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Changes in the oxidative metabolism of cassava (*Manihot esculenta* Crantz) roots associated with cultivation managements

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Abstract

The sweet cassava root age and pre-harvest managements can modulate the oxidative metabolism and provide a longer shelf life. Moreover, the evaluation of enzymes envolved in oxidative protection regarding the phenolic metabolism can be an additional approach to understand how the cultivation affects the post-harvest physiological deterioration tolerance in sweet cassava roots. Thus, the aim of this study was to evaluate the oxidative enzymatic protection associated to oxidation mediate by PPO and POD, in response to population density and harvest time of sweet cassava roots, cv. "Mossoró". The roots were cultivated in field conditions in the Brazilian Semiarid, under irrigation. The experimental design was a randomized complete block with three replications. Treatments corresponded to four harvest times (240, 300, 360, 420 days after planting - DAP) and four population densities (10,000, 12,500, 15,000 and 17,500 plants ha^{-1}). Sweet cassava roots were sampled and the total soluble phenol content as well as soluble protein content were measured. Also, polyphenol oxidase (PPO), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) activity was evaluated. The roots showed higher total soluble phenol content at 300 and 360 DAP and higher protein content at 240, 300 and 360 DAP, for all densities avaluated. Planting densities extremes, of 10,000 and 17,500 plants ha^{-1}, resulted in higher levels of phenolics compounds and SOD activity. However, did not promote difference in the levels of soluble proteins. There was a significant reduction at PPO, SOD and CAT activity with late harvest, which did not occur for POD activity. Therefore, younger roots have higher oxidative enzymatic protection when compared to older roots in Semiarid conditions.

Keywords: *Manihot esculenta*; oxidative protection; phenolic metabolism; post-harvest physiological deterioration.

Introduction

Sweet cassava (*Manihot esculenta* Crantz) is a crop of worldwide importance, being the primary food source to about 750 million people, secondary only to rice and maize (Silva et al., 2013). Brazil is the fourth largest producer of cassava, behind Nigeria, Thailand and Indonesia (FAO, 2016). According to data from IBGE (2016), cassava production in Brazil reached 23.9 million tons by November 2016, with the highest contributions from the north (42.2%), south (21.9%) and northeast (20.6%) regions.

Much of the Brazilian production can be ruined even before reaching the consumer's table, mainly due to the high post-harvest physiological deterioration (PPD) of roots. PPD can occur within 24 to 48 hours after harvest (Buschmann et al., 2000); then, microbiological deterioration happens, culminating in total decomposition of the roots (Vieites et al., 2012).

PPD is a disorder that occurs in large quantities of edible roots, as well as in other prominent organs such as fruits and vegetables. Browning is the main PPD type in the post-harvest of cassava roots. The symptoms begin as darkened streaks in vascular tissues, reducing the acceptance and quality of the product (Uarrota et al., 2015). The main cause of this process is the oxidation of phenolic compounds by polyphenoloxidase (PPO) and peroxidase (POD) activities, which convert phenols to quinones. The latter, in turn, undergo polymerization, resulting in darkened pigments (Tomás-Barberán and Spín, 2001; Ramos et al., 2013).

Phenol oxidation is closely related to the typical changes in plant response to injury, which is characterized by increased production of reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) (Zidenga et al., 2012). The H_2O_2 is a substrate to POD enzyme in the oxidation of phenols, linking ROS accumulation to browning (Tomás-Barberán and Spín, 2001). In the case of roots, the main process by which ROS are produced is cellular respiration, wich can be potentialized by the presence of cianidric acid (HCN) (Zidenga et al., 2012).

Several studies have demonstrated the importance of the performance of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzymes in the tolerance to oxidative stress. This leads to the elimination of excess ROS, particularly the superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), which can cause irreversible damage to cellular metabolism (Neill et al., 2002).

Xu et al. (2013) demonstrated that overexpression of SOD and CAT enzymes reduced browning rate and prolonged the shelf life of cassava roots. This suggests that tissues with higher activity of these enzymes show less susceptibility to enzymatic browning and, consequently, reduction of PPD intensity. Therefore, in sweet cassava, ROS accumulation is

correlated with the intensification of the enzymatic browning of roots, which is regulated by oxidative defense mechanisms.

Planting density and harvest time have been relatively well studied regarding agronomic characteristics. Studies report that, to a certain extent, increasing population density influences the increase in root yield (Aguiar et al., 2011) and the shape and yield of commercial roots (Silva et al., 2013). Notwithstanding, increased population density favors competition for water, light and nutrients, which may lead to a stress condition for the plant (Maia-Almeida et al., 2011).

Couto (2013) evidenced that root harvest time can modify the activity of PPO and POD enzymes, which act in the plant defense against pathogens attack of and in response to wounding. These enzymes appear to be some of the main involved in browning in cassava roots (Rickard, 1985; Freire et al., 2015; Coelho et al., 2017). Additionaly, there are divergences in the PPD tolerance of different cassava genotypes harvested at 180 (Zidenga et al., 2012), 300 (Canto et al., 2013), and 330 DAP (Venturini, 2015).

