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Evaluation of phenolics and antioxidant activity of black sorghum hybrids

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ABSTRACT

Black sorghums possess very high levels of the rare 3-deoxyanthocyanidins which can be used as natural food colorants with health benefits. However, these sorghum genotypes have undesirable agronomic properties (i.e. high height, low yield, increased weathering). Black sorghum hybrids with improved agronomic properties were developed and their phenolic profiles and antioxidant activity were compared with black sorghum lines. Black sorghum hybrids were significantly lighter in colour than the lines ($P < 0.001$). All hybrids had a pigmented testa which was indicated by the presence of condensed tannins, which significantly increased total phenol levels and antioxidant activity. The 3-deoxyanthocyanidin, flavan-4-ol, and flavone levels were significantly lower in the hybrids ($P < 0.001$) and were strongly correlated to pericarp colour ($P < 0.001$). Flavanone levels were not significantly different among the lines and hybrids ($P > 0.05$) and pericarp colour did not affect their levels ($P > 0.05$). Even though the 3-deoxyanthocyanidin levels were lower in black sorghum hybrids than in the lines, the presence of condensed tannins in the hybrids significantly increased their antioxidant activity. Since 3-deoxyanthocanidin levels were dependent on pericarp colour, hybrids with increased blackness intensity should be developed to increase the stable 3-deoxyanthocyanidins.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal grain originating in Africa and is grown in tropical, subtropical, and arid regions (Waniska and Rooney, 2000) and is the fifth leading crop in the world after wheat, maize, rice, and barley (FAO, 2007). It is used primarily as a food in Africa and Asia and as feed in the western hemisphere (Dykes et al., 2005). There is now an increased interest in the United States in using sorghum in foods due to its gluten-free and other health properties such as slow digestibility, cholesterol-lowering, anti-inflammatory, and anti-cancer properties (Bralley et al., 2008; Burdette et al., 2010; Dykes and Rooney, 2006; Moraes et al., 2012; Turner et al., 2006; Yang et al., 2009).

Sorghum has a wide array of phenolic compounds concentrated in the pericarp which include phenolic acids, flavonoids, and condensed tannins. Their composition and levels are affected by the genotype (Dykes and Rooney, 2006; Dykes et al., 2005, 2009, 2011; Hahn and Rooney, 1986; Waniska and Rooney, 2000). Sorghum genetics related to pericarp colour and the presence of a pigmented testa are summarised by Rooney (2000). Pericarp colour is controlled by the *R* and *Y* genes and can be classified into four distinct colours: white, lemon-yellow, red, and black. Sorghum with a white pericarp has homozygous recessive *Y* genes (*R_yy* or *rryy*) whereas sorghum with a lemon-yellow sorghum has recessive *R* and dominant *Y* genes (*rrY_*). Red and black pericarp has both homozygous *R* and *Y* genes (*R_Y_*). Black sorghum is a special red sorghum that turns black in the presence of sunlight and has high levels of 3-deoxyanthocyanidins located in the pericarp (Awika et al., 2004; Dykes and Rooney, 2006; Dykes et al., 2009). The mechanism of pericarp colour change in the black sorghum when exposed to sunlight is unknown. Pericarp colour does not control the presence of a pigmented testa or the presence of tannins. The presence of a pigmented testa is solely controlled by the $B_1\_B_2\_$ genes ($B_1\_B_2\_$ = pigmented testa; $B_1\_b_2b_2$ or $b_1b_1B_2\_$ = non-pigmented testa) and is expressed in sorghums regardless of pericarp colour.

The high levels of 3-deoxyanthocyanidins in black sorghums make this genotype valuable since it is the only dietary source of those compounds (Wu and Prior, 2005). Decorticating black sorghum grains to obtain bran concentrates 3-deoxyanthocyanidin levels four-fold (Awika et al., 2004). Sorghum 3-deoxyanthocyanidins have good potential as natural food colorants due to their stability (Awika et al., 2004; Mazza and Brouillard, 1987; Sweeny and Iocobucci, 1983). They also have potential health benefits such as antioxidant and anti-cancer properties (Devi et al., 2011; Shih et al., 2007; Yang et al., 2009).

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Tx3362 (Rooney et al., 2013), Shawaya Black, and Black PI Tall are three lines developed by the Texas A&M AgriLife Research Sorghum Breeding Program at College Station, TX, USA. Each of these lines has limited production potential due to various problems. For example, Tx3362 has low grain yield and is susceptible to weathering and mould damage (Rooney et al., 2013) whereas Shawaya Black and Black PI Tall are tall (2–3 m) and relatively low in grain yield (Texas A&M Agrilife Research Sorghum Breeding Program, unpublished results). To mitigate these problems, this program has developed a set of experimental black sorghum hybrids with improved agronomic properties (Rooney et al., in press).

Information on phenolic levels and potential antioxidant activities in black sorghum hybrids is limited. Thus, it was necessary to determine whether hybrids could be used as a substitute over the lines as a source of phenolics for the production of grain-based foods (i.e. breads, cookies, cakes) with potential health benefits. Taleon et al. (2012) investigated the relative effect of environment and genotypes on major flavonoids in black sorghum grains. From their study, 3-deoxyanthocyanidins and flavones were lower in the hybrids than in the lines. In addition, it was further suggested that pericarp colour did not affect flavonoid levels due to the low correlation between pericarp colour and total flavonoids. However, correlations between pericarp colour and each flavonoid class (i.e. 3-deoxyanthocyanidins, flavones) were not made. Dykes et al. (2009) demonstrated that a black sorghum grown in the presence of sunlight has a darker pericarp and higher levels of 3-deoxyanthocyanidins but the correlation could not be made since only one genotype was used. Thus, correlations between pericarp colour and phenolic levels (i.e. flavonoids, condensed tannins) should be investigated to determine whether levels of these compounds are affected by pericarp colour. The objectives of this study were to compare phenolic and potential antioxidant activity levels between black sorghum lines and hybrids and to determine the effect of pericarp colour on phenolic levels.

