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Scientific Article

Morphological and biochemical changes in squash fruits with fruiting induced by cytokinin and auxin

Alterações morfológicas e bioquímicas em abóbora com frutificação induzida por citocinina e auxina

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ABSTRACT - The use growth regulators from the cytokinins and auxins group has been shown to increase the productivity of certain crops, as they may be involved in the expression of plant sex, although little is known about the action of auxin IBA and cytokinin CPPU on the fruiting of squash, as well as the interaction between both, which makes this field of study promising. Thus, the objective of this study was to induce the fruiting in Atlas squash, by means of different forms of application and proportions of cytokinin and auxin and to evaluate their effects on the physical-chemical characteristics of the fruits. The treatments were composed of six proportions associated with growth regulators and of two forms of application. The experimental design was a randomized block design with four replicates. At 72 days after transplanting, growth and fruit quality analyses were performed. The behavior of the hormones is modulated by their concentration and place of application, and the effect may be beneficial or not. The proportions 50/50% via leaf and 75/25% via ovary were the ones that increased the growth and parameters of fruit quality. The application of CPPU via leaf at higher concentration in proportion decreases quality characteristics such as vitamin C, carotenoids and starch.

Keywords: *Cucurbita moschata* Duch. Growth regulators. Quality. Productivity.

RESUMO - O uso de reguladores de crescimento do grupo das citocininas e auxinas tem demonstrado aumentar a produtividade de algumas culturas, pois podem estar envolvidos na expressão do sexo da planta, embora pouco se saiba sobre a ação da auxina IBA e da citocinina CPPU na frutificação da abóbora, bem como a interação entre ambas, o que torna este campo de estudo promissor. Assim, o objetivo deste estudo foi induzir a frutificação em abóbora Atlas, por meio de diferentes formas de aplicação e proporções de citocinina e auxina e avaliar seus efeitos nas características físico-químicas dos frutos. Os tratamentos foram compostos por seis proporções associadas a reguladores de crescimento e duas formas de aplicação. O delineamento experimental foi em blocos casualizados com quatro repetições. Aos 72 dias após o transplante, foram realizadas análises de crescimento e qualidade dos frutos. O comportamento dos hormônios é modulado por sua concentração e local de aplicação, podendo o efeito ser benéfico ou não. As proporções 50/50% via folha e 75/25% via ovário foram as que aumentaram o crescimento e os parâmetros de qualidade dos frutos. A aplicação de CPPU via foliar em maior concentração em proporção diminui características de qualidade como vitamina C, carotenoides e amido.

Palavras-chave: *Cucurbita moschata* Duch. Reguladores de crescimento. Qualidade. Produtividade.

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INTRODUCTION

Atlas squash presents itself as a profitable option, as it allows better use of area and ease of management during cultivation, due to the shorter branch and cycle minor. However, productivity is dependent on the initial production of a large number of flowers, together with the correct pollination. Such processes are highly sensitive to environmental conditions such as temperatures, rain and wind speeds (LIU et al., 2018).

The use of growth regulators has been reducing pollination problems in several fruit species, mainly those integrated into the group of cytokinins and auxins. Taiz et al. (2017) confirm that cytokinin contributes to fruiting by favoring the establishment of sinks in the plant, promoting a better mobilization of assimilates. In turn, auxins are present in pollen, endosperm and seed embryo, which suggests that they actively participate in fruit development (FIGUEIREDO et al., 2015).

The use of the synthetic cytokinin forchlorfenuron (CPPU) resulted in good quality production for several fruit species, this effect being confirmed by Ding et al. (2013) in tomatoes, Li et al. (2017) in cucumber and Zhang et al. (2017) in kiwi. As well as the use of synthetic auxins, aiming at asexual production for the formation of parthenocarpic fruits, as stated by Ferreira et al. (2017) with the use of dichlorophenoxyacetic acid (2,4-D) in Japanese pumpkin. Pereira et al. (2014), working with 'Gefner' atemoya, observed that the exogenous application of 1-naphthalene acetic acid (NAA), associated with gibberellic acid promoted high fruiting in a similar way to artificial pollination.

Such statements suggest that growth regulators can be used as an alternative in the quest to optimize fruiting, although little is known about the action of indole-3-butyric acid (IBA) in this physiological process, as well as its interaction with cytokinin CPPU. Thus, the objective was to induce fruiting in Atlas squash through different proportions and forms of application of growth regulators in the plant and to evaluate their effect on the growth and quality of fruits.

MATERIAL AND METHODS

This study was carried out in the period from March 3 to June 30, 2018, at the Center for Science and Agri-Food Technology, at the Universidade Federal de Campina Grande, Pombal-PB, Brazil. The municipality of Pombal is located in the western region of the state of Paraíba, at the geographical coordinates 6º 48' 16″ S, 37º 49' 15″ W and an average altitude of 148 m. Throughout the experimental period, we recorded an average temperature of around 30 °C, along with a relative humidity of 80%.

Atlas squash (Sakata®) seeds were duly sown in polystyrene trays for seedling formation, and when they obtained two true leaves, they were transplanted into pots with capacity of 2 L, filled with coconut fiber and commercial substrate Tropstrato HT® in a 3:1 ratio (SANTOS el al., 2020).

The treatments consisted of six different proportions of regulators (0/0, 0/100, 25/75, 50/50, 75/25, 100/0% CPPU/ IBA) and two application methods: via leaf and directly on the ovary. In the 0/0% or control ratio, pollination occurred through natural pollinating agents. The concentrations of CPPU and 100% IBA corresponded to 2.5 and 10 mg L-1, respectively (SANTOS et al., 2020).

The application via ovary was performed on all female flowers exogenously during anthesis, using flexible sticks with cotton at the ends (Cotonetes®), in order to moisten the entire wall of the ovary. The first application via leaf was also carried out during anthesis, with the aid of a manual sprayer in order to wet all the leaves of the plant; in all there were two applications with a weekly interval from one to the other. Both applications were performed between 6:00 and 8:00 am, 20 days after transplantation. All plants that received growth regulators had their female flowers protected with TNT bags 24 h before and after application of treatments, in order to avoid interference from natural pollination. (SANTOS et al., 2020.

Irrigation was performed daily with the determination of quantity from drainage lysimetry, adopting a leaching fraction of 10%, according to the methodology used by Bernardo, Soares and Mantovani (2008). To guarantee the nutrition of the plants, all the irrigation water supplied was composed of a nutrient solution diluted to 75% of the original concentration (N:15.0, P:1.0, K:6.0, Ca:5.0, Mg:2.0, S:2.0, Fe:0.05, Mg:0.01, B:0.05, Cu: 0.003, Zn: 0. 0008 and Mo:0.001 mmol L^{-1}), according to Hoagland and Arnon (1950). The volume of water used per plant/cycle was approximately 75 L, considering the daily values provided.

