

# Gibberellin spraying in the production of male flowers of squash (*Cucurbita pepo* L.) and their postharvest quality

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## ABSTRACT

**Objective:** To evaluate the effect of gibberellic acid (GA<sub>3</sub>) spraying on the production of male flowers and their physical and chemical quality for fresh sale of pumpkin (*Cucurbita pepo* L.).

**Methodology:** A pumpkin crop was established under open-air conditions, where two GA<sub>3</sub> sprays were applied at doses of 0, 30, 60, and 90 mg L<sup>-1</sup> during the first 45 days after planting. The number of male and female flowers accumulated over 31 days after GA<sub>3</sub> application was recorded, and selected physical and chemical quality parameters were determined. A randomized complete block design with four blocks was used.

**Results:** The proportion of male to female flowers increased from 2:1 at 0 mg L<sup>-1</sup> to 4:1 at 90 mg L<sup>-1</sup> during the evaluation period. Chlorophylls, carotenoids, phenolic compounds, antioxidant activity, and vitamin C were not negatively affected by GA<sub>3</sub> spraying.

**Limitations/Implications:** This experiment should be replicated under different climatic conditions and with other varieties.

**Conclusions:** GA<sub>3</sub> spraying increased the proportion of male flowers without affecting the quality of pumpkin flowers.

**Keywords:** Phenolic compounds, carotenoids, vitamin C.

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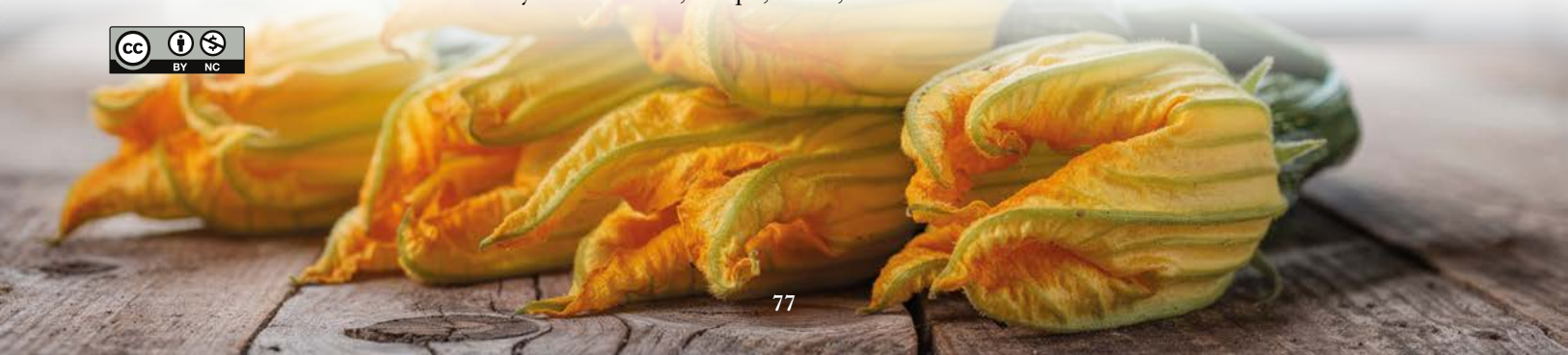
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## INTRODUCTION

Squashes (*Cucurbita* spp.) have been part of the diet of the Americas for more than 1,000 years and are traditionally included in the milpa production system, associated with maize, beans, and chili, which were the dietary staples of Mesoamerican civilizations. Today, squash remains an important crop in traditional Mexican agriculture, a region recognized as the center of origin and diversity for four of the five species of the genus: *Cucurbita argyrosperma*, *Cucurbita ficifolia*, *Cucurbita moschata*, and *Cucurbita pepo* (Basurto-Peña *et al.*, 2010). In 2023, Mexico cultivated 77,745.98 ha of squash for seed, zucchini, and other edible squashes, producing 744,295.5 t, with a market value of 6,163,102 thousand pesos (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2024).