Thus, it is believed that the standardization of pre-harvest factors, root age and planting density can help in the identification of PPD-tolerant in roots. Moreover, the enzymes of oxidative protection (SOD and CAT) associated to oxidation mediate by PPO and POD can be an additional tool for understanding the PPD process in sweet cassava roots.

Therefore, the objective of this study was to evaluate the oxidative protection associated to oxidation mediate by PPO and POD, in response to population density and harvest time of sweet cassava roots, cv. "Mossoró". This approach will be an additional tool for agronomic management to minimize PPD.

Results and Discussion

Data analysis showed a significant interaction between the factors, at 5% probability, for total soluble phenols (TSP) and for the enzymes activity: polyphenol oxidase (PPO) peroxidase (POD) and catalase (CAT). The analyses of soluble proteins and SOD activity did not show interaction and their results were presented alone.

Harvest time changed the total soluble phenols and protein content

The TSP contents of the cassava roots presented quadratic behavior, except for the density of 12,500 plants ha^{-1}, where it remained constant (Fig. 1B). Moreover, TSP values were maximum from 300 to 360 DAP (Fig. 1A). This variation may be related to seasonality, since the metabolism of phenylpropanoids and flavonoids was stimulated by lower temperatures (Grace et al., 1998; Rodziewicz et al., 2014). This fact was verified in the present study, in which the highest values of TSP coincided with the time of lower environmental temperatures (Fig. 2).As for the 420 DAP, a redction of TSP was detected, which can be explained as a consequence of the process of root development, since an increase in the fiber content is reported with increasing age in sweet cassava (Cerada, 2005). Thus, probably some soluble phenols are allocated to lignin synthesis pathway (Boerjan, Ralph and Baucher, 2003; Vanholme et al., 2010).

Regarding population density, it was observed a higher content of soluble phenols at the 10,000 plants ha^{-1} and 17,500 plants ha^{-1}densities of, which were statically higher than the other densities (Fig. 1B). As the number of plants per area increases, some plants are expected to demonstrate some stress signs, resulting from intra- and interspecific competition for water, light and nutrients. In cassava, this is evident when related to root yield (Aguiar et al., 2011). In contrast, in the case of secondary metabolism, such response was not predictable, given that in the population density of 10,000 and 17,500 plants ha^{-1}, which represented the situations of least and most competition, respectively, the roots presented higher TSP content (Fig. 1B). This suggests a behavior not yet explained.

In the first three harvest times, the soluble protein content remained around 2.26 mg g^{-1} FW, and decreased about 21.8% at 420 DAP (1.65 mg g^{-1} FW), adjusting to a quadratic model (Fig. 3A). Changes in protein content occur due to changes in synthesis and/or degradation, in this case atributed to root senescence, which consequently affects protein balance (Ishihara et al., 2015). The soluble protein content did not change as a function of the increase in planting density and there was no regression adjustment (Fig. 3B).

In general, these results suggest that very low and high densities (10,000 and 17,500 plants ha^{-1}), in addition to harvests occurring at periods of low ambient temperature, contributed to the highest levels of total phenolic compounds, but do not affect protein levels. In addition, late harvest reduced the levels of soluble proteins.

Enzymes of the phenolic metabolism presented contrasting behavior

PPO activity showed a considerable reduction from 300 to 420 DAP, for all densities (Fig. 4A). In the present study, a 33.7% reduction, in average, was observed with increasing harvest time. This was also observed by Couto (2013). On the other hand, POD activity increased significantly at 420 DAP, when it was 53.6% higher than at 360 DAP (Fig. 5A). This indicates that POD activity was more active in older roots, in contrast to PPO, which was more active in young roots (Fig. 4A and 5A). This suggests that POD activity can be related to lignin synthesis in older roots of sweet cassava (Cantos et al., 2002; Ramos et al., 2013), considering that cassava roots tend to become more fibrous with age progress. As for densities, no difference was observed for POD activity (Fig. 5B).

Freire et al. (2015) observed that PPO and POD activities were the main cause of browning in processed sweet cassava roots. Furthermore, there is the possibility of a synergy between these enzimes, consisting in the production of ROS by PPO, which during the oxidation of some phenolic compounds also produces H_2O_2 (Subramanian et al., 1999; Oms-Oliu et al., 2010). In turn, POD uses the reducing power of phenolic compounds to reduce H_2O_2 so the performance of these enzymes results in oxidation of phenols, cumminating in thehe production of browned compounds and, consequently, PPD (Tomás-Barberán and Espín, 2001).

There is controversy regarding the involvement of PPO activity in browning (Vitti et al., 2011), since for the reaction to occur, a high cell membranes integrity loss is necessary, so that the phenols that are usually located in the vacuole can contact the PPO, placed in plastids (Boeckx et al., 2015). Thus, considering the increase of POD activity (Fig. 5A), it would be reasonable to say that possibly one of the enzymes responsible for PPD in older cassava roots would be POD.

