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Original Research Article

Water stress increased the flavonoid content in tannin-free sorghum grains

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ABSTRACT

The effect of water stress on the profile of flavonoids was evaluated in three tannin-containing (SC115, SC60, and SC720) and in three tannin-free (B.Tx635, SA 5330-MARTIN, and SC373) sorghum genotypes. Flavones, flavanones and 3-deoxyanthocyanidins were analyzed by high-performance liquid chromatography with diode array detection. The water stress x genotype interaction increased the sorghum flavones (ranging from 11.3–85.5 μg/g), flavanones (0.14–157.2 μg/g) and 3-deoxyanthocyanidins (5.2–245.3 μg/g) content. The effect of water stress on 3-deoxyanthocyanidins was related to the presence of tannin in sorghum genotypes. Water stress increased the functional potential of sorghum, especially in tannin-free genotypes, by increasing the flavone, flavanone and 3-deoxyanthocyanidin content.

1. Introduction

Drought is the most important abiotic stress in modern agriculture as it causes several physiological disorders in plants reducing nutrient uptake and, consequently, plant growth and yield (Jedmowski et al., 2013; Santana et al., 2020). Exposure to water deficit stimulates the production of reactive oxygen species, which causes oxidative damage to the plant (Sánchez-Rodríguez et al., 2011). Plants have different adaptive mechanisms to reduce this damage through a cascade of antioxidants that inhibits the spread of the chain of oxidative reactions (Sánchez-Rodríguez et al., 2011). In this sense, phenolic compounds constitute one of the main classes of antioxidants that can act to detoxify free radicals (Ksouri et al., 2007).

Sorghum is more resistant to drought than other cereals, which favors its cultivation in arid regions of the world (Carvajal et al., 2019). Studies investigated the content and profile of polyphenols in six sorghum genotypes grown under either irrigation or a deficit irrigation regime (Wu et al., 2017a,b), or three levels of irrigation (Wu et al., 2017a). Hoshino and Duncan (1982) investigated the effects of water stress and other environmental conditions on sorghum tannin content and verified that water stress during the late ripening stage caused a decrease in this compound. In Brazil, Embrapa Milho e Sorgo (Brazilian Agricultural Research Corporation) and partner institutions have been conducting breeding programs seeking the selection of sorghum genotypes with improved quality for human consumption. They also investigated the effect of water stress on the proximal composition and mineral content of 100 sorghum genotypes (Queiroz et al., 2015; Paiva et al., 2017). However, there has been no investigation on the effect of water stress on the phenolic content of these sorghum genotypes. This study, is expected to contribute to the development and selection of sorghum cultivars that accumulate higher levels of antioxidant compounds in an adverse cultivation condition.

The profile of antioxidants in the sorghum grain, especially phenolic compounds, is determined by genetic factors (Taleon et al., 2014) and can be affected by grain processing (Xiong et al., 2019). In addition, biotic and abiotic stresses can affect the accumulation of these compounds in the sorghum grain (Taleon et al., 2014), increasing the content of flavonoids. There are evidence *in vitro* and in animals that phenolic compounds isolated from sorghum improve parameters related to cardiovascular diseases, cancer, dyslipidemia and other chronic diseases associated with increased oxidative stress (Cardoso et al., 2017; Taylor et al., 2014). The sorghum phenolic compounds are mainly

Abbreviations: 3-DXAs, 3-deoxyanthocyanidins; 5-MeO-LUT, 5-methoxy-luteolinidin; 7-MeO-API, 7-methoxy-apigeninidin; DAD, diode array detector; HPLC, high performance liquid chromatography.

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responsible for its high antioxidant activity, which can minimize or inhibit the action of free radicals (Cardoso et al., 2017; Taylor et al., 2014).

Thus, this study aimed to evaluate the influence of water stress on flavonoids (3-deoxyanthocyanidins, flavones and flavanones) of tannin-containing and tannin-free sorghum genotypes.

