

# Phenotypic Diversity of Anthocyanins in Sorghum Accessions with Various Pericarp Pigments

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## Abstract

Anthocyanins, a sub-class of flavonoids, are natural pigments known to have functional health benefits. Sorghum is a rich source of various phytochemicals including anthocyanins. This study was to identify and quantify the profiles of anthocyanins by HPLC-DAD in the selected 25 sorghum accessions with various phenotypic pericarp pigments. The predominant anthocyanins found in sorghums were 3-deoxyanthocyanidins including the unique leuteolinidin and apigeninidin analogs. The high levels of total anthocyanins were found in the red pericarp PI297139 and the brown pericarp PI221723, followed by the brown pericarp PI35038 and the yellow pericarp PI229838. There were moderate to low levels of anthocyanins observed in all the other accessions except for the white pericarp that generally contained least to undetectable amount. Although anthocyanins appeared to be associated with the pericarp color in the sorghum accessions with the highest contents in each pericarp pigment category, a distinguishable diversity of anthocyanin contents was presented among and between the phenotypic pericarp colors, suggesting other colorful phytochemicals such as carotenoids might be contributed. Establishing a database of anthocyanin profile and diversity in sorghum accessions with various pericarp pigments may lead to the development of novel functional sorghum products with active anthocyanin-enriched health benefits.

**Keywords:** Anthocyanins; Sorghum; Pericarp pigments; Phytochemicals

## Introduction

Sorghum (*Sorghum bicolor*) ranks the fifth most staple crop all over the world in term of world grain production [1] and is the mainstay of people in the warmer temperatures and tropical regions of the world such as South Asia and Africa [2]. It is also a good source of proteins, calories and minerals in developing countries [3,4]. The United States is the largest producer and exporter of sorghum, consisting of 25% of world production and approximately 70% of sorghum export in 2015-2016 [5].

Among cereals, sorghum contains the highest phytochemical contents with up to 6% (w/w), including anthocyanins, carotenoids, phenolic acids, and condensed tannins, etc. [6-8]. These phytochemicals are widely distributed in the pericarp and testa of the sorghum [9-11]. The color of sorghum pericarp shows a wide range of different colors from red to white. Although both anthocyanins and carotenoids may attribute to the color, the red color of sorghum accessions has been reported due to anthocyanins [12-14]. Furthermore, the pericarp color was considered a dependable indicator for the sorghum varieties and the levels of anthocyanins [12,15].

Anthocyanins are one of the most important water-soluble plant pigments [16]. They are synthesized by the flavonoid branch of the phenylpropanoid pathway through secondary metabolism in the plants. Among the over 600 types of anthocyanins [17], the majority of anthocyanin aglycone found in the food items usually consists of six anthocyanidins, i.e., cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin [18,19]. However, sorghum anthocyanins are unique 3-deoxyanthocyanidins, which include the orange leuteolinidin and the yellow apigeninidin [20,21]. Figure 1 shows the chemical structures of four common sorghum 3-deoxyanthocyanins, i.e., luteolinidin, apigeninidin, 5-methoxyluteolinidin, and 7-methoxyapigeninidin. It appears that sorghum grain is the only

known dietary source of 3-deoxyanthocyanidins except for the flowers of sinningia (*Sinningia cardinalis*), the silk tissues of maize (*Zea mays*), and the stalks of sugarcane (*Saccharum* sp.) [22-24]. The exceptional 3-deoxyanthocyanins in sorghum seems more stable than other anthocyanins, making them a desired natural food colorant [9,15]. While both luteolinidin and apigeninidin analogs were identified in sorghum [21] and the genetic expression of 3-deoxyanthocyanidin synthesis enzymes was investigated [13], the quantitative contents were not reported yet [20]. Furthermore, both luteolinidin and apigeninidin in sorghum belong to phytoalexin due to their responsibilities of anti-fungal invasion and/or anti-stressful activities [13,25]. Dietary sorghum 3-deoxyanthocyanidins have been also associated with human health benefits such as prevention of obesity and diabetes through antioxidant and anti-inflammatory mechanisms [26].

Considering the relatively little data published documenting the phenotypic diversity of anthocyanins in sorghum and virtually nothing known about the relationship with the pericarp colors, the objective of this study was to identify phenotypic profile and diversity of anthocyanins in the selected 25 sorghum accessions with various pericarp pigments.

