School of Public Health

Implications of Genotype and Climate Variability on Phenolic Compounds of Grain: a Sorghum Case Study

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DECLARATION

To the best of my knowledge and belief this thesis titled “IMPLICATIONS OF GENOTYPE AND CLIMATE VARIABILITY ON PHENOLIC COMPOUNDS OF GRAIN: A SORGHUM CASE STUDY” contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is a drought and heat tolerant cereal crop that is widely used to manufacture staple foods in semi-arid areas such as sub-Saharan Africa. It is consumed by humans mainly in developing countries, such as Sudan and India, while it is mainly utilized for animal feed or biofuel in developed countries like the USA and Australia. Increasing global climate variability, including rising temperatures, increased water scarcity in some already arid regions and solar ultraviolet (UV) radiation changes, has the potential to modify the composition of sorghum grain, for instance polyphenols, and therefore may have implications for the health of those humans who consume sorghum foods. This research investigates how polyphenols in sorghum grain will be affected by sorghum genotype, and growth temperature, irrigation and exposure to solar ultraviolet (UV) radiation during plant growth.

The first study investigated the way in which how sorghum polyphenols are affected by genotype, growth temperatures and their interaction. The profile and level of polyphenols and antioxidant activity were evaluated in the wholegrains of six sorghum genotypes: CCH2, PI563516, AQL33/QL36, CCH1, Ai4 and IS 8525, under two day/night temperature regimes, 32/21°C (optimum temperature, OT) and 38/21°C (high temperature, HT). Sorghum IS 8525 and PI563516 had the highest and lowest concentrations of total polyphenols, respectively. The HT regime significantly reduced total concentrations of polyphenols in sorghum IS 8525, while the other five genotypes were not significantly influenced by the temperature treatments. High performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry (HPLC-DAD-ESIMS) was used to identify individual polyphenols and 23 were positively or tentatively identified in these grains. Compared to the white sorghum PI563516, more individual polyphenols were identified in the other five coloured genotypes. Results also indicated that HT could significantly decrease both free luteolinidin and apigeninidin concentrations, which indicated that the synthesis of 3-deoxyanthocyanins was inhibited. According to 2, 2'–azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) and 2-2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) assays, the highest and lowest values of antioxidant activity

were found in IS 8525 and PI563516, respectively. In addition, polyphenol content of sorghum grain was positively correlated with antioxidant activity.

To find out how *in vitro* iron availability is affected by genotype, growth temperatures and their interaction, the contents of mineral, phytate and tannin were evaluated in the six sorghum genotypes under both OT and HT regimes. The tannin concentration was significantly reduced under HT. The phytate content was significantly influenced by genotype, while diverse effects of the growth temperature regime were observed. An analysis of the effect of cooking showed that the tannin content was significant reduced, but that the phytate content was unaffected across all genotypes. The *in vitro* iron availability was significantly influenced by sorghum genotypes, and HT significantly reduced the *in vitro* iron availability, with the exception of Ai4, which showed a significant increase ($p \leq 0.05$).

In the second study, the influence of water availability on the content and profile of polyphenols and antioxidant activity in six sorghum genotypes was assessed. Liberty, CHH1, Alpha, B963676, IS8237C and Shawaya Short Black 1 were grown under three irrigation regimes: full irrigation (100%), deficit irrigation (50%) and severe deficit irrigation (25%). The values of polyphenols and antioxidant activity were significantly influenced by irrigation, genotype and irrigation × genotype interaction ($p \leq 0.05$). Compared to full irrigation and severe deficit irrigation regimes, the values of total phenolics total phenolics and antioxidant capacity of sorghum wholegrain were higher under the deficit irrigation regime. In addition, the contents of individual polyphenols were also significantly affected by irrigation. For example, luteolinidin and apigeninidin, were higher in sorghum grown under the deficit irrigation regime as compared to the other two regimes.

In the third study, the effect of blocking solar UV radiation on the profile and concentration of polyphenol and antioxidant activity in the wholegrain of six sorghum genotypes was investigated. Shawaya Short Black 1, QL33, QL12, B923296, QL33/QL36 and IS1311C were grown under UV-transmitting and UV-blocking treatments. Genotype, UV treatment and genotype×UV treatment showed a significant effect on levels of polyphenol and antioxidant activity, with the wholegrain of Shawaya Short Black 1 and QL12 having the highest and lowest values of total polyphenol and antioxidant activity, respectively. Total polyphenol concentrations were significantly

reduced in grain from sorghums grown under the UV-blocking treatment. Specifically, the contents of 3-deoxyanthocyanidins were significantly reduced, which suggested that UV radiation may affect 3-deoxyanthocyanidin formation in sorghum.

These results provide new information on the ways in which temperature, water availability and exposure to UV radiation modify the phytochemical properties of sorghum grain. This information can be used to assist the selection of both optimum genotype and optimum environment for sorghum production. This will be particularly important given the projected rapid changes in climate, so that breeding and growing of sorghum with specific concentrations of polyphenols can be well managed. For instance, sorghum grain with high values of polyphenols and antioxidant activity would be important for health protective functional foods, or in other cases, low levels may be advantageous for maximising nutrient (e.g., iron) dialysability.

Given that polyphenols in general are greatly modified upon their ingestion and absorption by humans, an understanding of the gastrointestinal handling of sorghum polyphenols (e.g., the unique 3-deoxyanthocyanins) is also important. Additionally, measurements of the cellular antioxidant activity (CAA) are needed to evaluate antioxidant activity of sorghum polyphenolics and the grain and food products in which they are contained. This cellular assay incorporates some aspects of uptake, metabolism, and location of antioxidant compounds within cells, which may provide more in-depth and biologically relevant understanding of the bioactivity of sorghum polyphenols. Therefore, further research is required to better elucidate antioxidant capacity of sorghum grain by chemical assays as well as cellular based assays.

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PUBLICATIONS

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CONFERENCE PRESENTATIONS

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AWARDS

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LIST OF ABBREVIATIONS

ABTS	2, 2'–azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
AP	Apigenin
API	Apigeninidin
CA	Caffeic acid
DAD	Diode array detector
DAFWA	Department of Agriculture and Food Western Australia
db	Dry basis
DF	Deficit irrigation
DPPH	2-2-diphenyl-1-picrylhydrazy
ESIMS	Electrospray ionization mass spectrometry
FA	Ferulic acid
FAI	Ferulic acid isomer
FI	Full irrigation
HPLC	High performance liquid chromatography
HT	High temperature
LUT	Luteolinidin
LU	Luteolin
NAR	Naringenin
OT	Optimun temperature
SD	Standard deviation
SDF	Severe deficit irrigation
TA	Taxifolin
TAI	Taxifolin isomer
UV	Ultraviolet
WHO	World Health Organization

CHAPTER ONE: GENERAL INTRODUCTION

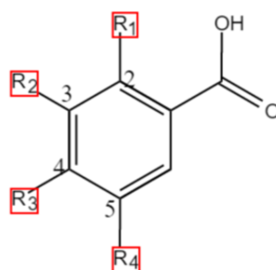
1.1. BACKGROUND

Sorghum (*Sorghum bicolor* (L.) Moench), a member of the grass family, is the fifth largest cereal crop in the world after rice, wheat, barley and maize. The global production of sorghum was 63.89 million metric tons in 2015, and reached 66.71 million metric tons in 2016 (United States Department of Agriculture, 2016a). In comparison with other cereal crops, sorghum is widely planted in tropical and semi-arid areas, especially in Asia, India and Africa, due to its high yields under diverse environmental stresses (e.g., high temperature and drought) (de Oliveira et al., 2017). The majority of sorghum grain produced in developing countries is consumed by humans, and the demand for it is still growing, particularly in West Africa and India, due to the growing population and its industrial utilization, such as for ethanol (Dicko, Gruppen, Traoré, Voragen, & Van Berkel, 2006; Prasad, Singh, Jain, & Joshi, 2007). For instance, the production of sorghum has increased significantly from 10 million to 26 million metric tons in Africa over a period of 40 years (Dicko et al., 2006; Jacob, Fidelis, Salaudeen, & Queen, 2013). In addition, a wide variety of foods and beverages are prepared from sorghum grain, such as porridge, couscous, baked goods, beer and spirits (Anglani, 1998; Kayodé, Hounhouigan, Nout, & Niehof, 2007; Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Sorghum is also widely grown in some developed countries such as the USA, which is the leading global sorghum grain producer (United States Department of Agriculture, 2016a). In Australia, about 60% of the sorghum grown is planted in Queensland, with the remaining 40% grown in northern New South Wales (United States Department of Agriculture, 2016b). Around 65% of Australian sorghum grain has been exported to other countries, while the remainder is mainly utilized for animal feed or biofuel production (United States Department of Agriculture, 2016b; Stefoska-Needham et al., 2015). However, the number of people, who consume food that includes sorghum grain, is gradually and steadily increasing in Australia and other developed countries, probably because sorghum grain is gluten free and contains high levels of health-promoting components, such as polyphenols and carotenoids (Khan, Yousif, Johnson, & Gamlath, 2013; Khan,

Yousif, Johnson, & Gamlath, 2014).

Polyphenols are a group of chemical compounds containing one or more aromatic rings bearing one or more hydroxyl substituents. Polyphenols are mainly concentrated in the pericarp of cereal grains (Shirley, 1998) and can affect the flavour, appearance and antioxidant activities of sorghum grains (Dykes & Rooney, 2007). Wide variations among the total polyphenol content of different cereals have been reported, and sorghum grain can contain a higher total polyphenol content than other cereal grains depending on the genotype. There are three main categories of polyphenols in sorghum grains, namely phenolic acids, flavonoids and condensed tanninsSorghum grain genotypes with different levels of polyphenols have been developed and grown in countries other than Australia (Dlamini, Taylor, & Rooney, 2007; Flores-Naveda, 2016), but with limited information available on polyphenolic compounds of Australian sorghum genotypes.

The phenolic acids are composed of two classes: hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids have a C_6–C_1 structure and those in sorghum include gallic, protocatechuic, vanillic, *p*-hydroxybenzoic and syringic acids. The common C_6–C_3 structure is presented in hydroxycinnamic acids, which in sorghum include sinapic, ferulic, *p*-coumaric and caffeic acids (Awika & Rooney, 2004). The structures of these compounds are presented in Figure 1.1. Both free and bound forms of phenolic acids have been found in sorghum grains. Free phenolic acids are in the outer layer of the pericarp, and organic solvents (e.g., methanol) are adequate to extract them effectively. Since bound phenolic acids can form covalent bonds with the cell wall structural compounds (e.g., cellulose and hemicellulose) (Wong, 2006), acidic or alkaline solutions are required to hydrolyze and release them for extraction and analysis (Stalikas, 2007).



Hydroxybenzoic acids

Gallic acid: R_1= H, R_2 = R_3 = R_4 = OH

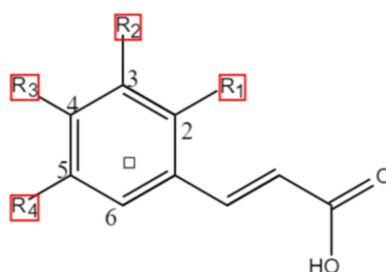
p-hydroxybenzoic acid: R_1= R_2 = R_4 = H, R_3 = OH

Protocatechuic acid: R_1= R_4 = H, R_2 = R_3 = OH

Gentisic acid: R_1= R_4 = OH, R_2 = R_3 = H

Syringic acid: R_1= H, R_2 = R_4 = OCH_3, R_3 = OH

Salicylic acid: R_1= OH, R_2 = R_3 = R_4 = H



Hydroxycinnamic acids

Caffeic acid: R_1= R_4 = H, R_2 = R_3 = OH

Ferulic acid: R_1= R_4 = H, R_2 = OCH_3, R_3 = OH

p-coumaric acid: R_1= R_2 = R_4 = H, R_3 = OH

o-coumaric acid: R_1= OH, R_2 = R_3 = R_4 = H

Sinapic acid: R_1= H, R_2 = R_4 = OCH_3, R_3 = OH

Figure 1.1. The chemical structures of some common phenolic acids identified in sorghum (Dykes & Rooney, 2006).

Flavonoids constitute the majority of polyphenols in plant tissues, and more than 6500 have been identified in nature (Corradini et al., 2011). Flavonoids have the common chemical structure：C_6-C_3-C_6 skeleton with two aromatic rings (A and B) linked through three carbons (Fig 1.2). Flavonoids mainly include flavanols, flavonones

flavonols, flavones, and anthocyanins, most of which have been identified in sorghum grains (Dykes & Rooney, 2007). Sorghum also contains unique anthocyanidins, called 3-deoxyanthocyanidins due to a lack of the hydroxyl group at the C-3 position (Fig 1.3). This unique character makes 3-deoxyanthocyanidins more stable in acidic conditions than anthocyanidins reported in other plants (Awika, Rooney, & Waniska, 2004a). Luteolinidin and apigeninidin are the two main 3-deoxyanthocyanidins identified in sorghum grains (Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2004). In addition to sorghum grain, 3-deoxyanthocyanidins have only been found in a limited number of other plants such as in the silk tissues of maize (*Zea mays*), flowers of sinningia (*Sinningia cardinalis*) and the stalks of sugar cane (*Saccharum sp.*) (Boddu et al. 2006; Malathi et al., 2008; Sharma et al., 2012; Winefield et al., 2005).

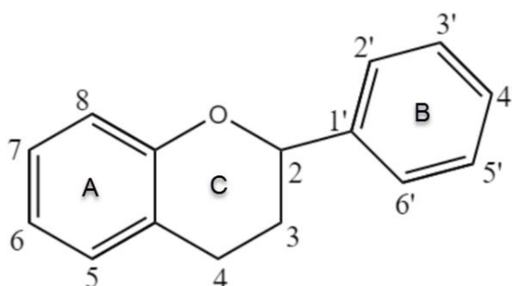
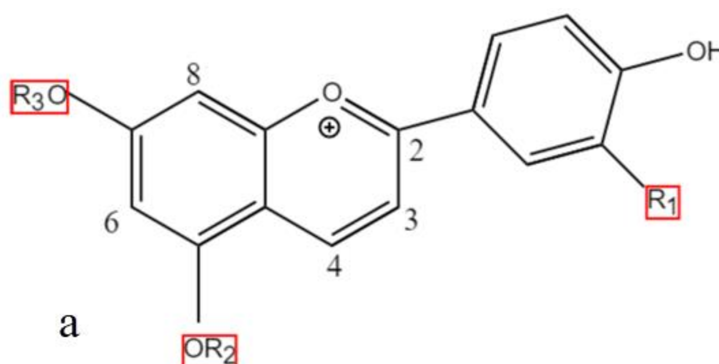


Figure 1.2. The common chemical structure of flavonoids (Bravo, 1998).



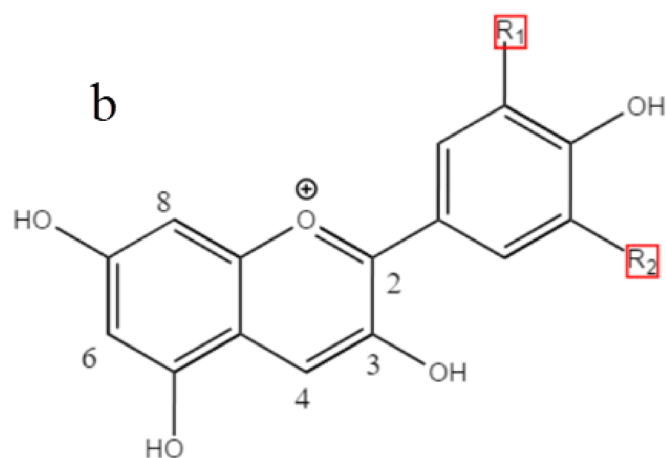
Apigeninidin: $R_1 = R_2 = R_3 = H$

Luteolinidin: $R_1 = OH$, $R_2 = R_3 = H$

Apigeninidin-5-glucoside: $R_1 = R_3 = H$, $R_2 = Glc$

Luteolinidin-5-glucoside: $R_1 = OH$, $R_2 = Glc$, $R_3 = H$

7-*O*-methyl apigeninidin: $R_1 = R_2 = H$, $R_3 = CH_3$



Pelargonidin	$R_1 = H$	$R_2 = H$
Cyanidin	$R_1 = OH$	$R_2 = H$
Delphinidin	$R_1 = OH$	$R_2 = OH$
Peonidin	$R_1 = OCH_3$	$R_2 = H$

Figure 1.3. The chemical structure of 3-deoxyanthocyanidins and their glucosides identified in sorghum (a) and common anthocyanidins found in fruits, vegetables, and some cereals (b) (Dykes & Rooney, 2006).

Condensed tannins, also called procyanidins or proanthocyanidins, have the higher molecular weight than the above two described subgroups of polyphenols (Schofield, Mbugua, & Pell, 2001). The monomeric units include flavan-3-o1 (e.g., catechin, and epicatechin) or flavan-3,4-diol units (Fig 1.4). One of the characteristics of tannin-rich foods is the astringent taste, due to the precipitation of salivary proteins which can form insoluble complexes with tannin (Bravo, 1998). Tannins are traditionally considered as an anti-nutritional factor because they can from insoluble compounds with minerals, carbohydrates and proteins to decrease their digestibility (Dykes & Rooney, 2006). In order to increase feed efficiency of tannin-rich foods, several pre-treatments have been developed to decrease tannin contents, such as heating, malting and fermentation (Taylor & Duodu, 2015). However, condensed tannins have high antioxidant activity and anti-carcinogenic, cardiovascular and cholesterol-lowering properties (Brglez Mojzer, Knez Hrnčič, Škerget, Knez, & Bren, 2016; Hagerman et al., 1998). Consequently, the daily intake of foods with abundant amounts of condensed tannins may improve human health (Dykes & Rooney, 2007). In addition,

it is erroneously believed that sorghum pericarp colour can be used as an indication of the levels of tannin in sorghum grains, e.g., by relating the highest tannin content with black coloured sorghum. Boren and Waniska (1992) measured correlations between sorghum pericarp colour and tannin level in 24 sorghum genotypes and from their findings suggested that seed colour is a poor indicator of tannin content in sorghum grain.

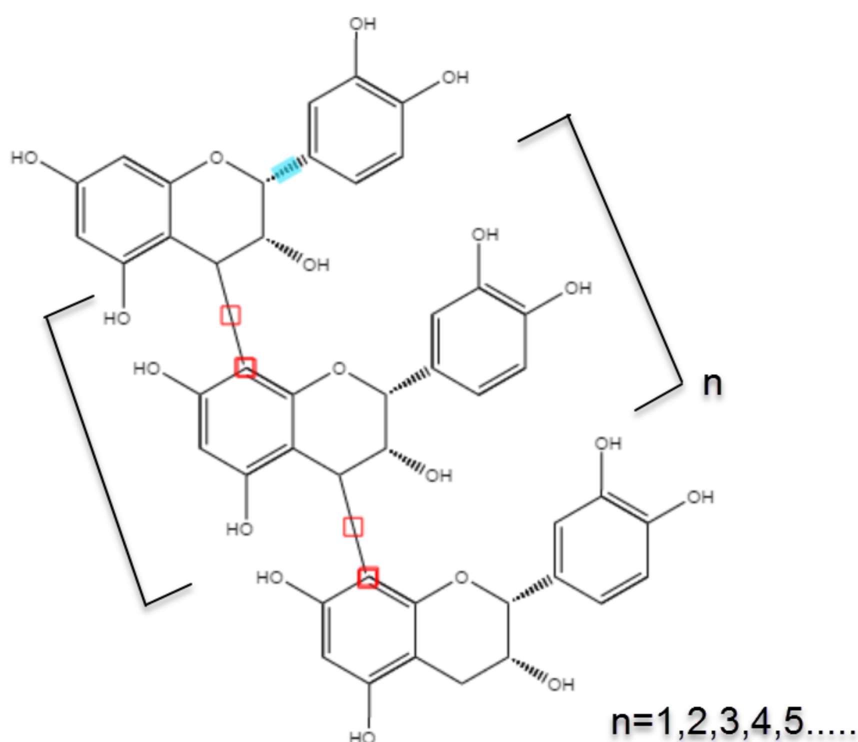


Figure 1.4. The chemical structure of condensed tannins identified in sorghum (Dykes & Rooney, 2005).

Antioxidants are compounds that can diminish oxidative stress and scavenge free radicals to prevent, inhibit or delay the oxidation of oxidisable materials (Dai & Mumper, 2010). From a human health point of view, oxidative stress, which is an imbalanced state, leads to oxidation of biomacromolecules, including DNA, lipids and proteins and enzymes, and is a factor leading to the development of chronic degenerative diseases. Polyphenols have been considered as a main group of natural antioxidants. The mechanisms of antioxidant activity of polyphenols include scavenging of free radicals, suppressing of free radical formation and stimulation of

the body's own antioxidant mechanisms (Cotelle, 2001; Lambert & Elias, 2010; Zheng & Wang, 2001). Consuming wholegrains with abundant polyphenols may be beneficial for those at risk of chronic diseases associated with over-nutrition, such as type 2 diabetes, heart disease and some cancers (Dykes & Rooney, 2007; Stefoska-Needham et al., 2015). For instance, in a large prospective study of 75,521 adult females who were followed for 10 years, Liu et al. (1999) concluded that the risk of coronary heart disease was strongly inversely correlated with the amount of wholegrain foods consumed, and a 50% reduction in risk was observed among women who had the highest level of wholegrain consumption in comparison with the lowest. Dykes, Rooney, Waniska, & Rooney (2015) reported a positive correlation between the antioxidant activity of sorghum grain and concentrations of polyphenols, the levels of which in turn mainly depended on genotype. However, there are few studies available on the antioxidant activities of phenolic compounds in Australian grown sorghum genotypes.

The average optimum temperature ranges for sorghum in different plant development stages are: 21 to 35°C for seed germination, 26 to 34°C for vegetative growth and development, and 25 to 28°C for reproductive growth (Prasad, Pisipati, Mutava, & Tuinstra, 2008). Due to carbon dioxide emissions, it is predicted that the global average temperature will increase 1.8 to 4.0°C by 2100 (Intergovernmental Panel on Climate Change, 2014). Increasing temperatures could have negative effects on sorghum production. In the semi-arid tropics where air temperatures can exceed 40°C, leaf temperatures of sorghum could be around 55°C (Prasad, Boote, & Allen, 2006). In these circumstances seed set, number, size and yields of sorghum were significantly decreased, and the possible reason is that the high temperature decreases pollen viability and seed-set (Singh et al., 2015). Currently there is no information about the specific effect of growth temperature on sorghum grain polyphenolics, although Rascio et al. (2015) has investigated the influence of growth temperature on polyphenolics of wheat grain. Studies have been carried out also on plants other than cereals. For example, it was shown that in leaves of sweet potato (*Ipomoea batatas L.*), growth temperature affected phenolic compounds such that the total content in leaves of plants grown at low temperatures was always greater than that of leaves under high temperatures (Islam, Yoshimoto, Ishiguro, Okuno, and Yamakawa 2003). In contrast,

the content of phenolic compounds in tomato and watermelon increased at high temperature compared to that found at low temperature (Rivero et al. 2001). Although there are several reports on the effect of high temperature on physiological and growth processes of sorghum plants (Ercoli, Mariotti, Masoni, & Arduini, 2004; Nguyen et al., 2013; Olufayo, Baldy, & Ruelle, 1996), the effect on phenolic compounds in sorghum grain is unknown.

Water deficit has been considered as an important factor in restricting crop growth during the growing season in arid and semiarid areas, because of irregular annual rainfall. Irrigation treatments have been used to provide sufficient water for crops in low rainfall areas (Alderfasi, Selim, & Alhammad, 2016; Vasilakoglou, Dhima, Karagiannidis, & Gatsis, 2011). There are many reports describing the relationships between sorghum yield and irrigation level in low rainfall environments (Farah, Salih, Taha, Ali, & Ali, 1997; Farré & Faci, 2006). For example, O'Shaughnessy, Evett, Colaizzi, and Howell (2012) found that sorghum yields decreased with reduced irrigation. Currently no information is available on the specific effect of water availability on sorghum grain polyphenolics, although polyphenols of maize seed oil were significantly reduced by irrigation treatment (Ali, Ashraf, & Anwar, 2010). For non-cereal plants such as tomato fruits, flavonoid and antioxidant activities were significantly enhanced under no irrigation and reduced irrigation regimes (Pernice et al., 2010). For fruit from young olive trees, the concentrations of polyphenol and oxidative stability were significantly increased under water stress conditions, but the consumer acceptance was negatively affected due to increases in the bitterness index (Romero, Tovar, Girona, & Motilva, 2002). Similar results were also found by Servili et al. (2007), who planted olive trees under different water stress regimes. However, how water availability during plant growth can affect polyphenols of sorghum grain is still unknown.

Solar ultraviolet (UV) radiation is classified according to wavelength: UV-A (315-400 nm), UV-B 280 - 315 nm and UV-C (< 280 nm). UV-C radiation is completely absorbed by the stratospheric ozone layer and thus does not reach the Earth’s surface, while most of the solar UV-A radiation reaches Earth. UV-B radiation is largely absorbed by stratospheric ozone. In the past 50 years, 3–6% of stratospheric ozone has

been depleted by human activities due to release of bromine- and chlorine-containing compounds into the atmosphere (Martínez-Lüscher et al., 2013). Many plants can be negatively affected by increased exposure to UV-B radiation, including damage to DNA, alteration of plant physiological functions (e.g., respiration and reproduction) reduced biomass reduction or alterations in protein synthesis (Kakani, Reddy, Zhao, & Sailaja, 2003; Lidon, 2012; Zlatev, Lidon, & Kaimakanova, 2012). The adaptive and acclimative mechanisms for plants such as increased levels of UV-screening of phenolic substances protect the plants from elevated UV-B radiation (Williamson et al., 2014). However, due to the implementation of the Montreal Protocol, it is predicted that the ozone layer will recover to 1980 levels in this century (Bais et al., 2015). Several studies have already determined the influences of lower levels of UV radiation on polyphenols and antioxidant activity of plants (Josuttis et al., 2010; Luthria, Mukhopadhyay, & Krizek, 2006). No published information is available on the relationships between polyphenol content of sorghum grain and exposure to UV radiation during plant growth and development.

Iron is one of the essential trace minerals required for the body's diverse metabolic functions, with its deficiency leading to anemia (Haas & Brownlie, 2001). The bioavailability of iron can in part be reduced by the presence of polyphenolic tannins in plant foods. According to the World Health Organization (WHO), iron deficiency causing anemia was defined by a hemoglobin level less than 11 g/dL, 12 g/dL and 13 g/dL in pregnant women, non-pregnant women over the age of 15 years and men, respectively. Iron deficiency leading to anemia has been ranked as the most important nutritional deficiency in the world, and affected around 2 billion people globally, the majority of whom are older people, pregnant women and children (Quintaes, Barberá, & Cilla, in press). One of the most abundant micro-elements in sorghum grain is iron, but its availability is low, due to the presence of tannins and phytate in sorghum (Afify, Beltagi, Salam, & Omran, 2012; Pontieri et al., 2014). Phytate is the salt form of phytic acid, and both tannins and phytate can form indigestible or insoluble complexes with iron that inhibits its dialysability (Kruger et al., 2013). Since the concentrations of iron, tannins and phytate vary with sorghum genotype (Kruger, Taylor, & Oelofse, 2012; Kruger et al., 2013), and sorghum may be the main dietary source of iron in some communities, it is important to understand the iron dialysability of food that is made

using new sorghum genotypes. Kayodé, Linnemann, Hounhouigan, Nout, & van Boekel (2006) determined the contents of iron and phytate of sorghum grain grown in three different locations with differing environmental conditions, and found that their concentrations showed significant changes across field locations, indicating an environmental effect. However, little information is available on how temperature, water availability and UV radiation exposure during plant growth can affect the levels of iron, tannins, in *vitro* iron availability and phytate in sorghum grain.

1.2. AIMS

The overall object of this thesis was to investigate the polyphenols, antioxidant activity, and *in vitro* iron availability of Australian sorghum genotypes under different environmental conditions. Specifically, the study aimed to:

1. Investigate how sorghum genotype and temperature during plant growth affects the profile and levels of the grain phenolic compounds, antioxidant activity and *in vitro* iron availability.

2. Determine the effect of sorghum genotype and water availability on the profiles and levels of the sorghum grain phenolic compounds and antioxidant activity.

3. Assess the role of UV radiation on the profiles and levels of the sorghum grain phenolic compounds and antioxidant activity in different genotypes.

CHAPTER TWO: REVIEW OF LITERATURE

2.1. ABSTRACT

Sorghum grain has abundant levels of phytochemicals, especially polyphenols, which are considered powerful antioxidants and potential functional food components that may reduce the risk of some chronic diseases. However, polyphenols may also have adverse nutritional effects including reducing dialysability of essential nutrients such as iron. Some sorghum genotypes contain higher contents of total phenolics total phenolics when compared to other cereal grains with their profile, concentration and inhibitory effect on iron dialysability being dependent on genotype. However, an increasingly variable climate including rising temperatures, reduced water availability and changing UV radiation, may alter the polyphenols in sorghum grain. This review provides an overview of the influences of some of the environmental conditions and different genotypes on the profile and level of polyphenols and antioxidant properties of sorghum grain along with the potential impact of these on mineral dialysability. Understanding factors that affect these polyphenols can assist sorghum breeders in developing new genotypes with the desired concentration and profile of the polyphenols to either (a) enhance antioxidant capacity for sorghum food products or (b) to minimise polyphenolics to maximise dialysability of nutrients such as iron in foods containing sorghum.

2.2. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), which belongs to the grass family, originated in north-eastern Africa, and is now widely grown in many countries, due to its adaptation to a diverse range of harsh environmental conditions. The total world annual sorghum production by country in 2014 is shown in Figure 2.1. Most of the sorghum in 2014 was produced by USA, Mexico and Nigeria. For example, the sorghum production in Australia is ranked the eighth in the world. Around 65% of the sorghum grain is exported to other countries, while the remainder is mainly utilised for local animal feed with a very small amount used for local biofuel production (United States Department of Agriculture, 2016b; Stefoska-Needham et al., 2015).

Sorghum grain makes a vital contribution to the staple diet in some developing countries, such as in sub-Saharan Africa, because it provides starch, protein, vitamins, minerals and energy for local people. However, the nutritional content can vary widely according to genotype and grain production environment (de MoraisCardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2015). Human consumption of sorghum-based food products is slowly but steadily increasing in developed countries, due in part to its gluten-free status as well as the grain's abundant levels of polyphenols that can act as antioxidants. For example, several gluten-free sorghum products with high antioxidant activity have been developed, such as flaked breakfast cereal (released commercially in Australia as gluten-free Weetbix^{TM}), bread and pasta (Khan et al., 2013; Stefoska-Needham, Beck, Johnson, Chu, & Tapsell, 2016; Yousif, Nhepera, & Johnson, 2012). According to an epidemiological study, increased daily intake of whole cereal grains, including whole sorghum grain, was associated with a reduction in certain types of cancers, such as esophageal cancer (van Rensburg, 1981). More recently, it has been suggested that some potential health benefits, including anti-microbial, anti-allergenic and antioxidant properties, may be provided by polyphenols of wholegrains including sorghum (Balasundram, Sundram, & Samman, 2006; Dykes & Rooney, 2007). Several *in vivo* studies have indicated that rats, treated with polyphenol extracts from sorghum grains, had lower biomarkers of risk of diabetes, cardiovascular disease and increased anti-inflammatory activities (Chung, et al., 2011; Lee et al., 2014; Shim, Kim, Jang, Ko, & Kim, 2013).

Polyphenols are ubiquitous in plants (Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). Polyphenols can affect flavour, appearance, nutritional quality and health-promoting properties of plant-derived food (Croft, 2016; Joseph, Edirisinghe, & Burton-Freeman, 2016). They also have a series of important functions in the plant itself, e.g., acting as free radical scavengers to reduce photogeneration of reactive oxygen species, as plant defence compounds against pathogens and herbivores, and providing plant antimicrobial and antifungal protection, as well as playing important roles in protecting the plant against abiotic stress factors such as changes in UV radiation, drought and elevated temperature (Bornman et al., 2015; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). Sorghum is genetically very diverse with many different grain colours (Fig 2.2), which have been associated with a widely

varied concentration and profile of polyphenols depending on the genotype (Awika & Rooney, 2004; Dykes et al., 2005). However, the digestibility of sorghum proteins is negatively affected by polyphenols, especially condensed tannins, which can form indigestible protein–tannin complexes reducing protein availability (Duodu, Taylor, Belton, & Hamaker, 2003). In addition, polyphenols can strongly chelate minerals (e.g., iron), to form insoluble complexes, which may reduce dialysability of the iron (Kruger et al., 2012).

Climate change-associated shifts in environmental conditions during the sorghum growing season will likely pose considerable challenges for sorghum production and grain quality in the future. It is projected that global average surface temperatures will increase between 1.8 and 4.0℃ in 2100 (Intergovernmental Panel on Climate Change, 2014). Apart from temperature changes, the changing climate is also leading to changes in annual rainfall with subsequent reduced availability of water during the growing season in many regions: solar UV radiation is also changing (Alinian, Razmjoo, & Zeinali, 2016; Bornman et al., 2015). The biosynthesis of phenolic compounds in plants can be modified by environmental stressors, such as temperature and UV radiation, although specific information on the mechanisms and extent of this regulation is limited (Cohen & Kennedy, 2010) and absent for sorghum. Previous reviews on polyphenols of sorghum grains have mainly focused on the structure, classification and antioxidant activity of these compounds in different sorghum genotypes (Althwab, Carr, Weller, Dweikat, & Schlegel, 2015; Awika & Rooney, 2004; Dykes & Rooney, 2007; Stefoska-Needham et al., 2015), while information of the effect of environmental conditions on the profile and concentration of sorghum grain polyphenols is scarce.

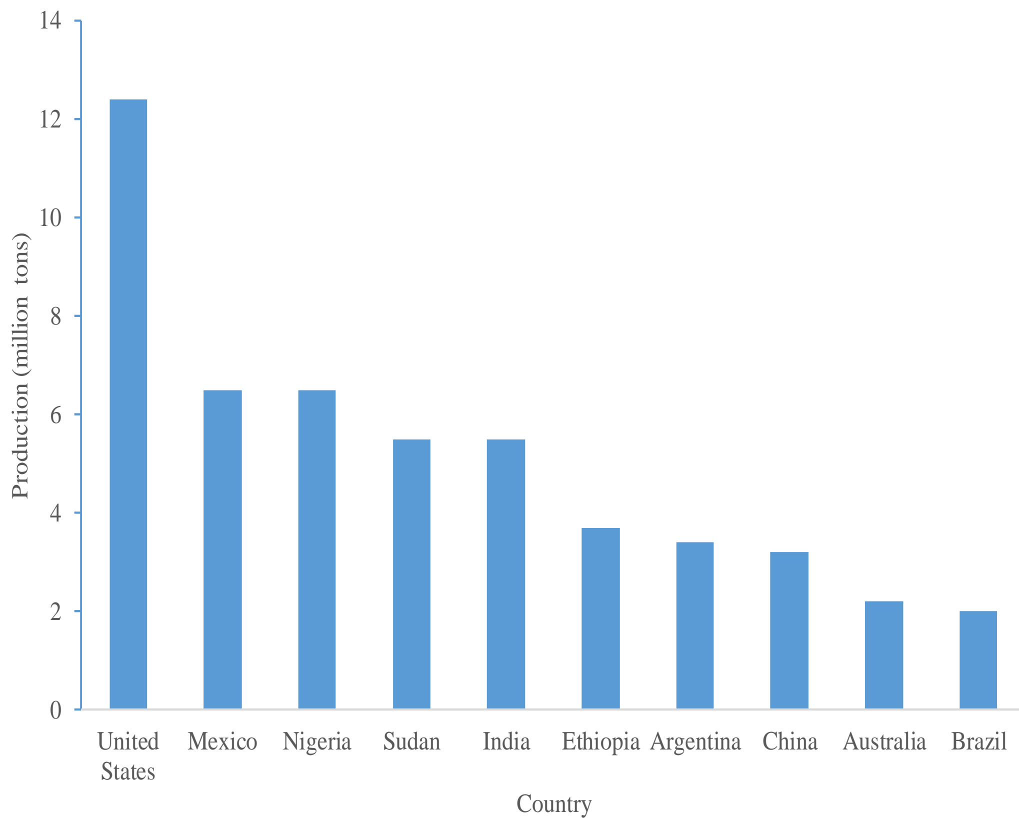


Figure 2.1. Sorghum production by country in 2014 (United States Department of Agriculture, 2016a).

Understanding the role of genotype and environment and their interaction in modifying polyphenols of sorghum grain, and thus nutrient dialysability, will be essential to fully understand the nutritional properties of new sorghum-based foods in the future, especially with predictions of future climate change around the world (Intergovernmental Panel on Climate Change, 2014). This review looks at the evidence for how genotype and environmental conditions have the potential to affect polyphenols in sorghum grain. In addition, the contribution of these phytochemicals to the nutritional and health properties of the grain, with emphasis on antioxidant capacity will be discussed.

Figure 2.2. Sorghum genotypes showing three different grain colours.

2.3. EFFECTS OF GENOTYPE ON SORGHUM POLYPHENOLS AND ANTIOXIDANT ACTIVITY

All sorghum grains contain polyphenols, most of which have also been identified in other plants (Dykes & Rooney, 2007). Four major parts: pericarp, testa, germ and endosperm, are included in the sorghum grain. The polyphenols are mainly concentrated in the pericarp, which is the outermost layer of the grain (Dykes & Rooney, 2007; Earp & Rooney, 1982). Polyphenols in sorghum grains include: phenolic acids, flavonoids and condensed tannins. Dykes and Rooney (2006) have previously reviewed the molecular structures of these compounds in detail, and the structures of some common individual polyphenols of sorghum are also presented in Figure 1.1 – 1.4 of this thesis. A range of the approximate levels of total phenolics total phenolics in different cereal grains are shown in Table 2.1, from which it is apparent that sorghum can have a far higher total polyphenol content than other cereal grains. The content of total phenolics total phenolics in sorghum varies as much as ten-fold with genotype (Table 2.1). It is therefore important to know the content of polyphenols in sorghum grain when preparing sorghum food products with specific target nutritional or health properties. The ways in which genotype affects the profiles and contents of polyphenols in sorghum grain will now be addressed.

Table 2.1. The range of total polyphenol content of selected cereal wholegrains [α].

Cereals	Total polyphenol content (mg GAE/g, dbdb) [β]	Reference
Sorghum	1.4-37.3	Dykes, Hoffmann, Portillo-Rodriguez, Rooney, and Rooney (2014)
Wheat	1.3-5.3	Beta, Nam, Dexter, and Sapirstein (2005)
Rice	0.3-0.4	Iqbal, Bhanger, and Anwar (2005)
Oats	0.4-0.5	Viscidi, Dougherty, Briggs, and Camire (2004)
Millet	3.1-4.4	Hag, Tinay, and Yousif (2002)
Barley	2.2-2.6	Zhao et al. (2008)
Maize	1.3-4.9	Del Pozo-Insfran, Brenes, Serna Saldivar, and Talcott (2006)

[α] measured with the Folin-Ciocalteu method

[β] GAE, gallic acid equivalents

2.3.1. Total polyphenols

Numerous studies have investigated the content of polyphenols in grains of different sorghum genotypes (Table 2.2), and several methods have been proposed for classifying sorghum based on their grain polyphenol content. For example, according to the levels of extractable tannins, sorghum genotypes are divided into three types. Type one： sorghums without pigmented testa containing no significant levels of tannin; types two and three: sorghums with pigmented testa containing tannins. The main difference between types two and three is that 1% acidified methanol is used to extract tannins in Type two sorghum, while those in Type three are extractable with methanol alone (Price, Van Scoyoc, & Butler, 1978). However, this classification does not take into consideration other major polyphenolic constituent levels, such as phenolic acids and flavonoids (Awika & Rooney, 2004). Sorghum genotypes have also been classified according to their total phenolics total phenolics combined with appearance, i.e., black and brown sorghum with high levels of tannin (plus varying levels of total extractable polyphenols), red sorghum without tannins (but with varying

levels of total extractable polyphenols) and white sorghum without tannins (but with low levels of total extractable polyphenols) (Awika & Rooney, 2004). However, these classification systems fail to consider the significant genetic variability in the profile and levels of individual polyphenols that may significantly affect the nutritional and health properties of the grain.

The high variabiliy in phenolic levels, even within sorghum genotypes of the same grain colour classification, is illustrated by the study of Chiremba, Taylor, Rooney, and Beta (2012), who investigated the total polyphenol and phenolic acid contents of eight red sorghum genotypes without pigmented testa. Although these sorghum genotypes had the same grain colour, the total polyphenol and phenolic acid contents varied significantly. For example, for phenolic acids, some genotypes had about three times the concentration of others.

Dykes et al. (2005) evaluated total phenolics total phenolics of 13 sorghum genotypes with five colours: white, red, orange, black and brown; genetic information of these genotypes was also provided. Three of these with a pigmented testa, black, red and brown, had higher concentrations of total phenolics total phenolics than those without the pigmented testa.

