
CS	A05028/Black Tx430	25.4	5.5	30.9
WE	A05028/Black Tx430	18.0	5.1	23.1
HW	A05028/Black Tx430	8.8	ND	8.8
CC	A05030/Black Tx430	21.3	4.5	25.7
CS	A05030/Black Tx430	16.1	3.5	19.6
WE	A05030/Black Tx430	13.5	2.9	16.4
HW	A05030/Black Tx430	5.4	ND	5.4
CC	A05028/Shawaya	16.2	2.5	18.7
CS	A05028/Shawaya	16.5	2.6	19.1
WE	A05028/Shawaya	13.3	2.1	15.3
HW	A05028/Shawaya	8.2	ND	8.2
CC	A05030/Shayawa	20.4	1.6	22.0
CS	A05030/Shayawa	10.7	1.2	11.9
WE	A05030/Shayawa	16.1	1.5	17.6
HW	A05030/Shayawa	9.5	ND	9.5

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 1.3 and 0.3 µg/g for luteolin and apigenin respectively, among genotypes. $\alpha = .05$

values with different letter are statisticaly different for each column.

[c] ND = non detectable.

Table 43

Effect of Location on Average Flavones of 8 Black Sorghums Grains

Location[1]	Luteolin[2] (µg /g)	Apigenin[2] (µg/g)	Total (µg/g)
CC	23.8[a]	4.4[a]	28.2 [a]
WE	23.1[a]	4.0[a]	27.1 [a]
CS	20.2[a]	3.7[a]	23.9 [a]
HW	10.6[b]	0.5[b]	11.1 [b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 5.0, 1.2 and 6.1 for luteolin, apigenin and total, respectivelly.α = .05

values with different letter are statisticaly different.

Table 44

Average Flavones of 8 Black Sorghums Grown at Different Locations

Line or hybrid	µg/g[1,2]		
	Luteolin	Apigenin	Total
Tx430 Black	40.7	7.5	48.2
B05030 Black	24.0	5.8	26.9
A05028/Black Tx430	20.5	7.0	25.7
Shawaya Black	15.9	3.1	18.2
A05030/Black Tx430	14.1	3.6	16.8
A05028/Shawaya	13.5	2.4	15.3
A05030/Shayawa	14.2	1.4	15.2
B05028 Black	12.5	2.2	14.1

[1] Average of 4 locations.

[2] Minimum Significant Difference = 8.4 and 2.1 µg/g for luteolin and apigenin. α = .05

values with different letter are statisticaly different for each column.

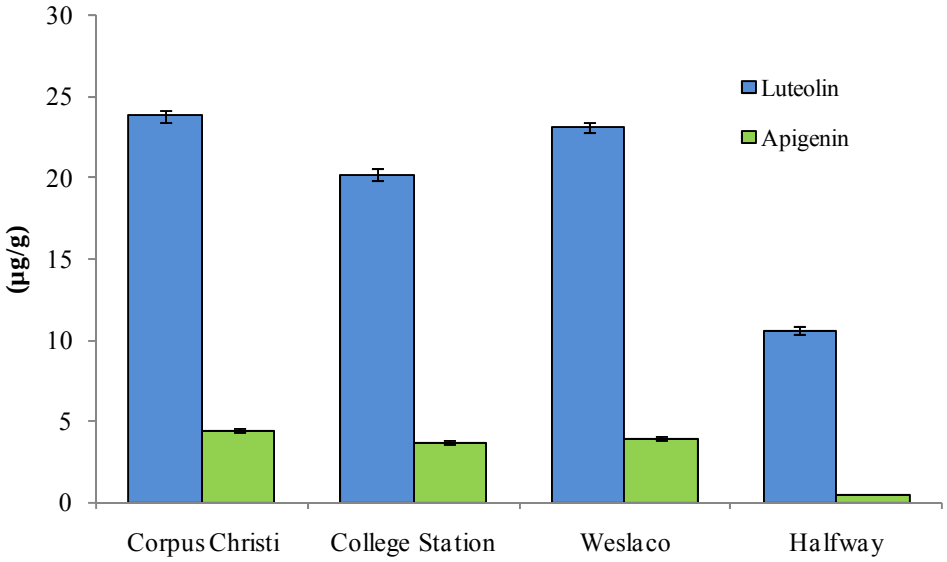

Fig. 12. Effect of environment on average flavones concentration in 8 black sorghums grown in different locations.

Flavones in Red Sorghum

Two flavones luteolin and apigenin were identified and quantified in red sorghums in all locations (Table 45). Levels of luteolin were detected on four samples from Halfway only and in the genotype 99LGWO50 from all locations. In Corpus Christi, College Station and Weslaco, only the genotype 99LGWO50 had significant levels of luteolin but in Halfway, four genotypes had significant levels of luteolin (between 2.0 and 147.2 μg/g) which indicates an environment effect (Table 46). Genotype effect was observed for flavones (Table 47). Interaction of variety with environment also was observed (Table B36 and B.36.1). Sorghums from Halfway had the higher amount of apigenin (52.8 μg/g), while the other three locations had values between 18.8 and 40.4 μg/g (Fig. 13) (Table 46). An interaction of variety with environment was observed (Table B.37 and B.37.1). There is no evidence that stress by molds affects synthesis of flavones. These results show that the reaction of the synthesis of flavones were similar to that of 3-deoxyanthocyanins in red sorghums.

Table 45

Flavones in Red Sorghums Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]		
		Luteolin	Apigenin	Total
CC	SC719-11E	ND[c]	2.5	2.5
CS	SC719-11E	ND	2.7	2.7
WE	SC719-11E	ND	ND	2.1
HW	SC719-11E	ND	ND	0.0
CC	Tx2911	ND	7.1	7.1
CS	Tx2911	ND	7.3	7.3
WE	Tx2911	ND	4.4	4.4
HW	Tx2911	10.8	11.6	22.4
CC	99LGWO50	84.7	187.3	272.1
CS	99LGWO50	92.2	119.4	211.6
WE	99LGWO50	60.6	84.2	144.9
HW	99LGWO50	147.2	246.8	394.0
CC	98CA4779	ND	2.9	2.9
CS	98CA4779	ND	ND	0.0
WE	98CA4779	ND	3.3	3.3
HW	98CA4779	13.4	3.2	16.6
CC	B9904	ND	2.1	2.1
CS	B9904	ND	1.4	1.4
WE	B9904	ND	ND	0.0
HW	B9904	2.0	2.5	4.4

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 1.2 and 3.1 µg/g for luteolin and apigenin respectively, among genotypes. $\alpha = .05$

values with different letter are statisticaly different for each column.

[c] ND = non detectable.

Table 46

Effect of Location on Average Flavones of 5 Red Sorghum Grains

Location[1]	Luteolin[2] (µg /g)	Apigenin[2] (µg/g)	Total (µg/g)
HW	34.7[a]	52.8[a]	87.5 [a]
CC	16.9[b]	40.4[ab]	57.3 [ab]
CS	18.4[b]	26.2[b]	44.6 [b]
WE	12.1[b]	18.8[b]	30.9 [b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 12.3, 25.8 and 37.0 for luteolin, apigenin and total, respectivelly.α = .05

values with different letter are statisticaly different.

Table 47

Average Flavones of 5 Red Sorghums Grown at Different Locations

Line or hybrid	µg/g[1,2]		
	Luteolin	Apigenin	Total
99LGWO50	96.2	159.5	255.6
Tx2911	ND[c]	7.6	7.6
98CA4779	ND[c]	3.2	3.2
SC719-11E	ND[c]	2.6	2.6
B9904	ND[c]	2.0	2.0

[1] Average of 4 locations.

[2] Minimum Significant Difference = 14.4 and 30.7 µg/g for luteolin and apigenin. α = .05

values with different letter are statisticaly different for each column.

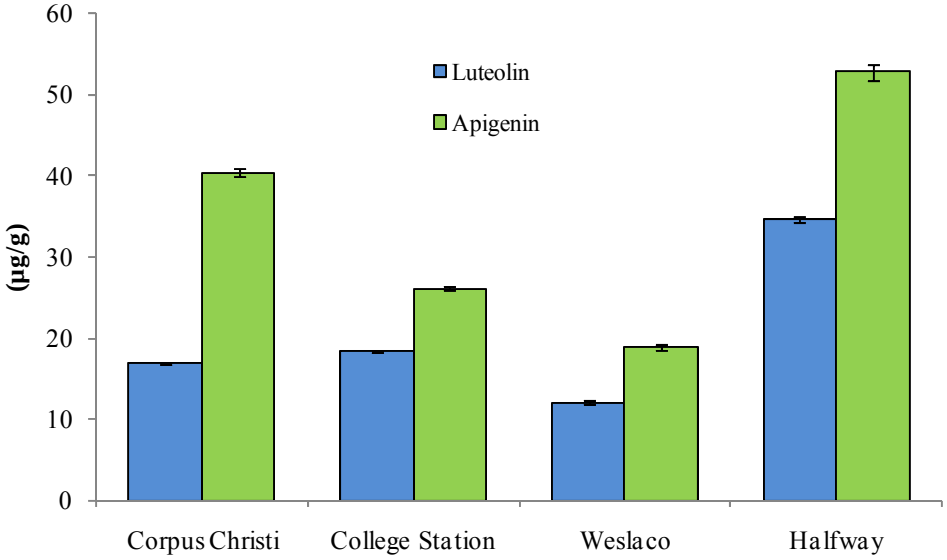

Fig. 13. Effect of environment on average flavone concentration in 5 red sorghums grown in different locations.

Flavones of Yellow Sorghum

Two flavones luteolin and apigenin were identified and quantified in yellow sorghums in all locations (Table 48). Sorghums from Halfway and Corpus Christi had the lowest levels of luteolin (21.9 and 22.9 μg/g, respectively), while Weslaco and College Station had 31.2 and 35.3 μg/g, respectively (Fig. 14) (MSD = .4 α = .05) (Table 49). An interaction of variety with environment was observed (Table B.38 and B.38.1). Genotype effect was observed in flavones (Table 50). The effect of weathering was not the most important factor in the variability of flavones, since sorghums from Corpus Christi which were not affected by weathering had similar levels of flavones as Halfway. Sorghums from College Station had the lower amount of apigenin (4.2 μg/g), while the other three locations had values between 5.0 and 5.7 μg/g (Fig. 14) (MSD = 8.4 α = .05) (Table 49). Like with luteolin, weathering of the grain was not the major environmental effect in apigenin concentration. An interaction of variety with environment was observed (Table B.39 and B.39.1).