The cultivated *Cucurbita* species intended for human consumption exhibit great diversity in fruit size, shape, color, and ornamentation. Their fruits are not bitter and



can be consumed along with their seeds, flowers, and shoots (Eguiarte *et al.*, 2018). In particular, squash flowers are a popular ingredient in the USA, Asia, and Europe due to their appealing yellow color, soft texture, and delicate flavor. In Mexico, they are extremely popular and are sold in local markets, mainly as an ingredient in soups or incorporated into various dishes such as tamales, tacos, quesadillas, and stuffed peppers, among others (Mulík & Ozuna, 2020).

One hundred grams of squash flowers contain 93.2% moisture, 46 mg of carbohydrates, 18.1 mg of protein, 5 mg of fat, 15.9 mg of ash, and 10.5 mg of fiber (Sotelo *et al.*, 2007). Beyond their culinary qualities, squash flowers have beneficial properties for human health. Each 100 g provides 11 mg of Na, 18.2 mg of K, 17.6 mg of Ca, 17.4  $\mu$ g of phenolic compounds, 17.1  $\mu$ g of flavonoids, 10.3 mg of anthocyanins, an antioxidant activity of 51.6% (DPPH), and between 15.2 and 45.8 mg of carotenoids. The phytochemical compounds present make squash flowers a functional food, as some of these molecules have demonstrated antimicrobial activity (Ghosh & Sing, 2021; Biezanowska-Kopec *et al.*, 2022).

The sexual expression of *Cucurbita pepo* is monoecious, with large, showy orange flowers. Female flowers are produced in lower proportion than male flowers. At the beginning of the crop cycle, only male flowers are produced; later, male and female flowers are produced alternately, and finally, female flowers dominate (Costa-Silva *et al.*, 2019). In Morelos, Mexico, there is a market for squash flower production, where it is desirable to maintain or increase the proportion of male flowers for a longer period.

Hormones play a key role in regulating sex expression in cucurbits. In general, ethylene and auxins promote feminization, whereas gibberellins induce masculinization (Rudich, 1990). Gibberellins act as regulators of plant growth and development, promoting cell elongation, seed germination, flowering transition, pollen development, pollen tube growth, and fruit development (Taiz *et al.*, 2023). In Morelos, Mexico, no specific studies have been conducted to determine the appropriate gibberellin application rates to maintain a higher production of male flowers relative to female flowers in squash crops. Likewise, the quality, functional metabolites, and antioxidant activity of flowers treated with gibberellic acid have not been evaluated. Therefore, this study assessed the effect of different GA<sub>3</sub> doses on the number of male and female flowers, as well as on the quality, functional metabolites, and antioxidant activity of squash flowers.

## MATERIALS AND METHODS

The study was conducted on a 0.4 ha area located in Cuentepec, Temixco, Morelos, Mexico (18° 86' 02.7" N, 99° 32' 63.8" W, 1,390 m a.s.l.), which has a warm-subhumid climate. The average annual temperature ranges from 20.7 °C to 26 °C, with an annual rainfall of 891.9 mm (Díaz *et al.*, 2008). *Grey zucchini* squash seeds (Seminis, USA) were used as plant material.

### Experimental Design and Agronomic Management

Sowing was carried out in furrows spaced 80 cm apart, with a distance of 50 cm between plants. Two seeds were sown per planting hole, and 20 g of fertilizer (NPK 18-4.5-

3) was applied midway between the plants. A randomized complete block design was used, consisting of four blocks. The four treatments were randomized within each block.

The treatments consisted of the application of GA (Biogib<sup>®</sup> 10 PS, Arysta). Four treatments were evaluated: Treatment 1 served as the control, with no GA<sub>3</sub> application; Treatments 2, 3, and 4 consisted of two foliar sprays (at 30 and 45 days after sowing) with 30, 60, and 90 mg L<sup>-1</sup> GA<sub>3</sub>, respectively. The solution was prepared by dissolving the product in tap water, and applications were performed in the morning at 9:00 a.m. The experimental unit consisted of three rows, each 2 m long; the central plant in each row was sampled, and three plants were evaluated per experimental unit. Blocks were separated by one row.