Information on the genetics of sorghum grain colour has been documented in the literature. Both *R* and *Y* genes control the pericarp colour of sorghum grain. The pericarp of sorghum grain is yellow when genes are expressed as *rrY_,* red when expressed as *RRYY* and colourless or white when the genes are expressed as *R-yy* or *rryy* (Dykes & Rooney, 2006). Both B_1 and B_2 genes control the testa pigmentation of sorghum grain, and only when genes are expressed as $B_1B_1B_2B_2$, is the testa pigmented (Rooney, Miller, & Mertin, 1981). In addition, the three genotypes with the pigmented testa in the report of Dykes et al. (2005) contained high amounts of condensed tannins, which suggested that sorghum genotypes, expressing both dominant B_1 and B_2 genes, contain significant contents of condensed tannins (Awika & Rooney, 2004).

Table 2.2 presents examples of the range in total polyphenol content of sorghum genotypes with different colours. Total polyphenol content of a large number of differently coloured sorghum genotypes was determined by Rhodes et al. (2014), who

found that the visual colour of sorghum grain is not a good indicator of total polyphenol content, since although the appearance of sorghum grain colour is mainly contributed by polyphenols, it can also be affected by endosperm colour and may be modified by plant growth temperature. This potential effect of temperature on grain colour suggests the possibility of temperature affecting polyphenolic levels in sorghum grain. There is, however, a scarcity of literature investigating this hypothesis.

Sorghums with high concentrations of flavonoid polyphenols may not only provide human health benefits, but also are a potential source of natural colorants. The correlation between grain colour and total flavonoid concentrations of sorghum was evaluated by Taleon, Dykes, Rooney, and Rooney (2012), and was found to be very low. Taleon et al. (2012) suggested that this weak correlation may be caused by the heterogeneous colour of the grain surface, due to the glume (husk) covering some parts of the kernel that in turn do not show pigmentation due to lack of sunlight exposure. This suggestion indicates that the UV spectral component in sunlight may modify the levels of polyphenols in sorghum grain.

Table 2.2. The range of total polyphenol content of different sorghum genotypes.

Number of genotype included in study	Colour of grain[a]	Level of processing	Total polyphenol content (mg GAE/g, (db))[b]	Reference
50	W, R, Y	Wholegrain	3.1-30.1	Dicko, Gruppen, Traoré, van Berkel, and Voragen (2005); Dicko et al. (2002)
3	R, Y, Br	Wholegrain	2.2-6.0	de Morais Cardoso et al. (2015)
4	ND	Wholegrain	0.8-8.2	Bvochora, Reed, Read, and Zvauya (1999)
9	W, R	Wholegrain	1.0-23.0	Bröhan, Jerkovic, and Collin (2011)
13	W, R, Y, O, B, Br	Wholegrain	2.1-8.9	Dykes et al. (2005)
1	R	Wholegrain	0.4	Bvochora, Danner, Miyafuji, Braun, and Zvauya (2005)
373	W, R, Y, B, Br	Wholegrain	0-37.5	Rhodes et al. (2014)
3	W	Decorticated grain	1.1-1.2	Afify et al. (2012)
8	B	Wholegrain	5.3-19.8	Dykes, Rooney, and Rooney (2013)
6	W, R	Wholegrain	0.6-6.1	Hahn and Rooney (1986)
3	W, R, Br	Sorghum bran	3.7-41.4	Ayala-Soto, Serna-Saldívar, Welti-Chanes, and Gutierrez-

				Uribe (2015)
287	W, R, Y, B	Wholegrain	1.4-37.3	Dykes et al. (2014)
7	R	Decorticated grain	8.9-27.2	Dóka, Bicanic, Dicko, and Slingerland (2004)
3	W, R, Br	Decorticated grain	0.7-4.5	Moraes et al. (2012)
2	W, R	Sorghum bran	3.1-10.8	Buitimea-Cantúa et al. (2013)
52	W, R, Y	Wholegrain	3.2-13.6	Flores-Naveda (2016)
5	W, R, Y	Wholegrain	2.4-19.3	Woo et al. (2011)
7	ND	Wholegrain	0.2-0.4	Pasha, Riaz, Saeed, and Randhawa (2015)
2	W, R	Wholegrain	2.2-6.7	Khan et al. (2013)
8	ND	Wholegrain	0.9-1.6	Chavan, Pansare, Patil, and Shinde (2015)
6	ND	Wholegrain	0.4-0.9	Thaddi and Nallamilli (2014)
20	W, R, Br	Decorticated grain	0.3-0.8 [c]	Palavecino, Penci, Calderón-Domínguez, and Ribotta (2016)
8	R	Decorticated grain	2.7-4.9 [d]	Chiremba et al. (2012)
6	W, R	Wholegrain	1.7-17.5 [c]	Kobue-Lekalake, Taylor, and De Kock (2007)
5	W, R	Wholegrain	2.7-24.5 [c]	Dlamini et al. (2007)

3	W, Br	Wholegrain	4.5-5.6 [c]	Babiker (2013)

[a] B, black; Br, brown; O, orange; R, red; Y, yellow; W, white; ND, no data

[b] GAE, gallic acid equivalents

[c] mg tannic acid equivalents /g

[d] mg catechin equivalents /g

2.3.2. Individual polyphenols

In addition to total polyphenol content, the antioxidant activity of sorghum grain is influenced by the content and profile of individual polyphenols, due to their different antioxidant potentials, which have been previously reviewed in detail by Rice-Evans, Miller, and Paganga (1996). Table 2.3 shows the contents of the most common phenolic acids and flavonoids of sorghum grain, and illustrates the great diversity of their levels among different genotypes.

In order to understand how sorghum genotype influences individual phenolic acids, Hahn, Faubion, and Rooney (1983) studied contents of individual phenolic acids, including vanillic, caffeic, gallic, ferulic, cinnamic, *p*-hydroxybenzoic, *p*-coumaric and protocatechuic acids in seven sorghum genotypes. The study indicated that some sorghum genotypes without a pigmented testa showed higher concentrations of individual phenolic acids than those with a pigmented testa, and suggested that there may be no relationship between the concentrations of individual phenolic acids and pigmented testa.

The relationships between individual phenolic acids and sorghum colours were also determined by Chiremba et al. (2012) who measured caffeic, *p*-coumaric, ferulic and sinapic acids concentrations in eight sorghum genotypes. The concentrations of these acids in both the free and bound forms were significantly different, even though their grain colour was all visually red. In addition, Afify et al. (2012) evaluated some individual phenolic acid concentrations of three white sorghum genotypes, and similar high variability in levels was found. Overall, these results suggest that the individual phenolic acid concentrations in sorghum genotypes cannot be simply evaluated from the grain colour.

Dykes, Peterson, Rooney, and Rooney (2011) found that white and red sorghum grains did not contain the flavonoid eriodictyol, whereas grains of other colours did. This finding illustrates how flavonoid composition is influenced by genotype. Several other studies have evaluated the influence of sorghum genotype on the unique sorghum flavonoids, the 3-deoxyanthocyanidins. Awika, Rooney, & Waniska (2004b) reported that a black genotype was a very good source of 3-deoxyanthocyanidins. In another

study, the concentration of 3-deoxyanthocyanidins in three black genotypes was much higher than in three brown and one red genotype (Awika et al., 2004a). Dykes et al. (2005) found similar results, and showed that of 13 sorghum genotypes, the 3-deoxyanthocyanidin concentration of two black genotypes was the highest. By using Pearson correlation coefficient analysis, Dykes et al. (2013) also found that the sorghum pericarp colour, as determined instrumentally by the "Lightness" value showed a significantly negative correlation with 3-deoxyanthocyanidin concentration, indicating that higher 3-deoxyanthocyanindin concentrations were associated with darker colouration.

In summary, these results suggest that sorghum grain colour is not a good indicator for total or individual polyphenol content of sorghum, and thus it is necessary to evaluate the profile and content of individual polyphenols when considering which sorghum genotypes to produce or use for specific human food applications.

2.3.3 Antioxidant activity

Polyphenols in the whole sorghum grain have been considered as anti- nutritional factors, due to their effect on nutrient dialysability, such as iron dialysability in sorghum (Kruger et al., 2013). However, it has been demonstrated that whole sorghum grain is a good source of powerful free radical scavenging, largely due to the antioxidant activity provided by polyphenols (de MoraisCardoso et al., 2015). The development of chronic diseases is closely correlated with the excessive and chronic production of reactive oxygen species, such as the superoxide free radicals, peroxyl and highly reactive hydroxyl, which can damage cellular DNA, lipids and proteins (Kim, Hyun, & Kim, 2010; Lee, Park, Zuidema, Hannink, & Zhang, 2011). Animal and *in vitro* studies have indicated that the gut microbiota could be beneficially modified by consumption of polyphenols isolated from sorghum grains, and therefore potentially the risk of some chronic diseases, like dyslipidemia, diabetes, obesity and cardiovascular disease might be reduced (Chung, Kim, et al., 2011; Chung, Yeo, et al., 2011; Farrar, Hartle, Hargrove, & Greenspan, 2008; Suganyadevi, Saravanakumar, & Mohandas, 2013). Given the well-established positive correlation between polyphenolic levels and antioxidant capacity for sorghum grain, the antioxidant capacity as well as polyphenolic levels are strongly influenced by genotype (Dykes et al., 2005).

Chemical-based antioxidant assays can be classified into two types: the hydrogen atom transfer (HAT)- and electron transfer (ET)- based assays. The results obtained from these two types of assays are difficult to compare, due to different redox potentials, solvent dependencies, pH and mechanisms. HAT-based assays evaluate the capacity of an antioxidant to quench peroxylradicals by H-atom donation, which have been considered to be biologically relevant, , while ET-based assays determine the capability of an antioxidant in the reduction of an oxidant, which changes color when reduced (Apak et al., 2013). The HAT-based assays include oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assays, while ET-based assays include 2,2$^{/}$-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet +}$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), Folin-Ciocalteu reagent (FCR) as well as ferric ion reducing antioxidant power (FRAP). All these methods have been used to measure the antioxidant activity of polyphenols, and the advantages and disadvantage of these methods have been well reviewed by Huang, Ou, & Prior (2005). Mechanistically, it is difficult to distinguish electron transfer from hydrogen atom transfer reaction, so the multitude of assays available have been used to messure the antioxidant activity of cereals, fruits and vegetables(Apak et al., 2013).

For sorghum grain, $ABTS^{\bullet +}$ and $DPPH^{\bullet}$ assays are commonly used to determine antioxidant capacity. In a study by Dykes et al. (2005) the values of $ABTS^{\bullet +}$ and $DPPH^{\bullet}$ antioxidant capacity of 13 sorghum genotypes were positively correlated with the content of total polyphenols, and those sorghum genotypes with the pigmented testa gene, B_1B_2, and spreader gene, *S,* had higher levels of antioxidant capacity when compared to those lacking these genes. Additionally, increased levels of antioxidant activity were also observed in sorghums with the thick pericarp (*z*) gene. The likely reason for these observations is that these genes may be responsible for high polyphenol contents of sorghum grain and that this was responsible for the increase in antioxidant activities.

Some detoxifying enzymes within the human body, such as glutathione reductase and quinone oxidoreductase, can be activated by polyphenols of sorghum grains, potentially providing health benefits for humans (Althwab et al., 2015). Polyphenols isolated from three sorghum genotypes led to higher levels of quinone oxidoreductase

in mouse hepatoma cells, suggesting that the polyphenols may have chemopreventive properties through increasing these detoxifying enzymes (Yang, Browning, & Awika, 2009). The effect of polyphenol extracts from black sorghum was more potent than that of extracts from white or red sorghum genotypes, possibly due to the higher 3-deoxyanthocyanidins content in the black sorghum.

Lewis (2008) investigated the antioxidant activity of different sorghum genotypes in an *in vivo* experiment by measuring superoxide dismutase activity and glutathione peroxidase activity levels in rats. Both enzymes could protect the organism from oxidative damage. Thus, the increased activity of these two enzymes would likely be beneficial for human health. Rats were fed with bran from white, brown or black sorghum genotypes, and all treatments significantly enhanced glutathione peroxidase activity and superoxide dismutase activity, with the black sorghum being the most potent. Again, the higher concentrations of 3-deoxyanthocyanidin in black sorghum may have been responsible for this beneficial effect.

There are several reports in the literature on food products prepared from different sorghum genotypes with their associated antioxidant activity. For example, flat bread incorporating wholegrain red sorghum flour had higher levels of antioxidant activity compared to that incorporating wholegrain white sorghum or the control wheat flour bread (Yousif et al., 2012). Another study by Khan et al. (2013) added wholegrain white and red sorghum flours to durum wheat to prepare pasta, resulting in products with increased antioxidant properties compared to the durum wheat-only pasta. A post-prandial clinical study in healthy subjects using these pasta formulations (Khan et al., 2014) showed that plasma total polyphenols, superoxide dismutase activity and antioxidant activity levels were significantly increased, while the level of protein carbonyl, a biomarker for oxidative protein damage, was significantly lower after consuming the pasta with red wholegrain sorghum flour compared to white wholegrain sorghum flour or the durum wheat-only pasta. This suggests that consuming sorghum grains with high antioxidant activity is likely to effectively reduce biomarkers of oxidative stress and increase *in vivo* antioxidant status.

Table 2.3. The range of individual polyphenol content of different sorghum genotypes.

Individual polyphenol	Number of genotypes included in study	Colour of grain[a]	Level of processing	Content (μg/g, (db))	Reference
			Phenolic acids		
Hydroxybenzoic acids					
p-Hydroxybenzoic	7	W, R, Y	Wholegrain	15.4-43.8	Hahn et al. (1983)
Vanillic	7	W, R, Y	Wholegrain	8.3-126.7	Hahn et al. (1983)
Protocatechuic	7	W, R, Y	Wholegrain	19.5-228.0	Hahn et al. (1983)
Gallic	7	W, R, Y	Wholegrain	13.2-46.0	Hahn et al. (1983)
Syringic	3	W	Decorticated grain	15.7-17.5	Afify et al. (2012)
Gentistic	17	W, R	Wholegrain	ND	Waniska, Poe, and Bandyopadhyay (1989)
Salicylic	17	W, R	Wholegrain	ND	Waniska et al. (1989)
Hydroxycinnamic acids					
Caffeic	7	W, R, Y	Wholegrain	23.3-50.6	Hahn et al. (1983)
Ferulic	7	W, R, Y	Wholegrain	104.6-287.0	Hahn et al. (1983)

Sinapic	8	R	Decorticated grain	41.4-78.6	Chiremba et al. (2012)
p-coumaric	7	W, R, Y	Wholegrain	72.7-232.1	Hahn et al. (1983)
Cinnamic	7	W, R, Y	Wholegrain	2.0-19.7	Hahn et al. (1983)
			Flavonoids		
Flavanols					
Catechin	9	W, R	Wholegrain	0.1-84.5	Bröhan, Jerkovic, Wilmotte, and Collin (2011)
Epicatechin	9	W, R	Wholegrain	0.2-1.8	Bröhan et al. (2011)
Procyanidin B1	9	W, R	Wholegrain	0.2-7.0	Bröhan et al. (2011)
Catechin hexoside	3	W, R, Br	Wholegrain	ND	Kang et al. (2016)
7-*O*-methyl catechin	3	W, R, Br	Wholegrain	ND	Kang et al. (2016)
7-*O*-methyl afzelechin	3	W, R, Br	Wholegrain	ND	Kang et al. (2016)
Flavonols					
Quercetin	3	W	Decorticated grain	22.3-29.4	Afify et al. (2012)
Kaempferol	3	W	Decorticated grain	17.9-36.4	Afify et al. (2012)

Kaempferol 3-rutinoside-7- glucuronide	3	R	Wholegrain	ND	Nip and Burns (1969)
<u>Flavanones</u>					
Naringenin	6	W, R, Br	Wholegrain	4.9-22.2	Wu et al. (2016)
Eriodictyol	7	R, Y	Wholegrain	0.9-1148.3	Taleon, Dykes, Rooney, and Rooney (2014)
Naringenin-7-*O*-glucoside	1	R	Wholegrain	ND	Svensson, Sekwati-Monang, Lutz, Schieber, and Gänzle (2010)
<u>Flavones</u>					
Luteolin	6	W, R, Br	Wholegrain	0.7-15.9	Wu et al. (2016)
Apigenin	6	W, R, Br	Wholegrain	2.2-4.0	Wu et al. (2016)
Luteolin hexoside	3	W, R, Br	Wholegrain	ND	Kang, Price, Ashton, Tapsell, and Johnson, (2016)
Apigenin-7-*O*-hexoside	3	W, R, Br	Wholegrain	ND	Kang et al. (2016)

Apigenin-6-*C*-glucoside	3	W, R, Br	Wholegrain	ND	Kang et al. (2016)
Flavan-4-ols					
Dihydroflavonols	3	W, R, Br	Wholegrain	ND	Kang et al. (2016)
Taxifolin	6	W, R, Br	Wholegrain	1.2-54.2	Wu et al. (2016)
Taxifolin7- glucoside	1	ND	Wholegrain	ND	Gujer, Magnolato, and Self (1986)
3-deoxyanthocyanidins					
Apigeninidin	6	W, R, Br	Wholegrain	2.5-27.9	Wu et al. (2016)
Luteolinidin	6	W, R, Br	Wholegrain	1.6-15.0	Wu et al. (2016)
7-Methoxyapigenidin	7	R, Y	Wholegrain	1.0-89.2	Taleon et al. (2014)
5-Methoxyluteolinidin	7	R, Y	Wholegrain	0.6-16.0	Taleon et al. (2014)
Apigeninidin-5-glucoside	3	R	Wholegrain	ND	Nip, and Burns (1969)
Luteolinidin-5-glucoside	3	R	Wholegrain	ND	Nip, and Burns (1969)

[a] Br, brown; O, orange; R, red; Y, yellow; W, white

[b] ND: no data.

Very recently a new hypothesis of sorghum polyphenol bioactivity, aside from antioxidant activity, was raised by Stefoska-Needham et al. (2016). They showed that red sorghum flaked breakfast cereal was more highly satiating and affected appetite hormones in a manner supporting the satiety findings than white or brown sorghum or wheat-based equivalent products in a human post-prandial clinical study. The authors suggested that the higher 3-deoxyanthocyanidins concentrations in the red sorghum product may have contributed to these satiety-related effects (Stefoska-Needham et al., 2016)

2.4. EFFECTS OF ENVIRONMENT ON SORGHUM POLYPHENOLS AND ANTIOXIDANT ACTIVITY

Understanding how the polyphenolic content and antioxidant activity of sorghum grain is influenced by environmental conditions during plant growth is important for breeders and growers to develop and grow sorghum genotypes for human food consumption. In addition, this knowledge is valuable for food manufacturers in order to select appropriate sorghum grain for use in the manufacture of nutritional and health functional foods, for example, sorghum-containing bread with high antioxidant activity (Dahir, Zhu, Guo, Aboshora, & Peng, 2015; Yousif et al., 2012).

Numerous studies have investigated if environmental stresses, such as limited water availability and increased exposure to UV-B radiation, could negatively affect the development and yield of cereal plants including sorghum (Jamieson, Martin, & Francis, 1995; Kataria & Guruprasad, 2012; O'Shaughnessy et al., 2012; Yao, Chu, He, & Si, 2014). However, environmental stress may also improve food quality of cereal grains as a result of changes in several physiological and biochemical parameters, including the level of antioxidant phytochemicals (Wang & Frei, 2011; Wargent & Jordan, 2013).

Currently, there are limited studies focusing on the response of sorghum grain polyphenolics or their antioxidant activity to changes in the environment. Therefore, some examples of the influence of environment on these properties of other cereal crops and horticultural crops will also be discussed.

2.4.1. Temperature

The development and growth rate of plants are affected by temperature (Prasad, Pisipati, Mutava, & Tuinstra, 2008). Elevated temperature not only may affect photosynthesis, but can also cause other biochemical, molecular and physiological changes, which can modify the concentrations of secondary metabolites of plants, such as polyphenols, as was found in strawberry (Wang, & Zheng, 2001; Zobayed, Afreen, & Kozai, 2005). However, in one study on wheat grown under different temperatures, total polyphenol and antioxidant activity levels of the grain were not significantly influenced by the temperature (Rascio et al., 2015). Studies investigating the effect of temperature on polyphenols of sorghum grain and their antioxidant activity are currently lacking.

Genotypes respond differently to varying conditions of temperature and water availability combined. Waniska et al. (1989) analyzed concentrations of polyphenols and phenolic acids in 17 sorghum genotypes planted in two seasons: post-rainy (control) and rainy season (+ irrigated). Compared to the post-rainy regime, the rainy season regime was irrigated with the overhead sprinkler system 1 h in the morning and evening, respectively, when it did not rain in the field. In comparison to the post-rainy season, the average temperature was warmer during the rainy season. The results showed that white pericarp sorghum genotypes had higher concentrations of free polyphenol, under the warmer and moister conditions (rainy season) when compared to the control regime (post-rainy). However, for free polyphenols of red sorghum genotypes the effect of conditions were reversed. For the red genotypes, the bound phenolic acid content did not change consistently between the two treatments. In the study Waniska et al. (1989) planted sorghums in different seasons to get different growth temperatures and water availability. However, in order to elucidate the effects of each environmental condition, well-controlled sorghum production trials are necessary.

The level and profile of flavonoids of sorghum grains may also be affected by a combination of temperature and water availability. Taleon et al. (2014) planted sorghums in two different locations, and the difference between these two locations was their rainfall and temperature. They found that most sorghum genotypes (10 out of 12) in their experiment that were planted in conditions of increased rainfall and

temperature showed higher 3-deoxyanthocyanidin concentrations than those from sorghum grown under lower rainfall and temperature. In addition, the profile of individual 3-deoxyanthocyanidins was significantly influenced by genotype and environment. Four major 3-deoxyanthocyanidins (luteolinidin, apigeninidin, 5-methoxyluteolinidin and 7-methoxyapigeninidin) were present in the genotype, Dorado, under warm and moist conditions, while only apigeninidin and 7-methoxyapigeninidin were detected in the same genotype when grown in a lower rainfall and cooler temperature environment. Luteolin and apigenin concentrations in all sorghum genotypes were not significantly affected by the environment treatment. These data suggest that temperature and water availability in combination can change the profile and concentration of flavonoids in sorghum grain, and that individual flavonoids have a variable response to changes in the environment. However, it is not possible to separate the influences of temperature and water in this study because of the change in both parameters at the two locations.

Although both Waniska et al. (1989) and Taleon et al. (2014) indicated that total or individual polyphenol concentrations of sorghum grain were significantly affected by environment and genotype, all sorghums were planted in the open field and the growth temperature or the amount of water were not well controlled, and thus it was not possible to separate the individual environmental effects. Therefore, the variations in polyphenols of sorghum grain need to be evaluated under well-controlled growth conditions in which temperature and water availability are well controlled individually and in combination, and then an attempt made to extrapolate the results back to field conditions.

2.4.2. Water availability

Water deficiency is a significant agricultural constraint in many parts of the world, and is increasing, with future predictions of reduced rainfall, and more variable rainfall events, combined with rising temperatures in many parts of the world (Farré & Faci, 2006). Plant development and growth are affected by a limited water supply, and many studies have reported that the concentrations of polyphenols and antioxidant activity in vegetables and fruits, such as cherry tomato, peach and grape berries were significantly increased by water stress (Buendía, Allende, Nicolás, Alarcón, & Gil, 2008; Esteban, Villanueva, & Lissarrague, 2001; Sánchez-Rodríguez, Moreno,

Ferreres, del Mar Rubio-Wilhelmi, & Ruiz, 2011). However, no information is available on sorghum, but some is available for other cereal crops. For example, Alexieva, Sergiev, Mapelli, and Karanov (2001) planted wheat under different water availability treatments, and found that water deficit had no significant influence on the content of anthocyanins, but resulted in a significant increase in total polyphenol levels in the grain. In another investigation on drought stress of two genotypes of wheat, the contents of total flavonoids and anthocyanin were significantly affected by genotype, temperature and their interaction, and their contents were significantly increased under the drought treatments (Ma, Sun, Wang, Li, & Guo, 2014). Also, changes in flavonoid content under drought stress were related to expression levels of certain key genes, such as those encoding for the enzymes flavanone 3-hydroxylase (F3H), chalcone isomerase (CHI) and chalcone synthase (CHS). In three maize genotypes under different irrigation regimes, the concentrations of total polyphenol and ferulic acid in the drought-resistant genotype (Tina) were shown to be higher under drought stress when comparing to high water availability (Hura, Hura, & Grzesiak, 2008). The activity of the enzyme L-phenylalanine ammonia-lyase (PAL), one of the key enzymes in the synthesis of polyphenols, was significantly increased under drought stress, and the contents of these compounds had a positive correlation with its activity in the grain samples.

There are only very limited studies reporting the effect of water availability on the antioxidant properties of grains. The antioxidant activity of maize seed oil in two genotypes grown under different water availability treatments in the field was reduced significantly after the imposition of water stress (Ali et al., 2010). This may have been due to high levels of water stress. However, no information is available of the effects of water availability on sorghum grain antioxidant activity.

2.4.3. Solar ultraviolet radiation

Sunlight provides the primary energy for plant photosynthesis. Although solar UV radiation, comprising wavebands of UV-C, UV-B and UV-A, is part of sunlight, only UV-A and a small amount of the UV-B radiation reaches the Earth's surface because of the efficient absorption by the stratospheric ozone layer (Antón et al., 2014; Bornman et al., 2015; Solomon et al., 2016). At ground level, UV-B radiation only makes up ca 0.5% of the total light energy, but has the highest energy wavelength

region of the daylight spectrum, and is both a regulator of plant development and can potentially cause deleterious effects (Wargent & Jordan, 2013). Due to human activities, large amounts of bromine, chlorine and fluorine compounds have been emitted into the atmosphere. These compounds have depleted the stratospheric ozone layer since the mid-1970s, leading to enhanced amounts of UV-B radiation reaching the Earth's surface. However, due to compliance with the Montreal Protocol, production of ozone depleting substances has been phased out and a slow ozone recovery is expected (Solomon et al., 2016). At the same time, interactions with other rapid climate changes, such as increased carbon dioxide (CO_2) concentrations, are likely to result in either an increase or decrease in UV radiation at ground level, depending on the location (Bais et al., 2015; Meul et al., 2016). An important adaptive mechanism of plants exposed to high UV radiation environments is an increase in the amounts of UV-screening phenolic substances (Bornman et al., 2015; Williamson et al., 2016). Likewise, a decreased UV radiation regime generally results in a lower level of UV-screening phenolic substances. Therefore, under future scenarios it is possible that the polyphenols of sorghum grains will change.

There are few reports of the effect of changing UV radiation exposure on sorghum grain polyphenolics. In a field study where sorghum was grown under ambient and increased amounts of UV-B radiation, corresponding to a 20% ozone depletion (Ambasht & Agrawal, 1998) using UV-B 313 fluorescent lamps, results showed that the enhanced UV-B radiation significantly increased contents of flavonoids in sorghum leaves. However, compared to those findings, a field study of 20 wheat genotypes under enhanced UV-B radiation showed that the response of the leaf flavonoids varied with genotype UV-B radiation and their interaction (Yuan et al. 2000). Seven of the genotypes were not significantly affected by the enhanced UV-B radiation, and only one genotype showed an increased flavonoid content, with a reduction in levels in the other twelve genotypes. These genotypic differences in flavonoid response to changing UV-B radiation levels highlight the need for well-controlled studies in sorghum to better understand the effect of genotype, level of UV radiation exposure, and their interaction on the grain polyphenolics. No studies have so far determined how UV radiation affects sorghum grain antioxidant capacity.

2.5. CONCLUSION

Polyphenols in sorghum grain have attracted much attention, primarily as a result of their proposed health benefits, including antioxidant properties. However, as already mentioned, there may also be negative effects of their inclusion in foods such as reduced dialysability of essential minerals. The polyphenol profile and content of sorghum grains grown in different regions of the world are generally modified by environmental conditions. The information presented in this review has endeavoured to provide some insights into the effect of genotype and environment on sorghum polyphenols.

Since the concentration and profile of sorghum polyphenols are highly dependent on genotype, evaluation of the concentration and profile of these compounds in a wider range of sorghum genotypes globally would be useful. Sorghum with high levels of polyphenols can then be selected to produce health foods high in antioxidants, while low polyphenol sorghum can be selected for sorghum foods with maximum energy and nutrient dialysability.

To date, studies evaluating the effects of environmental conditions on sorghum grain polyphenols are limited. There are also few studies available on the mechanism(s) resulting in the changes in sorghum grain polyphenols in response to environmental conditions. Therefore, there is a need to evaluate, in well-controlled environments, how key environmental constraints such as temperature, water availability, and exposure to UV-B radiation can modify sorghum grain polyphenols and antioxidant activity taking genotypic differences into account.

CHAPTER THREE: EXPERIMENTAL

Effect of genotype and growth temperature on polyphenol, antioxidant activity and *in vitro* iron dialysability of sorghum grain

Information contained in this chapter has been published as follows:

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ABSTRACT

It has been predicted that the global temperature will continue to rise in the future, which means crops including sorghum will likely be grown under higher temperatures than those currently, and consequently this may affect the nutritional properties of the grain. This chapter reports how genotype and growth temperature affected polyphenol and antioxidant activity and *in vitro* iron availability in sorghum grains. Six sorghum genotypes (CCH1, CCH2, AQL33/QL36, Ai4, PI563516 and IS 8525) were grown in a controlled growth chamber under two day/night temperature regimes, 32/21 ℃ (optimum temperature, OT) and 38/21 ℃ (high temperature, HT). The effects of temperature regimes on physical characteristics (hardness, weight and diameter) were also investigated. Additionally, the effects of the two growth temperatures on tannin, phytate, mineral, and *in vitro* iron availability of raw and cooked grains (as porridge) of the six sorghum genotypes were also determined. The results indicated that the weight and diameter of the sorghum kernels significantly increased in almost all genotypes (except for CCH1) with the higher growing temperature. Genotype had a

significant influence on total polyphenol content, while temperature was not found to have a significant effect on total polyphenol content, with the exception of genotype IS 8525, which was significantly reduced under HT. A total of 23 phenolic compounds were positively or tentatively identified by HPLC-DAD-ESIMS. Tannin content significantly decreased across all sorghum genotypes under high growth temperature ($p \leq 0.05$), while the phytate and mineral contents maintained the same level, or showed an increase or decrease, depending on the genotype. The *in vitro* iron availability in most sorghum genotypes was also significantly reduced under high temperature, except for Ai4, which showed a pronounced increase ($p \leq 0.05$). The cooking process significantly reduced tannin content in all sorghum genotypes ($p \leq 0.05$) while the phytate content and *in vitro* iron availability were not significantly affected. The observed variations in phenolic compounds and antioxidant activities of different sorghum genotypes under different growing temperatures provide valuable information for the selection of different genotypes under expected future increased temperatures. With these projected increasing temperatures, sorghum genotypes with high amounts of phenolic compounds and antioxidant activity have the potential to contribute to health-functional foods, while those with lower contents of phenolic compounds will be useful grains for foods requiring maximum available energy, protein and increased iron dialysability.

3.1. INTRODUCTION

Polyphenols are plant secondary metabolites that help to defend plants against pests and diseases among other functions (Snyder & Nicholson, 1990; Lattanzio, Cardinali, & Linsalata, 2012). A range of potential health benefits, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, and antioxidant activities are thought to be provided by phenolic compounds (Robards et al., 1999; Shahidi, & Ambigaipalan, 2015). Some sorghum genotypes have high concentrations of phenolic compounds and strong antioxidant activities (Stefoska-Needham et al., 2015). The consumption of foods with high concentrations of phenolic compounds may assist in prevention of certain chronic diseases, such as obesity and type 2 diabetes (Proteggente et al., 2002; Visioli et al., 2011). Results of epidemiological studies have suggested that dietary intake of phenolic abundant foods may reduce the incidence of cardiovascular disease, coronary heart disease and a variety of cancers (Edmands et al., 2015; total polyphenols,

Feskens, Hollman, Katan, & Kromhout, 1994; Hertog, Feskens, Kromhout, Hollman, & Katan, 1993; Zamora-Ros et al., 2016). In addition, the phenolic compounds, such as 3-deoxyanthocyanidins, reportedly have good anti-inflammatory properties (Taleon et al., 2014). The concentrations of phenolic compounds in sorghum grains vary widely with genotype (Dicko et al., 2005; Dykes et al., 2005). However, most studies only evaluated polyphenols as a collective group, and information on the differences in specific polyphenols among sorghum genotypes is limited. Although some specific polyphenols in several sorghum genotypes have been quantified by high-performance liquid chromatography diode array detection (HPLC-DAD) using authentic standards (Khan et al., 2013), others without authentic standards require mass spectrometry for elucidation of their structures.

However, the presence of anti-nutritional factors, such as phytate and tannins, has significant negative effects on the nutritional quality of sorghum foods. For example, both phytate and tannins can bind to minerals, such as iron (Fe), and reduce its digestibility and dialysability leading to iron deficiency in humans (Dykes & Rooney, 2006; Idris, AbdelRahaman, ElMaki, Babiker, & Tinay, 2006; Towo, Matuschek, & Svanberg, 2006). Low iron dialysability may cause some physical problems such as tiredness and shortness of breath (Martınez-Navarrete, Camacho, Martınez-Lahuerta, Martınez-Monzó, & Fito, 2002). Phytate and tannin contents in sorghum grain vary with genotype (Proietti, Mantovani, Mouquet-Rivier, & Guyot, 2013). As discussed in Chapter 2, sorghum genotypes with the B_1B_2 gene contain high contents of tannin, which can have important consequences for the dialysability of iron in the grain (Dykes & Rooney, 2006; Kruger et al., 2012). However, little information is available on tannin, phytate and iron variability in the sorghum genotypes from the Australian sorghum breeding program.

The Intergovernmental Panel on Climate Change, Fourth Assessment, states that global temperature may increase by 1.8 to 4.0 ℃ by 2100 (Intergovernmental Panel on Climate Change, 2014). Understanding variability of phenolic compound composition under increasing temperatures is essential because the concentration and profile of phenolic compounds and the chemical composition can affect the nutritional value of sorghum grain. Published studies investigating how increased temperature influences the phenolic compounds in plants, have mainly focused on fruits and

vegetables. For example, total phenolic compounds in strawberry increased under high temperature treatments (Wang & Zheng, 2001), whereas higher temperatures significantly decreased total phenolic contents in sweet potato leaves (Islam et al., 2003). To date, no published information is available on the effects of genotype and temperature during plant growth on sorghum grain polyphenols, antioxidant activity, tannin, phytate and mineral contents, or the associated iron availability.

To our knowledge, no previous studies have been conducted on the effects of high temperatures during plant growth on sorghum grain polyphenols. Therefore, the purpose of this chapter was to (1) investigate how different growth temperatures can affect the grain polyphenols and antioxidant activities in different sorghum genotypes, (2) identify individual polyphenols with high performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry (HPLC-DAD-ESIMS), (3) determine the effects of two different temperature regimes during plant production on grain phytate, tannin and mineral concentrations and *in vitro* iron availability in six different sorghum genotypes, (4) identify genotypes with high levels of phenolic compounds that could be used as a healthy food source high in antioxidant levels, and (5) identify genotypes with low levels of tannins and phytate as a source of available energy and enhanced iron availability when grown at high temperatures.

3.2. MATERIALS AND METHODS

3.2.1. Experimental design

Six sorghum genotypes including three red pericarp hybrid lines (CCH1, CCH2 and AQL33/QL36) and three inbred lines (Ai4 red pericarp; PI563516, white pericarp; IS 8525, brown pericarp) were used in this study (Fig. 3.1). The details of sorghum growing conditions have been described previously (Nguyen et al., 2013). Briefly, two separate growth chambers were used to control all environmental factors and provide two temperature regimes: optimal temperature (OT, 32 ℃ day /21 ℃ night) and high temperature (HT, 38 ℃ day /21 ℃ night) at the Controlled Environment Facility, Queensland Bioscience Precinct, The University of Queensland, Brisbane, Australia, 2012. The night temperature was gradually increased at a rate of 3 ℃/h to the maximum temperatures commencing from 1 h after the light period began. The maximum temperatures were kept constant for 7 h and 3 h in the OT and HT chambers,

respectively. The temperature was then decreased at a rate of 3 °C/h until the minimum temperature was obtained in both chambers 21 °C. Therefore, the main environmental difference between the two treatments was only the temperature in the middle of the light period. The experiment was a completely randomised block design (using 6 genotypes) within two temperature treatments and three replications (individual plants). Grains were harvested at maturity, manually cleaned and air-dried until the moisture content of around 10% was reached. The dried samples from each replicate were individually vacuum-packed in moisture-proof packaging and kept at -20 ℃ in the dark until analysis.

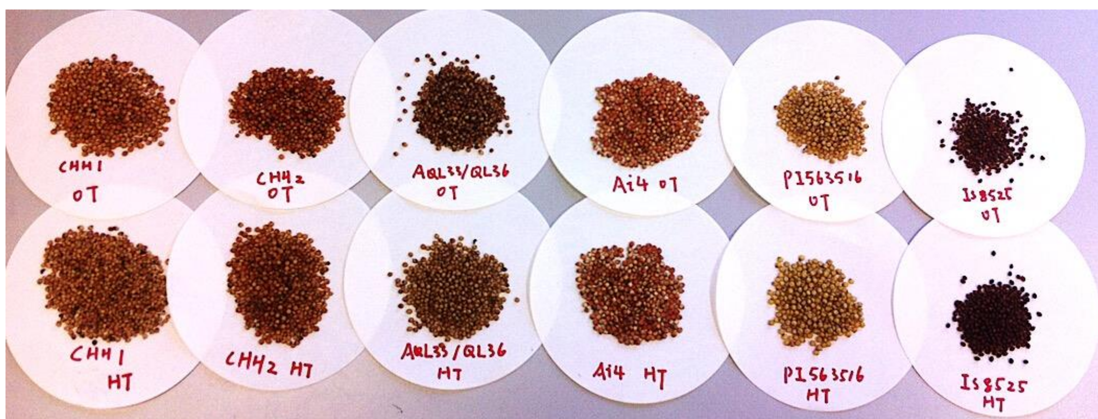


Figure 3.1. Six genotypes of mature sorghum grain under optimal temperature (OT) and high temperature (HT) growth conditions

3.2.2. Sample preparation

3.2.2.1. Flour

Sorghum wholegrain samples were ground for 5 min using a grain mill CEMOTEC 1090 (Foss Tecator, Höganäs, Sweden), to enable 100% of the flour to pass through a 500 µm sieve. The flour was vacuum-packed and stored at 4 ℃ in the dark until analysis and porridge preparation.

3.2.2.2. Porridge

The sorghum wholegrain flours were made into a thick porridge with three replications by the method described by Kruger et al. (2012). The porridge was freeze-dried with a rotational vacuum concentrator 2-18 CDplus freeze drier (Christ, Osterode,,

Germany) at -30 ℃ and 0.37 mbar for 20 h. The dried porridge samples were passed 100% through a 500 μm sieve to break down agglomerates before analysis. Ground samples were vacuum packed and stored at 4 ℃ until analysis.

3.2.3. Analytical methods

3.2.3.1. Physical characteristics of the grain

The physical characteristics of hardness, weight (mg) and diameter (mm) were measured on the wholegrains using the Single Kernel Characterization System 4100) (Perten Instruments, Hägersten, Sweden). Approximately 300 sorghum grains per genotype were evaluated in triplicate.

3.2.3.2. Moisture content

The oven method was used to determine the moisture content in sorghum grain (Wu, Zhang, Mujumdar, & Wang, 2010). Briefly, samples were placed in an oven at 103 – 104 ℃ and dried for 5 h. Samples were then removed and transferred to a vacuum desiccator, and weighed after cooling to room temperature. Samples were re-dried for a further 1h and weighed. If the loss of moisture was more than 0.1%, samples were dried and weighed again until within 0.1%, at which time the moisture content was calculated.

3.2.3.3. Extraction of free phenolic compounds

The free phenolic compounds were extracted from the milled grains according to Svensson et al. (2010) with some modification. Briefly, 2 g of the ground sample were mixed with 15 mL of 80% (v/v) aqueous methanol under N_2 for 2 h in a shaking water bath at 25 ℃. The sample was centrifuged at 3, 220 × g for 10 min after extraction at 4 ℃. The supernatant was decanted, and the residue was extracted twice more as described above. The residue was retained for bound phenolic extraction. The three supernatants were combined and evaporated to dryness using a vacuum rotary evaporator. The resulting solid was re-dissolved in methanol to a final volume of 10 mL and stored under N_2 at -20 ℃ in the dark until analysis. Each sample was extracted in duplicate.

3.2.3.4. Extraction of bound phenolic compounds

For the extraction of bound phenolic compounds, the residues after the extraction of free phenolic compounds were re-extracted with 15 mL of 2 M hydrochloric acid (HCl) under N_2 in a boiling water bath for 1 h (Svensson et al., 2010). This was followed by addition of 15 mL ethyl acetate to the extraction solution, which was mixed thoroughly. After partitioning, the ethyl acetate fraction was collected. The ethyl acetate extraction was repeated five times with the ethyl acetate fractions being combined and then evaporated to dryness. The resulting solids were re-dissolved in 10 mL methanol and kept at -20 ℃ under N_2 in the dark until analysis.

