LWT - Food Science and Technology 161 (2022) 113367

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Germinated sorghum (*Sorghum bicolor* L.) and seedlings show expressive contents of putrescine

Caroline Liboreiro Paiva [a,b], Déa A.M. Netto [c], Valéria A.V. Queiroz [c], Maria Beatriz A. Gloria [a,d,*]

[a] LBqA & LCQ, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 31270-901, Belo Horizonte, Minas Gerais, Brazil
[b] ICA – Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Av. Universitária, 1000, Bairro Universitário, 39404-547, Montes Claros, Minas Gerais, Brazil
[c] Embrapa Milho e Sorgo, Caixa Postal 151, 35701-970, Sete Lagoas, Minas Gerais, Brazil
[d] Departamento de Ciências do Consumo, Universidade Federal Rural de Pernambuco, Campus Dois Irmãos, 21020-231, Recife, Pernambuco, Brazil

ARTICLE INFO

Keywords:
Polyamines
Sorghum bicolor L.
Germination
Spermine
Spermidine

ABSTRACT

Free bioactive amines were quantified during germination of tannin and tannin-free sorghum seeds (8 genotypes) and seedlings (2 genotypes). Among the ten amines investigated, three (putrescine, spermidine and spermine) were found. Tannin-free sorghum seeds had higher levels of spermidine, spermine, putrescine and total amines (1.28, 1.27, 0.35, 2.91 mg/100 g dwb, respectively), compared to tannin sorghum (0.91, 1.13, 0.06, 2.09 mg/100 g dwb, respectively). Throughout germination, in all seedling parts, putrescine was prevalent followed by spermidine. Spermine was only detected in the 7th germination day in the cotyledon and radicle of tannin sorghum. In the cotyledon, putrescine levels increased (24- and 30- fold for tannin and tannin-free sorghum, respectively). Spermine decreased, but it increased reaching initial levels (~1 mg/100 g dwb) in two tannin-free genotypes. Spermidine changes were not significant. Germinated sorghum was characterized by high putrescine and low spermidine levels, with higher levels for tannin-free (29.55 mg/100 g putrescine; 2.79 mg/100 g spermidine) compared to tannin (24.41 mg/100 g putrescine, 1.40 mg/100 g spermidine) sorghums. Different from other seeds, germinated sorghum is rich in putrescine. By selecting the proper genotype one can modulate polyamine contents in germinated sorghum, thereby obtaining different profile of amines for specific food application.

1. Introduction

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop in the world, overcome only by wheat, rice, maize and barley in production. Sorghum shows high production performance in a wide range of adverse edaphoclimatic conditions (Arouna, Gabriele, & Pucci, 2020; Espitia-Hernández et al., 2020; Rashwan, Yones, Karim, Taha, & Chen, 2021). Sorghum has been extensively used as animal feed and human food, mainly in arid and semi-arid areas of Africa, Asia and Latin America (Espitia-Hernández et al., 2020; Kimani, Zhang, Wu, Hao, & Jing, 2020; Palacios, Nagai, Torres, Rodrigues, & Salatino, 2021; Teferra, Amoako, Rooney, & Awika, 2019). It has been used as a staple for millions of people in several countries, mainly in Africa and Asia. Recently, this cereal has gained momentum in developed countries, due to its high nutritional value, health promoting properties, and potential use in the production of gluten-free foods which are especially important for people with celiac disease (Arouna et al., 2020; Espitia-Hernández et al., 2020; Gaytán-Martínez, 2020; Kimani et al., 2020).

Sorghum varieties vary based on genetics, color, and chemical composition (Dykes & Rooney, 2006; Dykes, Hoffmann Jr., Portillo-Rodriguez, Rooney & Rooney, 2014). Sorghum classification based on the presence/absence of a pigmented testa and its association with condensed tannin is widely used as an agronomic trait, resulting in tannin-free and tannin sorghum. This phenotypic characteristic is controlled by B_1B_2 genes and affected by the spreader gene S. Tannin-free sorghums (type I), without a pigmented testa, have low levels of phenols, and no condensed tannin, whereas tannin-sorghums (types II and III), have a pigmented testa and contain condensed

Abbreviations: CE, catechin equivalent; dwb, dry weight basis; LOQ, Limit of quantification; OPA, *o*-phthalaldehyde; TCA, trichloroacetic acid.

* Corresponding author. LBqA & LCQ, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 31270-901, Belo Horizonte, Minas Gerais, Brazil.
E-mail address: mbeatriz@ufmg.br (M.B.A. Gloria).

https://doi.org/10.1016/j.lwt.2022.113367
Received 10 October 2021; Received in revised form 27 December 2021; Accepted 18 March 2022
Available online 5 April 2022

tannin (Palacios et al., 2021; Xiong, Zhang, Warner, & Fang, 2019). Tannin sorghum varieties play important agronomic roles as it is a chemical barrier against pathogens, herbivores, insects, birds (Dykes & Rooney, 2006; Kimani et al., 2020; Palacios et al., 2021). Currently, tannin-free sorghum cultivars are grown in the United States (99%), whereas distinct sorghum varieties are grown in other countries (Palacios et al., 2021). Condensed tannin levels vary among tannin sorghum genotypes, from 0.02 to 2.5 and 6.7–15.5 mg/100 mg catechin equivalent for types II and III, respectively, whereas they are not detected in tannin-free sorghums (Dykes & Rooney, 2006). Tannins are associated with health benefits including antioxidant (radical scavenging), immunomodulatory, anticancer, anti-inflammatory, cardioprotective, vasodilating and antithrombotic effects (Palacios et al., 2021; Wu et al., 2012). However, tannin can bind to proteins, carbohydrates, and minerals, resulting in reduced digestibility, caloric bioavailability, and feed efficiency (Dykes & Rooney, 2006). It also imparts a bitter and astringent taste which can affect palatability (Palacios et al., 2021). According to FAO/WHO an upper limit of 0.5% tannins (dry weight) for sorghum grains is recommended for human consumption (Codex, 1989).

Sorghum has been valued as a potential source of dietary fiber (Gaytán-Martínez, 2020; Rashwan et al., 2021) and minerals – magnesium, iron and zinc (Espitia-Hernández et al., 2020; Kimani et al., 2020; Paiva et al., 2017). In addition, sorghum is rich in bioactive compounds, which are responsible for health effects including the prevention of oxidative stress, obesity, dyslipidemia, cardiovascular disease, and hypertension (Gaytán-Martínez, 2020; Lee et al., 2020). These beneficial effects can be associated with the presence of phenolic compounds and biologically active amines. In fact, phenolic compounds have anti-inflammatory, anti-carcinogenic and anti-diabetic properties and can prevent oxidative stress (Espitia-Hernández et al., 2020; Gaytán--Martínez, 2020; Ghimire, Seo, Yu, Kim, & Chung, 2021; Kimani et al., 2020; Luo et al., 2018; Ofosu et al., 2021; Teferra et al., 2019). Among bioactive amines, sorghum is rich in spermine and spermidine (Paiva, Evangelista, Queiroz, & Glória, 2015). These polyamines participate in the synthesis and stabilization of DNA, RNA, and protein, and in the prevention of oxidative stresses (Handa, Fatima, & Mattoo, 2018; Muñoz-Esparza et al., 2019; Sagar, Tarafdar, Agarwal, Tarafdar, & Sharma, 2021). They play relevant roles in the prevention of cardiovascular disease (Handa et al., 2018; Hirano, Shirasawa, & Kurihara, 2021; Sagar et al., 2021), and are anti-aging agents (Dala-Paula, Starling, & Gloria, 2021; Sagar et al., 2021). However, sorghum also show anti-nutritional properties, due to the presence of phytic acid, trypsin inhibitor, and oxalates (Arouna et al., 2020).