The plants were grown in a protected environment using a shade net with a 30% reduction in solar radiation. Manual weeding was performed between the pots and around the experimental area, in order to keep the environment free from spontaneous vegetation.

When the fruits reached full maturity (72 days after transplanting), identified by means of intense cream color, their collections were carried out with subsequent evaluation of growth and quality parameters.

The fruits were weighed on a semi-analytical balance to determine their fresh weight; the values being expressed in g $plant^{-1}$. The length of the fruit was measured using a graduated ruler and expressed in cm. The diameters of the bulb and the neck were measured using a digital caliper and expressed in mm.

The pulp thickness in the bulb was obtained considering the distance from the post-peel to the internal cavity of the fruit. For the neck part of the fruit, because it is completely filled with pulp, it was measured horizontally from one end to the other. Both characteristics were measured with a digital caliper and expressed in mm.

For the pulp thickness, the fruit was divided longitudinally into two parts and then two readings were made in the equatorial region of the pulp by means of a texturometer (Fruit Hardness Tester®), with a 2 cm depth of penetration, speed of 2 mm s^{-1} and 8 mm tip, and the results obtained were expressed in Newtons (N).

Soluble solids were obtained using a digital refractometer (model PR - 100, Palette, Atago Co., LTD., Japan), expressed in % (AOAC, 2006); Titratable acidity (TA) was determined according to the methodology recommended by the Instituto Adolfo Lutz (IAL, 2008), using 5 mL of homogenized pulp and diluted in 50 mL of distilled water, followed by titration with a standardized 0.1 N NaOH solution, using phenolphthalein as an indicator of the turning point. The results were expressed in % citric acid.

Total sugars were determined according to IAL (2008). After diluting 0.2 g of the pulp sample in 100 mL of distilled water, an aliquot of 0.15 mL was taken from this solution in a test tube together with 0.85 mL of water and 2 mL of anthrone, with subsequent stirring of the solution. Then it was placed in a water bath at 100 ºC for 5 minutes and, after cooling, the spectrophotometer readings were performed at 620 nm wavelength. Values are expressed in mg 100 g^{-1}.

Vitamin C was obtained by stirring 1 mL of juice diluted in 49 mL of oxalic acid, proceeding to titration with DFI solution, according to Tilman's method (AOAC, 2006), with results expressed in % ascorbic acid.

For carotenoids, the pigments were extracted in 80% acetone and quantified by spectrophotometry, as described by Lichtenthaler (1987), with some adaptations. 200 mg of juice was used, plus 0.2 mg of calcium carbonate and further dilution in 5 mL of acetone. Then, the sample was centrifuged for 10 min at 3,000 rpm and readings were taken in the supernatant at 470, 646 and 663 nm. The values were expressed in µg g^{-1} of dry matter.

$$\text{Chlorophyll a} = 12.21 * A_{663} - 2.81 * A_{646}$$
$$\text{Chlorophyll b} = 20.13 * A_{646} - 5.03 * A_{663}$$
$$\text{Carotenoids} = (1000 * A_{470} - 1.82 * Ca - 85.02 * Cb)/198$$

where:
A - absorbance at the wavelength used;
Ca - chlorophyll a;
Cb - chlorophyll b.

Starch was determined according to the methodology adapted from Dubois et al. (1956) and Mccready et al. (1950).

A 500 mg sample of the pulp was collected and then macerated in 5 mL of 80% ethanol until a homogeneous mixture was formed. Subsequently, the samples were stored in test tubes and kept in a water bath (80 ºC) for 30 minutes. After cooling, the samples were centrifuged for 10 min at 3,000 rpm and the supernatants were discarded. To the residue, precipitated at the base of the test tube, 5 mL of 30% perchloric acid were mixed, vortexed and kept at rest for 30 min, followed by centrifugation (previous programming). This procedure was repeated once more, with the supernatants collected in 10-mL test tubes. Aliquots of 0.1 and 0.5 mL of the extracts were taken in threaded test tubes, which were completed up to 1 mL with distilled water and received 2.5 mL of the anthrone reagent. The tubes were shaken and absorbance was read at 620 nm. The standard curve for determining the starch content was prepared with D-glucose considering the standard glucose solution 100 µg of GLU mL^{-1}. For readings on the spectrophotometer, volumes of 3 mL were used, combining glucose solution (0; 300; 600; 900 and 1200 µL) and distilled water (3000; 2700; 2400; 2100; 1800 and 1500 µL), respectively. An absorbance of 490 nm was used and the result was multiplied by the factor 0.9.

The data referring to the measured variables were submitted to the F test, through analysis of variance, and compared by the Tukey test ($p \leq 0.05$), both performed in the SISVAR® software version 5.6 (FERREIRA, 2014).

RESULTS AND DISCUSSION

All characteristics of growth and quality of the fruit showed significant interaction between the factors: place of application and proportions of the regulators CPPU and IBA, by the F test ($p \leq 0.01$). For treatment 0/100% (CPPU/IBA) via ovary, all fruits aborted and it was not possible to include this treatment in the statistical analysis. Thus, the hormonal proportions were analyzed separately considering each application site, in order to evaluate the 0/100% via leaf. In the evaluation of the interaction, only the proportions 0/0, 25/75, 50/50, 75/25 and 100/0% were considered, for both application sites.

In this study, the foliar application of growth regulators in the proportion 0/100%, that is, the isolated use of IBA synthetic auxin, did not cause the abortion of fruits. On the other hand, the application directly to the ovary caused total abortion, so probably the concentration applied was high for the ovary. It is known that the increase in auxin can induce ethylene synthesis, which has abscission as one of its main effects on the plant. Li et al. (2017) claim that the absence of certain hormones can cause abortion of fruits.

For fresh weight of the fruit, there was a significant effect of the proportions, when evaluated separately in each place of application of regulators in the plant (Figure 1A). The highest values were observed using the proportion 75/25% when applied via ovary with an increase of 61.50% in the control and using the proportion of 50/50% when applied via leaf with an increase of 59.26%. In the interaction of proportion *versus* location of the hormonal application in the plant, it was observed that the application via ovary was more efficient than via leaf, with gains of 37.13%, 26.82% and 23.55%, respectively, for the proportions 100/0%, 25/75% and 75/25% (Figure 1B). However, in the use of the 50/50% proportion, the application via leaf was 22.07% better than that via ovary.