Table 48

Flavones in Yellow Sorghums Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]		
		Luteolin	Apigenin	Total
CC	RO7007	21.9	3.7	25.7
CS	RO7007	21.4	2.5	23.9
WE	RO7007	27.9	2.9	30.9
HW	RO7007	15.5	3.9	19.4
CC	SC748-5	23.9	7.7	31.6
CS	SC748-5	49.3	6.0	55.3
WE	SC748-5	34.5	7.2	41.7
HW	SC748-5	28.3	7.2	35.5

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 2.9 and 0.5 µg/g for luteolin and apigenin respectively, among genotypes. $\alpha = .05$

values with different letter are statisticaly different for each column.

Table 49

Effect of Location on Average Flavones of 2 Yellow Sorghums Grains

Location[1]	Luteolin[2] (µg /g)	Apigenin[2] (µg/g)	Total (µg/g)
CS	35.3[a]	4.2[c]	39.5 [a]
WE	31.2[ab]	5.0[b]	36.2 [ab]
CC	22.9[bc]	5.7[a]	28.6 [b]
HW	21.9[c]	5.6[a]	27.5 [b]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 9.1, 0.4 and 8.8 for luteolin, apigenin and total, respectivelly.$\alpha = .05$

values with different letter are statisticaly different.

Table 50

Average Flavones of 2 Yellow Sorghums Grown at Different Locations

Line or hybrid	µg/g[1,2]		
	Luteolin	Apigenin	Total
SC748-5	34.0 [a]	7.0 [a]	41.0 [a]
RO7007	29.2 [b]	5.1 [b]	34.3 [b]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 4.8 and 0.2 µg/g for luteolin and apigenin. α = .05

values with different letter are statisticaly different for each column.

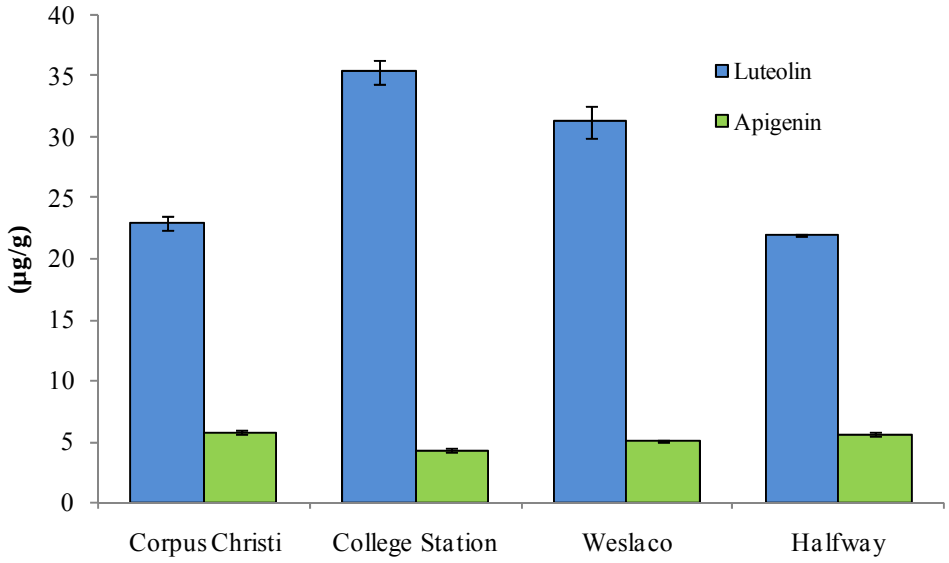

Fig. 14. Effect of environment on average flavone concentration in 2 yellow sorghums grown in different locations.

Effect of Environment on Flavanones of Sorghum

Flavanones in Black Sorghum

Eriodictyol and naringenin were the two flavanones identified and quantified in all black sorghum grains (Table 51). Grains from Halfway had the lowest levels of eriodictyol (52.0 μg/g) while the other three locations had values between 86.7 to 114.1 μg/g (Fig. 15) (Table 52) (MSD = 8.6, $\alpha = .05$). The environmental effect was greater than the genotype effect (Table B.40), and a low interaction of genotype by environment was observed (Table B.40.1). Levels of naringenin also were lower at Halfway (25.9 μg/g) while in the other three locations the values were between 41.1 to 52.0 μg/g (Fig. 15) (Table 52) (MSD = 4.7, $\alpha = .05$). The environmental effect for naringenin also was higher than the genotype effect (Table B.41). An interaction of genotype by environment was observed but was low compared to the location and genotype effect (Table B.41.1).

Table 51

Flavanones in Black Sorghums Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]		
		Eriodictyol	Naringenin	Total
CC	Tx430 Black	67.0	40.4	107.4
CS	Tx430 Black	66.1	37.0	103.1
WE	Tx430 Black	64.2	44.7	108.9
HW	Tx430 Black	33.0	31.9	65.0
CC	Shawaya Black	92.2	55.0	147.2
CS	Shawaya Black	55.0	36.7	91.7
WE	Shawaya Black	84.4	46.9	131.2
HW	Shawaya Black	45.0	25.3	70.4
CC	B05028 Black	143.7	32.8	176.5
CS	B05028 Black	106.8	30.0	136.8
WE	B05028 Black	128.1	31.2	159.3
HW	B05028 Black	50.5	16.0	66.5
CC	B05030 Black	124.6	52.1	176.7
CS	B05030 Black	120.4	41.8	162.3
WE	B05030 Black	127.2	43.4	170.6
HW	B05030 Black	75.2	31.5	106.7
CC	A05028/Black Tx430	147.4	63.0	210.5
CS	A05028/Black Tx430	98.2	47.8	146.0
WE	A05028/Black Tx430	143.5	61.0	204.5
HW	A05028/Black Tx430	56.1	22.1	78.2
CC	A05030/Black Tx430	135.19	75.50	210.7
CS	A05030/Black Tx430	106.5	60.6	167.2
WE	A05030/Black Tx430	129.1	70.0	199.0
HW	A05030/Black Tx430	52.0	26.4	78.4
CC	A05028/Shawaya	101.4	51.2	152.6
CS	A05028/Shawaya	71.4	43.9	115.3
WE	A05028/Shawaya	88.3	42.3	130.6
HW	A05028/Shawaya	48.9	22.3	71.1
CC	A05030/Shayawa	101.2	45.8	147.0
CS	A05030/Shayawa	69.1	30.9	100.0
WE	A05030/Shayawa	91.9	37.9	129.8
HW	A05030/Shayawa	55.2	31.6	86.8

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 14.0 and 7.2 µg/g for eriodictyol and naringenin respectively, among genotypes. $\alpha = .05$

values with different letter are statisticaly different for each column.

Table 52

Effect of Location on Average Flavanones of 8 Black Sorghum Grains

Location[1]	Eriodictyol[2] (µg /g)	Naringenin[2] (µg/g)	Total[2] (µg/g)
CC	114.1[a]	52.0[a]	166.1 [a]
WE	107.1[a]	47.2[b]	154.2 [a]
CS	86.7[b]	41.1[c]	127.8 [b]
HW	52.0[c]	25.9[d]	77.9 [c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 8.6, 4.8 and 12.0 for eriodictyol, naringenin and total, respectivelly.α = .05 values with different letter are statisticaly different. α = .05.

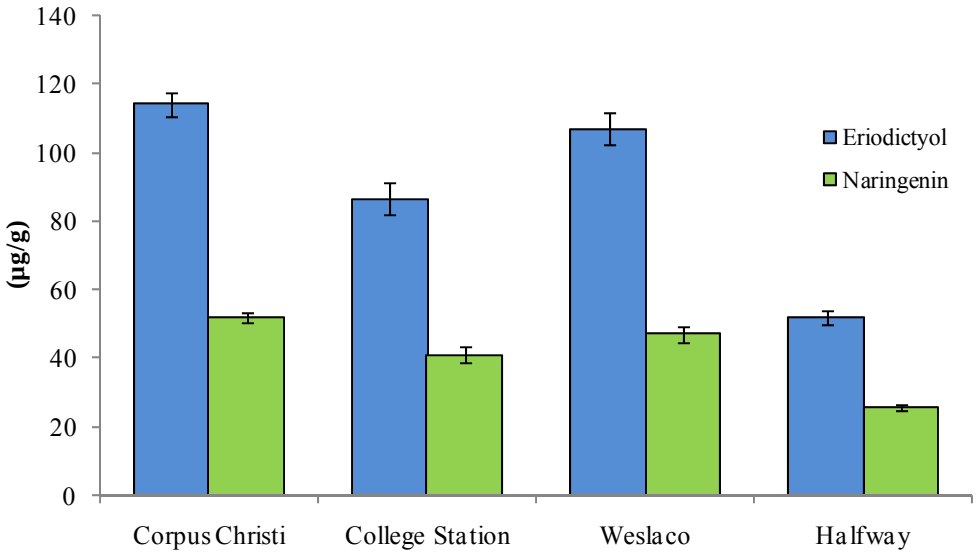

Fig. 15. Effect of environment on average flavanone concentration in 8 genotypes of black sorghum grains grown in different locations.

Table 53

Average Flavanones of 8 Black Sorghums Grown at Different Locations

Line or hybrid	µg/g[1,2]		
	Eriodictyol	Naringenin	Total
A05030/Black Tx430	105.7 [a]	58.1 [a]	163.8 [a]
A05028/Black Tx430	111.3 [a]	48.5 [b]	159.8 [a]
B05030 Black	111.8 [a]	42.2 [bc]	154.1 [ab]
B05028 Black	107.3 [a]	27.5 [d]	134.8 [bc]
A05028/Shawaya	77.5 [b]	39.9 [c]	117.4 [cd]
A05030/Shayawa	79.3 [b]	36.6 [c]	115.9 [cde]
Shawaya Black	69.2 [bc]	41.0 [bc]	110.1 [de]
Tx430 Black	57.6 [c]	38.5 [c]	96.1 [e]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 14.4, 8.0 and 20.0 µg/g for eriodictyol, naringenin and total. α = .05 values with different letter are statisticaly different for each column.

Flavanones in Red Sorghum

Eriodictyol and naringenin were the two flavanones identified and quantified in red sorghum grains (Table 54). Eriodictyol was found only in 98CA4779 and B9904 genotypes where Corpus Christi was the location with the highest levels (Table 55) (Fig.16); interaction of variety with environment was observed (Table B.42 and B.42.1). Levels of naringenin were lower in Weslaco (135.5 µg/g) compared to the other three locations which had values between 168.7 and 224.3 µg/g (Fig. 16) (Table 55); interaction of variety with environment was observed (Table B.43 and B.43.1), for example, genotype B9904 from Halfway had lower levels of naringenin than at Weslaco.

