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Antioxidant activity in a set of sorghum landraces and breeding lines

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Abstract

Sorghum (*Sorghum bicolor* L) is becoming an increasingly important crop in the developed world especially as a cereal grain option for patients with celiac disease, being also characterized by a high level of bioactive compounds. It is a good source of phenolic compounds, including phenolic acids, flavonoids and condensed tannins, that express antioxidant capacity and potential health benefits. A group of 210 sorghum genotypes was evaluated in terms of physical parameters and resulted to be characterized by a wide range of 1000-seeds weight (6.93 - 42.67 g) and kernel colour. A sub-set of 121 samples were selected by near infrared spectroscopy for chemical analyses, and revealed a wide range of variability for total antioxidant capacity (6.89 - 172.02 mmol TE kg^{-1} dm^{-1}), phenols (0.60 - 20.73 g GAE kg^{-1} dm^{-1}), condensed tannins (0 - 28,362.63 μg CE g^{-1} dm^{-1}) and flavonoids (0 - 8,138.22 μg CE g^{-1} dm^{-1}). A high negative correlation was observed between antioxidant compounds and the colour parameters L* and b*; on the contrary, correlation of the same parameters with a* was low and positive. The results of these preliminary analyses highlighted genotypes characterized by light-coloured grains (white or yellow), large seeds, high antioxidant properties but absence of condensed tannins, all traits which make them suitable for food industry.

Keywords: *sorghum, total antioxidant capacity, phenols, breeding lines*

Introduction

Sorghum (*Sorghum bicolor* L) is listed among the most cultivated cereal crops worldwide. It is mainly grown for animal feed, industrial products and biomass, only about 35% being directly used for human consumption (Awika and Rooney, 2004). In particular, its use as a staple food is reported in Eastern and Southern Africa, Latin America, China, and India.

The interest in sorghum as a food plant also for developed countries has been increasing in recent years due to some considerations. First, sorghum is a crop with a good yield potential and resistance to biotic and abiotic stresses, and can contribute providing food for the growing world population (Dicko et al, 2006a). Moreover, currently grown sorghum hybrids are non-GMO, a point which favour people's acceptance of this crop. Second, there is a large, still unexplored, reservoir of genetic variability in sorghum, represented by landraces, mainly present in Africa and Asia. Landraces are heterogeneous and can express resilience to adverse growing conditions, high production stability and favourable quality characteristics, in terms of high content of compounds with a nutritional value (Habyarimana et al, 2016). Third, sorghum grain is gluten-free, a quite interesting characteristic for the consumers in Western countries, where different forms of gluten intolerance are spreading. Finally, the chemical composition of sorghum grain is particularly interesting, due to the presence of a significant content of phytochemicals, that express antioxidant activities, cholesterol-lowering properties and other potential health benefits (Awika and Rooney, 2004). The bioactive compounds that most contribute to total antioxidant capacity in sorghum are mainly phenols, in addition to plant sterols and policosanols. Phenols fall under two major categories: phenolic acids and flavonoids. Phenolic acids, which are located in the pericarp, testa, aleurone layer, and endosperm, are benzoic or cinnamic acid derivatives, whereas flavonoids include tannins (proanthocyanidins) and anthocyanins as the most important constituents isolated from sorghum to date (Awika and Rooney, 2004).

However, in spite of these considerations, the utilization of sorghum to improve the nutritional value of foods is quite restricted. The main limiting factor in this context is the presence of tannins, which are considered to be responsible of the reduction of the availability of minerals and digestibility of proteins and starch in sorghum. As an example, polymeric tannins strongly interact with amylose, contributing to the formation of resistant starch (Cardoso et al, 2017).

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As tannins are especially present in sorghum varieties with a pigmented testa, white genotypes with no detectable level of tannins are generally selected for food industry. On the other hand, condensed tannins have been associated with various benefits for human health, such as antioxidant and anti-carcinogenic properties (Dicko et al, 2006b; Dykes et al, 2014). Traditional sorghum varieties with moderate tannin content are in fact widely grown and used for staple food and alcoholic beverages in various parts of the world, including Africa, Central and South America, China, and India. Moreover, in many parts of the world where pests and diseases are common, tannin sorghums are still grown in significant quantities, since they are more tolerant of such conditions than the non-tannin varieties (Awika and Rooney, 2004).

