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Influence of plant biostimulant and spacing on production and postharvest conservation of watermelons cv. Quetzali

V.F.S. de Paula, J.C. Vilvert (*), N.O. de Aráujo, I.B. do Nascimento, J.F. de Medeiros, E.M.M. Aroucha
Federal Rural University of the Semi-Arid Region, Mossoró, Rio Grande do Norte, Brazil.

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(*) **Corresponding author:**
jcvilvert@gmail.com

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Abstract: **The aim of this study was to evaluate the influence of pre-harvest application of plant biostimulant Crop Set® and different plant spacings on the production attributes and postharvest quality of watermelon 'Quetzali'. The experiment was set up in a completely randomized split-plot (3 × 2 × 4) design, corresponding to three plant spacings (0.40, 0.45 and 0.50 m), application of the plant biostimulant (with and without) and four storage periods at 10°C and RH 90% (0, 14, 21 and 28 days). Fruits were assessed after harvest in terms of average mass of fruits, number of fruits per plant and yield, and throughout the storage periods for flesh firmness, soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio, pH and total soluble sugars (TSS). The average mass of fruit (4.02 kg) was higher in the larger spacing without application of biostimulant. The pre-harvest application of plant biostimulant negatively influenced SSC of fruits, depending on the plant spacing and storage periods. For TA and TSS content, the effect of this product varies with plant spacing and storage days. The lower plant spacing provided higher TSS to the fruits.**

1. Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] is a vegetable belonging to the Cucurbitaceae family that has great economic and social expression, with a world production of 118,413,465 tonnes in 2017. Among the four largest producers are China, Turkey, Iran and Brazil, which together are responsible for 76% of global watermelon production (FAOSTAT, 2019).

In Brazil, the Northeast region is an important pole of agricultural crop, with soil and climate conditions favorable for watermelon cultivation throughout the year. Cultural practices are always studied to increase yield and quality of the products. Watermelon is the second most exported vegetable in Brazil and Quetzali is an early commercial cultivar, with the harvest at 70 days. This cultivar has average mass of 2.5 to 6.0 kg, green skin color with dark and thin streaks, red pulp with few seeds and high soluble solids content, which are desirable characteristics for consumers (Dia *et al.*, 2016).

During the vegetable development an unable cultural management of plant, in the field, can cause irreversible damage in fruit cells, which can affect their shelf life. The management techniques can morphologically and physiologically alter the plant, intervening in its productive potential and affecting the quality and the conservation of the fruits are plant spacing (Gomes *et al*., 2017) and use of plant biostimulants (Martins *et al*., 2013).

Species grown in high densities, especially cucurbits, produce a large number of fruits per area, but with small size, weight and number per plant, which may affect their development, and consequently, the final quality of fruits (Sabo *et al*., 2013; Oga and Umekwe, 2016).

On the other hand, plant biostimulants are substances applied to plants that enhance their nutrition efficiency, abiotic stress tolerance and/or quality traits (Jardin, 2015). They can be defined as mixtures of one or more plant growth regulators with other compounds of a different chemical nature, such as mineral salts (Castro and Pereira, 2008), which are applied in various species of fruits and vegetables with the aim of increasing its production and quality (Leão *et al*., 2005; Costa *et al*., 2008; Martins *et al*., 2013; Aroucha *et al.*, 2018).

Crop Set® (Improcrop-Kentucky-USA) is a commercial product registered in Brazil as foliar fertilizer, containing 1.5% manganese, 1.5% iron and 1% copper, it is a composed of agave (*Yucca shidigera*) extracts and mineral micronutrients with cytokinin-like action (Leão *et al*., 2005). The use of plant regulators belonging to the cytokinin group can increase the fruit size (Tecchio *et al*., 2006; Ainalidou *et al*., 2016) because inducing cell division and thus stimulating cell growth in plant tissues (Taiz *et al*., 2015). Nevertheless, its influence on the yield and watermelon conservation was not reported yet. The watermelon shelf life is around 2-3 weeks at 10-15°C (Maynard, 2001), depending on cultivar. A good quality is reached as soluble solid is above 8% (Tlili *et al*., 2011).

The aim of this study was to evaluate the influence of pre-harvest application of plant biostimulant Crop Set® and different plant spacings on the production attributes and postharvest quality of watermelon cv. Quetzali.

2. Materials and Methods

The experiment was carried out in Mossoró, state of Rio Grande do Norte, Brazil (4° 39’ 39” S, 37° 23’ 13” W, and 20 m of altitude). The climate of the region according to Köppen climate classification is BSwh type (hot and dry). The region has average annual temperature of 27°C, average annual precipitation of 673.9 mm, unevenly distributed, and air relative humidity of 68.9%.