Table 54

Flavanones in Red Sorghums Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]		
		Eriodictyol	Naringenin	Total
CC	SC719-11E	ND[c]	231.5	231.5
CS	SC719-11E	ND	281.3	281.3
WE	SC719-11E	ND	201.1	201.1
HW	SC719-11E	ND	215.4	215.4
CC	Tx2911	ND	189.5	189.5
CS	Tx2911	ND	302.9	302.9
WE	Tx2911	ND	178.5	178.5
HW	Tx2911	ND	231.7	231.7
CC	99LGWO50	ND	257.3	257.3
CS	99LGWO50	ND	382.1	382.1
WE	99LGWO50	ND	165.4	165.4
HW	99LGWO50	ND	421.4	421.4
CC	98CA4779	23.9	117.2	141.2
CS	98CA4779	ND	110.9	110.9
WE	98CA4779	7.0	97.2	104.3
HW	98CA4779	1.3	134.0	135.3
CC	B9904	71.1	47.9	118.9
CS	B9904	58.3	44.4	102.7
WE	B9904	60.6	35.2	95.8
HW	B9904	43.5	24.0	67.5

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 4.8 and 26.3 µg/g for eriodictyol and naringenin respectively, among genotypes. $\alpha = .05$

values with different letter are statisticaly different for each column.

[c] ND = non detectable.

Table 55

Effect of Location on Average Flavanones of 5 Red Sorghum Grains

Location[1]	Eriodictyol[2] (µg /g)	Naringenin[2] (µg/g)	Total[2] (µg/g)
CS	11.7[b]	224.3[a]	236[a]
HW	9.1[b]	205.3[ab]	214.5[ab]
CC	19.0[a]	168.7[bc]	187.7[bc]
WE	13.5[b]	135.5[c]	149[c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 5.3, 42.3 and 44.7 for eriodictyol, naringenin and total, respectivelly.α = .05 values with different letter are statisticaly different. α = .05.

Table 56

Average Flavanones of 5 Red Sorghums Grown at Different Locations

Line or hybrid	µg/g[1,2]		
	Eriodictyol	Naringenin	Total
99LGWO50	ND[3]	306.6 [a]	306.6 [a]
SC719-11E	ND[3]	232.3 [b]	232.3 [b]
Tx2911	ND[3]	225.7 [b]	225.7 [b]
98CA4779	10.7 [b]	114.9 [c]	125.6 [c]
B9904	58.4 [a]	37.9 [d]	96.2 [c]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 6.3, 50.4 and 53.2 µg/g for eriodictyol, naringenin and total. α = .05 values with different letter are statisticaly different for each column.

[3] ND = non detectable.

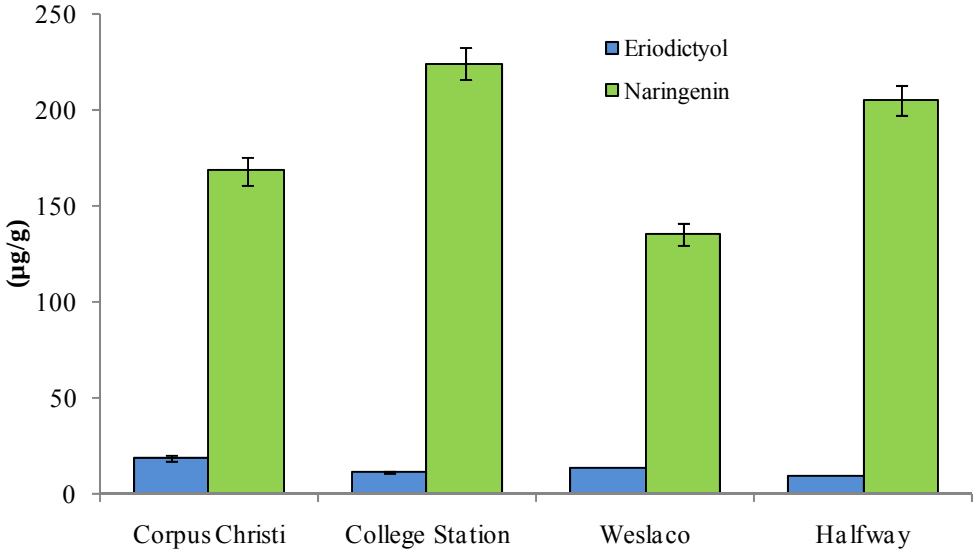

Fig. 16. Effect of environment on flavanone concentration in 5 red sorghums grown in different locations.

Flavanones in Yellow Sorghum

Eriodictyol and naringenin were the flavanones identified and quantified in yellow sorghum grains (Table 57). Grains from Halfway had the lowest levels of eriodictyol (283.2 µg/g) while the other three locations had values between 780.6 and 1177.3 µg/g (Fig. 17) (MSD = 253.2 α = .05) (Table 58). An interaction of variety with environment was observed (Table B.44 and B.44.1). Levels of naringenin also were lower in Halfway (242.5 µg/g) while the other three locations had values between 302.2 and 429.4 µg/g (Fig. 17) (MSD = 95.3 α = .05) (Table 58). An interaction of variety with environment was observed (Table B.45 and B.45.1).

Table 57

Flavanones in Yellow Sorghums Grown at Different Locations

Location[1]	Line or hybrid	µg/g[2]		
		Eriodictyol	Naringenin	Total
CC	RO7007	1483.8	339.3	1823.1
CS	RO7007	1154.8	243.1	1397.9
WE	RO7007	1084.3	241.0	1325.3
HW	RO7007	264.8	43.9	308.8
CC	SC748-5	870.8	519.6	1390.3
CS	SC748-5	406.3	376.4	782.7
WE	SC748-5	648.2	363.4	1011.5
HW	SC748-5	301.5	441.1	742.6

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 43.5 and 24.8 µg/g for eriodictyol and naringenin respectively, among genotypes. α = .05

values with different letter are statisticaly different for each column.

Table 58

Effect of Location on Average Flavanones of 2 Yellow Sorghum Grains

Location[1]	Eriodictyol[2] (µg /g)	Naringenin[2] (µg/g)	Total (µg/g)
CC	1177.3[a]	429.4[a]	1606.7 [a]
WE	866.2[b]	302.2[b]	1168.4 [b]
CS	780.6[b]	309.7[b]	1090.3 [b]
HW	283.2[c]	242.5[b]	525.7 [c]

[1] CC = Corpus Christi, CS = College Station, WE = Weslaco, HW = Halfway.

[2] Minimum Significant Difference = 271.7, 102.3 and 365.8 for eriodictyol, naringenin and total, respectivelly.α = .05

values with different letter are statisticaly different. α = .05.

Table 59

Average Flavanones of 2 Yellow Sorghums Grown at Different Locations

Line or hybrid	µg/g[1,2]		
	Eriodictyol	Naringenin	Total
RO7007	996.9 [a]	216.8 [b]	1213.8 [a]
SC748-5	556.7 [b]	425.1 [a]	981.8 [b]

[1] Average of 4 locations.

[2] Minimum Significant Difference = 143.0, 53.8 and 192.5 µg/g for eriodictyol, naringenin and total. α = .05

values with different letter are statisticaly different for each column.

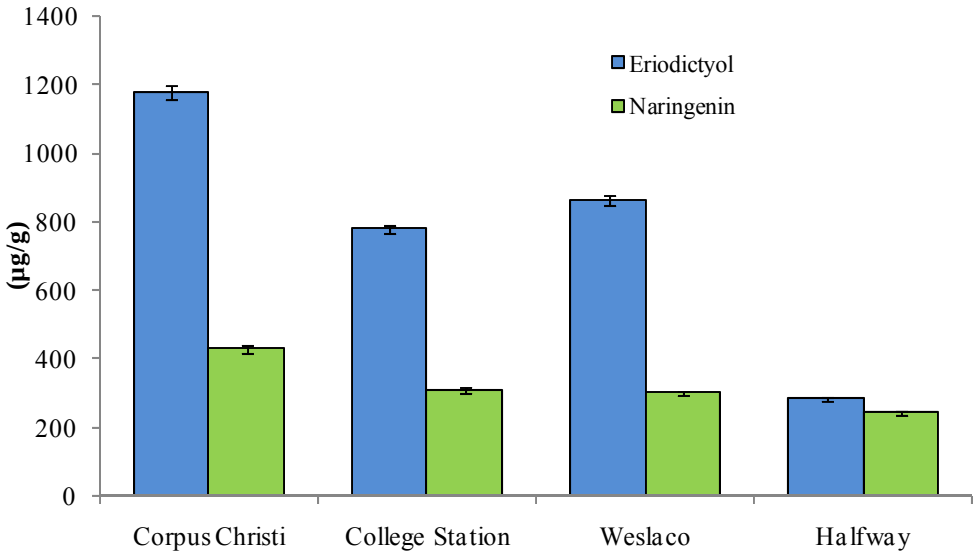

Fig. 17. Effect of environment on average flavanone concentration in 2 yellow sorghums grown in different locations.

DISCUSSION

Hardness of Sorghum Grains

Differences on hardness of the grains were found in all sorghum types. In general, black sorghums were softer and white sorghums were harder (Table 60). Weathering affected the hardness index values negatively, with r values of -0.44 for black sorghums, 0.98 for red sorghums and 0.96 for yellow sorghums, indicating that softer grains were observed in grains with higher weathering damage. The effect of weathering on hardness index value was higher in yellow sorghums were the range for hardness was between 45.2 and 101.0 with most of this variation caused by environment. Black sorghums high in flavonoids can be milled using less energy than red, yellow and white sorghums. Sorghums damaged by weathering can be easily milled.

Table 60

Average Hardness of Sorghum Types

Sorghum Type	Hardness Index	TADD Value	Weathering Score
Black	65.7 ± 8.2	57.9 ± 9.3	4.3 ± 1.8
Red	79.0 ± 10.4	73.0 ± 11.1	3.1± 1.0
Yellow	78.5 ± 18.9	79.5 ± 9.0	4.0 ± 1.8
White	85.1 ± 4.8	78.3 ± 5.2	2.5 ± 1.0

Total Phenols and Antioxidant Activity of Sorghum Grains

Total phenols and antioxidant activity of black sorghum with tannins was higher than black sorghums without tannins (Table 61) which support the data of Dykes (2008) that shown that black sorghum with tannins had higher total phenols and antioxidant activity values. Red and yellow sorghums had lower values of total phenols and antioxidant activity compared to black sorghum. Total phenols correlated poorly with total flavonoids measured (r = 0.3) while the same correlation in red and yellow sorghums was 0.71 and 0.77, respectively, such values suggest that flavonoids measured in black sorghums are just a small part of the total phenols of the grains, while in red and yellow sorghums, the flavonoids quantified represents a considerably amount of the total phenols present in these grains. From the antioxidant prospective, black sorghums shows to have a great potential as a nutraceutical food.

