Eur Food Res Technol (2013) 236:753–758
DOI 10.1007/s00217-013-1933-x

SHORT COMMUNICATION

Antioxidant enzyme activity and hydrogen peroxide content during the drying of Arabica coffee beans

Mery Y. Rendón · Priscila L. Gratão · Terezinha J.G. Salva · Ricardo A. Azevedo · Neura Bragagnolo

Received: 24 July 2012 / Revised: 24 January 2013 / Accepted: 26 January 2013 / Published online: 2 March 2013

Abstract The development of the germination process and drought stress during the drying of coffee can generate reactive oxygen species, which can be neutralized by way of antioxidant mechanisms. No studies related to antioxidant enzymes during the drying of coffee were found in the literature, and considering their importance, the enzymatic activities of superoxide dismutase (SOD), guaiacol peroxidase (GPOX) and glutathione reductase (GR), and also the hydrogen peroxide content were evaluated during the drying of two types of coffee bean, one processed as natural coffee and the other as pulped natural coffee. The results showed a reduction in the SOD, GPOX and GR enzymatic activities of the natural coffee as compared to the pulped natural coffee during the drying period. Moreover, the hydrogen peroxide content of the natural coffee was greater than that of the pulped natural coffee. These results suggest the development of oxidative stress during the coffee drying process, controlled more efficiently in pulped natural coffee by the early action of GPOX during the drying process. Nevertheless, differential responses by SOD isoenzymes and possibly the role of other peroxidases also appear to be involved in the responses observed.

Keywords *Coffea arabica* · Coffee drying · Antioxidant enzymes · Hydrogen peroxide · Reactive oxygen species

M. Y. Rendón · N. Bragagnolo (✉)
Faculdade de Engenharia de Alimentos, Universidade de Campinas, Campinas, SP 13083-862, Brazil
e-mail: neura@fea.unicamp.br

P. L. Gratão
Departamento de Biologia Aplicada à Agropecuária, Universidade Estadual de São Paulo "Julio de Mesquita Filho", Jaboticabal, SP 14884-900, Brazil

T. J.G.Salva
Instituto Agronômico de Campinas, Centro do Café, Campinas, SP 13012-970, Brazil

R. A. Azevedo
Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP 13418-900, Brazil

Introduction

The coffee drying process is a fundamental aspect and determinant for the quality of the beverage, such that critical care taken cultivation, harvesting and processing of the fruits may be lost if the drying process is not carried out correctly. The moisture content of the beans in freshly picked coffee fruit is about 49–58 %, whereas after drying it drops to approximately 12 % [1, 2].

Coffee fruits can be processed as natural coffee (dried non-pulped fruit), pulped natural coffee (dried pulped fruit) and pulped coffee (dried after fermentation of the pulped fruit). The basic difference among these processes is the preparation of the coffee fruits before the drying phase; for natural coffee, the whole harvested fruits are dried to the desired moisture; for pulped natural coffee (CD), the harvested fruits are peeled and the grains that are covered by the parchment and by most of the mucilage are dried; for pulped coffee, the harvested fruits are also peeled, but the mucilage that covers the grains is hydrolyzed, usually by natural fermentation under water. After the fermentation period, the water is drained and the wet parchment grains are left to dry. Some studies carried out during the drying of coffee beans have shown the development of the germination process and drought stress [3–5]. This means that the respiration process continues in the coffee beans, which could facilitate the generation of reactive oxygen species

(ROS), such as the superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), singlet oxygen (O_2^1) and hydrogen peroxide (H_2O_2), leading to significant damage to the cell structure [6].

Plants have developed enzymatic and non-enzymatic defense systems for the detoxification of ROS. The non-enzymatic system consists of ascorbate, glutathione, alkaloids, phenolics and carotenoids, among others [7], whereas the enzymatic system includes a wide range of enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APX) and glutathione reductase (GR) [7–10], among others.