Younger roots have higher oxidative enzymatic protection

The SOD activity decreased with advanced harvest time, which was confirmed by the difference of 20.4% between 240 and 420 DAP (Fig. 6A). Furthermore, SOD activity was statistically higher in the densities of 10,000 and 17,500

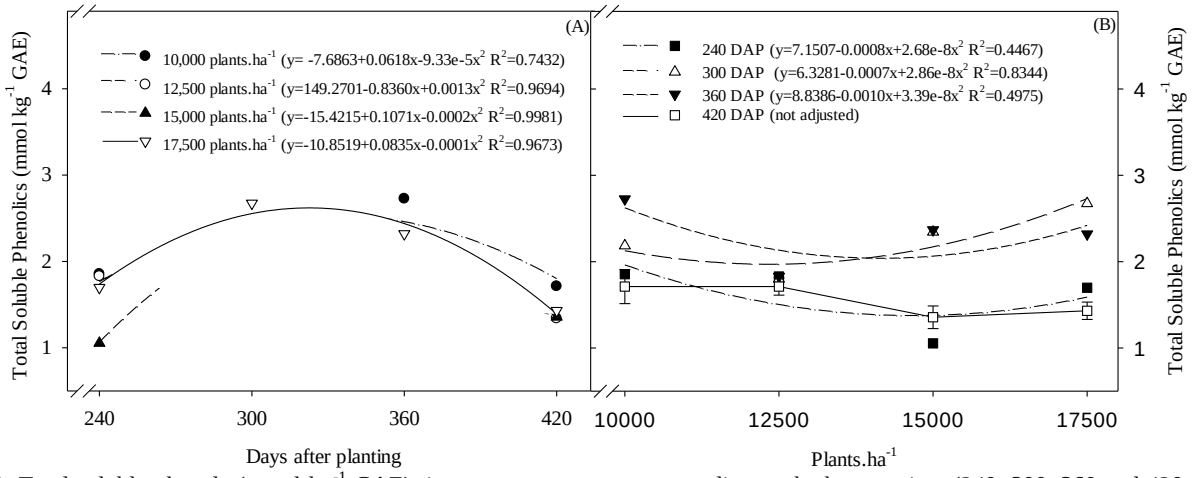

Fig 1. Total soluble phenols (mmol kg^{-1} GAE), in sweet cassava roots, according to the harvest time (240, 300, 360 and 420 days after planting, A) and the population density (10000, 12500, 15000 and 17500 plants ha^{-1}, B). The vertical bars represent the standard deviation from the mean. Data from four replications.

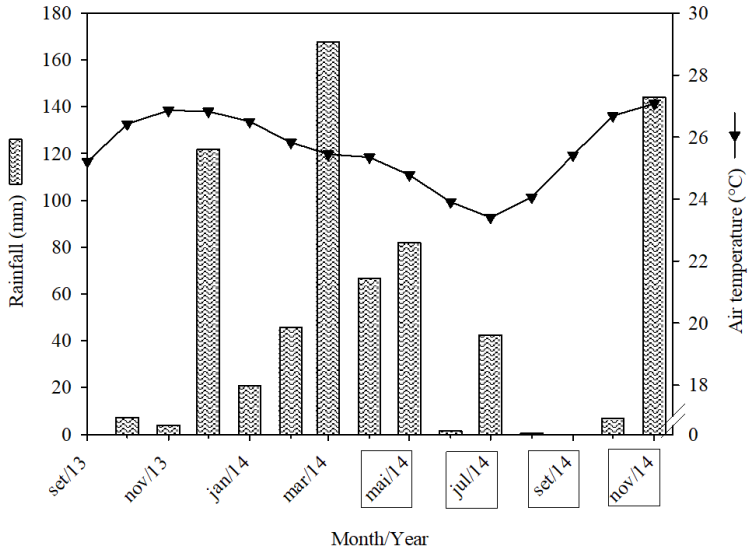

Fig 2. Mean air temperature (°C) and accumulated monthly rainfall (mm), during cultivation of sweet cassava, between September 2013 and November 2014. The rectangle (□) indicates the month of harvest.