Table 61

Total Phenols and Antioxidant Activity of Sorghum Types

Sorghum Type	Total Phenols (mg GAE/g)	Antioxidant Activity (µmol TE/g)
Black	16.9 ± 2.0	222.9 ± 24.0
Black non tannin	5.7 ± 1.0	69.3 ± 9.9
Red	3.2 ± 1.0	54.7 ± 22.7
Yellow	2.0 ± 0.3	57.2 ± 8.8

Color of Sorghum Grains

*L** values of black sorghums affected by weathering were lower because 3-deoxyanthocyanins were distributed homogeneously which increased the general black appearance of the grains (Figure 3). Black sorghums affected by molds also had low *a** and *b** values because yellow/red areas were not present in the grains. Pigments of metabolites of molds also contributed to the reduction of the values *L**, *a** and *b**. Correlation between *L** values and total anthocyanidins and flavonoid content was not observed but a low negative correlation (r = -0.55) was observed between total anthocyanidins and *a** value.

*L** values of red sorghums were not affected on weathered grains. Lower *a** values occurred in weathered grains because more 3-deoxyanthocyanins were present producing darker colors and masking the bright red tones. *b** values of red sorghums affected by molds also were low, because higher levels of 3-deoxyanthocyanins and pigments of molds reduced the expression of yellow pigments.

Environment had a significant effect on *L** values of yellow sorghums. The increase of 3-deoxyanthocyanins in yellow sorghums affected by weathering caused in part the reduction in *L** values. The color of the molds grown in the grains during maturation also contribute to the low *L** values.

For yellow sorghums, the effect of molds on *a** values was not significant because the distribution of red pigments was not homogeneous and the differences in concentration of anthocyanins and flavones were not significant to modify the intensity of the red spots that occurred in yellow grains. The *b** values of yellow sorghums

affected by weathering were low, because higher levels of 3-deoxyanthocyanins and pigments of molds reduce the expression of yellow pigments. The positive correlation (r = 0.81) was observed between total flavanones and *L** value. Low negative correlation (r^2 = -0.49) was observed between total flavanones and *a** value. High correlation was observed between flavanones and b* value (r = 0.93).

Flavonoids of Sorghum Grains

Variability of flavonoids among locations was 33 % for the average value for 3-deoxyanthocyanins, 51 % for flavones and 40 % for flavanones in black sorghums. Variability among grains not affected by weathering was 24, 9 and 14 % for total 3-deoxyanthocyanins, flavones and flavanones, respectively. In grains affected by weathering, the reduction of total 3-deoxyanthocyanins compared to the other three locations was 27.1%, while for flavones and flavanones were 58.1 and 47.8 %, respectively.

Even though non-methoxyanthocyanidins were reduced under weathering stress (46.3% reduction), total 3-deoxyanthocyanins did not decrease extensively, in part because the levels of methoxylated 3-deoxyanthocyanins were not affected by weathering. Stability of methoxylated 3-deoxyanthocyanins indicates that these compounds can be obtained from black sorghums even if the total 3-deoxyanthocyanins levels are reduced because of stress by weathering. These findings are important because studies in cell cultures demonstrated that methoxylated 3-deoxyanthocyanins have greater anticancer activity than non-methoxylated anthocyanins (Yang et al 2009).

Flavones and flavanones levels were reduced in sorghums affected by weathering, but the levels of these compounds were not too high in black sorghums.

Correlation between flavonoids, total phenolic compounds and antioxidant activity was low in black sorghums because the flavonoids measured correspond just to a small portion of the phenolic compounds present in black sorghums (ratio mg GAE/g to mg flavonoids/g was 31:1).

In red sorghums the variability in flavonoids levels was 77, 44 and 24 % for 3-deoxyanthocyanins, flavones and flavanones, respectively. While the variability among sorghums not affected by weathering was 20, 30 and 24 % for total 3-deoxyanthocyanins, flavones and flavanones, respectively.

Among 3-deoxyanthocyanins, methoxylated 3-deoxyanthocyanins increased considerable in grains of red (210%) sorghums affected by weathering compared to the non-methoxylated (123%). Thus, methoxylated 3-deoxyanthocyanins appear to be formed more when damage by weathering occurred in red sorghums.

Luteolin and apigenin levels also increased in sorghums affected by weathering though only the 99LGWO50 genotype had considerable amounts of both compounds (96.2 and 159.5 µg/g, respectively).

Naringenin levels of the red sorghum 99LGWO50 were not affected by weathering. This is important because the levels of naringenin in this sorghum are similar to other sources with high levels of naringenin (USDA, 2007).

Correlation between flavonoids, total phenolic compounds and antioxidant activity also was low in red sorghum; because the flavonoids measured were just a small

portion of the phenolic compounds present in black sorghums (ratio mg GAE/g to mg flavonoids/g was 8:1).

Variability in yellow sorghums was 71, 19 and 52 % for anthocyanins, flavones and flavanones, respectively. While the variability among sorghums not affected by weathering was 24, 18 and 25 % for total 3-deoxyanthocyanins, flavones and flavanones, respectively.

Among 3-deoxyanthocyanins, methoxylated 3-deoxyanthocyanins increased considerably in yellow sorghums affected by weathering (280%) compared to the non-methoxyanthocyanidins (107%). The increase of total 3-deoxyanthocyanins may be due to relatively high damage by molds. Also, Njongmeta (2009) found higher levels of 3-deoxyanthocyanins in glumes of sorghum, leading to the conclusion that with the rain, some of these compounds migrated from the glumes to the grains, but analysis of migration of methoxy and non-methoxyanthocyanidins in glumes is needed to confirm the contribution of these compounds since the ratio of methoxylated to non-methoxylated 3-deoxyanthocyanins in glumes is high, contrary to the values found in grains of red and yellow sorghums.

Flavone levels in yellow sorghum were not affected by weathering.

Yellow grains affected by weathering had a high reduction in flavanone levels, the reason could be that low levels of 3-deoxyanthocyanins were present making the grains more susceptible to weathering.

Comparison of Flavonoid Content

Black sorghum had the highest amount of 3-deoxyanthocyanins while yellow sorghums had the highest amount of flavanones in all environmnets. For all sorghum types the range of each flavonoid group was higher than previous results, (Table 62). Despite the environment had considerable changes in the concentration of each group of flavonoid, black and yellow sorghums consistently showed high amount of 3-deoxyanthocyanins and flavanones, respectively. This must be taken into consideration when specialty sorghums are used as a source of colorants and natural antioxidants.

Table 62
Comparison of Flavonoid Content of Sorghums Types in Different Years

Sorghum Type	Year	µg/g					
		3-Deoxyanthocyanins		Flavones		Flavanones	
		Range	Average	Range	Average	Range	Average
Black (8 hybrids)	2008	151.6 - 1047.5	373.5	5.4 - 65.7	22.6	65.0 - 210.7	131.5
	Previous[a]	172.9 - 804.4		6.1 - 43.7		58.8 - 155.9	
Red (5 hybrids)	2008	7.2 - 250.4	56.6	0 - 394.5	55.1	67.5 - 421.4	196.7
	Previous[a]	0 - 139.1		0 - 385.9		0 - 63.2	
Yellow (2 hybrids)	2008	10.3 - 46.4	24.5	19.4 - 55.3	33.0	308.8 - 1823.1	1097.8
	Previous[a]	13.7 - 74.7		19.4 - 55.3		1488.9 - 1779.6	

[a] Dykes, 2008.

CONCLUSIONS

Among sorghum genotypes, black sorghums had the highest amount of total 3-deoxyanthocyanins (373.5 μg/g), the red sorghum 99LGWO50 had the highest amount of flavones (255.6 μg/g) and yellow sorghums had the highest amount of flavanones (1097.8 μg/g), such values are similar with those reported by Dykes (2008).

Red and yellow sorghums increased the synthesis of 3-deoxyanthocyanins under weathering stress, probably because indigenous levels of 3-deoxyanthocyanins were not enough to reduce attack by molds, leading to the production of more 3-deoxyanthocyanins. To confirm this, we need to evaluate the effect of mold stress on the activity of the flavanone 3-hydroxylase, anthocyanidin synthase and dihydroflavonol 4-reductase enzymes in genotypes with low and high levels of 3-deoxyanthocyanins which will indicate the gene expression changes that produce this compound during stress conditions as found in potatoes (Andre et al 2009). Levels of 3-deoxyanthocyanins were lower in grains damaged by weathering. Since the levels of 3-deoxyanthocyanins in red and yellow sorghums are lower compared with black sorghums, the higher values of 3-deoxyanthocyanins observed in these sorghums under weathering conditions are not enough to conclude that red or yellow sorghum can produce more 3-deoxyanthocyanins that black sorghums even under weathering conditions.

Considerable reduction of flavanones was observed in weathered grains of yellow sorghums compared to black and red sorghums. We need to determine if the

reduction of flavanones in yellow sorghum under stress conditions can be prevented by high levels of 3-deoxyanthocyanins as occurs in red sorghums.

Only yellow sorghums had a high correlation between total flavonoids and total phenols and antioxidant activity value (r = 0.91 and 0.92, respectively) possibly because they do not contain high levels of other flavonoids (ratio mg GAE/g to mg flavonoids/g was 1.8:1).

This study showed the magnitude of the variation in the profile of sorghum flavonoids for each type of sorghum, in which clear effects of stress by weathering were observed, in a wide range of environments.

The magnitude of the changes of L^*, a^* and b^* values within black, red and yellow sorghums were different among genotypes, indicating a genotype by environment interaction.

Crosses between black and yellow sorghums might produce grains with high levels of both 3-deoxyanthocyanins and flavanones; these crosses can be evaluated to determine if the levels of 3-deoxyanthocyanins protect the flavanones when the grains are affected by weathering.

The variation in flavonoid profiles of sorghums probably affect color and flavor of foods prepared with specialty sorghums. This must be evaluated.

This study can be utilized to give an estimated variation of the results that can be obtained in studies related to health benefits of black, red and yellow sorghums.

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APPENDIX A

Figure A.1. Weathering Scores of Black Sorghums.

