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Phenolic profile and content of sorghum grains under different irrigation managements

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ABSTRACT

Sorghum grain is widely consumed in Sub-Saharan Africa and Asia, as a staple food due to its adaptation to harsh environments. The impact of irrigation regime: full irrigation (100%); deficit irrigation (50%); and severe deficit irrigation (25%) on phenolic profile and content of six sorghum grain genotypes was investigated by high performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS). A total of 25 individual polyphenols were unequivocally or tentatively identified. Compared to the colored-grain genotypes, the white grained sorghum var. Liberty had a simpler polyphenol profile. The concentrations of the sorghum-specific 3-deoxyanthocyanidins luteolinidin and apigeninidin, were higher under deficit irrigation compared to the other two regimes in all genotypes. These findings will be valuable for the selection of sorghum genotypes for grain production as human food under water deficit conditions, since polyphenol levels can affect the grain's nutritional value and health properties.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most valuable global cereal crop, widely grown in semi-arid and arid regions of the world because of its tolerance to drought and high temperatures (Taylor, Schober, & Bean, 2006). In many parts of Africa and Asia, sorghum grain provides nutrients and energy for millions of local people, whereas in the developed countries such as the USA and Australia, it is used primarily as an animal feed or for biofuel production (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). However, the number of people consuming sorghum grain is slowly but steadily increasing in developed countries mainly due to sorghum's gluten-free property and antioxidant potential from polyphenolic phytochemicals (Taylor et al., 2006).

Polyphenols have antioxidant activity due to their free-radical scavenging capability, and thus may protect against some chronic diseases, such as coronary heart disease and type 2 diabetes (Dykes & Rooney, 2007). Polyphenols in sorghum grain consist of simple phenolic acids (e.g. ferulic and *p*-coumaric acids), 3-deoxyanthocyanidins, flavanones, flavones and other flavonoids, as well as condensed tannins (Awika & Rooney, 2004). In particular, the 3-deoxyanthocyanidins, including apigeninidins, luteolinidins, 5-methoxyluteolinidin and 7-methoxyapigeninidin, are at high levels in some sorghum grain genotypes, but are absent in other cereal grains (Awika & Rooney, 2004; L Dykes & Rooney, 2007). The amounts and profiles of polyphenols in sorghum grain vary significantly between genotypes. For example, it has been reported that red and yellow sorghum genotypes contained high amounts of flavones, and sorghum genotypes with pigmented testa have higher content of condensed tannins (Taleon, Dykes, Rooney, & Rooney, 2014; Wu, Johnson, Bornman, Bennett, Singh, Simic, et al., 2016).

Under a changing climate, annual mean precipitation is projected to decrease in many mid-latitude and subtropical dry regions, in which crops, such as sorghum, maize and pearl millet, will invariably suffer from moisture stress (Pachauri et al., 2014). Polyphenol content and antioxidant activity of plant materials may be affected by water deficit, and their changes depend on plant species (Cohen & Kennedy, 2010). Tovar, Motilva, and Romero (2001) planted young olive trees under seven irrigation treatments. They found that the concentration of the dialdehydic form of elenolic acid and oleuropein aglycon of the olive

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oils and the antioxidant activity significantly increased as the amount of irrigation water decreased to deficit levels. Buendía, Allende, Nicolás, Alarcón, and Gil (2008) investigated the effects of regulated deficit irrigation and full irrigation on polyphenols and antioxidant activity of peaches, and reported that the content of phenolics, mainly anthocyanins and procyanidins, and antioxidants increased under regulated deficit irrigation. In another study, comparing irrigated and non-irrigated grapevines, the levels of proanthocyanidins and flavonols increased in fruit from irrigated vines (Zarrouk et al., 2012). There is little information in the literature from controlled studies investigating how level of irrigation influences profile and concentrations of polyphenols of sorghum grain. In our recent study, it was found that the levels of total polyphenol and antioxidant activity of sorghum grain significantly increased when the amount of water was reduced (Wu, Johnson, Bornman, Bennett, & Fang, 2017). However, individual polyphenols of sorghum grain were not measured in the previous study, and it is also still unknown how irrigation treatment influences the profile of polyphenols in sorghum grain.

Therefore, in the present study, using an as yet unreported trial, the effects of three levels of irrigation treatments on the individual phenolic compounds of six different sorghum genotypes were determined by the powerful analytical technique of high performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS).

2. Materials and methods

2.1. Plant material and treatments

The sorghum field experiment was conducted at Curtin University's Field Trials Area, Western Australia (latitude 32°00′S, longitude 115°53′E, altitude 20 m). Daily rainfall and minimum/maximum air temperature were obtained from the Perth Airport Bureau of Meteorology weather station 9.6 km away from the experimental site (Supplementary Fig. S1) (BOM, 2013).

Six sorghum genotypes comprised of two hybrid lines (‘Liberty’ white pericarp and ‘MR Bazley’ red pericarp) and four inbred lines (‘Alpha’ red pericarp; ‘IS1311C’ and ‘IS8237C’ both brown pericarp; and ‘Shawaya Short Black 1’, dark red-black pericarp). All seeds were provided from the Australian sorghum pre-breeding program, a partnership between the University of Queensland, the Queensland Department of Agriculture and Fisheries and the Grains Research and Development Corporation, courtesy of Professor David Jordan. All samples were planted in 1 m × 1 m fibre glass pots with a depth of 0.5 m. One row each of three sorghum genotypes were planted in each pot, with a row spacing of 0.25 m. Each row was sown on 9th January 2014 with 10 seeds of the nominated variety and thinned to five plants spaced 0.2 m apart after two weeks. The experiment of 6 genotypes × - three levels of irrigation was carried out in two replications with a randomised complete block design.

