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# Individual polyphenolic profiles and antioxidant activity in sorghum grains are influenced by very low and high solar UV radiation and genotype

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ABSTRACT

Sorghum is becoming more widely recognised around the world as a valuable crop for its polyphenol antioxidant health-promoting properties and adaptation to harsh environments. The antioxidant capacity of diverse polyphenols in sorghum grain can vary with climatic conditions and genotype. To explore this further, the potential role of UV radiation on the profile and concentration of polyphenols was investigated in six diverse sorghum genotypes in a controlled environment facility using natural UV and visible radiation under either UV-transmitting or UV-blocking treatments. The polyphenol content and antioxidant activity were significantly reduced under the UV-blocking treatment, with contents of individual polyphenols differing among the genotypes. This information is valuable for sorghum breeders enabling them to select genotypes and UV growth conditions to produce sorghum grain with target levels of polyphenols and antioxidant capacity for human foods to meet the nutritional and health needs of different consumers.



## 1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth largest most important cereal globally. It is widely planted in more than 30 countries of the semi-arid tropics and is tolerant to drought and high temperature (Stefoska-Needham et al., 2015). Sorghum is also part of the dietary staple for more than 300 million people in developing countries, representing their major source of energy and nutrients (Althwab et al., 2015). In contrast to developing countries, sorghum is primarily used as animal feed or for ethanol production in developed countries, such as in the USA and Australia (Stefoska-Needham et al., 2015). Recently, particularly in developed countries, several sorghum products have been developed, including bread, pasta and biscuits (Khan et al., 2013; Stefoska-Needham et al., 2016; Yousif et al., 2012), due in large part to the gluten-free and antioxidant activity of the sorghum grain (Althwab et al., 2015; Taylor et al., 2006).

In sorghum grain, polyphenols are the main contributors to the antioxidant capacity (Dykes and Rooney, 2007). The consumption of sorghum foods with high levels of polyphenols may play a positive role in reducing the risk of chronic diseases, such as breast and colon cancer, and diabetes (Stefoska-Needham et al., 2015). Sorghum genotypes vary widely in their profile and levels of phenolics with those having a pigmented testa and spreader genes ($B_1B_2S$) showing particularly high levels of phenols and antioxidant activity (Dykes et al., 2005). A simpler profile of polyphenols occurs in unpigmented white sorghum compared to that in pigmented coloured sorghums (Wu et al., 2016); and the profile and level of polyphenol expression also changes with genotype and growth conditions, such as temperature and water stress (Wu et al., 2016, 2017).

An increasingly variable climate coupled with frequent episodes of prolonged extreme events such as drought or high temperatures,

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pose a challenge to agriculture and food security, making the study of stress-tolerant crop plants and selected genotypes important for future agriculture and sustainable livelihoods. Although the Montreal Protocol has been an unmitigated success in curbing further drastic decreases in stratospheric ozone, future levels of UV-B radiation (280–315 nm) – that part of the spectrum most responsive to absorption by ozone – may be modulated by changing climate factors as a consequence of accelerated global warming (Andrady et al., 2017). This will likely result in decreased cloud cover over some regions, reduced expanses of snow and ice, as well as changes in global circulation patterns. Projected decreases in aerosols over some areas of the globe will likely increase the amount of UV radiation reaching ground level. On the other hand, the predicted recovery of the stratospheric ozone layer due to the Montreal Protocol to historical levels, coupled with renewed global efforts to reduce greenhouse gases may result in an increase in ozone concentration above that of historical levels in some regions. This in turn would lead to less UV radiation reaching ground level, which therefore could have consequences for natural and agricultural ecosystem growth and development (Butler et al., 2016).

One example of the impact of a change in exposure to UV radiation is the production of secondary plant metabolites, such as antioxidant polyphenols, which are generally enhanced by exposure to UV-B radiation (Schreiner et al., 2012). For instance, in a study where tomato grown under both UV-transmitting and UV-blocking filters, the total concentration of caffeic acid, *p*-coumaric acid, and ferulic acid was approximately 20% higher under UV-transmitting compared to UV-blocking filters (Luthria et al., 2006). Similarly, a study of strawberries grown under two films that differed in their ultraviolet transparency showed that total polyphenol and antioxidant activity were significantly lower in plants grown under the 100% UV-blocking film compared to the 70% UV-transmitting film (Josuttis et al., 2013). However, the effect of exposure to different levels of UV radiation on polyphenols of sorghum grain is still unknown.

In the present study, we investigated the potential of UV radiation to change the individual polyphenol content and antioxidant activity in different genotypes under controlled growth conditions using ambient radiation. High performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry (HPLC-DAD-ESIMS) was used to identify and quantify individual polyphenols.