Sorghum germination is traditionally used for the preparation of several sorghum-based products (Arouna et al., 2020; Rashwan et al., 2021). It can decrease anti-nutritional factors (phytate and protease inhibitors) and enhance the bioavailability of other nutrients and bioactive compounds (Arouna et al., 2020). Germinated sorghum has higher protease and free amino nitrogen levels, increased albumin, and globulin and decreased kafirin levels (Arouna et al., 2020; Bueno et al., 2020; Rashwan et al., 2021). In addition, sorghum germination can improve riboflavin availability, nutrient digestibility, antioxidant activity and sensory acceptance of products (Pinheiro et al., 2021; Singh, Sharma, Singh, & Kaur, 2019). The polyamines – spermine and spermidine – have been reported to increase during germination of soybean and corn (Bandeira, Evangelista, & Glória, 2012; Bueno et al., 2020; Gloria, Tavares-Neto, Labanca, & Carvalho, 2005); however, no information is available regarding changes on amines during sorghum germination. In addition, the biotransformation of the various forms of polyamines in sorghum seedling components as a function of germination time has not been explored. In this context, the objective of this study was to investigate the influence of germination on the profile and levels of free bioactive amines in different sorghum seedling parts and genotypes with and without tannin.

2. Material and methods

2.1. Material

2.1.1. Samples

Eight sorghum genotypes developed and grown at the experimental fields of Embrapa Milho & Sorgo, in Sete Lagoas, Minas Gerais, Brazil (June to October 2010), were used in this study. The samples included four genotypes of tannin (BR9929026, SC59, SC603, BAZ9504) and four of tannin-free (BRS310, SC42, RTx2783, SC192) sorghum. The sorghum samples were grown in two replications. At maturity, the grains were harvested, threshed, fumigated and stored under controlled atmosphere (11 °C and 40% relative humidity) until the experiment was undertaken (2013). Tannin sorghums contained tannin at levels of 38.19–83.65 (mean = 53.73) mg catechin equivalent (CE)/g dry weight basis (dwb), whereas tannin was not detected (<1.75 mg CE/g) in tannin-free sorghum, determined by the vanillin method (Price, Van Scoyoc, & Butler, 1978). The samples and respective tannin contents (Personal Communication) were provided by Embrapa.

2.1.2. Reagents, solvents and amines standards

The reagents were of analytical grade, except the HPLC solvent (acetonitrile), which was chromatographic grade. Ultra-pure water was from Milli-Q Plus system (Millipore Corp., Milford, MA, USA).

Bioactive amines standards (spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride, agmatine sulphate, cadaverine dihydrochloride, 5-hydroxitryptamine (serotonin), histamine dihydrochloride, tyramine hydrochloride, 2-phenylethylamine hydrochloride and tryptamine) and *o*-phthalaldehyde (OPA) were ≥98% and purchased from Sigma Chemical Co. (St. Louis, MO, USA). The mobile phases were filtered through 0.45 μm pore size membranes (Millipore Corp.), types HAWP and HVLP for aqueous and organic solvents, respectively.

2.2. Methods

2.2.1. Germination

Germination was accomplished as determined by Brasil (2009). Briefly, sorghum seeds (n = 100) were germinated in paper towel under conditions simulating night and day periods: 20 ± 2 °C for 16 h in the dark and 30 ± 2 °C for 8 h in the presence of light (fluorescent lamp), maintained in a thermostatically controlled incubator (Stults Scientific Engineering Corporation, Springfield, IL, USA) at 92 ± 2% relative humidity. Germination was accomplished with three sheets of paper per roll (28 × 38 cm Germitest®, J. Prolab, Curitiba, PR, Brazil) moistened with water. Samples were collected for analysis at 48-h intervals for up to 7 days.

Two experiments were conducted. In the first experiment, two sorghum genotypes, one with tannin (BR9929026) and the other without tannin (BRS310) were used. Throughout germination, samples were collected, separated into radicle, cotyledon and leaf materials and the different parts were analyzed for free bioactive amines and moisture content. In the second experiment, six genotypes were used: three with tannin (SC59, SC603, BAZ9504) and three without tannin (SC42, RTx2783 and SC192). Throughout germination (days 1, 3, 5 and 7), the cotyledons were taken and analyzed for free bioactive amines and moisture content. Each experiment was performed in triplicate.

2.2.2. Methods of analysis

2.2.2.1. Moisture content. The moisture content was determined according to AOAC (2019). The ground samples were dried until constant weight in an air circulating oven at 105 ± 2 °C. This information was used to calculate and express amine levels on a dry weight basis.

2.2.2.2. Free bioactive amines. The amines were extracted from the samples (1 g ground and sieved – 420 μm) with 7 mL 5% trichloroacetic acid – TCA (Paiva, Evangelista, Queiroz, & Gloria., 2015). The solutions were homogenized for 10 min in a shaker (140-TE Tecnal, Piracicaba, SP, Brazil), centrifuged at 11,180×*g* at 4 °C for 21 min, and the supernatant was collected. The solid residue was extracted with additional 2 × 7 mL TCA and the supernatants were combined, filtered through qualitative filter paper and the volume was brought up to 25 mL with 5% TCA. It was filtered through a 0.45 μm pore size membrane (HAWP, Millipore Corp. Milford, MA, USA) prior to HPLC analysis (Paiva et al., 2015).

The amines were determined by ion-pair reverse phase HPLC, post-column derivatization and fluorometric detection (Paiva et al., 2015). Briefly, liquid chromatography was carried out in a Shimadzu HPLC (LC-10AD) connected to a RF-10 AXL spectrofluorometric detector at 340 and 450 nm of excitation and emission, respectively. A Nova Pak® C18 (3.9 × 300 mm, 4 μm, Waters, Milford, MA, USA) column was used and kept at 25 ± 1 °C. The mobile phases were: (A) 0.2 mol/L sodium acetate buffer and 15 mmol/L sodium octanesulphonate (pH 4.9) and (B): acetonitrile. The flow rate was 0.5 mL/min and the gradient was as follows: 0 min 15% B, 1 min 6% B, 14 min 6% B, 16 min 26% B, 22 min 16% B, 25 min 16% B, 26 min 33% B, 27 min 18% B, 29 min 18% B, 30 min 38% B, 65 min 38% B. The post column derivatization reagent (1.5 mL Brij-35, 1.5 mL mercaptoethanol and 0.2 g OPA dissolved in 500 mL 5% boric acid and 4.4% KOH, pH 10.5) was delivered at 0.3 mL/min. The amines were identified by comparison of retention times of amines in the sample with those of standard solutions and by spiking the suspected amine to the sample. The levels of amines were determined by interpolation in external calibration curves built at concentrations from 0.1 to 6.0 μg/mL ($r^2 \geq 0.997$).