2. Materials and methods

2.1. Samples

Three lines (Tx3362, Black PI Tall, and Shawaya Black) and five hybrids (Hyb107, Hyb115, Hyb116, Hyb117, and Hyb118) were grown in the sorghum breeding nursery in College Station, TX, USA in 2006. The three lines represent different phases of black colour sorghum improvement with Black PI Tall and Shawaya Black being photoperiod insensitive selections made in Texas. Tx3362 was selected from a cross of Tx430/Shawaya Black for acceptable height maturity and black colour (Rooney et al., 2013). The five hybrids were created by pollinating five various experimental black-coloured seed parents with Tx3362 (Table 1). All sorghum samples were collected at maturity, air-dried, and threshed. All glumes were removed from the kernels and all broken kernels were discarded. Whole sorghum grains were ground for 2 min using a Cuisinart DCG-20 coffee grinder (East Windsor, NJ, USA). All samples were ground to achieve a particle size that passed through a 500-μm sieve.

Table 1
CIELAB L^*, a^*, b^* values of black sorghums.

Variety	Pedigree	L^*	a^*	b^*
Tx3362	Tx430/Shawaya Black	34.2 ± 0.2a	3.8 ± 0.1a	2.8 ± 0.1a
Shawaya Black	–[a]	31.5 ± 0.1b	2.5 ± 0.0b	0.9 ± 0.0b
Black PI Tall	–	31.5 ± 0.1b	3.1 ± 0.0c	1.3 ± 0.0c
Hyb107	A05023/Tx3362	34.7 ± 0.5c	6.0 ± 0.1d	4.7 ± 0.2d
Hyb115	A05027/Tx3362	35.3 ± 0.2d	6.2 ± 0.2df	5.0 ± 0.1e
Hyb116	A05029/Tx3362	35.9 ± 0.1e	6.6 ± 0.2e	5.7 ± 0.2f
Hyb117	A05030/Tx3362	35.6 ± 0.2de	6.0 ± 0.1d	5.4 ± 0.2f
Hyb118	A05555/Tx3362	34.2 ± 0.1a	6.3 ± 0.2f	3.7 ± 0.2g

Values followed by different letters within a column are significantly different by Fisher's LSD ($P < 0.05$).
[a] Original accession.

2.2. Standards and reagents

Gallic acid, catechin hydrate, naringenin, and 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) were from Sigma–Aldrich (St. Louis, MO, USA). The 2-2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Acros Organics (Morris Plains, NJ, USA), and Trolox was obtained from Aldrich (Milwaukee, WI, USA). Apigenin and luteolin were obtained from Indofine Chemical Co., Inc. (Hillsborough, NJ, USA). Eriodictyol, luteolinidin chloride, and apigeninidin chloride were obtained from ALSACHIM (Strasbourg, France) and 7-methoxy-apigeninidin chloride was obtained from ChromaDex (Santa Ana, CA, USA). Methanol, acetonitrile, and HCl (12 M) were purchased from VWR (West Chester, PA, USA). Formic acid and *sec*-butanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water was purified using the Simplicity® UV water purification system (Millipore, Billerica, MA, USA). All reagents were HPLC or analytical grade.

2.3. Pericarp colour determination

Colour measurements of whole grains were obtained using a Minolta CR-310 Colorimeter equipped with a CR-A50 granular materials attachment (Konica Minolta, Osaka, Japan). Sorghum kernels were fully packed into the granular materials attachment (6 cm × 0.8 cm, inner diameter × depth) prior to colour measurement. Colour measuring area was Φ5 cm. Values were expressed as Commission Internationale de l'Éclairage L^*, a^*, and b^* (CIELAB, 1986). L^* values represent lightness (0 = black, 100 = white). The a^* values represent redness ($-a^*$ = greenness) whereas the b^* values represent yellowness ($-b^*$ = blueness).

2.4. Sorghum phenolics and antioxidant activity evaluations using colorimetric assays

2.4.1. Preparation of phenolic extracts

For all assays with the exception of the DPPH assay, ground samples (0.1–0.5 g) were extracted in 25 mL of 1% HCl in methanol (v/v) for 2 h while shaking at low speed using an Eberbach shaker (Eberbach Corp., MI, USA). For the DPPH assay, samples (0.10–0.15 g) were extracted in 25 mL of aqueous 70% acetone (v/v) for 2 h while shaking at low speed. All extracts were centrifuged at 2790*g* for 15 min in a Sorvall SS-34 centrifuge (DuPont Instruments, Wilmington, DE) and were decanted.

2.4.2. Colorimetric assays

Total phenols of the acidified methanol extracts were measured using the modified Folin–Ciocalteu method of Kaluza et al. (1980). Condensed tannins were measured using the modified vanillin/HCl assay as described by Price et al. (1978). Flavan-4-ol content was measured using the butanol/HCl assay of Govindarajan and Mathew (1965). The 3-deoxyanthocyanidin content was obtained using the colorimetric method of Fuleki and Francis (1968). The extinction coefficient of luteolinidin chloride dissolved in the extraction solvent was determined (ε = 27,400) at 485 nm and concentration of 3-deoxyanthocyanidin pigments were calculated using the Lambert–Beer's Law equation. Details on the aforementioned procedures are found in Dykes et al. (2005). Antioxidant activity of sorghum extracts were assessed *in vitro* by the DPPH and ABTS assays as described by Awika et al. (2003). Antioxidant

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