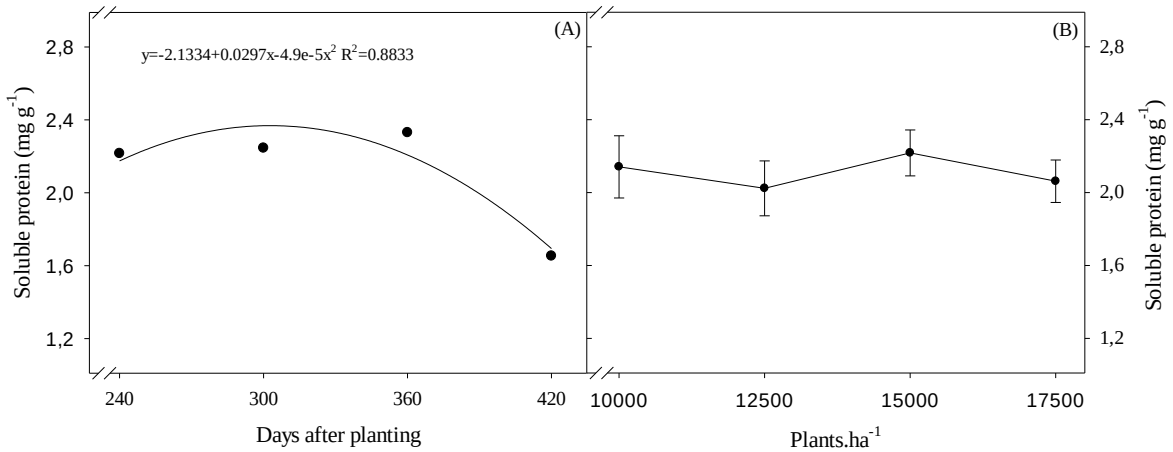

Fig 3. Soluble protein (mg g^{-1}), in sweet cassava roots, according to the harvest time (240, 300, 360 and 420 days after planting, A) and the population density (10000, 12500, 15000 and 17500 plants ha^{-1}, B). The vertical bars represent the standard deviation from the mean. Data from four replications.

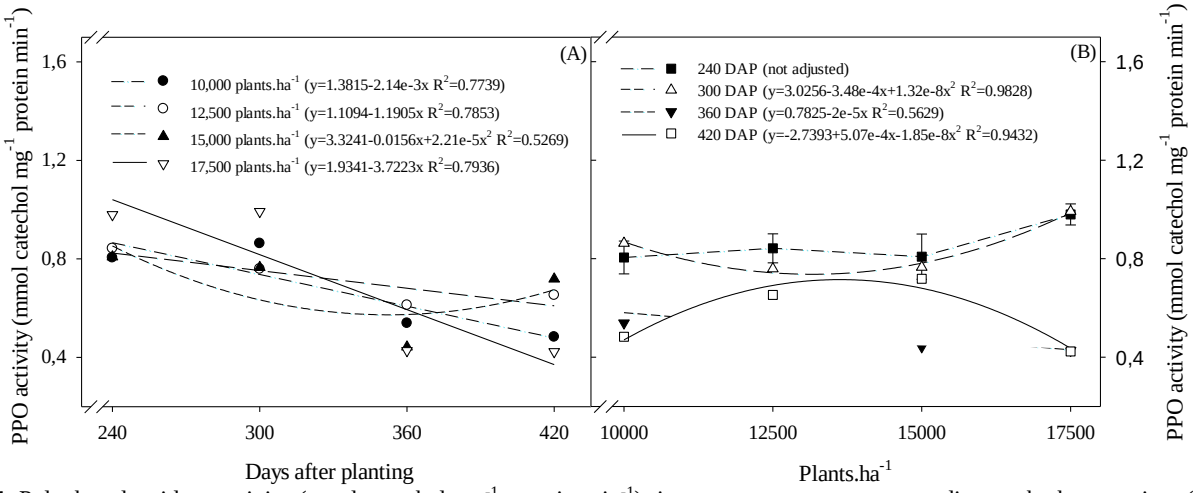

Fig 4. Polyphenol oxidase activity (µmol catechol mg^{-1} protein min^{-1}), in sweet cassava roots, according to the harvest time (240, 300, 360 and 420 days after planting, A) and the population density (10000, 12500, 15000 and 17500 plants ha^{-1}, B). The vertical bars represent the standard deviation from the mean. Data from four replications.