3.2.3.5. Determination of total phenolic content

The modified Folin–Ciocalteau method was used to measure total phenolics of the extracts (Sellappan, Akoh, & Krewer, 2002). Briefly, 100 µL of the free or bound phenolic extracts and methanol (as blank) were diluted with 400 µL ultrapure water. Then 2.5 mL of 0.2 N Folin–Ciocalteau reagent were added, followed by 2 mL of saturated sodium carbonate solution (75 g/L), and the sample mixed well. After reaction for 2 h at room temperature in the dark, the absorbance of the sample was recorded at 765 nm with a UV-1800 Spectrophotometer (Shimadzu, Canby, Oregon, USA). The results were expressed as mg gallic acid equivalents (GAE)/g grain (on a db, db). The gallic acid standard ranged between 0-144 mg/L. Each sample was analyzed in triplicate.

3.2.3.6. HPLC-DAD-ESIMS analyses

An Agilent 1290 UHPLC system equipped with binary pump, autosampler, thermostated column compartment, degasser, and diode array detector (DAD) was coupled to an Agilent 6460 LC-QQQ LC-MS/ MS system (Agilent Technologies, Palo Alto, CA, USA). The separation of phenolic compounds was performed on a Kinetex XB-C 18 reversed phase-HPLC column (5 µm, 250 × 4.6 mm, Phenomenex, Torrance, CA, USA). The DAD was set to scan between 190 and 600 nm at steps of 2 nm. Solvent A consisted of 0.1% formic acid in LC-MS grade water (Honeywell Burdick & Jackson, Gillman, SA, Australia), and solvent B was LC-MS grade acetonitrile (Honeywell Burdick & Jackson, Gillman, SA, Australia). The sample injection volume was 5 µL with the following linear gradient elution: 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 flow rate was 0.5 mL/min.

Mass spectra was performed in the ESI negative mode with a scan time of 2000 MS under the following conditions: gas (N2) 5 L/min at 300 °C, nebulizer 45 psi, sheath gas (N_2) 11 L/min at 250 °C, capillary voltage -3.5kV and nozzle voltage -500V. Phenolic compounds were detected by full scan ranging from m/z 50 to 1300.

3.2.3.7. Identification and quantification of polyphenols

The phenolic compounds in sorghum were identified by their UV-Vis and ESIMS spectra. The authentic standards were also used to identify phenolic compounds based on the chromatographic comparisons. The external standard method was used to quantify phenolic compounds in samples under the above HPLC-DAD conditions. Standards were dissolved in methanol (1 mg/mL each), and diluted to several concentrations ranging from 0.001 to 0.1 mg/mL. Data acquisition, peak integration and calibrations were performed with the Agilent ChemStation software. Peak areas compared with calibration curves of the respective standards were performed to calculate levels of phenolic compounds, and results were expressed as μg/g sample (db, db). Quantification of taxifolin isomer and ferulic acid isomer were calculated using the calibration curve for taxifolin and ferulic acid, respectively.

3.2.3.8. Determination of antioxidant activities

The antioxidant activity of the polyphenolic extracts was evaluated using 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt ($ABTS^{\bullet+}$) and the 2-2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) assays following the procedures of Thaipong, Boonprakob, Crosby, Cisneros-Zevallos and Hawkins Byrne (2006). For the $DPPH^{\bullet}$ assay, a stock solution was prepared by dissolving 24 mg $DPPH^{\bullet}$ in 100 mL methanol that was kept at -20 ℃ in the dark until use. The working $DPPH^{\bullet}$ solution was made from 10 mL of this stock solution with 45 mL methanol, with the working solution having an absorbance of 1.1 ± 0.02 units at 515 nm. An aliquot of 60 μL of the free or bound phenolic extracts or methanol (as blank) was diluted with 90 μL ultrapure water and reacted with 2850 μL of the $DPPH^{\bullet}$ working solution for 8 h in the dark at room temperature. The absorbance was then measured at 515 nm using a UV-1800 spectrophotometer. The results were expressed as mg Trolox equivalents (TE)/g db. The trolox standard ranged between 20-250 mg/L.

For the ABTS$^{\bullet+}$ assay, a stock solution was prepared by mixing equal amounts of 7.4 mM ABTS$^{\bullet+}$ and 2.6 mM potassium persulphate solution, which were left to react for 12 h at room temperature in the dark. The stock solution was freshly prepared before each assay. After incubation, 1 mL of this stock solution was diluted with 60 mL methanol, and the absorbance confirmed as 1.1±0.02 units at 734 nm to give the ABTS$^{\bullet+}$ working solution. An aliquot of 60 µL of the free or bound phenolic extracts or methanol (as blank) was diluted with 90 µL ultrapure water and reacted with 2,850 µL of the ABTS$^{\bullet+}$ working solution for 2 h in the dark. The absorbance was then measured at 734 nm using a UV-1800 spectrophotometer. The results were expressed as mg Trolox equivalents (TE)/g db. The Trolox standard ranged between 20-250 mg/L.

3.2.3.9. Determination of condensed tannin content

Condensed tannin content of the raw flours and dried porridge was determined by the vanillin HCl assay as described by Price et al. (1978). Ten mL acidified methanol (1% HCl in methanol) were added to 1 g of sorghum flour or dried porridge in triplicate, and the mixture extracted for 20 min with constant shaking at room temperature. The extracted sample was centrifuged at 3, 220 × g for 10 min at room temperature and the supernatant collected for analysis. The residue was re-extracted twice with 5 mL acidified methanol. Vanillin HCl reagent (equal volumes of 2% vanillin in methanol and 8% concentrated HCL in methanol) was prepared daily. To 1 mL of extract was added 5 mL of vanillin HCl reagent. The solution was mixed and incubated for 20 min at room temperature. The absorption of the resulting solution was measured at 500 nm on a UV-1800 spectrophotometer (Shimadzu, Canby, USA). Catechin was used as the standard for the calibration curve. Condensed tannin content was expressed as mg catechin equivalents (CE)/g sample (, db).

3.2.3.10. Determination of phytate content

Phytate content of the raw flours and dried porridge was measured using the spectrophotometric method of Haug and Lantzsch (1983). In brief, the sample solution of ground sorghum (about 0.5 g) was extracted from both raw flour and dried porridge with 20 mL of 0.2 M HCl for 90 min in a shaking water bath at 25 ℃. The solution was centrifuged at 3, 220 × g for 10 min after extraction at room temperature, and the supernatant collected. The residue was washed twice with 10 mL 0.2 M HCl,

centrifugation was repeated between each washing and the resulting supernatants combined. The combined supernatants were made up to 50 mL with 0.2 M HCl. Each sample was extracted in duplicate.

An 0.5 mL aliquot of the supernatant or a standard phytate solution (phytate phosphorus concentrations from 3-30 µg/mL) was pipetted into capped test tubes. A 2 mL ferric solution (0.2 g FeNH4(SO4)2 ·12 H2O in 1L 0.2 M HCl) was added to each tube and mixed thoroughly. The capped tubes were heated in a boiling water bath for 30 min, after which they were cooled in ice water to room temperature. Two mL of 2,2′-bipyridyl solution (10 g 2,2′-bipyridyl and 10 mL thioglycollic acid in 1 L distilled water) were added. After mixing thoroughly, the absorption of the solutions was measured at 519 nm on a UV-1800 spectrophotometer (Shimadzu, Canby, Oregon, USA). Absorption must be read within 0.5 – 1 min because reactions between bipyridine and iron phytate can change the colour with time. Distilled water was used as a blank. Phytate phosphorus was used as the standard. Phytate content was expressed as mg/g sample db.

3.2.3.11. Determination of mineral content

Mineral content of the raw flours was measured using a Vista Pro inductively coupled plasma optical emission spectrometer (ICP-OES) (Varian, Palo Alto, California, USA) at the National Measurement Institute, Perth, Western Australia. Sorghum flour (1 g) was digested with 3 mL of concentrated HNO_3 plus 3 mL of concentrated HCl in a DEENA automated digestion block (Thomas Cain, Omaha, Nebraska, USA) at 95 ℃ for 2 hours.

After digestion, the sample tubes were made up to 40 mL with distilled water, and solutions were left to settle. Samples were diluted 5-fold with distilled water before they were analysed using the ICP-OES. Appropriate emission wavelengths that had higher sensitivity and lower interferences were chosen to analyse elements of Fe, Ca, P and Zn. The wavelengths used to determine Fe, Ca, P and Zn were 238.204 nm, 327.395 nm, 117.434 nm and 213.857 nm, respectively. All results were expressed as mg/kg sample db.

3.2.3.12. Determination of in vitro iron dialysability

The dialysis method as described by Luten et al. (1996) was used to estimate the *in vitro* iron dialysability of raw flour and dried porridge samples. Two samples of equal amounts (around 1 g) were weighed, and each sample was mixed with 5 mL distilled water, and the pH adjusted to 2 with 6 M HCl. For simulated gastric digestion, 0.15 mL of freshly prepared pepsin solution (4 g pepsin, 2,500 units/mg, from Toxfree (Bedford St, Gillman, South Australia, Australia)) dissolved in 25 mL 0.1 M HCl were added to each sample and the mixture incubated at 37 ℃ for 2 h in a shaking water bath. Titratable acidity was determined in one sample of the gastric digest in which a freshly prepared pancreatin-bile extract mixture (400 mg pancreatin from Toxfree (Bedford St, Gillman, South Australia, Australia) and 2.5 g bovine bile extract from Sigma (Sigma, St. Louis, MO, USA) in 100 mL 0.1 M NaHCO3) was added, and the pH adjusted to 7.5 with 0.2 M NaOH. Titratable acidity of the samples was defined as the amount of 0.2 M NaOH required to reach pH 7.5.

In the simulated intestinal stage, segments of dialysis tubing with a molecular mass cut off at 10 kDa (Thermo Scientific, Massachusetts, Illinois,USA) containing 25 mL of sodium bicarbonate (equivalent in moles to the NaOH used to measure the titratable acidity), were placed in tubes with the gastric digest and incubated at 37 ℃ for 30 min, after which 5 mL pancreatin-bile solution were added to the tubes and incubated for a further 2 h. The iron content in the dialysis bag was determined as described in Section 3.2.3.10. The *in vitro* iron dialysability was expressed as percentage.

3.2.4. Statistical analysis

All data were expressed as means ± SD and analyzed using SPSS Statistics V20 (IBM Corp., Armonk, NY, USA). The main effects of genotype and temperature and their interaction were investigated by two-way ANOVA. One-way ANOVA with Tukey's post-hoc tests were used to separate the means when the main effect or interaction was significant. The associations between sorghum phenolic content and antioxidant activities were determined using Pearson's rank correlation. For all analyses, $p \leq 0.05$ was considered significant.

3.3. RESULTS

3.3.1. Grain physical characteristics

Hardness of grain from the genotype × temperature treatments are shown in Table 3.1. Both genotype and temperature had a significant influence on grain hardness ($p \leq 0.05$). The lowest grain hardness ($p \leq 0.05$) under both temperature treatments was found in IS 8525. Grain weight and diameter differed among the genotypes and between the temperature treatment (Table 3.1), with CCH1 having the highest and IS 8525 the lowest weight and diameter at OT. In contrast, at the HT, CCH2, CCH1 and IS 8525 had lower weights than the other genotypes, whereas CCH1 had the lowest diameter. Grain weight and diameter were significantly higher ($p \leq 0.05$) for all genotypes under HT compared to OT, except for CCH1 which showed significantly lower values under HT ($p \leq 0.05$).

Table 3.1. Sorghum grain hardness, weight and diameter in relation to growth temperature and genotype [α].

Genotype	Hardness		Weight (mg)		Diameter (mm)	
	OT	HT	OT	HT	OT	HT
CCH2	83.4±0.9 ^{cA}	82.2±2.7 ^{cA}	27.9±0.3 ^{bA}	32.5±0.3 ^{aB}	2.05±0.15 ^{bA}	2.38±0.10 ^{bB}
CCH1	77.4±0.5 ^{bA}	85.6±1.3 ^{cB}	40.7±0.3 ^{dB}	29.0±0.5 ^{aA}	2.60±0.15 ^{dB}	2.11±0.04 ^{aA}
AQL33/QL36	88.4±1.9 ^{dB}	82.1±0.8 ^{cA}	32.9±0.9 ^{cA}	37.4±0.2 ^{bB}	2.45±0.05 ^{cA}	2.64±0.02 ^{cB}
Ai4	86.4±1.8 ^{cB}	75.1±0.3 ^{bA}	35.4±0.5 ^{cA}	41.3±0.6 ^{bB}	2.47±0.02 ^{cA}	2.69±0.02 ^{cB}
PI563516	85.5±0.7 ^{dB}	76.2±0.5 ^{bA}	36.7±0.6 ^{cA}	42.9±0.3 ^{bB}	2.46±0.02 ^{cA}	2.71±0.06 ^{cB}
IS 8525	69.7±0.5 ^{aB}	62.5±0.4 ^{aA}	16.9±0.4 ^{aA}	29.9±0.5 ^{aB}	1.55±0.02 ^{aA}	2.34±0.03 ^{bB}

[α]OT: optimum temperature (32/21℃), HT: high temperature (38/21℃). a-d values with different superscripts in the same column are significantly different ($p \leq 0.05$). A, B values with different superscripts in the same row in the same dependent variable are significantly different ($p \leq 0.05$).

Table 3.2. Free, bound and total polyphenol content (mg GAE/g sample db) in the wholegrain of six genotypes grown at optimum temperature (OT) and high temperature (HT) $^{\alpha}$.

Genotype	Free polyphenols		Bound polyphenols		Total polyphenols	
	OT	HT	OT	HT	OT	HT
CCH2	1.59±0.11 ^{bA}	1.26±0.20 ^{bA}	0.66±0.04 ^{aA}	0.83±0.13 ^{aA}	2.25±0.12 ^{aA}	2.09±0.32 ^{aA}
CCH1	1.03±0.01 ^{aA}	1.28±0.11 ^{bA}	2.31±0.29 ^{fA}	2.68±0.12 ^{eA}	3.34±0.28 ^{cA}	3.95±0.17 ^{cA}
AQL33/QL36	1.09±0.20 ^{aA}	1.10±0.26 ^{abA}	1.57±0.09 ^{dA}	1.52±0.05 ^{dA}	2.67±0.28 ^{bA}	2.62±0.29 ^{bA}
Ai4	1.97±0.34 ^{bA}	1.92±0.12 ^{cA}	1.01±0.06 ^{bA}	1.05±0.13 ^{bA}	2.98±0.39 ^{bA}	2.97±0.22 ^{bA}
PI563516	0.73±0.05 ^{aA}	0.62±0.06 ^{aA}	1.24±0.12 ^{cA}	1.20±0.15 ^{cA}	1.97±0.17 ^{aA}	1.82±0.21 ^{aA}
IS 8525	7.47±0.65 ^{cB}	6.31±0.09 ^{dA}	2.07±0.06 ^{eA}	2.16±0.01 ^{cA}	9.56±0.60 ^{dB}	8.47±0.10 ^{dA}

$^{\alpha}$OT: optimum temperature (32/21℃), HT: high temperature (38/21℃). GAE, gallic acid equivalent. a-f values with different superscripts in the same column are significantly different ($p \leq 0.05$). A, B values with different superscripts in the same row in the same dependent variable are significantly different ($p \leq 0.05$).

3.3.2. Free, bound and total polyphenols

The free polyphenol contents in the grains of the six sorghum genotypes ranged from 0.73 to 7.47 mg GAE/g db under OT and 0.62 to 6.31 mg GAE/g db under HT (Table 3.2). Genotype had a significant effect on free polyphenol content ($p \leq 0.05$) with IS 8525 (brown pericarp) being about four times higher ($p \leq 0.05$) than Ai4 (red) and CCH2 (red) which in turn were higher ($p \leq 0.05$) than CCH1 (red), AQL33 (red) and PI563516 (white) at OT. A similar pattern of free polyphenol content was seen under HT.

Genotype had a significant effect on bound polyphenols ($p \leq 0.05$), but no significant changes were observed with increasing temperature ($p > 0.05$). The contents of bound polyphenols ranged from 0.66 to 2.31 mg GAE/g db at OT and 0.83 to 2.68 mg GAE/g db at HT (Table 3.2).

The content of total phenolics also showed a significant genotypic effect with IS 8525 showing the highest ($p \leq 0.05$) content, followed by CCH1, which had a higher content than Ai4 and AQL33/QL36, which in turn were higher than those levels in CCH2 and PI 563516 under both temperature conditions ($p \leq 0.05$). No significant differences in total polyphenol content were observed between temperature treatments in the genotypes tested ($p > 0.05$), with the exception of IS 8525, where the level was significantly lower ($p \leq 0.05$) under HT.

3.3.3. Identification of phenolic compounds

The HPLC retention time (Rt), UV absorption maxima and MS parent and fragment ions of 23 different phenolic compounds positively or tentatively identified in the samples are listed in Table 3.3. Representative HPLC-DAD chromatograms of the free polyphenolics in the red-pericarp genotype CHH2 are presented in Fig. 3.2. To assist in the interpretation of MS fragmentation patterns, the 23 compounds were classified into five groups according to their chemical structures (Table 3.3), and mass spectra of these compounds were presented in Appendix.

Phenolic acids

Phenolic acids consist of hydroxybenzoic acids and hydroxycinnamic acids. Peak 3

was tentatively identified as a derivative of hydroxybenzoic acids. Its MS spectra showed a pseudomolecular ion $[M-H]^-$ at *m/z* 137 and a prominent fragment ion at *m/z* 109 via loss of a CO group, corresponding to protocatechuic aldehyde. The UV spectra with λ_{max} at 280 and 310 nm were consistent with those of protocatechuic aldehyde reported in the literature (Svensson et al., 2010).

Table 3.3. Identification of individual polyphenols in sorghum wholegrains and their respective standards by HPLC-DAD-ESIMS[a].

Peak No.	Rt[b] (min)	λ_{max} (nm)	*m/z* [M - H]$^-$	*m/z* MS^2 (Abundance %)	Tentative identification
1	10.9	280	577	425 (60), 289 (26)	Procyanidin B1
2	13.1	298sh[c], 326	253	179 (1), 161 (78), 135 (55)	2-*O*-caffeoylglycerol
3	13.5	280. 310	137	109 (15)	Protocatechuic aldehyde
4	14.4	298sh, 326	253	179 (1), 161 (78), 135 (55)	1-*O*-caffeoylglycerol
5	15.0	297, 322	179	135 (100)	Caffeic acid (std[d])
6	15.8	282	449	287 (8), 151 (100), 135 (41)	Eriodictyol-7-*O*-glucoside
7	16.1	280, 490	269	241 (41), 225 (27), 169 (19), 133 (30)	Luteolinidin (std)
8	17.7	310	237	163 (20), 145 (50), 119 (31)	2-*O*-*p*-coumaroylglycerol
9	18.2	275, 470	253	225 (10), 209 (70), 179 (40), 117 (65)	Apigeninidin (std)
10	19.1	283	433	287 (100), 151 (59)	Eriodictyol deoxyhexoside
11	19.2	310	163	119 (100)	*p*-coumaric acid
12	20.8	295, 325	193	178 (42), 134 (100)	Ferulic acid (std)
13	21.6	288	303	285 (100), 217 (9), 177 (18), 125 (35)	Taxifolin (std)
14	24.3	285	303	285 (10), 177 (18), 125 (35)	Taxifolin isomer
15	26.7	295, 325	193	134 (100)	Ferulic acid isomer
16	28.5	300sh, 326	415	253 (100), 179 (100) , 161 (11), 135 (85)	1,2-*O*-dicaffeoylglycerol

17	29.4	300sh, 326	415	253 (100), 179 (100) , 161 (11), 135 (85)135(85),	1,3-*O*-dicaffeoylglycerol
18	30.6	287	287	151(10)	Eriodictyol
19	31.1	252, 347	285	241 (1), 217 (3), 199 (3), 175 (3), 151 (17), 133 (13), 107 (3)	Luteolin (std)
20	33.4	219, 315	399	253 (80), 235 (11), 179 (25), 163 (86), 145 (35), 119 (100),135 (57)	Coumaroyl-caffeoylglycerol
21	34.2	295sh, 325	429	253 (70), 235 (11), 193 (100), 175 (32), 161 (53), 135 (41)	Feruloyl-caffeoylglycerol
22	35.9	266, 322	269	225 (10), 201 (30), 183 (10), 149 (32), 117 (100)	Apigenin (std)
23	36.2	295	271	177 (10), 151 (50), 119 (20), 107 (20)	Naringenin (std)

[a] HPLC-DAD-ESIMS = High performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry

[b] Rt = Retention time; [c] sh = shoulder; [d] std = standard

Three hydroxycinnamic acids, namely caffeic acid (peak 5), *p*-coumaric acid (peak 11) and ferulic acid (peak 12), were identified, giving the deprotonated molecule $[M-H]^-$ at *m/z* 179, 163, and 193 and distinguished fragment ions at m/z 135, 119 and 135, respectively (Svensson et al., 2010). These hydroxycinnamic acids showed the loss of a CO_2 group from the carboxylic acid functional group. Ferulic acid (peak 12) also lost a CH_3 group demonstrating a $[M-H-15]^-$ anion at m/z 178 (Table 3.3). The caffeic and ferulic acids in the samples were further identified by comparison with the HPLC Rt, UV and MS spectra of the authentic standards. Peak 15 had similar UV and MS spectra to ferulic acid, and was tentatively identified as ferulic acid isomer (Luthria & Liu, 2013).

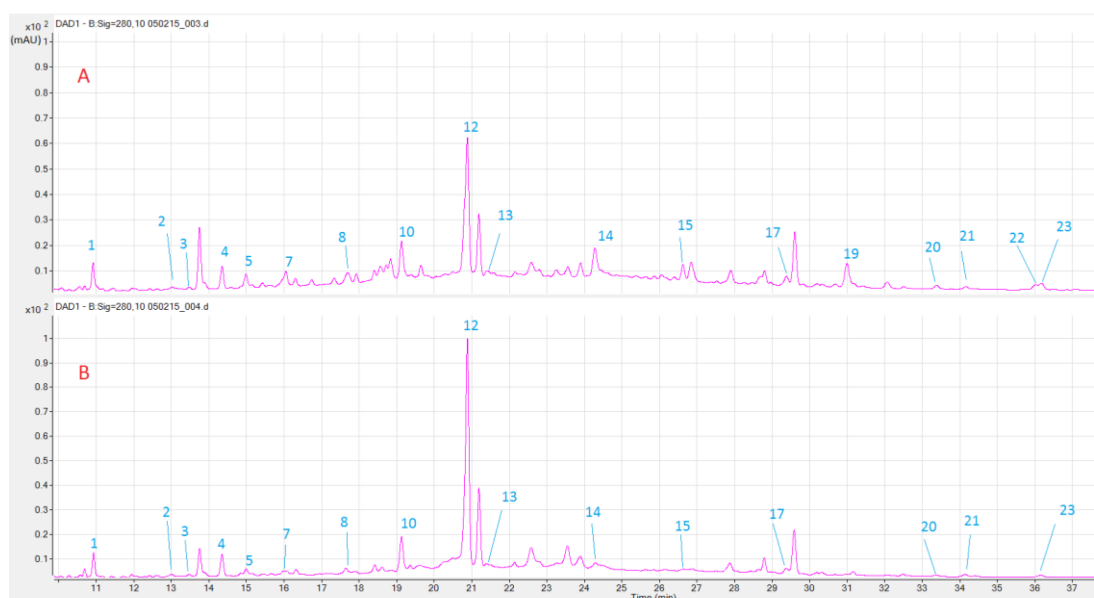


Figure 3.2. Representative HPLC-DAD chromatograms of sorghum wholegrain phenolic compounds A: Free phenolic compounds from genotype CHH2 grown at optimum temperature (OT). B: Free phenolic compounds from CHH2 grown at high temperature (HT).

Several derivatives of hydroxycinnamic acids were identified. Peaks 2 and 4 had a deprotonated molecule $[M-H]^-$ at *m/z* 253 and fragmentation patterns with ions at m/z 179 [caffeic acid –H]$^-$ and 135 [caffeic acid –H-CO_2]$^-$, suggesting the possible occurrence of a caffeic acid residue. The UV λ_{max} of these two peaks were at 297 and 326 nm, which was the same as that of caffeic acid. The $[M-H-74]^-$ ion at *m/z* 179 in peaks 2 and 4 is typical of that produced by the loss of a glycerol residue. Hence, peaks

2 and 4 were tentatively deduced as either 1-*O*-caffeoylglycerol or 2-*O*-caffeoylglycerol (Ma, Xiao, Li, Wang, & Du, 2007), since it was not possible to ascertain the substituent group positions. Peak 8 had the same UV λ_{max} of 310 nm as that of *p*-coumaric acid. MS spectra showed a deprotonated molecule $[M\text{-}H]^-$ at *m/z* 237 with fragment ions at *m/z* 163, 145 and 119. Fragment ions at *m/z* 163 and 119 appeared to correspond to deprotonated and decarboxylated *p*-coumaric acid, indicating the presence of *p*-coumaric acid. According to the above, this compound which lost the glycerol residue can form a 163 $[M\text{-}H\text{-}74]^-$ anion. Therefore, this compound was tentatively identified as 1-*O*-*p*-coumaroylglycerol or 2-*O*-*p*-coumaroylglycerol. The UV spectral characteristics of peaks 16 and 17 were similar to that of caffeic acid with λ_{max} at about 326 nm, suggesting derivatives of caffeic acid. They had a deprotonated molecule $[M\text{-}H]^-$ at *m/z* 415 and fragmentation patterns with ions at *m/z* 253, 179 [caffeic acid –H]$^-$, 161 [caffeic acid –H-H_2O]$^-$ and 135 [caffeic acid –H-CO_2]$^-$, which supported the presence of a caffeic acid residue. The fragment ion of *m/z* at 253 (415-162) could correspond to dehydrated caffeic acid attached to the caffeoylglycerol. From a comparison of literature data of UV and MS spectra, these two peaks were tentatively identified as either 1,2-*O*-dicaffeoyglycerol or 1,3-*O*-dicaffeoyglycerol. The deprotonated molecule ion $[M\text{-}H]^-$ showed peaks of 20 and 21 at m/z 399 and 429, respectively. Both peaks contained the same ions at *m/z* 253, indicating that these two compounds may also contain a caffeoylglycerol residue. Peak 20 also showed two prominent ions at m/z 163 [*p*-coumaric acid-H]$^-$ and 235 [M-H-*p*-coumaric acid]$^-$, suggesting it could have been derived from the loss of a *p*-coumaric acid residue. For peak 21, some fragment ions at *m/z* 235 and 193 were detected. The ion at *m/z* 235 [M-H-194] indicated the loss of a ferulic acid moiety, while another ion at *m/z* 193, corresponded to deprotonated ferulic acid, suggesting the presence of a ferulic acid moiety. After comparisons with the literature (Ma et al., 2007), peaks 20 and 21 were tentatively identified as *p*-coumaroyl-caffeoylglycerol and feruloyl-caffeoylglycerol, respectively.

Flavanols

Peak 1 had a deprotonated molecular ion $[M\text{-}H]^-$ at *m/z* 577 and fragment ions at *m/z* 425 and 289 (Table 3.3). The ion at *m/z* 425 [M-H-152] can be formed by the removal of a B-ring from the heterocyclic ring of catechin derivatives with the Retro-Diels-

Alder rearrangement in the ESI negative ion mode (Sun & Miller, 2003). The most intense ion at *m/z* 289 $[M-H-289]^-$ was considered to be specific for a C—C interflavan linkage in the catechin dimer (Guyot, Vercauteren, & Cheynier, 1996). The UV spectra of peak 1 were similar to that of (+)-catechin with λ_{max} at 280 nm. Therefore, this peak was tentatively identified as procyanidin B, which was supported by its MS and UV spectra in the literature (Sun, Liang, Bin, Li, & Duan, 2007).

Flavones

Most flavones have two characteristic UV absorption bands in the region 250-285 nm and 320-385 nm. Band I (320-385 nm) is produced by the B-ring, while Band II (250-285 nm) by the A-ring (Rice-Evans et al., 1996). Peaks 19 and 22 (Fig. 3.3) were positively identified as the flavones luteolin and apigenin respectively by comparison with Rt, UV and mass spectral data (Table 3.3) of the authentic standards.

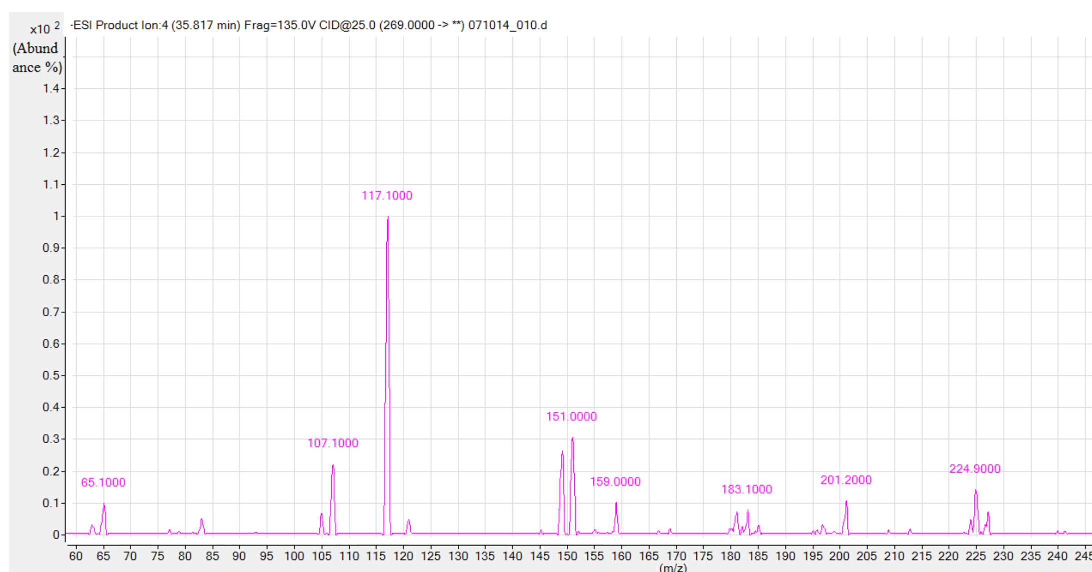


Figure 3.3. Mass spectra of apigenin of sorghum grain.

Flavanones and dihydroflavonols

Several flavanones were identified in sorghum grains. Peak 23 was positively identified as naringenin by comparing its Rt, UV and mass spectral data with the authentic standard. The deprotonated molecule ion $[M-H]^-$ of peak 18 was at m/z 287, suggesting this compound might be eriodictyol or dihydrokaempferol with the

molecular mass of 288. Peak 18 also showed the characteristic fragmentation ion at *m/z* 151 and UV spectra of eriodictyol, which exhibited a strong absorption in the region of 270-295 nm. Referring to MS and UV spectra of eriodictyol from the literature, peak 18 was tentatively identified as eriodictyol (Svensson et al., 2010). Peaks 6 and 10 showed the deprotonated molecule ions $[M-H]^-$ at *m/z* 449 and 433, respectively. The fragmentation ions at *m/z* 287 and 151, corresponding to an eriodictyol moiety, were also observed. The UV spectra of these two peaks also showed similar absorption in the region 270-295 nm as that for eriodictyol, indicating they might be derivatives of eriodictyol. It is well known that most of the flavonoids can conjugate sugars, and their derivatives are present as the glycosides of *O*- or *C*-forms (Stobiecki, 2000). Peak 6 produced a fragmentation ion at *m/z* 287 $[M-H-162]^-$ by possible loss of a glucoside, and the same fragmentation ion 287 $[M-H-146]^-$ was also produced via the possible loss of a deoxyhexoside from peak 10. Therefore, peaks 6 and 10 were tentatively identified as eriodictyol-7-*O*-glucoside and eriodictyol deoxyhexoside, respectively (Fang, Zhang, & Wang, 2007).

The compound of peak 13 was positively identified as the dihydroflavonol: taxifolin by comparison of Rt, UV and mass spectral data (Table 3.3) with the authentic standard. Since peak 14 had similar UV and MS spectra to that of peak 13, it was tentatively identified as taxifolin isomer.

3-Deoxyanthocyanidins

Two compounds, corresponding to peaks 7 and 9, exhibited the distinctive UV spectra of 3-deoxyanthocyanidins. Typical 3-deoxyanthocyanidins produce two UV absorption peaks in the 450-560 nm region due to the B ring hydroxy cinnamoyl system and in the 240-280 nm region due to the A ring benzoyl system (Rice-Evans et al., 1996). Compared with the Rt, MS and UV spectra of available standards, peaks 7 and 9 were unambiguously identified as luteolinidin and apigeninidin respectively.

3.3.4. Profiles of phenolic compounds

The list of phenolic compounds found in the free and bound forms of the different sorghum genotypes under OT and HT are shown in Table 3.4. Nearly all phenolic compounds identified across the sample collection (Table 3.4) were also identified in

each individual sorghum sample, except for the white sorghum PI563516, which contained fewer phenolic compounds. In addition, the range of phenolic compounds in the sorghum grains was larger in free form, and only brown sorghum IS 8525 showed a similar number of phenolic compounds in both the free and bound fractions. For all the sorghum genotypes, phenolic profiles for free and bound phenolics were essentially similar except for a few cases where some phenolic compounds were present or absent depending on the temperature regime (Table 3.4). For example, the free forms of luteolin and apigenin were not detectable in CHH2 genotype under HT, although they were present under OT (Fig. 3.2 A and B). These results indicated that the phenolic profile in sorghum grains was strongly influenced by both genotype and growth temperature.

Table 3.4. Profiles of polyphenols in the wholegrain of six sorghum genotypes grown at optimum temperature (OT) and high temperature (HT).

Polyphenol class and temperature treatment	Peak number [a]			
	OT		HT	
	Free	Bound	Free	Bound
CCH1	1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 15, 16, 17, 19, 20, 21, 23	5, 7, 9, 11, 12, 18, 19, 20, 23	1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 15, 16, 17, 19, 20, 21, 23	5, 7, 9, 11, 12, 18, 19, 20, 23
CCH2	1, 2, 3, 4, 5, 7, 8, 10, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23	5, 7, 9 11, 12, 15, 19, 20, 22, 23	1, 2, 3, 4, 5, 7, 8, 10, 12, 13, 14, 15, 17, 20, 21, 23	5, 7, 9 11, 12, 15, 19, 20, 22, 23
AQL33/QL36	1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, 15, 16, 17, 20, 21, 22, 23	5, 7, 9, 11, 12, 17, 18, 19, 20, 22, 23	1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, 15, 16, 17, 20, 21, 23	5, 7, 9, 11, 12, 17, 18, 19, 20, 22, 23
Ai4	1, 2, 4, 5, 7, 8, 9, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23	5, 7, 9, 11, 12, 18, 19, 20, 22, 23	1, 2, 4, 5, 7, 8, 9, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23	5, 7, 9, 11, 12, 18, 19, 20, 22, 23
PI563516	1, 2, 4, 5, 7, 8, 9, 12, 16, 17, 20, 21	5, 11, 12, 18	1, 2, 4, 5, 8, 12, 16, 17, 20, 21	5, 11, 12, 18
IS 8525	1, 2, 3, 4, 5, 6, 9, 10, 12, 13, 14, 15, 16, 17, 20, 21, 23	2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 15, 16, 18, 20, 23	1, 2, 3, 4, 5, 6, 9, 10, 12, 13, 14, 15, 16, 17, 20, 21, 23	2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 15, 16, 18, 20, 23

[a] Peak number identified in Table 3.3.

Table 3.5. Free, bound and total hydroxycinnamic acid content (µg/g db) in the wholegrain of six sorghum genotypes grown at optimum temperature (OT) and high temperature (HT).

Content		Temperature treatment	Genotypes					
			CHH1	CHH2	AQL33/QL36	Ai4	PI563516	IS 8525
CA	Free	OT	5.98±0.23 ^{aA}	7.19±0.54 ^{bB}	7.86±0.49 ^{bB}	13.12±0.74 ^{cB}	6.54±0.32 ^{aB}	33.72±4.22 ^{dB}
		HT	7.63±0.50 ^{cB}	4.45±0.66 ^{aA}	6.17±0.49 ^{bA}	9.98±0.65 ^{dA}	4.22±0.97 ^{aA}	18.84±1.46 ^{eA}
	Bound	OT	12.14±1.05 ^{cA}	12.92±0.76 ^{cA}	9.37±0.35 ^{bA}	5.56±0.47 ^{aA}	4.80±0.22 ^{aA}	13.89±0.34 ^{cA}
		HT	15.89±0.81 ^{dB}	11.38±1.87 ^{cA}	9.54±0.03 ^{bA}	6.88±0.17 ^{aA}	5.01±1.12 ^{aA}	14.18±0.15 ^{dA}
	Total	OT	18.12±1.27 ^{bA}	20.10±0.21 ^{cB}	17.22±0.83 ^{bB}	18.67±0.27 ^{bB}	11.34±0.54 ^{aB}	47.61±3.89 ^{dB}
		HT	23.51±0.31 ^{cB}	15.83±2.53 ^{bA}	15.71±0.52 ^{bA}	16.68±0.82 ^{bA}	9.23±0.97 ^{aA}	33.02±1.61 ^{dA}
FA	Free	OT	11.06±1.16 ^{cA}	33.16±0.35 ^{eB}	0.63±0.06 ^{aA}	1.06±0.13 ^{bA}	0.53±0.11 ^{aA}	26.82±2.94 ^{dB}
		HT	10.21±0.17 ^{cA}	26.00±3.35 ^{eA}	1.11±0.11 ^{bB}	1.18±0.01 ^{bA}	0.61±0.12 ^{aA}	16.74±5.17 ^{dA}
	Bound	OT	71.07±1.77 ^{bA}	86.09±0.40 ^{cB}	35.43±0.97 ^{aA}	35.85±0.33 ^{aA}	34.88±1.75 ^{aA}	125.50±1.96 ^{dB}
		HT	73.18±0.81 ^{cA}	69.02±0.24 ^{cA}	45.22±0.16 ^{bB}	40.45±1.62 ^{aB}	37.17±0.51 ^{aB}	116.13±5.30 ^{dA}
	Total	OT	82.13±0.61 ^{bA}	119.25±0.05 ^{cB}	36.05±0.91 ^{aA}	36.91±0.45 ^{aA}	35.41±1.84 ^{aA}	152.32±7.06 ^{dB}
		HT	83.39±0.64 ^{cA}	95.02±3.11 ^{dA}	46.32±0.06 ^{bB}	41.62±1.61 ^{aB}	37.78±0.46 ^{aB}	132.88±10.48 ^{eA}
FAI	Free	OT	1.76±0.04 ^{aA}	7.62±0.44 ^{cB}	3.40±0.16 ^{bA}	1.65±0.14 ^{aA}	nd	3.60±0.20 ^{bB}
		HT	2.74±0.13 ^{bB}	4.48±0.97 ^{cA}	4.17±0.48 ^{cA}	1.38±0.07 ^{aA}	nd	1.79±0.38 ^{aA}
	Bound	OT	nd	0.24±0.01 ^{aB}	nd	nd	nd	5.34±0.25 ^{bB}
		HT	nd	0.14±0.02 ^{aA}	nd	nd	nd	4.19±0.35 ^{bA}
	Total	OT	1.76±0.04 ^{aA}	7.86±0.45 ^{cB}	3.40±0.16 ^{bA}	1.65±0.14 ^{aB}	na	9.00±0.63 ^{dB}
		HT	2.74±0.13 ^{bB}	4.62±0.97 ^{cA}	4.17±0.48 ^{cB}	1.38±0.07 ^{aA}	na	5.98±0.73 ^{dA}

$^{a, b, c, d, e}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$). $^{A, B}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

Abbreviations: nd=not detected; na= data not available; CA= caffeic acid; FA= ferulic acid; FAI= ferulic acid isomer.

Table 3.6. Free, bound and total individual flavonoids content (µg/g db) in the wholegrain of six sorghum genotypes grown at optimum temperature (OT) and high temperature (HT).