Sorghum grain composition has been described in different works, reporting data on proximates (Dicko et al, 2006a; Ragaee et al, 2006; Pontieri et al, 2010, 2011), antioxidant compounds (Dicko et al, 2006b; Ragaee et al, 2006; López-Contreras et al, 2015), total antioxidant activity (Awika et al, 2003; Pontieri et al, 2016). NIRS calibration curves have also been developed for different chemical compounds (De Alencar Figueiredo et al, 2006; Dykes et al, 2014) and for pericarp thickness (Guindo et al, 2016). Finally, the use of sorghum flour in gluten-free products has been explored (Winger et al, 2014).

In the present study the antioxidant capacity was described in a set of breeding lines derived from crosses between *S. bicolor* and *S. halepense*, and in a group of landraces, with the aim to identify those which could be better suited to food industry.

Materials and Methods

Plant material

A set of breeding lines from *S. bicolor* x *S. halepense* hybrids, and *S. bicolor* lines and landraces were fully described in Habyarimana et al (2017).

Physical analyses of the grain

Grain colour parameters L*, a*, and b* (CieLab system) were evaluated with the use of a CR300 Chroma Meter (Konica Minolta, Osaka, Kapan). Both grain colour and 1,000-seeds weight are the average of three replicates.

Sample preparation

A 10 g sample from each genotypes was ground using a Cyclotec Udy Mill (sieve: 0.5 mm). Moisture was determined in an oven overnight at 105°C. The flours were then analysed by Near InfraRed Spectroscopy, using a NIRSystem 6500 (Foss, Padova, Italy) in the range 400 - 2,500 nm, and the spectra were collected as the average of two replicates. Statistical analysis of the spectra (Winisi II) allowed to select the lines which best represented the genetic variability of the set.

Chemical analyses

Samples extraction, quantification of antioxidants and total antioxidant capacity (TAC) were carried out according to López-Contreras et al (2015) with minor modifications. One hundred milligrams of flours were extracted with 3 ml of 80% (v/v) methanol and stirred for 4 h at 200 rpm. Samples were centrifuged at 2,600 g, supernatants were recovered and conserved at -20 °C until analysis. Each sample was extracted in duplicate. Total phenolic compounds were determined by a reaction with Folin-Ciocalteu reagent at 750 nm and expressed as g GAE kg^{-1} dm^{-1}. Extractable condensed tannins were determined using vanillin-H_2SO_4 at 500 nm, and total flavonoids by a reaction with aluminium chloride at 510 nm. For condensed tannins and total flavonoids assays, catechin was used as standard (0 - 50 µg ml^{-1}) and results were expressed as µg CE g^{-1} dm^{-1}. TAC was determined on extracts by ABTS assay at 734 nm using ethanol as solvent, and expressed as mmol TE kg^{-1} dm^{-1}. Two replicates for each analysis were carried out.

Statistical analyses

Principal component analysis (PCA) and Pearson correlation coefficient were carried out with PAST (Hammer et al, 2001).

Results and Discussion

Physical analyses and PCA

The results of seed weight and colour measurement of 210 sorghum genotypes are shown in Table 1. These materials were characterized by a large variability of 1,000 seeds weight, which ranged from 6.93 to 42.67 g, with 22.54 ± 7.75 g as a mean value. A large variability was also observed for kernel colour, as shown by the range of variation of parameters L*, a* and b* (Table 1). The seeds of the sorghum genotypes analysed in this work ranged from white to black, also including yellow, red and brown (Figure 1).

Principal component analysis (PCA) was carried out on seed weight and colour parameters (Figure 2). Four principal components explained more than 97% of total variance; they are listed in Table 2, with the corresponding eigenvalues, variance percentages and sources of variation. PC1, which explained 52.76% of the variance, was mainly related to colour parameters L* and b*. PC2 accounted for 27.56% of total variance, which was attributed to seed weight and colour parameter a*. An additional 17.08% was

Table 1 - Thousands seeds weight and colour measurement in the whole set of genotypes (n=210).

	TSW (g)	Colour parameters		
		L*	a*	b*
Mean ± SD	22.54 ± 7.75	49.40 ± 10.00	7.53 ± 3.34	18.14 ± 3.77
Range	6.93 – 42.67	28.00 – 69.18	2.50 - 19.85	5.93 - 28.01

TSW = thousands seeds weight

Figure 1 - Colour variability of the grains in the set of sorghum genotypes.

contributed by PC3, related to seed weight and b*.

The light-coloured genotypes (white or yellow) were distributed in regions I and IV of the scatterplot (Figure 2); the red-brown ones in region II, whereas the few black genotypes were grouped in region III. A low but significant correlation was observed between seed weight and L* ($r = 0.25$, $p \leq 0.01$) and b* ($r = 0.31$, $p \leq 0.01$), suggesting that larger seeds often had a lighter colour.