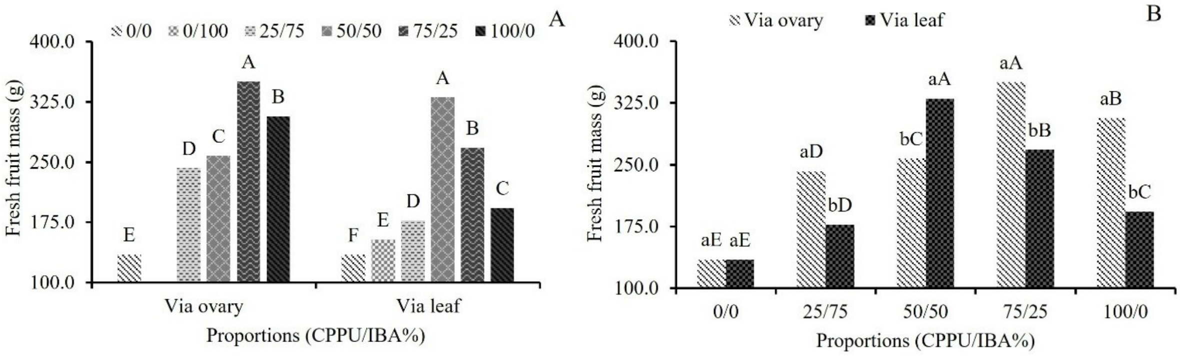

Figure 1. Fresh fruit mass of squash (A and B) with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test ($p \leq 0.05$). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

The behavior of the regulators is apparently modulated by their route of application and the proportion used, since the increase in the fresh weight of the fruit (Figure 1) was greater when the values of the regulators within the proportion were equivalent in the application via leaf, or when CPPU was elevated in proportion to IBA applied via ovary. Auxin is relevant for fruit standardization, while cytokinin is involved in the growth of the placenta and ovules. In other words, both regulators can act simultaneously or dependently to improve productivity (MARSCH-MARTÍNEZ et al., 2012).

The results show that the isolated use and in a greater proportion of CPPU through the leaf route does not favor satisfactory yields for the fresh mass of the fruit; however, if associated with 50% and 25% of IBA, through the leaf and through the ovary, respectively, there is a significant increase in this variable. In normal fruit development, embryos and seeds control the rate and maintenance of cell division through the production of plant hormones such as auxins, gibberellins and cytokinins. Therefore, the exogenous application of these regulators in adequate proportions causes better fruit development (PEREIRA et al., 2014).

For the length of the fruit, there were increases of

28.24% with the proportion 50/50% applied via leaf and 27.88% with the proportion 75/25% applied in the ovary when compared to the values obtained by the control (Figure 2A). The greatest discrepancy in the length of the fruit (13.33%) between the application routes in the plant was found with the proportion 100/0% (Figure 2B).

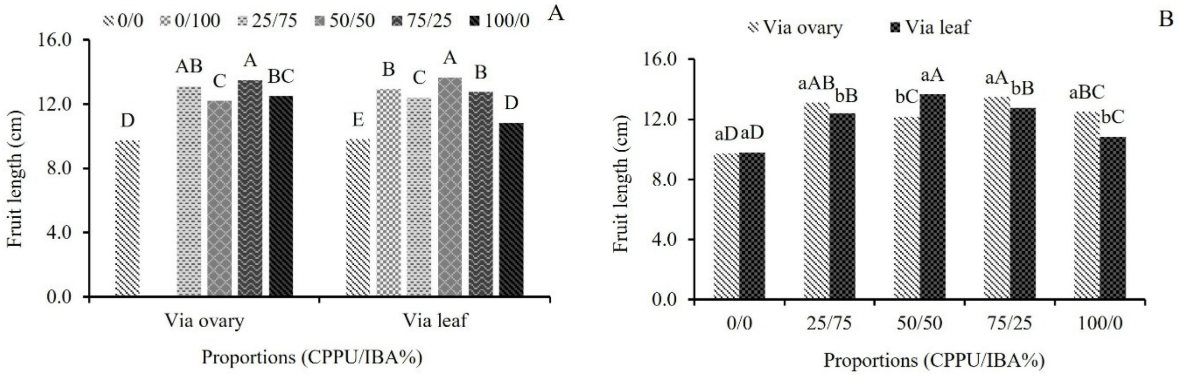

Figure 2. Length of squash fruits (A and B) with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

Plant hormones increase fruit growth, as they alter the production and allocation of photoassimilates during the development of reproductive organs, with greater demands on the levels of auxin and cytokinin during fruiting (KUMAR; KHURANA; SHARMA, 2014). According to Taiz et al. (2017), photoassimilates are preferably transported and accumulated in tissues treated with cytokinin, giving rise to a new source-sink relationship. This statement corroborates the results obtained in this study, where the application of CPPU in the ovary stimulated greater fruit length (Figure 2) when compared to application via leaf.

For the bulb diameter, increases of 28.33% and 27.67% were observed with the proportions 75/25% and 100/0% via ovary, respectively, in comparison to the control (Figure 3A). Via leaf the largest increments were verified with the proportions 75/25% and 50/50%, with respective values of 25.50% and 24.92%, in comparison to the control. In the interaction between the factors, it was found that the application via ovary stood out from that via leaf in the proportions 25/75 (18.91), 100/0 (15.76) and 75/25% (2.79%) (Figure 3B).

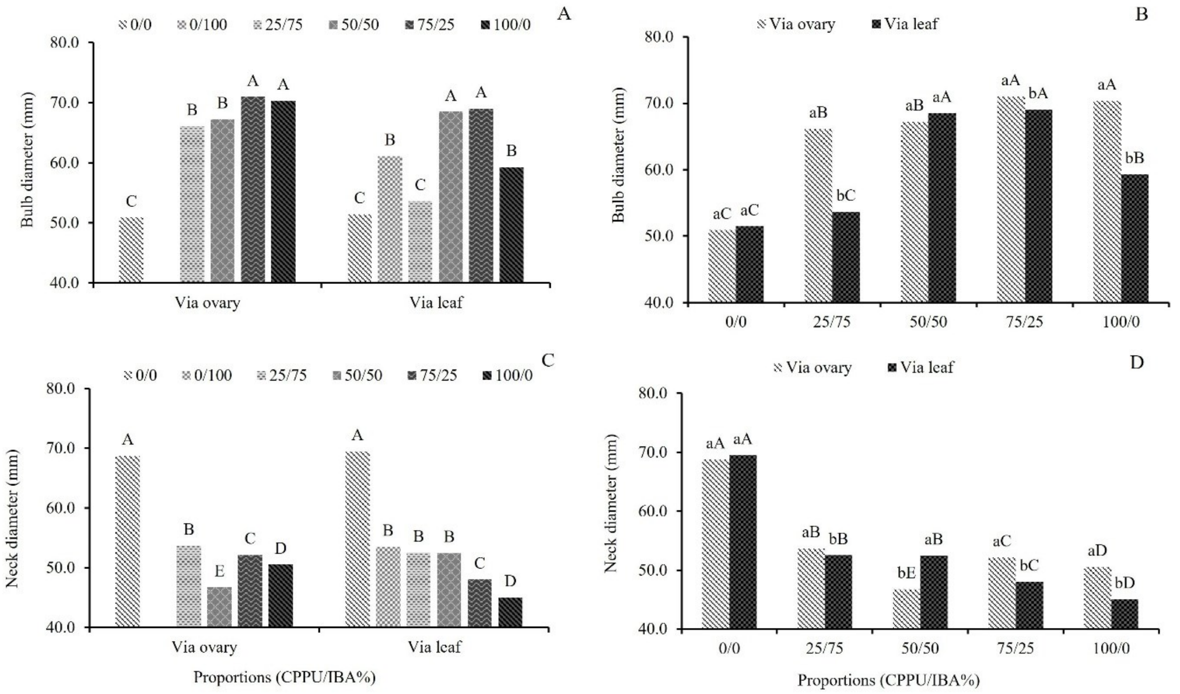

Figure 3. Diameter of the bulb (A and B) and of the neck (C and D) of squash fruits with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