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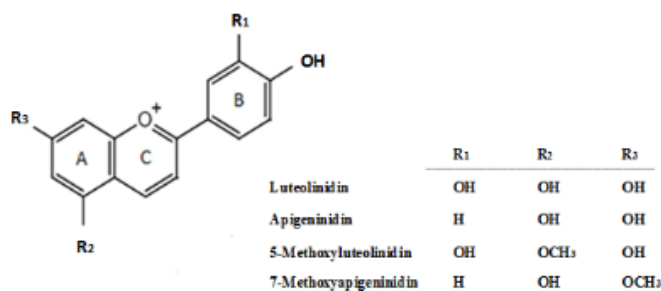


Figure 1: Chemical structures of anthocyanins detected in sorghum: Luteolinidin, apigeninidin, 5-methoxyluteolinidin, and 7-methoxyapigeninidin.

## Material and Methods

### Chemicals

Acetonitrile, methanol, and formic acid at either HPLC grade or analytic grade used in this study were purchased from Thermal Fisher Scientific (Suwanee, GA). Water was purified through Barnstead E-Pure Deionization System (Dubuque, IA) and filtered by Millipore 0.45  $\mu\text{m}$  membrane (Bedford, MA). Commercial standard of Peonidin-3-glucoside chloride and apigeninidin were purchased from Sigma-Aldrich (St. Louis, MO).

### Sample preparation and extraction

Sample material has been described previously [27]. In brief, accessions were grown in South Carolina in 2012, a subset was selected based on variation on polyphenol content, and bran was obtained by decortication with a tangential abrasive dehulling device. Thresher (Precision Machine Company, Lincoln, NE). Prepared bran was then stored at  $-80^{\circ}\text{C}$  until further extraction. For preparation of anthocyanin extracts, 1 g of the bran was extracted with 10 mL of acidified MeOH with 1N of formic acid at 95:5 (v/v). The flasks containing bran/solvent mixture were wrapped with aluminum foil to avoid light exposure. After a 12-h extraction, the samples were centrifuged at 2,800 rpm for 30 min and then the supernatant was collected and dried by vacuum drier at  $25^{\circ}\text{C}$  overnight. One mL of the acidified MeOH was added and then the dissolved extract was filtered by Whatman syringe filter (Whatman 0.45  $\mu\text{m}$  PVDF) for further HPLC-DAD analysis.

### Identification and quantification of anthocyanins by HPLC-DAD

According to our previous publications [19,28], Shimadzu HPLC system (Kyoto, Japan) was used for chromatographic analysis and separation. This system employed a DGU-20A3 built in degasser, a LC-20AB solvent delivery pump, a SIL-20A8T auto-sampler, a CTO-20AC column-holding oven, a CBM-20A communicator module, and a SPD-M20A Photodiode Array Detectors. A Waters (Milford, MA)  $\text{C}_{18}$  reversed phase column (250 mm length, 4.6 mm diameter) was used for anthocyanin separation. Data was analyzed using LC solution software (Kyoto, Japan). Elution was performed with mobile phase A (5% formic acid in de-ionized water) and mobile phase B (5% formic acid in acetonitrile/water 1:1 v:v). An optimum column temperature of  $25^{\circ}\text{C}$  was set. At a flow rate of 0.6 mL/min, the gradient conditions were set with solvent B volume as 10-30% for 30 min, 30-55% the following 20 min before returning to 10% at 60 min. The detector performed a full spectrum scan between 190-800 nm, where 480 nm was used for monitoring anthocyanins. Peonidin-3-glucoside was used as an internal standard for estimation of extraction recovery. The contents of anthocyanins were quantitated based upon standard

curve of apigeninidin, and the results were expressed as Apigeninidin Equivalent (APGE).

### Statistical analysis

Data were analyzed using SAS statistical software 9.3 (SAS Institute, Cary, NC, USA). Data were analyzed by one-way ANOVA using a general linear model procedure followed by Turkey's post-hoc test. The results were presented as means  $\pm$  SD, and a probability of  $p \leq 0.05$  was considered significant.