Figure A.1. Weathering Scores of Black Sorghums. - Continued

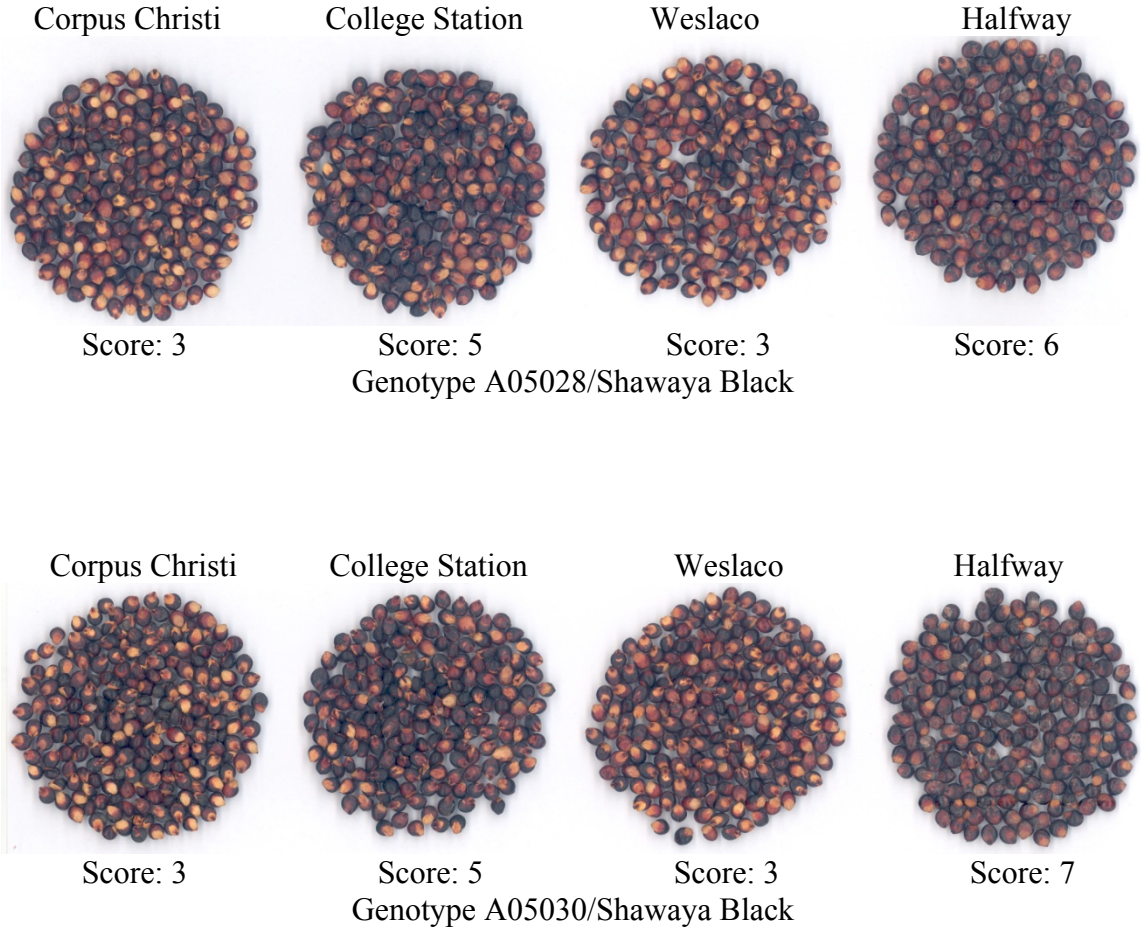

Figure A.1. Weathering Scores of Black Sorghums. - Continued

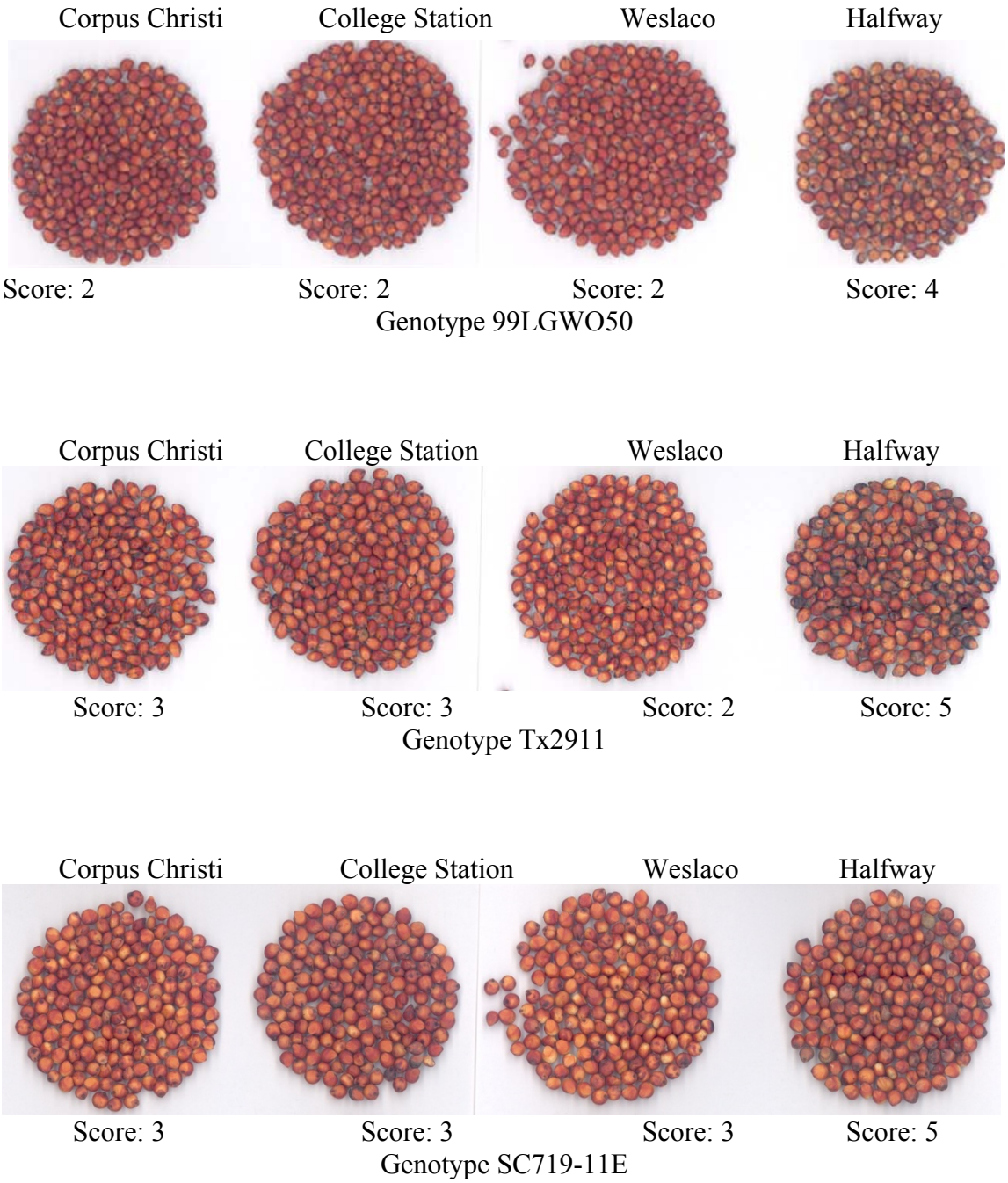

Figure A.2. Weathering Scores of Red Sorghums.

