

# Preharvest treatment with 1-aminoethoxyvinylglycine and gibberellin on the quality and physiology of cashew peduncles

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**Abstract** – The objective of this work was to evaluate the effects of the preharvest treatment with gibberellic acid (GA<sub>3</sub>) and aminoethoxyvinylglycine (AVG) on the quality and physiological attributes of ripe 'CCP 76' cashew (*Anacardium occidentale*) peduncles at different developmental stages. Sprays of 180 mg L<sup>-1</sup> GA<sub>3</sub> and 180 mg L<sup>-1</sup> AVG were applied, combined and isolated, at 34, 40, and 44 days after anthesis (DAA), and peduncles were harvested ripe at 46 DAA and evaluated for physical and physiological variables. The treatment with GA<sub>3</sub> resulted in firmer peduncles with a greater apical diameter, but did not affect the physiological variables activity of the pectin methylesterase and polygalacturonase cell wall enzymes and degree of lipid peroxidation of the biological membranes, nor the physical attributes total mass (peduncles and nut) and nut size. At early developmental stages (34 DAA), GA<sub>3</sub> increased the activity of the antioxidant enzymes superoxide dismutase and catalase, and, at later stages (40 DAA), promoted polyphenol and carotenoid accumulation. The application of AVG at the final stages (44 DAA) provided greater total antioxidant activity, despite the lower ascorbate peroxidase activity. The application of GA<sub>3</sub> pre-harvest increases the firmness and diameter of the cashew peduncles, and the treatment with AVG increases the total antioxidant activity of the peduncles.

**Index terms:** *Anacardium occidentale*, AVG, GA<sub>3</sub>, phenolic compounds.

## Influência do tratamento pré-colheita com 1-aminoetoxivinilglicina e ácido giberélico na fisiologia e na qualidade de pedúnculos de caju

**Resumo** – O objetivo deste trabalho foi avaliar o efeito do tratamento pré-colheita com ácido giberélico (GA<sub>3</sub>) e 1-aminoetoxivinilglicina (AVG) nos atributos de qualidade e fisiológicos de pedúnculos maduros de cajueiro (*Anacardium occidentale*) 'CCP 76', em diferentes estádios de desenvolvimento. Foram realizadas pulverizações com 180 mg L<sup>-1</sup> GA<sub>3</sub> e 180 mg L<sup>-1</sup> AVG, combinadas e isoladas, aos 34, 40 e 44 dias após a antese (DAA), e os pedúnculos foram colhidos maduros aos 46 DAA para avaliações de variáveis físicas e fisiológicas. O tratamento com GA<sub>3</sub> proporcionou pedúnculos mais firmes e com maior diâmetro apical, mas não influenciou as variáveis fisiológicas atividades das enzimas pectinametilesterase e poligalacturonase da parede celular e grau de peroxidação lipídica das membranas biológicas, nem os atributos físicos massa total (pedúnculo e castanha) e comprimento e largura da castanha. Em estádios iniciais (34 DAA), GA<sub>3</sub> aumentou a atividade das enzimas antioxidantes superóxido dismutase e catalase, e, nos finais (40 DAA), promoveu o acúmulo de polifenóis e carotenoides. A aplicação de AVG, ao final do desenvolvimento (44 DAA), proporcionou maior atividade antioxidante, apesar da menor atividade da ascorbato peroxidase. A aplicação de GA<sub>3</sub> em pré-colheita promove aumento da firmeza e do diâmetro dos pedúnculos de cajueiro, e o tratamento com AVG aumenta a atividade antioxidante dos pedúnculos.

**Termos para indexação:** *Anacardium occidentale*, AVG, GA<sub>3</sub>, compostos fenólicos.

### Introduction

Cashew (*Anacardium occidentale* L.) is constituted of a nut (true fruit) and of a fleshy peduncle, also referred to as cashew apple, a non-climacteric pseudofruit, which should be harvested ripe. In Brazil,

its production is concentrated in the dry season, from August to December, in the Northeastern region.

Although the nut is the most relevant economically, the peduncle is a rich source of vitamin C, carotenoids, and phenolic compounds, which contribute to its high antioxidant capacity (Lopes et al., 2012). However,

the short postharvest life of 48 hours, at ambient condition (24°C), represents a major obstacle to the marketing of cashew apples, as the accentuated loss of firmness compromises both their handling and quality (Moura et al., 2010).