Several studies have been carried out during drying to evaluate the contents of glutathione, ascorbate, free fatty acids, sugars and γ-aminobutyric acid and also to examine the integrities of the membrane and cell wall [1, 11–14]. However, to the best of our knowledge, studies related to the evaluation of antioxidant enzymes during the drying of coffee beans are not available.

Thus, the aim of the present study was to follow the behavior of some antioxidant enzymes, which have been shown to have a major role in oxidative stress responses, during coffee drying. *Coffea arabica* var. IAC Mundo Novo fruits were harvested and processed as natural coffee (CN) and pulped natural coffee (CD). During the drying process, the SOD, GPOX and GR activities were determined, and also the hydrogen peroxide content. In this study, natural coffee and pulped natural coffee were used because they represent the coffees of Brazil, and their production requires less water than pulped coffee.

Materials and methods

Sample preparation

Ripe *C. arabica* cv. IAC Mundo Novo fruits were picked and selected (5 kg) at the Campinas Agronomic Institute, Campinas, São Paulo, Brazil. Half of the fruits were processed as natural coffee and the other half as pulped natural coffee. The processed coffee samples were sun-dried to approximately 12 % moisture. During the drying period, the temperatures ranged between 20.7 and 38.6 °C and the air moisture between 21 and 62 %.

SOD, GPOX and GR activities and the production of hydrogen peroxide were determined in the beans from the freshly harvested fruits (start of drying) and at four different bean moisture levels. The samples of natural coffee were designated as: CN40, beans at 41 % moisture after 5 days of drying; CN30, beans at 25 % moisture after 6 days of drying; CN20, beans at 15.5 % moisture after 8 days of drying; and CN10, beans at 11.7 % moisture after 13 days of drying. The samples of pulped natural coffee were designated as: CD40, beans at 42.4 % moisture after 2 days of drying; CD30, beans at 29.5 % moisture after 5 days of drying; CD20, beans at 16.4 % moisture after 6 days of drying, and CD10, beans at 11.3 % moisture after 8 days of drying.

Moisture determination

The moisture content was determined by drying 5 g of sample at 105 °C to constant weigh. [15].

Protein extraction and quantification

Approximately 50 g of sample at each moisture content level was frozen in liquid nitrogen and maintained at −80 °C for subsequent protein extraction. The protein was extracted at 4 °C using 2 g of sample, 0.3 g polyvinylpolypyrrolidone (PVPP) and 10 mL 100 mmol L^{-1} potassium phosphate buffer (pH 7.5) containing 10 mmol L^{-1} ethylenediaminetetraacetic acid, 3 mmol L^{-1} dithiothreitol and 57 mmol L^{-1} ascorbic acid. The protein extract was eluted using a PD 10 column (Amersham Biosciences) and kept frozen at −80 °C [16, 17]. The protein content of each extract was determined according to Bradford [18] using bovine serum albumin (BSA) as the standard.

Determination of enzymatic activity

The assays for the determination of all enzyme activities were carried out with the samples prepared as described above and whose protein concentrations were determined by the Bradford method.

Superoxide dismutase activity (SOD) was determined on native PAGE as described by Andrade et al. [19]. The electrophoretic analysis was carried out in 12 % acrylamide gels (w/v) and 50 μg of protein applied to each lane.

Guaiacol peroxidase (GPOX) activity was determined according to Matsuno and Uritani [20] using 50 μL of protein extract. The results were expressed in units (U) where one (1) enzyme activity unit corresponded to an increase of 0.001 in absorbance per min.

Glutathione reductase (GR) activity was determined according to Cia et al. [16] using 50 μL of protein extract, and the results were expressed as μmol min^{-1} mg^{-1} protein.

Hydrogen peroxide quantification

The hydrogen peroxide and other peroxy species that might eventually be present in the extracts were extracted and described previously by Monteiro et al. [21] and quantified in 1 g of sample ground in a mortar with liquid nitrogen

and extracted with 6 mL of 1 % (w/v) trichloroacetic acid and 0.2 g of PVPP. Briefly, after extraction, the samples were centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant was removed and a 0.2 mL aliquot mixed with 0.8 mL 1 mol L^{-1} potassium iodide and 0.2 mL 100 mmol L^{-1} potassium phosphate buffer (pH 7.5). The hydrogen peroxide was quantified spectrophotometrically at 390 nm [22], and the results were expressed as mg 100 g^{-1} dry weight (d.w.).