2. Materials and methods

2.1. Raw material

Six sorghum genotypes (*Sorghum bicolor* L.) selected from a panel of drought-resistant lines belonging to Embrapa Milho e Sorgo (Brazil) were used: SC115 (brown pericarp, tannin-containing); SC60 (red pericarp, tannin-containing); SC720 (light brown pericarp, tannin-containing); SC373 (yellow pericarp, tannin-free); B.Tx635 (cream pericarp, tannin-free) and SA5330-MARTIN (brown pericarp, tannin-free) (Queiroz et al., 2015).

2.2. Standards and reagents

Authentic standards of flavonoids (3-deoxyanthocyanidins: luteolinidin chloride and apigeninidin chloride; flavones: luteolin and apigenin; flavanones: naringenin and eriodictyol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The purity of all standards was above 98 %, according to the supplier's information.

Analytical grade reagents (VETEC, São Paulo, Brazil) were used to extract flavonoids. For analysis, HPLC grade reagents (acetonitrile and formic acid) were obtained from Tedia (São Paulo, Brazil).

2.3. Cultivation of sorghum grains and obtaining sorghum flour

The selected genotypes were grown in the experimental field of Embrapa Milho e Sorgo (Nova Porteirinha, MG, Brazil), from June to October 2010. The climate of this region is semi-arid, with regular rainfall. The rainless period of the year lasts for 4.7 months, from April 28 to September 19. The minimum rainfall occurs around July 26, with an average total accumulation of 0 mm. Each genotype was subjected to two conditions: 1) with irrigation (control) and 2) without irrigation (water stress). Supplemental water was applied by sprinkler irrigation for 2 h once a week. In the control condition, the irrigation remained until the grain-filling phase was complete and in the water stress condition, irrigation was suspended 50 days after planting, at the boot stage, that is just prior to the emergence of the panicle where the panicle is extended into flag leaf sheath. The other production practices (fertilizer, pesticide and management) were similar in both conditions. The experimental plots consisted of two rows three meters long, spaced 0.50 m between rows. Three hundred kg/ha of the NPK (nitrogen, phosphorus and potassium) formula 08−28-16 was applied at planting and twenty-five days after planting, 150 kg/ha of urea was applied. This is the recommended fertilizer rate for the grain sorghum production system in this region. All experiments were performed in triplicate.

After harvesting, the grains were sent to Embrapa Milho e Sorgo in Sete Lagoas, Minas Gerais, Brazil, where they were sorted and stored in a cold chamber (5 ± 2 °C), for up to two weeks. Subsequently, the grains were transported by land, at room temperature, to the Department of Nutrition and Health of the Universidade Federal de Viçosa (Minas Gerais, Brazil). In the laboratory, the grains were ground in a rotor mill (Marconi, MA 090, Brazil) to obtain flours with a particle size of 850 μm. Subsequently, the flours were packed in polyethylene bags covered with aluminum foil and stored at -18 ± 1 °C, until the time of analysis.

2.4. Flavonoid analysis

The flavonoid content (3-deoxyanthocyanidins, flavones and flavanones) was analyzed simultaneously in the sorghum grains in 3 replicates.

For extraction, 1 g of sorghum flour was added to 10 mL of 1% methanol/HCl (v: v) and stirred in a metabolic bath (Marconi, MA231, Brazil) for 2 h at 180 rpm. Then, the suspension was centrifuged (FANEM centrifuge, Excelsa Baby II, Brazil) at 2790 g, for 5 min, with the supernatant collected and its volume made up to 10 mL with 1% methanol/HCl (v: v). Subsequently, the extract was transferred into an amber bottle and stored at -18 ± 1 °C until analysis (Dykes et al., 2009), which occurred within 2 h.