The neck diameter decreases with the application of the regulators, regardless of the proportion when compared to the control (0/0%), with the largest decrease (35.20%) verified with the use of the hormonal proportion of 100/0% via leaf (Figure 3C). In the interaction, the application via ovary showed superiority compared to that via leaf for all hormonal proportions, except for 50/50% (Figure 3D).

In the proportion 50/50%, there was no significant difference between the places of application of the regulators in the plant. The use of CPPU in the ovary in the proportions 75/25 and 100/0% and in the leaf in the proportions 50/50 and 75/25% of the concentration 2.5 mg L^{-1} promoted increases in bulb diameter and smaller decreases in neck diameter (Figure 3). It is suggested, therefore, that the larger neck diameter in the control is due to the deficiency in natural pollination and seed formation, which increased the neck disproportionately compared to the bulb.

Comparing the fruits based on visual aspects (Figure 4), it is possible to affirm that each treatment influences their shape in a different way, with great deformity in those that received natural pollination (0/0%). It was also observed that the appearance of male flowers was delayed by 15 days, hindering the correct pollination by pollinating agents and delaying the formation of fruits in plants that did not receive hormonal treatments. Climatic conditions, such as temperature and humidity, during the work may also have disadvantaged the correct pollination by pollinating agents, resulting in malformation of fruits in control plants (Figure 4). Generally, the plant has high levels of auxins in the pollen grain found in the ovary and, because of this characteristic, well-pollinated flowers have high ovary growth, while poorly pollinated ones have deformed ovaries (TAIZ et al., 2017).

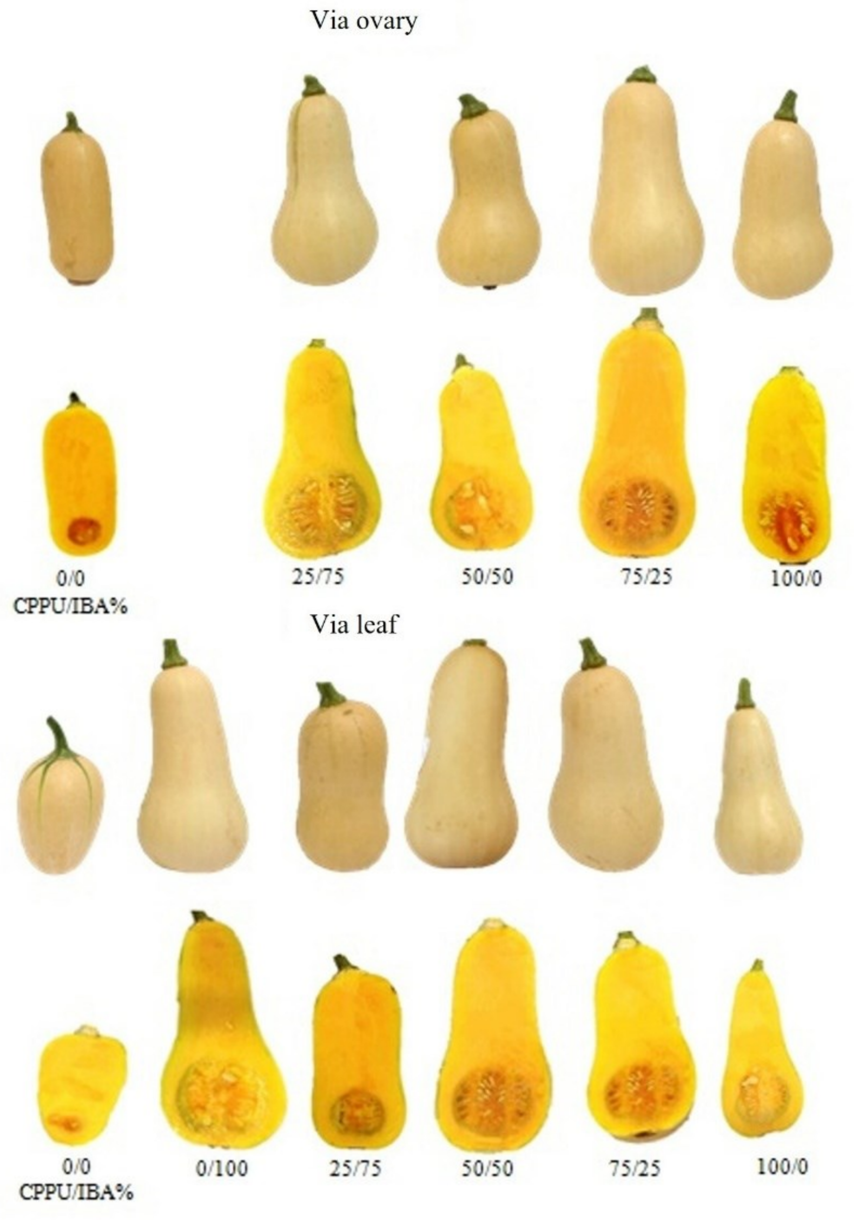

Figure 4. Visual aspects of squash fruits subjected to proportions of growth regulators (CPPU/IBA%) applied via ovary and leaf.

The greatest increase in pulp thickness in the bulb was observed in the proportion 0/100% via leaf, with 50.65% gain compared to the control (Figure 5A). For application via ovary, the proportions 25/75, 75/25 and 100/0%, statistically equal, increased pulp thickness by up to 47.70%. In the interaction between the proportions and places of application of the regulators, there was a higher value for foliar application compared to the ovary application only for the proportion 50/50% (Figure 5B). The greatest difference between the routes of application was found in the proportion 75/25%, with an increase of 34.97% via ovary compared to the application via leaf.