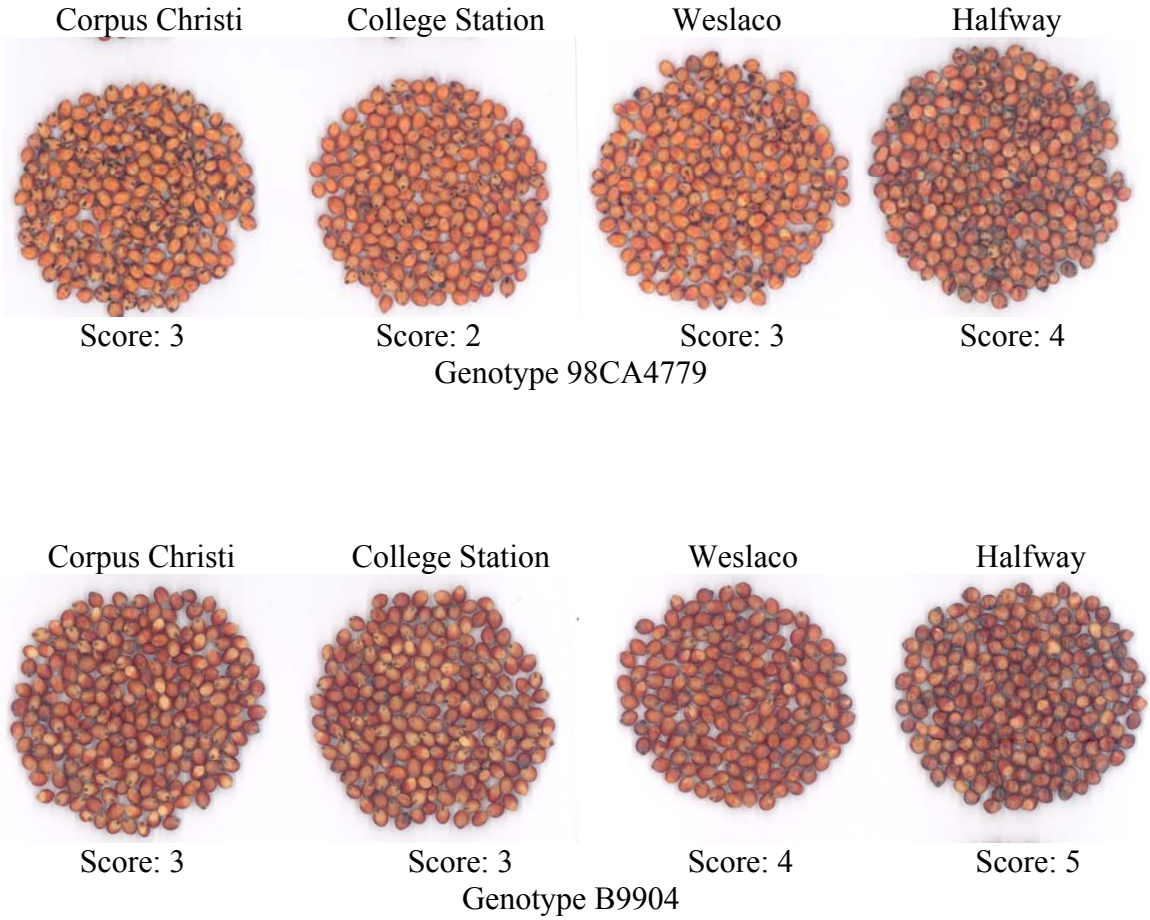

Figure A.2. Weathering Scores of Red Sorghums. - Continued

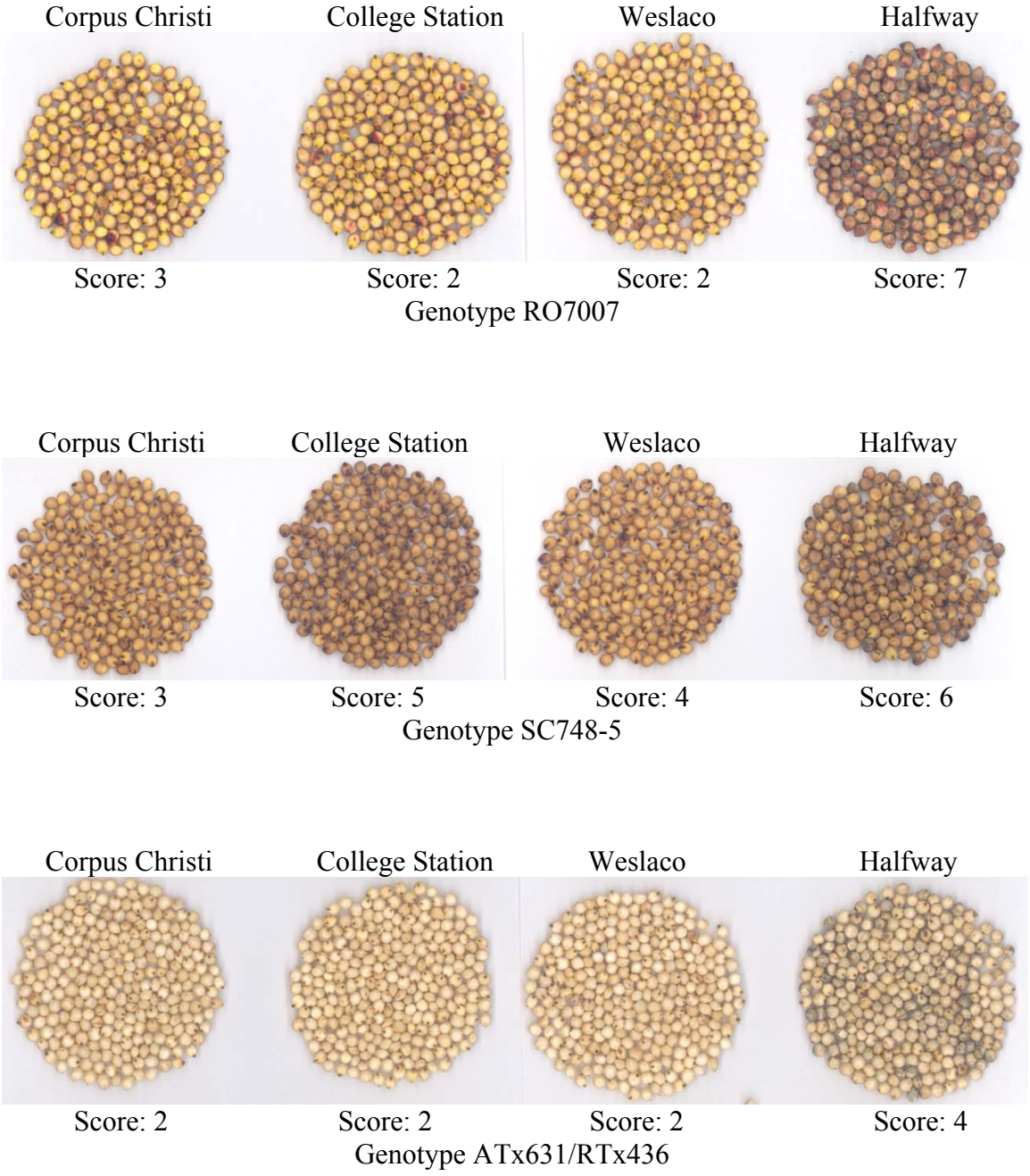

Figure A.3. Weathering Scores of Yellow Sorghums.

APPENDIX B

Table B.1

ANOVA of the Hardness Index (SKHT) of Grains of 8 Black Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	351.71	3	117.24	4.21	0.01
Sample	2376.61	7	339.52	12.19	0.00
Error	1476.76	53	27.86		
Corrected Total	4205.08	63			

[a] R Squared = .65 (Adjusted R Squared = .58)

Table B.1.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Hardness Index (SKHT) in Black Sorghum Grains[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	2376.61	7	339.52	249.47	0.00
Location	351.71	3	117.24	86.14	0.00
Sample * Location	1433.21	21	68.25	50.15	0.00
Error	43.55	32	1.36		
Corrected Total	4205.08	63			

[a] R Squared = .97 (Adjusted R Squared = .96)

Table B.2

ANOVA of the Hardness (TADD) of Grains of 8 Black Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	887.18	3	295.73	10.88	0.00
Sample	3076.78	7	439.54	16.18	0.00
Error	1440.10	53	27.17		
Corrected Total	5404.06	63			

[a] R Squared = .73 (Adjusted R Squared = .68)

Table B.2.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Hardness (TADD) in Black Sorghum Grains[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	3076.78	7	439.54	161.21	0.00
Location	887.18	3	295.73	108.46	0.00
Sample * Location	1352.85	21	64.42	23.63	0.00
Error	87.25	32	2.73		
Corrected Total	5404.06	63			

[a] R Squared = .971 (Adjusted R Squared = .956)

Table B.3

ANOVA of the Hardness Index (SKHT) of Grains of 5 Red Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	4762.31	4	1190.58	165.12	0.00
Location	224.13	3	74.71	10.36	0.00
Error	230.74	32	7.21		
Corrected Total	5217.18	39			

[a] R Squared = .96 (Adjusted R Squared = .95)

Table B.3.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Hardness (SKHT) in Black Sorghum Grains[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	3381.42	4	845.35	973.07	0.000
Location	859.48	3	286.49	329.78	0.000
Sample*Location	284.76	12	23.73	27.32	0.000
Error	17.38	20	0.87		
Corrected Total	4543.04	39			

[a] R Squared = .994 (Adjusted R Squared = .991)

Table B.4

ANOVA of the Hardness (TADD) of Grains of 5 Red Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	3381.42	4	845.35	89.53	0.00
Location	859.48	3	286.49	30.34	0.00
Error	302.14	32	9.44		
Corrected Total	4543.04	39			

[a] R Squared = .93 (Adjusted R Squared = .92)

Table B.4.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Hardness (TADD) in Red Sorghum Grains[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	4762.31	4	1190.58	1654.73	0.000
Location	224.13	3	74.71	103.84	0.000
Sample*Location	216.35	12	18.03	25.06	0.000
Error	14.39	20	0.72		
Corrected Total	5217.18	39			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.5

ANOVA of the Hardness Index (SKHT) of Grains of 2 Yellow Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	3110.85	1	3110.85	945.24	0.00
Location	1895.95	3	631.98	192.03	0.00
Error	36.20	11	3.29		
Corrected Total	5043.00	15			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.5.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Hardness (SKHT) in Yellow Sorghum Grains[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	3110.85	1	3110.85	1298.55	0.00
Location	1895.95	3	631.98	263.81	0.00
Sample * Location	17.04	3	5.68	2.37	0.15
Error	19.17	8	2.40		
Corrected Total	5043.00				

[a] R Squared = 1.00 (Adjusted R Squared = .99)

Table B.6

ANOVA of the Hardness (TADD) of Grains of 2 Yellow Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	641.36	1	641.36	105.00	0.00
Location	424.63	3	141.54	23.17	0.00
Error	67.19	11	6.11		
Corrected Total	1133.17	15			

[a] R Squared = .94 (Adjusted R Squared = .92)

Table B.6.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Hardness (TADD) in Yellow Sorghum Grains[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	641.36	1	641.36	788.75	0.00
Location	424.63	3	141.54	174.07	0.00
Sample * Locatio	60.69	3	20.23	24.88	0.00
Error	6.51	8	0.81		
Corrected Total	1133.17	15			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.7

ANOVA of the Color *L** in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	114.65	7	16.38	40.65	0.00
Location	139.47	3	46.49	115.38	0.00
Error	34.25	85	0.40		
Corrected Total	288.37	95			

[a] R Squared = .88 (Adjusted R Squared = .87)

Table B.7.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Color *L** in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	139.47	3	46.49	267.24	0.00
Variety	114.65	7	16.38	94.15	0.00
Location*variety	23.12	21	1.10	6.33	0.00
Error	11.13	64	0.17		
Corrected Total	288.37	95			

[a] R Squared = .96 (Adjusted R Squared = .94)

Table B.8

ANOVA of the Color *a** in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	139.60	7	19.94	33.99	0.00
Location	66.61	3	22.20	37.84	0.00
Error	49.87	85	0.59		
Corrected Total	256.08	95			

[a] R Squared = .81 (Adjusted R Squared = .78)

Table B.8.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Color *a** in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	66.61	3	22.20	262.43	0.00
Variety	139.60	7	19.94	235.71	0.00
Location*variety	44.46	21	2.12	25.02	0.00
Error	5.41	64	0.08		
Corrected Total	256.08	95			

[a] R Squared = .98 (Adjusted R Squared = .97)

Table B.9

ANOVA of the Color *b** in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	199.21	7	28.46	44.45	0.00
Location	154.54	3	51.51	80.46	0.00
Error	54.42	85	0.64		
Corrected Total	408.17	95			

[a] R Squared = .87 (Adjusted R Squared = .85)

Table B.9.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Color *b** in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	154.54	3	51.51	273.83	0.00
Variety	199.21	7	28.46	151.27	0.00
Location*variety	42.38	21	2.02	10.73	0.00
Error	12.04	64	0.19		
Corrected Total	408.17	95			

[a] R Squared = .97 (Adjusted R Squared = .96)

Table B.10

ANOVA of Color *L** in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	18.24	3	6.08	9.08	0.00
Sample	236.75	4	59.19	88.35	0.00
Error	34.84	52	0.67		
Corrected Total	289.83	59			

[a] R Squared = .88 (Adjusted R Squared = .86)

Table B.10.1
Genotype (5 Varieties) x Environment (4 Locations) Interaction of Color *L** in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	236.75	4	59.19	1293.42	0.00
Location	18.24	3	6.08	132.88	0.00
Variety*Location	33.01	12	2.75	60.11	0.00
Error	1.83	40	0.05		
Corrected Total	289.83	59			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.11

ANOVA of Color *a** in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	216.34	3	72.11	96.48	0.00
Sample	204.97	4	51.24	68.56	0.00
Error	38.87	52	0.75		
Total	18334.63	60			
Corrected Total	460.17	59			

[a] R Squared = .92 (Adjusted R Squared = .90)

