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Original Research Article

Environmental effect on flavonoid concentrations and profiles of red and lemon-yellow sorghum grains

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ABSTRACT

Pigmented sorghum genotypes high in flavonoids have been developed in recent years. Flavonoid levels of seven sorghum genotypes grown in four locations in Texas, USA were evaluated to assess the relative genotype, environment and genotype × environment effects. Sorghums from Halfway were highly weathered which affected flavonoid levels. Two flavanones, four 3-deoxyanthocyanidins, and two flavones were detected and quantified. Weathered grains had the lowest flavanone levels (303 μg/g) and the highest 3-deoxyanthocyanidins levels (82 μg/g). Only genotype 99LGWO50 had high levels of flavones (255 μg/g). For all flavonoids there was a genotype × environment interaction effect ($p < 0.01$), which suggested that environment had a different effect on flavonoid levels depending on the genotype. Red and lemon-yellow genotypes must be evaluated in multiple environments to identify the best genotype to obtain the highest yields of specific flavonoid compounds.

1. Introduction

Sorghum (*Sorghum bicolour* (L.) Moench) is an important cereal crop in the world mainly because of its adaptation to adverse environmental conditions, especially drought (Maunder, 2000; Blum, 2004). In Asia and Africa, sorghum grain is used mostly for human foods like flat breads, fermented or unfermented porridges, couscous, and fried products (Léder, 2004). The types of sorghum used vary from white, lemon-yellow or red sorghums. In the United States, South America, and Australia, sorghum is produced primarily for animal feed and ethanol production while a small proportion of white sorghum is used in the production of beer and gluten-free foods.

In recent years, black, red, and lemon-yellow sorghums high in flavonoids with or without condensed tannins have been developed (Rooney et al., 2013a,b). These sorghums are a source of natural colorants and beneficial phytochemicals for human health (Shih et al., 2007; Bralley et al., 2008; Burdette et al., 2010; Devi et al., 2011). Most sorghum flavonoids and condensed tannins are located in the bran and their concentrations and profiles are influenced by pericarp color, secondary plant color, and/or the presence of a pigmented testa (Dykes et al., 2009, 2011; Shirley, 1998). Pericarp color is controlled by the *R* and *Y* genes. The pericarp is white when *Y* is homozygous recessive (*R_yy or rryy*), lemon-yellow when only *R* is homozygous recessive (*rrY_*), and red when both *R* and *Y* are dominant (*R_Y_*) (Dykes and Rooney, 2006). Black sorghums are special red sorghums that become black when exposed to sunlight during maturation (Dykes et al., 2009).

The metabolism of sorghum flavonoids is regulated by genotype and environmental conditions (Boddu et al., 2005; Shih et al., 2006). Three groups of flavonoids have been found in high amounts in sorghum grains of different genotypes: 3-deoxyanthocyanidins (32–680 μg/g), flavones (60–386 μg/g), and flavanones (134–1780 μg/g) (Dykes et al., 2009, 2011). In black genotypes, the predominant flavonoids are the 3-deoxyanthocyanidins, which have good potential as natural colorants in low pH food systems and have shown anti-inflammatory and anticancer properties (Awika et al., 2004; Shih et al., 2007; Yang et al., 2009; Burdette et al., 2010). The sorghum 3-deoxyanthocyanidins are methoxylated and non-methoxylated (Dykes et al., 2009; Taleon et al., 2012). Yang et al. (2009) demonstrated that the methoxylated forms have stronger anticarcinogenic activity. Flavanones, which have also shown anticancer activity (Ko et al., 2002; Lee et al., 2008), are found in high concentrations in lemon-yellow sorghum genotypes (Dykes et al., 2011). Flavones were found in high concentrations in red and lemon-yellow sorghums with tan

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secondary plant color (Dykes et al., 2009, 2011). In cereals, abiotic factors such as light and temperature (Christie et al., 1994; Han et al., 2009) affect flavonoid synthesis. Biotic factors, such as fungal infection, are known to affect flavonoid synthesis in sorghum (Weiergang et al., 1996; Lo and Nicholson, 1998).

Information regarding the variability of flavonoid composition based on environmental conditions is useful because the use of sorghum as a nutraceutical or colorant depends on flavonoid concentration and profile. Recently, our research group characterized flavonoids from elite genetic material from black, red, and lemon-yellow sorghums (Dykes et al., 2009, 2011). High environmental variability was found on flavonoid levels in black sorghum grains (Taleon et al., 2012) but the effect of environment on individual flavonoids of elite red and lemon-yellow sorghum genotypes has not been evaluated. Consequently, this study was done to determine the relative effect of environment on major flavonoids in red and lemon-yellow sorghum grains.

