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Bioactive amines in sorghum: Method optimisation and influence of line, tannin and hydric stress

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ABSTRACT

The profile and levels of bioactive amines in different sorghum lines were reported for the first time. The amines were quantified by ion-pair HPLC, post-column derivatisation with *o*-phthalaldehyde and fluorimetric detection. The extraction procedure was optimised: 420 μm particle size, extraction with 5% trichloroacetic acid and three extractions. The screening of 22 sorghum lines showed that four of the ten amines investigated were detected. Spermine and spermidine were the prevalent amines (100%), followed by putrescine (77%) and cadaverine (14%). Total amines ranged from 5.8 to 41.4 mg/100 g, and the polyamines represented 60–100% of the total. Sorghum without tannin had higher amines levels compared to sorghum with tannin and cadaverine was specific to samples without tannin. Hydric stress caused accumulation of spermidine in the grains and affected the levels of other amines at rates depending on the presence or not of tannin. Sorghum is a significant source of polyamines.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench), a cereal of the *Poaceae* family, is the fifth most important cereal in the world, after wheat, rice, maize and barley. It has been widely used in animal feed and also as a staple food for millions of people, mainly in Africa, India and Asia. Sorghum is a promising cereal as it is easy to produce and it can grow under adverse environmental conditions, such as very dry, saline and hot areas, where the production of other cereals is uneconomical. There have been several breeding programs seeking the selection of sorghum genotypes with improved quality for animal and human consumption. Bird-resistant varieties, related to the amount of condensed tannins, have been bred. There are several ongoing researches aimed at the development of varieties for use in semi-arid zones to increase grain yields and the understanding of metabolic strategies adopted by higher plants to adapt to water stress (Ahmed, Abdalla, Inoue, Ping, & Babiker, 2014; Awika, Yang, Browning, & Faraj, 2009; Dicko, Gruppen, Traoré, Van Berkel, & Voragen, 2006; Moraes et al., 2012; Paterson et al., 2009).

Sorghum is very versatile as it has potential use for food, feed, fibre and fuel. Furthermore, sorghum has been valued as a potential source of fibre, resistant starch, minerals (calcium, iron and potassium) and some bioactive compounds, such as polyphenols. Moreover, there is now an increased interest in using sorghum in foods worldwide due to its gluten-free and other health promoting properties, such as slow digestibility, cholesterol-lowering,

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anti-inflammatory, and anti-cancer properties (Awika et al., 2009; Dykes, Rooney, & Rooney, 2013; Moraes et al., 2012; Paterson et al., 2009). Several studies were undertaken to investigate the components of sorghum; however no information is available regarding the levels of bioactive amines in sorghum.

Bioactive or biologically active amines are nitrogenous bases of low molecular weight which play important metabolic and physiological functions in living cells. Bioactive amines can be classified as biogenic amines and polyamines. The polyamines spermidine and spermine occur ubiquitously in the plant kingdom, together with their diamine precursor putrescine. Polyamines have numerous physiological functions in plants. They are associated with plant growth and development, playing roles in embryogenesis, root and shoot formation, floral initiation, fruit development, fruit ripening and senescence. In recent years there has been an increasing interest in the roles polyamines play in the protection of plants against environmental stresses, such as potassium deficiency, osmotic shock, drought and pathogen infection. Moreover, the radical-scavenging properties of polyamines can protect membranes from lipid peroxidation and other oxidative stresses (Calzadilla, Gazquez, Maiale, Ruiz, & Menendez, 2014, chap. 7; Cao, Hu, Zhu, Hu, & Knapp, 2010; Chai, Jiang, Shi, Shi, & Gu, 2010; Erdei, Szegletes, Barabas, & Pestenacz, 1996; Gloria, 2005; Hunter & Burritt, 2012, chap. 12; Kalač, 2014; Kusano, Yamaguchi, Berberich, & Takahashi, 2007).

There are several biogenic amines naturally found in the plant kingdom, among them phenylethylamine, serotonin, tryptamine, histamine, putrescine, cadaverine, agmatine, octopamine, and synephrine. Histamine and serotonin have a protective role in deterring predators and they are some of the active principles in stings. Some amine conjugates are important as antifungal and antiviral agents. Some aliphatic amines, such as putrescine and cadaverine, mimic the smell of rotten meat, therefore attracting pollinating insects. Tryptamine, tyramine and phenylethylamine are precursors of compounds of biological significance, like some plant hormones and alkaloids (Dey & Harborne, 1997; Gloria, 2005, chap. 13).

