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Susceptibility of different types of sorghums during storage to *Sitophilus zeamais* Motschulsky

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ABSTRACT

Caryopses belonging to twelve different sorghum cultivars (two red, one brown or high-tannin, two white heterowaxy, two white waxy, two white high-digestible protein and three white with regular endosperm) were selected to study their resistance to *Sitophilus zeamais* during storage. Five resistance parameters were evaluated: Dobie Index, Total Emerged Insects, Median Development Time, Ratio final/initial insects and Weight Loss. Biophysical characteristics (test weight, 1000 kernel weight, endosperm texture, flotation index, true density, percentage of kernel removed with TADD, anatomical parts, color index and kernel size), chemical composition (starch, amylose, protein, free amino nitrogen and ash), and nutraceutical traits (free and bound phenolics and antioxidant capacity for the free and bound fractions) were obtained. The most resistant cultivars were both red sorghums (RR1 and RR2) and a white cultivar with regular endosperm (WR1) whereas the most susceptible were the brown high-tannin (Sumac), a white waxy (Waxy1) and a white high digestible (HD1). Correlation coefficients among resistance parameters and physicochemical characteristics were calculated, yielding a clear relationship amongst different endosperm texture indicators, endosperm, ash, amylose and free amino nitrogen content, and susceptibility traits. The harder kernels (in terms of vitrousness), higher endosperm percentage, low ash, increased amylose content and reduced free amino nitrogen concentration had more resistance to *S. zeamais*. Significant relationships among nutraceutical profiles and resistance were not detected, despite the wide range of phenolics in the array of kernels. These results indicated that endosperm structure is predominant in sorghum resistance to *S. zeamais*.

1. Introduction

Sorghum is the fifth cereal most produced in the world with an annual output of 55.7 million tons (FAOSTAT, 2012) and also the main source of calories and protein in some regions of Africa and Asia (Waniska and Rooney, 2000). The main producer is the United States of America with almost 9.0 million ton yearly, followed by Mexico and India with 6.9 and 6.7 million ton respectively (FAOSTAT, 2012). Sorghum is a drought resistant crop, with a high tolerance to salinity and an outstanding performance in areas where soil nutrients are limited (Serna-Saldívar, 2010).

Despite the high sorghum production in the world and its high stability to abiotic and biotic factors, during the postharvest stages some qualitative and quantitative losses occur. According to Ramputh et al. (1999), cereal postharvest losses in small-farm tropical agriculture usually exceeds 30% and concurring to García-Lara and Bergvinson (2007), the range of worldwide postharvest losses in subsistence farming is between 10 and 40%. One of the main biotic factors associated to losses during postharvest are insects such as weevils, being *Sitophilus zeamais* the main pest in tropical and subtropical regions (García-Lara and Bergvinson, 2007). Despite the importance of the postharvest management in the overall production cycle of cereals, there are few studies about resistance mechanisms of sorghum facing insect infestation, in particular to *S. zeamais*.

There are simple tests as Test Weight, Flotation Index and True Density, used as indicators of overall grain quality. These grain physical traits are related to performance during dry and wet-milling operations as well as food quality in end products (Chiremba et al., 2011; Pomeranz, 1986; Serna-Saldívar, 2010).

Abbreviations: AOXC, Antioxidant Capacity; CI, Color Index; DI, Dobie Index; ET, Endosperm Texture; FAN, Free Amino Nitrogen; FI, Flotation Index; GAE, Gallic Acid Equivalent; HD, High Digestible; HTW, Heterowaxy; MDT, Median Development Time; RH, Relative Humidity; TADD, Tangential Abrasive Dehulling Device; TD, True Density; TEI, Total Emerged Insects; TKW, Thousand Kernel Weight; TW, Test Weight; WL, Weight Loss.

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Furthermore, these parameters are related to endosperm texture or hardness mainly affected by the ratio between vitreous to floury or chalky endosperms (Pomeranz, 1986). Moreover, according to Doggett (1982) there is a direct relationship between kernel hardness and its resistance to molds and insects. García-Lara et al. (2004) reported in maize a negative correlation ($r = -0.84$) between hardness and the kernel susceptibility to weevils. Thus, also in sorghum, physical parameters (simple, cheap, and quick to determine) could yield valuable information to predict the performance of a specific cultivar during storage facing *S. zeamais* infestation. In cereal grains, in addition to mechanical resistance, there are also other protective schemes associated to their chemical composition such as the presence of phenolic acid amides (Burt, 2003; García-Lara et al., 2004). Chandrashekar and Satyanarayana (2006) reported that phenolic compounds such as ferulic acid and tannins are potent inhibitors of pests and pathogens. In sorghum, the effect of tannins in bird resistance and protein digestibility is already well documented (Dicko et al., 2005; Serna-Saldívar and Rooney, 1995), but there are scarce reports about the role of different types of phenolics in insect resistance. The function of phenolics bound to cell walls in resistance against *S. zeamais* has been demonstrated in maize (García-Lara et al., 2004) but not in sorghum.

Therefore, the objectives of this research were: 1) to study the susceptibility of different sorghum cultivars with different physical and chemical profiles during storage inoculated with maize weevil (*S. zeamais*) and; 2) to associate the physical, chemical and nutraceutical characteristics of the sorghum kernels with resistance/susceptibility to *S. zeamais* during storage.