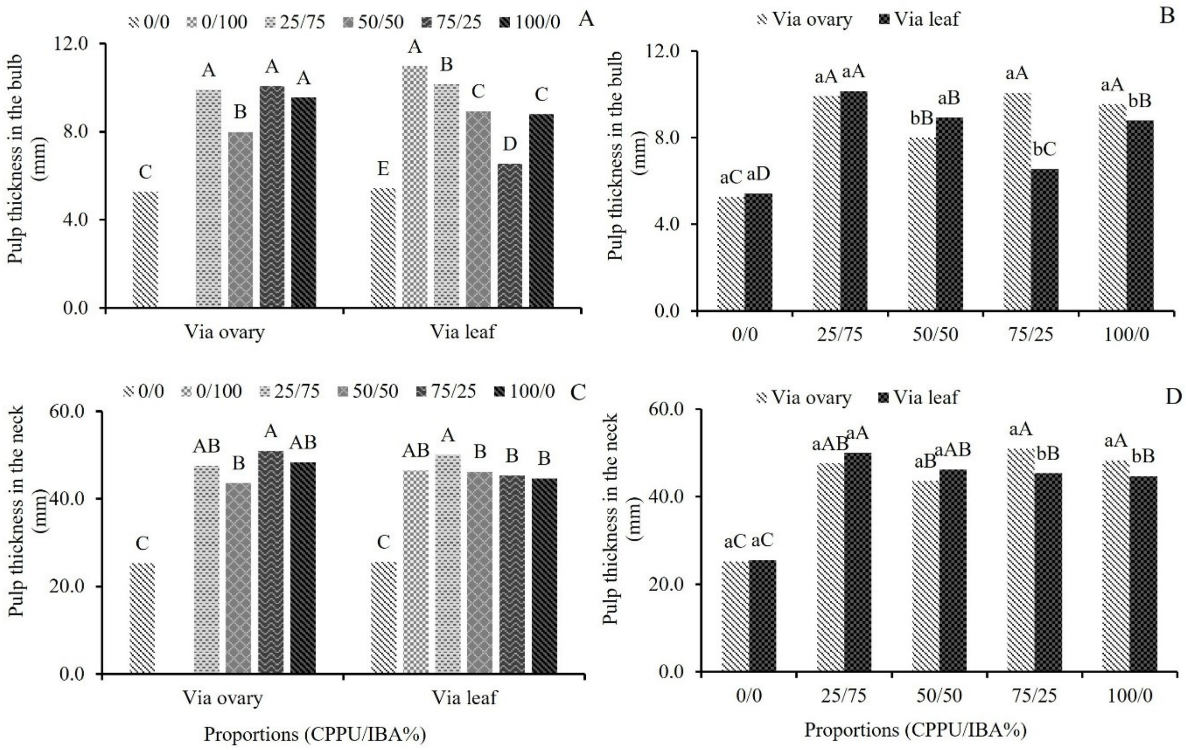

Figure 5. Pulp thickness in the bulb (A and B) and neck (C and D) of squash fruits with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test ($p \leq 0.05$). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

All proportions regardless of the route of application increased the thickness of the neck pulp compared to the control (Figure 5C). These increases were up to 50.46%, achieved with the use of the proportion 75/25% via ovary, and 48.96% obtained with the proportion 25/75% via leaf. Differences between the forms of application in the plant were found only with the use of the proportions 75/25 and 100/0%, where the application via ovary promoted increases of 11.00 and 7.53%, respectively, compared to the foliar application (Figure 5D).

According to Ding et al. (2013), the foliar application of CPPU can favor its accumulation in the pericarp, leading to an active cell division and increase of cells in that region, and finally a greater thickness compared to the pollinated fruits. However, in this study, the increase in the CPPU to IBA ratio applied via ovary proved to be more efficient in terms of pulp gain (Figure 5). On the other hand, the IBA applied through the leaves in a greater proportion than the CPPU led to a more satisfactory result.

The firmness of the pulp increased with the application of regulators with action of cytokinin and auxin, regardless of the proportion and the place of application, when compared to the control (Figure 6A). The greatest increase was 37.21%, obtained with the proportion 0/100% applied via leaf. For the application via ovary, the largest increase was 34.89%, obtained when the 75/25% ratio was used. For the interaction between the studied factors, there was a difference in fruit firmness in relation to the application site, with the highest values being observed when the applications of the regulators were performed via ovary (Figure 6B).

The isolated use of IBA through the leaves promoted greater firmness in the pulp (Figure 6) of squash fruits, followed by the 75/25% proportion by both forms of application. This response is significant, since firmer fruits are more resistant to mechanical injuries and their post-harvest life is increased. According to Chitarra and Chitarra (2005), the change in the structure of the tissues caused by the lower firmness of fruits can compromise the texture and aroma, and this effect is not desired.

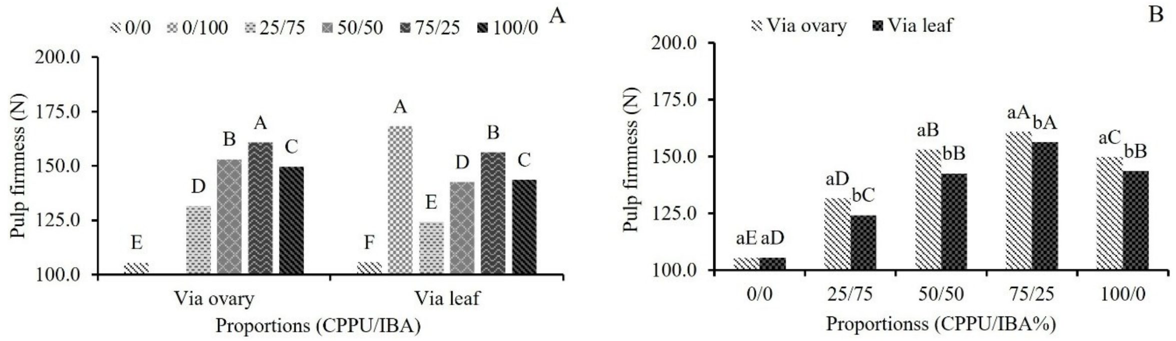

Figure 6. Pulp firmness of squash fruits (A and B) with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

The highest values in the total soluble solids content were obtained with the proportion 0/100% applied via leaf, with increase of 14.49% in comparison with the control (Figure 7A). In the application via ovary the proportion 50/50% was the one that promoted greater increase (11.84%), compared to the control. It was also observed that, for the proportions 25/75 and 75/25% in the application via leaf, there was superiority when compared to application via ovary (Figure 7B). On the other hand, the proportion 100/0% applied via ovary stood out with an increase of 12.01% compared to application via leaf.