Content			Temperature treatment	CHH1	CHH2	AQL33/QL36	Ai4	PI563516	IS 8525
				Genotypes					
3-Deoxyanthocyanidins									
	LUT	Free	OT	0.65±0.04 ^{aB}	2.33±0.13 ^{bB}	nd	14.24±0.98 ^{dB}	4.40±0.34 ^{c}	nd
			HT	0.13±0.02 ^{aA}	0.36±0.01 ^{bA}	nd	9.22±0.18 ^{cA}	nd	nd
		Bound	OT	1.92±0.55 ^{bA}	3.43±0.06 ^{cB}	3.88±0.23 ^{cA}	0.69±0.07 ^{aA}	nd	1.62±0.25 ^{bA}
			HT	3.47±0.28 ^{cB}	1.96±0.21 ^{bA}	4.10±0.48 ^{dA}	1.37±0.13 ^{aB}	nd	1.58±0.51 ^{abA}
		Total	OT	2.57±0.51 ^{bA}	5.76±0.08 ^{eB}	3.88±0.23 ^{cA}	14.93±1.05 ^{fB}	4.40±0.34 ^{dB}	1.62±0.25 ^{aA}
			HT	3.60±0.30 ^{cB}	2.31±0.21 ^{bA}	4.10±0.48 ^{cA}	10.59±0.31 ^{dA}	na	1.58±0.51 ^{aA}
	API	Free	OT	2.74±0.21 ^{bB}	2.49±0.01 ^{abB}	2.25±0.18 ^{aB}	23.24±1.29 ^{cB}	2.48±0.21 ^{ab}	2.05±0.08 ^{aB}
			HT	0.38±0.02 ^{aA}	0.64±0.08 ^{bA}	0.33±0.01 ^{aA}	14.52±0.71 ^{cA}	nd	0.60±0.02 ^{bA}
		Bound	OT	4.66±0.14 ^{bA}	23.84±1.73 ^{dB}	12.16±1.32 ^{cA}	4.70±0.16 ^{bA}	nd	2.27±0.18 ^{aA}
			HT	7.79±0.08 ^{cB}	16.88±3.79 ^{eA}	11.57±1.10 ^{dA}	6.20±0.31 ^{bB}	nd	2.43±0.65 ^{aA}
		Total	OT	7.40±0.06 ^{cA}	26.33±1.71 ^{eB}	14.41±1.51 ^{dB}	27.93±1.46 ^{eB}	2.48±0.21 ^{a}	4.33±0.26 ^{bB}
			HT	8.16±0.06 ^{bB}	17.52±3.71 ^{dA}	11.89±1.09 ^{cA}	20.72±0.40 ^{eA}	na	3.03±0.63 ^{aA}
Flavones									
	LU	Free	OT	0.92±0.17 ^{aA}	10.91±0.57 ^{c}	nd	4.04±0.07 ^{bA}	nd	nd
			HT	0.84±0.10 ^{aA}	nd	nd	5.57±0.35 ^{bB}	nd	nd
		Bound	OT	3.70±0.03 ^{bA}	5.05±0.74 ^{cB}	0.73±0.06 ^{aB}	0.91±0.02 ^{aB}	nd	nd
			HT	3.92±0.12 ^{bA}	3.62±0.13 ^{bA}	0.54±0.03 ^{aA}	0.52±0.03 ^{aA}	nd	nd
		Total	OT	4.62±0.14 ^{bA}	15.96±1.30 ^{cB}	0.73±0.06 ^{aB}	4.95±0.09 ^{bA}	na	na
			HT	4.76±0.22 ^{cA}	3.62±0.13 ^{bA}	0.54±0.03 ^{aA}	6.09±0.37 ^{dB}	na	na
	AP	Free	OT	nd	1.42±0.00 ^{b}	0.36±0.08 ^{a}	1.45±0.13 ^{bA}	nd	nd
			HT	nd	nd	nd	2.09±0.37 ^{B}	nd	nd
		Bound	OT	nd	2.61±0.13 ^{cB}	1.80±0.21 ^{bB}	0.86±0.02 ^{aA}	nd	nd
			HT	nd	1.20±0.17 ^{abA}	1.27±0.04 ^{bA}	1.16±0.02 ^{aB}	nd	nd
		Total	OT	na	4.03±0.13 ^{bB}	2.16±0.30 ^{aB}	2.30±0.11 ^{aA}	na	na
			HT	na	1.20±0.17 ^{aA}	1.27±0.04 ^{aA}	3.24±0.40 ^{bB}	na	na
Dihydroflavonols									
	TA	Free	OT	1.78±0.15 ^{bA}	7.92±0.82 ^{dB}	2.39±0.05 ^{cB}	1.21±0.01 ^{aA}	nd	12.85±0.78 ^{eA}
			HT	2.35±0.11 ^{cB}	4.15±0.21 ^{dA}	1.85±0.10 ^{bA}	1.55±0.01 ^{aA}	nd	12.36±2.38 ^{eA}

		Bound	OT	nd	nd	nd	nd	nd	41.33±1.10 ^{B}
			HT	nd	nd	nd	nd	nd	31.91±0.03 ^{A}
		Total	OT	1.78±0.15 ^{bA}	7.92±0.82 ^{dB}	2.39±0.05 ^{cB}	1.21±0.01 ^{aA}	nd	54.18±1.88 ^{eB}
			HT	2.35±0.11 ^{cB}	4.15±0.21 ^{dA}	1.85±0.10 ^{bA}	1.55±0.01 ^{aA}	nd	44.27±2.34 ^{eA}
	TAI	Free	OT	0.86±0.07 ^{aA}	12.97±0.54 ^{cB}	nd	11.21±0.08 ^{cB}	nd	3.60±0.20 ^{bB}
			HT	1.55±0.04 ^{aB}	5.45±0.48 ^{bA}	nd	9.41±0.28 ^{cA}	nd	1.66±0.50 ^{aA}
		Bound	OT	nd	nd	nd	nd	nd	nd
			HT	nd	nd	nd	nd	nd	nd
		Total	OT	0.86±0.07 ^{aA}	12.97±0.54 ^{cB}	na	11.21±0.08 ^{cB}	na	3.60±0.20 ^{bB}
			HT	1.55±0.04 ^{aB}	5.45±0.48 ^{bA}	na	9.41±0.28 ^{cA}	na	1.66±0.50 ^{aA}
Flavanone									
	NAR	Free	OT	1.37±0.53 ^{bA}	2.03±0.16 ^{cB}	0.62±0.08 ^{aA}	1.63±0.04 ^{bcA}	nd	1.97±0.10 ^{cB}
			HT	1.54±0.21 ^{dA}	4.15±0.21 ^{dA}	0.64±0.01 ^{aA}	1.47±0.06 ^{dA}	nd	1.25±0.03 ^{cA}
		Bound	OT	9.40±1.41 ^{cA}	6.46±0.25 ^{bB}	4.28±0.44 ^{aA}	20.58±0.75 ^{dB}	nd	10.34±0.93 ^{cB}
			HT	9.32±0.91 ^{cdA}	4.87±0.09 ^{aA}	6.45±0.83 ^{bB}	10.54±0.59 d^{A}	nd	8.80±0.38 ^{cA}
		Total	OT	10.76±1.94 ^{cA}	8.48±0.08 ^{bB}	4.90±0.35 ^{aA}	22.21±0.78 ^{eA}	nd	12.31±0.83 ^{dB}
			HT	10.86±1.12 ^{cA}	5.75±0.04 ^{aA}	7.09±0.84 ^{bB}	12.01±0.53 ^{dB}	nd	10.05±0.42 ^{cA}

$^{a, b, c, d, e}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$). $^{A, B}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

Abbreviations: nd=not detected; na= data not available; LUT= luteolinidin; API= apigeninidin; LU= luteolin; AP= Apigenin; TA= taxifolin; TAI= taxifolin isomer; NAR= naringenin.

3.3.5. Quantification of free, bound and total individual polyphenolic compounds

In order to more easily evaluate the results of the individual compounds, they were classified into five groups. Each compound was quantified in free and bound fractions and as total (free + bound).

Hydroxycinnamic acids

Three hydroxycinnamic acids were quantified in the sorghum genotypes: caffeic acid, ferulic acid and ferulic acid isomer (Table 3.5). The concentrations of the free, bound and total fractions of these were significantly modified by treatment and genotype ($p \leq 0.05$). HT resulted in significantly lower free caffeic acid concentration than OT across the sorghum genotypes, except for CHH1, which showed higher concentration at HT. No effect of treatment was found for free levels of ferulic acid and ferulic acid isomer of genotype Ai4. HT showed no significant impact on bound caffeic acid concentration in the different genotypes except for CHH1, which showed higher concentration at HT ($p \leq 0.05$). The total concentrations of caffeic acid and ferulic acid of PI563516 and IS 8525 had the lowest and highest respectively under both HT and OT (Table 3.5). The total ferulic acid concentration of all genotypes was higher than the other two phenolic acids, irrespective of temperature.

3-Deoxyanthocyanidins

Temperature, genotype and their interaction had a significant influence on free, bound and total concentrations of free luteolinidin ($p \leq 0.05$), which was lower under HT in four genotypes (not including the two genotypes which did not contain the free luteolinidin). The free fraction of luteolinidin could not be measured in PI 563516 at HT. Changes in free apigeninidin content due to temperature treatments followed the same pattern as those for free luteolinidin. Ai4 contained the highest free luteolinidin and apigeninidin concentrations under both temperatures. The levels of bound luteolinidin and apigeninidin in the different genotypes showed varying differences between the two temperature regimes. AQL33/QL36 and CHH2 accumulated the highest concentrations of bound luteolinidin and apigeninidin, respectively, under both treatments.

Flavones

Two flavones, luteolin and apigenin were identified and quantified in the sorghum samples and their concentrations are shown in Table 3.6. Temperature, genotype and the interaction between these two factors had a significant effect on the flavone concentrations ($p \leq 0.05$). Neither free nor bound luteolin and apigenin were detected in the two sorghum genotypes of PI563516 and IS 8525. CHH2 contained detectable concentrations of free luteolin and apigenin at OT, but these free flavones were not detected in this genotype at HT. Bound flavones accumulation also appears to be genotype-dependent under HT. Generally, its concentration had no significant change with increased temperature in CHH1, but significantly decreased in CHH2, AQL33/QL36 and Ai4. In fact, total luteolin and apigenin contents of CHH2 were higher than for the other genotypes under OT, but those of Ai4 reached the highest concentrations under HT.

Dihydroflavonol

The concentrations of two dihydroflavonols, taxifolin and the taxifolin isomer are presented in Table 3.6 and their concentrations were significantly affected by temperature, genotype and their interaction ($p \leq 0.05$). None of them in either the free or bound form were detectable in PI563516 under OT or HT. The free taxifolin and taxifolin isomer contents showed diverse changes in the other genotypes with the different temperatures. However, the bound form of taxifolin was only detected in sorghum IS 8525 and was around 3 times higher than its free form in this genotype; HT significantly reduced its concentration ($p \leq 0.05$). Regardless of treatment, no bound taxifolin isomer was detected across any of the genotypes. The results suggest that these two compounds were mainly in the free form. The total amounts of taxifolin were generally higher in IS 8525 as compared to other genotypes under both treatments.

Flavanones

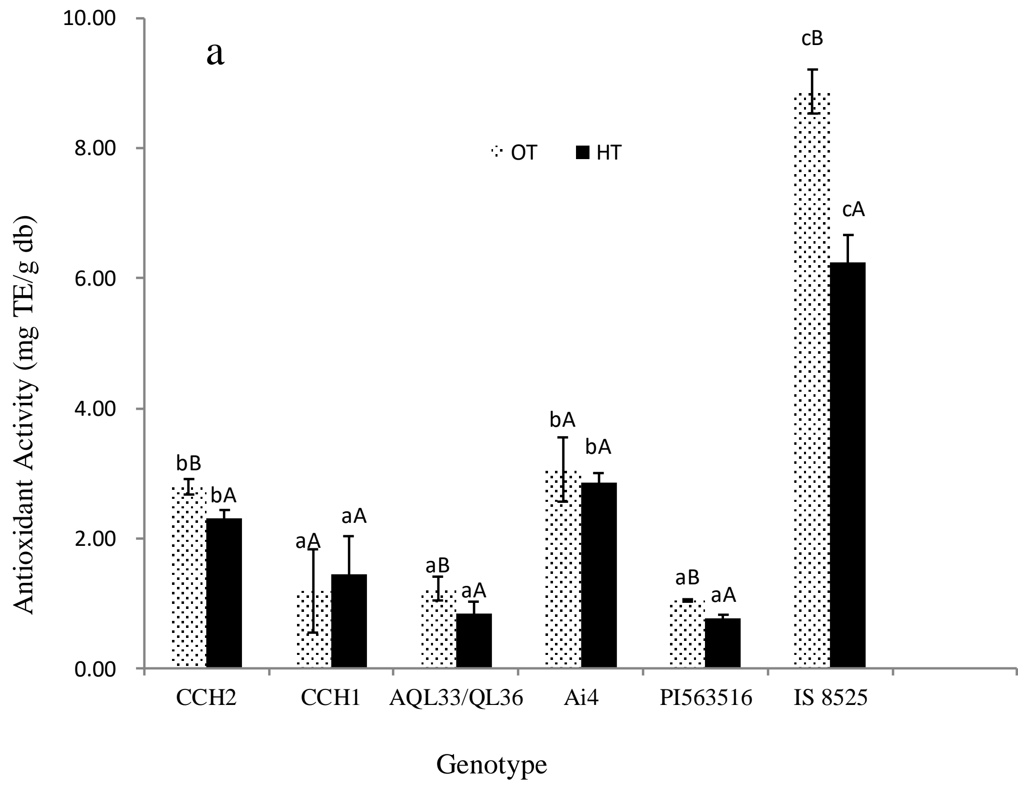
Several flavanones (Table 3.3) were identified in the sorghum samples but only naringenin was quantified, due to a lack of available standards. Values of naringenin were significantly affected by genotype, temperature and their interaction ($p \leq 0.05$). Apart from sorghum PI563516, all other sorghum genotypes contained naringenin in free and bound forms (Table 3.6). The bound naringenin concentration was significantly higher than free forms in five genotypes, irrespective of treatments. Total

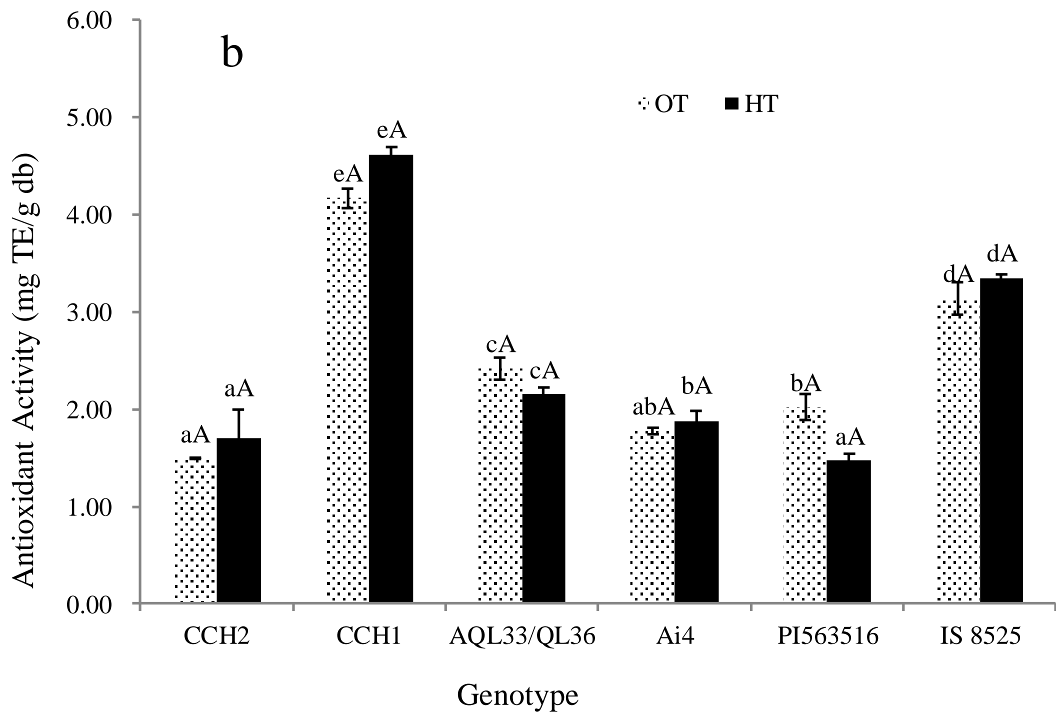
naringenin concentration in CHH2, Ai4 and IS 8525 was significantly lower at HT than OT, but significantly higher in AQL33/QL36 ($p \leq 0.05$).

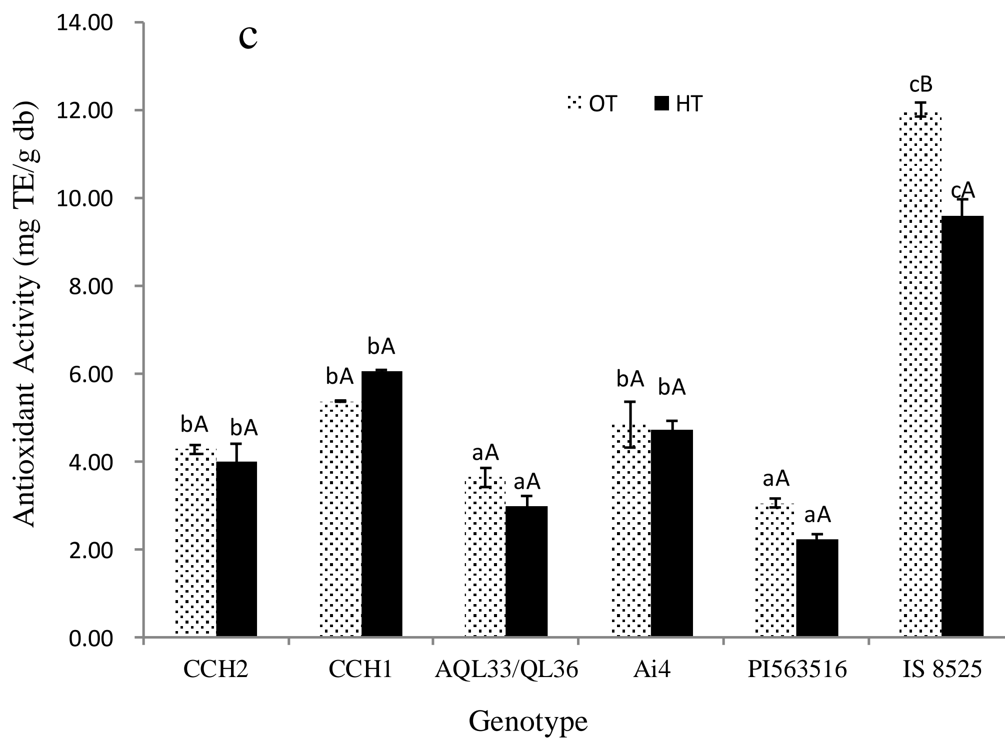
3.3.6. Antioxidant activity

The DPPH• scavenging activities of the free, bound and total phenolics total phenolics from the six sorghum genotypes grown under OT and HT are shown in Figure 3.4. The DPPH• scavenging activities of free polyphenol extracts was highest in IS 8525, and lowest in CCH1, AQL33/QL36 and PI523516 ($p \leq 0.05$) at both temperatures. For the bound fraction, the highest value was for CCH1 at both temperatures, whereas lowest values were for CCH2 and Ai4 at OT but CCH2 and PI523516 at HT. The DPPH• scavenging activities of total polyphenol extracts was highest in IS 8525 and lowest for AQL33/QL36 and PI523516 at both temperatures ($p \leq 0.05$).

The HT treatment gave lower DPPH• free antioxidant activity than the OT for CCH2, AQL33/QL36 and PI523516. Temperature did not significantly affect DPPH• values for bound fraction ($p > 0.05$), whereas the HT resulted in a lower total DPPH• antioxidant activity only for IS 8525 ($p \leq 0.05$).

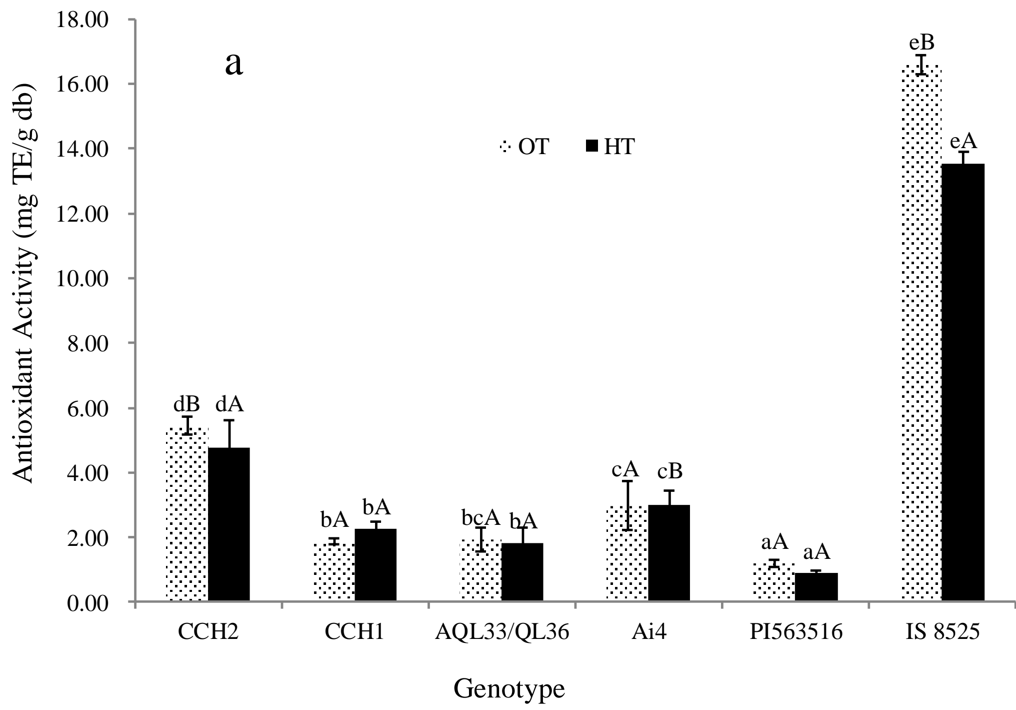

a
10.00
8.00
6.00
4.00
2.00
0.00
Antioxidant Activity (mg TE/g db)
OT HT
bB
bA
aA
aA
aB
aA
bA
bA
aB
aA
cB
cA
CCH2
CCH1
AQL33/QL36
Ai4
PI563516
IS 8525
Genotype

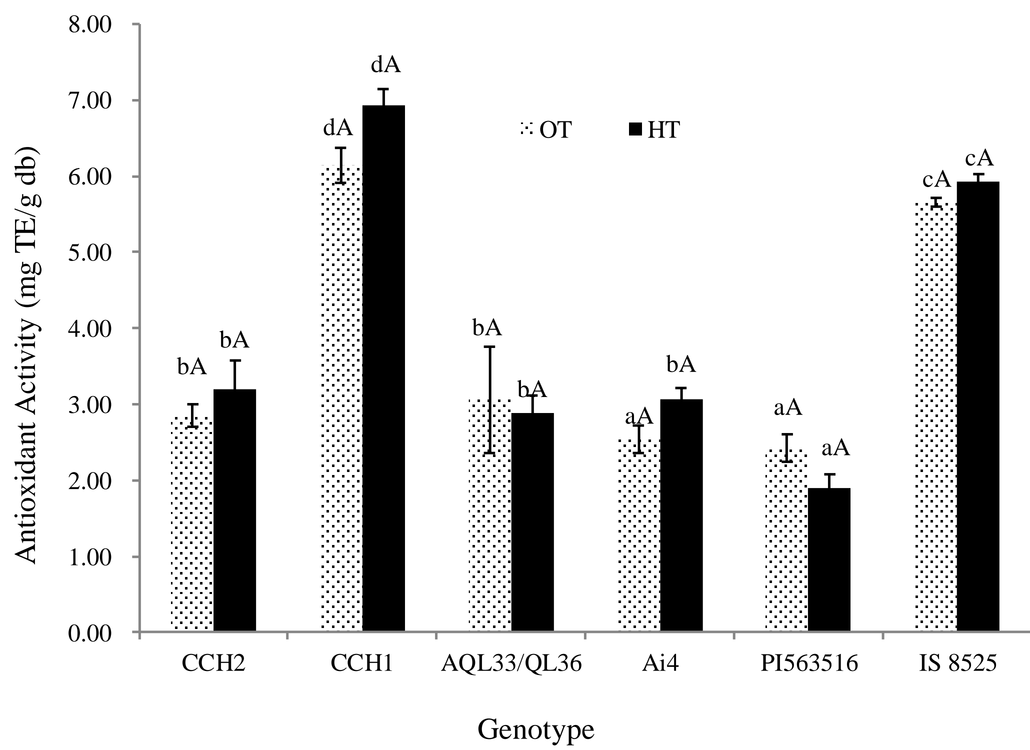

b
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Antioxidant Activity (mg TE/g db)
OT HT
aA
aA
eA
eA
cA
cA
abA
bA
bA
aA
dA
dA
CCH2
CCH1
AQL33/QL36
Ai4
PI563516
IS 8525
Genotype

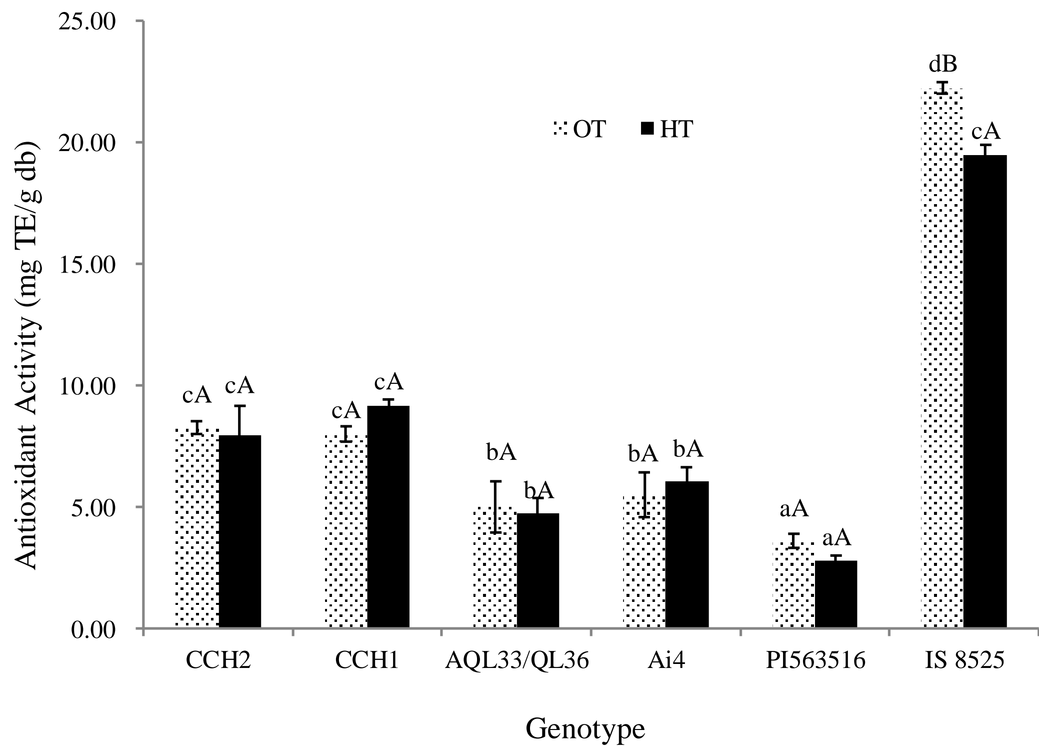


Optimal temperature, OT, 32/21°C; high temperature, HT, 38/21°C. TE, Trolox equivalents. a-e values with different letters in the same temperature treatment are significantly different ($p \leq 0.05$). A, B values with different letters in the same genotype are significantly different ($p \leq 0.05$).

Figure 3.4. Antioxidant capacity of free (a), bound (b) and total (c) extracts of sorghum grains as affected by two growth temperatures using the DPPH$^{\bullet}$ assay across six genotypes.


a
OT
HT
Antioxidant Activity (mg TE/g db)
Genotype
CCH2
CCH1
AQL33/QL36
Ai4
PI563516
IS 8525
dB
dA
bA
bA
bcA
bA
cA
cB
aA
aA
eB
eA
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00


OT
HT
Antioxidant Activity (mg TE/g db)
Genotype
CCH2
CCH1
AQL33/QL36
Ai4
PI563516
IS 8525
bA
bA
dA
dA
bA
bA
aA
bA
aA
aA
cA
cA
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00



Optimal temperature, OT, 32/21°C; high temperature, HT, 38/21°C. a-e values with different letters in the same temperature treatment are significantly different (p ≤0.05). A, B values with different letters in the same genotype are significantly different (p ≤0.05).

Figure 3.5. Antioxidant capacity of free (a), bound (b) and total (c) extracts of sorghum grains as affected by two growth temperatures using the ABTS$^{\bullet+}$ assay across six genotypes.

Table 3.7. Pearson correlation coefficients between sorghum polyphenol contents and antioxidant activity under optimum temperature (OT) $^{\alpha}$.

	Bound PP	Total PP	DPPH$^{\bullet}$ AC free	DPPH$^{\bullet}$ AC bound	DPPH$^{\bullet}$ AC total	ABTS$^{\bullet+}$ AC free	ABTS$^{\bullet+}$ AC bound	ABTS$^{\bullet+}$ AC total
Free PP	0.363	**0.978** ***	**0.986** ***	0.231	**0.971** **	**0.979** ***	0.498	**0.962** **
Bound PP		0.550 *	0.255	**0.951** **	0.523 *	0.293	**0.879** **	0.468
Total PP			**0.941** **	0.421	**0.987** **	**0.943** **	0.644 *	**0.967** **
DPPH$^{\bullet}$ AC free				0.131	**0.953** **	**0.983** ***	0.430	**0.949** **
DPPH$^{\bullet}$ AC bound		.			0.425	0.179	**0.915** **	0.379
DPPH$^{\bullet}$ AC total				.		**0.952** ***	0.672 *	**0.982** ***
ABTS$^{\bullet+}$ AC free		.					0.483	**0.976** **
ABTS$^{\bullet+}$ AC bound								0.683 *

$^{\alpha}$ PP, polyphenols; AC, antioxidant capacity.

Significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$

Table 3.8. Pearson correlation coefficients between sorghum polyphenol contents and antioxidant activity under high temperature (HT) [α].

	Bound PP	Total PP	DPPH$^{\bullet}$ AC free	DPPH$^{\bullet}$ AC bound	DPPH$^{\bullet}$ AC total	ABTS$^{\bullet +}$ AC free	ABTS$^{\bullet +}$ AC bound	ABTS$^{\bullet +}$ AC total
Free PP	-0.383	**0.964** **	**0.964** **	0.353	**0.910** **	**0.970** ***	0.322	**0.941** ***
Bound PP		0.213	-0.237	**0.957** *	-0.119	-0.295	**0.893** *	-0.533 *
Total PP			**0.891** *	0.234	**0.956** **	**0.913** *	0.142	**0.957** **
DPPH$^{\bullet}$ AC free				0.262	**0.896** *	**0.964** ***	0.466 *	**0.920** **
DPPH$^{\bullet}$ AC bound					0.664 *	0.297 *	**0.967** ***	0.559 *
DPPH$^{\bullet}$ AC total						**0.884** ***	**0.807** *	**0.970** ***
ABTS$^{\bullet +}$ AC free							0.320	**0.957** ***
ABTS$^{\bullet +}$ AC bound								0.724 *

[α] PP, polyphenols; AC, antioxidant capacity

Significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$

Figure 3.5 presents the effects of temperature on the values of $ABTS^{\bullet+}$ scavenging activities of the free, bound and total polyphenolic fractions of the sorghum grain. The pattern of genotype and temperature effects on $ABTS^{\bullet+}$ scavenging activities was similar to those for $DPPH^{\bullet}$ activity. To determine the relationships between sorghum polyphenol content and antioxidant activity, Pearson's correlation coefficients were determined, which demonstrated that the free, bound and total polyphenol contents had strong positive correlations with antioxidant activities of the free, bound and total phenolics ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$), respectively, under both OT and HT conditions. Significant positive correlations between $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ activity were also found (Table 3.7 and 3.8).

3.3.7. Tannin content

The influence of genotype and temperature during plant growth on the tannin content of the grain is presented in Table 3.9. Temperature, genotype and their interaction had a significant effect on tannin content of the uncooked wholegrains ($p \leq 0.05$). There were large variations in the tannin content ranging from 2.16 to 42.01 mg CE/g, with genotypes AQL33/QL36 and CHH1 having the lowest ($p \leq 0.05$) and IS 8525 having the highest ($p \leq 0.05$) content under OT, respectively. However, under HT, the tannin contents in the six sorghum wholegrain genotypes were significantly lower than under OT and ranged from 0.79 to 32.42 mg CE/g, with PI563516 and IS 8525 having the lowest and highest contents ($p \leq 0.05$), respectively (Table 3.9). The tannin content in the sorghum porridge ranged from 0.72 to 5.08 mg CE/g under OT, and from 0.38 to 4.02 mg CE/g under HT. After cooking, there was a large reduction in tannin content (about 87%) in IS 8525, while in the other five genotypes it was reduced by between 30 and 76%. The tannin contents in the porridge were still the highest in the genotype IS 8525, irrespective of growing temperature, and were the lowest in PI563516 and CHH2 ($p \leq 0.05$) for the OT, whereas at HT, porridge of all of the genotypes except IS 8525 had similar contents ($p > 0.05$).

Table 3.9. Influence of genotype and temperature during plant growth under optimum temperature (OT) and high temperature (HT) on tannin content (mg catechin equivalents/g db) in sorghum wholegrain flour (uncooked) and porridge.

Genotype	Wholegrain flour (OT)	Wholegrain flour (HT)	Porridge (OT)	Porridge (HT)
CHH2	2.73±0.11 ^{bC}	1.49±0.07 ^{cB}	1.91±0.12 ^{dB}	0.34±0.04 ^{aA}
CHH1	2.29±0.03 ^{aD}	1.67±0.06 ^{cC}	1.14±0.18 ^{bB}	0.68±0.05 ^{aA}
AQL33/ QL36	2.16±0.09 ^{aD}	1.00±0.05 ^{bC}	0.84±0.05 ^{aB}	0.49±0.02 ^{aA}
Ai4	4.13±0.13 ^{cD}	3.40±0.27 ^{dC}	1.46±0.04 ^{cB}	0.38±0.03 ^{aA}
PI563516	3.02±0.43 ^{bC}	0.79±0.03 ^{aB}	0.72±0.05 ^{aB}	0.46±0.07 ^{aA}
IS 8525	42.01±2.22 ^{dD}	32.42±0.66 ^{eC}	5.08±0.07 ^{eB}	4.02±0.03 ^{bA}

$^{a, b, c, d, e}$ Values with different letters in the same column are significantly different ($p \leq 0.05$).
$^{A, B,C, D}$ Values with different letters in the same row are significantly different ($p \leq 0.05$)

3.3.8. Phytate content

The phytate content of the wholegrain flours among the six sorghum genotypes varied between 10.60 mg/g (CHH2) and 17.44 (IS 8525) mg/g under OT (Table 3.10). There was a significant effect of genotype, temperature and their interaction on phytate content in the uncooked flours ($p \leq 0.05$). Increased temperature had diverse effects on phytate content of the sorghum grain. Considering the three hybrid lines with the same red colour (Fig 3.1), the phytate in CHH1 and CHH2 increased 32.24% and 26.13%, respectively, but showed no significant changes in AQL33/QL36 when growth temperature was increased. The white inbred line PI563516 had the higher content of phytate, while the other two genotypes (brown and white) showed no differences under the two temperature treatments. The phytate content in the porridge was not significantly different to that in the raw wholegrain flour for all sorghum genotypes ($p > 0.05$) at both growth temperatures (Table 3.10).

Table 3.10. Influence of genotype and temperature during plant growth under optimum temperature (OT) and high temperature (HT) on the phytate content (mg/g db) of sorghum wholegrain flour (uncooked) and porridge.

Genotype	Wholegrain flour (OT)	Wholegrain flour (HT)	Porridge (OT)	Porridge (HT)
CHH2	10.60±0.39 ^{aA}	13.73±0.13 ^{aB}	10.61±1.13 ^{aA}	12.98±0.50 ^{aB}
CHH1	12.25±0.49 ^{bA}	16.20±0.74 ^{cB}	12.37±0.15 ^{bA}	16.74±0.47 ^{cB}
AQL33/QL36	15.80±0.23 ^{dA}	15.02±0.82 ^{bA}	14.89±0.21 ^{dA}	14.37±0.17 ^{bA}
Ai4	15.56±0.23 ^{dA}	15.92±0.82 ^{bcA}	15.53±0.52 ^{dA}	15.70±1.62 ^{bcA}
PI563516	14.07±0.10 ^{cB}	12.96±0.94 ^{aA}	13.68±0.92 ^{cB}	12.87±0.63 ^{aA}
IS 8525	17.44±0.40 ^{eA}	16.39±0.41 ^{cA}	16.47±0.89 ^{eA}	16.24±0.13 ^{cA}

$^{a, b, c, d, e}$ Values with different letters in the same column are significantly different ($p \leq 0.05$).
$^{A, B}$ Values with different letters in the same row are significantly different ($p \leq 0.05$).

3.3.9. Mineral content

The iron content of the wholegrain flour ranged from 14.07 to 47.83 mg/kg db, with PI563516 having the lowest and CHH1 having the highest level ($p \leq 0.05$) under OT (Table 3.11). Iron content was significantly affected by genotype and temperature ($p \leq 0.05$). It is interesting to note that the iron content in the hybrid lines (36.9-47.83 mg/kg db) was significantly higher than that in the inbred lines (14.07-37.43 mg/kg db) ($p \leq 0.05$). Under HT the iron content in the wholegrain flours varied between 19.57 and 54.53 mg/kg , with the content in CHH1 and PI563516 remaining the lowest and highest respectively ($p \leq 0.05$) (Table 3.11). Temperature was found to have a significant effect on the iron content in some of the sorghum flours ($p \leq 0.05$). For example, the iron content in CHH1 and IS 8525 was significantly higher ($p \leq 0.05$) in HT than in OT, whereas that of AQL33/QL36 was lower under HT ($p \leq 0.05$).

The calcium content of the sorghum is also shown in Table 3.11, and was significantly affected by genotype and temperature ($p \leq 0.05$). The six sorghum genotypes varied significantly ($p \leq 0.05$) in their calcium content, ranging from 50.87 to 99.47 mg/kg db flour under OT and 63.26 to 105.33 mg/kg db flour under HT. Calcium content in only two of the sorghum genotypes (AQL33/QL36 and Ai4) was significantly reduced under high growing temperatures, while in the others they increased

significantly ($p \leq 0.05$), demonstrating a genotype × treatment interaction.

Phosphorus is the major mineral in sorghum and its content in the sorghum grains was significantly modified by genotype and temperature ($p \leq 0.05$). A 1.015 mg/kg flour difference was recorded between AQL/QL36 (the highest content) and PI563516 (the lowest content) under OT. An increase in temperature resulted in a decrease in the phosphorus content in the grains of AQL33/QL36 and PI563516 ($p \leq 0.05$).

Zinc content was significantly affected by genotype and temperature ($p \leq 0.05$), and ranged from 12.33 to 27.03 mg/kg flour under OT, and from 16.10 to 26.80 mg/kg flour under HT, respectively (Table 3.11). Zinc levels were unchanged in Ai4 and CHH2, decreased in AQL33/QL36 ($p \leq 0.05$), but increased in the other three genotypes ($p \leq 0.05$) when the growing temperature was increased.

Table 3.11. Influence of genotype and growing temperature during plant growth under optimum temperature (OT) and high temperature (HT) on mineral (Fe, Ca, P, Zn) contents (mg/kgdb) in sorghum wholegrain flour (uncooked).

Genotype	Fe		Ca		P		Zn	
	OT	HT	OT	HT	OT	HT	OT	HT
CHH2	36.90 ± 0.10^{cB}	33.47 ± 0.15^{bA}	99.47 ± 0.55^{eA}	105.33 ± 2.51^{dB}	3156 ± 55^{aA}	3636 ± 38^{bB}	20.17 ± 0.21^{bA}	21.06 ± 1.00^{cA}
CHH1	47.83 ± 0.47^{eA}	54.53 ± 1.16^{eB}	66.00 ± 3.21^{cA}	85.37 ± 3.57^{cB}	3460 ± 25^{bA}	4490 ± 36^{dB}	20.70 ± 0.35^{bA}	24.57 ± 0.75^{dB}
AQL33/QL36	45.37 ± 0.38^{dB}	35.57 ± 1.21^{cA}	88.57 ± 2.37^{dB}	63.26 ± 0.53^{aA}	4215 ± 13^{cB}	3957 ± 31^{cA}	19.30 ± 0.17^{bB}	17.50 ± 0.46^{bA}
Ai4	37.43 ± 0.75^{cA}	35.57 ± 0.31^{cA}	96.47 ± 1.32^{eB}	87.17 ± 1.92^{cA}	3480 ± 10^{bA}	3680 ± 43^{bB}	27.03 ± 0.84^{dA}	26.80 ± 0.63^{eA}
PI563516	14.07 ± 0.15^{aA}	19.57 ± 0.31^{aB}	50.87 ± 0.77^{aA}	65.74 ± 0.15^{aB}	3200 ± 36^{aB}	2980 ± 75^{aA}	12.33 ± 0.15^{aA}	16.10 ± 0.20^{aB}
IS 8525	30.53 ± 0.25^{bA}	40.37 ± 0.66^{dB}	58.40 ± 0.26^{bA}	70.30 ± 1.18^{bB}	3963 ± 51^{bA}	3593 ± 15^{cB}	24.40 ± 0.26^{cA}	30.63 ± 0.06^{fB}

$^{a, b, c, d, e, f}$ values with different letters in the same column are significantly different ($p \leq 0.05$).
$^{A, B}$ values with different letters in the same genotype and the same mineral are significantly different ($p \leq 0.05$).

3.3.10. *In vitro* iron dialysability

Genotype, temperature and their interaction had a significant effect on the *in vitro* iron dialysability ($p \leq 0.05$) of the uncooked wholegrain flours (Table 3.12.). Under OT the highest *in vitro* iron dialysability ($p \leq 0.05$) was found in CHH2, while the lowest ($p \leq 0.05$) occurred in IS 8525. Values varied from 0.87 to 5.94% for the genotypes grown under OT but ranged from 0.47 to 3.15% under HT. The *in vitro* iron dialysability in most tested sorghum genotypes was decreased, with the exception of genotype Ai4 which was significantly increased, under the HT regime. The flour from IS 8525 had the lowest levels of *in vitro* iron dialysability under both growing temperatures.

