OCCURRENCE OF OXIDATIVE STRESS IN CARIOCA BEANS

Maria E. de O. Ferreira[1], Priscila Z. Bassinello[1], Rosângela N. Carvalho[1], Anna C. Lanna[1]

[1]Embrapa Rice and Beans, Caixa Postal 179, 75375-000, Santo Antônio de Goiás, GO, Brazil

INTRODUCTION: The accumulation of Reactive Oxygen Species (ROS) in the cells of the seed coat and cotyledon of carioca bean grain can cause: (a) oxidation of compounds present, mainly, in the tegument, which culminates in the formation of compounds responsible for the color change of the grains; (b) lipid peroxidation (damage to the cell membranes) and, consequently, electrolyte leakage as Ca^{2+} and Mg^{2+} with the probable formation of insoluble pectates, in the case of seeds of legumes[1] and (c) changes in texture, which is considered one of the most important attributes of legume seeds, since it affects the palatability and, consequently, the consumer acceptability[2]. Due to the lack of information about the primary event triggering of the hardening and darkening processes of the carioca bean grains, this study evaluated the SOD (Superoxide Dismutase) activity, enzyme responsible for the production of ROS, specifically, hydrogen peroxide (H_2O_2), hydrogen peroxide content, lipid peroxidation, effect originated by accumulation of H_2O_2 and technological parameters usually evaluated by consumers, such as color and cooking time.

METHODS: Freshly harvested naturally dried grains of carioca genotypes (BRS Pontal, BRS Madrepérola, CNFC 10467, BRS Estilo) and a Pinto Bean slow-darkening line 1533-15[3] (Canadian genotype used as negative control), were cultivated at the Capivara farm of Embrapa Rice and Beans (Oct 2013). 200 g of grains were peeled using rice dehuller (TM05C, SATAKE), and the cotyledon and seed coat were separated by using sieves of 9, 14 and 16 mesh, followed by grinding and maceration with liquid nitrogen. The extracts were prepared according to Lee & Lee[4]. The supernatant (crude extract of cotyledon and seed coat) was used for determination of SOD activity[5], hydrogen peroxide content[6] and for lipid peroxidation level[7]. The specific activity of SOD was obtained on the basis of the total soluble protein content with BSA as standard[8]. Color assessments of whole grains were held at the Colorimeter Color Quest XE, Hunter Lab, using CIELAB system[9], thus obtaining the values of 10 readings for coordinates L * (lightness), a* (yellowish) and b* (reddish). The cooking time was determined in Mattson cooker[10]. Mean values were compared by Tukey test at 5% probability and analysis of Pearson correlation coefficient using Statistica 7.0 (STATSOFTINC, Tulsa, Ok, USA).

RESULTS AND DISCUSSION: In stored grains, the presence of SOD and its activity indicate that oxidative stress may occur during this period. In the tegument of grains of BRS Pontal and BRS Estilo, genotypes with known fast darkening, the SOD activity was higher compared to that found in BRSMG Madrepérola and CNFC 10467, known as slower darkening beans. The Pinto Bean line, in turn, which is considered resistant to darkening, presented SOD activity significantly reduced in tegument compared to other samples (Table 1). Regardless of the genotype, the SOD activity in the cotyledon was lower than that found in the tegument and, among the genotypes, there was no significant difference. A higher SOD activity, mainly in the carioca bean grain hull, may indicate the occurrence of oxidative process and, ultimately, the darkening process. The hydrogen peroxide content was higher in the BRS Estilo seed coat and Pinto Beans cotyledon. As a result of the generation of H_2O_2 from SOD activity, damage may occur to the constituents of membranes of bean grain (lipid peroxidation) and favor the formation of insoluble pectates. It is observed that the higher level of lipid peroxidation took place in