Statistical analysis

All the determinations were carried out in triplicate and analyzed independently for each coffee (CN and CD) by ANOVA and the Tukey test ($p < 0.05$), using the Statistica software package (v10, Statsoft).

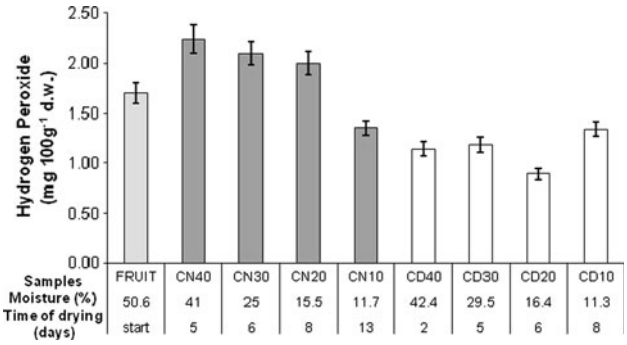

Fig. 1 Hydrogen peroxide content (mg 100 g^{-1}, d.w.) in the natural coffee (CN) and pulped natural coffee (CD) beans during drying: start of drying (Fruit), natural coffee after 5 days of drying (CN40), natural coffee after 6 days of drying (CN30), natural coffee after 8 days of drying (CN20), natural coffee after 13 days of drying (CN10), pulped natural coffee after 2 days of drying (CD40), pulped natural coffee after 5 days of drying (CD30), pulped natural coffee after 6 days of drying (CD20) and pulped natural coffee after 8 days of drying (CD10)

Results and discussion

The coffee beans of the freshly harvested fruits had a moisture content of 50.6 %, which was reduced to 11.7 and 11.3 % in the natural and pulped natural coffees, respectively, by the end of the drying period. These values are within the range used for trading coffee [23].

Some studies reported in the literature have shown the development of the germination process and drought stress during the drying of coffee beans [3–5]. These events represent a level of metabolic activity that could lead to the generation of ROS, including hydrogen peroxide (H_2O_2), which was quantified in the present study not only because it is a ROS and its increased production may indicate an oxidative stress condition, but also due to its important role in cell metabolism as a signaling molecule. A higher concentration of H_2O_2 was found during the drying of natural coffee as compared to that of pulped natural coffee (Fig. 1), but beans from both processes showed lower concentrations at the end of the drying process than at the beginning (fruit). This decrease may be associated with some antioxidant protection mechanism [6]. Hydrogen peroxide production has also been found in other foods such as tea (3.6–4.8 μM mg^{-1} of fresh weight) [24], apple (1.03 μmol g^{-1} of fresh weight), strawberry fruit (2.45 μmol g^{-1} of fresh weight) and spinach (1.98 μmol g^{-1} of fresh weight) [25]. Values of H_2O_2 between 15 and 20 μmol g^{-1} fresh weights for pea and wheat leaves have been reported before a stressful condition, while these values were increased (25–30 μmol g^{-1} fresh weights) after UV-B irradiation [22]. The results found were recalculated in fresh weight to compare with the data of the literature. In this study, H_2O_2 concentrations for CN and CD before the drying process were 250 μmol g^{-1} fresh weight, while after drying, values of 390 and 350 μmol g^{-1} fresh weights, respectively, were obtained.