2.3. Statistical analysis

Statistical analyses were carried out employing analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% probability. The existence of correlations between the contents of tannin and those of putrescine, spermidine and spermine before and after germination (7 days germination) was investigated by Pearson correlation at 5% probability. The SISVAR Software, version 5.3 (UFLA, Lavras, MG, Brazil) was used.

3. Results and discussion

3.1. Sorghum germination

According to Fig. 1, the germination of sorghum seeds was as follows: in the first day (24 h), the seeds were turgid due to water absorption; however, no germination structures were observed. On the second and third days, it was possible to see hypocotyls and epicotyls coming out of the cotyledons. From the sixth day on, the sorghum seedling could be divided into radicle, hypocotyl, cotyledon, epicotyl, and leaf. These plant structures were developed until the mature seedling stage which was achieved on the seventh germination day.

The moisture content of the samples changed significantly during germination. Initially, they were 11.17 and 10.75 g/100 g for tannin and tannin-free sorghum seeds, respectively. On the first germination day, the seeds absorbed 2.8 times the initial moisture contents, reaching, respectively, 31.23 and 30.33 g/100 g, respectively. From the third day on, when different plant parts were visible, they were separated, and the moisture contents determined. The moisture content of the leaves and the radicles on the third day germination were ~90 g/100 g and remained constant up to the seventh day (Table 1). However, the moisture content of the cotyledon, which was ~40 g/100 g on the third

Table 1
Moisture content of the different plant parts of the seedling from tannin and tannin-free sorghum cultivars during germination.

Sample/seedling part	Moisture content (g/100 g) at germination day		
	3rd	5th	7th
Tannin sorghum (BR9929026)			
Leaf	89.52 ± 0.52 [a]	92.75 ± 0.40 [a]	91.33 ± 0.32 [a]
Cotyledon	39.97 ± 1.01 [c]	58.63 ± 0.74 [b]	67.14 ± 1.09 [a]
Radicle	93.10 ± 0.15 [a]	91.74 ± 0.83 [a]	88.39 ± 0.79 [a]
Tannin-free sorghum (BRS 310)			
Leaf	89.50 ± 0.62 a	91.37 ± 0.45 a	90.65 ± 0.47 a
Cotyledon	40.50 ± 0.19 [c]	55.16 ± 0.96 [b]	67.77 ± 0.62 [a]
Radicle	88.33 ± 0.61 [a]	88.34 ± 0.92 [a]	87.48 ± 1.48 [a]

n = 3.
Mean values with different superscripts in the same line are significantly different (Tukey test, $p < 0.05$).
Germination conditions: 20 ± 2 °C for 16 h in the dark and 30 ± 2 °C for 8 h in the presence of light, and 92 ± 2% relative humidity for 7 days.

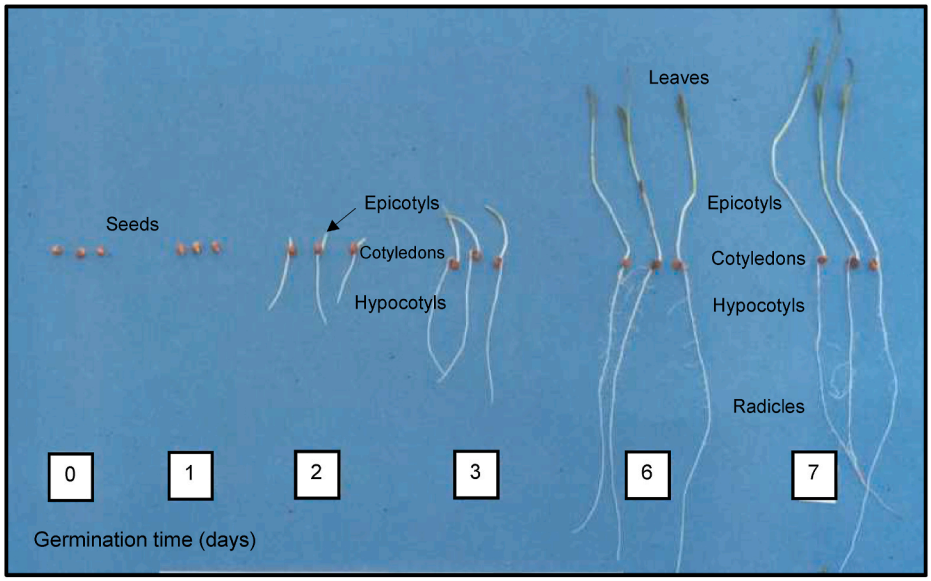

Fig. 1. Illustrative photograph of the germination of *Sorghum bicolor* L. at 20 ± 2 °C for 16 h in the dark and 30 ± 2 °C for 8 h in the presence of light and at 92 ± 2% relative humidity for 7 days, showing seeds, hypocotyls, epicotyls, cotyledons, radicals and leaves structures at 24 h (1 day) intervals.

germination day, increased significantly up to the 7th day at a logarithmic rate [$y = 32.431 \ln(x)+4.9365$, $R^2 = 0.9912$ and $y = 31.9 \ln(x)+4.989$, $R^2 = 0.9944$ for tannin and tannin-free seeds, respectively]. Based on these results, there was a significant change in moisture content of the seed on the first germination day and on the cotyledon from the 3rd up to the 7th germination days. Similar changes in the moisture content were reported during corn (Bandeira et al., 2012) and soybean germination (Bueno et al., 2020; Gloria et al., 2005). The change in moisture content of the sorghum seed during germination reinforces the need to express the changes of amines during germination on a dry weight basis.

In fact, water absorption is required during germination, as it is critical for the activation of metabolic processes in the seed culminated during germination (Rezende, Masetto, Oba, & Jesus, 2017). There is swelling of the embryos which speeds up germination by facilitating water absorption. Pre-germination metabolic processes are stimulated, and the seedlings emerge and grow vigorously (Alcázar, Bueno, & Tiburcio, 2020).

3.2. Bioactive amines in different parts of the sorghum seedling during germination

Among the ten amines investigated, only three – the polyamines spermidine and spermine and the diamine putrescine – were found in the different parts of the sorghum seedling before and during germination (Table 2). Prior to germination, total amines were higher ($p < 0.05$) in tannin-free sorghum compared to the tannin sorghum. This is probably due to the conjugation of free amines with the phenolic compounds and condensed tannins typical of tannin sorghum (Dykes & Rooney, 2006; Palacios et al., 2021). In fact, Paiva et al. (2015) and Adamczyk, Simon, Kitunen, Adamczyk, and Smolander (2017) demonstrated that spermine, spermidine and putrescine formed conjugates with tannin and that the amounts conjugated were higher for spermine, which has a larger number of amine groups and a higher molecular mass (Adamczyk et al., 2017).