Table B.11.1
Genotype (5 Varieties) x Environment (4 Locations) Interaction of Color *a** in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	204.97	4	51.24	921.61	0.00
Location	216.34	3	72.11	1296.99	0.00
Variety*Location	36.64	12	3.05	54.92	0.00
Error	2.22	40	0.06		
Corrected Total	460.17	59			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.12

ANOVA of Color *b** in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	62.20	3	20.73	18.67	0.00
Sample	339.10	4	84.77	76.34	0.00
Error	57.75	52	1.11		
Corrected Total	459.05	59			

[a] R Squared = .87 (Adjusted R Squared = .86)

Table B.12.1
Genotype (5 Varieties) x Environment (4 Locations) Interaction of Color *b** in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	339.10	4	84.77	1275.22	0.00
Location	62.20	3	20.73	311.90	0.00
Variety*Location	55.09	12	4.59	69.05	0.00
Error	2.66	40	0.07		
Corrected Total	459.05	59			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.13

ANOVA of Color *L** in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	187.82	1	187.82	42.98	0.00
Location	195.24	3	65.08	14.89	0.00
Error	83.03	19	4.37		
Corrected Total	466.09	23			

[a] R Squared = .82 (Adjusted R Squared = .78)

Table 13.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Color *L** in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	187.82	1	187.82	4767.11	0.00
Location	195.24	3	65.08	1651.75	0.00
Variety*Location	82.40	3	27.47	697.13	0.00
Error	0.63	16	0.04		
Corrected Total	466.09	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.14

ANOVA of Color *a** in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	0.51	1	0.51	3.36	0.08
Location	0.51	3	0.17	1.13	0.36
Error	2.89	19	0.15		
Corrected Total	3.91	23			

[a] R Squared = .262 (Adjusted R Squared = .106)

Table B.14.1
Genotype (2 Varieties) x Environment (4 Locations) Interaction of Color *a** in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	0.51	1	0.51	7.64	0.01
Location	0.51	3	0.17	2.56	0.09
Variety*Location	1.82	3	0.61	9.07	0.00
Error	1.07	16	0.07		
Corrected Total	3.91	23			

[a] R Squared = .727 (Adjusted R Squared = .607)

Table B.15

ANOVA of Color *b** in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	109.74	1	109.74	17.20	0.00
Location	268.00	3	89.33	14.01	0.00
Error	121.19	19	6.38		
Corrected Total	498.93	23			

[a] R Squared = .76 (Adjusted R Squared = .71)

Table B.15.1
Genotype (2 Varieties) x Environment (4 Locations) Interaction of Color *b** in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	109.74	1	109.74	1421.11	0.00
Location	268.00	3	89.33	1156.84	0.00
Variety*Location	119.96	3	39.99	517.81	0.00
Error	1.24	16	0.08		
Corrected Total	498.93	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.16

ANOVA of the Total Phenols in Grains of 8 Black Sorghum Genotypes[a]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	1515.01	7	216.43	167.42	0.00
Location	43.19	3	14.40	11.14	0.00
Error	109.88	85	1.29		
Corrected Total	1668.08	95			

[a] R Squared = .93 (Adjusted R Squared = .93)

Table B.16.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Total Phenols in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	43.19	3	14.40	35.82	0.00
Variety	1515.00	7	216.43	538.44	0.00
Location*variety	84.16	21	4.01	9.97	0.00
Error	25.73	64	0.40		
Corrected Total	1668.08	95			

[a] R Squared = .98 (Adjusted R Squared = .98)

Table B.17

ANOVA of the Antioxidant Activity Values in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	279455.65	7	39922.24	256.06	0.00
Location	6630.23	3	2210.08	14.18	0.00
Error	13252.51	85	155.91		
Corrected Total	299338.39	95			

[a] R Squared = .96 (Adjusted R Squared = .95)

Table B.17.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Antioxidant Activity Values in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	6630.23	3	2210.08	35.07	0.00
Variety	279455.65	7	39922.24	633.51	0.00
Location*variety	9219.40	21	439.02	6.97	0.00
Error	4033.11	64	63.02		
Corrected Total	299338.39	95			

[a] R Squared = .99 (Adjusted R Squared = .98)

Table B.18

ANOVA of Total Phenols in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	3.14	3	1.05	15.59	0.00
Sample	45.40	4	11.35	168.99	0.00
Error	3.49	52	0.07		
Total	659.43	60			
Corrected Total	52.03	59			

[a] R Squared = .93 (Adjusted R Squared = .92)

Table B.18.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Total Phenols in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	45.40	4	11.35	421.81	0.00
Location	3.14	3	1.05	38.91	0.00
Sample * Locatior	2.42	12	0.20	7.48	0.00
Error	1.08	40	0.03		
Corrected Total	52.03	59			

[a] R Squared = .98 (Adjusted R Squared = .97)

Table B.19

ANOVA of the Antioxidant Activity Values in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	208.57	3	69.52	7.54	0.00
Sample	38924.59	4	9731.15	1055.64	0.00
Error	479.35	52	9.22		
Corrected Total	39612.51	59			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.19.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Antioxidant Activity Values in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	38924.59	4	9731.15	10462.08	0.00
Location	208.57	3	69.52	74.74	0.00
Variety*Location	442.14	12	36.85	39.61	0.00
Error	37.21	40	0.93		
Corrected Total	39612.51	59			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.20

ANOVA of Total Phenols in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	0.01	1	0.01	0.20	0.66
Location	1.78	3	0.59	14.89	0.00
Error	0.76	19	0.04		
Corrected Total	2.55	23			

[a] R Squared = .70 (Adjusted R Squared = .64)

Table B.20.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Total Phenols in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	0.01	1	0.01	2.11	0.17
Location	1.78	3	0.59	156.72	0.00
Sample * Locatior	0.70	3	0.23	61.33	0.00
Error	0.06	16	0.00		
Corrected Total	2.55	23			

[a] R Squared = .97 (Adjusted R Squared = .97)

Table B.21

ANOVA of Antioxidant Activity Values in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	0.00	1	0.00	0.00	0.99
Location	1270.67	3	423.56	18.77	0.00
Error	428.81	19	22.57		
Corrected Total	1699.48	23			

[a] R Squared = .75 (Adjusted R Squared = .70)

Table B.21.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Antioxidant Activity Values in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	0.00	1	0.00	0.00	0.99
Location	1270.67	3	423.56	92.25	0.00
Variety*Location	355.35	3	118.45	25.80	0.00
Error	73.46	16	4.59		
Corrected Total	1699.48	23			

[a] R Squared = .96 (Adjusted R Squared = .94)

Table B.22

ANOVA of Luteolinidin levels in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Genotype	208461.42	7	29780.20	32.59	0.00
Location	118685.06	3	39561.69	43.30	0.00
Error	77663.49	85	913.69		
Corrected Total	404809.97	95			

[a] R Squared = .81 (Adjusted R Squared = .79)

Table B.22.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Luteolinidin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	118685.06	3	39561.69	7562.24	0.00
Variety	208461.42	7	29780.20	5692.50	0.00
Variety*Location	77328.67	21	3682.32	703.88	0.00
Error	334.81	64	5.23		
Corrected Total	404809.97	95			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.23

ANOVA of Apigeninidin levels in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	148705.14	7	21243.59	27.79	0.00
Location	138859.82	3	46286.61	60.55	0.00
Error	64971.71	85	764.37		
Corrected Total	352536.66	95			

[a] R Squared = .82 (Adjusted R Squared = .79)

Table B.23.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Apigeninidin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Location	138859.82	3	46286.61	18844.00	0.00	0.999
Variety	148705.14	7	21243.59	8648.60	0.00	0.999
Variety*Location	64814.50	21	3086.40	1256.52	0.00	0.998
Error	157.20	64	2.46			
Corrected Total	352536.66	95				

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.24

ANOVA of 5-methoxyluteolinidin levels in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	384673.01	7	54953.29	113.81	0.00
Location	49709.91	3	16569.97	34.32	0.00
Error	41042.24	85	482.85		
Corrected Total	475425.16	95			

[a] R Squared = .91 (Adjusted R Squared = .90)

Table B.24.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of 5-methoxyluteolinidin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	49709.91	3	16569.97	6786.24	0.00
Variety	384673.01	7	54953.29	22506.15	0.00
Variety*Location	40885.97	21	1946.95	797.37	0.00
Error	156.27	64	2.44		
Corrected Total	475425.16	95			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.25

ANOVA of 7-methoxyapigeninidin levels in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	102702.86	7	14671.84	115.02	0.00
Location	11463.68	3	3821.23	29.96	0.00
Error	10842.64	85	127.56		
Corrected Total	125009.18	95			

[a] R Squared = .91 (Adjusted R Squared = .90)

Table B.25.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of 7-methoxyapigeninidin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	11463.68	3	3821.23	4085.47	0.00
Variety	102702.86	7	14671.84	15686.42	0.00
Variety*Location	10782.77	21	513.47	548.97	0.00
Error	59.86	64	0.94		
Total	294997.23	96			
Corrected Total	125009.18	95			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.26

ANOVA of Luteolinidin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	5122.60	4	1280.65	39.24	0.00
Location	1650.50	3	550.17	16.86	0.00
Error	1697.16	52	32.64		
Corrected Total	8470.25	59			

[a] R Squared = .80 (Adjusted R Squared = .77)

Table B.26.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Luteolinidin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Variety	5122.60	4	1280.65	5478.56	0.00	0.998
Location	1650.50	3	550.17	2353.58	0.00	0.994
Variety*Location	1687.81	12	140.65	601.70	0.00	0.994
Error	9.35	40	0.23			
Corrected Total	8470.25	59				

[a] R Squared = 1.00 (Adjusted R Squared =1.00)

Table B.27

ANOVA of Apigeninidin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	3864.40	3	1288.13	9.53	0.00
Sample	15014.77	4	3753.69	27.76	0.00
Error	7030.95	52	135.21		
Corrected Total	25910.13	59			

[a] R Squared = .73 (Adjusted R Squared = .69)

Table B.27.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Apigeninidin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	15014.77	4	3753.69	2612.42	0.00
Location	3864.40	3	1288.13	896.49	0.00
Variety*Location	6973.48	12	581.12	404.44	0.00
Error	57.47	40	1.44		
Corrected Total	25910.13	59			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.28

ANOVA of 5-methoxyluteolinidin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	1490.53	3	496.84	24.64	0.00
Sample	2431.34	4	607.83	30.15	0.00
Error	1048.32	52	20.16		
Corrected Total	4970.19	59			

[a] R Squared = .79 (Adjusted R Squared = .76)

Table B.28.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of 5-methoxyluteolinidin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	2431.34	4	607.83	8008.00	0.00
Location	1490.53	3	496.84	6545.74	0.00
Variety*Location	1045.29	12	87.11	1147.61	0.00
Error	3.04	40	0.08		
Corrected Total	4970.19	59			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.29