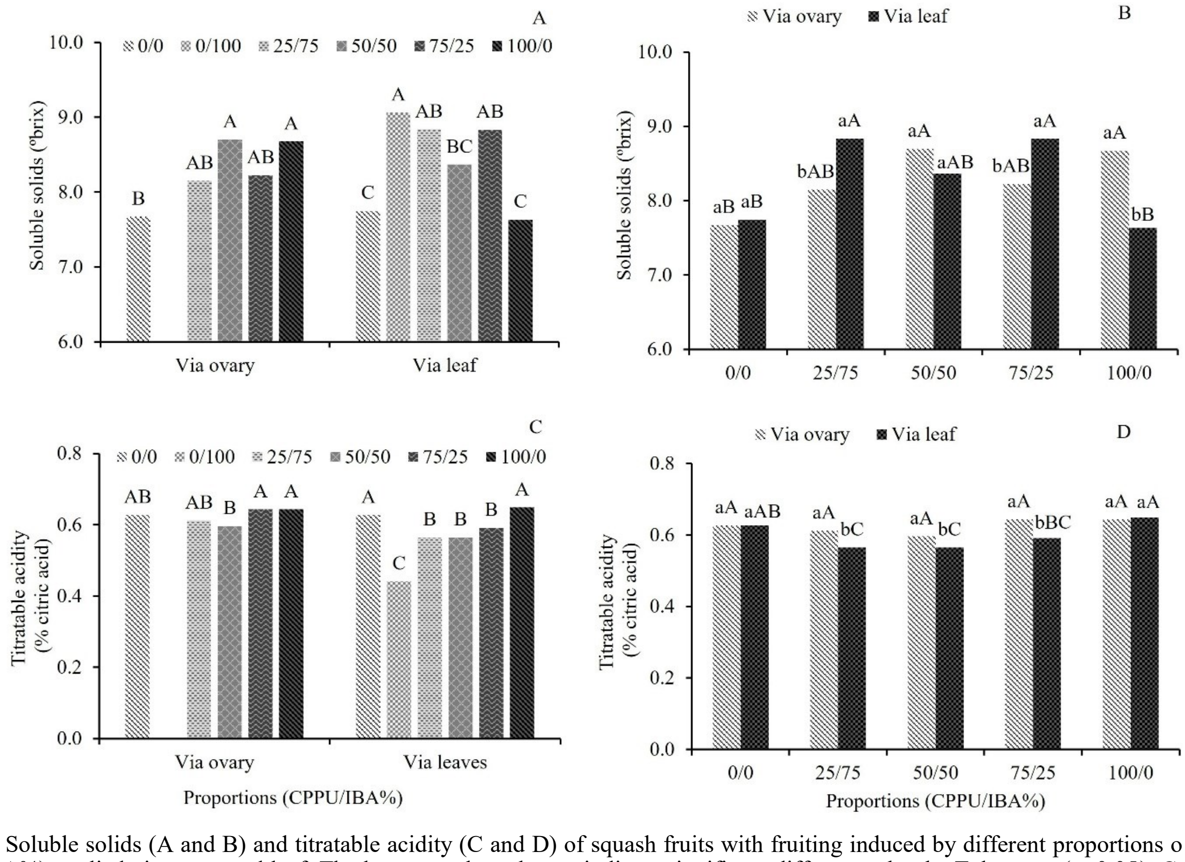

Figure 7. Soluble solids (A and B) and titratable acidity (C and D) of squash fruits with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

For the percentage of citric acid, there was a greater reduction of 29.75% in the proportion 0/100% via leaf compared to the control (Figure 7C). At the same time, for the application via ovary, no statistical differences were observed between the control and the hormonal proportions. The greatest difference (8.13%) between the application forms was observed in the proportion 75/25%, followed by 25/75 and 50/50%, with higher values of acidity when the regulators were applied via ovary (Figure 7D).

The increase in the soluble solids content (Figures 7A and 7B) with the use of IBA was expected, since auxin contributes to the accumulation of carbohydrates (KUMAR; MISHRA; SINGH, 2018). Possibly, because the use of IBA alone through the leaf promoted greater elevation of soluble solids, there were greater reductions in acidity (Figures 7C and 7D). A greater reduction in acidity may also be due to the increase in ethylene concentration (SILVA et al., 2014). An opposite effect was observed with exogenous application of 2,4-D by Ashraf et al. (2013), who reported that it increases acidity.

There was an increase of 30.91% in total soluble sugars for the proportion 25/75% via leaf in comparison to the control (Figure 8). For the proportion 0/100%, also applied via leaf, there was a reduction of 41.86%. When applied via ovary, the proportions 75/25, 50/50 and 25/75%, which were statistically equal, also led to greater gains compared to the control (Figure 8A). The greatest difference between the routes of application was identified in the use of the proportion 25/75%, with an increase of 21.86% for the application through the leaf compared to that through the ovary (Figure 8B). The association of 25% CPPU and 75% IBA by both routes optimized the total sugar content.

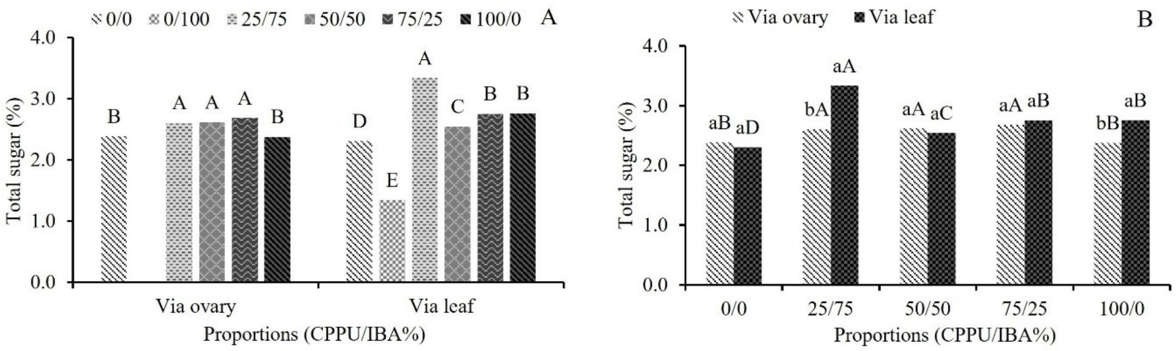

Figure 8. Total sugar of squash fruits (A and B), with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

As for the content of total sugars (Figure 8), results different from that of this study were obtained by Qian et al. (2018), who observed that in cucumber the use of CPPU and NAA synthetic auxin did not cause a significant effect on this variable. However, the increase in this quality index by the addition of CPPU was also observed by Susila et al. (2013) in watermelon. Through these responses it is possible to state that the cytokinin CPPU combined with IBA auxin are associated with an increase in the total sugar content in squash.