On the first germination day, there were changes on amines in the turgid seeds, which differed for tannin and tannin-free sorghum. In tannin sorghum, there was a decrease in the contribution of spermine to the total levels of amines and an increase in putrescine, resulting in significantly lower total amine levels in the turgid seed (Fig. 2A). In tannin-free sorghum (Fig. 2B), the levels of spermine and putrescine decreased and there was an increase in spermidine. According to these changes, the prevalence of spermine decreased resulting in an increase in the prevalence of spermidine and putrescine. The reduction in spermine levels was common to both genotypes, suggesting its use by the seed in adaptation to the germination process (Tao et al., 2018).

From the third day on, when the different parts of the sorghum seedling were visible, the radicle, cotyledon and leaves were separated and analyzed individually. In all seedling parts, throughout germination, putrescine was the prevalent amine followed by spermidine. Spermine was only detected in the 7th germination day in the cotyledon and radicle of tannin sorghum (Fig. 2A), reaching levels of 1.08 and 3.17 mg/100 g dwb, respectively, which represented 3.6 and 5.9% of the total amine level. The low levels of spermine in the seedling parts suggests its use by the plant during germination, as this polyamine participates in the synthesis and stabilization of DNA, RNA, and protein (Muñoz-Esparza et al., 2019; Sagar et al., 2021), which are typical during germination.

In the third germination day, total amines were higher in the cotyledon and radicle of tannin-free sorghum compared to the tannin sorghum, whereas total levels were similar in the leaves of both sorghum types. Putrescine was the prevalent amine in the 3rd germination day of tannin sorghum (Fig. 2A) in the leaf (85.9%) and cotyledon (66.9%), whereas spermidine was prevalent in the radicle (56.2%). However, in tannin-free sorghum (Fig. 2B), putrescine was the prevalent amine in every part of the seedling (75.9–87.2%).

Table 2
Levels of free bioactive amines in different parts of the seedling from tannin and tannin-free sorghum cultivars from the third until the seventh germination day.

Sample/ germination day	Amine levels (mg/100 g dwb)			
	Spermidine	Spermine	Putrescine	Total
Tannin sorghum (BR99290266)				
Leaf				
3rd	4.31 ± 0.25^{xB}	0.00	26.34 ± 3.48^{bx}	30.65 ± 3.73^{x}
5th	5.79 ± 1.59^{x}	0.00	26.66 ± 4.27^{bx}	32.45 ± 5.86^{x}
7th	6.13 ± 0.33^{xA}	0.00^{y}	34.69 ± 0.75^{ayA}	40.82 ± 1.08^{yA}
Cotyledon				
3rd	1.45 ± 0.24^{bz}	0.00	2.93 ± 0.33^{cyB}	4.38 ± 0.57^{czB}
5th	3.02 ± 0.40^{ay}	0.00	16.28 ± 1.50^{byB}	19.30 ± 1.90^{bzB}
7th	1.15 ± 0.22^{byB}	1.08 ± 1.86^{y}	27.61 ± 1.34^{azB}	29.84 ± 3.42^{azB}
Radicle				
3rd	3.18 ± 0.33^{ayB}	0.00^{b}	2.48 ± 0.33^{cyB}	5.66 ± 0.66^{cyB}
5th	0.00^{bz}	0.00^{b}	24.99 ± 0.33^{bxB}	24.99 ± 0.33^{byB}
7th	0.00^{bzB}	3.17 ± 0.15^{axA}	50.51 ± 0.33^{axA}	53.68 ± 0.48^{axA}
Tannin-free sorghum (BRS310)				
Leaf				
3rd	5.27 ± 0.32^{ayA}	0.00	23.24 ± 3.16^{bx}	28.51 ± 3.48^{y}
5th	5.67 ± 0.91^{ax}	0.00	23.36 ± 3.59^{by}	29.03 ± 1.23^{y}
7th	0.00^{byB}	0.00	29.43 ± 0.48^{azB}	29.43 ± 0.48^{zB}
Cotyledon				
3rd	1.40 ± 0.36^{cz}	0.00	9.52 ± 1.27^{cyA}	10.92 ± 1.63^{czA}
5th	2.88 ± 0.88^{by}	0.00	32.77 ± 0.55^{bxA}	35.65 ± 1.43^{bxA}
7th	5.00 ± 0.07^{axA}	0.00	54.22 ± 2.48^{axA}	59.22 ± 2.55^{axA}
Radicle				
3rd	9.65 ± 1.30^{axA}	0.00	30.42 ± 5.27^{xA}	40.07 ± 6.57^{xA}
5th	0.00^{bz}	0.00	34.21 ± 3.28^{xA}	34.21 ± 3.28^{xA}
7th	0.00^{by}	0.00^{B}	36.64 ± 3.03^{yB}	36.64 ± 3.03^{yB}

n = 3.
dwb = dry weight basis.
Mean values (± standard deviation), calculated using nd (LOQ - 0.04 mg/100 g) = 0.00, with different letters for the same amine and seedling part but at different germination time (a,b,c), and in the same germination time but at different seedling parts (x,y,z) and in the same seedling part but with or without tannin (AB) are significantly different (Tukey test, $p < 0.05$).

Overall, the levels of putrescine increased in every seedling part during germination. The levels of spermidine decreased, except in the leaves of tannin sorghum, where it remained constant. At the end of germination (7th day), putrescine was the prevalent amine in every seedling part, followed by spermidine (up to 15%). However, putrescine was the only amine detected in the leaf and radicle of tannin-free sorghum. Spermine, which was not detected in any seedling part, showed up in the cotyledon and radicle of tannin sorghum. A significant increase in the levels of putrescine was also observed during rice germination (Hayat et al., 2015; Shu, Frank, Shu, & Engel, 2008). Overall, the changes in spermidine during germination were minor, compared to the significant changes observed for putrescine. The changes in spermidine occurred in the radicle of both tannin and tannin-free sorghum. It was detected only in the third day decreasing to non-detected levels from the 5th day on.