The potential reference crop evapotranspiration (PET_0) from the nearby weather station was 822.7 mm from sorghum sowing date to maturity 10th May. In the same period, the crop potential evapotranspiration under standard conditions (PETc) was calculated from PET_0 and the Food and Agricultural Organization (FAO) crop coefficient (Kc) for sorghum, giving a PETc of 576.25 mm (Allen, Pereira, Raes, & Smith, 1998). The experimental irrigation implementation was based on PETc. Three irrigation regimes were applied: full irrigation (FI, 100% PETc), deficit irrigation (DI, 50% PETc) and severe deficit irrigation (SDI, 25% PETc).

The sowing date was defined as 0 day after sowing, and all plants received unlimited water in the first two weeks. After that, all irrigation treatments were applied by hand watering. Sorghum was irrigated every 3–4 days with a total of 24 irrigations during the growing season. All grains were harvested at maturity, air-dried to a moisture content of around 10%, manually cleaned, vacuum packed and stored at − 20 °C until analysis.

2.2. Physical characteristics of grain

The Single Kernel Characterization System (SKCS 4100, Perten Instruments, Hägersten, Sweden) was used to evaluated the physical characteristics of sorghum grain, and all samples were evaluated in duplicate.

2.3. Phenolic extraction

All sorghum whole grains were milled to pass 100% through a 500 μm sieve using a grain mill (CEMOTEC 1090, Foss Tecator, Hoganäs, Sweden). For free and bound polyphenols, extraction was conducted according to the method of Svensson, Sekwati-Monang, Lutz, Schieber, and Gänzle (2010) with some modifications. In brief, the 15 mL of 80% (*v*/v) aqueous methanol was mixed with around 2 g of the ground sample under N_2, and the mixture was shaken in the water bath at 25 °C for 2 h. The supernatant was collected after centrifuging at 3220 × *g* for 10 min at 4 °C. The residue was extracted with 20 mL 80% (*v*/v) aqueous methanol two times more, and all supernatants were combined after centrifuging. Rotary vacuum evaporation was carried out to evaporate supernatants to dryness. The resulting solid was re-dissolved in 10 mL of methanol and stored at − 20 °C under N_2. The residue remaining after free polyphenol extraction was the used for bound phenolic extraction.

For the extraction of bound phenolic compounds, the residue after free polyphenol extraction was mixed with 15 mL of 2 M hydrochloric acid (HCl) under N_2 in the water bath at 100 °C for 1 h. After hydrolyzing, the 15 mL ethyl acetate was added and thorough mixed. Then, the ethyl acetate fraction was collected after partitioning. The hydrolysate was re-extracted with the 15 mL ethyl acetate four times more, and all ethyl acetate fractions were combined and evaporated to dryness. The resulting solid was re-dissolved in 10 mL of methanol and stored at − 20 °C under N_2 before analysing.

2.4. HPLC-DAD-ESI-MS^n analysis

An Agilent 1290 UHPLC system with diode array detector (DAD) and Agilent 6460 LC-QQQ LC-MS/MS System (Agilent Technologies, Palo Alto, CA, USA) were used to separate polyphenols according to the procedure described in detail previously (Wu, Johnson, Bornman, Bennett, Clarke, et al., 2016). Briefly, the Kinetex XB-C 18 reversed phase-HPLC column (5 μm, 250 × 4.6 mm, Phenomenex, Torrance, CA, USA) was used to separate individual phenolic compounds, and the scanning range of the DAD was set between 190 and 600 nm at steps of 2 nm. Solvent A was 0.1% formic acid in LC-MS grade water (Honeywell Burdick & Jackson, Gillman, SA, Australia), and solvent B consisted of LC-MS grade acetonitrile (Honeywell Burdick & Jackson, Gillman, SA, Australia). Extract (5 μL) was injected and the following linear gradient elution was used: 5%–15% B (5 min), 15%–50% B (40 min), 50%–70% B (2 min), 70%–100% B (1 min), 100% B (7 min), 100%–5% B (1 min), 5% B (9 min). The rate of flow was 0.5 mL/min.

Mass spectra were performed in the ESI negative mode with a scan time of 2000 MS under the following conditions: gas (N_2) 5 L/min at 300 °C, nebulizer 45 psi, sheath gas (N_2) 11 L/min at 250 °C, capillary voltage − 3.5 kV and nozzle voltage − 500 V. Phenolic compounds were detected by full scan ranging from m/z 50 to 1300.

2.5. Quantification of polyphenols

The following individual authentic standards were used for quantitation. Ferulic acid, caffeic acid, luteolin, apigenin, taxifolin and naringenin were purchased form Sigma–Aldrich (St. Louis, MO, USA). Luteolinidin chloride, and apigeninidin chloride were purchased from Alsachim (Strasbourg, France). Results were expressed as μg/g sample

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