The method proposed by Yang et al. (2012) and modified by Cardoso et al. (2014) was used to identify and quantify 3-deoxyanthocyanidins (luteolinidin, apigeninidin, 7-methoxy-apigeninidin and 5-methoxy-luteolinidin), flavones (luteolin and apigenin) and flavanones (naringenin and eriodictyol) from sorghum. The analyses were performed in a high-performance liquid chromatography (HPLC) system (Shimadzu, SCL 10AT VP, Japan) equipped with diode array detector (DAD) (Shimadzu, SPD-M10A, Japan), quaternary pump for high-pressure gradient (Shimadzu, LC-10AT VP, Japan), autosampler with a 500 μL loop (Shimadzu, SIL-10AF, Japan), and mobile phase degassing system with helium gas (Shimadzu, DGU-2 A, Japan).

The chromatographic conditions used included the HPLC system, column C-18 Kinetex (150 × 4.6 mm id, 5 μm) equipped with guard column C-18 (4 mm x 3 mm) (Phenomenex, Torrance, CA), temperature column at 35 °C, injection volume of 15 μL, scanning the spectrum from 200 to 700 nm with detection at 480 nm for 3-deoxyanthocyanidins, 360 nm for flavones and 280 nm for flavanones. The mobile phase was composed of 2% formic acid in ultrapure water (line A) and 2% formic acid in acetonitrile (line B). The elution gradient for B was as follows: 0−3 min, 10 % isocratic; 3−4 min, 10−12%; 4−5 min, 12 % isocratic; 5−8 min, 12–18 %; 8−10 min, 18 % isocratic; 10−12 min, 18–19 %; 12−14 min, 19 % isocratic; 14−18 min, 19–21 %; 18−22 min, 21–26 %; 22−28 min, 26–28 %; 28−32 min, 28–40 %; 32−34 min, 40-60 %; 34−36 min, 60 % isocratic; 36−38 min, 60−10%; 38−45 min, 10 % isocratic. To increase the repeatability of peak retention time, the mobile phase was degassed with helium gas (50 kPa) during the runs and the following flow gradient was used: 0−36 min, 1.0 mL/min; 36−38 min, 1.0–2.0 mL/min; 38−44 min, 1.0 mL/min; 44−45 min, 1.0–2.0 mL/min.

The identification of the flavonoids was performed by comparing the retention time and the absorption spectrum of the peaks of the standards and samples, analyzed under the same conditions. The quantification of each compound was used analytical curves constructed by injecting, in duplicate, six different concentrations of standard solutions. The 5-methoxy-luteolinidin (5-MeO-LUT) and 7-methoxy-apigeninidin (7-MeO-API) were quantified using luteolinidin and apigeninidin standards, respectively, considering the appropriate molecular weight correction factor (Dykes et al., 2009). The R^2 of the analytical curves ranged from 0.9939 to 0.9999, the detection limits were from 18.98 to 35.12 ng/mL, and the quantification limits from 94.90–175.60 ng/mL. The compound concentrations were expressed in μg/g of sample, as isolated components and as the sum of 3-deoxyanthocyanidins (3-DXAs), flavones and flavanones.

2.5. Experimental design and statistical analysis of the data

The completely randomized design was used, in a 6 × 2 factorial scheme (6 sorghum genotypes and 2 water conditions), with 3 replicates. The normality of the data was assessed using the Shapiro-Wilk test. The data were analyzed by Analysis of Variance (ANOVA) followed by Duncan's test to compare the six genotypes within the same water condition. Student's *t*-test was used to compare the means of the same genotype between water conditions. Statistical analyses were performed using the IBM SPSS software version 11.0, adopting a significance level (α) of 5%.

3. Results and discussion

3.1. Flavonoids

3.1.1. 3-Deoxyanthocyanidins

The total 3-DXAs content in sorghum grains not subjected to water stress ranged from 5.2 (B.Tx635) to 245.3 μg/g (SC60) (Table 1). These values are within the range reported by other authors in grains with different pericarp colorations (Dykes et al., 2009; Taleon et al., 2014). The total 3-DXAs content increased significantly in the tannin-free genotypes (B.Tx635: 84.2 %; SA 5330-MARTIN: 91.2 %; and SC373: 26.2 %) but remained constant in the tannin-containing genotypes. Although the genotypes had a cream- to red-colored pericarp, the 3-DXAs content increased only in tannin-free genotypes.