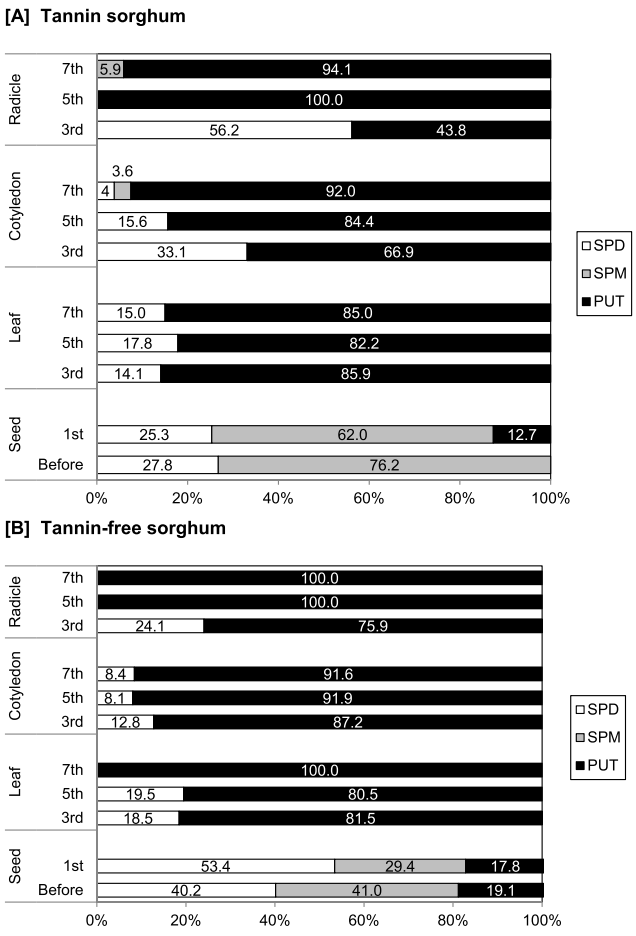

Fig. 2. Contribution of each amine to total levels in tannin sorghum ([A] – BR99290266) and tannin-free ([B] – BRS310) sorghum before germination, on the first germination day (1st), and during germination (3rd, 5th and 7th days) on seed, leaf, cotyledon and radicle. [SPD–spermidine; SPM–spermine; PUT–putrescine].

These results indicated that the profile of amines during germination was distinct for each seedling part, probably associated with the physiological role of each one of them. In addition, results indicated that the presence of tannin affected the profile of amines during germination in the different seedling parts. However, to ascertain the role of tannin on amines, in the cotyledon, which is the portion of the germinated sorghum most widely used, a larger number of samples from each group should be used. Therefore, additional tannin and tannin-free sorghum genotypes, three of each, were germinated and the respective cotyledons were analyzed for amines.

3.3. *Bioactive amines in the cotyledon of several sorghum genotypes during germination*

Prior to germination, the profile of amines in the tannin and tannin-free sorghum genotypes (Table 3), showed that only three amines were found – putrescine, spermidine and spermine. Spermidine and spermine were detected in every sample, whereas putrescine was only detected in 25% and 50% of the tannin and tannin-free genotypes, respectively. None of the other seven amines investigated were detected. Overall, the levels of total and individual amines were higher in tannin-free sorghum compared to the tannin sorghums, which follows the same trend reported by Paiva et al. (2015) for a broader range of sorghum genotypes. This tendency corroborates with the findings that free amines can be bound to tannins (phenolic compounds) in tannin sorghum (Adamczyk

Table 3
Levels of free bioactive amines in the seed of different genotypes of tannin and tannin-free sorghum cultivars during germination for up to 7 days.

Genotype	Day	Amine level (mg/100 g dwb)			
		Putrescine	Spermidine	Spermine	Total
Tannin sorghum					
BR9929026	0	0.00^{dw}	0.76 ± 0.03^{bcxy}	2.08 ± 0.32^{v}	2.84 ± 0.35^{cvw}
	1	0.36 ± 0.28^{dv}	0.38 ± 0.02^{cx}	0.93 ± 0.09^{v}	1.67 ± 0.09^{dv}
	3	2.83 ± 0.33^{cw}	1.45 ± 0.24^{bwx}	0.00	4.32 ± 0.43^{cw}
	5	16.28 ± 1.50^{bx}	3.02 ± 0.40^{avw}	0.00	19.30 ± 1.10^{b}
	7	27.61 ± 1.34^{aw}	1.15 ± 0.22^{by}	1.08 ± 1.86	29.84 ± 1.47^{awx}
BAZ9504	0	0.00^{cw}	0.73 ± 0.02^{bxy}	0.97 ± 0.13^{aw}	1.70 ± 0.12^{cxy}
	1	0.10 ± 0.11^{cyx}	0.96 ± 0.24^{abx}	0.00^{bw}	1.06 ± 0.63^{cw}
	3	2.81 ± 0.36^{cw}	1.25 ± 0.14^{ax}	0.00^{b}	3.80 ± 0.49^{cwx}
	5	20.73 ± 1.13^{bw}	1.28 ± 0.00^{aw}	0.05 ± 0.09^{b}	22.06 ± 1.21^{bwx}
	7	32.03 ± 8.82^{aw}	1.32 ± 0.13^{axy}	0.00^{b}	33.35 ± 8.82^{aw}
SC603	0	0.23 ± 0.01^{cw}	1.28 ± 0.07^{v}	0.41 ± 0.18^{aw}	1.92 ± 0.26^{bxy}
	1	0.28 ± 0.01^{cw}	1.34 ± 0.07^{wx}	0.00^{bw}	1.62 ± 0.08^{bw}
	3	0.81 ± 0.10^{cwx}	1.76 ± 0.07^{vwx}	0.00^{b}	2.58 ± 0.15^{bwx}
	5	16.37 ± 0.41^{bx}	1.73 ± 0.93^{w}	0.00^{b}	18.11 ± 1.32^{awx}
	7	18.46 ± 0.76^{aw}	1.38 ± 0.00^{xy}	0.00^{b}	19.82 ± 0.76^{axy}
SC59	0	0.00^{cw}	0.85 ± 0.14^{bxy}	1.07 ± 0.17^{aw}	1.92 ± 0.31^{dxy}
	1	0.19 ± 0.08^{cw}	0.95 ± 0.08^{bx}	0.00^{bw}	1.14 ± 0.11^{dw}
	3	0.92 ± 0.13^{cwx}	2.50 ± 0.46^{avw}	0.00^{b}	3.31 ± 0.47^{cwx}
	5	6.97 ± 1.61^{bz}	2.58 ± 1.18^{aw}	0.00^{b}	10.91 ± 0.92^{by}
	7	19.52 ± 0.21^{aw}	1.73 ± 0.10^{ay}	0.00^{b}	21.27 ± 0.31^{axy}
Tannin-free sorghum					
BRS310	0	0.68 ± 0.27^{dv}	1.43 ± 0.29^{cv}	1.43 ± 0.29^{aw}	3.57 ± 0.32^{dv}
	1	0.57 ± 0.17^{dv}	1.71 ± 0.22^{cvw}	0.94 ± 0.06^{av}	3.22 ± 0.59^{dv}
	3	9.52 ± 1.27^{cv}	1.40 ± 0.36^{cvwx}	0.00^{b}	11.11 ± 1.63^{cv}
	5	32.77 ± 0.55^{bv}	2.88 ± 0.08^{bvw}	0.00^{b}	35.65 ± 0.47^{bv}
	7	54.22 ± 2.48^{av}	5.00 ± 0.07^{av}	0.00^{b}	59.20 ± 2.52^{av}
RTx2783	0	0.00^{bw}	0.58 ± 0.08^{by}	1.35 ± 0.84^{w}	1.93 ± 0.08^{by}
	1	0.00^{bx}	2.24 ± 0.51^{av}	0.00^{w}	2.24 ± 0.50^{bvw}
	3	0.31 ± 0.01^{bx}	2.17 ± 0.29^{avwx}	0.00	2.59 ± 0.30^{bwx}
	5	14.02 ± 2.00^{axy}	1.62 ± 0.61^{aw}	0.00	15.99 ± 2.08^{axy}
	7	15.38 ± 0.72^{aw}	2.42 ± 0.00^{awx}	0.84 ± 0.40	18.67 ± 1.08^{ay}
SC42	0	0.00^{cw}	1.04 ± 0.18^{cdvx}	1.33 ± 0.23^{bw}	2.37 ± 0.44^{cwx}
	1	0.00^{cx}	0.86 ± 0.04^{dx}	0.00^{cw}	0.86 ± 0.04^{cw}
	3	0.54 ± 0.09^{cw}	1.30 ± 0.30^{cvwx}	0.00^{c}	2.05 ± 0.39^{cx}
	5	13.94 ± 0.68^{bxy}	4.60 ± 0.37^{av}	0.00^{c}	18.54 ± 0.81^{bwx}
	7	24.83 ± 2.33^{aw}	2.85 ± 0.63^{bw}	1.83 ± 0.24^{a}	29.51 ± 2.65^{awx}