## Results

### HPLC chromatographic separation and identification

Anthocyanin extracts from the representative sorghum accessions were separated by HPLC as shown in Figure 2. While anthocyanins were undetectable in the white pericarp sorghum PI656079, a total of four anthocyanins were eluted at the retention times between 16 and 27 min in the yellow PI229838, brown PI 1221723, and red PI 297139. The sorghum anthocyanins or 3-deoxyanthocyanidins were identified based on the retention times of the commercial standards, peak UV-vis spectra, and published LC-MS data [20]. Four major 3-deoxyanthocyanins were identified, i.e., luteolinidin, apigeninidin, 5-methoxyluteolinidin, and 7-methoxyapigeninidin. Their retention times were 17.25, 22.23, 22.92, and 27.77 min, respectively (Figure 2). Both apigeninidin and 7-methoxyapigeninidin were predominant, which counted approximately 60-80% of the total 3-deoxyanthocyanins.

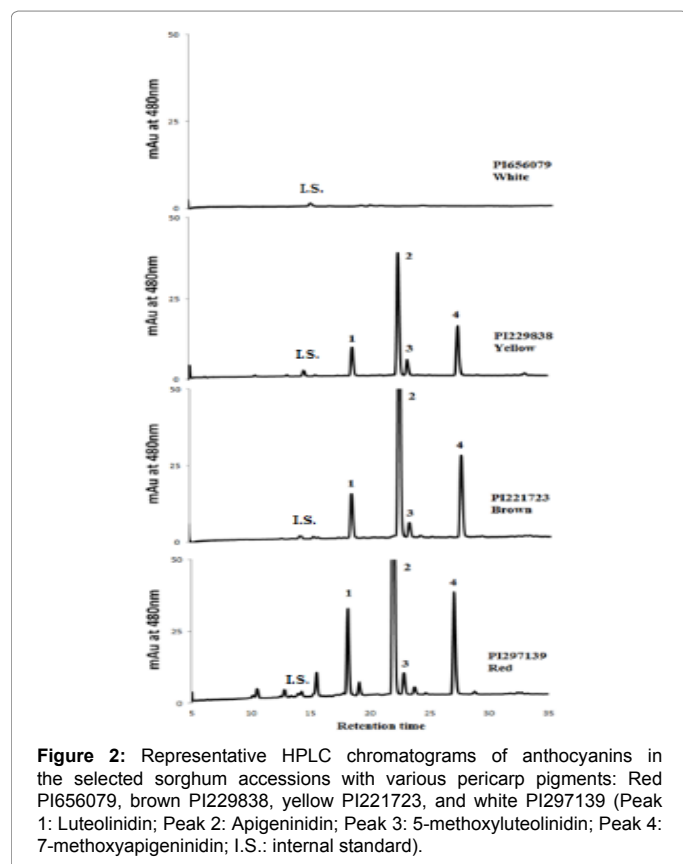
### Quantification of anthocyanin in 25 sorghum accessions

The contents of each anthocyanin and total anthocyanins in 25 sorghum accessions with various pericarp pigments were presented in Table 1. Based up the categories of the pericarp colors from red, brown, yellow to white, the high levels of anthocyanins in each category were found in a red pericarp PI297139 ( $1461.4 \pm 98.7$  g/kg), followed by two brown pericarp accessions PI221723 and PI35038 ( $1376.4 \pm 33.2$ ,  $937.3 \pm 29.4$  g/kg, respectively) and a yellow pericarp accession PI229838 ( $574.8 \pm 105.4$  g/kg). While anthocyanins were undetectable in most of the white sorghum accessions, the moderate to low levels of anthocyanins were observed in all the pericarp pigment categories.

## Discussion

The objective of the present study was to identify phenotypic diversity of anthocyanins in the selected 25 sorghum accessions with various pericarp pigments that were postulated to be associated with the colored anthocyanins.

The profiles of anthocyanins in various categories of the pericarp colors were similar, but the contents varied remarkably. In spite



of four 3-deoxyanthocyanin peaks, some unidentified peaks with a similar spectral characteristic of apigeninidin or luteolinidin were revealed. These minor peaks may most likely be derivatives of the 3-deoxyanthocyanidins as suggested by Lafayette [29]. Four 3-deoxyanthocyanins were detected in most of the sorghum accessions in each pigment category except for the white accessions that generally contained least to undetectable anthocyanins. If the contents of anthocyanins were compared among the highest one in each pericarp color category as shown in Table 1, it would appear that the contents of anthocyanins were associated with the pericarp colors clearly. That is to say, the contents of anthocyanins in sorghum accessions seemed to be predicted by the pericarp color.