Table 3.12. Influence of genotype and temperature during plant growth under optimum temperature (OT) and high temperature (HT) on *in vitro* iron dialysability (%) of sorghum wholegrain flour and porridge.

Genotype	Wholegrain flour (OT)	Wholegrain flour (HT)	Thick porridge (OT)	Thick porridge (HT)
CHH2	5.94±0.13 ^{fB}	1.79±0.09 ^{cA}	5.76±0.45 ^{fB}	1.67±0.10 ^{cA}
CHH1	4.70±0.07 ^{eB}	3.15±0.17 ^{dA}	4.54±0.15 ^{eB}	3.21±0.11 ^{dA}
AQL33/QL36	1.54±0.11 ^{bB}	1.10±0.24 ^{bA}	1.47±0.12 ^{bB}	0.89±0.04 ^{bA}
Ai4	2.61±0.06 ^{cA}	3.10±0.08 ^{dB}	2.42±0.23 ^{cA}	3.18±0.23 ^{dB}
PI563516	3.09±0.21 ^{dB}	1.07±0.15 ^{bA}	2.92±0.13 ^{dB}	0.95±0.12 ^{bA}
IS 8525	0.87±0.09 ^{aB}	0.47±0.08 ^{aA}	0.83±0.06 ^{aB}	0.44±0.05 ^{aA}

$^{a, b, c, d, e, f}$ values with different letters in the same temperature column are significantly different ($p \leq 0.05$).
$^{A, B}$ values with different letters in the same row are significantly different ($p \leq 0.05$).

The *in vitro* iron dialysability in the porridge varied between 0.83 and 5.76% for the OT samples and between 0.44 and 3.21% for the HT samples ($p \leq 0.05$). The cooking process had no significant effect on the iron dialysability compared to the raw wholegrain flours ($p > 0.05$). Levels of *in vitro* iron dialysability in sorghum grains were negatively correlated with tannin (Pearson's correlation: r = 0.463, $p \leq 0.05$) and phytate (r = 0.694, $p \leq 0.05$) content.

3.4. DISCUSSION

3.4.1 Grain physical characteristics

Grain physical characteristics are important quality attributes for food processing. The range of grain hardness in the present study was similar to that reported by Schober, Messerschmidt, Bean, Park, and Arendt (2005), with values of grain hardness between 76.1 and 95.1. The effect of increased temperature on grain hardness showed genotype-dependent differences with a significant increase in four genotypes, a decrease in one genotype and no effect in another genotype. These results differ from a study by Griess et al. (2010), who found that sorghum grain was harder under warmer growth temperatures, possibly because grain hardness changes may be related to changes in the contents of starch and protein in sorghum grains under high temperature (Li et al., 2013).

Flour yield of wheat grain is positively correlated with grain weight and diameter (Gibson, McCluskey, Tilley, & Paulsen, 1998). Therefore the low grain weight and diameter of CCH1 under HT suggests that this genotype may not be suited for use in flour manufacture under predicted future elevated temperatures. The higher grain weight and diameter under HT of the other genotypes suggests they may be better suited. A study by Nguyen et al. (2013), using the same plants from which the grain in the present study was obtained, found that high temperatures affected seed-set, with Ai4 and PI563516 attaining high seed-set under HT. This further supports the potential of these genotypes to obtain good yields in high temperature environments.

3.4.2 Polyphenol and antioxidant activity

Waniska et al. (1989) found that free polyphenol content increased in white pericarp sorghum but decreased in red pericarp sorghum under warmer and wetter environments, a finding different to that in the present study. However, the study by Waniska et al. (1989) was not performed under highly controlled conditions and any individual effect of temperature was not separated from that of water availability. In addition, bound polyphenolic compounds in plants are bound to cell wall structural components and therefore alkaline or acid hydrolyses are required to release them during extraction prior to their analysis (Acosta-Estrada, Gutiérrez-Uribe, & Serna-

Saldívar, 2014). In the present study, the acid hydrolyses assay was used to extract bound polyphenols. Other cereal grains, such as barley, contain similar amounts of bound polyphenols ranging from 1.78-2.95mg GAE/g (db) (Madhujith & Shahidi, 2009). Widely different levels of total phenolics can occur in different sorghum genotypes (Dykes et al., 2005; Afify et al., 2012). According to sorghum classifications by Awika and Rooney (2004), tannin brown sorghum grain typically has the highest levels of polyphenols, as was also found in the current study for the genotype IS 8525.

In the current study, several compounds were identified for the first time in sorghum grain. For example, feruloyl-caffeoylglycerol, which is also present in *Ananas comosus* L. leaves (Ma et al., 2007), was tentatively identified. Similarly, two flavonoid compounds of eriodictyol deoxyhexoside and taxifolin isomer were also tentatively identified. Although catechin has been found in the red sorghum PAN 3860 by Svensson et al. (2010), this compound was not detected in any of the six sorghum genotypes used in the present study. The two-ubiquitous sorghum 3-deoxyanthocyanidins, apigeninidin (yellow) and luteolinidin (orange) were identified in our sorghum genotypes. The two other 3-deoxyanthocyanidins of 5-methoxyluteolinidin and 7-methoxyapigeninidin were not detected in our samples, but were present in another study of sorghum genotypes with lemon-yellow pericarp colour (Dykes et al., 2011). Additionally, certain phenolic acids, such as gallic and cinnamic acids were previously identified in sorghum grain (Hahn et al., 1983), but were not detected in the present study. Some phenolic acid esters of glycerol, including dicaffeoylglycerol and caffeoylglycerol, were detected by Svensson et al. (2010), while the isomers of these phenolic acid esters of glycerol are reported for the first time in the present study. There were some putative polyphenol peaks that were present in the HPLC-DAD-ESIMS chromatograms (Fig. 3.2) that could not be identified, likely due to their low UV signals in the DAD or unknown molecular mass fragments.

The free, bound and total contents of individual polyphenols in the sorghum genotypes in the current study were within the range of those previously reported (Dykes et al., 2011; Hahn et al., 1983; Taleon et al., 2012, 2014). Most of the phenolic compounds in sorghum grain were present in both free and bound forms. For several individual compounds, such as ferulic acid and naringenin, they mainly occurred in the bound form, which was in agreement with other studies (Dykes & Rooney, 2006; Svensson

et al., 2010). It has been suggested that dietary intake of free forms rather than bound forms of phenolic compounds may have health benefits in protecting against cardiovascular disease and certain types of cancer, since their gastrointestinal absorption is more rapid (Bravo, 1998). However, epidemiological studies also indicate that consuming wholegrains with bound phenolic compounds could reduce the risk of colon cancer and other digestive cancers. This may be related to bound phenolic compounds being degraded by the microflora and their degradation products being absorbed in the colon (Adom & Liu, 2002). In the large intestine, these bound polyphenols can be enzymatically degraded by colonic bacteria to produce metabolites with different physiological significance, and the metabolism of this degradation includes the breakdown of the heterocyclic backbone and the cleavage of glycosidic linkages (Cardona, F., Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Saura-Calixto, 2012).The profiles and levels of phenolic compounds cannot be simply attributed to the sorghum grain colour. Taleon et al. (2014) grew five different red sorghum genotypes under the same growth condition, but some individual phenolics, such as luteolin, were only detected in red 99LGWO50. In the present study, luteolin was absent in red sorghum AQL33/QL36 in comparison with other red genotypes. White sorghum PI563516 contained the least number of flavonoids, with some common flavonoids, such as luteolin, apigenin and naringenin being absent in this genotype. In additon, two of the 3-deoxyanthocyanidins, luteolinidin and apigeninidin, were present in white sorghum 02CA4796 (Dykes et al., 2005), although the concentrations were lower than PI563516 in the present study.

The biosynthesis of phenolic compounds in plants is regulated by enzymes such as phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI) and flavonoid-3'-hydroxylase (F3'H) (Cohen & Kennedy, 2010). Enzyme activities, which may modify phenolic biosynthesis, have varied among plant genotypes in previous studies. Results from Mccallum and Walker (1991) showed that red wheat genotypes had higher PAL and CHI activities than white genotypes during early grain development, which can lead to higher synthesis of flavonoids. Similar results were also observed in two different apple genotypes, with PAL, CHI and flavonoid 3-*O*-glucosyltransferase (UFGT) activities of the red-skinned genotype being several-fold higher than for the green-skinned genotype at all stages of fruit development, leading to a greater accumulation of flavonoids in the red-skinned genotype (Lister, Lancaster, & Walker,

1996). In the present study, the total 3-deoxyanthocyanidins, flavones, dihydroflavonol and flavanone concentrations of coloured sorghum were higher than those in white sorghum, indicating that the PAL, CHI and UFGT enzyme activities may have been higher in these genotypes during grain development.

The effect of high temperature on sorghum grain phenolic compounds was diverse across the six sorghum genotypes. The possible reason could be that the phenolic metabolism pathway was also influenced by temperature. In tomato high temperature increased PAL activity and total phenolic compounds showed an increase under high temperature stress (Rivero et al. 2001). Based on the present study, it can be proposed that the phenolic changes under high temperature in sorghum grain may have been caused by changes in PAL activity. For individual polyphenols, the changes may have been related to some specific enzyme activity changes. For example, anthocyanin concentrations of grape berries grown at a constant 30 ℃ was lower than those grown at 30 ℃/15 ℃(day/night), and anthocyanin accumulation correlated strongly with UFGT activity (Mori, Sugaya, & Gemma, 2005). However, the biosynthetic pathways of 3-deoxyanthocyanidins in sorghum are different from anthocyanin biosynthetic pathways in other plants, and the enzymes involved in 3-deoxyanthocyanidin synthesis are flavonoid-3'-hydroxylase (F3'H) and chalcone synthase (CHS) (Cohen & Kennedy, 2010; Shih, Chu, Yip, & Lo, 2006). In the present research, it was noted that the abundance of free luteolinidin and apigeninidin was markedly decreased under high temperature, and these changes could be related to these enzymes changes. Further research is required to study these enzymes in the metabolic pathway of 3-deoxyanthocyanidin of sorghum grains under different temperature conditions.

Polyphenols act as antioxidants and much research has been carried out on their health benefits. Sorghum is an important cereal crop in many parts of the world, and with its high levels of polyphenols has potential to be developed as a health-enhancing functional food. To explore this further, antioxidant activities of the sorghum grain polyphenols were evaluated in the present study using the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays; two common methods used to assess antioxidant activities (Thaipong et al., 2006). Results of the antioxidant activity of total phenolic compounds of sorghum grain determined by the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays indicated that both assays can rank sorghum grain samples for their antioxidant activity and that the antioxidant activity

was primarily due to sorghum polyphenol compounds, which is consistent with previous work on sorghum (Dykes et al., 2005; Dykes & Rooney, 2007).

3.4.3. Tannin, phytate, mineral and i*n vitro* iron availability

The tannin content of the sorghum grains in this study was significantly reduced by increased temperature. The condensed tannins in sorghum develop sequentially from a monomer through dimer and trimers of procyanidins, until they reach high molecular weight forms during the plant growth cycle (Glennie, Kaluza, & Van Niekerk, 1981). It is proposed that this biosynthesis process may be inhibited under high temperature, since HT significantly reduced the condensed tannin content.

It has previously been reported that sorghum genotypes with a pigmented testa contained significantly higher levels of tannin than those lacking a pigmented testa (Dykes et al., 2005). In the present study, the tannin content of IS 8525 (brown) was about 10-15 times higher than for the other genotypes, suggesting that IS 8525 may have both dominant B_1 and B_2 genes, which control the presence or absence of a pigmented testa.

Sorghum grains are usually cooked prior to human consumption, so that understanding changes in tannins during the cooking process is important. The tannin contents in the six genotypes were significantly reduced in the cooked porridge, which is in agreement with that of Dlamini et al. (2007) and Wedad, El Tinay, Mustafa, and Babiker (2008) who showed that the tannin content in sorghum grain was reduced by 36% - 80% through cooking. This may be a result of the phenolic hydroxyl groups of tannin reacting with food compounds (protein, and minerals such as iron) to form insoluble complexes during the heating process (Matuschek, Towo, & Svanberg, 2001).

However, tannin has been commonly referred to as an anti-nutritional factor, because of its high affinity for proteins and tendency to bind with metal ions, thus decreasing their dialysability (Nyachoti, 1997; Radhakrishnan & Sivaprasad, 1980). Nevertheless, these negative effects can be reduced or eliminated by decreasing the tannin content in sorghum grains through cooking. As discussed, in the present experiment, the tannin content of sorghum was significantly reduced by growing under the higher temperatures. This suggests that sorghum could contain lower contents of tannin under

the expected higher future temperatures, which would help increase its nutritional value as human food or animal feed. However, tannin has also been identified as the major contributor to the antioxidant activity of some genotypes of sorghum grains (Brglez et al., 2016); the tannins may also have gastro-protective and cholesterol-lowering properties (Dykes & Rooney, 2007). For future production of high tannin sorghum for manufacture into high antioxidant foods, the genotype IS 8525 could be a good candidate since it contained the highest contents of tannin in both OT and HT conditions in the present study.

Apart from tannin, phytate chelates metal ions, such as iron, to form insoluble complexes in the gastrointestinal tract, and the iron cannot then be digested due to lack of intestinal phytase enzymes (Gibson, Bailey, Gibbs, & Ferguson, 2010). The effect of temperature during plant growth on the phytate content in sorghum grains has not been reported previously. In the present study, HT could significantly increase or had no influence on phytate content. Compared to other crops, including maize, however, Horvatic and Balint (1996) found that lower temperatures and increased irrigation decreased the phytate levels, but they did not separate the influence of temperature and irrigation.

In addition, the phytate content in the cooked porridge was not significantly different to that in the raw wholegrain flour for all sorghum genotypes within each growth temperature. Endogenous phytases that are present in sorghum grains have the potential to break down phytate. However, they may have been deactivated by heat during the porridge preparation (Duodu, Minnaar, & Taylor, 1999). Previous research found that phytate in sorghum seeds was reduced under the traditional porridge preparation method (Wedad et al., 2008), probably because of longer cooking time than that used in the present research. In the present study, cooking into a simple porridge did not reduce the phytate content. These results indicate that the cooking process used may not have alleviated the mineral binding properties of the phytate.

The mineral content of sorghum reported in the present study was in the range of previous reports (Kruger et al., 2013; Afify, El-Beltagi, El-Salam, & Omran, 2011). However, Martino et al. (2012) measured the mineral concentrations of eight sorghum genotypes planted in Brazil, and results indicated that the contents of Fe, Zn and P ranged from 4.7 to 14.9 mg/kg , 13.2-27.0 mg/kg , and 1790-2798 mg/kg , respectively,

which was significantly lower than present studies. One of the possible reasons for the difference between these two studies is that the mineral content may have been influenced by soil, climate variations and genotype. As shown in Table 3.11, HT significantly increased the content of Fe, Ca, P, Zn in genotypes CHH1 and IS 8525, but decreased it in AQL33/QL36. Based on these results, it is proposed that the transport of nutrients in sorghum may be affected by high temperature, but also varies depending on genotype. As sorghum is one of the staple cereal grains in Africa, sorghum grain may provide a major source of minerals for local people. Consequently, knowledge of variation in mineral content in different sorghum genotypes under high temperature treatments can significantly support plant breeding programs to breed cultivars for human consumption with high nutrient minerals. In this research, the two sorghum hybrid lines CHH2 and CHH1 with high mineral content are likely suitable genotypes to be grown in a high temperature environment as a good dietary mineral source.

Evaluation of the way in which genotype and increased temperature influence *in vitro* iron dialysability of sorghum is important before preparing foods. For *in vitro* iron dialysability, sorghum IS 8525 had the lowest values compared to the other genotypes. This may be related to the fact that IS 8525 had extremely high levels of tannin, which may have exerted a significant inhibitory effect on iron absorption (Brune, Rossander, & Hallberg, 1989). One of the postulated mechanisms of the action of tannins is chelation of pro-oxidant metals such as iron (Kayodé, Hounhouigan, & Nout, 2007).

Across all genotypes, the *in vitro* iron dialysability was not improved after cooking. This could partly be due to the phytate not being affected by the heat treatment, since phytate can also chelate iron to form insoluble complexes to decrease iron dialysability. Kruger et al. (2012) observed similar results when they prepared thick porridge using four different sorghum genotypes. However, some researchers have shown that heat treatment could slightly increase (Kayodé et al., 2006) or decrease (Hemalatha, Platel, & Srinivasan, 2007) the *in vitro* iron dialysability. The *in vitro* iron dialysability is strongly affected by the levels of polyphenols and phytate, and it has previously been proposed that a reduction of the phenolic content without a reduction of phytate would not improve iron dialysability (Towo et al., 2006). Some researchers have also investigated the effect of reducing phytate in tannin and tannin-free sorghum

genotypes by fermentation, and reported that only tannin-free sorghum genotype increased their iron dialysability after phytate reduction (Wedad et al., 2008). Their results suggest that the *in vitro* iron dialysability in sorghum grain was increased by reducing both phytate and tannin content. In the present experiment, cooking significantly decreased tannin content but had no effects on the phytate content, such that the iron dialysability was not improved after cooking.

From the present study, growth under HT gave reduced *in vitro* iron dialysability of the raw wholegrain and porridge of some sorghum genotypes. Therefore the nutritional value of these foods, from an iron dialysability point of view, could decrease under predicted higher temperatures in the future. In terms of sorghum porridge as a staple food for people living in malnourished communities in rural Africa, the sorghum genotype CHH2, which had higher *in vitro* iron dialysability than the other sorghum genotypes, could be a suitable available iron source. However, under high temperature growth conditions, the sorghum genotypes CHH1 and Ai4 have potential for high *in vitro* iron dialysability.

3.5. CONCLUSION

This study successfully identified the wholegrain characteristics, polyphenol profiles and amounts, antioxidant activities, tannin, phytate, mineral (Fe, Ca, P, Zn) contents and *in vitro* iron dialysability, in response to growth under optimal and high temperature regimes for six sorghum genotypes. These genotypes were of 3 different grain colours (red for CCH2, CCH1, AQL33/QL36 and Ai4, white for PI563516 and brown for IS 8525). Wholegrain characteristics were significantly modified by temperature and genotype. The genotypes included in this study had significantly different total polyphenol levels regardless of growth temperature. Temperature, however, was not found to have a significant effect on total polyphenol content or antioxidant capacities with the exception of IS 8525 for which these parameters were lower under HT than OT. Genotype, temperature and their interaction also had a significant effect on tannin, phytate, mineral contents and *in vitro* iron dialysability of raw grain flours. The tannin content in all sorghum grains investigated decreased under the higher growth temperature. At the same time, the high growth temperatures had diverse effects on the phytate, mineral contents and *in vitro* iron dialysability of the

sorghum grains, depending on genotype. These results suggest that of the genotypes investigated, IS 8525 may be the most suitable for use in health-functional food formulation, while CCH2, CHH1 and Ai4 are recommended for use in staple foods for population groups requiring high iron dialysability.

Additionally, increasing global climate change will result not only in elevated temperatures, but also in reduced availability of seasonal rainfall, due to increased evaporation in response to increased temperatures and changing rainfall patterns with more rainfall falling outside the growing season. However, no research is available on how the level of water availability can influence the polyphenol and antioxidant activity of sorghum grain and therefore this is the topic of the next chapter of this thesis.

CHAPTER FOUR: EXPERIMENTAL

Impact of water deficit on polyphenol composition and antioxidant activity of sorghum grains

Information contained in this chapter has been submitted as follows:

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ABSTRACT

Sorghum, being adapted to harsh environments, is often grown in conditions of water deficit that may impact on the phytochemical composition of the grain. In this study, the effect on six sorghum grain genotypes of an irrigation regime was investigated. This included full irrigation (100%, (FI)), deficit irrigation (50%, (DI)), and severe deficit irrigation (25%, (SDI)) imposed during the growing season. The amounts of free, bound and total phenolics and their antioxidant capacity as well as the phenolic profile and content were determined by high performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS). Polyphenol and antioxidant activity levels were significantly influenced by genotype, irrigation and genotype × irrigation interaction ($p \leq 0.05$). Compared to FI and SDI regimes, the values of total polyphenol and antioxidant capacity were higher under the DI regime. A total of 25 individual polyphenols were unequivocally or tentatively identified. Compared to the red-grained genotypes, the white-grained sorghum genotype hybrid line, Liberty, had a simpler polyphenol profile. Concentrations of the sorghum-specific 3-deoxyanthocyanidins, apigeninidin and luteolinidin, were significantly increased under DI in comparison to the other two regimes in all genotypes. Sorghum genotypes that show increased levels of polyphenols and antioxidant capacity under water deficit conditions may be desirable for breeding programs, since these changes can affect the nutritional value and health properties of sorghum grain as a human food.

4.1. INTRODUCTION

It is predicted that annual summer precipitation will be reduced due to elevated temperature in some cereal growing regions, with a reduction in intensity of rainfall events leading to greater water stress for plants (Diffenbaugh, Ashfaq, & Scherer, 2011; Knapp et al., 2008). In addition, increased temperature also can increase plant water use through its effects on vapour pressure deficit, which is defined as the difference between the saturation and actual vapour pressure (Allen et al., 2010; Williams et al., 2013). Higher temperature can lead to increased transpiration, and therefore to vapour pressure deficit. Vapour pressure deficit may be enhanced by 45% when temperatures increase by 3 °C, suggesting that much more water will be needed by plants to maintain growth (Will, Wilson, Zou, & Hennessey, 2013). Therefore, high temperatures may exacerbate the effects of drought.

Drought has become one of the most important global problems, especially in areas where rain is a primary source of water for agriculture, and it is predicted that water scarcity will become even more serious due to elevated temperature and reduced rainfall (Devnarain, Crampton, Chikwamba, Becker, & O'Kennedy, 2016). As agricultural yields are highly reliant on water availability, agricultural production is significantly negatively impacted by drought. The increased demand for agricultural yields with limited water availability has become a crucial challenge in the supply of food to feed the expanding global population, and irrigation has been widely used to increase or maintain agricultural yields (Liu et al., 2013; Pachauri et al., 2014). Irrigation has been used as a solution in many areas where there is access to water for irrigation purposes, but is becoming an increasingly unavailable resource. There is therefore a need to grow crops under water deficit conditions. However, the levels of polyphenol and antioxidant activity within food crops may be modified by water deficits (Cohen & Kennedy, 2010) which may negatively affect their nutritional quality. For example, maize grown under water stress conditions, had significantly lower levels of polyphenol and antioxidant activity than non-water stressed plants (Ali et al., 2010). In young olive trees, the dialdehydic form of elenolic acid and oleuropein aglycone contents showed a positive correlation with the oxidative stability of the oils, and the antioxidant activity significantly increased as application of irrigation water decreased (Tovar, Motilva, & Romero, 2001). In another study on peaches, Buendía, Allende,

Nicolás, Alarcón, and Gil (2008) evaluated the effects of full irrigation and regulated deficit irrigation on polyphenols and antioxidant activity. They found that the content of phenolic compounds, mainly anthocyanins and procyanidins, and antioxidants increased under regulated deficit irrigation. A study comparing non-irrigated grapevines showed that the levels of anthocyanin increased in deficit irrigation and regulated deficit irrigation vines (Zarrouk et al., 2012). However, little information is available on how water availability influences the levels of polyphenol and antioxidant activity of sorghum grains.

Therefore, the purpose of this chapter is to investigate the effect of three water treatments on levels of phenolic compounds and antioxidant activity of six different sorghum genotypes. In addition, the individual phenolic compounds were identified and measured by HPLC-DAD-ESI-MS. This study has relevance for breeding trials and future selection of sorghum genotypes suitable for producing specific levels of polyphenolics and antioxidant capacity under water stress conditions to meet the nutritional and health needs of different consumers under increasing climate constraints. In addition, knowledge of the effect of water deficit and genotype on polyphenols will help to determine end-uses of sorghum being grown, and may help farmers in targeting specific markets depending on the genotypes available, growing conditions on their farm and season.

4.2. MATERIALS AND METHODS

4.2.1. Experimental design

All sorghum genotypes were planted at Curtin University's Field Trials Area, Western Australia (latitude 32 ° 00/S, longitude115 ° 53/E, altitude 20 m). Daily minimum/maximum air temperature and rainfall data were collected from the Perth Airport Bureau of Meteorology weather station 9.6Km away from the experimental site (Fig 4.1) (Bureau of Meteorology, 2014).

All seeds were provided by 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. Two hybrid lines ('Liberty' white pericarp and

'MR Bazley' red pericarp) and four inbred lines ('Alpha' and 'B963676' red pericarp; 'IS8237C' both brown pericarp; and 'Shawaya Short Black 1', dark red-black pericarp) were selected and planted in 1 m x 1 m fibre glass pots with a depth of 0.5 m. One row each of three sorghum genotypes was planted in each pot, with a row spacing of 0.25 m. Each row was sown on 9th January 2014 with 10 seeds of each genotype that were thinned to five plants spaced 0.2 m apart after two weeks. The experiment of six genotypes x three levels of irrigation was carried out in two replications in a completely randomised block design.

The potential reference crop evapotranspiration (PET_0) from the nearby weather station was 822.7 mm from the sorghum sowing date to maturity on10th May. In the same period, the Food and Agricultural Organization crop coefficient (Kc) and PET_0 were used to calculate the crop potential evapotranspiration under standard conditions (PETc) for sorghum, giving a PETc of 576.25mm (Allen, Pereira, Raes, & Smith, 1998). According to the PETc, three irrigation regimes were applied: full irrigation (FI, 100% PETc), deficit irrigation (DI, 50% PETc) and severe deficit irrigation (SDI, 25% PETc).

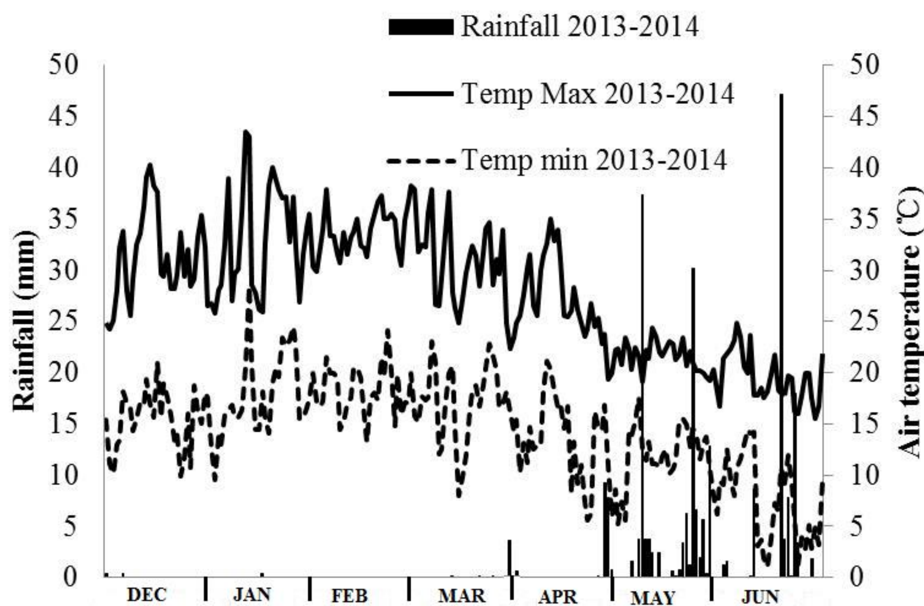

Figure 4.1. Daily minimum/ maximum air temperature and rainfall between December 2013 and June 2014 (Bureau of Meteorology, 2014).

All plants received unlimited water in the first two weeks after planting. Thereafter all irrigation treatments were applied. During the growing season, all sorghums were watered every 3–4 days with a total of 24 irrigations. Sorghum grain was harvested at physiological maturity. Grain was cleaned manually and dried. All grain was then vacuum-packed and kept at -20℃ prior to analysis.

4.2.2. Sample preparation

4.2.2.1. Flour

The procedure for wholegrain flour preparation by milling has been previously described in detail in Section 3.2.2.1.

4.2.3. Analytical methods

4.2.3.1. Moisture content

The procedure for moisture content by oven drying to constant weight has been described in detail in Section 3.2.3.2.

4.2.3.2. Extraction of free phenolic compounds

The procedure for extraction of free phenolic compounds by 80% (v/v) aqueous methanol has been described in detail in Section 3.2.3.3.

4.2.3.3. Extraction of bound phenolic compounds

The procedure for extraction of bound phenolic compounds by acid hydrolyses has been described in detail in Section 3.2.3.4.

4.2.3.4. Determination of total phenolic content

The procedure for determination of total phenolic content by Folin-Ciocalteu assay has been described in detail in Section 3.2.3.5.

4.2.3.5. Determination of profile of phenolic compounds

The procedure for determination of profile of phenolic compounds by *HPLC-DAD-ESIMS analyses* has been described in detail in Section 3.2.3.6.

4.2.3.6. Determination of total flavonoid content

The content of total flavonoids was measured by a spectrophotometry method with some modifications (Schieber, Keller, and Carle, 2001). The sample extracts or a standard of catechin (between 20 and 100mg/L) of 250µl were placed in tubes, and 1ml water and 75µl 5% Na_2NO_2 were added. A further 75µl 10% $AlCl_3$ were added after 5 min. After a reaction time of 6 min, 500µl 1mol/L NaOH and 600µl water were mixed. Then, a UV-1800 Spectrophotometer (Shimadzu, Canby, Oregon, USA) was used to determine the absorbance of samples immediately at 510nm. Results were expressed as mg catechin equivalents (CE)/g db. All samples were determined in duplicate.

4.2.3.7. Quantification of phenolic compounds

The procedure for quantification of phenolic compounds by *HPLC-DAD-ESIMS analyses* has been described in detail in Section 3.2.3.7.

4.2.3.8. Determination of antioxidant activities

The procedure for determination of antioxidant activities by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays has been described in detail in Section 3.2.3.8.

4.2.4. Statistical analysis.

All data were expressed as means ± SD and analyzed using SPSS Statistics V20 (IBM Corp., Armonk, NY, USA). The main effects of genotype and irrigation and their interaction were investigated by two-way ANOVA. One-way ANOVA with Tukey's post-hoc tests were used to separate the means when the main effect or interaction was significant. For all analyses, $p \leq 0.05$ was considered significant.

4.3. RESULTS

4.3.1. Polyphenol content

The contents of free, bound and total phenolics were significantly influenced by irrigation regime, genotype and irrigation × genotype interaction ($p \leq 0.05$), and all data is presented in Table 4.1. Contents of free polyphenols were significantly higher

in the DI regime than in FI and SDI regimes. Grains of sorghum grown under SDI showed lower contents of bound polyphenol as compared with those under FI and DI, with the exception of MR-Bazley and IS8237C, for which irrigation had no significant effect. Total polyphenol content was lower in samples from FI and SDI compared to those from DI, with the genotype of Shawaya Short Black 1 and Liberty with the highest and lowest concentrations across all irrigation regimes, respectively.

Table 4.1. Free, bound and total polyphenol (mg GAE/g db) and flavonoid (mg CE/g db) content in the wholegrain of six sorghum genotypes grown under three irrigation treatments.

Content	Irrigation treatment	Genotype					
		Liberty	MR-Bazley	Alpha	B963676	IS8237C	Shawaya Short Black 1
Polyphenols							
Free	FI	0.45±0.10 ^{aA}	1.15±0.09 ^{bA}	0.97±0.30 ^{bA}	1.16±0.08 ^{bA}	4.95±0.48 ^{cA}	9.57±1.03 ^{dB}
	DI	1.27±0.12 ^{aC}	2.34±0.10 ^{bC}	1.31±0.33 ^{aB}	2.61±0.11 ^{cB}	6.19±0.23 ^{dB}	11.67±1.10 ^{eC}
	SDI	0.69±0.06 ^{aB}	1.40±0.11 ^{cB}	0.98±0.24 ^{bA}	1.27±0.08 ^{cA}	na	8.24±0.17 ^{dA}
Bound	FI	1.44±0.16 ^{aB}	1.61±0.13 ^{bA}	1.36±0.42 ^{aA}	1.66±0.19 ^{bB}	2.77±0.25 ^{cA}	2.93±0.33 ^{cB}
	DI	1.35±0.06 ^{aAB}	1.58±0.24 ^{abA}	1.64±0.39 ^{bB}	2.27±0.06 ^{cC}	2.75±0.34 ^{dA}	3.17±0.21 ^{dC}
	SDI	1.29±0.10 ^{abA}	1.76±0.23 ^{cA}	1.22±0.28 ^{aA}	1.40±0.23 ^{bA}	na	1.95±0.08 ^{dA}
Total	FI	1.90±0.26 ^{aA}	2.77±0.24 ^{bA}	2.32±0.12 ^{aA}	2.82±0.28 ^{bA}	7.72±0.39 ^{cA}	12.50±0.70 ^{dB}
	DI	2.62±0.08 ^{aB}	3.92±0.34 ^{cC}	2.95±0.11 ^{bB}	4.87±0.17 ^{dB}	8.94±0.70 ^{eB}	14.83±1.31 ^{fC}
	SDI	1.98±0.16 ^{aA}	3.16±0.24 ^{dB}	2.23±0.14 ^{bA}	2.65±0.18 ^{cA}	na	10.19±0.98 ^{eA}
Flavonoids							
Free	FI	0.10±0.06 ^{aA}	0.59±0.11 ^{bA}	0.66±0.16 ^{bA}	1.44±0.13 ^{cA}	4.49±0.28 ^{dA}	5.72±0.48 ^{eB}
	DI	0.42±0.21 ^{aB}	1.08±0.08 ^{bB}	0.96±0.09 ^{bB}	2.86±0.11 ^{cB}	5.67±0.14 ^{dB}	7.27±0.35 ^{eC}
	SDI	0.19±0.04 ^{aA}	0.55±0.08 ^{bA}	0.61±0.11 ^{bA}	1.70±0.17 ^{cA}	na	3.08±0.30 ^{dA}
Bound	FI	0.23±0.04 ^{aA}	0.77±0.05 ^{bA}	0.71±0.25 ^{bA}	1.79±0.37 ^{cA}	2.12±0.12 ^{cA}	3.32±0.16 ^{dA}
	DI	0.49±0.07 ^{aB}	1.65±0.08 ^{cC}	0.80±0.08 ^{bB}	3.72±0.13 ^{eC}	2.16±0.23 ^{dA}	4.92±0.28 ^{fC}

	SDI	0.61±0.03 ^{aC}	1.00±0.16 ^{bB}	0.92±0.05 ^{bC}	2.08±0.04 ^{cB}	na	3.58±0.08 ^{dB}
	FI	0.33±0.11 ^{aA}	1.35±0.16 ^{bA}	1.36±0.08 ^{bA}	3.22±0.51 ^{cA}	6.61±0.50 ^{dA}	9.04±0.67 ^{eB}
Total	DI	0.91±0.08 ^{aC}	2.73±0.01 ^{cB}	1.75±0.02 ^{bB}	6.58±0.25 ^{dB}	7.83±0.37 ^{eB}	12.21±0.60 ^{fC}
	SDI	0.80±0.01 ^{aB}	1.55±0.23 ^{bA}	1.52±0.06 ^{bA}	3.78±0.13 ^{cA}	na	6.67±0.21 ^{dA}

$^{a-f}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$).

$^{A, B, C}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

na= data not available

4.3.2. Flavonoid content

The concentrations of free, bound and total flavonoids were significantly affected by irrigation regime, genotype and irrigation × genotype interaction ($p \leq 0.05$). The white-grained sorghum (Liberty) contained the lowest contents of free, bound and total flavonoids when compared with coloured grain genotypes, irrespective of irrigation treatment. Free flavonoid concentration was higher in the grain from the DI regime than for FI and SDI regimes in all genotypes. Water deficit treatments (DI and SDI) showed a higher bound flavonoid concentration than with the full irrigation treatment, except for IS8237C, which had no significant differences between irrigation treatments. Under all three irrigation regimes, Shawaya Short Black 1 contained the highest concentration of total flavonoid, followed by IS8237C, while Liberty had the lowest.

4.3.3. Identification of polyphenols of grain

Individual phenolic compounds in the six sorghum genotypes from three irrigation regimes were monitored by HPLC-DAD and mass spectrometry (MS). Representative HPLC chromatograms of phenolic compounds of the genotype of MR-Bazley are presented in Figure 4.2. The MS and UV characteristics of the chromatographic peaks, along with their proposed structures, are shown in Table 4.2. Up to 25 different phenolic compounds were detected in the extracts.

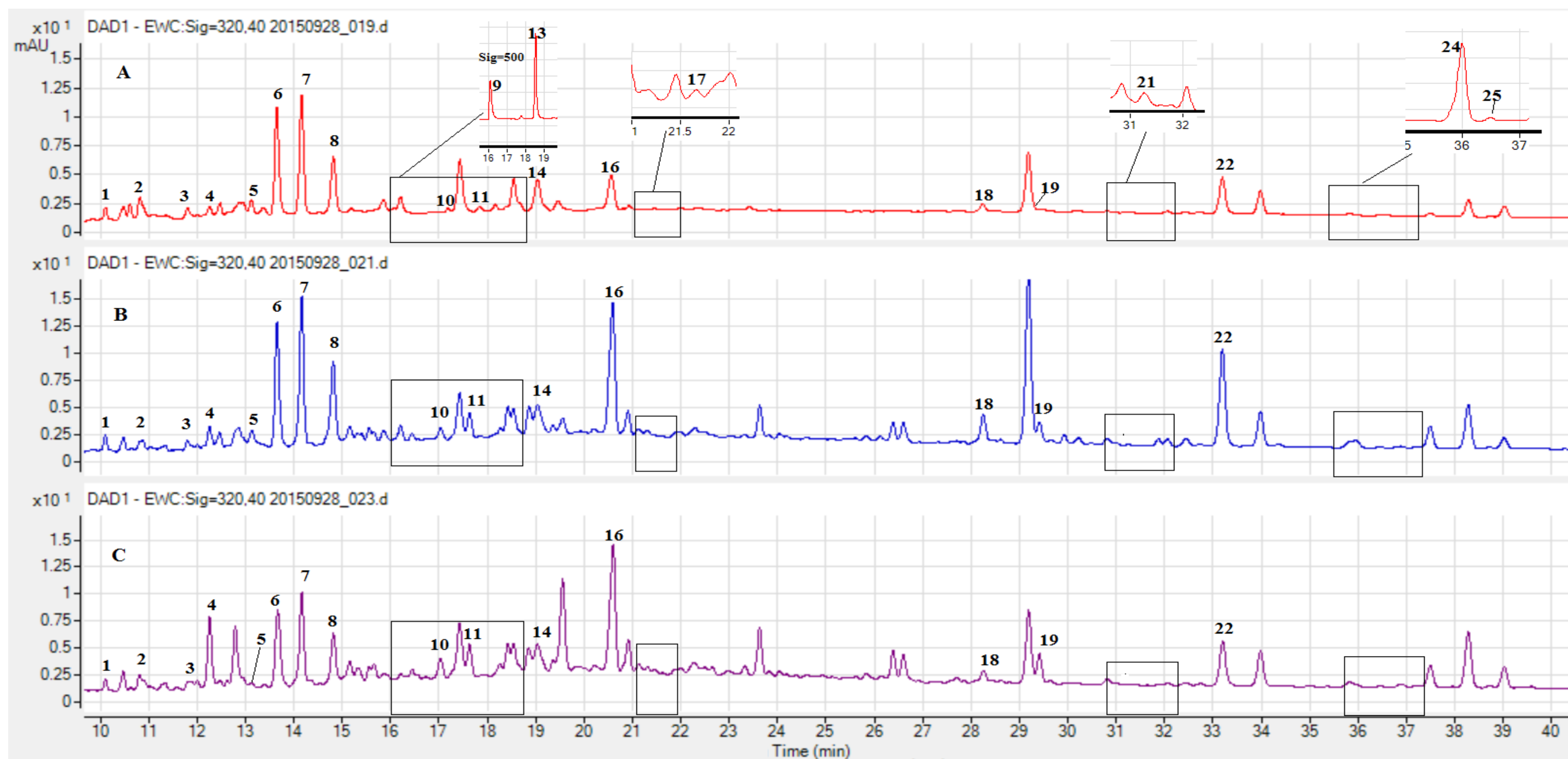

Figure 4.2. Representative HPLC-DAD chromatograms of sorghum wholegrain free polyphenols from genotype MR-Bazley under A: full irrigation regime; B: deficit irrigation regime; C: severe deficit irrigation regime.

Table 4.2. Identification of individual polyphenols in sorghum wholegrains and their respective standards by HPLC-DAD-ESIMS[a].