(*continued on next page*)

Table 3 (*continued*)

Genotype	Day	Amine level (mg/100 g dwb)			
		Putrescine	Spermidine	Spermine	Total
SC192	0	0.73 ± 0.04^{cv}	2.06 ± 0.13^{aw}	0.97 ± 0.27^{aw}	3.76 ± 0.41^{cv}
	1	0.66 ± 0.11^{cv}	1.68 ± 0.20^{bvw}	0.00^{bw}	2.34 ± 0.57^{cv}
	3	1.93 ± 0.09^{cy}	2.62 ± 0.52^{av}	0.00^{b}	4.25 ± 0.60^{cw}
	5	12.21 ± 0.92^{by}	1.50 ± 0.32^{bcw}	0.00^{b}	13.72 ± 1.14^{by}
	7	25.77 ± 2.74^{aw}	0.87 ± 0.02^{cy}	0.00^{b}	24.46 ± 4.67^{awxy}

n = 3.
nd = not detected. Quantification limit: 0.04 mg/100 g.
Mean values (calculated with nd = 0) (± standard deviation) with different superscripts (a,b,c,d) for the same amine and genotype but at different germination times and (v,w,x,y,z) in the same amine and germination time but at different genotypes are significantly different (Tukey test, $p < 0.05$).

et al., 2017). The absence of agmatine in sorghum prior to and during germination, suggests that the synthesis of putrescine in sorghum does not follow the arginine pathway, which is common for other plants, for example brassica (Hamana, Hayashi, & Niitsu, 2015).

In the first germination day, the changes on the levels of amines varied between tannin and tannin-free samples. In tannin sorghum, there was a decrease in spermine, an increase in putrescine and spermidine levels remained the same. In tannin-free sorghum, the levels of spermine decreased, whereas the levels of spermidine and putrescine remained the same. Common to both sorghum (tannin and tannin-free) was the reduction in spermine levels, and the maintenance of spermidine levels.

From the third up to the 7th germination day, there was a similar pattern of changes in the cotyledons for putrescine, which increased significantly, resulting in concomitant increases in total amines (Table 3). The rates at which putrescine increased ranged from 8.4 to 19.3 in tannin sorghum and from 7.9 to 12.1 in tannin-free sorghum. These results indicate that putrescine increased significantly up to the 7th day. The changes in putrescine levels followed linear regression ($R^2 \geq 0.8247$ for tannin sorghum and $R^2 \geq 0.9348$ for tannin-free sorghum).

Overall, the levels of spermidine in the cotyledon of both types of sorghum kept unchanged or underwent minor changes. On the other side, spermine levels decreased to non-detected levels in most samples both tannin and tannin-free. However, in two genotypes of tannin-free sorghum (RTx2783 and SC42), spermine levels were recovered, reaching initial levels in the 7th germination day. In only one genotype of tannin sorghum, the levels of spermine increased in the 7th germination day, however they only reached 50% of initial values. The increase in spermine levels in the 7th germination day, suggests that at this point it was not needed anymore for germination, thereby, it accumulated. Further studies are needed to ascertain the role of these amines during germination.

Based on these results and considering the role of the polyamines – spermidine and spermine – as well as their obligate precursor putrescine (diamine) in plant growth and health (Alcázar et al., 2020; Taie, El-Yazal, Ahmed, & Rady, 2019), it is likely that tannin sorghum would have lower germination power compared to tannin-free. Lower germination power of tannin sorghum was reported in the literature (Almeida et al., 2014), and the authors reasoned that the presence of phenolic substances in the seed coat inhibited sorghum seed germination, due to conjugation with proteins. However, it is likely that the binding of phenolic compounds (tannin) to polyamines will also negatively affect sorghum germination.

The increase in putrescine levels during germination can be associated with its role in the response to a wide range of abiotic and biotic stress conditions, including water and temperature stresses and K-deficiency (Chen et al., 2016; Gohari et al., 2021; Hasanuzzaman, Nahar, Alam, Roychowdhury, & Fujita, 2013). In fact, putrescine can modulate higher plant photosynthetic proton circuit (Ioannidis, Cruz, Kotzabasis, & Kramer, 2012), improve somatic embryogenesis and plant regeneration (Sakhanokho, Ozias-Akins, May, & Chee, 2005).

The increase in the levels of putrescine during sorghum germination was similar to the one reported for rice (Hayat et al., 2015; Shu et al., 2008). However, during corn and soybean germination, significant increases were observed for the levels of putrescine, but also for spermine and spermidine (Bandeira et al., 2012; Bueno et al., 2020; Gloria et al., 2005). However, results for sorghum showed that depending on the genotype, there could also be increases in spermine during germination. Therefore, with the proper selection of a genotype, the desirable profile of polyamines and putrescine can be achieved, thereby allowing the modulation of the amines profile in the germinated product. This is an advantage compared to other germinated products.

3.4. Correlation between the contents of tannin and free amines in sorghum before and after germination

The existence of correlation between the levels of tannin and the respective contents of putrescine, spermidine, and spermine in the sorghum genotypes before and after seven days germination was investigated by means of Pearson correlation. According to Table 4, before germination, there was a weak to moderate positive significant correlation ($r = 0.35$, $p < 0.05$) between putrescine and tannin contents. For the germinated sorghum, there was significant positive moderate correlation between tannin contents and the levels of both spermidine ($r = 0.51$ $p < 0.05$) and spermine ($r = 0.56$ $p < 0.05$). Based on these results, putrescine seems to be positively affected by tannin in non-germinated sorghum, whereas spermidine and spermine are positively affected by tannin after germination. A larger number of samples should be analyzed to better understand the correlation between tannin and amines levels during germination.

4. Conclusions

Among the ten amines investigated, only spermine, spermidine and putrescine were detected in the sorghum seeds. Tannin-free sorghum had higher levels of free polyamines (spermidine and spermine), putrescine and total amines compared to tannin sorghum, probably due to the binding of free amines to tannin. Putrescine was the prevalent amine followed by spermidine in all seedling parts, throughout germination. Spermine was only detected in the 7th germination day in the cotyledon and radicle of tannin sorghum. The profile and levels of amines in the leaves were similar for both tannin and tannin-free sorghum. During sorghum germination there was an increase in putrescine and a decrease in spermine levels, at rates affected by the presence or not of tannin in the seed and by the genotype. In two genotypes of tannin-free sorghum, spermine levels were recovered to initial values. Spermidine changes were also affected by tannin and genotype. Different from what happens with corn and soybean, during sorghum germination there is an increase in putrescine levels. By selecting the proper genotype one can modulate spermine contents in the germinated sorghum, thereby obtaining

Table 4
Estimates of Pearson correlations *between the contents of amines and tannins in sorghum samples before (seed) and after germination (7 days germination)*.