The increase of 3-DXAs only in tannin-free sorghum genotypes suggests that the presence of tannin, a potent antioxidant, in sorghum grain reduces the plant's need to produce 3-DXAs. In the absence of tannins, probably the plant used other defence mechanisms against water stress, increasing the 3-DXA content.

The increase in total 3-DXAs contents was demonstrated in plants submitted simultaneously to different environmental weather conditions, including the lower water availability. This effect varied with the color of the genotypes, occurring only in grains with red and lemon-yellow pericarp, but not in those with black pericarp (Taleon et al., 2012, 2014). According to these authors, these results suggest that grains with red and lemon-yellow pericarp have insufficient 3-DXAs content to react against the damage caused by environmental weather, increasing the production of 3-DXAs.

In the present study, the 3-DXAs luteolinidin, apigeninidin, 5-MeO-LUT and 7-MeO-API were found in all genotypes, in both growing conditions. These are the main 3-DXAs of sorghum and can occur concurrently in varieties with yellow to black color pericarp (Dykes et al., 2011, 2009; Taleon et al., 2012, 2014). The non-methoxylated 3-DXAs were the most prevalent forms in the analyzed genotypes in our study (luteolinidin: SC115, B.Tx635 and SC373; apigeninidin: SC720). The genotypes SC60 and SA 5330-MARTIN showed similar proportions of luteolinidin and apigeninidin. The sorghum 3-DXA profile varies widely depending on the genotype, being affected even by the secondary color of the plant (Dykes et al., 2011, 2009).

Water stress differently affected the profile of 3-DXAs in the present study, with the methoxylated forms being more susceptible to alterations. In general, there was an increase in the content and proportion of 5-methoxy-luteolinidin and 7-methoxy-apigeninidin in grains grown under water restriction. However, the contents of non-methoxylated forms of 3-DXA were maintained in four of the six sorghum genotypes analyzed (SC60, SC720, B.Tx635 and SC373). Unlike the present study, Taleon et al. (2014) found an increase in the content of all forms of 3-DXAs in sorghum grains with red and lemon-yellow pericarp, with an increase in the proportion of non-methoxylated forms. Wu et al. (2017a) also verified that the contents of the two individual 3-deoxyanthocyanidin (luteolinidin and apigeninidin) assessed increased under water stress.

The increase of 3-DXAs could be interpreted as a defense mechanism developed by some genotypes to minimize the effects of water stress, such as losses in grain quality and plant productivity. 3-DXAs are phytoalexins produced in response to the invasion of fungi or other stresses (Waniska and Rooney, 2000) and, therefore, may indicate susceptibility to these adverse conditions. In addition, water stress reduced the size of the grains of these sorghum genotypes (Queiroz et al., 2015) with an increase in the surface of the pericarp that contains these compounds. Data on grain size of these sorghum genotypes are available in supplementary Table 2 in Queiroz et al. (2015).

3.1.2. Flavanones

The total flavanone content of sorghum grains not subjected to water stress ranged from 0.14 (SC373) to 157.2 μg/g (SC115) (Table 2). Water stress significantly increased the total flavanone content of the analyzed genotypes (12.1–80.3%; on average, 46.6 %). Since flavanones are also phytoalexins, the mechanisms involved in these changes are unknown but may be similar to those described for 3-DXAs.

Naringenin was identified in all genotypes and eriodictyol in only four (SC115, SC720, B.Tx635 and SA 5330-MARTIN). The water stress x genotype interaction significantly affected the content and proportion of eriodictyol and naringenin in the grains. The observed changes were an increase in eriodictyol (B.Tx635) content, increase in naringenin (SC60, SC115 and SC373) content, and increase in both flavanones (SC720). Due to these modifications, the proportion of eriodictyol reduced 35.5 %, on average, in SC115 and SC720 genotypes.