Peak No.	Rt[b] (min)	λ_{max} (nm)	*m/z* $[M - H]^-$	*m/z* MS^2 (Abundance %)	Tentative identification
1	10.1	275	479	149 (4), 121 (8), 105 (4)	Unknown
2	10.9	280	577	425 (60), 289 (26)	Procyanidin B1
3	11.8	325, 300sh[c]	415	253 (100), 161 (11), 135 (85)	1,2-*O*-dicaffeoylglycerol isomer
4	12.3	330	353	191 (100), 173 (48), 85 (17)	Chlorogenic acid
5	13.1	326, 298sh	253	179 (1), 161 (78), 135 (55)	2-*O*-caffeoylglycerol
6	13.5	280. 310	137	109 (15)	Protocatechuic aldehyde
7	14.4	326, 298sh	253	179 (1), 161 (78), 135 (55)	1-*O*-caffeoylglycerol
8	15.0	297, 322	179	135 (100)	Caffeic acid (std[d])
9	16.1	280, 490	269	241 (11), 225 (27), 169 (19), 133 (30)	Luteolinidin (std)
10	17.0	290	447	285 (45)	Luteolin hexoside
11	17.7	310	237	163 (20), 145 (50), 119 (31)	2-*O*-*p*-coumaroylglycerol
12	18.0	285	433	271 (86);151 (56)	Naringenin hexoside
13	18.3	275, 470	253	225 (10), 209 (70), 179 (40), 117 (65)	Apigeninidin (std)
14	19.1	283	433	287 (100), 151 (59)	Eriodictyol deoxyhexoside
15	19.2	310	163	119 (100)	*p*-coumaric acid
16	20.8	295, 325	193	178 (42), 134 (100)	Ferulic acid (std)
17	21.6	288	303	285 (100), 217 (9), 177 (18), 125 (35)	Taxifolin (std)
18	28.5	326, 300sh	415	253 (100), 179 (100) , 161 (11), 135 (85)	1,2-*O*-dicaffeoylglycerol

19	29.4	326, 300sh	415	253 (100), 179 (100) , 161 (11), 135 (85)	1,3-*O*-dicaffeoylglycerol
20	30.6	287	287	135 (85), 151 (10)	Eriodictyol
21	31.1	252, 347	285	241 (1), 217 (3), 199 (3), 175 (3), 151 (17), 133 (13), 107 (3)	Luteolin (std)
22	33.3	219, 315	399	253 (80), 235 (11), 179 (25), 163 (86), 145 (35), 119 (100),135 (27)	Coumaroyl-caffeoylglycerol
23	34.2	325, 295sh	429	253 (70), 235 (11), 193 (100), 175 (32), 161 (53), 135 (81)	Feruloyl-caffeoylglycerol
24	35.9	266, 322	269	225 (21), 201 (10), 183 (10), 149 (32), 117 (100)	Apigenin (std)
25	36.3	295	271	177 (10), 151 (50), 119 (20), 107 (10)	Naringenin (std)

[a] HPLC-DAD-ESIMS = High performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry

[b] Rt = retention time; [c] sh = shoulder; [d] std = standard

Peaks 2, 5-9, 11 and 13-25 are described in detail in section **3.3.3** of this thesis. Of the other peaks, peak 1 represents an unknown complex polyphenol. It was noted that peak 3 had similar UV and MS spectra to peaks 18 and 19, and was tentatively identified as 1,2-*O*-dicaffeoylglycerol or 1,3-*O*-dicaffeoylglycerol isomer. Peak 4 had identical $[M - H]^-$ ions at *m/z* 353, and prominent fragment ions at *m/z* 191 and 173, corresponding to deprotonated quinic acid and caffeic acid fragments, respectively. After comparisons with the literature (Han et al., 2008), peak 4 was tentatively identified as chlorogenic acid. A deprotonated molecule $[M-H]^-$ at *m/z* 447 was observed in the peak 10. The $[M - H- 162]^-$ ion at *m/z* 285 is typical of that produced by losing a hexoside residue, and the prominent fragment ions at *m/z* 285 would correspond to the deprotonated luteolin fragment. Hence, peak 10 was tentatively identified as luteolin hexoside. Peak 12, with a deprotonated molecule $[M-H]^-$ at *m/z* 433, also lost a hexoside residue (433-162) to produce the prominent fragment ions at *m/z* 271, corresponding to the deprotonated naringenin fragment. Therefore, peak 12 was tentatively identified as naringenin hexoside (Table 4.2).

4.3.4. Influence of genotype and irrigation on the profile of polyphenols of sorghum grain

The representative HPLC chromatograms of free polyphenols in the MR-Bazley genotype are shown in Figure 4.2. From the HPLC chromatograms of all samples it was evident that in all genotype × irrigation combinations the polyphenolic species present did not differ; only changes in their concentrations were found. The profiles of both free and bound forms of individual polyphenols of the six sorghum genotypes under three irrigation regimes are shown in Table 4.3.

4.3.5. Influence of genotype and irrigation on the individual polyphenol concentration of sorghum grain

The antioxidant properties of sorghum grain relate to both polyphenol concentration and profile. In the present study, eight phenolic compounds were quantified based on the availability of authentic standards. Based on their structural characteristics, the quantified phenolic compounds were classified into five groups.

Hydroxycinnamic acids

The hydroxycinnamic acids identified and quantified were caffeic and ferulic acids (Table 4.4). The free, bound and total contents of these compounds were significantly influenced by irrigation regime, genotype and their interaction ($p \leq 0.05$). Under the FI regime, the highest and lowest free and total caffeic acid concentrations were found in Alpha and B963676, respectively. For total ferulic acid concentration under the FI regime, IS8237C had the lowest while MR-Bazley had the highest concentration. Water deficit treatments significantly increased the level of free ferulic acid as compared to the FI treatment. A reduction in the amount of irrigation water resulted in varying changes in both bound caffeic acid and bound ferulic acid concentration amongst the genotypes. Under the SDI regime, Liberty had the lowest concentrations of total caffeic acid, while B963676 had the highest total caffeic acid content and MR-Bazley the highest total ferulic acid content.

Table 4.3. Profiles of polyphenols in the wholegrain of six sorghum genotypes grown under irrigation treatments.

Polyphenol class and irrigation treatment		Peak number [a]					
		Liberty	MR-Bazley	Alpha	B963676	IS8237C	Shawaya Short Black 1
Free	FI	1, 3, 5, 6, 7, 8, 11, 14, 16, 18, 19, 21	1, 2, 3, 4, 5, 6, ,7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 24, 25	5, 6, 7, 8, 9, 11, 13, 14, 16, 17, 18, 19, 22, 23, 24, 25	1, 4, 5, 6, ,7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25	2, 3, 5, 6, 8, 9, 11, 12, 13, 14, 16, 17, 21, 22, 24, 25	2, 5, 6, 7, 10, 11, 8, 9, 13, 13, 16, 17, 21, 22, 23, 24, 25
	DI	1, 3, 5, 6, 7, 8, 11, 14, 16, 18, 19, 21	1, 2, 3, 4, 5, 6, ,7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 24, 25	5, 6, 7, 8, 9, 11, 13, 14, 16, 17, 18, 19, 22, 23, 24, 25	1, 4, 5, 6, ,7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25	2, 3, 5, 6, 8, 9, 11, 12, 13, 14, 16, 17, 21, 22, 24, 25	2, 5, 6, 7, 10, 11, 8, 9, 13, 13, 16, 17, 21, 22, 23, 24, 25
	SDI	1, 3, 5, 6, 7, 8, 11, 14, 16, 18, 19, 21	1, 2, 3, 4, 5, 6, ,7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 24, 25	5, 6, 7, 8, 9, 11, 13, 14, 16, 17, 18, 19, 22, 23, 24, 25	1, 4, 5, 6, ,7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25	na	2, 5, 6, 7, 10, 11, 8, 9, 13, 13, 16, 17, 21, 22, 23, 24, 25
Bound	FI	8, 15, 16, 21	6, 8, 9, 13, 15, 16, 17,20, 21, 24, 25	8, 9, 13, 15, 16, 17, 20, 22, 24, 25	8, 9, 13, 15, 16, 17, 20, 21, 22, 24, 25	6, 8, 9, 13, 15, 16, 17, 19, 20, 21, 22, 24, 25	6, 8, 9, 11, 13, 15, 16, 17, 20, 21, 24, 25
	DI	8, 15, 16, 21	6, 8, 9, 13, 15, 16, 17,20, 21, 24, 25	8, 9, 13, 15, 16, 17, 20, 22, 24, 25	8, 9, 13, 16, 17, 21, 24, 25	6, 8, 9, 13, 15, 16, 17, 19, 20, 21, 22, 24, 25	6, 8, 9, 11, 13, 15, 16, 17, 20, 21, 24, 25
	SDI	8, 15, 16, 21	6, 8, 9, 13, 15, 16, 17,20, 21, 24, 25	8, 9, 13, 15, 16, 17, 20, 22, 24, 25	8, 9, 13, 16, 17, 21, 24, 25	na	6, 8, 9, 11, 13, 15, 16, 17, 20, 21, 24, 25

[a] Peak numbers were identified in Table 4.2.
na= data not available

Table 4.4 Free, bound and total contents of hydroxycinnamic acids (μg/g db) in the wholegrain of six sorghum genotypes grown under three irrigation treatments.

Content	Irrigation treatment	Genotype					
		Liberty	MR-Bazley	Alpha	B963676	IS8237C	Shawaya Short Black 1
CA							
Free	FI	7.18±0.29 ^{eB}	5.04±0.10 ^{bA}	12.55±0.29 ^{fA}	4.51±0.38 ^{aA}	5.54±0.26 ^{cA}	6.36±0.44 ^{dC}
	DI	10.64±0.82 ^{cC}	9.84±0.21 ^{bC}	19.76±0.67 ^{eC}	9.34±0.62 ^{bB}	14.97±0.25 ^{dB}	5.08±0.17 ^{aA}
	SDI	6.44±0.18 ^{bA}	6.78±0.32 ^{bB}	13.00±0.18 ^{dB}	11.38±0.48 ^{cC}	na	5.44±0.08 ^{aB}
Bound	FI	5.76±0.38 ^{cA}	9.54±0.11 ^{dB}	10.47±0.34 ^{eC}	4.63±0.10 ^{bA}	4.18±0.09 ^{aB}	5.01±0.18 ^{cA}
	DI	9.97±0.53 ^{dC}	7.20±0.23 ^{cA}	7.56±0.10 ^{cB}	10.38±1.17 ^{dB}	3.66±0.12 ^{aA}	5.13±0.10 ^{bA}
	SDI	6.98±1.03 ^{aB}	14.71±0.88 ^{cC}	6.97±0.28 ^{aA}	15.59±0.88 ^{cC}	na	10.05±0.49 ^{bB}
Total	FI	12.94±0.68 ^{dA}	14.58±0.01 ^{eA}	23.21±0.77 ^{fB}	9.14±0.28 ^{aA}	9.72±0.16 ^{bA}	11.55±0.76 ^{cA}
	DI	20.61±1.40 ^{dB}	17.04±0.43 ^{bB}	27.49±0.70 ^{eC}	19.73±1.79 ^{dB}	18.62±0.16 ^{cB}	10.13±0.34 ^{aA}
	SDI	13.42±1.21 ^{aA}	21.48±0.57 ^{dC}	19.98±0.05 ^{cA}	26.97±1.90 ^{eC}	na	16.11±0.70 ^{bB}
FA							
Free	FI	1.18±0.20 ^{aA}	1.98±0.13 ^{dA}	1.58±0.21 ^{bA}	2.03±0.01 ^{dA}	1.55±0.12 ^{bA}	1.65±0.10 ^{aA}
	DI	1.97±0.11 ^{aC}	2.87±0.01 ^{cC}	2.78±0.16 ^{cC}	4.83±0.20 ^{dC}	2.34±0.24 ^{bB}	2.36±0.07 ^{bB}
	SDI	1.82±0.06 ^{aB}	2.31±0.13 ^{bB}	2.46±0.03 ^{cB}	2.48±0.22 ^{cB}	na	2.97±0.21 ^{dC}
Bound	FI	45.87±2.14 ^{cB}	82.60±3.57 ^{eB}	60.47±1.70 ^{dB}	38.11±5.68 ^{bA}	28.87±0.75 ^{aA}	46.91±2.29 ^{cA}
	DI	50.97±1.78 ^{bC}	78.75±2.10 ^{dA}	40.56±0.77 ^{aA}	114.82 ± 3.59	52.69±2.23 ^{bcB}	55.18±0.69 ^{cB}

					^{eC}		
	SDI	35.51±3.15 ^{aA}	100.23±1.20 ^{eC}	42.66±0.59 ^{bA}	61.01±1.67 ^{cB}	na	66.85±2.49 ^{dC}
	FI	47.05±2.33 ^{cB}	84.58±3.43 ^{eB}	62.48±1.82 ^{dC}	40.14±5.69 ^{bA}	30.43±0.83 ^{aA}	47.87±2.94 ^{cA}
Total	DI	52.94±1.89 ^{bC}	81.62±2.11 ^{dA}	43.17±0.75 ^{aA}	119.65±3.79 ^{eC}	55.03±2.47 ^{bcB}	57.72±0.76 ^{cB}
	SDI	37.33±3.22 ^{aA}	102.54±1.37 ^{eC}	45.26±0.58 ^{bB}	63.49±1.45 ^{cB}	na	70.61±3.32 ^{dC}

$^{a\text{-}f}$ Values with different superscripts in the same row are significantly different ($P \leq 0.05$).

$^{A, B, C}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($P \leq 0.05$).

Abbreviations: CA= caffeic acid; FA= ferulic acid; nd= no data

Table 4.5. Free, bound and total individual flavonoid content (μg /g, db) in the wholegrain of six sorghum genotypes grown in three irrigation treatments.

Content		Irrigation treatment	Genotypes: Liberty	MR-Bazley	Alpha	B963676	IS8237C	Shawaya Short Black 1
3-Deoxyanthocyanidins								
LUT	Free	FI	nd	0.26±0.04 ^{aB}	0.48±0.09 ^{bA}	1.58±0.08 ^{cA}	4.81±0.18 ^{dA}	13.44±0.76 ^{eB}
		DI	nd	1.29±0.11 ^{aC}	2.30±0.04 ^{bC}	2.42±0.44 ^{cB}	6.90±0.13 ^{dB}	18.25±0.98 ^{eC}
		SDI	nd	0.04±0.01 ^{aA}	1.61±0.20 ^{dB}	1.49±0.20 ^{cA}	na	1.34±0.10 ^{bA}
	Bound	FI	nd	3.31±0.16 ^{dB}	0.71±0.05 ^{aA}	0.84±0.06 ^{bC}	2.15±0.04 ^{cA}	11.72±0.73 ^{eB}
		DI	nd	8.91±0.57 ^{dC}	3.11±0.24 ^{bB}	0.66±0.15 ^{aB}	4.41±0.10 ^{cB}	11.60±1.40 ^{eB}
		SDI	nd	2.86±0.13 ^{cA}	0.68±0.10 ^{bA}	0.30±0.02 ^{aA}	na	0.34±0.07 ^{aA}
	Total	FI	nd	3.56±0.20 ^{cA}	1.20±0.05 ^{aA}	2.41±0.01 ^{bB}	5.95±0.21 ^{dA}	25.16±0.04 ^{eB}
		DI	nd	10.21±0.23 ^{bB}	5.41±0.20 ^{cC}	3.08±0.59 ^{aC}	7.36±0.21 ^{dB}	29.98±0.51 ^{eC}
		SDI	nd	2.90±0.55 ^{cC}	2.33±0.12 ^{bB}	1.79±0.22 ^{aA}	na	1.73±0.21 ^{aA}
API	Free	FI	nd	1.02±0.11 ^{bB}	0.68±0.07 ^{aB}	1.63±0.11 ^{cB}	5.17±0.09 ^{dA}	16.42±0.35 ^{eB}
		DI	nd	3.67±0.18 ^{bC}	0.39±0.02 ^{aA}	3.96±0.30 ^{bC}	8.17±0.15 ^{cB}	18.89±0.28 ^{dC}
		SDI	nd	0.08±0.02 ^{aA}	1.41±0.14 ^{cC}	1.37±0.16 ^{cA}	na	1.18±0.08 ^{bA}
	Bound	FI	nd	10.49±0.37 ^{eC}	4.42±0.30 ^{cB}	1.71±0.39 ^{aA}	3.09±0.03 ^{bB}	9.04±0.35 ^{dB}
		DI	nd	9.62±0.49 ^{bB}	15.66±0.44 ^{cC}	19.62±0.93 ^{dC}	1.29±0.06 ^{aA}	9.85±0.43 ^{bC}
		SDI	nd	2.53±0.13 ^{bA}	3.85±0.24 ^{cA}	9.70±1.74 ^{dB}	na	1.26±0.10 ^{aA}

		FI	nd	11.51±0.48 ^{dB}	5.22±0.46 ^{bA}	3.34±0.28^{aA}	8.25±0.13 ^{cA}	25.26±0.86 ^{eB}
	Total	DI	nd	13.28±0.31 ^{bC}	16.05±0.41 ^{cB}	23.57±1.23 ^{dC}	9.47±0.08 ^{aB}	28.95±0.88 ^{eC}
		SDI	nd	2.61±0.11 ^{aA}	5.19±0.24 ^{bA}	11.07±1.89 ^{cB}	na	2.49±0.21 ^{aA}
Flavones								
		FI	0.38±0.06^{aA}	3.48±0.10 ^{cA}	1.98±0.08 ^{bA}	8.20±0.04 ^{dB}	15.11±0.03 ^{eA}	12.59±0.54 ^{eB}
	Free	DI	0.74±0.13 ^{aC}	6.62±0.04 ^{cC}	3.37±0.16 ^{bB}	10.79±0.13 ^{dC}	19.14±0.09 ^{eB}	19.74±1.23 ^{eC}
		SDI	0.67±0.04 ^{aB}	4.08±0.02 ^{cB}	1.94±0.13 ^{bA}	7.33±0.08 ^{eA}	na	5.59±0.08 ^{dA}
		FI	1.72±0.08 ^{bB}	3.58±0.30 ^{cB}	0.38±0.02 ^{aA}	0.40±0.08 ^{aA}	4.13±0.13 ^{dA}	3.23±0.23 ^{cB}
LU	Bound	DI	3.83±0.22 ^{cC}	5.53±0.09 ^{eC}	0.55±0.01 ^{aB}	1.35±0.13 ^{bC}	3.61±0.09 ^{cB}	4.37±0.25 ^{dC}
		SDI	1.41±0.12 ^{cA}	1.71±0.19 ^{cA}	0.86±0.12 ^{aC}	1.09±0.06 ^{bB}	na	1.49±0.32 ^{cA}
		FI	2.10±0.02 ^{aA}	6.06±0.40 ^{cB}	2.33±0.12 ^{bA}	8.60±0.13 ^{dA}	19.24±0.10 ^{eA}	15.82±0.30 ^{eB}
	Total	DI	4.57±0.35 ^{bB}	12.15±0.13 ^{cC}	3.92±0.18 ^{aC}	12.14±0.26 ^{cB}	22.75±0.17 ^{dB}	24.11±0.98 ^{eC}
		SDI	2.07±0.15 ^{aA}	5.78±0.17 ^{cA}	2.79±0.01 ^{bB}	8.42±0.14 ^{eA}	na	7.07±0.40 ^{dA}
AP		FI	nd	2.16±0.24^{bA}	nd	2.28±0.26 ^{bA}	4.59±0.11 ^{cA}	0.98±0.35 ^{aA}
	Free	DI	nd	4.97±0.35 ^{bB}	nd	6.04±0.54 ^{cC}	6.70±0.19 ^{dB}	1.94±0.39 ^{aB}
		SDI	nd	2.10±0.31 ^{bA}	nd	4.61±0.17 ^{cB}	na	0.92±0.13 ^{aA}
		FI	nd	8.97±0.08 ^{dB}	nd	3.39±0.24 ^{bA}	7.48±0.22 ^{cA}	1.83±0.51 ^{aC}
	Bound	DI	nd	23.14±1.34 ^{dC}	nd	5.71±0.46 ^{bC}	13.60±0.37 ^{cB}	1.08±0.40 ^{aB}
		SDI	nd	7.05±0.35 ^{cA}	nd	4.03±0.08^{bB}	na	0.91±0.36 ^{aA}
		FI	nd	11.13±0.32 ^{cB}	nd	5.67±0.02 ^{bA}	12.07±0.33 ^{cA}	2.81±0.88 ^{aB}
	Total	DI	nd	28.10±1.73 ^{dC}	nd	11.74±1.00 ^{bC}	20.30±0.37 ^{cB}	3.02±0.78 ^{aC}
		SDI	nd	9.15±0.04 ^{cA}	nd	8.64±0.05^{bB}	na	1.83±0.49 ^{aA}

Dihydroflavonol								
		FI	nd	6.93±0.08 ^{bB}	9.35±0.24 ^{dC}	5.43±0.06 ^{aA}	8.19±0.30 ^{cA}	14.56±0.34 ^{eB}
	Free	DI	nd	11.47±0.31 ^{bC}	8.20±0.21 ^{aB}	28.64±1.03 ^{eC}	14.93±0.27 ^{cB}	16.02±0.57 ^{dC}
		SDI	nd	0.31±0.04 ^{aA}	3.01±0.11 ^{bA}	11.18±0.36 ^{dB}	na	8.86±0.25 ^{cA}
		FI	nd	2.41±0.40 ^{aA}	6.12±0.14 ^{dA}	4.53±0.47 ^{bA}	5.11±0.29 ^{cA}	10.92±1.14 ^{eB}
TA	Bound	DI	nd	9.58±0.69 ^{aC}	9.73±0.20 ^{aC}	12.33±1.34 ^{bC}	16.48±0.29 ^{cB}	18.50±0.30 ^{dC}
		SDI	nd	6.53±0.13 ^{bB}	8.51±0.54 ^{cB}	10.24±0.59 ^{bB}	na	4.67±0.43 ^{aA}
		FI	nd	9.34±0.32 ^{aB}	15.50±0.13 ^{cB}	9.96±0.53 ^{aA}	13.30±0.56 ^{bA}	25.48±0.82 ^{dB}
	Total	DI	nd	21.05±1.00 ^{bC}	17.93±0.01 ^{aC}	40.97±2.34 ^{dC}	31.41±0.02 ^{cB}	34.52±0.26 ^{cC}
		SDI	nd	6.83±0.17 ^{aA}	11.34±0.80 ^{bA}	21.41±0.23 ^{dB}	na	13.53±0.67 ^{cA}
Flavanone								
		FI	nd	1.70±0.06 ^{aB}	15.66±0.05 ^{dA}	5.80±0.42 ^{bA}	11.32±0.44 ^{cA}	23.39±0.80 ^{eB}
	Free	DI	nd	2.92±0.07 ^{aC}	31.88±0.11 ^{eC}	12.47±0.31 ^{bC}	13.19±0.13 ^{cB}	30.60±2.23 ^{dC}
		SDI	nd	1.15±0.05 ^{aA}	22.51±0.07 ^{dB}	7.69±0.87 ^{bB}	na	17.63±1.55 ^{cA}
		FI	nd	3.48±0.25 ^{aB}	5.54±0.19 ^{bA}	6.11±0.28 ^{cB}	8.04±0.10 ^{dA}	16.64±1.18 ^{eB}
NAR	Bound	DI	nd	4.07±0.22 ^{aC}	8.88±0.23 ^{bC}	9.09±0.31 ^{cC}	10.47±0.21 ^{dB}	17.87±0.43 ^{eC}
		SDI	nd	2.74±0.07 ^{aA}	6.96±0.15 ^{cB}	4.58±0.45 ^{bA}	na	12.50±2.11 ^{dA}
		FI	nd	5.18±0.19 ^{aB}	21.14±0.29 ^{cA}	11.91±0.46 ^{bA}	19.36±1.13 ^{cA}	40.02±1.62 ^{dB}
	Total	DI	nd	6.99±0.27 ^{aC}	40.76±0.31 ^{dC}	21.56±0.72 ^{bB}	23.65±0.07 ^{cB}	48.46±2.66 ^{eC}
		SDI	nd	3.89±0.12 ^{aA}	29.49±0.10 ^{cB}	12.27±0.66 ^{bA}	na	30.13±1.86 ^{cA}

$^{a-e}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$).

$^{A, B, C}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

Abbreviations: LUT= luteolinidin; API=apigeninidin; LU= luteolin; AP= Apigenin; TA= taxifolin; NAR= naringenin; nd= not detected; na= data not available.

3-Deoxyanthocyanidins

The concentration of the 3-deoxyanthocyanidins, luteolinidin and apigeninidin, is presented in Table 4.5. Again, genotype, irrigation regime and their interaction had a significant effect on the concentrations of these compounds ($p \leq 0.05$). The white sorghum, Liberty, did not have any detectable content of apigeninidin and luteolinidin. In the other genotypes the lowest detectable concentrations of free luteolinidin and apigeninidin were in MR-Bazley and Alpha under the FI regime, respectively, while Shawaya Short Black 1 had the highest values of these two compounds under the FI regime. The intermediate DI regime resulted in a higher concentration of luteolinidin than under the FI and SDI regimes in all genotypes. The concentration of both bound luteolinidin and bound apigeninidin varied with decreasing water supply. The genotype Shawaya Short Black 1 had the highest contents of total luteolinidin and apigeninidin under both FI and DI regimes, but became the lowest under the SDI regime when compared with the other genotypes

Flavones

Two flavones were quantified in the samples, namely luteolin and apigenin (Table 4.5), with significant changes in concentrations ($p \leq 0.05$) due to irrigation regime, genotype and irrigation × genotype interaction. No apigenin in free or bound form was detected in Liberty or Alpha under the three irrigation regimes. Free and total luteolin and apigenin concentrations were the highest ($p \leq 0.05$) under the DI regime across all genotypes. In those genotypes in which they were detected, Shawaya Short Black 1 had the lowest concentrations of free, bound and total apigenin, but the highest concentrations of free and total luteolin under the FI regime. However, differing irrigation regimes resulted in different bound luteolin and apigenin concentrations. Compared to the FI and SDI regimes, higher contents of free, bound and total apigenin and luteolin were detected in all sorghum genotypes under the DI regime. Irrespective of treatment, Shawaya Short Black 1 had the lowest concentrations of free, bound and total apigenin.

Dihydroflavonol and Flavanones

One dihydroflavonol taxifolin and one flavanone naringenin were identified and

quantified, and irrigation regime, genotype and their interaction had a significant effect on their concentrations ($p \leq 0.05$), as shown in Table 4.5. Free, bound and total naringenin and taxifolin concentrations were highest under the intermediate DI regime when compared to the FI and SDI regimes across all genotypes, with the exception of Liberty, where taxifolin and naringenin were not detected. The lowest detected concentration of total taxifolin was in Alpha, while B963676 had the highest detected concentration of total naringenin under the DI regime.

Table 4.6. Free, bound and total antioxidant capacity in the wholegrain as affected by irrigation treatments using the DPPH$^{\bullet}$ (mg TE/g db) and ABTS$^{\bullet+}$ (mg TE/g db) assays across six sorghum genotypes.

Content	Irrigation treatment	Genotype					
		Liberty	MR-Bazley	Alpha	B963676	IS8237C	Shawaya Short Black 1
DPPH$^{\bullet}$							
	FI	1.07±0.16 ^{aA}	1.44±0.07 ^{bA}	1.38±0.20 ^{bA}	1.51±0.26 ^{bA}	7.28±0.19 ^{cA}	13.09±0.71 ^{dB}
Free	DI	2.01±0.20 ^{aC}	2.62±0.13 ^{bC}	1.90±0.09 ^{aB}	2.93±0.11 ^{cB}	8.82±0.18 ^{dB}	15.61±0.19 ^{eC}
	SDI	1.32±0.11 ^{aB}	1.92±0.08 ^{cB}	1.60±0.25 ^{bA}	1.84±0.14 ^{cA}	na	11.83±0.23 ^{dA}
	FI	1.92±0.26 ^{aB}	2.54±0.16 ^{bA}	1.81±0.18 ^{aA}	2.52±0.22 ^{bB}	3.91±0.13 ^{cA}	3.86±0.52 ^{cB}
Bound	DI	1.77±0.13 ^{aAB}	2.01±0.27 ^{abA}	2.08±0.26 ^{bB}	3.10±0.18 ^{cC}	4.09±0.14 ^{dA}	4.17±0.02 ^{dC}
	SDI	1.48±0.31 ^{aA}	2.28±0.36 ^{cA}	1.64±0.01^{aA}	2.09±0.09 ^{bA}	na	2.84±0.10 ^{dA}
	FI	2.99±0.23 ^{aA}	3.98±0.29 ^{bA}	3.19±0.19 ^{aA}	4.03±0.33 ^{bA}	11.19±0.32 ^{cA}	16.95±1.22 ^{dB}
Total	DI	3.79±0.33 ^{aB}	4.63±0.21 ^{cC}	3.97±0.16 ^{bB}	6.03±0.29 ^{dB}	12.91±0.94 ^{eB}	19.77±1.18 ^{fC}
	SDI	2.80±0.21 ^{aA}	4.20±0.16 ^{dB}	3.24±0.33 ^{bA}	3.93±0.11 ^{cA}	na	14.67±2.33 ^{eA}
ABTS$^{\bullet+}$							
	FI	1.34±0.03 ^{aA}	1.98±0.08 ^{bA}	2.03±0.21 ^{bA}	2.11±0.05 ^{bA}	9.65±0.26 ^{cA}	16.09±0.29 ^{dB}
Free	DI	3.01±0.18 ^{aC}	4.14±0.11 ^{bC}	2.86±0.15 ^{aB}	4.54±0.11 ^{cC}	11.70±0.32 ^{dB}	19.36±0.34 ^{eC}
	SDI	1.85±0.16 ^{aB}	2.37±0.32 ^{cB}	2.12±0.13 ^{bA}	2.69±0.28 ^{cB}	na	13.03±0.45 ^{dA}
Bound	FI	2.79±0.21 ^{aB}	3.61±0.34 ^{bA}	2.50±0.28 ^{aA}	3.77±0.12 ^{bB}	5.42±0.37 ^{cA}	5.21±0.22 ^{cB}
	DI	2.90±0.17 ^{aAB}	3.15±0.13 ^{abA}	3.49±0.19 ^{bB}	5.04±0.13 ^{cC}	5.56±0.28 ^{dA}	5.82±0.31 ^{dC}

	SDI	2.49±0.13 aA	3.54±0.26 cA	2.68±0.13 aA	3.05±0.18 bA	na	3.98±0.21 dA
	FI	4.13±0.24 aA	5.59±0.14 bA	4.53±0.17 aA	5.88±0.17 bA	15.07±0.04 cA	21.30±0.37 dB
Total	DI	5.91±0.16 aB	7.29±0.63 cC	6.35±0.15 bB	9.58±0.29 dB	17.26±0.59 eB	25.18±0.27 fC
	SDI	4.34±0.12 aA	5.91±0.15 dB	4.90±0.31 bA	5.74±0.10 cA	na	17.01±0.66 eA

[a-f] Values with different superscripts in the same row are significantly different ($p \leq 0.05$).

[A, B, C] Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

na= data not available

4.3.6. Influence of genotype and irrigation on the antioxidant activity of sorghum grains

The antioxidant capacity of polyphenol extractions from sorghum grain using the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays varied significantly with genotype, irrigation and their interaction ($p \leq 0.05$). The highest free, bound and total antioxidant activities by both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays were recorded for Shawaya Short Black 1, and the lowest in Liberty under all three irrigation regimes (Table 4.6). Compared with the white sorghum Liberty, all coloured sorghum genotypes had higher values of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ antioxidant activity. The free and total $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ antioxidant activity in all sorghum samples was significantly higher in the DI regime samples compared to those in the FI and SDI regime samples, while bound activity showed a varied response in the free radical scavenging activity.

The highest $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ levels of three forms of polyphenols were recorded for Shawaya Short Black 1, and the lowest in Liberty under the three irrigation regimes (Table 4.6). Compared with white sorghum, Liberty, all coloured sorghum genotypes had higher values of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. The free and total $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging activity in all sorghum samples were significantly higher in the DI regime samples compared to those in the FI and SDI regime samples, while bound activity showed a varied response in free radical scavenging.

4.4. DISCUSSION

In the present study, the results indicated that total polyphenol and flavonoid concentration were higher under the intermediate DI regime as compared with either FI or SDI regimes. This may have been due to modulation of the biosynthetic pathway of polyphenols by water stress conditions in the case of SDI (Zarrouk et al., 2012), whereas for the full irrigation regime, a lower amount of total phenolics and flavonoids would likely have reflected the low stress conditions. The biosynthesis of polyphenols in plants is through the phenylpropanoid pathway, where L-phenylalanine is deaminated by the enzyme phenylalanine ammonia lyase (PAL) to biosynthesize various polyphenols (Cohen & Kennedy, 2010). It has been shown that PAL is the key enzyme in phenolic and flavonoid biosynthesis, and has a predominant role in the biosynthesis of polyphenols under conditions of abiotic stress, such as temperature and

irrigation (Cohen & Kennedy, 2010; D. Ma et al., 2014). As there is no information on sorghum or other cereals on the role of water availability during plant growth on grain polyphenolics and antioxidant activities or the mechanism behind any changes, examples from other species are briefly discussed. Machado, Felizardo, Fernandes-Silva, Nunes, and Barros (2013) planted olive trees of the cultivar Cobrançosa under different irrigation regimes, and found that both total phenolics and the PAL activity significantly increased as the amount of water supplied decreased. Thus, in the present study, the result suggested that the synthesis of polyphenols in particular was likely triggered under DI treatment, due to stimulation of PAL activity.

The biosynthesis of individual polyphenols is modulated by key enzymes, such as flavonoid-3'-hydroxylase (F3'H), chalcone synthase (CHS) and PAL (Cohen & Kennedy, 2010). Synthesis of 3-deoxyanthocyanidin has been previously shown to be catalysed by CHS and F3'H enzymes, and studies found that the synthesis of these two enzymes was enhanced under biotic stress in sorghum, which led to increased 3-deoxyanthocyanidin concentration (Boddu et al., 2004; Szecc, De Verdier, & Nicholson, 1999). In the present study, total 3-deoxyanthocyanidin concentration was the highest under the DI regime across all genotypes when compared with other regimes. Therefore, it is proposed that more CHS and F3'H enzymes were synthesized when the irrigation level was reduced from FI to DI. However, more research work will be needed to confirm this. It is still unclear how the water deficit affects the metabolic pathways of other individual polyphenols in sorghum, and further research is required to understand these pathways.

In the present study, some individual polyphenols significantly decreased in concentration when irrigation was decreased from DI to SDI regime. These findings were in contrast with other plants, in which polyphenol concentration increased when the level of irrigation decreased (Artajo, Romero, Tovar, & Motilva, 2006; Buendía et al., 2008; Martinelli, Basile, Morelli, d'Andria, & Tonutti, 2012; Tovar et al., 2001). However, normal physiological and biochemical plant processes can be inhibited by severe water deficit through reduced cell elongation and reduced photosynthesis (Jahanzad et al., 2013; Saeed & El-Nadi, 1998). In the present study, sorghum grain yield was the lowest under SDI treatment (data not shown), with no sorghum grain set in sorghum IS8237C, which indicated that the SDI regime may have seriously

inhibited physiological and biochemical processes compared with FI and DI regimes. It is therefore proposed that although the synthesis of polyphenols increases under marginal water stress, it is inhibited under severe water stress.

The average maximum daytime temperature during the growth of the sorghum plants in the present study was in fact in excess of 35℃ (Fig 4.1), a threshold temperature where decreases in the concentration of total and individual polyphenols in sorghum grain usually occur. As the sorghum was most likely unable to regulate the severe water stress, the high temperature may have exacerbated the stress and led to decreased biosynthesis of polyphenols, flavonoids and some individual polyphenols in the sorghum grain under the SDI regime. These findings are of practical importance especially under a Mediterranean climate, such as the Mediterranean Basin, and some regions of Australia, and North and South Africa, in which plant biochemical and physiological processes are mainly regulated by water deficit coupled with other interacting factors, such as high temperatures.

All sorghum genotypes contained bound phenolic and flavonoid fractions (Table 4.1). The percentage of bound phenolic and flavonoid fractions to the total was about 50% in some genotypes, such as Liberty and Alpha, which indicated that total phenolic contents may be underestimated by commonly cited analytical methods that do not carefully hydrolyse the sample and extract the bound fraction (Acosta-Estrada et al., 2014; Dietrych-Szostak & Oleszek, 1999; Saura-Calixto, 2012; Velioglu, Mazza, Gao, & Oomah, 1998; Zieliński & Kozłowska, 2000). In the human gastrointestinal tract, the bound fraction of polyphenolics is only slowly released in the stomach and small intestinal treatments, but on reaching the colon these compounds could be released by microfloral fermentation (Adom & Liu, 2002). Bound flavonols can be degraded to simpler phenolics by the microflora *Clostridium spp.,* and *Eubacterium spp.* in the colon (Selma, Espin, & Tomas-Barberan, 2009). These findings indicate that intake of whole sorghum grain with high content of bound polyphenols may still provide health benefits (Liu, 2007).

Results in Table 4.1 showed that white sorghum (Liberty) had lower flavonoid concentrations than the other, coloured-grain genotypes, which is in agreement with Afify et al. (2012). Dykes et al. (2005) found that sorghum genotypes, which express the pigmented testa gene, *B1B2,* and the spreader gene, *S,* contained the highest total

polyphenol concentrations. The total polyphenol concentrations of Shawaya Short Black 1 and IS8237C (brown) were about five and three times higher than those of the other genotypes under the FI regime, respectively, suggesting that these two genotypes might express both B_1B_2 and *S* genes.

The concentration of individual polyphenols determined in this study is in agreement with previous studies (Dykes, Seitz, Rooney, & Rooney, 2009; Taleon et al., 2012). The concentrations of 3-deoxyanthocyanidins were highest in Shawaya Black Short 1 compared to the other genotypes, highlighting that black sorghum is an abundant source of this group of compounds (Taleon et al., 2012). The accumulation of 3-deoxyanthocyanidins in sorghum grain is regulated by *P1*, which is controlled by *Yellow seed1* gene (Ibraheem, Gaffoor, & Chopra, 2010). As no 3-deoxyanthocyanidins were detected in the white sorghum, Liberty, it is proposed that the *P1* gene was either absent or not expressed in this genotype.

The present study indicates that the individual flavonoid concentration of sorghum grain cannot be simply predicted by grain colour. The luteolin concentration of the two red genotypes, MR-Bazley and Alpha was significantly different (Table 4.5). However, previously published data on two other red sorghum genotypes, Tx2911 and 98CA4779 showed that they contained 10.8 and 13.4 µg/g luteolin, respectively (Dykes et al., 2009), more than two times greater than in MR Bazley or Alpha. These results again underline the importance of determining the profile and concentration of the individual polyphenols in a genotype before producing sorghum products for target levels of specific polyphenols, such as 3-deoxyanthocyanidins.

The antioxidant activity of food products has been evaluated by a variety of chemical methods, with $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical methods being widely used (Pérez-Jiménez et al., 2008). The $DPPH^{\bullet}$ assay is generally used to measure the antioxidant activity of hydrophilic compounds while the $ABTS^{\bullet+}$ method can be used for both hydrophilic and lipophilic compounds (Moo-Huchin et al., 2015). Sorghum Shawaya Short Black 1 contained the highest concentrations of total phenolics and flavonoids, followed by IS8237C, a brown-coloured grain (Tables 4.1, 4.4 and 4.5); these two genotypes also had higher values of antioxidant activity (Table 4.6). Therefore, of the genotypes investigated in the present study, Shawaya Short Black 1 and IS8237C may be suitable in particularly arid climatic regions as a hardy crop type that would also contribute to

a nutritious food source.

4.5. CONCLUSION

Total polyphenol concentrations and antioxidant activity of sorghum grain were higher under the DI regime as compared to either FI or SDI regimes, across all genotypes. Irrigation had a significant influence on individual polyphenols, and most of them showed the highest concentration under the DI regime. The present study suggested that it is necessary to consider both genotype and water availability when selecting sorghum genotypes to plant for the production of grain for either (a) higher antioxidant capacity health foods and/or nutraceuticals or (b) low polyphenol levels to ensure maximum energy and nutrient availability from the grain for those who are -malnourished and for efficient animal feed.

As well as temperature and water, plants require solar radiation for photosynthesis and subsequent plant growth. UV radiation only makes up a small component of solar radiation, but is a principle regulator of plant development and growth (Bornman et al., 2015 and references therein). However, ozone depletion has led to an increase in solar UV radiation, especially, UV-B radiation, reaching the Earth's surface in many regions. Even though this trend is projected to decrease, changes in exposure to UV radiation will likely continue to occur because of the modifying influence of rapid climate change. Plant growth and biomass may be negatively affected by increased levels of solar UV-B radiation, while the quality of certain crops may in some cases be improved by increased UV-B radiation through induced secondary metabolites and increased antioxidant activity (Williamson et al., 2014). Due to the successful implementation of the Montreal Protocol, the concentrations of ozone depleting substances, which reached a peak in the 1990s, have been decreasing, and depletion of stratospheric ozone also has been reduced. Additionally, the ozone layer is beginning to recover, although several decades will be needed for ozone to return to pre-1980 levels (Bais et al., 2015; McKenzie et al., 2011), and as mentioned above, certain climate change factors are likely to modify future levels of UV radiation in different regions. It is therefore necessary to evaluate how variations in solar UV radiation will influence the polyphenolic and antioxidant properties of sorghum grains, and therefore this is the topic of the next chapter of this thesis.