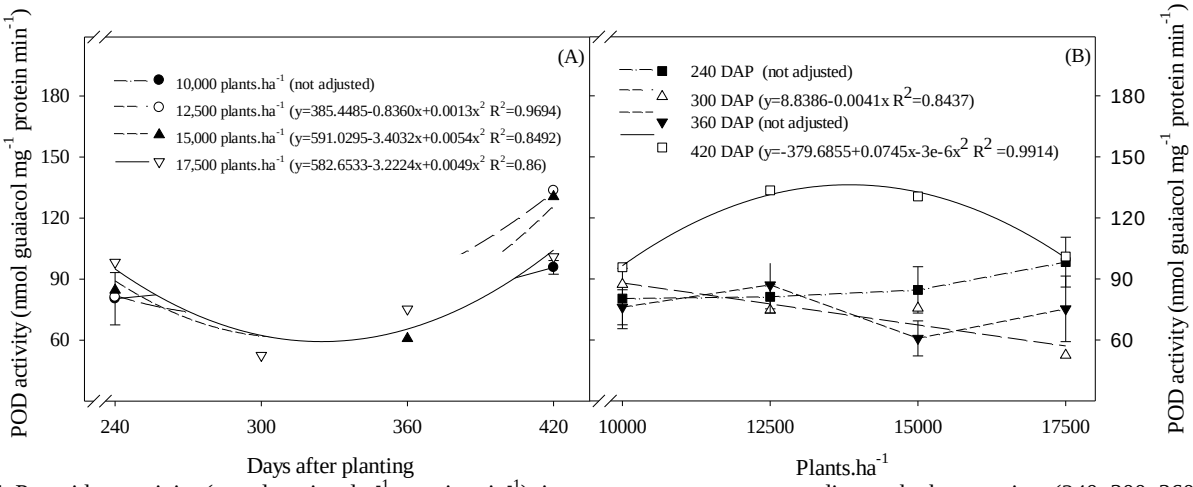

Fig 5. Peroxidase activity (mmol guaiacol g^{-1} protein min^{-1}), in sweet cassava roots, according to the harvest time (240, 300, 360 and 420 days after planting, A) and the population density (10000, 12500, 15000 and 17500 plants ha^{-1}, B). The vertical bars represent the standard deviation from the mean. Data from four replications.

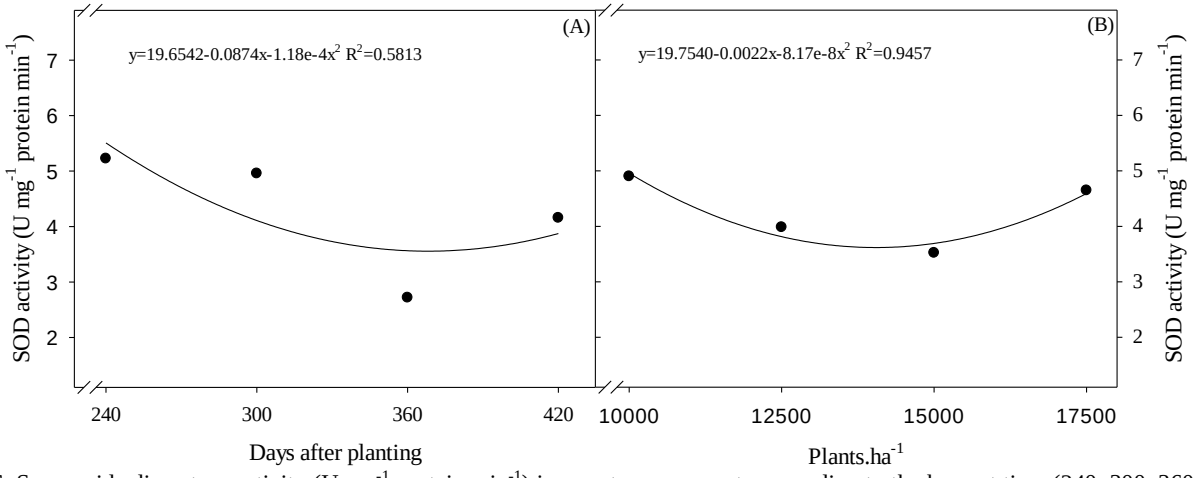

Fig 6. Superoxide dismutase activity (U mg^{-1} protein min^{-1}) in sweet cassava roots, according to the harvest time (240, 300, 360 and 420 days after planting, A) and the population density (10000, 12500, 15000 and 17500 plants ha^{-1}, B). Data from four replications.

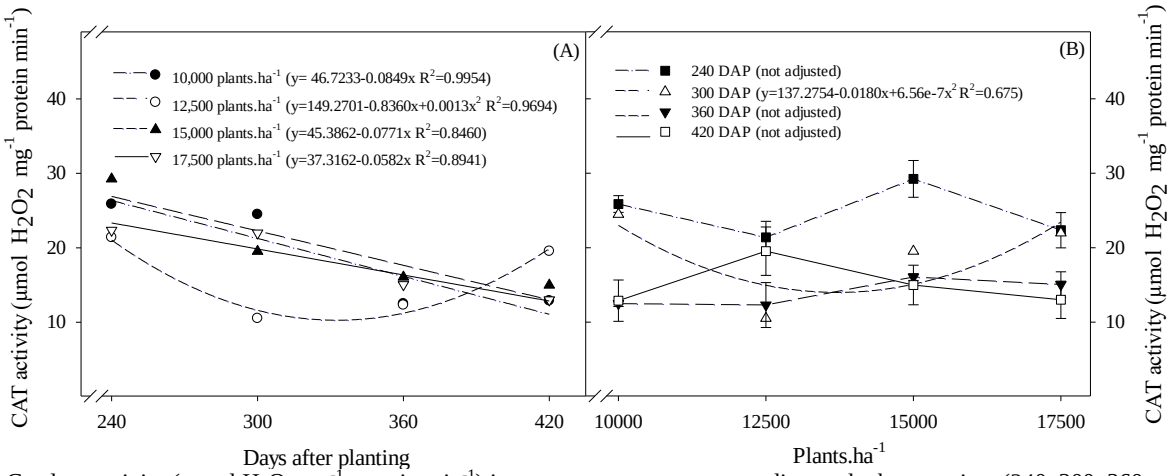

Fig 7. Catalase activity (mmol H_2O_2 mg^{-1} protein min^{-1}) in sweet cassava roots, according to the harvest time (240, 300, 360 and 420 days after planting, A) and the population density (10000, 12500, 15000 and 17500 plants ha^{-1}, B). The vertical bars represent the standard deviation from the mean. Data from four replications.

plants ha^{-1} compared to the other densities (Fig. 6B). This evidenced that both the lowest and highest densities studied, 10,000 and 17,500 plants ha^{-1}, increased the oxidative protection of roots.