The total flavanones were within the range observed by other authors (0−241 μg/g) (Dykes et al., 2011, 2009). Our result differed from that observed by Taleon et al. (2014), who found a reduction in flavanones in sorghum grains grown in areas with greater environmental stress. In another study (Wu et al., 2017a), only naringenin was found in six sorghum genotypes, and their concentrations were significantly affected by genotype, irrigation regime and their interaction: naringenin concentrations were highest under the intermediate deficit irrigation regime when compared to the full irrigation and severe deficit irrigation regimes across all genotypes analyzed. The difference in results can be attributed to environment interaction and the color of the pericarp of the sorghum grains.

Table 1
Effect of water stress on the profile and content of 3-deoxyanthocyanidins (μg/g) of sorghum genotypes.

Sorghum genotype	Luteolinidin		Apigeninidin		5-Methoxy-luteolinidin		7-Methoxy-apigeninidin		Total 3-deoxyanthocyanidins	
	No water stress	Water stress	No water stress	Water stress	No water stress	Water stress	No water stress	Water stress	No water stress	Water stress
SC60 (tannin-containing)	110 ± 26 ^{aA}	107 ± 13 ^{aA}	110 ± 6 ^{aA}	110 ± 17 ^{aA}	9.0 ± 1.4 ^{aB}	16.6 ± 2.0 ^{aA}	16.8 ± 2.1 ^{aA}	17.1 ± 5.3 ^{aA}	245 ± 24 ^{aA}	230 ± 15 ^{aA}
SC115 (tannin-containing)	20.1 ± 2.2 ^{bA}	15.2 ± 1.7 ^{cB}	10.2 ± 0.5 ^{cA}	8.3 ± 1.1 ^{cB}	7.0 ± 1.5 ^{aB}	7.3 ± 1.3 ^{bA}	5.0 ± 1.1 ^{bA}	6.1 ± 0.3 ^{bA}	35.1 ± 5.8 ^{cA}	36.9 ± 4.7 ^{cA}
SC720 (tannin-containing)	22.2 ± 1.2 ^{bA}	21.7 ± 3.7 ^{bA}	28.5 ± 1.0 ^{bA}	27.2 ± 3.2 ^{bA}	1.5 ± 0.1 ^{cB}	4.2 ± 1.3 ^{cA}	3.1 ± 0.6 ^{cB}	6.5 ± 1.3 ^{bA}	55.3 ± 1.5 ^{bA}	59.6 ± 5.9 ^{bA}
B.Tx635 (tannin-free)	2.6 ± 0.1 ^{dA}	2.5 ± 0.0 ^{dA}	1.2 ± 0.7 ^{eA}	1.5 ± 0.7 ^{eA}	0.8 ± 0.2 ^{dB}	2.9 ± 0.3 ^{dA}	0.6 ± 0.3 ^{dB}	2.7 ± 0.7 ^{cA}	5.2 ± 1.0 ^{fB}	9.6 ± 1.1 ^{eA}
SA 5330-MARTIN (tannin-free)	9.9 ± 1.1 ^{cB}	23.2 ± 1.5 ^{bA}	10.4 ± 0.8 ^{cB}	22.2 ± 2.8 ^{bA}	7.1 ± 1.2 ^{aA}	6.6 ± 0.9 ^{bA}	2.5 ± 0.4 ^{cB}	5.2 ± 1.0 ^{bA}	29.9 ± 2.8 ^{dB}	57.2 ± 1.9 ^{bA}
SC373 (tannin-free)	12.2 ± 2.6 ^{cA}	14.4 ± 2.6 ^{cA}	3.9 ± 0.7 ^{dA}	4.3 ± 0.5 ^{dA}	4.1 ± 1.2 ^{bB}	7.0 ± 0.3 ^{bA}	2.2 ± 0.4 ^{cA}	1.1 ± 0.1 ^{dB}	19.5 ± 1.1 ^{eB}	24.6 ± 3.3 ^{dA}

The results were expressed on a fresh matter as the average of 3 replicates ± standard deviation; Means followed by the same lowercase letter in the columns are not statistically different at 5% probability by the Duncan test; Means followed by the same capital letters in the lines are not statistically different at 5% probability by Student's *t*-test.