In order to extend the postharvest storage period of cashew apples, several technologies have been adopted, such as refrigeration (Moura et al., 2010), gamma radiation (Souza et al., 2009), and postharvest calcium application (Figueiredo et al., 2007). At preharvest, plant growth regulator treatments have been used aiming to delay ripening and preserve postharvest fruit quality, considering that regulators affect plant growth and development, acting as messengers on a broad spectrum of metabolic processes (Marzouk & Kassem, 2011). Different doses of 1-aminoethoxyvinylglycine (AVG) or gibberellic acid (GA<sub>3</sub>), for example, have been applied to peach, banana, and plum, in different developmental stages, to extend their postharvest storage life (Amarante et al., 2005; Huang et al., 2014; Steffens et al., 2011). When applied at preharvest, GA<sub>3</sub> significantly influenced the ripening of both climacteric and non-climacteric fruit, including loquat [*Eriobotrya japonica* (Thunb.) Lindl.], grape (*Vitis vinifera* × *Vitis labrusca*), and sweet cherry [*Prunus avium* (L.) L.] (Mesejo et al., 2010; Zhang & Whiting, 2013). In preharvest treatments, AVG, as an inhibitor of ethylene synthesis, resulted in firmer fruit with higher titratable acidity and soluble solids contents, as well as in a lower incidence of physiological disorders (Steffens et al., 2011).

Although several preharvest treatments with growth regulators have been reported for fruit (Amarante et al., 2005; Huang et al., 2014; Mesejo et al., 2010; Steffens et al., 2011; Zhang & Whiting, 2013), only one was used to evaluate the effects of the preharvest application of growth regulators on cashew cultivars (Souza et al., 2016). In this study, 'CCP 76' and 'BRS 189' cashew were treated with 180 mg L<sup>-1</sup> AVG and GA<sub>3</sub> at maturation stage 1, and peduncles were harvested ripe at stage 7 and then stored under refrigeration for 20 days. The obtained results showed that, during the postharvest storage of cashew peduncles, GA<sub>3</sub> had a greater effect on 'BRS 189' reducing mass and firmness losses, while the effects of AVG on cashew peduncle physiology and quality were inconsistent.

The objective of this work was to evaluate the effects of the preharvest treatment with GA<sub>3</sub> and AVG on the quality and physiological attributes of ripe 'CCP 76' cashew peduncles at different developmental stages.

## Materials and Methods

The study was conducted at the experimental station of Embrapa Agroindústria Tropical, located in Pacajus, in the state of Ceará, Northeastern Brazil (4°11'27"S, 38°29'51"W). The soil of the experimental area is classified as a Neossolo quartzarênico, i.e., a Typic Quartzipsamment, according to the Brazilian system of soil classification (Santos et al., 2013). Early 'CCP 76' cashew trees with 20 years of age were spaced at 6x4 m in the experimental area, which had an annual average rainfall of 652 mm.

A preliminary preharvest experiment was carried out to evaluate the effects of different AVG and GA<sub>3</sub> concentrations (60, 120, and 180 mg L<sup>-1</sup>) on cashew apple physical attributes. Since the dose of 180 mg L<sup>-1</sup> resulted in firmer ripe peduncles, which is one of the main factors limiting the postharvest life of cashew peduncles, 150 mg g<sup>-1</sup> AVG and 100 mg g<sup>-1</sup> GA<sub>3</sub> of the commercial products ReTain (Valent BioSciences, Libertyville, IL, USA) and ProGibb (Sumitomo Corporation, Tokyo, Japan), respectively, were diluted in water plus surfactant 0.5% Tween 20 (v/v) to a concentration of 180 mg L<sup>-1</sup>, which was then manually applied with a backpack sprayer onto the canopy of the trees, at an amount enough to flow thoroughly over the entire plant. Two hundred trees were divided into six treatment plots; plots on the same row were separated by at least one tree, and rows of treated trees were separated by an untreated row, in order to avoid drift effects. The experiment was carried out in a completely randomized design, consisting of six treatments with three replicates of 15 fruit each.