Similar to SOD, CAT also reduced its activity with advanced harvest time (Fig. 7A), with a 39.2% reduction in activity at 420 DAP, compared to the first harvest. Regarding population densities, no significant difference was observed for CAT activity (Fig. 7B).

In the present study, the enzymes related to the oxidative protection showed reduced activity as the roots aged, except for POD, which increased its activity at the highest harvest time studied (Fig. 5A). This may imply a greater susceptibility to ROS accumulation, since part of the cellular defense system, represented by SOD and CAT (Ferreira-Silva et al., 2011), presented a activity decrease at the most advanced harvest times (Fig. 6A and 7A). This did not occur in young roots, in which the high PPO activity did not result in browning, possibly because SOD and CAT activities were also higher, evidencing oxidative protection. Additionally, ROS accumulation can provide a favorable environment for phenols oxidation, especially for POD activity, since its role as a connecting point between ROS accumulation and oxidation of phenolic compounds through H_2O_2 has been evidenced.

Hence, it was evident that older roots of sweet cassava presented smaller PPO, SOD and CAT activities, and an oxidative protection supposedly less efficient than younger roots, that presented higher activity for these enzimes. Moreover, POD activity was higher in older roots. This can be associated with lignin synthesis. Therefore, the sweet cassava harvest in a shorter period can result in roots with higher conservation potential and less prone to enzymatic browning.

Materials and Methods

Plant materials and experimental design

The roots of sweet cassava, cv. Mossoró, were cultivated at the experimental station of the Academic Unit of Serra Talhada - UAST, Federal Rural University of Pernambuco - UFRPE. The Unit is in a region classified as BSWh climate, according to the Köppen classification, being characterized by a hot and dry climate, with annual average rainfall of 632 mm, average air temperature of 25.2 °C and relative air humidity of 61% (DCA-UFCG, 2015). The monthly variation of average air temperature and rainfall during the experimental period can be visualized in Fig 2.

The experimental design was a randomized complete block with three replications, with treatments arranged in a subdivided plot. The treatments corresponded to four harvest times (240, 300, 360, 420 days after planting) and four planting densities (10,000, 12,500, 15,000 and 17,500 plants ha^{-1}).

Soil preparation, planting and harvest

Mechanized soil preparation was performed using a subsoiler and plow and disk harrow. Fertilization was done according to the recommendation determined to the state of Pernambuco (IPA, 2008). Then, stem cuttings were distributed at 1 m spacing between rows and 1, 0.8, 0.67 and 0.57 m between plants for the densities of 10,000, 12,500, 15,000 and 17,500 plants ha^{-1}, respectively. The estimate of the daily irrigation need was performed according to the method proposed by Hargreaves-Sarmmani, and distributed through a drip system. Weed control was done manually.
Subsequently, the roots of the useful area were harvested manually at 240, 300, 360 and 420 days after planting (DAP), randomly selected and peeled; then, samples of the root surface tissue were collected and stored at - 80 ° C (Ultrafreezer, - 86 °C, Panasonic/Sanyo).

Total Soluble Phenols and Soluble Protein

The roots were submitted to evaluation of total soluble phenol (TSP) content, determined by spectrophotometric method at 725 nm (spectrophotometer model libra S8; Biochrom), with extraction in concentrated methanol. The reaction used 0.25 N Folin-Ciocalteau's reagent, with results estimated by means of a gallic acid (Freire et al., 2015). The determination of soluble protein content was made according to the methodology proposed by Bradford (1976), in which a solution containing Coomassie Brilliant Blue G-250 (0.01%), ethanol (4.7%) and phosphoric acid (8.5%) promotes a colorimetric reaction quantified in a spectrophotometer at 595 nm, determined by means of a standard curve of BSA (bovine

serum albumin). Both results were expressed on a fresh weight basis.

Polyphenol Oxidase and Peroxidase

Polyphenol oxidase activity (PPO, EC 1.10.3.1) was determined by spectrophotometry at 425 nm and calculated based on the molar extinction coefficient of 3400 $M.cm^{-1}$. Extraction was performed by homogenizing 0.25 g of the tissue in phosphate buffer (0.2 M; pH 6.0), according to the methodology described by Junqueira et al. (2014) and in the presence of liquid nitrogen. Subsequently, the assay was performed, in which the enzymatic extract, in the presence of oxygen, promotes the oxidation of catechol (0.2 M), which causes the color change of the medium, at 25 °C. Peroxidase activity (POD, EC 1.11.17) was determined according to Freire et al. (2015), where the enzyme extract was reacted with guaiacol (40 mM), having H_2O_2 as a substrate (23 mM), at 25 °C. During the reaction, readings were carried out at 470 nm, every 30 seconds, for three minutes. Peroxidase activity was calculated based on the molar extinction coefficient of 26.6 $mM.cm^{-1}$ for guaiacol.