Table 2
Effect of water stress on the profile and content of flavanones (μg/g) of sorghum genotypes.

Sorghum genotype	Eriodictyol		Naringenin		Total flavanones	
	No water stress	Water stress	No water stress	Water stress	No water stress	Water stress
SC60 (tannin-containing)	nd	nd	2.6 ± 0.2 ^{cB}	4.1 ± 0.5 ^{eA}	2.6 ± 0.2 ^{cB}	4.1 ± 0.5 ^{eA}
SC115 (tannin-containing)	91.4 ± 5.4 ^{aA}	96.2 ± 6.3 ^{aA}	65.7 ± 4.2 ^{bB}	94.6 ± 12.0 ^{bA}	157 ± 5 ^{aB}	191 ± 6 ^{aA}
SC720 (tannin-containing)	0.7 ± 0.3 ^{cB}	1.7 ± 0.3 ^{cA}	2.5 ± 0.1 ^{eB}	17.9 ± 1.8 ^{cA}	3.2 ± 0.3 ^{cB}	19.7 ± 3.1 ^{cA}
B.Tx635 (tannin-free)	1.1 ± 0.2 ^{cB}	1.9 ± 0.2 ^{cA}	0.4 ± 0.3 ^{fA}	0.6 ± 0.2 ^{fA}	1.5 ± 0.3 ^{dB}	2.3 ± 0.2 ^{fA}
SA 5330-MARTIN (tannin-free)	23.8 ± 2.2 ^{bA}	25.5 ± 2.0 ^{bA}	104 ± 7 ^{aA}	118 ± 10 ^{aA}	120 ± 8 ^{bB}	135 ± 5 ^{bA}
SC373 (tannin-free)	nd	nd	0.14 ± 0.01 ^{dB}	0.72 ± 0.09 ^{dA}	0.14 ± 0.01 ^{dB}	0.72 ± 0.09 ^{dA}

The results were expressed on a fresh matter as the average of 3 replicates ± standard deviation; Means followed by the same lowercase letter in the columns are not statistically different at 5% probability by the Duncan test; Means followed by the same capital letters in the lines are not statistically different at 5% probability by Student's *t*-test; nd: not detected.

3.1.3. Flavones

The water stress x genotype interaction significantly affected the total flavone content of the grains with an increase in the total flavone content in most of the genotypes (SC115: 18.3 %, SC720: 40.0 %, SA 5330-MARTIN: 35.8 % and SC373: 18.1 %).

Sorghum grains not subjected to water stress had a total flavone content (Table 3) similar to that reported by other authors (Dykes et al., 2011, 2009). Taleon et al. (2014) found a significant increase in flavones in plants that showed physical damage due to environmental weather, including lower water availability.

Both flavones were identified in the six genotypes analyzed, in both cultivation conditions. Luteolin was the main flavone in three genotypes (SC60: 82.7 %; SC720: 72.2 %; SC373: 60.1 %) and apigenin was the main component in the other genotypes (SC115: 57.8 %; B.Tx635: 72.6 %; SA 5330-MARTIN: 58.4 %). The concomitant occurrence of luteolin and apigenin was observed in sorghum genotypes with black, red and lemon-yellow color grown in environments subject to simultaneous environmental weather conditions (Taleon et al., 2012, 2014).

The luteolin and apigenin content of sorghum was significantly affected by water stress, and this effect was strongly influenced by the genotype. In the present study, the luteolin content decreased and the apigenin content increased in the SC60 and SC720 genotypes. In addition, an increase in apigenin (SC115) or luteolin (SC373), simultaneous increase (SA 5330-MARTIN) or maintenance of luteolin and apigenin (B.Tx635) contents was observed in the sorghum grains. The different changes in the flavone profile resulted in an increase of, on average, 21 % in the proportion of apigenin in four genotypes (SC60, SC115, SC720 and SA 5330-MARTIN) and an increase (9.1 %) in the proportion of luteolin in the genotypes SC373 and B.Tx635. In another study (Wu et al., 2017b), luteolin concentration was higher in deficit than in full irrigation treatment across all genotypes analyzed.