Agronomic management included treating the seeds with a solution of N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide (Captan<sup>®</sup> WP, ADAMA) for 12 h prior to sowing. A second fertilization was applied 30 days after sowing with 30 g of NPK 80-40-20. Preventive pest control was carried out using Betacyfluthrin + Imidacloprid (Muralla Max<sup>®</sup> 300 OD, Bayer<sup>®</sup>), and preventive disease control was performed with Quintozene 30% + Thiram 30% (Interguzan 30-30<sup>®</sup>, AgroIQC). After 20 days of sowing, Methomyl 90% (Matador<sup>®</sup> 90 PS, ADAMA) was applied for the control of fruit borer (*Diaphania nitidalis*, *Diabrotica* spp.). Foliar fertilizers Foltron<sup>®</sup> (Arysta) and later Bayfolan<sup>®</sup> (Bayer) were applied weekly during the first 25 days after sowing.

### **Evaluated Variables**

The number of male and female flowers was recorded at 38, 46, 54, 62, and 70 days after sowing. Flower counts were performed daily over a 31-day period on three plants. Flower size and peduncle length were measured for each flower using a 30 cm ruler with a precision of 0.01 mm.

The total chlorophyll and carotenoid contents were determined following the methodology proposed by Rodés and Collazo (2006). One gram of squash flower tissue was homogenized with 5 mL of 80% acetone using an UltraTurrax (IKA<sup>®</sup>, USA). An additional 5 mL of acetone was added during homogenization, and the mixture was then filtered using filter paper. After filtration, absorbance readings were taken with a spectrophotometer (HACH DR 5000R, USA) at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 440.5 nm for carotenoids. Pigment concentrations were calculated using the following formulas:

$$Ca(\text{mgL}^{-1}) = 12.7(A_{663}) - 2.69(A_{645})$$

$$Cb(\text{mgL}^{-1}) = 22.9(A_{645}) - 4.68(A_{663})$$

$$Ccar(\text{gL}^{-1}) = 4.695(A_{440.5}) - 0.268(Ca + b)$$

Where: *Ca*=chlorophyll a concentration, *Cb*=chlorophyll b concentration, and *Ccar*=total carotenoid concentration. A663, A645, and A440.5=absorbance readings at 663, 645, and 440.5 nm, respectively.

The concentration of phenolic compounds was determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999). One gram of flower tissue was placed in a test tube and homogenized with 20 mL of distilled water using an Ultra Turrax (IKA<sup>®</sup>, USA R), then filtered. A 0.5 mL aliquot of the filtrate was mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10). After 5 minutes, 2 mL of sodium carbonate (7.5% w/v) were added, and the mixture was allowed to stand for 2 hours. Absorbance was then measured at 760 nm using a spectrophotometer (HACH DR 5000<sup>®</sup>). Results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh weight.

Using the same extract employed for phenolic determination, antioxidant activity was assessed through the FRAP, ABTS, and DPPH methods. The ferric reducing antioxidant power (FRAP) was determined following the methodology developed by Benzie and Strain (1996). The FRAP reagent (TPTZ, FeCl<sub>3</sub>, and acetate buffer) was prepared, and 1.8 mL of FRAP solution were mixed with 140  $\mu$ L of distilled water and 60  $\mu$ L of sample. The reaction mixture was incubated for 30 minutes at 37 °C, and absorbance was then measured at 593 nm. Results were expressed as mg ascorbic acid equivalents (AAE) per 100 g of fresh weight.

For the DPPH method, the procedure proposed by Brand-Williams, Cuvelier, and Berset (1995) was followed. One gram of pulp was homogenized with 10 mL of distilled water and filtered. From the filtrate, 0.10 mL was mixed with 3 mL of a methanolic DPPH solution ( $6.1 \times 10^{-5}$  M, Sigma-Aldrich, USA) and allowed to react for 30 minutes in the dark. The change in absorbance was measured at 517 nm. Antioxidant activity was determined using a standard curve of ascorbic acid, and results were expressed as mg ascorbic acid equivalents (AAE) per 100 g of fresh weight.