The treatments consisted of the applications of 180 mg L<sup>-1</sup> AVG and GA<sub>3</sub> on cashew trees at different developmental stages after anthesis (DAA): T1, GA<sub>3</sub> at 34 DAA; T2, GA<sub>3</sub> at 34 DAA + AVG at 44 DAA; T3, GA<sub>3</sub> at 40 DAA; T4, GA<sub>3</sub> at 40 DAA + AVG at 44 DAA; T5, AVG at 44 DAA; and a control, without GA<sub>3</sub> and AVG.

At 46 DAA, ripe orange-colored cashew peduncles at stage 7, according to Lopes et al. (2012), from all treatments, were manually harvested and transported to the laboratory, where they were selected based on color homogeneity (dark-orange peduncle), size, and absence of defects. Cashew peduncles were immediately evaluated for physical attributes and then processed with the RI6720 Walita domestic centrifuge (Philips do Brasil Ltda – Divisão Walita, Varginha, MG, Brazil). The pulp was stored at  $-18^{\circ}\text{C}$ , until analyzes for variables associated with firmness and antioxidant metabolism.

Total mass (nut with peduncle) was determined using the Mark 3100 analytical scale (Bel Engineering, Monza, Italy) and was expressed in grams. Peduncle and nut sizes were measured with the Ultra-Cal Mark III digital caliper (Sylvac/Fowler, Crissier, Switzerland), and results were expressed in millimeters.

Firmness was evaluated five times on opposite sides of each peduncle with the Magness-Taylor FT-011 manual penetrometer (Instron, Norwood, MA, USA) using an 8-mm diameter cylindrical flat-tipped steel plunger, and results were expressed in Newton.

Biological membrane integrity was estimated by the degree of lipid peroxidation, which was determined by the formation of malondialdehyde (MDA), based on the method described by Zhu et al. (2008). Absorbance at 532 nm was measured, corrected for unspecific turbidity by subtracting from absorbance at 600 nm, and MDA content – expressed as nmol MDA  $\text{g}^{-1}$  fresh weight (FW) – was calculated using an extinction coefficient of  $155 \text{ nmol cm}^{-1}$ .

Cell wall integrity was evaluated through the specific activities of cell wall hydrolases. Polygalacturonase (PG, E.C. 3.2.1.15) activity was assessed in 12-g samples homogenized with 25 mL ice-cold water. The homogenate was filtered through Whatman No. 1 filter paper and centrifuged at 3,248 g, for 10 min, at  $4^{\circ}\text{C}$ ; the precipitate was suspended in 10 mL distilled water and centrifuged as before. The reaction mixture was incubated for 3 hours at  $30^{\circ}\text{C}$ , followed by a boiling water bath to stop the reaction. The liberated reducing groups were determined (Pressey, 1986), and results were expressed as unit of enzyme activity (UA)  $\text{mg}^{-1}$  protein.

For the pectin methylesterase (PME, EC 3.1.1.11) assay, 5 g pulp were extracted by homogenization with 20 mL cold NaCl ( $0.2 \text{ mol L}^{-1}$ ) and then filtered through Whatman No. 1 filter paper; the filtrate was used as enzyme extract (Hagerman & Austin, 1986). Enzyme activity was measured in 5 mL of the extract plus 30 mL of 1% citrus pectin substrate in  $0.2 \text{ mol L}^{-1}$  NaCl (pH 7.0) titrated with 0.01 N NaOH. One unit of activity was defined as the amount of enzyme capable of removing a methyl group from pectin and was expressed as UA  $\text{mg}^{-1}$  protein.

Total protein content was obtained according to Bradford (1976) using bovine serum albumin as standard and was expressed as  $\text{mg g}^{-1}$  FW. Protein content was used to calculate the specific enzyme activity.

To determine bioactive antioxidant compounds and total antioxidant activity (anthocyanins and yellow flavonoids), 1 g pulp was extracted with a 95% ethanol:1.5 N HCl (85:15) solution, vortexed for 2 min, and then brought to 50 mL with the extracting solution. The mixture was protected from the light, refrigerated at  $4^{\circ}\text{C}$  for 12 hours, and filtered in Whatman No. 1 paper. The absorbance of the filtrate was measured at 535 nm for total anthocyanin content, using an absorption coefficient of  $98.2 \text{ mol cm}^{-1}$ , and at 374 nm for total yellow flavonoid content, using an absorption coefficient of  $76.6 \text{ mol cm}^{-1}$ ; both results were expressed as  $\text{mg } 100 \text{ g}^{-1}$  FW.