However, a distinguishable diversity of anthocyanin contents was found among and between the phenotypic pericarp colors. In the three red accessions tested, for example, one red accession contained the highest levels of anthocyanins, but the other two had much less levels than the most brown and yellow, even less than one of the white accessions. A high diversity of anthocyanins in sorghum is in agreement with the previous reports by others [9,11,30,31]. Although the capacity of anthocyanin biosynthesis is decided by the genotypic cultivar, the activity of anthocyanin biosynthesis can be actually influenced by many environmental factors.

Furthermore, a number of factors including genotype (cultivar accession) and environment (production practices and ecology, etc.) also determine the phenotypic pigment of a sorghum pericarp. One of the important genotypic factors that may affect the pericarp color is other colored phytochemicals biosynthesized such as carotenoids that may have interfered. Carotenoids are one of the colored phytochemicals that have been suggested to count for the phenotypic color of sorghum

Pericarp color	Accessions	Luteolinidin	Apigeninidin	5-methoxyluteolinidin	7-methoxyapigeninidin	Total
Red	PI297139	209.1 ± 5.3 <sup>a</sup>	978.8 ± 55.2 <sup>a</sup>	50.8 ± 1.3 <sup>b</sup>	222.8 ± 36.7 <sup>b</sup>	1461.4 ± 98.7 <sup>a</sup>
Red	PI576426	UD	UD	1.0 ± 0.03 <sup>d</sup>	0.8 ± 0.08 <sup>d</sup>	1.7 ± 0.1 <sup>f</sup>
Red	PI329440	UD	0.7 ± 0.02 <sup>f</sup>	UD	UD	0.7 ± 0.0 <sup>f</sup>
Brown	PI221723	144.6 ± 4.1 <sup>c</sup>	888.8 ± 8.7 <sup>b</sup>	51.1 ± 4.1 <sup>b</sup>	291.8 ± 16.3 <sup>a</sup>	1376.4 ± 33.2 <sup>b</sup>
Brown	PI35038	162.6 ± 7.9 <sup>b</sup>	552.3 ± 12.2 <sup>c</sup>	65.0 ± 1.9 <sup>a</sup>	157.4 ± 7.3 <sup>c</sup>	937.3 ± 29.4 <sup>c</sup>
Brown	PI208537	41.6 ± 0.4 <sup>hg</sup>	33.5 ± 2.3 <sup>ef</sup>	3.0 ± 0.1 <sup>d</sup>	1.5 ± 0.01 <sup>d</sup>	79.6 ± 2.9 <sup>f</sup>
Brown	PI221655	4.7 ± 0.2 <sup>hg</sup>	42.4 ± 0.1 <sup>ef</sup>	0.8 ± 0.1 <sup>d</sup>	2.9 ± 0.1 <sup>d</sup>	50.9 ± 0.4 <sup>f</sup>
Brown	PI533957	6.4 ± 0.9 <sup>hg</sup>	36.6 ± 1.5 <sup>ef</sup>	1.0 ± 0.5 <sup>d</sup>	6.5 ± 0.9 <sup>d</sup>	50.5 ± 3.8 <sup>f</sup>
Brown	PI533902	14.0 ± 0.01 <sup>g</sup>	11.7 ± 0.6 <sup>f</sup>	2.3 ± 0.3 <sup>d</sup>	2.5 ± 0.1 <sup>d</sup>	30.5 ± 0.9 <sup>f</sup>
Brown	PI542718	1.8 ± 0.6 <sup>hg</sup>	19.5 ± 3.9 <sup>ef</sup>	1.0 ± 0.2 <sup>d</sup>	2.9 ± 0.2 <sup>d</sup>	25.1 ± 4.9 <sup>f</sup>
Brown	PI656038	11.7 ± 1.7 <sup>hg</sup>	5.2 ± 0.6 <sup>f</sup>	1.2 ± 0.2 <sup>d</sup>	UD	18.1 ± 2.6 <sup>f</sup>
Brown	PI534105	5.4 ± 0.1 <sup>hg</sup>	5.1 ± 1.7 <sup>f</sup>	1.4 ± 0.1 <sup>d</sup>	1.1 ± 0.01 <sup>d</sup>	13.0 ± 1.8 <sup>f</sup>