Amines	Correlation coefficient (r)	
	Before germination	After germination
Putrescine	0.35*	0.11
Spermidine	0.30	0.51*
Spermine	0.14	0.56*

nd tannin levels were considered equal to zero.
* Significant *correlation - $p \leq 0.05$ (t-test)*.

different amines profile for specific food application.

Declaration of competing interest

The authors declare that there is no conflict of interest in connection with the work submitted.

CRediT authorship contribution statement

Caroline Liboreiro Paiva: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Déa A.M. Netto:** Methodology, Supervision, germination. **Valéria A.V. Queiroz:** Sample provision, Methodology, Formal analysis. **Maria Beatriz A. Gloria:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Brasília, DF, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (Belo Horizonte, MG, Brazil) for the financial support.

References

Adamczyk, B., Simon, J., Kitunen, V., Adamczyk, S., & Smolander, A. (2017). Tannins and their complex interaction with different organic nitrogen compounds and enzymes: Old paradigms versus recent advances. *Chemistry Open, 6*, 610–614. https://doi.org/10.1002/open.201700113

Alcázar, R., Bueno, M., & Tiburcio, A. F. (2020). Polyamines: Small amines with large effects on plant abiotic stress tolerance. *Cells, 9*, 2373–2393. https://doi.org/10.3390/cells9112373

Almeida, T. T., da Rosa, S. D. V. F., Oliveira, J. A., Oliveira, A. S., da Silva, A. A., & Pereira, D. S. (2014). Influence of tannin on sorghum seed germination. *African Journal of Food Science and Technology, 5*(6), 138–142. https://doi.org/10.1590/2317-1545v32n2923

AOAC. (2019). *Association of Official analytical chemists. Official methods of analysis of AOAC international* (21st ed.). Washington, MD: AOAC International.

Arouna, N., Gabriele, M., & Pucci, L. (2020). The impact of germination on sorghum nutraceutical properties. *Foods, 9*, 1218–1231. https://doi.org/10.3390/foods9091218

Bandeira, C. M., Evangelista, W. P., & Glória, M. B. A. (2012). Bioactive amines in fresh, canned and dried sweet corn, embryo and endosperm and germinated corn. *Food Chemistry, 131*, 1355–1359. https://doi.org/10.1016/j.foodchem.2011.09.135

Brasil Ministério da Agricultura, & e Abastecimento, P. (2009). *Regras para análise de sementes/Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. – Brasília: Mapa/ACS* (p. 399).

Bueno, D. B., da Silva Júnior, S. I., Chiarotto, A. B. S., Cardoso, T. M., Neto, J. A., dos Reis, G. C. L., et al. (2020). The germination of soybeans increases the water-soluble components and could generate innovations in soy-based foods. *LWT – Food Science and Technology, 117*, Article 108599. https://doi.org/10.1016/j.lwt.2019.108599

Chen, D., Cao, B., Qi, L., Yin, L., Wang, S., & Deng, X. (2016). Silicon-moderated K-deficiency-induced leaf chlorosis by decreasing putrescine accumulation in sorghum. *Annals of Botany, 118*(2), 305–315. https://doi.org/10.1093/aob/mcw111

Codex. (2019). *Codex alimentarius Official standard for sorghum grains FAO/WHO codex stan 1989 172*.

Dala-Paula, B. M., Starling, M. F. V., & Gloria, M. B. A. (2021). Vegetables consumed in Brazilian cuisine as sources of bioactive amines. *Food Bioscience, 40*, Article 100856. https://doi.org/10.1016/j.fbio.2020.100856

Dykes, L., Hoffmann, L., Jr., Portillo-Rodriguez, O., Rooney, W. L., & Rooney, L. W. (2014). Prediction of total phenols, condensed tannins, and 3-deoxyanthocyanidins in sorghum grain using near-infrared (NIR) spectroscopy. *Journal of Cereal Science, 60*(1), 138–142. https://doi.org/10.1016/j.jcs.2014.02.002

Dykes, L., & Rooney, L. W. (2006). Sorghum and millet phenols and antioxidants. *Journal of Cereal Science, 44*(3), 236–251. https://doi.org/10.1016/j.jcs.2006.06.007

Espitia-Hernández, P., González, M. L. C., Ascacio-Valdés, J. A., Dávila-Medina, D., Flores-Naveda, A., & Silva, T. (2020). Sorghum (*Sorghum bicolor* L.) as a potential source of bioactive substances and their biological properties. *Critical Reviews in Food Science and Nutrition*, 1–12. https://doi.org/10.1080/10408398.2020.1852389

Gaytán-Martínez, M. (2020). Sorghum: An important source of bioactive compounds. *American Journal of Biomedical Science & Research, 8*(2), Article 001253. https://doi.org/10.34297/AJBSR.2020.08.001253

Ghimire, B.-K., Seo, J.-W., Yu, C.-Y., Kim, S.-H., & Chung, I.-M. (2021). Comparative study on seed characteristics, antioxidant activity, and total phenolic and flavonoid contents in accessions of *Sorghum bicolor* (L.) Moench. *Molecules, 26*, 3964. https://doi.org/10.3390/molecules26133964

Gloria, M. B. A., Tavares-Neto, J., Labanca, R. A., & Carvalho, M. S. (2005). Influence of cultivar and germination on bioactive amines in soybeans (*Glycine max* L. Merril). *Journal of Agricultural and Food Chemistry, 53*, 7480–7485. https://doi.org/10.1021/jf0509310

Gohari, G., Panahirad, S., Sadeghi, M., Akbari, A., Zareei, E., Zahedi, S. M., et al. (2021). Putrescine-functionalized carbon quantum dot (put-CQD) nanoparticles effectively prime grapevine (*Vitis vinifera* cv. 'Sultana') against salt stress. *BMC Plant Biology, 21*, 120. https://doi.org/10.1186/s12870-021-02901-1

Hamana, K., Hayashi, H., & Niitsu, M. (2015). Polyamines in different organs of brassica crop plants with or without clubroot disease. *Plant Production Science, 18*(4), 476–480. https://doi.org/10.1626/pps.18.476

Handa, A. K., Fatima, T., & Mattoo, A. K. (2018). Polyamines: Bio-molecules with diverse functions in plant and human health and disease. *Frontiers of Chemistry, 6*, 10. https://doi.org/10.3389/fchem.2018.00010

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences, 14*(5), 9643–9684. https://doi.org/10.3390/ijms14059643