CHAPTER FIVE: EXPERIMENTAL

Reducing solar ultraviolet radiation exposure during the growing period changes polyphenols and antioxidant activity in sorghum grain

Information contained in this chapter has been submitted as follows:

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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 activity of the diverse polyphenols in sorghum grain can vary with climatic conditions and genotype. Thus, the main aim of this study was to explore sorghum genotype response to a key environmental factor, namely, UV radiation, in order to elucidate the potential for target plant properties, such as heightened antioxidants of nutritive value. The potential effects of variation in UV radiation exposure during plant growth on the profile and concentration of polyphenols were determined in the sorghum genotypes B923296, IS131C, QL33, QL12, QL33/QL36 and Shawaya Black Short 1. A controlled environment facility was used where plants were grown under either UV-transmitting or UV-blocking conditions. Compared to the UV-transmitting treatment, the UV-blocking treatment significantly reduced total polyphenol content and antioxidant activity, with sorghum genotypes Shawaya Black Short 1 and QL12 having the highest and lowest values of polyphenol and antioxidant activity, respectively. HPLC analysis showed that the response in concentrations of individual polyphenols was different under the two treatments. The concentration of total caffeic acid was significantly reduced, while diverse changes were observed in ferulic acid under UV-blocking conditions. Reduced UV exposure levels significantly decreased most of the flavonoid concentrations, such as luteolinidin, apigeninidin and naringenin. The results of this study further highlight the potential for improvements in crop nutritive value through informed selection of plant

genotype taking into consideration key environmental conditions and their interaction with the selected genotype. Therefore, selecting for UV-responsive genotypes for maximal adaptation to environmental conditions and desired nutritive improvements is an important step towards sustainable food production and contribution to healthy diets. The uncertainty of future climate conditions further strengthens this line of approach and it application in agricultural systems.

5.1. INTRODUCTION

An increasingly variable climate coupled with frequent episodes of prolonged extreme events such as drought or high temperatures, pose a challenge to agriculture and food security, making the study of stress-tolerant crop plants and 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 (280-315 nm) radiation, 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 (Williamson et al., 2014). Projected decreases in air pollution (aerosols) over some areas of the globe will likely result in higher levels of UV radiation reaching ground level. On the other hand, the predicted recovery of the stratospheric ozone layer to historical levels, due to the Montreal Protocol, coupled with renewed global efforts to reduce greenhouse gases may result in an increased ozone concentration above that of historical levels. This in turn would lead to less UV radiation reaching ground level, which may have consequences for natural and agricultural ecosystem growth and development (Butler et al., 2016).

One example of the effect of a change in exposure of crops to UV radiation that will likely alter food quality of staple crops are the changes in production of secondary plant metabolites, such as antioxidant polyphenols, which are generally enhanced by exposure to UV-B radiation (Czégény, Mátai, & Hideg, 2016; Rice-Evans, Miller, & Paganga, 1997; Schreiner et al., 2012). For instance, Luthria et al. (2006) planted tomatoes under both UV-transmitting and UV-blocking filters, and the total concentrations of caffeic, *p*-coumaric and ferulic acids were approximately 20% higher under the UV-transmitting filter. Similarly, strawberries grown under two films differing in their ultraviolet transparency had significantly lower levels of total

phenolics and antioxidant activity under a 100% UV-blocking film compared to a 70% UV-transmitting film (Josuttis et al. 2010). However, the effect of different UV exposure during plant growth on polyphenols and antioxidant activity of sorghum grain is still unknown.

The purpose of this chapter is to show the effect of two levels of solar UV radiation on the concentrations of polyphenols and the antioxidant activity of sorghum grain of diverse genotypes during plant growth. High performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry (HPLC-DAD-ESIMS) was used to identify and quantify individual polyphenols.

5.2. MATERIALS AND METHODS

5.2.1. Experimental design

Six sorghum genotypes were used in this experiment: white pericarp 'QL12'; brown pericarp 'IS131C'; black pericarp 'Shawaya Black Short 1'; and three red pericarp genotypes 'QL33', 'B923296' and 'QL33/QL36'. All seeds were provided by 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. The experiment was conducted at Curtin University Field Trials Area, Perth, Western Australia, in a controlled UV transparent glasshouse in which the treatments involved the use of two contrasting plastic films (British Polythene Industries, Greenock, UK). The UV-transmitting film was Lumisol Clear AF (200 µm thick), with the following specifications: a transparency of 94%, 84% and 93% for UV-A, UV-B and photosynthetically active radiation (PAR, 400-700 nm), respectively. The UV-blocking film was Lumivar Clear AF (200 µm thick), with 4%, 0% and 91% for UV-A, UV-B and PAR, respectively. The manufacturers' specifications of the UV and PAR transparency of the films were confirmed with MU-200 and MQ-200 quantum sensors (Apogee Instruments, Inc., Logan, Utah, USA), respectively, during the experimental trials. The visible and UV radiation measurements were carried out under clear sky conditions at noon, 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}. The average values of PAR under the UV-transmitting film were 1645 and 1726 W m^{-2} , and under the blocking filters were1443 and 1687 W m^{-2}, for February and March, respectively. 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, UV radiation was measured as 37.4 and 38.8 W m^{-2}, and 0.5 and 0.75 W m^{-2} , for February and March, respectively. We confirmed the manufacturer's 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 of the films was likely due to the plastic used in the glasshouse film.

All sorghum genotypes were planted on 9th December 2014. Five seeds were sown into each pot. Each genotype was planted in six pots under each UV treatment. Plants were thinned to one germinated seedling per pot two weeks after sowing. Plants were watered daily under both treatments so that water was not limited, and fertilized with Yates Thrive (Yates, Padstow, NSW, Australia) every two weeks. The 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, 2016. Grain was cleaned manually and dried to a moisture content around 10%. All grain was vacuum-packed and kept at -20°C prior to analysis.

5.2.2. Sample preparation

5.2.2.1. Flour

The procedure for wholegrain flour preparation by milling has been previously described in detail in Section 3.2.2.1.

5.2.3. Analytical methods

5.2.3.1. Moisture content

The procedure for moisture content by oven drying to constant weight has been described in detail in Section 3.2.3.2.

5.2.3.2. Extraction of free phenolic compounds

The procedure for extraction of free phenolic compounds by 80% (v/v) aqueous methanol has been described in detail in Section 3.2.3.3.

5.2.3.3. Extraction of bound phenolic compounds

The procedure for extraction of bound phenolic compounds by acid hydrolyses has been described in detail in Section 3.2.3.4.

5.2.3.4. Determination of total phenolic content

The procedure for determination of total phenolic content by Folin-Ciocalteu assay has been described in detail in Section 3.2.3.5.

5.2.3.5. Determination of profile of phenolic compounds

The procedure for determination of profile of phenolic compounds by *HPLC-DAD-ESIMS analyses* has been described in detail in Section 3.2.3.6.

5.2.3.6. Determination of total flavonoids

The procedure for determination of total flavonoids by spectrophotometry method has been described in detail in Section 4.2.3.6.

5.2.3.7. Quantification of polyphenols

The procedure for quantification of phenolic compounds by *HPLC-DAD-ESIMS analyses* has been described in detail in Section 3.2.3.7.

5.2.3.8. Determination of antioxidant activities

The procedure for determination of antioxidant activities by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays has been described in detail in Section 3.2.3.8.

5.2.4. Statistical analysis

All data were expressed as means ± SD and analyzed using SPSS Statistics V20 (IBM Corp., Armonk, NY, USA). The main effects of genotype, UV radiation and their interaction were investigated by two-way ANOVA. One-way ANOVA with Tukey's post-hoc tests were used to separate the means when the main effect or interaction was significant. For all analyses, $p \leq 0.05$ was considered significant.

5.3. RESULTS

5.3.1. Identification of grain polyphenols

The identity of individual polyphenols for which authentic standards were available was confirmed through retention time, UV absorbance and mass spectrum using HPLC-DAD-ESIMS. To tentatively identify those polyphenols for which we did not have authentic standards, the polyphenol UV-VIS absorbance and mass spectrum from data in the literature were used. Up to 25 individual polyphenols were identified across the six genotypes and UV treatments (Table 5.1). Table 5.1 presents the MS and UV characteristics of the identified compounds together with their proposed structures.

The MS and UV characteristics of caffeic acid, luteolinidin, ferulic acid, taxifolin, luteolin, apigenin and naringenin, corresponded with peaks 8, 10, 16, 17, 21, 24, 25, respectively, matching those of the authentic standards. Peaks 1 and 6 represent unknown compounds. Peaks 4 and 7 had a deprotonated molecule $[M-H]^-$ at m/z 253 and had lost a glycerol residue $[M-H-74]^-$ to produce an ion at m/z 179. The fragmentation patterns with ions at m/z 179 [caffeic acid –H]− and 135 [caffeic acid – H-CO2]− of peaks 4 and 7 indicated that these compounds might have a caffeic acid residue. These two peaks also had the same UV λ_{max} as caffeic acid. Therefore, peaks 4 and 7 were tentatively deduced as either 1-*O*-caffeoylglycerol or 2-*O*-caffeoylglycerol (Ma et al., 2007). Peak 11 had a deprotonated molecule $[M-H]^-$ at m/z 447 and the fragmentation pattern with ions at m/z 285 $[M - H- 162]^-$ through losing a hexoside residue. The prominent fragment ions at *m/z* 285 corresponded to the deprotonated luteolin fragment. Hence, peak 11 was tentatively identified as luteolin-hexoside. The rest of the peaks listed in Table 5.1 have been identified and described in section 3.3.3 of this thesis.

Table 5.1. Identification of individual polyphenols in sorghum wholegrains and their respective standards by HPLC-DAD-ESIMS[a].

Peak No.	Rt[b] (min)	λ_{max} (nm)	*m/z* $[M - H]^-$	*m/z* MS^2 (Abundance %)	Tentative identification
1	10.1	275	479	167(71),149(57),105(100)	Unknown
2	10.9	280	577	425 (13), 289 (100)	Procyanidin B1
3	12.3	330	353	191(94),85(13)	Chlorogenic acid
4	12.9	298sh[c], 326	253	179(5),161(100),135(64)	2-*O*-caffeoylglycerol
5	13.4	280, 310	137	109 (38)	Protocatechuic aldehyde
6	13.7	287	355	269(44),175(100),159(75)	Unknown
7	14.2	298sh, 326	253	179(5),161(100),135(64)	1-*O*-caffeoylglycerol
8	14.9	297, 322	179	135 (100)	Caffeic acid (std[d])
9	15.4	314	563	383(42),353(38)	Unknown
10	16.1	280, 490	269	241 (18), 225 (13), 169 (16), 133 (70)	Luteolinidin (std)
11	17.0	290	447	285 (95)	Luteolin- hexoside
12	17.7	310	237	163 (24), 145 (14), 119 (82)	2-*O*-*p*-coumaroylglycerol
13	18.4	275, 470	253	225 (36), 209 (65), 179 (59), 117 (100)	Apigeninidin (std)
14	19.1	283	433	287 (100), 151 (56)	Eriodictyol deoxyhexoside
15	19.2	310	163	119 (100)	*p*-coumaric acid

16	20.6	295, 325	193	178 (42), 134 (100)	Ferulic acid (std)
17	21.5	288	303	285 (100), 217 (7), 177 (22), 125 (41)	Taxifolin (std)
18	28.3	300sh, 326	415	253 (70), 179 (93) , 161 (17), 135 (70)	1,2-*O*-dicaffeoylglycerol
19	29.2	300sh, 326	415	253 (70), 179 (93) , 161 (17), 135 (70)	1,3-*O*-dicaffeoylglycerol
20	30.5	287	287	151 (13)	Eriodictyol
21	31.0	252, 347	285	241 (14), 217 (9), 199 (22), 175 (18), 151 (31), 133 (22), 107 (13)	Luteolin (std)
22	33.3	219, 315	399	253 (82), 235 (3), 179 (22), 163 (94), 145 (20), 135 (30),119 (100)	Coumaroyl-caffeoylglycerol
23	34.2	295sh, 325	429	253 (82), 235 (13), 193 (100), 175 (22), 161 (52), 135 (50)	Feruloyl-caffeoylglycerol
24	35.9	266, 322	269	225 (14), 201 (30), 183 (17), 149 (35), 117 (100)	Apigenin (std)
25	36.3	295	271	177 (17), 151 (60), 119 (24), 107 (24)	Naringenin (std)

[a] HPLC-DAD-ESIMS = High performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry

[b] Rt = Retention time; [c] sh = shoulder; [d] std = standard

5.3.2. Polyphenol profiles

Both free and bound forms of polyphenols identified in the sorghum grains are presented in Table 5.2. Representative HPLC chromatograms of free polyphenols in the red coloured B923296 genotype under both UV radiation treatments are shown in Figure 5.1. These chromatograms give an example of changes in polyphenol profiles under the UV radiation treatment. A reduced number of types of individual polyphenols were found with exposure to the lower level of UV radiation, compared to those under the UV-transmitting treatment, except for genotypes QL12 and QL33 which retained the same profile (Table 5.2).

The profiles of the polyphenols differed according to genotype under the two UV radiation regimes. In contrast to the five coloured sorghums, a smaller profile of polyphenols was detected in the white genotype, QL12. Among the three red sorghum genotypes, the polyphenol profile found in QL33/QL36 had fewer phenolic compounds than those for the other two genotypes. There were a greater number of individual polyphenols in the free form than in the bound form in all genotypes across all treatments (Table 5.2).

5.3.3. Polyphenol content

Genotype, UV radiation and their interactive effects significantly influenced free, bound and total polyphenol contents ($p \leq 0.05$), and their levels are shown in Table 5.3. The total polyphenol concentration significantly decreased under the UV-blocking treatment when compared to the UV-transmitting treatment, with Shawaya Short Black 1 and QL12 containing the highest and lowest concentrations under both treatments, respectively. Sorghum grain grown under the UV-blocking treatment had a significantly decreased free fraction of total polyphenol concentrations in all genotypes compared to those under the UV- transmitting treatment.

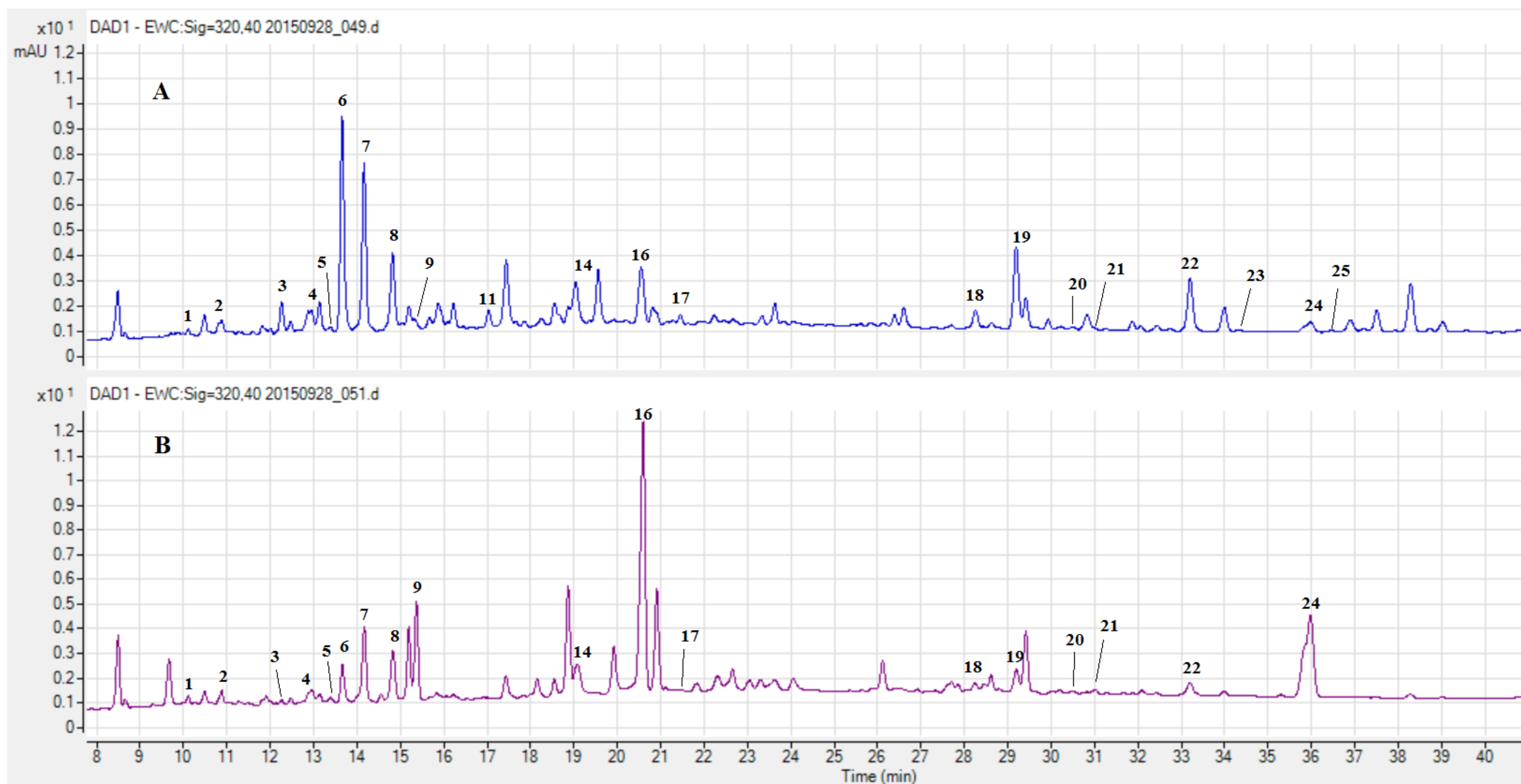

Figure 5.1. Representative HPLC-DAD chromatograms of sorghum wholegrain polyphenols A: Free polyphenols from B923296 grown under the UV-transmitting treatment. B: Free polyphenols from B923296 under the UV-blocking treatment.

Table 5.2. Profiles of polyphenols in the wholegrains of six sorghum genotypes grown under UV radiation treatments.

Polyphenol class and UV treatment		Peak number [a]					
		Shawaya Short Black 1	IS1311C	QL33/QL36	B923296	QL12	QL33
Free	Transmitting	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 21, 22, 24, 25	2, 3, ,5 ,6 ,7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 24, 25,	2, 3, 4, 5, 6, 7, 8, 11, 12, 14, 16, 17, 19, 20, 21, 24, 25	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25	2, 6, 7, 8, 14, 16, 18, 19	1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 14, 16, 18, 19, 21, 24, 25
	Blocking	2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 16, 17, 18, 19, 21,22, 23, 24, 25	2, 3, ,5 ,6 ,7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 25,	2, 3, 4, 5, 6, 7, 8, 11, 14, 16, 17, 19, 20, 21, 24, 25	1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 16, 17, 18, 19, 20, 21, 22, 24	2, 6, 7, 8, 14, 16, 18, 19	1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 14, 16, 18, 19, 21, 24, 25
Bound	Transmitting	5, 6, 8, 10, 13, 15, 16, 17, 19, 20, 21, 24, 25	6, 8, 10, 11, 13, 15, 16, 17, 19, 20, 21, 24, 25,	5, 6, 8, 10, 13, 15, 16, 17, 20, 21, 24, 25	6, 8, 13, 15, 16, 20, 21, 24, 25	6, 8, 15, 16, 20	6, 8, 10, 13, 15, 16, 20, 24, 25
	Blocking	5, 6, 8, 10, 13, 15, 16, 17, 19, 20, 21, 24, 25	6, 8, 10, 11, 13, 15, 16, 17, 19, 20, 21, 24, 25,	6, 8, 10, 13, 15, 16, 17, 20, 21, 24, 25	6, 8, 13, 15, 16, 20, 21, 24	6, 8, 15, 16, 20	6, 8, 10, 13, 15, 16, 20, 24, 25

[a] Identities of peak numbers are given in Table 5.1.

Table 5.3. Concentrations of free, bound and total phenolics (mg GAE/g db) and flavonoids (mg CE/g db) in the wholegrain of six sorghum genotypes grown under two UV radiation treatments.

Content		UV treatment	Genotypes					
			Shawaya Short Black 1	IS1311C	QL33/QL36	B923296	QL12	QL33
Polyphenols	Free	Transmitting	10.53±0.49 ^{fB}	7.85±0.34 ^{eB}	1.59±0.05 ^{cB}	1.32±0.10 ^{bB}	0.91±0.09 ^{aB}	2.73±0.08 ^{dB}
		Blocking	7.96±0.24 ^{eA}	5.94±0.18 ^{dA}	1.15±0.11 ^{bA}	1.19±0.08 ^{bA}	0.68±0.14 ^{aA}	1.52±0.10 ^{cA}
	Bound	Transmitting	2.68±0.15^{eB}	1.61±0.04 ^{dA}	1.36±0.09 ^{cB}	1.24±0.05 ^{bB}	0.74±0.04 ^{aB}	2.57±0.11 ^{eA}
		Blocking	1.90±0.11 ^{cA}	2.37±0.05 ^{dB}	1.16±0.07 ^{bA}	0.93±0.10 ^{bA}	0.48±0.07 ^{aA}	2.98±0.16 ^{eA}
	Total	Transmitting	13.21±0.33 ^{fB}	9.47±0.30 ^{eB}	2.95±0.14 ^{cB}	2.56±0.04 ^{bA}	1.64±0.18 ^{aB}	5.31±0.19 ^{dA}
		Blocking	9.86±0.14 ^{fA}	8.31±0.16 ^{eA}	2.31±0.05 ^{cA}	2.12±0.11 ^{bA}	1.16±0.07 ^{aA}	4.50±0.26 ^{dA}
Flavonoids	Free	Transmitting	4.17±0.10 ^{eB}	3.00±0.13 ^{dB}	1.45±0.11 ^{cB}	0.75±0.05 ^{bB}	0.34±0.01 ^{aB}	1.49±0.09 ^{cA}
		Blocking	3.55±0.16 ^{eA}	2.35±0.08 ^{eA}	1.01±0.17 ^{dA}	0.57±0.04 ^{cA}	0.03±0.01 ^{aA}	0.48±0.02 ^{bA}
	Bound	Transmitting	1.11±0.07 ^{dB}	1.22±0.29 ^{dA}	0.53±0.11 ^{bA}	0.65±0.07 ^{bcA}	0.08±0.03 ^{aA}	0.71±0.06 ^{cA}
		Blocking	0.88±0.09 ^{cA}	1.14±0.23 ^{dA}	0.77±0.14 ^{bcB}	0.68±0.15 ^{bA}	0.18±0.05 ^{aB}	0.86±0.17 ^{cA}
	Total	Transmitting	5.28±0.16 ^{fB}	4.22±0.42 ^{eB}	1.98±0.01 ^{cB}	1.40±0.02 ^{bB}	0.43±0.02 ^{aB}	2.20±0.07 ^{dB}
		Blocking	4.42±0.25 ^{eA}	3.49±0.15 ^{dA}	1.78±0.04 ^{cA}	1.25±0.19 ^{bA}	0.21±0.04 ^{aA}	1.33±0.14 ^{bA}

$^{a-f}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$).

$^{A, B}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

5.3.4. Flavonoid content

With respect to flavonoid concentrations, free, bound and total flavonoid concentrations were significantly affected by UV radiation, genotype and their interaction ($p \leq 0.05$) (Table 5.3). The free flavonoid concentrations were significantly reduced under the UV blocking treatment in all genotypes. Only genotype Shawaya Short Black 1 showed a significant change in bound flavonoid concentration, being lower under the UV-blocking treatment. The UV-blocking treatment significantly reduced total flavonoid concentration of all genotypes compared to the UV-transmitting treatment. Regardless of UV exposure level, Shawaya Short Black 1 and QL12 had the highest and lowest concentration of total flavonoids, respectively.

5.3.5. Individual polyphenol concentrations

The concentration of individual polyphenols in sorghum grain under the different UV radiation exposure levels was quantified by HPLC-DAD-MS with authentic standards. The contents of eight individual polyphenols were measured, and results are shown in Tables 5.4 and 5.5. These quantified compounds were divided into five groups, hydroxycinnamic acids, 3-deoxyanthocyanidins, flavones, dihydroflavonols and flavanones, according to their structural characteristics, of which white sorghum QL12 contained only hydroxycinnamic acids, but no polyphenols from the other groups.

Hydroxycinnamic acids of caffeic and ferulic acids were detected and quantified in sorghum samples. The concentrations of free, bound and total fractions of caffeic and ferulic acids were significantly influenced by genotype, UV radiation and their interaction ($p \leq 0.05$) as shown in Table 5.4. The free fraction of caffeic acid was lower under the UV-blocking treatment, while that of the free ferulic acid was higher in all genotypes except for Shawaya Short Black 1. This genotype contained the highest total ferulic and caffeic acids contents, whereas B923296 and QL12 had the lowest total caffeic and ferulic acids, respectively, under both UV treatments. These findings suggest that genotype is a more influential factor than UV radiation exposure in determining the hydroxycinnamic acid contents in these sorghums.

Two 3-deoxyanthocyanidins of luteolinidin and apigeninidin were identified and quantified (Table 5.5), and again, there were significant differences expressed

according to genotype, UV treatment and their interactive effect ($p \leq 0.05$). The UV-blocking treatment gave significantly lower concentrations of free fractions of both luteolinidin and apigeninidin in all genotypes in which this fraction was identified. However, in QL33/QL36 and B923296, no free form of luteolinidin and apigeninidin was detected. The changes in bound luteolinidin and apigeninidin contents under the UV treatments varied with genotype, and no bound luteolinidin was detected in B923296. Total luteolinidin and apigeninidin contents were significantly lower under the UV-blocking regime than for the UV-transmitting regime in all genotypes. Under both UV treatments Shawaya Short Black 1 and QL33 contained the highest and lowest content of total luteolinidin, respectively, while the content of total apigeninidin was highest and lowest in QL33/QL36 and IS1311C, respectively.

The contents of the two flavones of luteolin and apigenin (Table 5.5) were affected by genotype, UV radiation treatment and genotype × UV radiation interaction ($p \leq 0.05$). The genotypes responded differently in response to the UV treatments in relation to the free fractions of luteolin, but the UV-blocking treatment gave significantly lower contents of the free fractions of apigenin in all genotypes. For the bound forms of luteolin and apigenin, the UV-blocking treatment gave significantly higher concentrations than the UV-transmitting in all genotypes, with the exception of B923296, which had a lower concentration. The UV- blocking treatment showed a significantly higher total apigenin concentration in Shawaya Short Black 1, but a decreased level in all the other genotypes compared to the UV- transmitting treatment. Under both UV radiation exposure levels, IS1311C had the highest concentrations of both total luteolin and apigenin, whereas B923296 and QL33 contained the lowest contents of total luteolin and apigenin, respectively.

In the genotypes in which it was identified, the concentration of taxifolin of the dihydroflavonol group (Table 5.5) were significantly affected by UV radiation treatment, genotype and genotype × UV radiation interaction ($p \leq 0.05$). However, taxifolin was not detectable in either the free or bound form in QL12 or QL33. In those genotypes in which it was identified, the UV-blocking regime led to reduced concentration of free taxifolin when compared to the UV-transmitting regime. For values of bound taxifolin, no significant effect of UV treatment was observed for IS1311C, while for QL33/QL36 and Shawaya Short Black, their contents were lower

under the UV-blocking treatment compared to the UV-transmitting treatment. For the total taxifolin content, the UV-blocking treatment resulted in lower levels in all sorghums in which it was identified, with IS1311C and B923296 containing the highest and lowest values, respectively.

Of the flavanones, only naringenin was quantified in the sorghum genotypes (Table 5.5). For those genotypes containing this polyphenol, UV radiation treatment, genotype and genotype × UV radiation interaction had a significant influence on its free, bound and total contents ($p \leq 0.05$). However, it was not identified in the genotype QL12. In addition, only the free form of naringenin but not the bound was detected and quantified in B923296. For those genotypes containing the free form of naringenin, the UV-blocking treatment resulted in significantly lower levels than the UV-transmitting one in all genotypes except for IS1311C, which showed no difference. Likewise, bound naringenin content was lower in the UV-blocking treatment with the exception of Shawaya Short 1, for which it was significantly higher. The effect of UV treatment on total content of naringenin varied widely among the genotypes, with IS1311C and QL33 containing the lowest and highest values in both treatments respectively.

Table 5.4. Concentrations of free, bound and total hydroxycinnamic acids (μg/g db) in the wholegrain of six sorghum genotypes grown under UV radiation treatments.

Hydroxycinnamic acids content		UV treatment	Genotype					
			Shawaya Short Black 1	IS1311C	QL33/QL36	B923296	QL12	QL33
CA	Free	Transmitting	28.36±0.39 ^{eB}	21.10±0.39 ^{dB}	15.12±0.22 ^{cB}	6.35±0.12 ^{aB}	12.54±0.12 ^{bB}	12.06±0.14 ^{bB}
		Blocking	24.26±0.13 ^{eA}	15.42±0.07 ^{dA}	6.54±0.15 ^{bA}	4.45±0.19 ^{aA}	9.39±0.23 ^{cA}	9.18±0.12 ^{cA}
	Bound	Transmitting	13.42±0.14 ^{fB}	10.23±0.16 ^{eA}	1.86±0.12 ^{bA}	2.69±0.05 ^{cB}	1.55±0.08 ^{aA}	6.69±0.05 ^{dB}
		Blocking	10.19±1.22 ^{eA}	12.70±0.51 ^{fB}	2.68±0.15 ^{cB}	1.47±0.04 ^{aA}	1.96±0.15 ^{bB}	5.67±0.13 ^{dA}
	Total	Transmitting	41.77±0.25 ^{fB}	31.33±0.55 ^{eB}	16.98±0.34 ^{cB}	9.05±0.07 ^{aB}	14.10±0.04 ^{bB}	18.75±0.23 ^{dB}
		Blocking	34.44±1.35 ^{fA}	28.12±0.47 ^{eA}	9.23±0.11 ^{bA}	5.92±0.23 ^{aA}	11.34±0.38 ^{cA}	14.86±0.26 ^{dA}
FA	Free	Transmitting	18.53±0.32 ^{eB}	17.66±0.19 ^{dA}	5.11±0.02 ^{cA}	2.83±0.25 ^{bA}	2.66±0.10 ^{abA}	2.50±0.08 ^{aA}
		Blocking	14.37±0.34 ^{dA}	29.54±0.46 ^{eB}	6.18±0.25 ^{bB}	7.41±0.22 ^{cB}	4.64±0.06 ^{aB}	4.60±0.26 ^{aB}
	Bound	Transmitting	120.33±1.56 ^{fA}	103.53±2.41 ^{eA}	52.20±1.41 ^{bB}	80.89±0.45 ^{dB}	31.12±0.23 ^{aA}	58.54±0.31 ^{cA}
		Blocking	155.69±2.77 ^{fB}	141.64±0.47 ^{eB}	46.11±0.30 ^{bA}	68.01±1.25 ^{dA}	38.16±0.22 ^{aB}	61.35±0.20 ^{cA}
	Total	Transmitting	138.86±1.24 ^{fA}	121.21±2.60 ^{eA}	57.31±1.12 ^{bB}	83.72±0.70 ^{dB}	33.78±0.33 ^{aA}	61.05±0.39 ^{cA}
		Blocking	170.06±2.43 ^{eB}	171.18±0.93 ^{eB}	52.29±0.05 ^{bA}	75.42±1.03 ^{dA}	42.79±0.28 ^{aB}	65.95±0.06 ^{cB}

$^{a-f}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$).
$^{A, B}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).
Abbreviations: CA= caffeic acid; FA= ferulic acid

Table 5.5. Concentrations of free, bound and total individual flavonoids (µg/g db) in the wholegrain of six sorghum genotypes grown under two UV radiation treatments.

Flavonoids content		UV treatment	Genotypes					
			Shawaya Short Black 1	IS1311C	QL33/QL36	B923296	QL12	QL33
3-Deoxyanthocyanidins								
LUT	Free	Transmitting	6.46±0.23 ^{cB}	4.38±0.14 ^{bB}	nd	nd	nd	2.21±0.04 ^{aB}
		Blocking	1.72±0.12 ^{bA}	2.59±0.03 ^{cA}	nd	nd	nd	0.94±0.13 ^{aA}
	Bound	Transmitting	3.33±0.15 ^{bB}	0.63±0.06 ^{aA}	5.67±0.29 ^{cB}	nd	nd	0.54±0.04 ^{aA}
		Blocking	2.24±0.09 ^{cA}	1.27±0.15 ^{bB}	3.57±0.06 ^{dA}	nd	nd	0.88±0.12 ^{aB}
	Total	Transmitting	9.79±0.17 ^{dB}	5.01±0.03 ^{bB}	5.67±0.29 ^{cB}	na	na	2.75±0.09 ^{aB}
		Blocking	3.96±0.13 ^{cA}	3.65±0.01 ^{bA}	3.57±0.06 ^{bA}	na	na	1.82±0.08 ^{aA}
API	Free	Transmitting	5.30±0.04 ^{cB}	2.29±0.11 ^{bB}	nd	nd	nd	1.38±0.05 ^{aB}
		Blocking	1.98±0.09 ^{cA}	1.28±0.06 ^{bA}	nd	nd	nd	0.24±0.03 ^{aA}
	Bound	Transmitting	1.93±0.12 ^{bA}	0.70±0.05 ^{aA}	16.43±0.18 ^{eB}	13.95±0.28 ^{dB}	nd	8.03±0.13 ^{cB}
		Blocking	1.81±0.22 ^{bA}	0.94±0.16 ^{aB}	14.38±0.14 ^{eA}	7.64±0.16 ^{dA}	nd	5.01±0.17 ^{cA}
	Total	Transmitting	7.23±0.21 ^{bB}	2.99±0.17 ^{aB}	16.43±0.18 ^{eB}	13.95±0.28 ^{dB}	na	9.41±0.12 ^{cB}
		Blocking	3.79±0.18 ^{bA}	2.22±0.21 ^{aA}	14.38±0.14 ^{eA}	7.64±0.16 ^{dA}	na	5.25±0.10 ^{cA}
Flavones								
LU	Free	Transmitting	4.09±0.10 ^{bB}	11.82±0.29 ^{dB}	5.49±0.28 ^{cB}	0.69±0.05 ^{aA}	nd	0.62±0.19 ^{aA}
		Blocking	1.92±0.10 ^{bA}	4.36±0.19 ^{cA}	4.21±0.18 ^{cA}	1.55±0.15 ^{aB}	nd	1.42±0.05 ^{aB}

	Bound	Transmitting	1.28±0.09 ^{bA}	4.04±0.11 ^{dA}	0.83±0.02 ^{aA}	1.47±0.18 ^{cB}	nd	1.34±0.05 ^{cA}
		Blocking	1.71±0.32 ^{bB}	5.82±0.09 ^{dB}	2.36±0.19 ^{cB}	1.09±0.06 ^{aA}	nd	2.39±0.10 ^{cB}
	Total	Transmitting	5.37±0.01 ^{bB}	15.86±0.41 ^{dB}	6.32±0.22 ^{cA}	2.16±0.23 ^{aA}	na	1.96±0.24 ^{aA}
		Blocking	3.63±0.42 ^{bA}	10.17±0.28 ^{dA}	6.57±0.01 ^{cA}	2.64±0.21 ^{aB}	na	3.81±0.15 ^{bB}
AP	Free	Transmitting	2.67±0.17 ^{cB}	17.87±0.23 ^{eB}	3.16±0.07 ^{dB}	1.37±0.06 ^{bB}	nd	0.26±0.01 ^{aB}
		Blocking	1.84±0.12 ^{cA}	3.45±0.26 ^{eA}	2.08±0.04 ^{dA}	0.79±0.04 ^{bA}	nd	0.07±0.01 ^{aA}
	Bound	Transmitting	2.20±0.08 ^{dA}	1.50±0.15 ^{cA}	0.14±0.02 ^{aA}	0.71±0.03 ^{bB}	nd	nd
		Blocking	5.88±0.18 ^{cB}	4.88±0.14 ^{bB}	0.27±0.06 ^{aB}	0.32±0.01 ^{aA}	nd	nd
	Total	Transmitting	4.87±0.21 ^{dA}	19.37±0.38 ^{eB}	3.30±0.05 ^{cB}	2.08±0.09 ^{bB}	na	0.26±0.01 ^{aB}
		Blocking	7.72±0.30 ^{dB}	8.33±0.40 ^{eA}	2.35±0.10 ^{cA}	1.11±0.02 ^{bA}	na	0.07±0.01 ^{aA}
Dihydroflavonol								
TA	Free	Transmitting	7.22±0.13 ^{bB}	10.81±0.36 ^{cB}	12.66±0.30 ^{dB}	5.41±0.04 ^{aB}	nd	nd
		Blocking	2.57±0.06 ^{bA}	8.15±0.09 ^{dA}	6.81±0.38 ^{cA}	0.65±0.05 ^{aA}	nd	nd
	Bound	Transmitting	1.93±0.04 ^{bB}	1.03±0.07 ^{aA}	2.39±0.08 ^{cB}	nd	nd	nd
		Blocking	1.52±0.10 ^{bA}	1.28±0.24 ^{aA}	1.89±0.15 ^{cA}	nd	nd	nd
	Total	Transmitting	9.15±0.22 ^{bB}	11.83±0.43 ^{cB}	15.04±0.38 ^{dB}	5.41±0.04 ^{aB}	na	na
		Blocking	4.09±0.18 ^{bA}	9.43±0.18 ^{dA}	8.70±0.23 ^{cA}	0.65±0.05 ^{aA}	na	na
Flavanone								
NAR	Free	Transmitting	2.61±0.64 ^{cB}	1.23±0.12 ^{aA}	4.59±0.13 ^{dB}	1.61±0.18 ^{b}	nd	7.83±0.06 ^{eB}
		Blocking	0.32±0.04 ^{aA}	1.21±0.14 ^{bA}	3.32±0.10 ^{cA}	nd	nd	3.45±0.05 ^{cA}
	Bound	Transmitting	1.29±0.05 ^{aA}	1.57±0.16 ^{bB}	2.28±0.24 ^{cB}	2.77±0.32 ^{cd}	nd	2.90±0.02 ^{dB}

	Blocking	1.84±0.23 ^{dB}	0.98±0.07 ^{bA}	0.71±0.04 ^{aA}	nd	nd	1.41±0.06 ^{cA}
Total	Transmitting	3.90±0.09 ^{bB}	2.81±0.29 ^{aB}	6.87±0.11 ^{dB}	4.37±0.15 ^{c}	na	10.73±0.04 ^{eB}
	Blocking	2.16±0.19 ^{aA}	2.19±0.21 ^{aA}	3.96±0.14 ^{bA}	nd	na	4.86±0.11 ^{cA}

$^{a-e}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$).

$^{A, B}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

Abbreviations: nd= not detected; na= data not available; LUT= luteolinidin; API=apigeninidin; LU= luteolin; AP= Apigenin; TA= taxifolin; NAR= naringenin.

Table 5.6. Values of free, bound and total antioxidant capacity in sorghum wholegrains by genotype as affected by UV radiation treatments measured using the DPPH (mg TE/g, sample db) and $ABTS^{\bullet+}$ (mg TE/g sample db) assay across six genotypes.

Antioxidant capacity		UV treatment	Genotypes					
			Shawaya Short Black 1	IS1311C	QL33/QL36	B923296	QL12	QL33
$DPPH^{\bullet}$	Free	Transmitting	20.84±0.37 ^{fB}	15.79±0.10 ^{eB}	2.29±0.12 ^{cB}	1.83±0.11 ^{bB}	1.00±0.02 ^{aB}	4.38±0.07 ^{dB}
		Blocking	15.65±0.30 ^{eA}	11.09±0.27 ^{dA}	1.87±0.06 ^{bA}	1.59±0.21 ^{bA}	0.66±0.09 ^{aA}	2.17±0.06 ^{cA}
	Bound	Transmitting	3.89±0.18 ^{eB}	2.64±0.04 ^{dA}	2.04±0.06 ^{cB}	1.72±0.12 ^{bB}	1.06±0.14 ^{aB}	3.68±0.08 ^{eA}
		Blocking	2.93±0.07 ^{dA}	3.46±0.12 ^{eB}	1.73±0.08 ^{bA}	1.35±0.15 ^{bA}	0.45±0.03 ^{aA}	4.07±0.05 ^{fB}
	Total	Transmitting	24.73±0.19 ^{fB}	18.43±0.14 ^{eB}	4.33±0.06 ^{cB}	3.55±0.07 ^{bB}	2.06±0.17 ^{aB}	8.06±0.31 ^{dB}
		Blocking	18.57±0.38 ^{fA}	14.55±0.16 ^{eA}	3.59±0.01 ^{cA}	2.94±0.15 ^{bA}	1.11±0.06 ^{aA}	6.24±0.25 ^{dA}
$ABTS^{\bullet+}$	Free	Transmitting	31.92±0.32 ^{fB}	24.25±0.12 ^{eB}	3.24±0.07 ^{cB}	2.91±0.10 ^{bB}	1.69±0.12 ^{aB}	7.33±0.06 ^{dB}
		Blocking	23.96±0.14 ^{eA}	18.51±0.55 ^{dA}	2.72±0.06 ^{bA}	2.57±0.06 ^{bA}	0.91±0.09 ^{aA}	3.18±0.07 ^{cA}
	Bound	Transmitting	5.25±0.11 ^{eB}	3.23±0.05 ^{dA}	2.87±0.16 ^{cB}	2.23±0.17 ^{bB}	1.26±0.13 ^{aB}	5.04±0.09 ^{eA}
		Blocking	4.20±0.08 ^{dA}	4.89±0.02 ^{eB}	2.27±0.11 ^{bA}	1.73±0.08 ^{bA}	0.65±0.04 ^{aA}	6.50±0.17 ^{fB}
	Total	Transmitting	37.17±0.21 ^{fB}	27.48±0.07 ^{eB}	6.11±0.09 ^{cB}	5.14±0.23 ^{bB}	2.97±0.06 ^{aB}	12.37±0.04 ^{dB}
		Blocking	28.16±0.22 ^{fA}	23.40±0.58 ^{eA}	4.99±0.18 ^{cA}	4.30±0.18 ^{bA}	1.56±0.01 ^{aA}	9.68±0.15 ^{dA}

$^{a\text{-}f}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$).
$^{A, B}$ Values with different superscripts in the same column in the same dependent variable are significantly different ($p \leq 0.05$).