Total phenolics were determined with a colorimetric assay using the Folin-Ciocalteu reagent according to Obanda et al. (1997), while extracts were prepared as in Larrauri et al. (1997). Samples of 3 g each were homogenized in 4 mL methanol (50%) and allowed to stand in the dark, at room conditions, for 1 hour, before centrifugation at 4,000 g, for 30 min, at  $4^{\circ}\text{C}$ . The supernatant was collected, and the precipitate was extracted with 4 mL acetone (70%) under conditions similar to those previously described. After centrifugation, supernatants were joined and total volume was completed to 10 mL with distilled water. Extracts of 100  $\mu\text{L}$  were added to 100  $\mu\text{L}$  Folin-Ciocalteu reagent, 1 mL  $\text{Na}_2\text{CO}_3$  (20%), and 1  $\mu\text{L}$  distilled water, and were allowed to stand for 30 min, in the dark. Absorbance was measured at 700 nm, and results were expressed as gallic acid equivalents  $\text{mg } 100 \text{ g}^{-1}$  FW.

Total vitamin C was determined by titration with a 0.02% 2,6-dichloro-indophenol solution (Strohecker et al., 1967). One gram of pulp was diluted to 50 mL 0.5% oxalic acid and homogenized, and 2 mL of this solution were diluted to 50 mL with distilled water and then titrated. Results were expressed as mg 100 g<sup>-1</sup> FW.

Total carotenoids were extracted and determined as described by Higby (1962). Five grams of pulp were homogenized in 30 mL isopropyl alcohol and 10 mL hexane. The content was transferred to a separation funnel of 125 mL completed with distilled water, and it was let to rest for 30-min periods followed by subsequent filtrations for thrice. Absorbance was measured at 450 nm, and results were expressed as mg 100 g<sup>-1</sup> FW.

Total antioxidant activity (TAA) was determined using the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation method according to Re et al. (1999). Before the colorimetric assay, the samples were subjected to extraction in 50% methanol and 70% acetone as described by Larrauri et al. (1997). Once the radical was formed, the reaction was started by adding 30 µL extract in 3 mL radical solution, absorbance was measured at 734 nm after 6 min, and the decrease in absorption was used to calculate TAA. A calibration curve was prepared, and different Trolox concentrations (standard Trolox solutions ranging from 100 to 2,000 µmol L<sup>-1</sup>) were also evaluated against the radical. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC), i.e., µmol TEAC g<sup>-1</sup> FW.

The specific activity of antioxidant enzymes was also assessed. The extracts for antioxidant enzyme activity were prepared as in Yang et al. (2008). One gram of pulp was homogenized during 5 min with 10 mL phosphate buffer (0.1 mol L<sup>-1</sup>, pH 7) containing 0.1 mmol L<sup>-1</sup> ethylenediaminetetraacetic acid, filtered through Whatman No. 1 paper, and let to rest for 1 hour, prior to centrifugation at 12,000 g, for 15 min, at 4°C. The supernatant was then collected and used as enzyme extract.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to Giannopolities & Ries (1977), based on the inhibition of the photochemical reduction of nitroblue tetrazolium chloride (NBT). Absorbance was measured at 560 nm, and one unit of SOD activity

was defined as the amount of enzyme required to cause a 50% reduction in the NBT photoreduction rate; results were expressed as UA mg<sup>-1</sup> protein.

Catalase (CAT, EC 1.11.1.6) activity was measured using the method described by Beers & Sizer (1952). The reaction was started by adding the enzyme extract, and the decrease in H<sub>2</sub>O<sub>2</sub> was monitored through absorbance at 240 nm and quantified by its molar extinction coefficient (36 mol L<sup>-1</sup> cm<sup>-1</sup>). Results were expressed as µmol H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> protein.

Ascorbate peroxidase (APX, EC 1.11.1.1) activity was assessed according to Nakano & Asada (1981). The reaction was started by adding ascorbic acid, and ascorbate oxidation was measured by recording the absorbance readings at 290 nm. The APX activity was measured using the molar extinction coefficient for ascorbate (ε 290 = 2.8 mmol L<sup>-1</sup> cm<sup>-1</sup>), and results were expressed in µmol H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> protein.