According to the results of the electrophoretic analysis, the activity of SOD showed an oscillatory behavior during drying, as shown by the intensity of band IV in Fig. 2. Moreover, the enzyme appeared to be more active during the first days of drying in the pulped natural coffee (CD40) than in the natural coffee (CN40), which clearly exhibited a continuous increase in SOD band intensity during drying, indicating alteration of the redox balance and possibly an increase in H_2O_2 production. However, H_2O_2 exhibited a reduction in concentration during the drying process in CN (Fig. 1). These results for CN suggest that the drying process is inducing an oxidative condition based on SOD activity, but the expected H_2O_2 increased production as a result of the increased SOD activity appears to be under control by the action of other peroxidases, such as GPOX (Fig. 3), or even other peroxidases not measured in this study. When SOD activity in CD was concerned (Fig. 2), the trend was completely different from CN samples since SOD activity was higher at CD40 exhibiting a continuous reduction in activity by all four isoenzymes, but mainly SOD IV, at CD30 and CD20, but at CD10, a shift occurred with SOD IV isoenzyme activity recovering the activity level of CD40, while SOD I, II and III activities were reduced (Fig. 2). The results for CD samples appear to suggest that less H_2O_2 was produced during drying, indicating a reduced oxidative condition, nevertheless in the case of CD a high GPOX activity was observed and also varied, opposite to the SOD activity pattern observed (CD10), revealing that GPOX and possibly other peroxidases may have acted to maintain the H_2O_2 levels within the normal cell levels as observed in Fig. 1. Alterations in

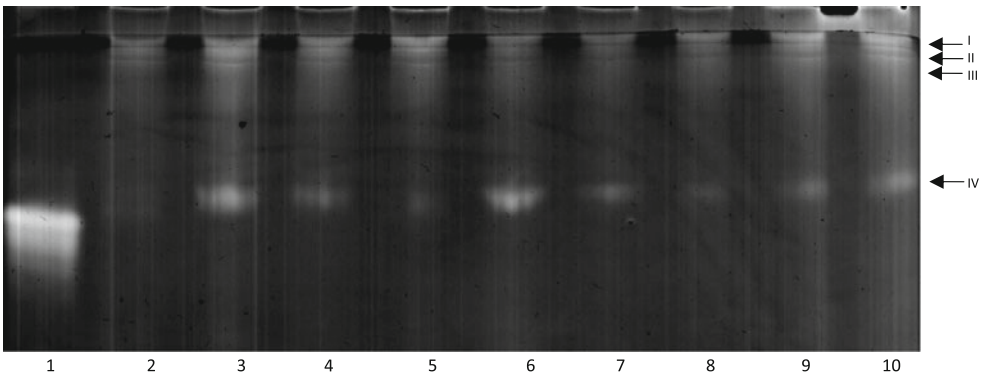

Fig. 2 Superoxide dismutase (SOD) activity in coffee beans processed as pulped natural coffee (CD) and natural coffee (CN) during drying. 1, standard bovine SOD; 2, start of drying (Fruit); 3, pulped natural coffee after 2 days of drying (CD40); 4, pulped natural coffee after 5 days of drying (CD30); 5, pulped natural coffee after 6 days of drying (CD20); 6, pulped natural coffee after 8 days of drying (CD10); 7, natural coffee after 5 days of drying (CN40); 8, natural coffee after 6 days of drying (CN30); 9, natural coffee after 8 days of drying (CN20); 10, natural coffee after 13 days of drying (CN10)

SOD activity also depend on the cellular compartment in which the isoenzymes are located. It has been widely reported in the literature that the different classes of SOD isoenzymes are located in distinct cell organelles so that it may also affect the response to the stress induced [7].

It is also important to comment that the number of SOD isoenzymes (4 bands) in the present study coincided with the number of bands reported in the literature at the end of the drying of coffee beans [26]. However, the number of SOD isoenzymes was significantly smaller than that reported for coffee cell suspension cultures, in which up to 9 distinct isoenzymes were observed [27].

The natural coffee (CN) exhibited GPOX and GR activities throughout the whole drying process (Fig. 3). Although statistically for GPOX activity no changes were observed during the drying period when compared with the sample with 50.6 % moisture, there is a trend for increased GPOX activity up to CN30 which is followed by reduction in activity at CN20 and CN10. On the other hand, a decrease in the GR activity was observed ($p < 0.05$) when comparing the samples with moisture contents of 41 and 11.7 % with those at the start of drying.