NIRS analysis

All ground samples were scanned by NIRS, and the spectra distribution was statistically analysed by software Winisi II to select the samples which best represented the variability available in the set of lines. The selection was performed using the neighbourhood Mahalanobis distance (NH). This statistic tells how a sample is different from the nearest neighbour and is used to eliminate nearly redundant samples in each neighbourhood (Shenk et al, 2008). For the selection a NH threshold of 0.3 was chosen. PCA was carried out on the spectra pre-treated with gap first derivative (1, 4, 4, 1) and standard normal variate (SNV) and detrend, in the near-infrared range of the instrument (1,100 - 2,500 nm). One hundred twenty-one samples were selected for chemical analyses.

Chemical analyses

Total antioxidant capacity (TAC) and the content of total phenols, total flavonoids and condensed tannins were determined in the 121 selected samples; the results are reported in Table 3.

Maximum TAC value was 172.2 mmol TE kg^{-1} dm^{-1}, and the mean value 42.87 ± 37.53 mmol TE kg^{-1} dm^{-1}, confirming sorghum as one of the richest cereals in antioxidant compounds. Lopez-Contreras and co-workers (2015) also reported high TAC values (up to 156.08 µmol TE g^{-1} dm^{-1}) in sorghum, and related them to its high content of condensed tannins and flavonoids and high variability in kernel colour. Antioxidant compounds were studied in the grains of different cereals: sorghum presented the highest values of total antioxidant capacity when compared to wheat, oats, barley and maize (Dykes and Rooney, 2007). In a recent study about total antioxidant capacity in different maize genotypes, including lines, hybrids and varieties, TAC values ranged from 9.88 to 32.35 mmol TE kg^{-1} dm^{-1} (Redaelli et al, 2016). A set of 20 oat cultivars, on the other hand, showed a range of TAC from 13.99 to 18.84 mmol TE kg^{-1} dm^{-1} (Alfieri and Redaelli, 2015).

In the present work total phenolic compounds ranged from 0.60 to 20.73 g GAE kg^{-1} dm^{-1}. A similar range of variation (1.35 – 37.73 mg GAE g^{-1} dm^{-1}) was reported by Dykes et al (2014) in 224 sorghum samples. Tafuri et al (2014), analyzing a total of 81

Table 2 - PCA components and sources of variation for the four parameters.

	PCA		Sources of variation			
Component	Eigenvalue	Variance %	TSW	L*	a*	b*
1	211.025	52.76	0.293	0.658	-0.462	0.517
2	110.252	27.56	0.683	-0.154	0.617	0.360
3	0.683	17.08	-0.667	0.075	0.390	0.630
4	0.104	2.60	-0.505	0.733	0.504	0.454