Table 3
Effect of water stress on the profile and content of flavones (μg/g) of sorghum genotypes.

Sorghum genotype	Luteolin		Apigenin		Total flavones	
	No water stress	Water stress	No water stress	Water stress	No water stress	Water stress
SC60 (with tannin)	47.0 ± 2.4 ^{aA}	33.9 ± 4.0 ^{bcB}	9.8 ± 8.1 ^{dB}	22.6 ± 2.5 ^{cA}	56.8 ± 9.6 ^{bA}	56.5 ± 3.0 ^{cA}
SC115 (with tannin)	36.0 ± 2.8 ^{bA}	38.0 ± 1.4 ^{bA}	49.4 ± 6.3 ^{aB}	66.5 ± 4.4 ^{aA}	85.5 ± 5.9 ^{aB}	104.5 ± 3.5 ^{aA}
SC720 (with tannin)	27.7 ± 2.4 ^{cB}	33.7 ± 2.1 ^{cA}	9.7 ± 3.4 ^{dB}	28.6 ± 2.5 ^{bA}	37.3 ± 4.6 ^{cB}	62.3 ± 2.5 ^{cA}
B.Tx635 (without tannin)	5.5 ± 0.9 ^{dA}	6.7 ± 0.8 ^{eA}	14.6 ± 3.2 ^{cA}	15.9 ± 0.1 ^{dA}	20.1 ± 4.1 ^{dA}	22.6 ± 1.0 ^{dA}
SA 5330-MARTIN (without tannin)	4.7 ± 0.9 ^{dB}	8.4 ± 0.5 ^{dA}	6.6 ± 1.8 ^{eB}	9.2 ± 1.0 ^{eA}	11.3 ± 2.3 ^{eB}	17.6 ± 1.5 ^{eA}
SC373 (without tannin)	38.6 ± 2.5 ^{bB}	52.2 ± 4.0 ^{aA}	25.0 ± 2.9 ^{bA}	25.5 ± 3.7 ^{bcA}	63.6 ± 4.9 ^{bB}	77.8 ± 2.7 ^{bA}

The results were expressed on a fresh matter as the average of 3 replicates ± standard deviation; Means followed by the same letter in the columns are not statistically different at 5% probability by Duncan's test; Means followed by the same capital letters in the lines are not statistically different at 5% probability by Student's t test.

The mechanisms by which water stress modifies the profile of 3-DXAs, flavanones and flavones in sorghum are still unknown. The results obtained in our study and also by other authors suggest that environmental stresses may affect differently the biosynthetic pathways of these compounds, which demonstrates the need for studies to evaluate these effects at the molecular level. Thus, the effects of abiotic agents on the expression of genes and proteins (enzymes) involved in the synthesis of flavonoids need to be evaluated. In addition, the interpretation of the results of water stress effect on bioactive compounds in sorghum must be linked to the analysis of plant productivity, allowing to infer how the increase in the content of these compounds is economically and commercially viable.

4. Conclusion

The effects of water stress on 3-deoxyanthocyanidin contents were conditioned to the presence of tannin in sorghum genotypes. Water stress increased the total of 3-deoxyanthocyanidins in tannin-free sorghum genotypes. Water stress increased the flavones in four genotypes and the flavanones in all genotypes analyzed. Since the effect of water stress on sorghum flavonoids profile varied according to the genotype, this study demonstrated the importance of selecting varieties adapted or tolerant to drought in order to achieve high yields in this adverse condition, as well as a high content of antioxidant compounds in sorghum grains.

Author statement

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Leandro de Morais Cardoso: Conceptualization, Methodology, Writing - review & editing

Pamella Cristine Anunciação: Conceptualization, Writing - review & editing

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Declaration of Competing Interest

The authors report no declarations of interest.

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