For the ABTS method, the ABTS reagent (Sigma-Aldrich<sup>®</sup>, USA) was prepared at 7 mM and potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) at 2.45 mM. Both solutions were mixed at a 1:1 ratio and allowed to stand for 16 hours, then diluted with 20% ethanol until reaching an absorbance of  $0.7 \pm 0.02$  at 734 nm. Subsequently, 3 mL of ABTS solution were mixed with 50  $\mu$ L of sample and allowed to react for 15 minutes. Absorbance was measured at 734 nm. Results were expressed as mg ascorbic acid equivalents (AAE) per 100 g of fresh weight, obtained from a calibration curve (Re *et al.*, 1999).

The vitamin C content was determined following the methodology proposed by Jagota and Dani (1982), a colorimetric technique for vitamin C estimation using the Folin-Ciocalteu reagent. One gram of sample was homogenized with 4 mL of 10% (w/v) trichloroacetic acid (TCA) and placed in an ice bath for 5 minutes. The mixture was then centrifuged at  $9,464 \times g$  for 20 minutes at 4 °C. For the reaction, 0.5 mL aliquots of the supernatant were mixed with 1.5 mL of double-distilled water and 200  $\mu$ L of Folin reagent, and the mixture was allowed to react in darkness for 15 minutes. Absorbance was measured at 760 nm. Vitamin C content was estimated using a standard curve prepared with ascorbic acid, and total concentration was expressed as mg g<sup>-1</sup> of fresh weight.

### Data Analysis

Data were analyzed and graphed using SigmaPlot v.14 (Systat Software, Inc., San José, California, USA). A one-way analysis of variance (ANOVA) was performed, followed by

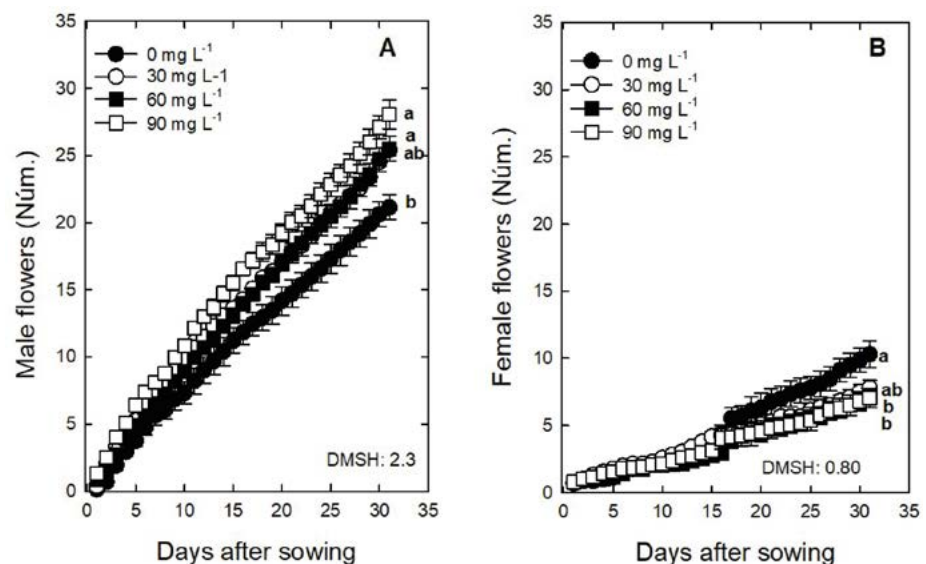
mean comparisons using Tukey's test. Simple correlations were evaluated using Pearson's method (Systat Software, Inc., San José, California, USA).