The highest value of vitamin C content in percentage of ascorbic acid was obtained by using the 100/0% ratio applied via ovary, with an increase of 10.53% compared to the control (Figure 9A). Gains of up to 9.47% were also obtained through the proportion 0/100% via leaf, which for the same route did not differ from the proportions 50/50 and 75/25%. In the interaction, there was a greater discrepancy between the routes of application (20%) by using the ratio 100/0% applied via leaf compared to the application via ovary (Figure 9B).

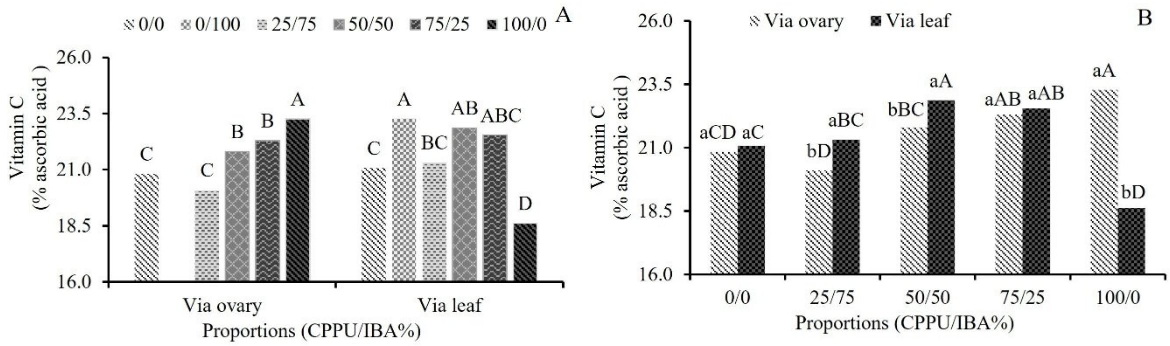

Figure 9. Vitamin C of squash fruits (A and B), with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

According to Kumar et al. (2014), the application of NAA (synthetic auxin) through the leaf raises the levels of ascorbic acid. Such statements associated with the results of this study reinforce that the use of synthetic auxins through the leaf contributes to the increase of quality indexes, mainly with the addition of CPPU in proportion. However, high concentrations of this auxin are not recommended for application via ovary.

Carotenoid values were reduced with the application of hormonal treatments, except for the application via ovary of the proportions 25/75 and 50/50%, with no statistical differences between both and the control (Figure 10A). In the interaction between the factors studied, a significant difference was observed between the routes of application only in the proportion 50/50%, with an increase of 32.63% for the route of application via ovary compared to that via leaf (Figure 10B). The concentrations in this study varied between 97.94 μg g^{-1} (obtained by the control) and 54.11 μg g^{-1} (obtained by the proportion 50/50% via leaf). The interrelationship between CPPU and IBA in equal proportions applied through the leaf may have caused a faster degradation in the content of carotenoids than compared to the application via ovary.

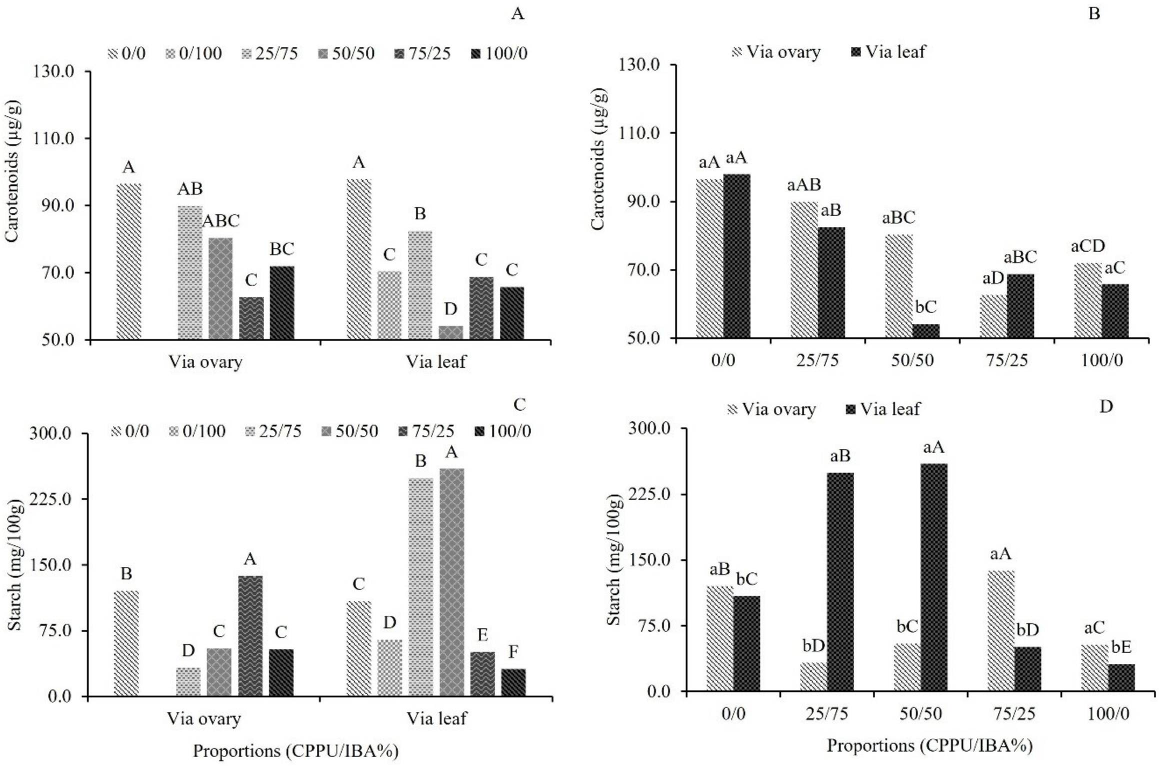

Figure 10. Carotenoids (A and B) and starch (C and D) of squash fruits with fruiting induced by different proportions of regulators (CPPU/IBA%) applied via ovary and leaf. The letters on the columns indicate significant differences by the Tukey test (p≤0.05). Capital letters compare different proportions and lowercase letters compare the application routes within each hormonal proportion.

The same treatment resulting from the greater loss of carotenoids was also responsible for the higher concentration of starch (259.57 mg/100g), with a gain of 58.08%, while the proportion 100/0% reduced it by 71.18%, both in contrast to the control (Figure 10C). For the application via ovary, the proportion 75/25% was the one that stood out from the control, with 12.67% increase, while at 25/75% it was the one that most reduced (72.73%). Nevertheless, a high difference (86.83%) was observed between the application routes in the proportion 25/75%, followed by 50/50% (78.88%), as CPPU increased and IBA decreased. Their application via ovary led to greater gains in starch than via leaf (Figure 10D).