## 2. Experimental

### 2.1. Plant material and treatments

Six sorghum genotypes: white pericarp 'QL12', brown pericarp 'IS131C', black pericarp 'Shawaya Short Black 1', and three red pericarp genotypes 'QL33/QL36', 'B923296' and 'QL33' were selected for this study. The experiment was conducted at Curtin University Field Trials Area, Perth, Western Australia, in a controlled environment facility in which the treatments involved shielding the plants from the solar radiation passing through the UV transparent "glasshouse" using two contrasting plastic films (BPI Polythene, British Polythene Industries, Greenock, UK). The UV-transmitting film was Lumisol Clear AF (200 μm thick, with the following specifications: a transparency of ca 94%, 84% and 93% for UV-A, UV-B and photosynthetically active radiation (PAR, 400–700 nm), respectively). Lumivar Clear AF was used for the UV-blocking film (200 μm thick, with ca 4%, 0% and 91% for UV-A, UV-B and PAR, respectively). The manufacturer specifications of the UV and PAR transparency of the films were checked with an MU-200 and MQ-200 quantum sensor (Apogee Instruments, Inc., Utah, USA), respectively, during the experimental trials. The visible and UV radiation measurements were carried out under clear sky conditions at noon in Perth, Western Australia, during the summer months of February and March, with four readings taken over each month. The average values of PAR, using the MQ-200 quantum sensor, outside of the experimental glasshouse for February were 1870 W $m^{-2}$, and for March 1940 W $m^{-2}$; and under the UV-transmitting and blocking filters for February and March, respectively, these were 1645 and 1726 W $m^{-2}$, and 1443 and 1687 W $m^{-2}$. Average solar UV radiation outside of the experimental glasshouse for February was 59.2 W $m^{-2}$, and for March, 57.9 W $m^{-2}$, using the MU-200 quantum sensor. Under the UV-transmitting and blocking filters for February and March, respectively, UV radiation was measured as 37.4 and 38.8 W $m^{-2}$, and 0.5 and 0.75 W $m^{-2}$. We confirmed the manufacturer specifications through finding similar values of PAR for the two types of plastic film, with the UV transparency of the transmitting and blocking films being around 70% and 1% of natural solar ultraviolet radiation, respectively. The difference in the UV transparency readings compared to the specifications was likely due to the glasshouse construction materials.

The sorghum seed was sown on 9th December 2014. Five seeds were sown into each pot, under which a larger plate was placed, such that water could be reserved in the plate, and each genotype was planted in six pots under each UV treatment. Plants were thinned to one germinated seedling per pot two weeks after sowing and irrigated daily with water under the UV-transmitting and UV-blocking treatments. Air temperature was recorded for the duration of the trials with thermometers positioned in different locations of the greenhouse, which showed that the greenhouse temperature was evenly controlled. The experiment was set up as a split-plot randomised design with six replications of each genotype randomised under two blocks of each treatment. All samples were harvested at grain maturity at the beginning of April 2015. After harvesting, grains were air-dried (to a moisture content of ca 10%), manually threshed and cleaned, vacuum packed and stored at −20 °C until analysis.

### 2.2. Standards and reagents

Potassium persulphate, trolox, 2,2′–azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2-2-diphenyl-1-picrylhydrazyl (DPPH), ethyl acetate, methanol, formic acid, hydrochloric acid, naringenin, apigenin, luteolin, caffeic and ferulic acids were all obtained from Sigma-Aldrich (St. Louis, MO, USA). LC-MS grade water and acetonitrile were purchased from Honeywell Burdick & Jackson (Gillman, SA, Australia). Taxifolin, luteolinidin chloride, and apigeninidin chloride were obtained from EXTRASYNTHESE (Neuville-sur-Saône, France). All chemicals were of analytical or HPLC grade.

### 2.3. Polyphenol extraction

A grain mill (CEMOTEC 1090, Foss Tecator, Hoganäs, Sweden) was used to grind sorghum grains ensuring 100% of the grain passed through a 500 μm sieve. The extraction of free and bound polyphenols followed the method of Svensson et al. (2010). In brief, 80% (v/v) aqueous methanol (15 ml) was mixed with the ground sample (2 g) under $N_2$ for 2 h in a shaking water bath at 25 °C. The mixture was centrifuged at 3, 220 × g for 10 min at 4 °C to collect the supernatant. Then, the residue was extracted twice more, and all supernatant were combined and evaporated to dryness with a vacuum rotary evaporator. The resulting solid was re-dissolved in 10 mL methanol and stored at −20 °C under $N_2$ in the dark until analysis. Each sample was extracted in duplicate.