Although increases ($p < 0.05$) in both the GR and GPOX activities were observed in the pulped natural coffee during drying, the change in GPOX activity was more pronounced (Fig. 4). As a matter of fact, the changes in GPOX and GR observed for CD are quite clear and exhibiting similar trends although slightly less synchronized in time. For instance, GR activity was quickly increased at CD40 which was then followed by a continuous reduction in activity, whereas for GPOX activity, the increase in activity was more significant already at CD40 continuing up to CD30, which was then followed by a drastic reduction in activity at CD20 and CD10. Although the trend in enzyme activity was similar for both enzymes,

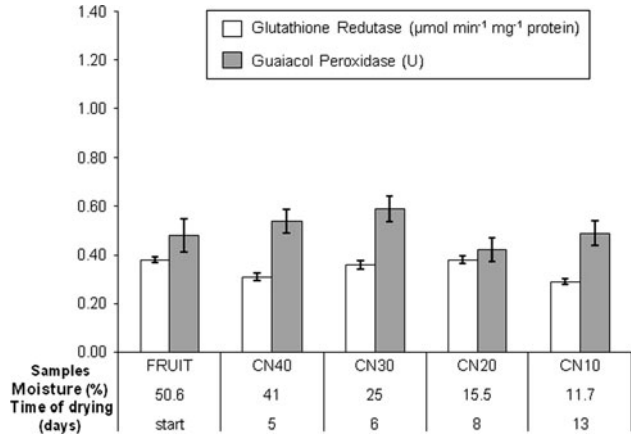

Fig. 3 Glutathione reductase (µmol min^{-1} mg^{-1} protein) and guaiacol peroxidase (U) activities in natural coffee endosperm during drying: start of drying (Fruit), natural coffee after 5 days of drying (CN40), natural coffee after 6 days of drying (CN30), natural coffee after 8 days of drying (CN20), and natural coffee after 13 days of drying (CN10)

these results initially indicate that the response was more rapid and intense for GPOX and that GPOX appears to have a more significant role in the response to the drying process in CD coffee.

The differences in the activities of GR and GPOX between the CN and CD coffees may have been a consequence of the absence of pulp in the CD coffee, favoring the effect of the sun directly on the endosperm during the drying process. A study related to the effect of UV-B illumination and drought on pea and wheat seedlings showed that a greater increase in antioxidant enzyme activity was caused by UV-B light than by drought [22]. In addition, the natural coffee pulp contains enzymes, phenolic compounds, sugars and water [28–30], which would provide different stimuli for the two types of coffee bean

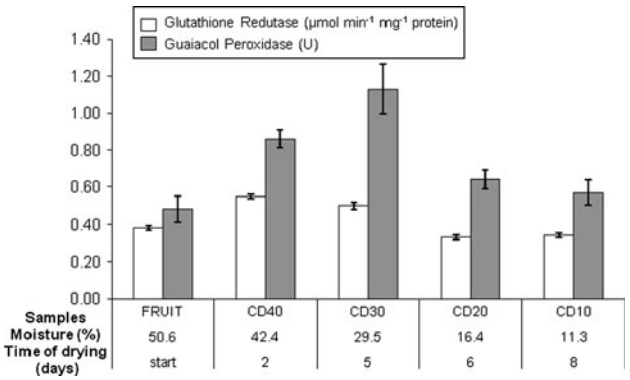

Fig. 4 Glutathione reductase (µmol min^{-1} mg^{-1} protein) and guaiacol peroxidase (U) activities in pulped natural coffee endosperm during drying: start of drying (Fruit), pulped natural coffee after 2 days of drying (CD40), pulped natural coffee after 5 days of drying (CD30), pulped natural coffee after 6 days of drying (CD20) and pulped natural coffee after 8 days of drying (CD10)

during drying, causing different enzymatic behaviors for the CN and CD coffees.