The knowledge of the types and levels of bioactive amines in foods is relevant from a nutritional and toxicological point of view. Biogenic amines are vasoactive and neuroactive and the polyamines are important in health and growth. Furthermore, polyamines have antioxidant properties, and are important in gut health. They are also involved in the regulation of inflammatory reactions, food allergy prevention and antiglycation. However, some amines, at high concentrations, can cause adverse effects to human health, including histamine poisoning, allergic effects, migraine, and hypertensive crisis in individuals under monoamine-oxidase therapy (Ali, Poortvliet, Stromberg, & Yngve, 2011; Gloria, 2005, chap. 13; Kalač, 2014).

Recently, two comprehensive reviews have been published on the levels of polyamines in different foods providing useful information for nutritionists (Ali et al., 2011; Kalač, 2014). However, no information was provided for sorghum. Several methods have been used for the analysis of bioactive amines in food. Among them, HPLC with post-column derivatisation is the most frequently used technique for the separation and quantification of bioactive amines (Onal, Tekkeli, & Onal, 2013). Prior to chromatographic separation, the amines are extracted from the matrix and additional purification steps can be required. Extraction is a critical step in terms of obtaining adequate amine recoveries and its efficiency is affected by the type of amines present, the food matrix and the solvent used. Generally extraction in acid medium is preferred and the most commonly used acids are hydrochloric acid (HCl) and trichloroacetic acid (TCA). It is widely known that the efficiency of the extraction procedure is affected by the structure of the matrix (mainly particle size) and extraction time. In general, smaller particle size and longer extractions facilitate mass transfer, but measurement of such effects for each matrix is required before optimisation can be performed (Gião, Pereira, Fonseca, Pintado, & Malcata, 2009).

The objective of this study was to determine the profile and levels of bioactive amines in different sorghum lines with and without tannin and to investigate the effect of hydric stress on the profile and levels of amines in sorghum. In order to do so, a procedure for the extraction of bioactive amines from sorghum (*S. bicolor* L.) was optimised for subsequent analysis by HPLC. Different particle sizes of the ground sorghum, the type of acid extraction and the number of extractions were investigated.

2. Materials and methods

2.1. Material

Different sorghum (*S. bicolor* L.) lines were provided by EMBRAPA Maize and Sorghum, Sete Lagoas, Minas Gerais, Brazil. These lines were grown in the experimental fields of EMBRAPA, located in Nova Porteirinha, Minas Gerais (MG), Brazil. The sorghum lines were grown with three replications under controlled cultivation conditions and harvested in October 2010. The grains were stored under refrigeration (4 ± 2 °C) after phosphine fumigation. Samples from each replicate were homogenised for analysis.

One sorghum line was used for the optimisation of the extraction procedure. Twenty-two different lines, 10 with tannin and 12 without tannin were screened for the profile and levels of amines.

To investigate the influence of hydric stress on the profile and levels of amines in sorghum, five lines, two with tannin and three without tannin were used. This experiment was also undertaken by EMBRAPA in Nova Porteirinha, MG, from June until October 2010. The plants were sprinkler irrigated for 2 h once a week. From the 50th day onwards, after leaf development, irrigation continued only in control samples up to the grain fill stage whereas hydric stressed samples were not irrigated after the 50th cultivation day. All experiments were performed in triplicate.

The reagents were of analytical grade, except HPLC solvents, which were chromatographic grade. The solutions were prepared with ultra pure-water (Milli-Q Plus system; Millipore Corp., Billerica, MA). The mobile phases were filtered through 0.45-μm pore size membranes (Millipore Corp.), types HAWP and HVWP for aqueous and organic solvents, respectively.

Standard solutions of the bioactive amines were prepared with spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, agmatine sulphate, 5-hydroxytryptamine (serotonin), histamine dihydrochloride, tyramine hydrochloride, 2-phenylethylamine hydrochloride and tryptamine (free base). These compounds, along with the derivatisation agent *o*-phthaladehyde (OPA), were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Methods

2.2.1. Grinding of sorghum grains

Sorghum grains (100 g) were ground in a knife mill (A11 Basic; IKA, Staufen, Germany) using two grinding times: 10 and 20 s. After grinding, the materials were sieved through a shaker (Solotest Indústria e Comércio Ltda, São Paulo, SP, Brazil) containing sieves of 4, 8, 16, 30, 50, 80 and 100 mesh at a frequency of 15 Hz for 30 min. The average particle diameter of the ground material was calculated according to ISO 2591 (ISO, 1988) by the geometric average of the masses retained and the opening of the sieves [average diameter = $(d_1 \times d_2)^{½}$, in which d_1 and d_2 are the openings, in mm, of the sieve with the largest and the second largest mass retained, respectively].