## RESULTS AND DISCUSSION

Control plants produced approximately 21 male flowers and 10 female flowers over 31 days, resulting in a male-to-female flower ratio of 2:1 (Figure 1). The application of gibberellic acid increased the number of male flowers and reduced the proportion of female flowers, with total flower production ranging from 32 to 35 when GA<sub>3</sub> was applied at 30-90 mg L<sup>-1</sup> (Figure 1). Male flowers increased by 4-7, while female flowers decreased by 2-3 as the GA<sub>3</sub> dose increased from 30 to 90 mg L<sup>-1</sup> (Figure 1). The male-to-female flower ratio was 3:1 when 30 or 60 mg L<sup>-1</sup> of GA was applied and 4:1 at 90 mg L<sup>-1</sup>. These results indicate that GA<sub>3</sub> application significantly increased the number of squash flowers and enhanced the proportion of male flowers.

Authors such as Babhare *et al.* (2020) reported that foliar application of GA<sub>3</sub> increased male flowers in papaya; however, they also noted that the male-to-female:hermaphrodite flower ratio remained unchanged. In the present study, it is evident that GA<sub>3</sub> application significantly increased the number of male flowers, making it a viable option for maintaining squash plants with male flower production for sale. Gupta and Chakrabarty (2013) reported that GA<sub>3</sub> treatments promote male sexual tendency in cucumber (*Cucumis sativa*), both in gynocious and hermaphrodite lines, although the exact mechanism of action is not yet understood. Future studies should evaluate a greater number of applications to maintain the flowering period with the observed ratios and achieve higher benefits.

Flower size did not show significant differences among treatments during the sampling periods, except on day 60, when plants treated with 30 mg L<sup>-1</sup> GA<sub>3</sub> had significantly larger flowers compared to the control plants and those treated with 60 or 90 mg L<sup>-1</sup> GA<sub>3</sub>



**Figure 1.** Number of male (A) and female (B) squash flowers accumulated during the evaluation period. Each point represents the mean of three observations  $\pm$  standard error. Different letters among treatments indicate significant differences according to Tukey's test ( $p \leq 0.05$ ). HSD: Honest significant difference.

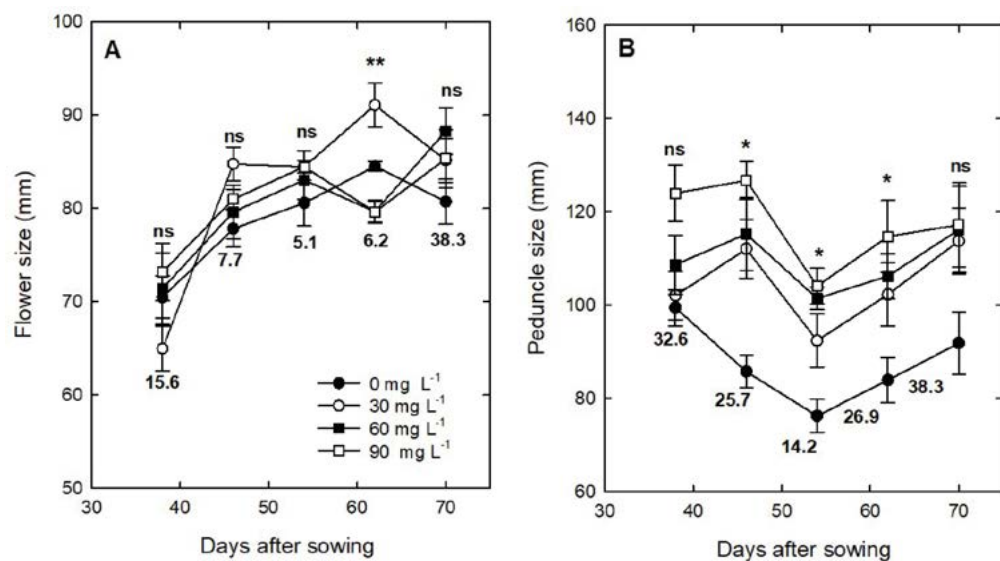
(Figure 2A). Flower dimensions increased from initial values of 65-73 mm to 80-88 mm (Figure 2A).