tegument of BRSMG Madrepérola, while in cotyledon it did not varied significantly among genotypes. However, the level of lipid peroxidation in cotyledon was greater than that observed in the tegument. This parameter seems to be highly related with the hardening than with the darkening process, therefore, more variables, such as the contents of Ca^{2+} and Mg^{2+}, need to be quantified in order to support such a hypothesis. In association with the analyses of oxidative stress level, luminosity (color) and the cooking time of the bean grains were assessed. While the color of the grains of BRS Madrepérola and CNFC 10467 remained clear, in comparison to BRS Pontal and BRS Estilo (Table 1), the cooking time was not different between genotypes, with exception of BRS Pontal which had a cooking time 3 times longer than others. The correlation analysis of data ($p \leq 0.05$) showed a moderate negative correlation between the average cooking time and the color of the beans ($r = -0.67$), which means that the higher the cooking time, the darker the grains. The activity of SOD in the tegument, and color (L) of whole grains also presented high negative correlation ($r = -0.93$), suggesting that biochemical events as SOD activity may explain, in part, the process of darkening of the carioca beans. In contrast, the evaluated parameters cannot explain the process of hardening, once there was low significant correlation ($r= -0.25$) in the cotyledon, between the index of lipid peroxidation and cooking time.

Table 1 – Oxidative stress parameters and color/cooking time of freshly harvested bean grains.

Analysis / Genotypes	**Madrepérola**	**BRS Estilo**	**CNFC 10467**	**BRS Pontal**	**Pinto Beans**
			SOD specific activity(Un SOD mg^{-1} proteína)		
Seed Coat	$193,2^{b} \pm 5,7$	$458,5^{a} \pm 2,9$	$158,0^{b} \pm 14,5$	$382,0^{a} \pm 54,4$	$45,1^{c} \pm 13,4$
Cotyledon	$55,9^{c} \pm 2,9$	$44,0^{c} \pm 4,8$	$47,2^{c} \pm 6,0$	$54,1^{c} \pm 12,3$	$57,8^{c} \pm 8,2$
			Hydrogen Peroxide (nmol g^{-1} MF)		
Seed Coat	$13,7^{c} \pm 1,3$	$60,7^{b} \pm 2,6$	$15,72^{c} \pm 0,9$	$8,5^{c} \pm 2,3$	$11,5^{b} \pm 0,1$
Cotyledon	$15,0^{c} \pm 2,5$	$33,9^{a} \pm 3,9$	$17,7^{c} \pm 0,4$	$8,4^{c} \pm 1,1$	$59,6^{b} \pm 7,4$
			MDA equivalents (nmol g^{-1} MF)		
Seed Coat	$10,2^{d} \pm 0,8$	$2,8^{e} \pm 0,4$	$4,4^{b,c} \pm 0,4$	$6,3^{b,c} \pm 0,7$	$6,7^{a,b} \pm 0,7$
Cotyledon	$12^{d,e} \pm 1,3$	$14,7^{e} \pm 3,4$	$13,2^{d,e} \pm 0,5$	$13,2^{d,e} \pm 0,5$	$14,3^{e} \pm 0,5$
Whole Grains			**Color**		
L*	$52,5^{d} \pm 1,3$	$48,9^{b} \pm 1,3$	$51,9^{d} \pm 1,3$	$47,0^{c} \pm 2,1$	$55,6^{a} \pm 2,1$
a*	$7,0^{e} \pm 0,3$	$9,7^{b} \pm 0,3$	$7,7^{d} \pm 0,4$	$10,2^{a} \pm 0,6$	$8,4^{c} \pm 0,6$
b*	$19,3^{c} \pm 0,7$	$20,0^{b} \pm 1,1$	$19,2^{c} \pm 0,9$	$20,4^{b} \pm 0,7$	$21,9^{a} \pm 1,0$
			Cooking time (min)		
	$43,6^{b} \pm 9,7$	$37,1^{b} \pm 3,7$	$46,5^{b} \pm 0,9$	$124,5^{a} \pm 35,9$	$30,8^{b} \pm 6,5$

Results are the mean of three repetitions ± SD. Within lines, means with same superscript are not significantly different by Tukey test ($p>0.05$). MDA = malonaldehyde. FM = fresh matter.

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