2. Materials and methods

2.1. Grain varieties

Twelve contrasting sorghum cultivars were selected based on their kernel color and endosperm characteristics. Genotypes used in this study were: 1) two red sorghums, commonly used as feed in the northern part of Mexico (*RR1* and *RR2*); 2) one high-tannin sorghum (*Sumac*); and 3) nine white cultivars (two heterowaxy −*HTW1*, *HTW2*-, two waxy −*Waxy1*, *Waxy2*-, two described as high protein digestible −*HD1*, *HD2*- and three regular −*WR1*, *WR2*, *WR3*-). The red and high-tannin sorghums were commercially available material in Northern Mexico and possessed an intermediate to soft endosperm texture. The white sorghum samples were kindly donated by Dr. Dirk Hays of the Texas A&M University Sorghum Breeding Program.

2.2. Sample preparation

Kernel samples, not previously treated with insecticides, were cleaned by air aspiration and sieves. The moisture was adjusted to 13% using the formula ([(100 − %Initial Moisture)/(100 − %Desired Moisture)] − 1] * Sample Weight) and allowed to equilibrate for at least 7 days at 27 ± 1 °C and 70 ± 5% RH. For physical, chemical, phenolics and antioxidant characterizations, the sorghum kernels were milled using a coffee mill (Krups, Model GX410011, Mexico) and stored at 4 °C prior to analysis. Whole kernels were used for susceptibility tests.

2.3. Kernel physical characterization

The physical properties of the array of sorghum kernels were determined using standard procedures: test weight (TW) according to Official US Grain Standard Procedures (AACC Method 55-10); thousand-kernel weight (TKW) by weighing 100 randomly selected whole kernels, and endosperm texture (ET) according to the subjective procedure previously reported by Chuck-Hernández et al. (2009). Flotation index (FI) was determined according to Salinas et al. (1992) and expressed as a percentage of floating kernels on an aqueous solution of sodium nitrate (1.25 g/cm^3 specific weight at 35 °C). A pycnometer was used to obtain true density (TD), whereas the Tangential Abrasive Dehulling Device (TADD) was employed to determine the percentage of kernel removed after a fixed decortication time as an indicator for hardness. Kernel size was measured with a digital micrometer (Mitutoyo, Model MDC-1, Japan) whereas volume calculated using the formula for an ellipsoid ($4/3 * \pi * R^2 * r$). R was the half of kernel's length and r = Thickness/2. L^*, a^*, b^*, and other CIE color parameters of ground samples were determined using a colorimeter (Minolta CR-300, Osaka, Japan) and Color Index (CI) was estimated with the formula reported by Vignoni et al. (2006) (a^*1000/L^*b). Tip cap, germ, pericarp and endosperm were obtained from dissected kernels, previously soaked for 2 min in water according to the method described by Gutiérrez-Uribe et al. (2010).

2.4. Chemical characterization

Crude Protein ($N^*6.25$) was determined using the micro-Kjeldahl method 46-13 (AACC, 2000) and Free Amino Nitrogen (FAN) with the ninhydrin procedure 945.30L (AOAC, 1980). Total and resistant starch and amylose were determined using enzymatic tests TStarch −AOAC 996.11-, RStarch −AOAC 2002.02- and Amylose/Amylopectin -K-AMYL- procedures, respectively (Megazyme International, Ireland). Ash was assayed according to method 08-01 (AACC, 2000). Presence of pigmented testa in all sorghums was performed using the Chlorox bleach procedure (Waniska et al., 1992) and condensed tannins were quantified with the vainillin-HCl assay (Price et al., 1978). Extracts for tannin assay were obtained using acidified methanol (1% HCl).

2.5. Extraction and determination of free and bound phenolics

Free and bound phenolics were extracted using the method described by Gutiérrez-Uribe et al. (2010). The extracts were used to determined total phenolics with the Folin-Ciocalteu method. Briefly 20uL of extracts were mixed with 200uL of Folin-Ciocalteu reagent (Sigma Aldrich, 2 N, diluted 1:9 in distilled water) and 30 uL of sodium carbonate (7.5% w/v) and maintained for 90 min at 37 °C in darkness. Phenolics were determined by absorbance at 765 nm (Microplate Reader, Sinergy, HT Multi-Detection, BioTek, Inc., VT, USA). Gallic acid was used as standard and phenolic content expressed as mg of Gallic Acid Equivalent (GAE)/g of flour in dry basis.

2.6. Antioxidant Capacity (AOXC)

Antioxidant capacity was determined using the Oxygen Radical Absorbance Capacity assay, using a standard of Trolox with fluorescein as a probe as described by Prior et al. (2003). Peroxyl radicals were generated by 2,2′ azobis (2-amidinopropane) dihydrochloride, and fluorescent loss was monitored in a Microplate Reader (Sinergy, HT Multi-Detection, BioTek, Inc., VT, USA). The absorbances of excitation and emission were set at 485 and 538 nm, respectively. Data was expressed as μmol of Trolox equivalents per each gram of ground sample (dry basis).

2.7. Susceptibility tests

2.7.1. Insect culture

The *S. zeamais* colony was maintained in the Postharvest Laboratory at Tecnológico de Monterrey, Monterrey, Mexico. Insects