The peduncle showed the most significant increase. When  $90 \text{ mg L}^{-1}$  GA was applied, peduncle length ranged from 104.1 to 126 mm, whereas in control plants it ranged from 76 to 99 mm (Figure 2B). Han *et al.* (2014) reported that GA<sub>3</sub> application in papaya increased the length of male flower peduncles, attributing this to a transient effect rather than a trait controlled by genes involved in gibberellic acid metabolism. However, in the present study, peduncle length remained significantly greater with GA<sub>3</sub> application, suggesting a residual effect of at least 32 days on this morphological characteristic of squash flowers.

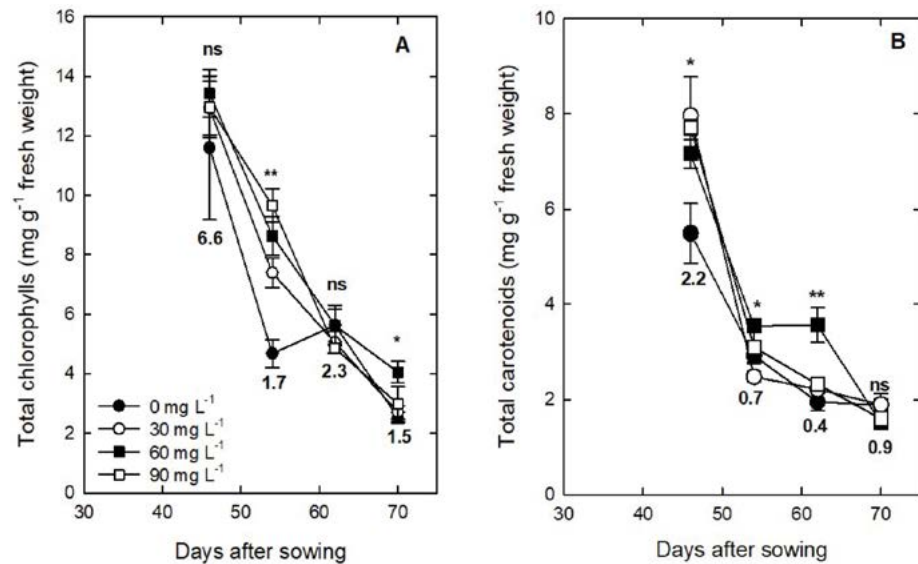
Total chlorophyll concentration decreased in all squash flowers as sampling dates progressed, with initial values ranging from 11.6 to 13.3  $\text{mg g}^{-1}$  fresh weight, declining to 2.5-4  $\text{mg g}^{-1}$  fresh weight in the last sampling (Figure 3A). GA<sub>3</sub> application significantly delayed the decrease in total chlorophyll, which was evident on days 54 and 70 (Figure 3A). Janowska and Jerzy *et al.* (2003) reported that applying a  $300 \text{ mg L}^{-1}$  GA<sub>3</sub> solution to calla lily leaves prevents chlorophyll degradation.

Total carotenoids decreased from initial values of 5.5 and 8  $\text{mg g}^{-1}$  fresh weight to 1.5 and 1.8  $\text{mg g}^{-1}$  fresh weight by the end of the evaluation period (Figure 3B). Aquino-Bolaños *et al.* (2013) reported that in squash flowers, carotenoid concentration decreases from 75 to 42  $\text{mg } 100 \text{ g}^{-1}$  fresh weight during eight days of storage at 5 °C. GA<sub>3</sub> application maintained total carotenoid concentration for a longer period, with the greatest effect observed at  $30 \text{ mg L}^{-1}$  GA<sub>3</sub> (Figure 3B). It has been reported that in 'Kinow' mandarins, GA<sub>3</sub> application at  $25 \text{ mg L}^{-1}$  helps to maintain total carotenoid content for a longer time (Talat *et al.*, 2020).