TSW = thousands seeds weight

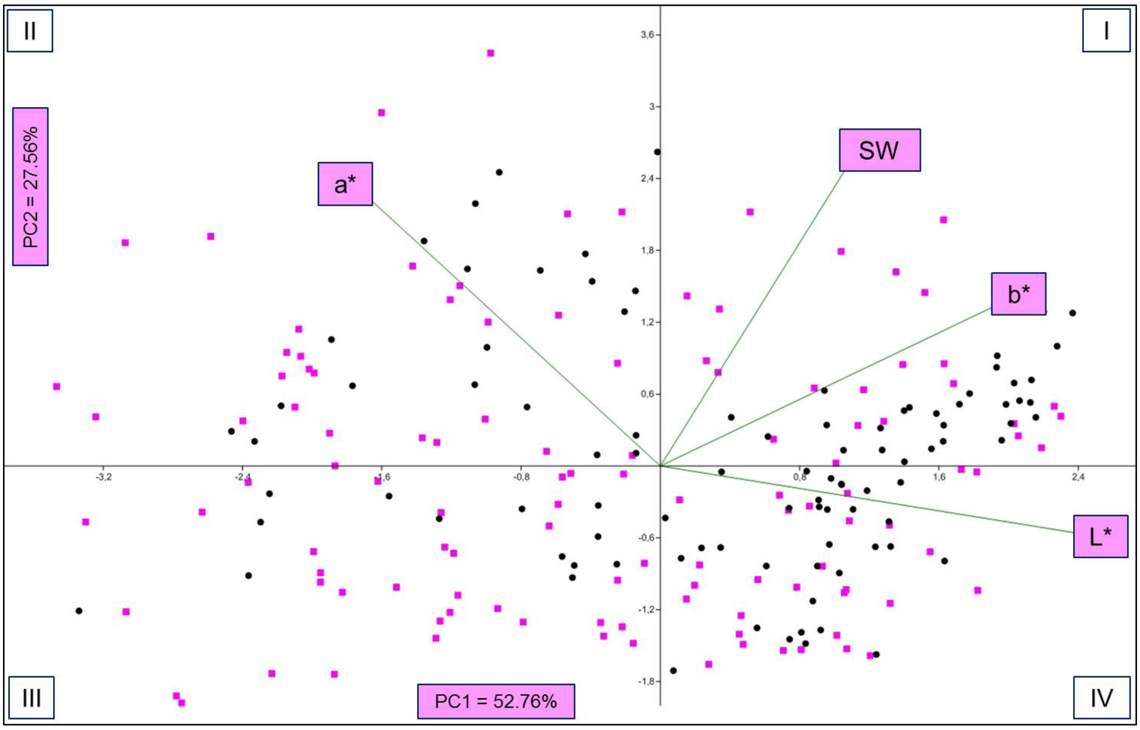

Figure 2 - PCA of thousands seeds weight (TSW) and colour parameters (L*, a* and b*) in 210 sorghum samples. The pink squares represent the samples selected for chemical analysis.

traditional and public maize lines, reported a range of variation for soluble phenolic content (SPC) from 0.82 to 1.92 g GAE kg^{-1} dm^{-1}. Among cereals, sorghum was shown to have the highest content of phenolic compounds, reaching up to 6% (w/w) in some varieties (Dicko et al, 2006b; Ragaee et al, 2006). This represents a favourable trait, as phenolic compounds are quality-grade markers for the preparation of several foods due to their enzyme inhibitory activities (Dicko et al, 2006b).

The analysis of condensed tannins (pro-anthocyanidins) and total flavonoids revealed that these compounds are present only in 104 genotypes, with a mean value of 3,826.49 µg CE g^{-1} dm^{-1} and 1,541.83 µg CE g^{-1} dm^{-1}, respectively. Condensed tannins bind to and reduce digestibility of various food/feed nutrients and were originally classified as anti-nutritionals factors (Awika and Rooney, 2004). Recent studies, on the contrary, indicated that they may have health benefits for human health, such as antioxidant and anti-carcinogenic properties (Dicko et al, 2006b; Dykes et al, 2014). Moreover, these secondary metabolites often act as a defence mechanism against pathogen and predators, increasing the plant resistance to biotic stresses. Flavonoids family include also anthocyanins. The most common anthocyanins in sorghum, called 3-deoxyanthocyanins, are unique since they do not contain the hydroxyl group in the 3-position of the C-ring. This unique feature increases their stability at high pH compared to the common anthocyanins which render these compounds as potential natural food colorants (Awika and Rooney, 2004).

Correlation coefficients.

All the chemical traits (TAC and antioxidant compounds) resulted to be positively and significantly correlated ($r \geq 0.9$, $p \leq 0.01$). In addition, a significant and negative correlation was observed between antioxidant compounds and the colour parameters L* ($r = -0.6$ on average, $p \leq 0.01$) and b* ($r = -0.5$ on average, $p \leq 0.01$); in effects, the white or yellow-seeded genotypes presented on average lower levels of antioxidant compounds.

On the other hand, a positive but moderate coefficient of correlation was reported between the content of antioxidant compounds and a* ($r = 0.25$, $p \leq 0.01$). This result suggests that the red or brown colour of the seeds cannot be used efficiently to indirectly select for antioxidants-rich genotypes. Among the lines

Table 3 - Mean values and ranges of TAC and antioxidant compounds in 121 sorghum genotypes.

	TAC (ABTS) mmol TE kg^{-1} dm^{-1}	Total phenolics g GAE kg^{-1} dm^{-1}	Condensed tannins µg CE g^{-1} dm^{-1}	Total flavonoids µg CE g^{-1} dm^{-1}
Mean ± SD	42.87 ± 37.53	4.31 ± 4.22	3826.49 ± 827.10	1541.83 ± 1785.14
Range	6.89 - 172.02	0.60 - 20.73	0 - 28362.63	0 - 8138.22
SE	2.30	0.22	245.68	140.15

Table 4 - Seed weight, TAC and antioxidant compounds in 36 light-coloured sorghum lines.