Data were subjected to the analysis of variance using the Sisvar computer software, version 5.3 (Ferreira, 2011), and the averages were compared by Tukey's test, at 5% probability.

## Results and Discussion

Combined or isolated GA<sub>3</sub> and AVG preharvest treatments did not significantly affect some of the evaluated physical attributes of 'CCP 76' cashew, such as total cashew (nut and peduncle) mass, peduncle mass, peduncle length, and nut length (Table 1). However, nut mass and width were lower in T2, T3, and T4, when compared with the control, which presented mass of 9.34 g and width of 26.4 mm. T1 significantly increased the peduncle apical diameter (51.24 mm) in comparison with the control (45.9 mm), which did not differ from the other treatments. It was reported that the active gibberellins GA<sub>1</sub> and GA<sub>3</sub> play an essential role in the growth regulating expansion of recently divided cells, influencing fruit size (Jong et al., 2009; Zhang & Whiting, 2013). This could explain the results found for the T1 treatment at an earlier developmental stage, which induced an increase in the peduncle apical diameter. Other fruits also had their size affected by treatments with gibberellins. Rufini et al. (2008), for example, observed that 20 mg L<sup>-1</sup> GA<sub>3</sub> promoted an increase in 'Ponkan' mandarin (*Citrus*

*reticulata* Blanco) diameter, while GA<sub>3</sub> concentrations from 250 to 1,000 mg L<sup>-1</sup> led to proportional increases in 'Gefner' atemoya (*Annona cherimola* Miller x *Annona squamosa* L.) size (Pereira et al., 2014).

Cashew peduncles subjected to GA<sub>3</sub> treatments (T1–T4) were significantly firmer, over 20%, than the control, whose value was 16.9 N, which did not differ from that of T5 (Table 2). Firmness is an important quality attribute of fresh fruits and may be affected by different factors, such as turgor, starch content, cell wall enzymes, and integrity of biological membranes (Jacomino et al., 2010). Previous studies with strawberry (*Fragaria x ananassa* Duch.) and Chilean strawberry [*Fragaria chiloensis* (L.) Mill.], which are non-climacteric fruit, subjected to exogenous GA<sub>3</sub> and abscisic acid treatments, resulted in increased firmness through the activation of the expression of the *FaXTH1* and *FaXTH2* genes (Opazo et al., 2013; Nardi et al., 2014). Ferri et al. (2002) proposed that the effect of gibberellin on the maintenance of fruit firmness

could be related to a reduction in the production of ethylene, a key hormone responsible for the ripening of climacteric fruits and, according to Steffens et al. (2009), also of non-climacteric fruits, as observed for cashew apple in the present study.

The lipid peroxidation degree of 'CCP 76' peduncles was not significantly influenced by the GA<sub>3</sub> and AVG treatments (Table 2). However, this variable may be an indicator of biological membrane integrity, which affects firmness. Moreover, the activities of the cell-wall hydrolases PME and PG were also not influenced by growth regulators in the peduncle. This shows that the effects of GA<sub>3</sub> in the T1–T4 treatments on cashew peduncle firmness may not be associated with tissue disintegration; however, it may be explained by other factors such as turgor control. Souza et al. (2016) reported that GA<sub>3</sub> positively affected 'BRS 189' cashew peduncles, which showed, besides lower loss of mass and firmness, a better visual appearance and

**Table 1.** Mean values of physical variables of 'CCP 76' cashew (*Anacardium occidentale*) nut and peduncles treated with 180 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) and 180 mg L<sup>-1</sup> aminoethoxyvinylglycine (AVG), isolated or combined, at different days after anthesis (DAA)<sup>(1)</sup>.

Treatment	Total mass (g)	Nut mass (g)	Nut length (mm)	Nut width (mm)	Peduncle mass (g)	Peduncle apical diameter (mm)	Peduncle length (mm)
Control	135.77a	9.34a	33.75a	20.49a	126.43a	45.98b	64.38a
T1 – GA <sub>3</sub> at 34 DAA	131.93a	8.59ab	33.78a	19.53ab	123.34a	51.24a	61.52a
T2 – GA <sub>3</sub> + AVG at 34 DAA	131.12a	7.90b	33.55a	19.21b	123.22a	48.25ab	64.31a
T3 – GA <sub>3</sub> at 40 DAA	136.59a	8.33b	33.96a	19.31b	128.26a	49.02ab	63.56a
T4 – GA <sub>3</sub> + AVG at 40 DAA	127.90a	8.23b	33.75a	19.26b	119.67a	47.98ab	60.94a
T5 – AVG at 44 DAA	122.77a	8.58ab	34.20a	19.67ab	114.19a	47.30ab	59.21a

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability.