Nardozza et al. (2017) state that the use of CPPU exogenously in kiwi reduced starch concentrations and resulted in loss of dry mass. Huang, Li and Zhao (2016) and Mcadam et al. (2017) indicate that auxins play a positive role in the synthesis of starch, but they also interfere in the ripening of fruits and in the accumulation of carotenoids (SU et al., 2015). Given these facts, it is assumed that the association of cytokinin and auxin causes changes in the metabolism of fruits, capable of decreasing the levels of carotenoids and increasing the level of starch, depending on their proportions and site of action. Other research can be conducted using the same proportions and application routes but with different sources of growth regulators with cytokinin and auxin action. These results could further elucidate endogenous and exogenous factors in the fruit.

CONCLUSIONS

When appropriate proportion of cytokinin and auxin induces fruiting in pumpkin and improves morphological and biochemical characteristics of the fruits.

The proportions 50/50% via leaf and 75/25% via ovary, respectively, were the ones that most increased the growth and quality of the fruits.

The application of CPPU via leaf at the highest concentration in the proportion (100/0%) decreases quality characteristics such as: vitamin C, carotenoids and starch.

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REFERENCES

AOAC - Association of Official Analytical Chemists. **Official methods of analysis**. 18. ed. Washington: AOAC international, 2006. 201 p.

ASHRAF, M. Y. et al. Improvement in yield, quality and reduction in fruit drop in Kinnow (*Citrus reticulata* B.) by exogenous application of plant growth regulators, potassium and zinc. **Pakistan Journal of Botany**, 45: 433-440, 2013.

BERNARDO, S.; SOARES, A. A.; MANTOVANI, E. C. **Manual de irrigação**. 8. ed. Viçosa, MG: UFV, 2008. 625 p.

CHITARRA, M. I. F.; CHITARRA, A. B. **Pós-colheita de frutos e hortaliças: fisiologia e manuseio**. 2. ed. Lavras, MG: UFLA, 2005. 783 p.

DING, J. et al. Cytokinin-induced parthenocarpic fruit development in tomato is partly dependent on enhanced gibberellin and auxin biosynthesis. **PloS one**, 8: 1-17, 2013.

DUBOIS, M. et al. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, 28: 350 -356, 1956.

FERREIRA, D. F. Sisvar: A guide for its bootstrap procedures in multiple comparisons. **Ciência e Agrotecnologia**, 38: 109-112, 2014.

FERREIRA, T. A. et al. Indução da frutificação paternocárpica de frutos em híbrido de abóbora japonesa com 2,4-D sob condições de temperatura elevada. **Nucleus**, 14: 145-152, 2017.

FIGUEIREDO, D. D. et al. Auxin production couples endosperm development to fertilization. **Nature Plants**, 1: 1-6, 2015.

HOAGLAND, D. R.; ARNON, D. L. **The water-culture methods for growing plants without soil**. Berkeley: California Agriculture Experiment Station, 1950. 32 p. (Circular, 347).

HUANG, J.; LI, Z.; ZHAO, D. Deregulation of the OsmiR160 target gene OsARF18 causes growth and developmental defects with an alteration of auxin signaling in rice. **Scientific Reports**, 6: 1-14, 2016.

IAL - Instituto Adolfo Lutz. **Normas Analíticas do Instituto Adolfo Lutz: Métodos Químicos e Físicos para Análise de Alimentos**. 4. ed. São Paulo, SP: IAL, 2008. 1020 p.

KUMAR, R.; KHURANA, A.; SHARMA, A. K. Role of plant hormones and their interplay in development and ripening of fleshy fruits. **Journal of Experimental Botany**, 65: 4561-4575, 2014.

KUMAR, R.; MISHRA, S.; SINGH, S. Improve the fruit setting and quality of pomegranate (*Punica granatum* L.), cv. Bhagwa by spraying the plant growth regulators under Allahabad agro climatic conditions. **International Journal of Chemical Studies**, 6: 2209-2211, 2018.

KUMAR, S. et al. Effect of foliar feeding of nutrients and plant growth regulators on vegetative growth and yield of phalsa (*Grewia subinaequalis* D.C.). **Research in Environment and Life Sciences**, 7: 293-294, 2014.

LICHTENTHALER, H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: PACKER, L., DOUCE, R. (Eds.). **Methods in Enzimology**. London: Academic Press, 1987, 350-382 p.

LI, J. et al. Proteomic insight into fruit set of cucumber (*Cucumis sativus* L.) suggests the cues of hormone-independent parthenocarpy. **BMC Genomics**, 18: 896-910, 2017.

LIU, L. et al. Histological, hormonal and transcriptomic reveal the changes upon gibberellin-induced parthenocarpy in pear fruit. **Horticulture Research**, 5: 1-13, 2018.

MARSCH-MARTÍNEZ, N. et al. Hormones talking: Does hormonal cross-talk shape the *Arabidopsis gynoecium*. **Plant Signaling & Behavior**, 7: 1698-1701, 2012.

MCADAM, E. L. et al. Evidence that auxin is required for normal seed size and starch synthesis in pea. **New Phytologist**, 216: 193-204, 2017.

MCCREADY, R. M. et al. Determination of starch and amylose in vegetables application to peas. **Analytical Chemistry**, 22: 1156-1158, 1950.

NARDOZZA, S. et al. Exogenous cytokinin application to *Actinidia chinensis* var. deliciosa 'Hayward' fruit promotes fruit expansion through water uptake. **Horticulture Research**, 4: 1-11, 2017.

PEREIRA, M. C. T. et al. Reguladores de crescimento na frutificação efetiva e qualidade de frutos partenocárpicos de atemoia 'Gefner'. **Pesquisa Agropecuária Brasileira**, 49: 281 -289, 2014.

QIAN, C. et al. Effects of exogenous application of CPPU, NAA and GA4+7onparthenocarpy and fruit quality in cucumber (*Cucumis sativus* L.). **Food Chemistry**, 243: 410-413, 2018.

SANTOS, G. L. et al. Growth and accumulation of dry mass in squashes with fruiting inducedby cytokinin and auxin. **Revista Brasileira de Ciências Agrárias**, 15: 1-8, 2020.

SILVA, D. F. et al. Ensacamento de frutos sobre a qualidade e produtividade de novas cultivares e seleções de pêssego no sul de Minas Gerais. **Revista Agrarian**, 7: 530-540, 2014.

SU, L. et al. Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. **BMC Plant Biology**, 15: 1-12, 2015.

SUSILA, T. et al. Studies on exogenous application of CPPU and GA on Yield, fruit quality characters and seedlessness in watermelon. **World Journal of Agricultural Sciences**, 9: 132-136, 2013.

TAIZ, L. et al. **Fisiologia e desenvolvimento vegetal**. 6. ed. Porto Alegre, RS: Artmed, 2017. 888 p.

ZHANG, Z. et al. Transformation products elucidation of forchlorfenuron in postharvest kiwifruit by time-of-flight mass spectrometry. **PloS one**. 12: 1-18, 2017.