Hayat, A., Jahangir, T. M., Khuhawar, M. Y., Alamgir, M., Hussain, Z., Haq, F. U., et al. (2015). HPLC determination of gamma amino butyric acid (GABA) and some biogenic amines (BAs) in controlled, germinated, and fermented brown rice by pre-column derivatization. *Journal of Cereal Science, 64*, 56–62. https://doi.org/10.1016/j.jcs.2015.04.014 0733-5210

Hirano, R., Shirasawa, H., & Kurihara, S. (2021). Health-Promoting effects of dietary polyamines. *Medical Science, 9*(8), 1–18. https://doi.org/10.3390/medsci9010008

Ioannidis, N. E., Cruz, J. A., Kotzabasis, K., & Kramer, D. M. (2012). Evidence that putrescine modulates the higher plant photosynthetic proton circuit. *PLoS One, 7*(1), Article e29864. https://doi.org/10.1371/journal.pone.0029864

Kimani, W., Zhang, L.-M., Wu, X.-Y., Hao, H.-Q., & Jing, H.-C. (2020). Genome-wide association study reveals that different pathways contribute to grain quality variation in sorghum (*Sorghum bicolor*). *BMC Genomics, 21*(1), 112. https://doi.org/10.1186/s12864-020-6538-8

Lee, S. H., Lee, J., Herald, T., Cox, S., Noronha, L., Perumal, R., et al. (2020). Anticancer activity of a novel high phenolic sorghum bran in human colon cancer cells. *Oxidative Medicine and Cellular Longevity*. , Article 2890536. https://doi.org/10.1155/2020/2890536

Luo, X., Cui, J., Zhang, H., Duan, Y., Zhang, D., Cai, M., et al. (2018). Ultrasound assisted extraction of polyphenolic compounds from red sorghum (*Sorghum bicolor* L.) bran and their biological activities and polyphenolic compositions. *Industrial Crops and Products, 112*, 296–304. https://doi.org/10.1016/j.indcrop.2017.12.019

Muñoz-Esparza, N. C., Latorre-Moratalla, M. L., Comas-Basté, O., Toro-Funes, N., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2019). Polyamines in food. *Frontiers in Nutrition, 6*, 108. https://doi.org/10.3389/fnut.2019.00108

Ofosu, F. K., Elahi, F., Daliri, E. B. M., Tyagi, A., Chen, X. Q., Chelliah, R., et al. (2021). UHPLC-ESI-QTOF-MS/MS characterization, antioxidant and antidiabetic properties of sorghum grains. *Food Chemistry, 337*, Article 127788. https://doi.org/10.1016/j.foodchem.2020.127788

Paiva, C. L., Evangelista, W. P., Queiroz, V. A. V., & Glória, M. B. A. (2015). Bioactive amines in sorghum: Method optimisation and influence of line, tannin and hydric stress. *Food Chemistry, 173*, 224–230. https://doi.org/10.1016/j.foodchem.2015.10.099

Paiva, C. L., Queiroz, V. A. V., Simeone, M. L. F., Schaffert, R. E., Oliveira, A. C., & da Silva, C. S. (2017). Mineral contents in sorghum genotypes: Influence of water stress. *Food Chemistry, 214*, 400–405. https://doi.org/10.1016/j.foodchem.2016.07.067

Palacios, C. E., Nagai, A., Torres, P., Rodrigues, J. A., & Salatino, A. (2021). Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. *Food Chemistry, 327*, Article 127970. https://doi.org/10.1016/j.foodchem.2020.127970

Pinheiro, S. S., Anunciação, P. C., de Morais Cardoso, L., Della Lucia, C. M., de Carvalho, C. W. P., Queiroz, V. A. V., et al. (2021). Stability of B vitamins, vitamin E, xanthophylls and flavonoids during germination and maceration of sorghum (Sorghum bicolor L.). *Food Chemistry, 345*, Article 128775. https://doi.org/10.1016/j.foodchem.2020.128775

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*, 1214–1218. https://doi.org/10.1021/jf60219a031

Rashwan, A. K., Yones, H. A., Karim, N., Taha, E. M., & Chen, W. (2021). Potential processing technologies for developing sorghum-based food products: An update and comprehensive review. *Trends in Food Science & Technology, 110*, 168–182. https://doi.org/10.1016/j.tifs.2021.01.087

Rezende, R. K. S., Masetto, T. E., Oba, G. C., & Jesus, M. V. (2017). Germination of sweet sorghum seeds in different water potentials. American. *Journal of Plant Sciences, 8*, 3062–3072. https://doi.org/10.4236/ajps.2017.812206

Sagar, N. A., Tarafdar, S., Agarwal, S., Tarafdar, A., & Sharma, S. (2021). Polyamines: Functions, metabolism, and role in human disease management. *Medical Science, 9*(44), 1–21. https://doi.org/10.3390/medsci9020044

Sakhanokho, H. F., Ozias-Akins, P. O., May, L., & Chee, P. W. (2005). Putrescine enhances somatic embryogenesis and plant regeneration in upland cotton. *Plant Cell, Tissue and Organ Culture, 81*, 91–95. https://doi.org/10.1007/s11240-004-1541-3

Shu, X. L., Frank, T., Shu, Q. Y., & Engel, K. H. (2008). Metabolite profiling of germinating rice seeds. *Journal of Agricultural and Food Chemistry, 56*(24), 11612–11620. https://doi.org/10.1021/jf802671

Singh, A., Sharma, S., Singh, B., & Kaur, G. (2019). In vitro nutrient digestibility and antioxidative properties of flour prepared from sorghum germinated at different conditions. *Journal of Food Science & Technology, 56*(6), 3077–3089. https://doi.org/10.1007/s13197-019-03804-8

Taie, H. A., El-Yazal, M. A. S., Ahmed, S. M., & Rady, M. M. (2019). Polyamines modulate growth, antioxidant activity, and genomic DNA in heavy metal–stressed wheat plant. *Environmental Science and Pollution Research, 26*(22), 22338–22350. https://doi.org/10.1007/s11356-019-05555-7

Tao, Y., Wang, J., Miao, J., Chen, J., Wu, S., Zhu, J., … Liang, G. (2018). The spermine synthase OsSPMS1 regulates seed germination, grain size, and yield. *Plant Physiology, 178*(4), 1522–1536. https://doi.org/10.1104/pp.18.00877

Teferra, T. F., Amoako, D. B., Rooney, W. L., & Awika, J. M. (2019). Qualitative assessment of 'highly digestible' protein mutation in hard endosperm sorghum and its functional properties. *Food Chemistry, 271*, 561–569. https://doi.org/10.1016/j.foodchem.2018.08.014

Wu, Y., Li, X., Xiang, W., Zhu, C., Lin, Z., Wu, Y., … Yu, J. (2012). Presence of tannins in sorghum grains is conditioned by different natural alleles of Tannin 1. *Proceedings of the National Academy of Sciences, 109*, 10281–10286. https://doi.org/10.1073/pnas.1201700109

Xiong, Y., Zhang, P., Warner, R. D., & Fang, Z. (2019). Sorghum grain: From genotype, nutrition, and phenolic profile to its health benefits and food applications. *Comprehensive Reviews in Food Science and Food Safety, 18*(6), 2025–2046. https://doi.org/10.1111/1541-4337.12506