Superoxide Dismutase and Catalase

The evaluation of superoxide dismutase activity (SOD, EC 1.15.1.1) was performed according to Giannopolitis and Ries (1977), in which the enzyme extract was submitted to a medium containing 1.6 ml of 50 mM sodium phosphate buffer (pH 7.8) containing 1 μM EDTA and 13 mM methionine, 40 μl of 1 mM riboflavin and 200 μL of 750 μM nitroblue tetrazolium chloride (NBT). The reaction was performed in a clear chamber, remaining under light incidence (two fluorescent lamps of 18 W) for five minutes. The readings were carried out at 540 nm. The activity was determined based on the inhibition of NBT reduction, a unit of activity being defined as the amount of enzyme required to inhibit 50% of photoreduction (Beauchamp and Fridovich, 1971). Catalase activity (CAT, EC 1.11.1.6) was determined according to Havir and Mchale (1987), by the addition, to the extract, of 50 mM sodium phosphate buffer (pH 7.0) containing H_2O_2 (20 mM). The reaction occurred at 30 °C with readings at 240 nm. Catalase activity was calculated based on the molar extinction coefficient of 36 $M^{-1}.cm^{-1}$ for H_2O_2.

Statistical analysis

Data were submitted to analysis of variance through the SISVAR 5.6 software followed by regression adjustment, when that data was not suitable to regression adjustiment, the means and their standard deviations were presented using SigmaPlot 12.0 Software.

Conclusion

Harvest time progression decrease PPO, SOD and CAT activity as well as reduce soluble proteins but increase POD activity. Thus, younger roots have higher oxidative enzymatic protection than older roots.

Extreme planting densities, of 10,000 and 17,500 plants ha^{-1}, resulted in higher levels of phenolics compounds and SOD activity, and had no influence in soluble proteins levels.

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References

Aguiar EB, Valle TL, Lorenzi JO, Kanthack RAD, Filho HM, Granja NP (2011) Efeito da densidade populacional e época de colheita na produção de raízes de mandioca de mesa. Bragantia. 70(3):561-569.

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 44(1):276-287.

Boeckx T, Winters AL, Webb KJ, Kingston-Smith AH (2015) Polyphenol oxidase in leaves: is there any significance to the chloroplastic localization? J Exp Bot. 66(12):3571-3579.

Bradford MM (1976) A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248-254.

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol. 54(1):519-546.

Buschmann H, Reilly K, Rodriguez MX, Tohme J, Beeching JR (2000) Accumulation of hydroxycoumarins during post-harvest deterioration of tuberous roots of cassava (*Manihot esculenta* Crantz). Ann Bot-London. 86(6):1153-1160.

Canto AR, Fonseca Júnior NS, Beleia A (2013) Alterações químicas e histológicas em mandiocas armazenadas das cultivares catarina amarela e catarina branca. Acta Agron. 62(2):105-113.

Cantos E, Tudela J A, Gil M I, Espín J C (2002) Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. J Agr Food Chem. 50(10):3015-3023.

Cerada MP (2005) Produtos e subprodutos. In: Souza LS, Farias ARN, Mattos PLP, Fukuda WMG. Processamento e utilização da mandioca. Embrapa Mandioca e Fruticultura Tropical, Cruz das Almas, 2005.

Coelho DG, Andrade MT, Mélo-Neto DF, Ferreira-Silva SL, Simões AN (2017) Application of antioxidants and edible starch coating to reduce browning of minimally-processed cassava. Rev Caatinga, 30(2):503-512.

Couto EM (2013) Caracterização de cultivares de mandioca no Semi-Árido Mineiro em quatro épocas de colheita. Tese (doutorado), Universidade Federal de Lavras, 2013.

DCA-UFCG (2015). Departamento de Ciências Atmosféricas – Universidade Federal de Campina Grande. Dados climatológicos do Estado de Pernambuco. Available at: <http://www.dca.ufcg.edu.br/clima/dadospe.htm>, 2015. Accessed on June 26, 2015.

FAO (Food and Agriculture Organization of the United Nations) (2016). Food outlook: biannual report on global food markets. 2016. Available at: < http://www.fao.org/3/a-i6198e.pdf >. Accessed on January 12, 2017.

Ferreira-Silva SL, Voigt EL, Silva EN, JM, Fontenele AV, Silveira JAG (2011) High temperature positively modulates oxidative protection in salt-stressed cashew plants. Environ Exp Bot. 74:162-170.