5.3.6. Antioxidant activity

The antioxidant activity of sorghum grains was evaluated by the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays (Table 5.6). The results using both assays were significantly affected by UV, genotype and genotype × UV interaction ($p \leq 0.05$). The UV-blocking treatment gave significantly lower free antioxidant activities than the UV-transmitting one for all genotypes, but the effect of the UV treatment on antioxidant activities of the bound fraction varied with genotype. The antioxidant activity of total phenolics was lower in all genotypes under the UV-blocking treatment, with the highest and lowest in Shawaya Short Black 1 and QL12, respectively.

5.4. DISCUSSION

The values of free, bound and total phenolics were in the range of previous studies (Dykes et al., 2005; Wu, Johnson, Bornman, Bennett, & Fang, 2017). The total polyphenol and flavonoid contents in the six sorghum genotypes in this study significantly decreased under the UV-blocking treatment compared to the UV-transmitting one. The change in secondary plant metabolites, such as polyphenols, in response to UV-B radiation likely evolved as a plant defence mechanism against the harmful effect of this radiation. For instance, polyphenols can act as scavengers of reactive oxygen species generated in the plant through exposure to UV-B radiation (Tapiero, Tew, Ba, & Mathe, 2002). The finding of the present study supported this, since lower polyphenol and flavonoid concentrations were found in the sorghum grains when UV radiation was reduced. It is well known that the biosynthesis of polyphenols in plants is modulated by the key enzymes, PAL and CHS (Cohen & Kennedy, 2010). The regulation of PAL and CHS activity is affected by environmental conditions (Rivero et al., 2001; Tomás-Barberán & Espin, 2001; Jesus Tovar, Romero, Girona, & Motilva, 2002). In the present study, the lower levels of polyphenols and flavonoids under the UV-blocking treatment may have been due to a UV-B radiation-induced reduction in PAL and CHS activity. However, the activities of these enzymes were not measured in the present study.

The influence of UV radiation on contents of polyphenols as a collective group and as individual compounds in plant tissues has been studied in a range of species (Josuttis et al., 2010; Josuttis, Verrall, Stewart, Krüger, & McDougall, 2013; Luthria et al., 2006;

Nenadis et al., 2015). However, to date, there have been no studies to our knowledge on sorghum grain. Evidence from other plant species indicates that the accumulation of individual polyphenolic compounds may show different patterns in response to UV radiation treatments, and therefore determining polyphenols as a collective group may be insufficient for evaluating important changes, due to differing exposure to UV radiation (Arróniz-Crespo, Núñez-Olivera, & Martínez-Abaigar, 2008). In the present study, diverse changes were observed in individual polyphenols in sorghum grain when comparing the two levels of UV radiation exposure. For example, decreased exposure to UV radiation reduced the concentration of extractable caffeic acid in all genotypes. This response has also been reported in tomato (Luthria et al., 2006). The ferulic acid content was significanltly increased in Shawaya Short Black 1, IS1311C, QL12 and QL33 under the UV-blocking treatment, which was also found in Malbec grapes (Alonso, Berli, Fontana, Piccoli, & Bottini, 2016).

UV radiation can modify the activity or synthesis of enzymes involved in the flavonoid biosynthesis pathway. In this pathway, chalcone is isomerized to naringenin, catalysed by the enzyme chalcone isomerase (Boddu et al., 2004). In our study, the UV-blocking treatment gave significantly lower levels of naringenin, suggesting that chalcone isomerase activity may have been lowered when exposure to UV radiation was low. In sorghum, this naringenin biosynthetic pathway can also lead to the production of various derivatives of naringenin, including luteoferol and apiferol, which are the precursors for luteolinidin and apigeninidin, respectively (Boddu et al., 2004). It is hypothesized that the reduced naringenin content under the UV-blocking treatment in the present study may have also resulted in lower concentrations of luteoferol and apiferol, which might have lead in turn to the lower concentrations of luteolinidin and apigeninidin observed.

The results of the polyphenolic profiles in the present study highlight their complexity and dependency on genotype (rather than only grain colour) and UV radiation treatment and their interaction. Thus, some of the polyphenol profiles for the newly tested genotypes in this study were different when compared to genotypes with similar grain colour. Again, it is important to identify the profiles of polyphenols in specific sorghum genotypes rather than rely solely on grain colour as a general indicator (Awika et al., 2004b; Taleon et al., 2014).

The consumption of sorghum grain polyphenolics, through their potential to reduce *in vivo* oxidative stress, may reduce the risk and progression of some chronic diseases, such as coronary heart disease and type 2 diabetes (Althwab et al., 2015). The antioxidant capacity of foods as a possible predictor of their potential to reduce *in vivo* oxidative stress is generally evaluated by numerous chemical methods, with the $ABTS^{\bullet+}$ and $DPPH^{\bullet\cdot}$ radicals being the most widely used. The antioxidant activity as determined by both methods has been positively correlated with polyphenol contents of sorghum (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). In the present study, sorghum genotypes Shawaya Short Black 1 and IS1311C, with higher concentrations of total phenolics showed higher antioxidant activity than the other genotypes. White sorghum QL12 had the lowest values of antioxidant activity, most likely due to the simpler profile and lower content of polyphenols (Table 5.3 and Table 5.6).

Sorghum is now being considered as a potentially important source of polyphenols, and some high antioxidant functional foods have been developed from sorghum, such as cookies, pasta, cakes and breads (Khan et al., 2013; Taylor et al., 2006; Yousif et al., 2012). Dietary intake of whole sorghum grain with abundant levels of polyphenol might prevent certain cancers, type 2 diabetes and cardiovascular disease, due to their antioxidant properties (Dykes & Rooney, 2007). Recently, several sorghum products, such as flaked breakfast cereal (Stefoska-Needham et al., 2016), bread (Yousif et al., 2012), pasta (Khan et al., 2013) and extruded snack food (Licata, Coorey, Zhao, Chu, & Johnson, 2015) have been developed using sorghums to increase their antioxidant capacity. Khan et al. (2015) found that in a human post-prandial study, compared with 100% durum wheat pasta and pasta containing 30% white wholegrain sorghum, the values of antioxidant capacity, plasma polyphenols and superoxide dismutase activity of the post-prandial blood of participants who consumed the pasta were significantly increased in pasta containing 30% red wholegrain sorghum flour. Superoxide dismutase is one of the most important enzymes in human cells, and can repair and protect cells from damage occurring due to free radicals in the body. An increase in its activity can enhance the antioxidant status of human body (Bresciani, da Cruz, & González-Gallego, 2015). These findings suggested that the antioxidant status of the participators was improved after eating pasta made from 30% red wholegrain sorghum. The presence of 3-deoxyanthocyanidins in red wholegrain sorghum flour but not the

other flours used in the study may have been responsible for the observed beneficial effects (Khan et al., 2013).

Results from the present study suggest that the two sorghum genotypes, Shawaya Short Black 1 and IS1311C, with the highest polyphenol content and antioxidant activity, have potential in the development of human food for enhancing antioxidant status. In addition, results presented in Chapter 4 indicated that Shawaya Short Black 1 also produced higher concentrations of polyphenols under mild water stress. Therefore, it is suggested that these two genotypes could be grown under conditions of mild water stress and in areas of high UV radiation to specifically increase their antioxidant activity for use in health functional foods. Therefore selecting for UV-responsive genotypes for maximal adaptation to environmental conditions and desired nutritive improvements is an important step towards sustainable food production and contribution to healthy diets. The uncertainty of future climate conditions further strengthens this line of approach and its application in agricultural systems.

5.5. CONCLUSION

Although the effect of the level of exposure to UV radiation on the pattern of individual polyphenols varied amongst genotypes, many of the individual polyphenolics were found at higher concentrations under the higher UV radiation treatment. These findings contribute valuable information for selecting suitable growth environments and genotypes.

OVERALL CONCLUSIONS

This thesis has attempted to further our understanding of the likely expected response of polyphenols in sorghum whole grains under the increasing climatic variability that is predicted in some important cereal growing regions. The research has highlighted the potential for particular sorghums to be used as a human food source due to their high antioxidant activity or high iron dialysability depending on environmental conditions and genotype. The results can also form the basis and serve as guidelines for selection of appropriate sorghum genotypes for targeting maximum levels of polyphenols or iron dialysability to increase the nutritive value of sorghum under current and future climatic conditions.

Phenolic compounds are one of the most abundant phytochemicals in sorghum grain, and their levels were shown to be strongly influenced by genotype. Although the antioxidant properties of these phenolic compounds may reduce risk factors of chronic diseases in humans, some of these compounds can negatively affect the nutritional quality of sorghum foods, through inhibition of digestive enzyme activity (e.g., amylase in starch digestion), as well as binding to dietary proteins and minerals, such as iron, reducing their digestibility and dialysability. Therefore, sorghum grain genotypes with lower levels of phenolic compounds, in particular tannins, are more suitable for foods where there is a consumer requirement for increased digestibility and mineral nutrition. On the other hand, sorghum genotypes with high levels of phenolic compounds are preferred in the preparation of high antioxidant foods for those who are at risk of chronic diseases associated with over-nutrition of high density foods low in micronutrients. For example, recently the first ever staple food in Australia made almost entirely from sorghum, Sanitarium's gluten-free Weetbix™, was commercially released (Sanitarium Health and Wellbeing, Cooranbong, NSW, Australia). This product carries a high antioxidant nutrient content claim.

The rapidly changing global climate, including rising temperatures, increased water scarcity in some already arid regions and exposure to changes in UV radiation in some regions, have the potential to have an impact on several chemical components of crops, including the phenolic compounds. Therefore, before preparing sorghum foods with

high antioxidant activity or high nutrient and energy availability, it is necessary to evaluate the phenolic compounds and antioxidant activity of sorghum genotypes grown under simulated future environmental conditions such as high temperature, water stress and different UV-B radiation. The results from this thesis demonstrate how sorghum genotype and climate conditions during plant growth can affect the profile and amounts of the grain phenolic compounds and their antioxidant properties.

The thesis aim to understand the effect of genotype and growth temperature on polyphenol, antioxidant activity and *in vitro* iron dialysability of sorghum grain was achieved by the finding reported, and the outcome of the studies has also been demonstrated in Chapter 3. The findings of this chapter demonstrated that the grain phenolic compounds, antioxidant properties and *in vitro* iron availability were significantly influenced by sorghum genotype, and high temperature. The brown sorghum genotype IS 8525 had the highest values of phenolic compounds and antioxidant activity, suggesting that this genotype would be a good choice for use to develop sorghum functional foods with higher antioxidant capacity. However, genotype IS 8525 also contained the highest content of condensed tannins, which could decrease the iron dialysability. The findings of Chapter 3, may assist farmers to plant specific sorghum genotypes to produce sorghum grains for manufacture of food with (a) high antioxidant capacity or (b) low levels of polyphenolics to maximize *in vitro* iron availability. The results of Chapter 3 also demonstrated that increased temperature could reduce polyphenol content of some sorghum genotypes. This information is valuable for farmers in semi-arid areas such as sub-Saharan Africa, where sorghum is widely used to manufacture staple foods for local people. Sorghums are grown under higher than optimal temperatures in semi-arid areas, and the reduced levels of phenolic compounds in the grain could help provide higher energy and iron dialysability foods for certain communities.

New understanding of impact of water deficit on polyphenol composition and antioxidant activity of sorghum grains was achieved in Chapter 4. The findings of Chapter 4 demonstrate that the values of grain phenolic compounds and antioxidant properties were significantly increased when irrigation levels were reduced to those representing water deficit across all sorghum genotypes. Plant breeders and farmers will benefit from this information to breed or grow varieties with increase polyphenol

and antioxidant levels in water stress conditions for use in foods and nutraceuticals. However, the results in Chapter 4 also showed that the levels of grain phenolic compounds and antioxidant properties were significantly decreased when under a severe water deficit regime, which highlights the need to control levels of water deficit when possible in order to achieve good yields of sorghum with high antioxidants for food consumption.

New knowledge of the changes in polyphenols and antioxidant activities in sorghum grain due to exposure to different levels of solar ultraviolet radiation during the growing period was presented in Chapter 5. The outcomes of that study showed that under the lower exposure to UV radiation, the levels of sorghum grain phenolic compounds and their antioxidant properties were significantly reduced. An application of this research could enable farmers to select for sorghum genotypes that are highly responsive to enhanced solar UV radiation for increasing grain phenolic compounds for processing into high antioxidant health-functional foods. However, in regions where the levels of UV radiation are comparatively low or predicted to decrease, the findings are also relevant for growing selected sorghums to produce reduced grain polyphenolics for higher energy and mineral availability.

The limitations of some of these findings are that the antioxidant activity of phenolic compounds in the sorghum grains was evaluated with *in vitro* chemical based $ABTS^{\bullet+}$ and DPPH● scavenging methods. Compared to these chemical methods, cellular methods may be more predictive of *in vivo* oxidative stress protective effects of the phytochemicals. Future recommended studies leading on from the work presented in this thesis include measuring antioxidants of sorghum grain with the more biological assay of cellular antioxidant activity (CAA). The principle of this assay, using cell cultures, is the measurement of the ability of antioxidants to prevent the formation of a fluorescent oxidative stress indicator induced by peroxyl radicals, which are reactive products of lipid oxidation (Wolfe & Liu, 2007). The antioxidant activity of some commonly consumed fruits and vegetables has previously been measured using both the CAA assay and the *in vitro* chemical-based method of ORAC (Song et al., 2010; Wolfe et al., 2008). It was observed that the total polyphenol content had a significant correlation with CAA values in both fruits and vegetables. However, CAA values were significantly correlated to ORAC values only in fruits, but not in the vegetables tested.

This finding suggests that the antioxidant capacity of samples measured by chemical-based assays may not correlate well in some foods with the more physiologically relevant cell-based indexes (López-Alarcón & Denicola, 2013). The CAA assay is considered as a more relevant testing method than the *in vitro* chemical methods for preliminarily screening the protective ability of potential food oxidative stress *in vivo*. No information is available on CAA assay for assessing antioxidant capacity of sorghum grain, and therefore, more research is needed in this area. However, this does not suggest that in comparison with cell-based assays, chemical methods are not useful. On the contrary, they should be understood as complementary methodologies and it is highly recommended to analyze the antioxidant capacity of natural products by using more than one chemical-based as well as cellular based assays, before venturing into oxidative stress protection studies of new foods such as those from high polyphenolic sorghum *in vivo*.

The results presented in this thesis suggested that sorghum grain has the important functional and health-promoting effects. However, this research work has only utilised in vitro assays and models, and no direct proof of these health-enhancing effects *in vivo* was provided. To provide the required evidence, it is necessary to design well-controlled human clinical studies in the future. Moreover, sensory evalution of any sorghum-based foods will also be required in order to select the "best" sorghum genotype for dietary purposes.

In conclusion, the new knowledge of variations in sorghum grain phenolic compounds and antioxidant properties of different genotypes grown under different growth conditions presented in this thesis is important for sorghum breeders, farmers and food processors for selections of genotypes with appropriate polyphenolic and antioxidant levels for their target consumers be they energy, protein or micronutrient malnourished or the opposite paradigm of being in energy oversupply and at risk of oxidative stress-related chronic diseases.

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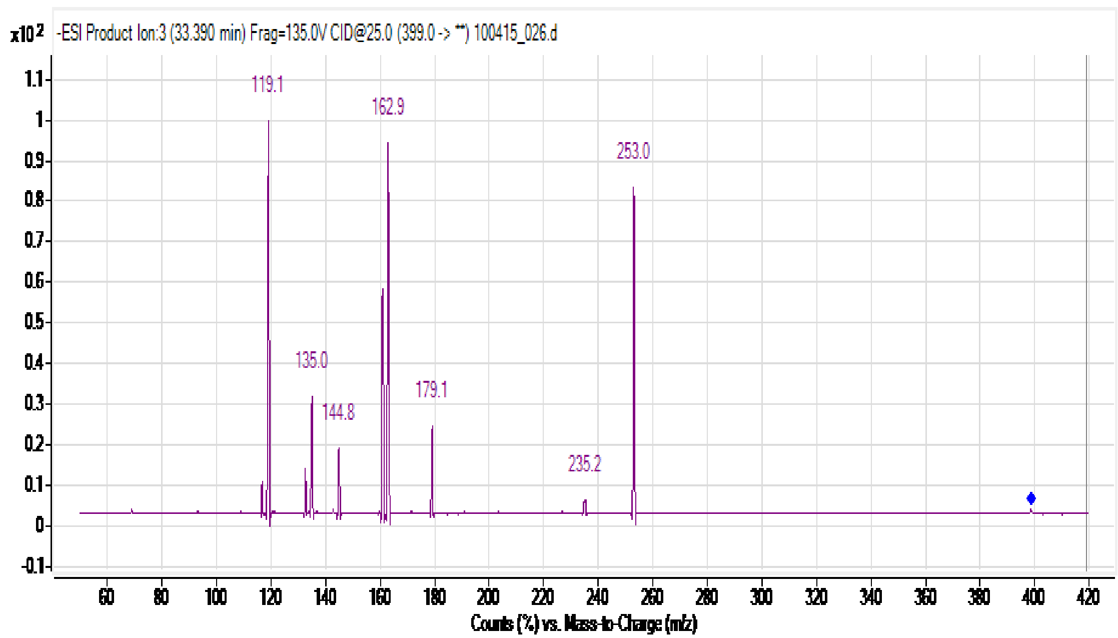
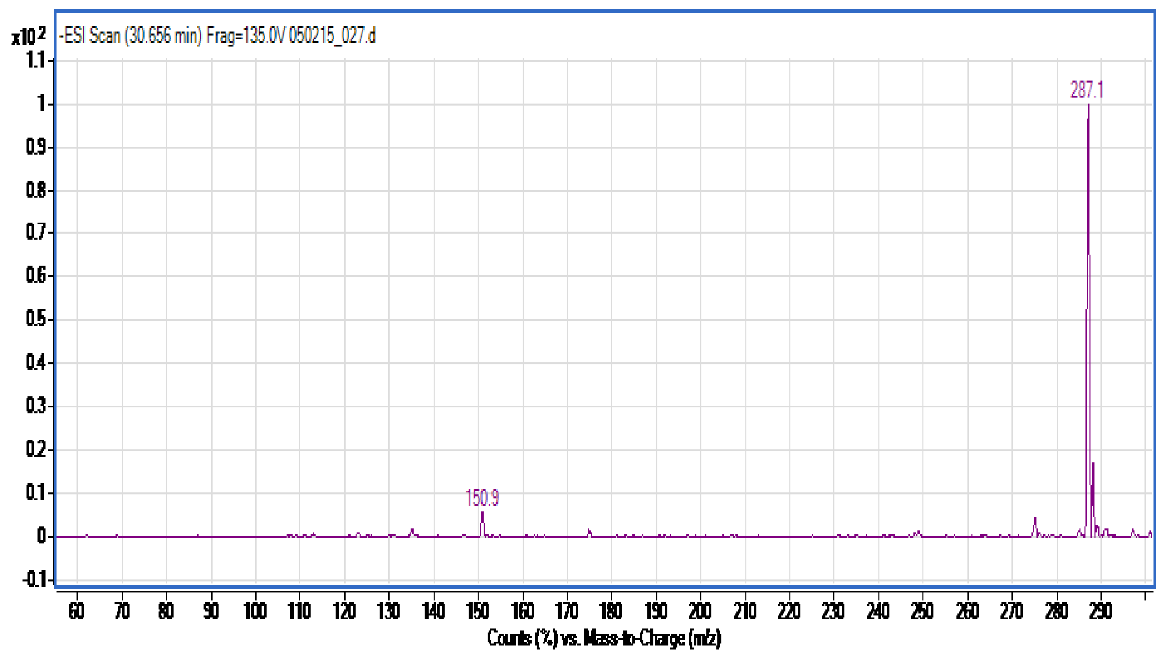
APPENDIX 1



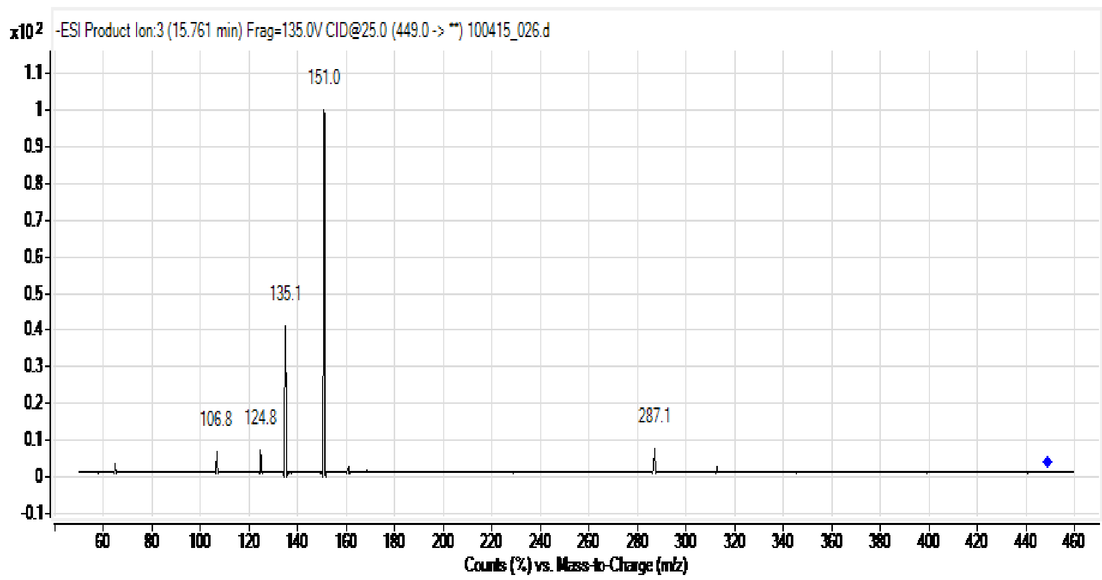
Mass spectra of coumaroyl-caffeoylglycerol of sorghum grain.

APPENDIX 2



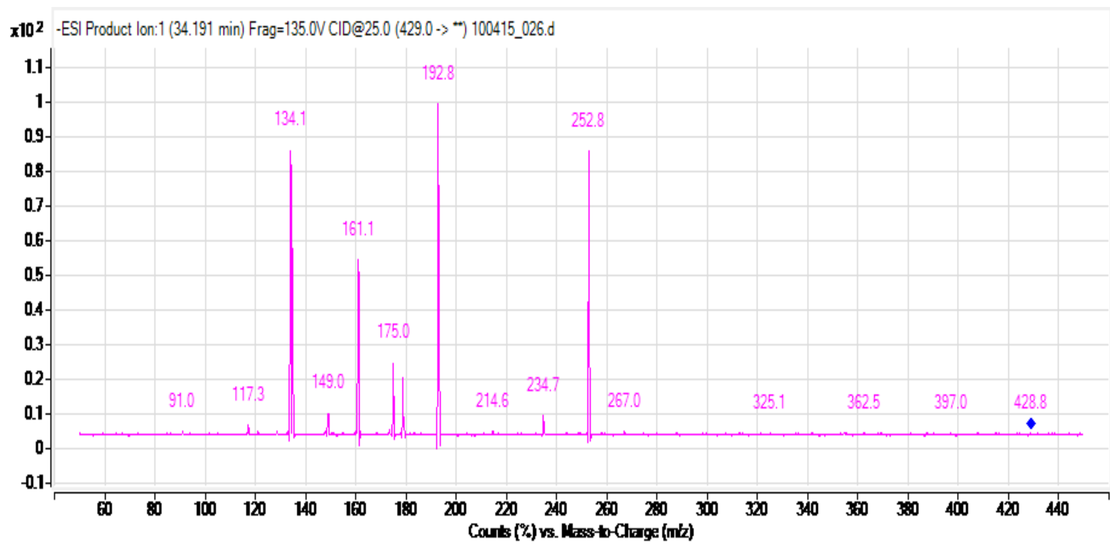
Mass spectra of eriodictyol of sorghum grain.

APPENDIX 3



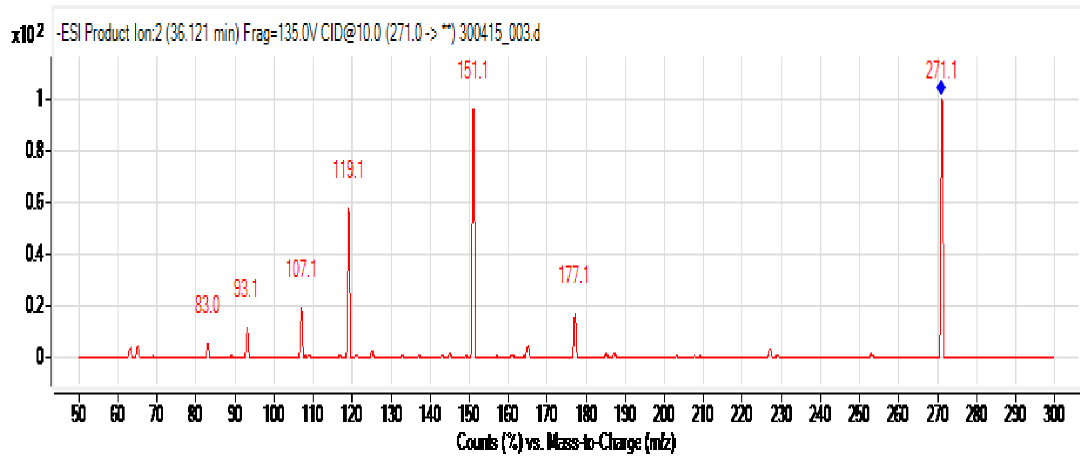
Mass spectra of eriodictyol-7-*O*-glucoside of sorghum grain.

APPENDIX 4



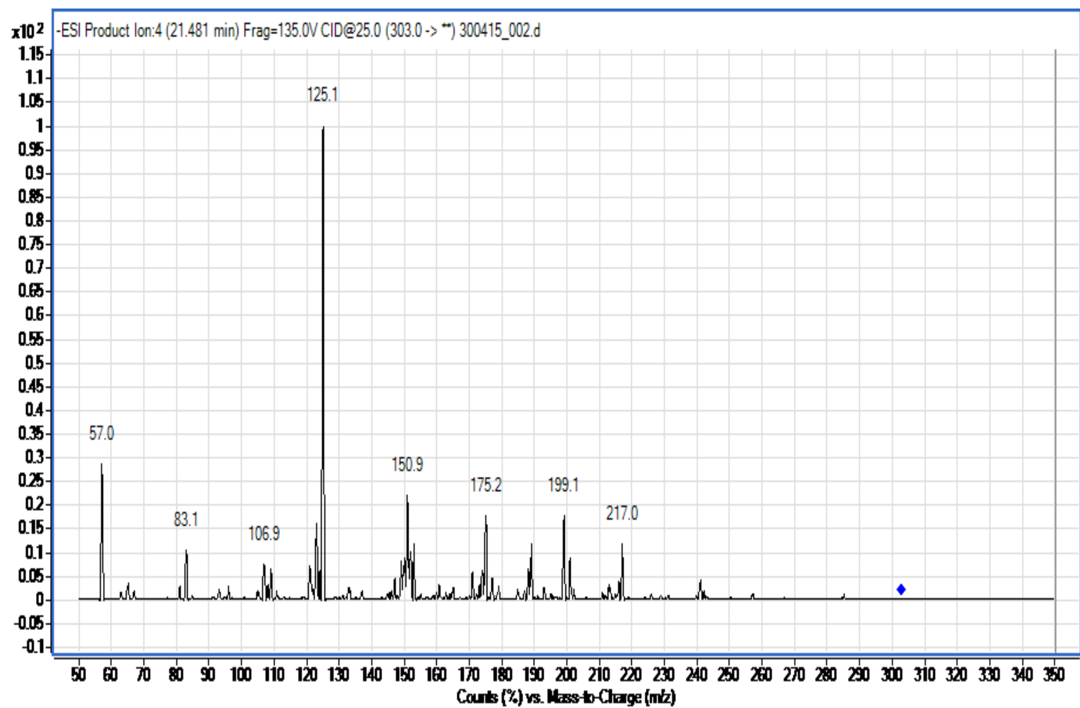
Mass spectra of feruloyl-caffeoylglycerolof sorghum grain.

APPENDIX 5



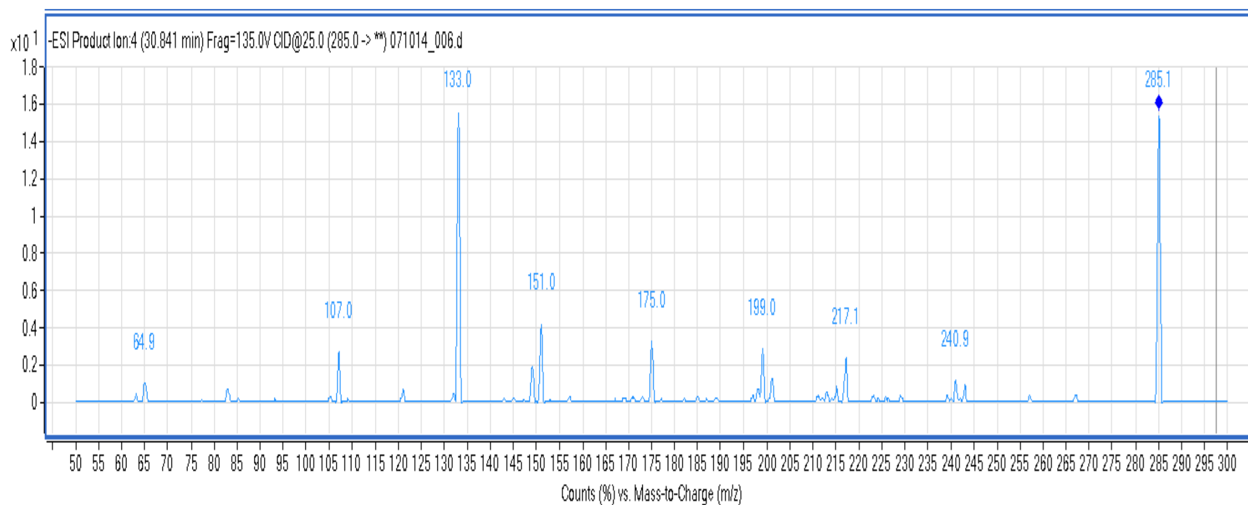
Mass spectra of naringenin sorghum grain.

APPENDIX 6



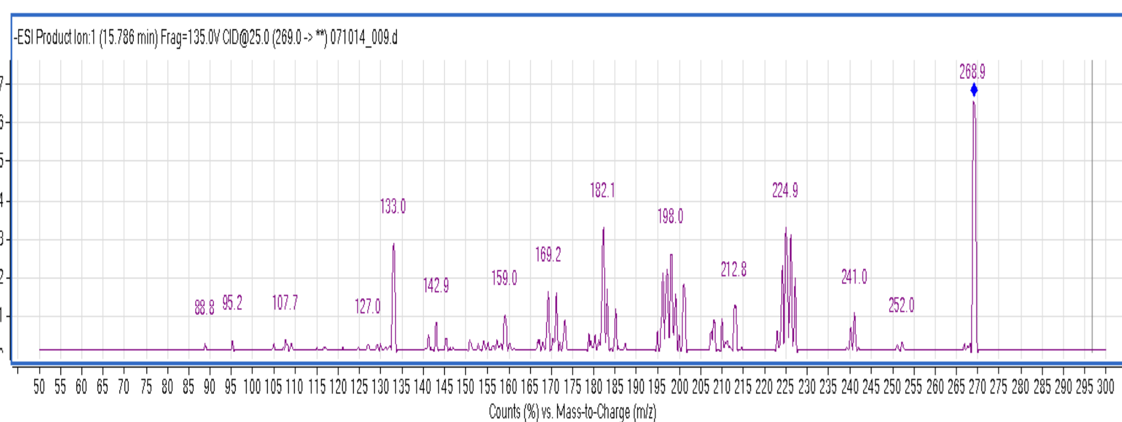
Mass spectra of taxifolin sorghum grain.

APPENDIX 7



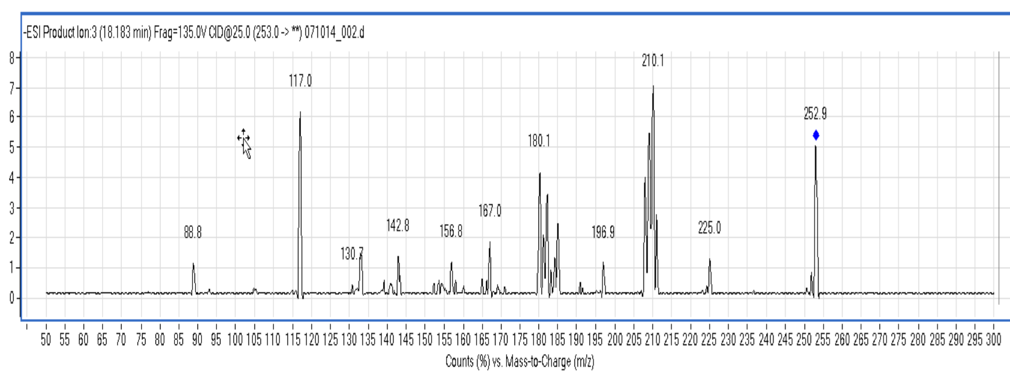
Mass spectra of luteolin of sorghum grain.

APPENDIX 8



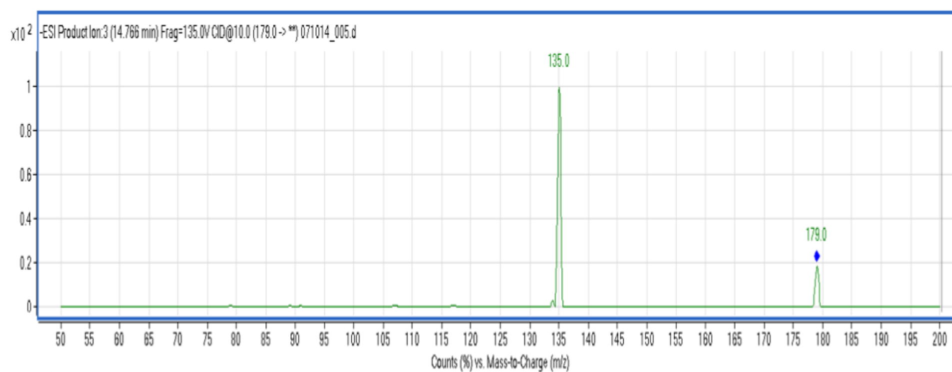
Mass spectra of luteolinidin of sorghum grain.

APPENDIX 9



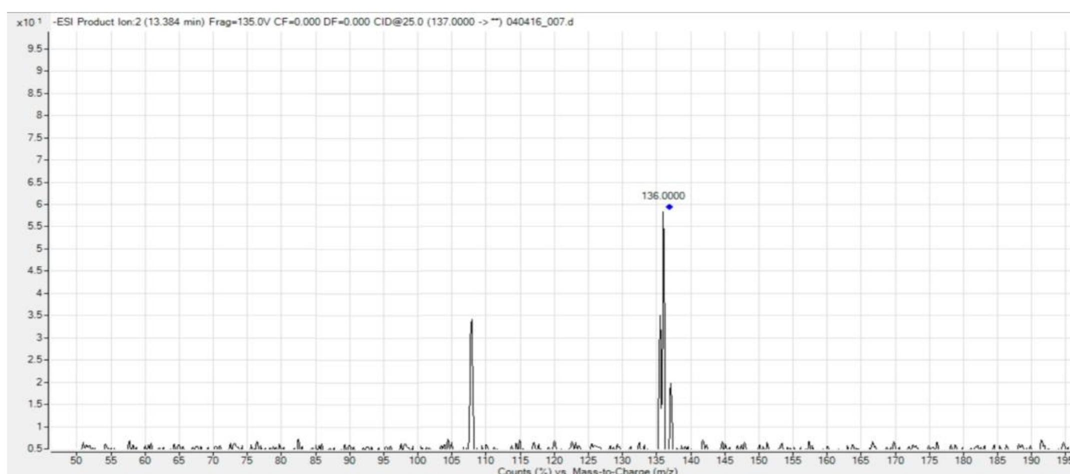
Mass spectra of apigeninidin of sorghum grain.

APPENDIX 10



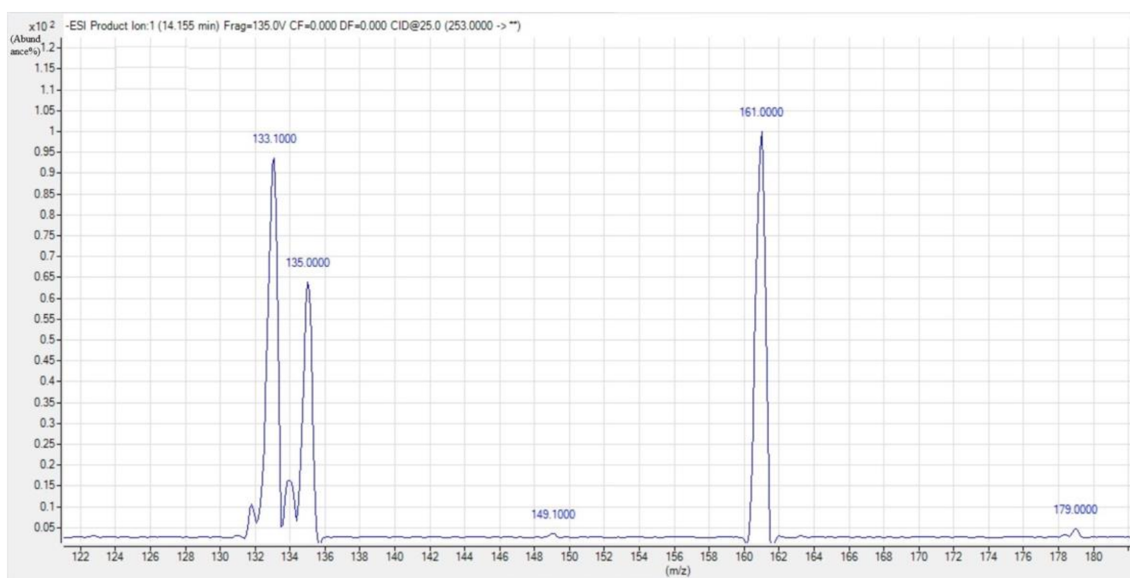
Mass spectra of caffeic acid of sorghum grain.

APPENDIX 11



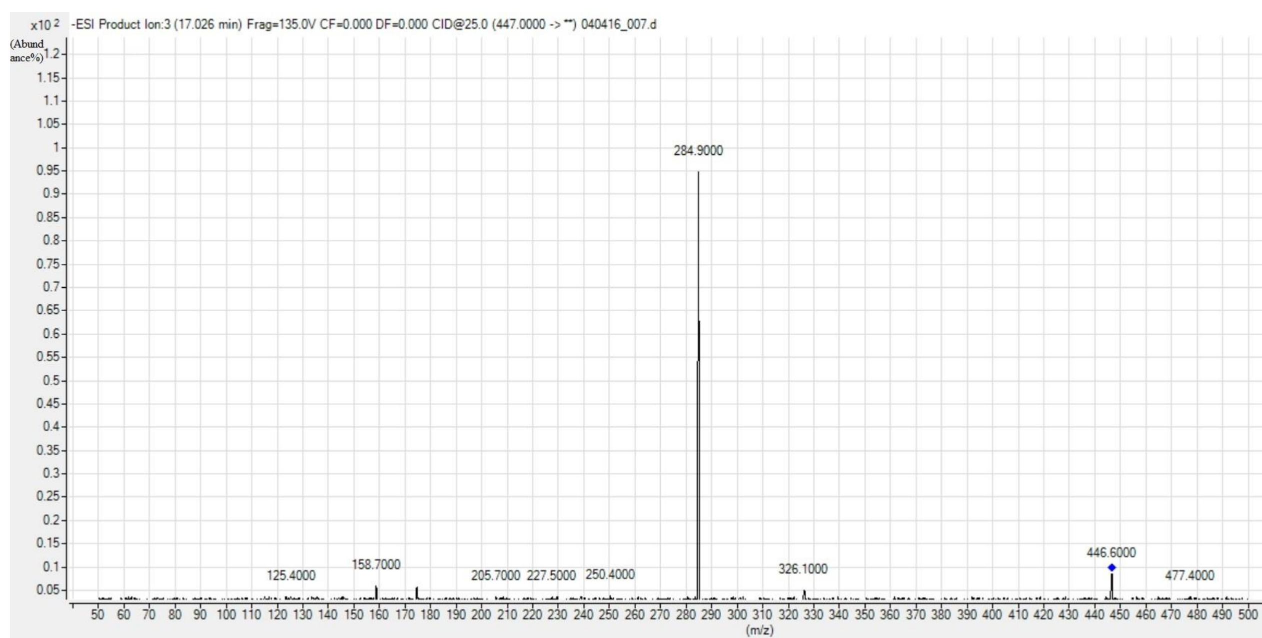
Mass spectra of potocatechuic aldehyde of sorghum grain.

APPENDIX 12



Mass spectra of 1-*O*-caffeoylglycerol of sorghum grain

APPENDIX 13



Mass spectra of luteolin hexoside of sorghum grain.