2. Materials and methods

2.1. Sorghum genotypes and locations

Grains from five red (Tx2911, 99LGWO50, SC719-11E, 98CA4779 and B9904) and two lemon-yellow (R07007 and SC748-5) sorghum genotypes were produced and harvested from field trials grown in 2008. All lines were selected for agronomic potential, adaptation, secondary plant color, and pericarp color. Genotype 99LGWO50 has tan secondary plant color. Genotypes Tx2911, SC719-11E, B9904, R07007 have red secondary plant color. Genotypes 98CA4779 and SC748-5 have purple secondary plant color. The four environments represented major ecological regions in Texas, USA where sorghum is produced (TASS, 2009): High Plains (Halfway), Central Texas Plains (College Station) and Western Gulf Coastal Plain (Corpus Christi and Weslaco, TX). At all locations, sorghum production practices (i.e. fertilization, pest management) standard to the region were used in the production of the grain and all trials were irrigated with the exception of Corpus Christi, which was rainfed. Average minimum/maximum temperatures and total precipitations were obtained from daily records from the National Climatic Data Center (2013). Average daylight hours was obtained from daily records from the United States Naval Observatory (2013). The climate data is presented in Table 1. At each location, all panicles from 4.6 m-long entries were hand-harvested at maturity, threshed, fumigated, combined, and stored at 4 °C until analysis. Samples were stored up to 4 months. A total of 28 independent samples were analyzed and 3 analytical replicates were analyzed for each genotype at each location.

2.2. Standards and reagents

Apigenin (98%) and luteolin (98%) were obtained from Indofine Chemical Co., Inc. (Hillsborough, NJ, USA). Naringenin (95%) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Eriodictyol (97%), luteolinidin chloride (97%), and apigeninidin chloride (97%) were obtained from ALSACHIM (Strasbourg, France). HPLC-grade methanol and acetonitrile were obtained from VWR (West Chester, PA, USA). Reagent-grade formic acid was obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.3. Grain weathering score

Grain weathering refers to the presence of deterioration on the pericarp of the grain and it results from both fungal infection and exposure to climatic conditions after physiological maturity of the grain. The grain weathering ratings were made by an experienced pathologist who, by visually evaluating each sample, rated it on a scale from 1 to 9. A rating of 1 indicated the sorghum was completely free of damage while a 9 represented grain showing symptoms of weathering (Rodriguez-Herrera et al., 2000).

2.4. Sample preparation for HPLC-DAD analysis

The extraction of flavonoids was performed as described by Dykes et al. (2011). Whole grain samples were ground using a Cuisinart DCG-20 coffee grinder (East Windsor, NJ, USA). The ground sample (1 g) was extracted in 10 mL of 1% HCl/methanol (v/v) for 2 h while shaking at low speed in an Eberbach shaker (Eberbach, Ann Arbor, MI, USA). The extracts were centrifuged at 2790 × *g* for 10 min. For 3-deoxyanthocyanidins and flavones, the extracts were decanted and filtered using a 0.45 μm nylon membrane filter (Whatman Inc., Maidstone, UK) prior to HPLC analysis. Since sorghum flavanones are present as unstable flavanone glycosides (Dykes et al., 2011), it was necessary to convert the flavanone glycosides to their aglycone forms. To do so, a second set of extracts were prepared using the aforementioned method. The acidified methanol extracts were then transferred to glass tubes, sealed, and placed in a water bath at 80 °C for 90 min. After equilibration at room temperature, the hydrolyzed extracts were filtered using the aforementioned filtration method prior to HPLC analysis.

2.5. Identification of flavonoids by HPLC-DAD

Extracts were analyzed on an Alliance 2695 system (Waters Corp., Milford, MA, USA) with a Waters 996 photodiode array

Table 1
Climate data for the Texas environments where sorghum grain development was evaluated in 2008.

Environment		April	May	June	July	August	September	October
Corpus Christi	Average temperature, min/max (°C)[a]		22.0/31.2	23.4/33.3	22.8/31.9			
	Total precipitation (cm)[a]		3.2	1.8	23.9			
	Average daylight duration (h)[b]		13.5	13.9	13.7			
College Station	Average temperature, min/max (°C)		19.7/29.9	23.7/35.7	23.2/36.4			
	Total precipitation (cm)		10.9	0.7	1.0			
	Average daylight duration (h)		13.7	14.1	13.9			
Halfway	Average temperature, min/max (°C)					18.8/32.0	13.6/27.0	7.9/23.1
	Total precipitation (cm)					8.0	7.0	8.2
	Average daylight duration (h)					13.4	12.4	11.3
Weslaco	Average temperature, min/max (°C)	18.3/30.5	22.7/33.1	24.0/35.6				
	Total precipitation (cm)	2.9	0.3	1.3				
	Average daylight duration (h)	12.8	13.4	13.7				

[a] Data obtained from the National Climatic Data Center (2013).
[b] Data obtained from the United States Naval Observatory (2013).