The decrease in GPOX and GR activities at the end of drying in CD samples in relation to CD40 and CD30 may be related to the effect of low moisture in the coffee bean (Fig. 4). Furthermore, the loss of moisture can cause dehydration of the proteins allowing for conformational changes, which can lead to loss of enzymatic function [8]. Another possibility is that due to the loss of water, the bonds between the enzymes and the phenolic compounds increased the molar ratio of protein versus phenolic compounds dramatically, which could inhibit the enzymatic activity by allosteric denaturation [31]. Moreover, the decrease in GR activity at CD20 and CD10 was even reduced to levels below the fruit start control.

The lower concentrations of H_2O_2 measured in the CD coffee during the drying (Fig. 1) may be due to the higher GR and GPOX activities observed. On the other hand, for the CN coffee, the decrease in H_2O_2 content at the end of drying could be explained by the presence of the enzyme catalase (CAT), which was shown to be active in Robusta coffee beans after drying [32]. Moreover, the participation of other peroxidases, such as ascorbate peroxidase, which have not been measured in this study, but could be involved in breaking down H_2O_2, cannot be ruled out. Another alternative for this behavior could be the formation of hydroxyl radicals from H_2O_2, considering the presence of Fe and the possibility of the formation of the superoxide anion during respiration in coffee beans.

The presence of stress markers, such as γ-aminobutyric acid, maximal expression of the dehydrin gene (marker correlated with several types of stress conditions) and losses of glutathione and ascorbate during coffee drying, has been reported in the literature [1, 3, 11]. Such changes in ascorbate and glutathione may be directly related to the changes observed in GR activity in the present study, but also suggest that other enzymes of the ascorbate–glutathione cycle may be affected and should hence be the focus of future investigations.

In conclusion, it appears that the drying process leads to an oxidative stress condition in both, CN and CD, which is better handled in the CD coffee primarily due to the action of GPOX, with a reduction in H_2O_2. Nevertheless, differential responses by SOD isoenzymes and possibly the role of other peroxidases appear to be also involved in the responses observed.

Acknowledgments This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Dussert S, Davey MW, Laffargue A, Doulbeau S, Swennen R, Etienne H (2006) Oxidative stress, phospholipid loss and lipid hydrolysis during drying and storage of intermediate seeds. Physiol Plant 127:192–204
2. Joët T, Laffargue A, Salmona J, Doulbeau S, Descroix F, Bertrand B, de Kochko A, Dussert S (2009) Metabolic pathway in tropical dicotyledonous albuminous seeds: *Coffea arabica* as a case study. New Phytol 182:146–162
3. Kramer D, Breitenstein B, Kleinwächter M, Selmar D (2010) Stress metabolism in green coffee beans (*Coffea arabica* L.): expression of dehydrins and accumulation of GABA during drying. Plant Cell Physiol 51:546–553
4. Selmar D, Bytof G, Knopp S-E, Breitenstein B (2006) Germination of coffee seeds and its significance for coffee quality. Plant Biol 8:260–264
5. Bytof G, Knopp S-E, Kramer D, Breitenstein B, Bergervoet JHW, Groot SPC, Selmar D (2007) Transient occurrence of seed germination processes during coffee post-harvest treatment. Ann Bot 100:61–66
6. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930
7. Gratão PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494
8. França MB, Panek AD, Eleutherio ECA (2007) Oxidative stress and its effects during dehydration. Comp Biochem Physiol 146:621–631
9. Ghelfi A, Gaziola SA, Cia MC, Chabregas SM, Falco MC, Kuser-Falcão PR, Azevedo RA (2011) Cloning, expression, molecular modelling and docking analysis of glutathione transferase from *Saccharum officinarum*. Ann Appl Biol 159:267–280
10. Queiroz CGS, Alonso A, Mares-Guia M, Magalhães AC (1998) Chilling-induced changes in membrane fluidity and antioxidant