Brown	PI533792	UD	UD	UD	1.6 ± 0.1 <sup>d</sup>	1.6 ± 0.1 <sup>f</sup>
Brown	PI576425	0.6 ± 0.1 <sup>h</sup>	UD	0.7 ± 0.03 <sup>d</sup>	UD	1.3 ± 0.1 <sup>f</sup>
Yellow	PI229838	73.7 ± 14.6 <sup>e</sup>	317.7 ± 55.5 <sup>d</sup>	42.1 ± 8.8 <sup>c</sup>	141.3 ± 26.6 <sup>c</sup>	574.8 ± 105.4 <sup>d</sup>
Yellow	PI221610	86.1 ± 2.8 <sup>d</sup>	71.4 ± 0.4 <sup>e</sup>	2.8 ± 0.1 <sup>d</sup>	3.0 ± 0.01 <sup>d</sup>	163.3 ± 3.3 <sup>e</sup>
Yellow	PI229830	3.0 ± 0.2 <sup>hg</sup>	36.5 ± 1.1 <sup>ef</sup>	1.5 ± 0.01 <sup>d</sup>	2.9 ± 0.3 <sup>d</sup>	43.9 ± 1.7 <sup>f</sup>
Yellow	PI221619	2.6 ± 0.8 <sup>hg</sup>	19.6 ± 0.4 <sup>ef</sup>	UD	1.3 ± 0.2 <sup>d</sup>	23.5 ± 1.4 <sup>f</sup>
Yellow	PI533991	3.7 ± 0.2 <sup>hg</sup>	6.4 ± 0.0 <sup>f</sup>	0.9 ± 0.1 <sup>d</sup>	0.8 ± 0.2 <sup>d</sup>	11.8 ± 0.5 <sup>f</sup>
Yellow	PI229875	2.9 ± 0.5 <sup>hg</sup>	1.3 ± 0.6 <sup>f</sup>	1.0 ± 0.1 <sup>d</sup>	1.5 ± 0.7 <sup>d</sup>	6.6 ± 1.6 <sup>f</sup>
Yellow	PI655978	UD	UD	UD	1.0 ± 0.04 <sup>d</sup>	1.0 ± 0.0 <sup>f</sup>
White	PI656079	3.0 ± 0.2 <sup>hg</sup>	1.1 ± 0.1 <sup>f</sup>	1.4 ± 0.1 <sup>d</sup>	UD	5.5 ± 0.4 <sup>f</sup>
White	PI561072	UD	UD	UD	UD	UD
White	PI656007	UD	UD	UD	UD	UD
White	PI565121	UD	UD	UD	UD	UD

\*Values are means ± S.D., n=2. Means with the different letter within same column differ significantly,  $p \leq 0.05$

**Table 1:** The contents of anthocyanins in 25 Sorghum Accessions (mg/kg DM APGE).

pericarp [27]. We conducted a pilot study by detecting carotenoid contents in the nine selected sorghum accessions with various pericarp colors. The highest contents of total carotenoids were found in the sorghum accessions with yellow pericarp, followed by brown pericarp. The lowest carotenoids were observed in the accessions with white pericarp [32]. It appeared that the phenotypic diversity of sorghum pericarp colors might be contributed, at least in part, by the contents of carotenoids.

## Conclusions

Taken together, the profile of anthocyanins was quantified in the selected 25 sorghum accessions with various phenotypic pericarp pigments. The predominant anthocyanins found in sorghums were 3-deoxyanthocyanidins that were high in one red accession and two brown accessions, followed by a yellow accession. The white accessions contained least to undetectable amount. However, a distinguishable diversity of anthocyanin contents was presented among and between the phenotypic pericarp colors, suggesting other colorful phytochemicals such as carotenoids might have interfered. Future studies by establishing a database of both anthocyanins and carotenoids in sorghum accessions with various pericarp pigments may be warranted.

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