Phenolic compound content decreased significantly during the evaluation period in all treatments, except in plants sprayed with  $90 \text{ mg L}^{-1}$  GA (Figure 4A). At the beginning of



**Figure 2.** Flower and peduncle size in squash following GA<sub>3</sub> application. Each point represents the mean of three observations  $\pm$  standard error. The HSD value is shown for each sampling.

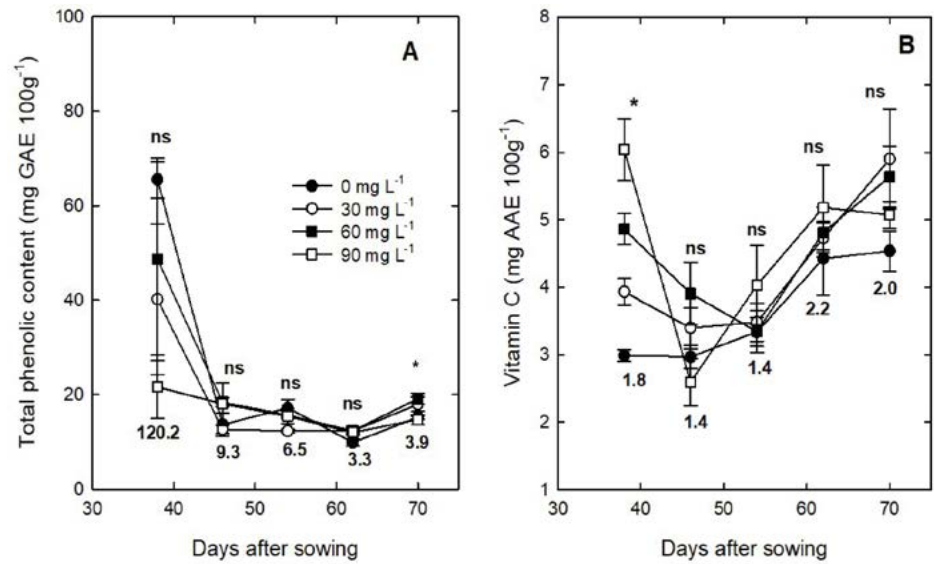


**Figure 3.** Chlorophyll (A) and total carotenoid (B) content in squash flowers following GA<sub>3</sub> application. Each point represents the mean of three observations  $\pm$  standard error. The HSD value is shown for each sampling.

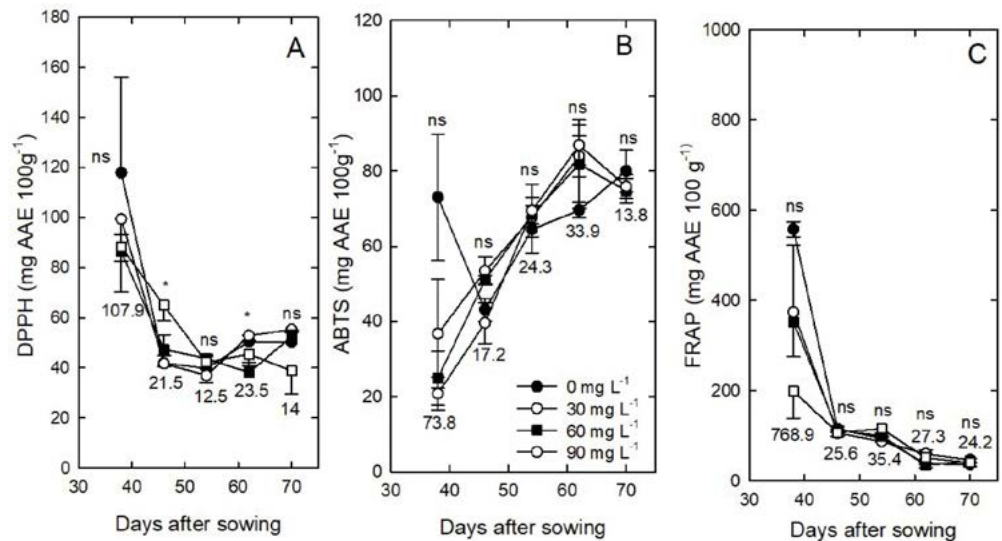
the evaluations, values ranged from 21 to 65 mg GAE 100 g<sup>-1</sup>, decreasing to 15-19 mg GAE 100 g<sup>-1</sup> by the last sampling. Differences were detected only at the beginning and end of the evaluations, with no clear trend observed (Figure 4A). Aquino-Bolaños *et al.* (2013) reported that phenolic compound concentration in squash flowers decreased from initial values of 334 mg 100 g<sup>-1</sup> to 238 mg 100 g<sup>-1</sup> after eight days at 5 °C. Toro-Vélez *et al.* (2022) indicated that phenolic content in squash flowers can decrease by up to 50% during postharvest.