**Table 2.** Firmness and associated variables of 'CCP 76' cashew (*Anacardium occidentale*) peduncles treated with 180 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) and 180 mg L<sup>-1</sup> aminoethoxyvinylglycine (AVG), isolated or combined, at different days after anthesis (DAA)<sup>(1)</sup>.

Treatment	Firmness (N)	Lipid peroxidation (nmol g <sup>-1</sup> )	PME activity (UA mg <sup>-1</sup> min <sup>-1</sup> protein)	PG activity (UA mg <sup>-1</sup> min <sup>-1</sup> protein)
Control	16.92b	36.24a	1657.43a	7.80a
T1 – GA <sub>3</sub> at 34 DAA	20.78a	76.20a	2438.31a	11.01a
T2 – GA <sub>3</sub> + AVG at 34 DAA	20.93a	57.37a	2458.72a	6.09a
T3 – GA <sub>3</sub> at 40 DAA	20.63a	45.67a	2521.94a	6.52a
T4 – GA <sub>3</sub> + AVG at 40 DAA	20.37a	42.72a	2042.48a	7.06a
T5 – AVG at 44 DAA	18.75ab	73.52a	2464.22a	8.97a

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. PME, pectin methylesterase; and PG, polygalacturonase.

lower cell-wall hydrolase PME activity, during 20 days under refrigerated storage.

The effects of AVG and GA<sub>3</sub> on the antioxidant compound contents and total antioxidant activity of 'CCP 76' peduncles varied (Table 3). Anthocyanins, yellow flavonoids, and vitamin C contents did not differ between the AVG and GA<sub>3</sub> treatments and the control, which presented contents of 0.03, 0.31, and 319.41 mg 100 g<sup>-1</sup> FW, respectively. However, the T1, T2, T3, and T5 treatments resulted in significantly greater total polyphenol contents; T3 presented the highest value of 224.19 g<sup>-1</sup> FW, which was >14% greater than the control and T4. The increase in total phenolics is probably due to the presence of other phenolics than anthocyanin and yellow flavonoids, whose values did not change. Contrasting effects of gibberellin on polyphenols were observed in other fruit, including 'Muscat' grapes (*Vitis vinifera* L.), in which 100 mg L<sup>-1</sup> GA<sub>3</sub> induced a reduction in phenolic compounds and total antioxidant activity in the pulp and peel (Tian, 2014), and

'Barhee' date palms (*Phoenix dactylifera* L.) treated with 50 and 100 mg L<sup>-1</sup> GA<sub>3</sub>, which presented total antioxidant activity 18% lower than that of the control (Mohamed et al., 2014).

Total carotenoid content in peduncles was higher (0.21 mg 100 g<sup>-1</sup> FW) in T3 and lower (0.13 mg 100 g<sup>-1</sup> FW) in T4 and T5, which showed similar results. Regarding total antioxidant activity, only T5 presented a value higher (20.53 μmol TEAC g<sup>-1</sup> FW) than that of the control, with 12.48 μmol TEAC g<sup>-1</sup> FW. Lopes et al. (2012) reported that the polyphenol contents of 'CCP 76' peduncles reduced during ripening and that they were strongly correlated to total antioxidant activity; however, except for T5, such association could not be evidenced in the present work.

Regarding the activities of antioxidant enzymes (Table 4), SOD activity in the peduncles was higher (916.40 UA mg<sup>-1</sup> protein) in T1, while the control showed a value of 564.14 UA mg<sup>-1</sup> protein. CAT activity was also higher (66.13 μmol H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup>) in the

**Table 3.** Antioxidant compounds and total antioxidant activity of 'CCP 76' cashew (*Anacardium occidentale*) peduncles treated with 180 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) and 180 mg L<sup>-1</sup> aminoethoxyvinylglycine (AVG), isolated or combined, at different days after anthesis (DAA)<sup>(1)</sup>.