Freire CS, Simões AN, Barros Júnior AP, Vieira MRS, Ferreira-Silva SL, Silva EF (2015) Activity of oxidative enzymes involved in the browning of minimally processed sweet cassava (*Manihot esculenta* Crantz). Aust J Crop Sci. 9(4):296-302.

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: i. occurrence in higher plants. Plant Physiol. 59(2):309-314.

Grace SC, Logan BA, Adams III WW (1998) Seasonal differences in foliar content of chlorogenic acid, a

phenylpropanoid antioxidante, in *Mahonia repens*. Plant Cell Environ. 21(5):513-521.

Havir EA, Mchale NA (1987) Biochemical and development characterization of multiples forms of catalase in tobacco-leaves. Plant Physiol. 84(2):450-455.

IBGE (Instituto Brasileiro de Geografia e Estatística) (2016). Levantamento Sistemático da Produção Agrícola. Available at: <ftp://ftp.ibge.gov.br/Producao_Agricola/Levantamento_Sistematico_da_Producao_Agricola_%5Bmensal%5D/Fasciculo/lspa_201611.pdf >. Accessed on January 11, 2017.

IPA – Instituto Agronômico de Pernambuco (2008). Recomendações de adubação para o estado de Pernambuco: 2ª aproximação. Ipa, Recife. p 198.

Ishihara H, Obata T, Sulpice R, Fernie AR, Stitt M (2015) Quantifying protein synthesis and degradation in *Arabidopsis* by dynamic 13CO2 labeling and analysis of enrichment in individual amino acids in their free pools and in protein. Plant Physiol. 168(1):74-93.

Junqueira MJ, Simões AN, Sediyama T, Correa PC, Puschmann R (2014) Biochemical and bioactive phytonutrients changes in tissues of two cultivars of fresh-cut cassava in stick form under refrigerated storage. Cienc Rural. 44(7):1284-1290.

Maia-Almeida CI, Ming LC, Marques MOM, Magalhães PM, Queiroz SCN, Scramim S, Mischan MM, Montanari-Jr I, Pereira B, Ferreira MI (2011) Densidade de plantio e idade de colheita de quebra-pedra [Phyllanthus amarus (Schumach. & Thonning) genótipo Unicamp-CPQBA 14] na produtividade de filantina. Rev Bras Plant Med. 13(spe):633-641.

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002) Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot. 53(372):1237-1247.

Oms-Oliu G, Rojas-Graü MA, González LA, Varela P, Soliva-Fortuny R, Hernando MIH, Munuera IP, Fiszman S, Martín-Belloso O (2010) Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: a review. Postharvest Biol Tec. 57(3):139-148.

Ramos PAC, Sedihyama T, Viana AES, Pereira DM, Finger FL (2013) Efeito de inibidores da peroxidase sobre a conservação de raízes de mandioca in natura. Braz J Food Technol. 16(2):116-124.

Rickard JE (1985) Physiological deterioration of cassava roots. J Sci Food Agr. 36:167-176.

Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Plant. 36(1):1-19.

Silva TS, Silva PSL, Braga JD, Silveira LM, Sousa RP (2013) Planting density and yield of cassava roots. Rev Cienc Agron. 44(2):317-324.

Subramanian N, Venkatesh P, Ganguli S, Sinkar VP (1999) Role of poliphenol oxidase and peroxidase in the generation of black tea theaflavins. J Agr Food Chem. 47(7):2571-2578.

Tomás-Barberán FA, Espín JC (2001) Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J Sci Food Agr. 81(9):853-876.

Uarrota VG, Moresco R, Schmidt EC, Bouzon ZL, Costa-Nunes E, Neubert EO, Peruchc LAM, Rocha M, Maraschin M (2016) The role of ascorbate peroxidase, guaiacol peroxidase, and polysaccharides in cassava (*Manihot esculenta* Crantz) roots under postharvest physiological deterioration. Food Chem. 197:737-746.

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol. 153(3):895-905.

Venturini MT, Santos LR, Oliveira EJ (2015) Development of a diagrammatic scale for the evaluation of postharvest physiological deterioration in cassava roots. Pesqui Agropecu Bras. 50(8):658-668.

Vieites RL, Daiuto ER, Carvalho LR, Garcia MR, Lozano MG, Watanabe LM (2012) Mandioca minimamente processada submetida a radiação gama. Semin-Cienc Agrar. 33(1):271-282.

Vitti MCD, Sasaki FF, Miguel P, Kluge RA, Moretti CL (2011) Activity of enzymes associated with the enzymatic browning of minimally processed potatoes. Braz Arch Biol Techn. 54(5):983-990.

Xu J, Duan X, Yang J, Beeching JR, Zhang P (2013) Enhanced reactive oxygen species scavenging by overproduction of superoxide dismutase and catalase delays postharvest physiological deterioration of cassava storage roots. Plant Physiol. 161(3):1517-1528.

Zidenga T, Leyva-Guerrero E, Moon H, Siritunga D, Sayre R (2012) Extending cassava root shelf life via reduction of reactive oxygen species production. Plant Physiol. 159(4):1396-1407.