Vitamin C content showed a decrease during the samplings at 46 and 54 days, and later increased in the final samplings (Figure 4B). Significant differences were detected only in the initial sampling, where higher GA<sub>3</sub> concentrations corresponded to higher vitamin C content. No significant differences were observed among treatments in the subsequent samplings, although flowers without GA<sub>3</sub> application had the lowest ascorbic acid concentration (Figure 4B). Aquino-Bolaños *et al.* (2013) report that vitamin C in squash flowers decreases after harvest from 16.51 mg 100 g<sup>-1</sup> to 9.09 mg 100 g<sup>-1</sup> during eight days of storage at 5 °C. There are no reports on the effect of gibberellins on vitamin C content in plants; however, in squash flowers a notable effect was the increase in its concentration, indicating that future studies should investigate this relationship in more detail.

Antioxidant activity measured by the DPPH and FRAP methods decreased during the sampling period (Figure 5A and C), whereas antioxidant activity measured by the ABTS method increased (Figure 5B). No significant effect of GA<sub>3</sub> application or control treatment was detected for any of the methods evaluated. Antioxidant activity ranged from 20 to 120 mg AAE 100 g<sup>-1</sup> for the DPPH and ABTS methods, and from 200 to 600 mg 100 g<sup>-1</sup> for FRAP, subsequently decreasing to 36 mg AAE 100 g<sup>-1</sup> (Figure 5A-C). Aquino-Bolaños *et al.* (2013) reported that postharvest squash flowers exhibit a decrease in antioxidant



**Figure 4.** Total phenolic (A) and vitamin C (B) content in squash flowers following GA<sub>3</sub> application. Each point represents the mean of three observations ± standard error. The HSD value is shown for each sampling.



**Figure 5.** Antioxidant activity of squash flowers measured by DPPH (A), ABTS (B), and FRAP (C) following GA<sub>3</sub> application. Each point represents the mean of three observations ± standard error. The HSD value is shown for each sampling.

activity from 62 mg AAE 100 g<sup>-1</sup> to 37.8 mg AAE 100 g<sup>-1</sup> after 8 days at 5 °C. Morittu *et al.* (2019) indicated that antioxidant activity in squash flowers, as measured by FRAP and ABTS, is substantial, and that the flowers also display antidiabetic activity.

A strong positive association was detected between total carotenoid content and antioxidant activity measured by FRAP ( $r=0.67^{***}$ ), and a negative association between total carotenoids and antioxidant activity measured by the ABTS method ( $r=0.71^{***}$ ). Phenolic compounds were positively and highly significantly associated with antioxidant

activity measured by DPPH and FRAP ( $r=0.72^{***}$  and  $r=0.88^{***}$ , respectively). These results indicate that, in squash flowers, phenolic compounds and carotenoids contribute the most to antioxidant capacity, compared to vitamin C.

## CONCLUSIONS

GA<sub>3</sub> application at doses of 30-60 mg L<sup>-1</sup> increased the number of male flowers without affecting morphological quality, functional molecules, antioxidants, or antioxidant activity.

## ACKNOWLEDGMENTS

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