enzyme activities in *Coffea arabica* L. roots. Biol Plant 41:403–413
11. Bytof G, Knopp S-E, Schieberle P, Teutsch I, Selmar D (2005) Influence of processing on the generation of γ-aminobutyric acid in green coffee beans. Eur Food Res Technol 220:245–250
12. Knopp S-E, Bytof G, Selmar D (2006) Influence of processing on the content of sugars in green Arabica coffee beans. Eur Food Res Technol 223:195–201
13. Borém FM, Marques ER, Alves E (2008) Ultrastructural analysis of drying damage in parchment Arabica coffee endosperm cells. Biosyst Eng 99:62–66
14. Kleinwächter M, Selmar D (2010) Influence of drying on the content of sugar in wet processed green Arabica coffees. Food Chem 119:500–504
15. MAPA - Ministério da Agricultura Pecuária e Abastecimento (2009) Regras para análise de sementes. Brasília, Brasil
16. Cia MC, Guimarães ACR, Medici LO, Chabregas SM, Azevedo RA (2012) Antioxidant response to water deficit by drought-tolerant and -sensitive sugarcane varieties. Ann Appl Biol 161:313–324
17. Mazzafera P, Robinson SP (2000) Characterization of polyphenol oxidase in coffee. Phytochemistry 55:285–296
18. Bradford MM (1976) Rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
19. Andrade SAL, Gratão PL, Schiavinato MA, Silveira APD, Azevedo RA, Mazzafera P (2009) Zn uptake, physiological response and stress attenuation in mycorrhizal jack bean growing in soil with increasing Zn concentrations. Chemosphere 75:1363–1370
20. Matsuno H, Uritani I (1972) Physiological behavior of peroxidase isoenzymes in sweet potato root tissue injured by cutting or with black rot. Plant Cell Physiol 13:1091–1101
21. Monteiro CC, Carvalho RF, Gratão PL, Carvalho G, Tezotto T, Medici LO, Peres LEP, Azevedo RA (2012) Biochemical responses of the ethylene-insensitive Never ripe tomato mutant subjected to cadmium and sodium stresses. Environ Exp Bot 71:306–320
22. Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344
23. Rojas J (2004) Green coffee storage. In: Wintgens JN (ed) Coffee: growing, processing, sustainable production. Wiley-VCH Verlag GmbH & Co KGaA Press, Weinheim, pp 733–750
24. Subramanian N, Venkatesh P, Ganguli S, Sinkar VP (1999) Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. J Agric Food Chem 47:2571–2578
25. Lu B, Song J, Campbell-Palmer L (2009) A modified chemiluminescence method for hydrogen peroxide determination in apple fruit tissues. Sci Hortic 120:336–341
26. Saath R (2010) Coffee quality natural and washed at different drying conditions and storage time. PhD-thesis Universidade Estadual Paulista "Julio de Mesquita Filho"
27. Gomes-Junior RA, Moldes CA, Delite FS, Pompeu GB, Gratão PL, Mazzafera P, Lea PJ, Azevedo RA (2006) Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. Chemosphere 65:1330–1337
28. Eira MTS, Da Silva EAA, De Castro RD, Dussert S, Walters C, Bewley JD, Hilhorst HWM (2006) Coffee seed physiology. Braz J Plant Physiol 18:149–163
29. Patui S, Braidot E, Peresson C, Tubaro F, Colussi A, Macrí F, Vianello A (2006) Lipoxigenase activity and hydroperoxide formation in coffee (*Coffea arabica* L.) cherries cultivated by different agronomic techniques. In: Proceeding of International Scientific Colloquium on coffee (ASIC), 11–15 September 2006, Montpellier, France, CDROM
30. Ramirez-Martinez JR (1988) Phenolic compounds in coffee pulp: quantitative determination by HPLC. J Sci Food Agric 43:135–144
31. Rawel HM, Rohn S (2010) Nature of hydroxycinnamate-protein interactions. Phytochem Rev 9:93–109
32. Montavon P, Bortlik K (2004) Evolution of Robusta green coffee redox enzymatic activities with maturation. J Agric Food Chem 52:3590–3594