Genotype	TAC (ABTS) mmol TE kg^{-1} dm^{-1}	Total phenolics g GAE kg^{-1} dm^{-1}	Condensed tannins μg CE g^{-1} dm^{-1}	Total flavonoids μg CE g^{-1} dm^{-1}	1,000-seeds weight g	grain colour*
2	10.89	0.79	0.00	0.00	12.00	w
5	9.24	0.80	0.00	0.00	25.33	w
8	10.31	0.71	270.68	0.00	25.73	w
11	10.75	0.97	0.00	0.00	31.47	w
22	16.88	1.35	903.98	377.23	32.07	y
25	14.90	1.32	0.00	0.00	31.33	w
30	21.93	2.56	1424.06	841.15	27.60	y
33	15.19	1.48	548.14	418.59	39.60	y
44	17.44	1.29	0.00	390.40	13.00	w
45	13.95	1.04	0.00	0.00	26.93	w
48	18.68	2.57	0.00	1138.95	28.87	y
49	8.53	0.78	0.00	0.00	31.60	w
52	22.07	1.75	0.00	741.93	19.80	y
56	17.53	1.19	675.05	373.98	33.80	w
63	8.88	0.68	0.00	0.00	34.00	w
64	25.87	1.82	0.00	452.94	21.20	w
66	8.60	0.71	0.00	715.89	17.00	w
67	11.86	0.95	0.00	0.00	21.53	y
71	9.09	0.76	0.00	0.00	35.00	w
79	12.86	0.91	368.76	0.00	31.80	y
80	10.03	0.75	0.00	0.00	19.73	w
81A	24.64	2.68	1678.17	644.47	7.53	w
100	14.35	1.11	629.20	0.00	11.47	w
126	27.43	2.04	0.00	503.21	29.00	y
130	9.25	0.77	0.00	0.00	31.80	y
131	11.46	1.04	242.94	405.18	15.40	w
133	39.21	1.90	0.00	373.58	23.87	w
143	22.12	1.89	0.00	414.76	7.47	w
144	6.89	0.60	0.00	0.00	36.07	w
146	11.59	1.10	0.00	564.57	22.13	y
161	14.76	1.27	546.30	509.29	29.07	w
169	15.67	1.23	446.59	0.00	22.93	y
178	9.27	0.60	0.00	0.00	32.60	w
205	10.26	0.72	466.71	0.00	13.73	w
223	11.08	1.04	335.62	0.00	26.27	w
224W	10.05	0.69	0.00	0.00	27.60	w

* w = white, y = yellow

with red or brown pericarp, in fact, TAC values varied between 12.95 (line 119) and 125.06 mmol TE kg^{-1} dm^{-1} (line 162); among those with a black pericarp a variation between 14.29 (line 267) and 147.41 mmol TE kg^{-1} dm^{-1} (line 98) was observed.

In a similar study carried out in maize flours by Redaelli and co-workers (2016), significant correlation coefficients between TAC and parameters L* (r = -0.73, $p \leq 0.01$) and a* (r = 0.49, $p \leq 0.05$) were observed. The results suggested that maize genotypes with black grains are surely characterized by high levels of TAC, whereas the selection of genotypes for antioxidant activity based on the yellow, orange or red colour of their grains is not efficient. Also in rice an association between colour parameters and TAC was highly significant in white rice cultivars, and less significant in a group of red cultivars (Shen et al, 2009).

Food-grade sorghum lines

As light-coloured, larger kernels are typically preferred for food industry, due to their high flour yield and reduced content of tannins (Tuinstra, 2008), a more detailed description of the phytochemicals present in 25 white and 11 yellow genotypes from our set is reported in Table 4. In this group TAC ranged between 6.89 (line 144) and 39.21 mmol TE kg^{-1} dm^{-1} (line 133). Line 144, together with line 178 had also the lowest content of total phenolics (0.60 g GAE kg^{-1} dm^{-1}), whereas the highest content (2.68 g GAE kg^{-1} dm^{-1}) was observed in line 81A. The level of condensed tannins was under the detection limit in 23 lines. Among the genotypes characterized by absence of condensed tannins, five lines (i.e. 48, 52, 64, 126, and 133) presented medium/high TAC values and contents of antioxidant compounds, associated to a suitable 1,000-seeds weight. They were therefore considered to be potentially interesting as raw materials for food production.

Conclusions

The data reported in the literature about sorghum grain composition highlighted its interesting potentiality as a raw material for food industry also in the Western countries, where the consumption of this cereal is still very limited. The genotypes analysed in the present study confirmed the good nutritional value of sorghum grain, especially in relation to the content of antioxidant compounds and total antioxidant capacity, which are well above the percentages usually reported in other crops like wheat, rice, oats or maize. This result highlights the importance of screening sorghum germplasm, with specific focus on the composition of the grain. At the same time, the data collected in this study could be exploited for the development of a near-infrared spectroscopy calibration curve, useful tool for a quick screening of progenies in breeding programs and for an efficient identification of the most suitable genotypes.

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