Treatment	Total anthocyanins (mg 100 g <sup>-1</sup> )	Yellow flavonoids (mg 100 g <sup>-1</sup> )	Total polyphenols (mg 100 g <sup>-1</sup> )	Total vitamin C (mg 100 g <sup>-1</sup> )	Total carotenoids (mg 100 g <sup>-1</sup> )	Antioxidant activity (μmol TEAC g <sup>-1</sup> )
Control	0.03a	0.31a	164.67c	319.41a	0.18bc	12.48b
T1 – GA <sub>3</sub> at 34 DAA	0.09a	0.51a	206.34ab	322.08a	0.18bc	10.72b
T2 – GA <sub>3</sub> + AVG at 34 DAA	0.05a	0.38a	188.69b	352.01a	0.19b	9.64b
T3 – GA <sub>3</sub> at 40 DAA	0.03a	0.41a	224.19a	362.23a	0.21a	16.51ab
T4 – GA <sub>3</sub> + AVG at 40 DAA	0.04a	0.35a	161.49c	326.31a	0.13c	15.84ab
T5 – AVG at 44 DAA	0.03a	0.36a	199.44ab	330.36a	0.14bc	25.03a

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. TEAC, Trolox equivalent antioxidant activity.

**Table 4.** Activities of antioxidant enzymes of 'CCP 76' cashew (*Anacardium occidentale*) peduncles treated with 180 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) and 180 mg L<sup>-1</sup> aminoethoxyvinylglycine (AVG), isolated or combined, at different days after anthesis (DAA)<sup>(1)</sup>.

Treatment	SOD activity (UA mg <sup>-1</sup> protein)	CAT activity (μmol H <sub>2</sub> O <sub>2</sub> mg <sup>-1</sup> min <sup>-1</sup> protein)	APX activity (μmol H <sub>2</sub> O <sub>2</sub> mg <sup>-1</sup> min <sup>-1</sup> protein)
Control	564.14d	46.82ab	0.15a
T1 – GA <sub>3</sub> at 34 DAA	916.40a	66.13a	0.16a
T2 – GA <sub>3</sub> + AVG at 34 DAA	565.02d	36.27b	0.12ab
T3 – GA <sub>3</sub> at 40 DAA	574.76d	40.15b	0.18a
T4 – GA <sub>3</sub> + AVG at 40 DAA	647.61c	36.70b	0.16a
T5 – AVG at 44 DAA	746.07b	48.67ab	0.07b

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. SOD, superoxide dismutase; CAT, catalase; and APX, ascorbate peroxidase.

peduncles subjected to T1, and APX activity was lower ( $0.07 \mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) in those treated with T5, when compared with the control, which showed a value of  $0.15 \mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$ . Ding et al. (2015) pointed out that  $0.20 \text{ mmol L}^{-1}$  gibberellins induced a reduction in lipid peroxidation degree and an increase in SOD activity, during 28 days of cold storage of cherry tomato (*Solanum lycopersicum* L.). Saeed et al. (2014) also found that the application of exogenous  $\text{GA}_3$  enhanced SOD activity during the storage of cut gladiolus flower (*Gladiolus hortulanus* L.H.Bailey).

The coordinated action of enzymes and non-enzymatic antioxidants is necessary to neutralize reactive oxygen species and, therefore, protect biological membranes from oxidative damage induced by adverse environmental conditions or by developmental processes, as ripening. This is an indicative that a greater antioxidant potential may result in the improvement of postharvest fruit quality.

### Conclusions

1. The gibberellic acid ( $\text{GA}_3$ ) preharvest treatment of 'CCP 76' cashew (*Anacardium occidentale*) peduncles at 34 days after anthesis (DAA) increases ripe peduncle apical diameter without any negative effects on nut mass and size, enhances fruit firmness and total polyphenol content, and induces higher activities of the antioxidant enzymes superoxide dismutase and catalase.

2. At 40 DAA, the treatment with  $\text{GA}_3$  increases total carotenoid and polyphenol contents of ripe 'CCP 76' cashew peduncles.

3. The aminoethoxyvinylglycine preharvest treatment of 'CCP 76' cashew peduncles at 44 DAA induces a greater total antioxidant activity in the ripe peduncles, despite the lowest ascorbate peroxidase enzymatic activity.

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