ANOVA of 7-methoxyapigeninidin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	3998.60	3	1332.87	11.23	0.00
Sample	12215.85	4	3053.96	25.72	0.00
Error	6174.00	52	118.73		
Total	33771.01	60			
Corrected Total	22388.45	59			

[a] R Squared = .72 (Adjusted R Squared = .69)

Table B.29.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of 7-methoxyapigeninidin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	12215.85	4	3053.96	7476.13	0.00
Location	3998.60	3	1332.87	3262.88	0.00
Variety*Location	6157.66	12	513.14	1256.17	0.00
Error	16.34	40	0.41		
Corrected Total	22388.45	59			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.30

ANOVA of Luteolinidin in Grains of 2 yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	44.72	1	44.72	149.58	0.00
Location	90.76	3	30.25	101.20	0.00
Error	5.68	19	0.30		
Corrected Total	141.15	23			

[a] R Squared = .96 (Adjusted R Squared = .95)

B.30.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Luteolinidin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	44.72	1	44.72	324.08	0.00
Location	90.76	3	30.25	219.25	0.00
Variety*Location	3.47	3	1.16	8.39	0.00
Error	2.21	16	0.14		
Corrected Total	141.15	23			

[a] R Squared = .98 (Adjusted R Squared = .98)

Table B.31

ANOVA of Apigeninidin in Grains of 2 yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F
Sample	612.36	1	612.36	45.67
Location	387.08	3	129.03	9.62
Error	254.76	19	13.41	
Corrected Total	1254.20	23		

[a] R Squared = .80 (Adjusted R Squared = .75)

Table B.31.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Apigeninidin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	612.36	1	612.36	2164.50	0.00
Location	387.08	3	129.03	456.06	0.00
Variety*Location	250.23	3	83.41	294.83	0.00
Error	4.53	16	0.28		
Corrected Total	1254.20	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.32

ANOVA of 5-methoxyluteolinidin in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	80.34	1	80.34	149.97	0.00
Location	59.93	3	19.98	37.29	0.00
Error	10.18	19	0.54		
Corrected Total	150.44	23			

[a] R Squared = .93 (Adjusted R Squared = .92)

Table B.32.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of 5-methoxyluteolinidin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	80.34	1	80.34	1047.36	0.00
Location	59.93	3	19.98	260.42	0.00
Variety*Location	8.95	3	2.98	38.90	0.00
Error	1.23	16	0.08		
Corrected Total	150.44	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.33

ANOVA of 7-methoxyapigeninidin in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	19.21	1	19.21	6.08	0.02
Location	152.73	3	50.91	16.12	0.00
Error	60.00	19	3.16		
Corrected Total	231.94	23			

[a] R Squared = .74 (Adjusted R Squared = .69)

Table B.33.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of 7-methoxyapigeninidin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	19.21	1	19.21	494.91	0.00
Location	152.73	3	50.91	1311.81	0.00
Variety*Location	59.38	3	19.79	510.05	0.00
Error	0.62	16	0.04		
Corrected Total	231.94	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table 34

ANOVA of Luteolin Levels in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	7503.66	7	1071.95	24.50	0.00
Location	2671.63	3	890.54	20.35	0.00
Error	3719.26	85	43.76		
Corrected Total	13894.55	95			

[a] R Squared = .73 (Adjusted R Squared = .70)

Table 34.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Luteolin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	2671.63	3	890.54	5877.28	0.00
Variety	7503.66	7	1071.95	7074.52	0.00
Variety*Location	3709.56	21	176.65	1165.80	0.00
Error	9.70	64	0.15		
Corrected Total	13894.55	95			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.35

ANOVA of Apigenin Levels in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	389.42	7	55.63	20.99	0.00
Location	233.67	3	77.89	29.39	0.00
Error	225.31	85	2.65		
Corrected Total	848.41	95			

[a] R Squared = .73 (Adjusted R Squared = .70)

Table B.35.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Apigenin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	233.67	3	77.89	6722.63	0.00
Variety	389.42	7	55.63	4801.45	0.00
Variety*Location	224.57	21	10.69	922.95	0.00
Error	0.74	64	0.01		
Corrected Total	848.41	95			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.36

ANOVA of Luteolin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	4317.16	3	1439.05	8.91	0.00
Sample	85934.92	4	21483.73	132.96	0.00
Error	8402.00	52	161.58		
Corrected Total	98654.08	59			

[a] R Squared = .92 (Adjusted R Squared = .90)

Table B.36.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Luteolin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	85934.92	4	21483.73	142049.01	0.00
Location	4317.16	3	1439.05	9514.93	0.00
Variety*Location	8395.95	12	699.66	4626.12	0.00
Error	6.05	40	0.15		
Corrected Total	98654.08	59			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.37

ANOVA of Apigenin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	10296.66	3	3432.22	4.84	0.00
Sample	234317.25	4	58579.31	82.58	0.00
Error	36887.34	52	709.37		
Corrected Total	281501.24	59			

[a] R Squared = .87 (Adjusted R Squared = .85)

Table B.37.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Apigenin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	234317.25	4	58579.31	60001.96	0.00
Location	10296.66	3	3432.22	3515.57	0.00
Variety*Location	36848.29	12	3070.69	3145.26	0.00
Error	39.05	40	0.98		
Corrected Total	281501.24	59			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table 38

ANOVA of Luteolin in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	911.06	1	911.06	29.20	0.00
Location	764.70	3	254.90	8.17	0.00
Error	592.86	19	31.20		
Corrected Total	2268.62	23			

[a] R Squared = .74 (Adjusted R Squared = .68)

Table 38.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Luteolin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	911.06	1	911.06	888.21	0.00
Location	764.70	3	254.90	248.51	0.00
Variety*Location	576.45	3	192.15	187.33	0.00
Error	16.41	16	1.03		
Corrected Total	2268.62	23			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table 39

ANOVA of Apigenin in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	84.26	1	84.26	1166.98	0.00
Location	8.01	3	2.67	36.96	0.00
Error	1.37	19	0.07		
Corrected Total	93.64	23			

[a] R Squared = .99 (Adjusted R Squared = .98)

Table 39.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Apigenin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	84.26	1	84.26	3077.15	0.00
Location	8.01	3	2.67	97.45	0.00
Variety*Location	0.93	3	0.31	11.37	0.00
Error	0.44	16	0.03		
Corrected Total	93.64	23			

[a] R Squared = .99 (Adjusted R Squared = .99)

Table B.40

ANOVA of Eriodictyol in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	38777.53	7	5539.65	42.93	0.00
Location	55825.55	3	18608.52	144.20	0.00
Error	10969.07	85	129.05		
Corrected Total	105572.15	95			

[a] R Squared = .90 (Adjusted R Squared = .88)

Table B.40.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Eriodictyol in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	55825.55	3	18608.52	993.48	0.00
Variety	38777.53	7	5539.65	295.75	0.00
Variety*Location	9770.31	21	465.25	24.84	0.00
Error	1198.76	64	18.73		
Corrected Total	105572.15	95			

[a] R Squared = .90 (Adjusted R Squared = .98)

Table B.41

ANOVA of Naringenin in Grains of 8 Black Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	6699.44	7	957.06	24.22	0.00
Location	9250.95	3	3083.65	78.05	0.00
Error	3358.32	85	39.51		
Corrected Total	19308.72	95			

[a] R Squared = .83 (Adjusted R Squared = .81)

Table B.41.1

Genotype (8 Varieties) x Environment (4 Locations) Interaction of Naringenin in Black Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	9250.95	3	3083.65	627.22	0.00
Variety	6699.44	7	957.06	194.67	0.00
Variety*Location	3043.67	21	144.94	29.48	0.00
Error	314.65	64	4.92		
Corrected Total	19308.72	95			

[a] R Squared = .98 (Adjusted R Squared = .98)

Table B.42

ANOVA of Eriodictyol in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	787.94	3	262.65	8.73	0.00
Sample	31016.33	4	7754.08	257.83	0.00
Error	1563.89	52	30.07		
Corrected Total	33368.16	59			

[a] R Squared = .95 (Adjusted R Squared = .95)

Table B.42.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Eriodictyol in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	31016.33	4	7754.08	3171.33	0.00
Location	787.94	3	262.65	107.42	0.00
Variety*Location	1466.09	12	122.17	49.97	0.00
Error	97.80	40	2.45		
Corrected Total	33368.16	59			

[a] R Squared = .997 (Adjusted R Squared = .996)

Table B.43

ANOVA of Naringenin in Grains of 5 Red Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	70024.66	3	23341.55	12.22	0.00
Sample	542744.40	4	135686.10	71.02	0.00
Error	99346.45	52	1910.51		
Corrected Total	712115.51	59			

[a] R Squared = .86 (Adjusted R Squared = .84)

Table B.43.1

Genotype (5 Varieties) x Environment (4 Locations) Interaction of Naringenin in Red Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	542744.40	4	135686.10	1870.59	0.00
Location	70024.66	3	23341.55	321.79	0.00
Variety*Location	96444.99	12	8037.08	110.80	0.00
Error	2901.47	40	72.54		
Corrected Total	712115.51	59			

[a] R Squared = 1.00 (Adjusted R Squared = .99)

Table B.44

ANOVA of Eriodictyol in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	1162832.33	1	1162832.33	41.51	0.00
Location	2472489.13	3	824163.04	29.42	0.00
Error	532314.23	19	28016.54		
Corrected Total	4167635.68	23			

[a] R Squared = .87 (Adjusted R Squared = .85)

Table B.44.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Eriodictyol in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	1162832.33	1	1162832.33	4916.24	0.00
Location	2472489.13	3	824163.04	3484.41	0.00
Variety*Location	528529.77	3	176176.59	744.84	0.00
Error	3784.46	16	236.53		
Corrected Total	4167635.68	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

Table B.45

ANOVA of Naringenin in Grains of 2 Yellow Sorghum Genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sample	260268.77	1	260268.77	65.57	0.00
Location	110369.67	3	36789.89	9.27	0.00
Error	75413.55	19	3969.13		
Corrected Total	446051.98	23			

[a] R Squared = .83 (Adjusted R Squared = .80)

Table B.45.1

Genotype (2 Varieties) x Environment (4 Locations) Interaction of Naringenin in Yellow Sorghum Grains

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	260268.77	1	260268.77	3370.16	0.00
Location	110369.67	3	36789.89	476.38	0.00
Variety*Location	74177.91	3	24725.97	320.17	0.00
Error	1235.64	16	77.23		
Corrected Total	446051.98	23			

[a] R Squared = 1.00 (Adjusted R Squared = 1.00)

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