The soil of the experimental area is classified as Neossolo quartzarenico (Santos *et al*., 2018) and its physical-chemical properties are: pH (H_20) = 5.52; organic matter: 5.5%; P (Mehlich) = 32 mg dm^{-3}; K = 96.5 cmol dm^{-3}; Ca = 1.60 cmol dm^{-3}; Mg = 0.43 cmol dm^{-3}; sand = 935.8 g kg^{-1}; silt = 26.5 g kg^{-1}; clay = 37.7 g kg^{-1}; bulk density = 1.48 g cm^{-3}; soil particle density = 2.69 g dm^{-3}; and total porosity = 0.45 m^3 m^{-3}. The results of the chemical analysis of the irrigation water are: pH = 7.70; electrical conductivity = 2.11 dS m^{-1}; K^+ = 0.12 mmol L^{-1}; Na^+ = 5.02 mmol L^{-1}; Ca^{2+} = 10.43 mmol L^{-1}; Mg^{2+} = 3.05 mmol L^{-1}; Cl^- = 11.48 mmol L^{-1}; CO_3^{-2} = 0.30 mmol L^{-1}; HCO_3^- = 3.70; Sodium adsorption ratio = 1.9 $(mmol^{-1})^{0.5}$; Hardness = 5.4 mg L^{-1}; Cations = 18.7 mmol L^{-1}; and Anions = 15.4 mmol L^{-1}.

The experiment was set up in a completely randomized split-plot (3 × 2 × 4) design, with six replications, each one corresponding to a plant. The plots consisted of plant spacings (2.0 x 0.4 m; 2.0 x 0.45 m and 2.0 x 0.5 m), application of the plant biostimulant Crop Set® (with and without), and postharvest storage (0, 14, 21 and 28 days) in the subplot (Fig. 1).

Seeds of watermelon cultivar Quetzali were used. Plant biostimulant was sprayed with a 20 L backpack sprayer, with stainless steel cone nozzle with flow rate of 615 mL/min, at 18 and 25 days after transplanting, applying 8 and 16 mL of Crop Set®, respectively, regularly on the plants, always in the same way. The dose of the biostimulant was determined according to the manufacturer’s recommendations.

The harvest was realized at 65 days after of seedling transplanting. Fruits were transported to Laboratory of Food Technology of the Federal Rural University of the Semi-Arid Region, where part of the fruits were characterized previously by sampling six fruits per treatment. The other part was stored in a cold chamber at 10±2°C and RH 90±1%, for 14, 21 and 28 days. After each storage periods, the fruit quality was evaluated.

Fruits were assessed after harvest for production in terms of number of fruits per plant, average mass of fruits and yield. At harvest and during cold storage, the quality characteristics were: flesh firmness (N), measured with a 12-mm tip manual penetrometer model 327 FT (McCormick, USA); soluble solids content (SSC, in °Brix), measured with a refractometer (PR - 100, Palette, Atago CO., LTD., Japan); titratable acidity (TA, in % of malic acid), analyzed by titrimetry; SSC/TA ratio; pH, evaluated using a digital potentiometer with glass membrane, calibrated with buffers of pH 7 and 4, according to the method of the Association of Official Analytical Chemists (AOAC, 2016); and total soluble sugars (TSS), measured by the Antrona method, as described by Yemn and Willis (1954), expressing results in percentage (%).

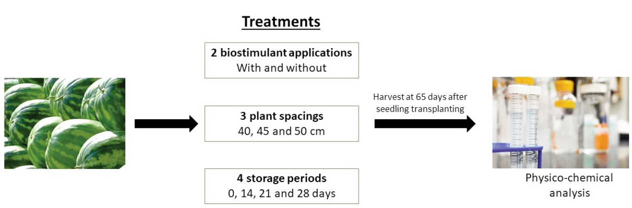

Fig. 1 - Scheme representing the treatments applied to the watermelon fruits.

Data were subjected to analysis of variance and means of the biostimulant and plant spacings factors were compared by the Tukey test ($p\leq0.05$). The effect of storage periods was evaluated by regression analysis. All statistical analysis were carried out using software Sisvar 5.3 (Ferreira, 2014).

3. Results

There was effect of plant spacing and biostimulant application on the average mass of fruits. While, the production attributes as number of fruits per plant and yield were not affected by plant spacing or application of plant biostimulant (Table 1).

During the fruit storage, there was a significant interaction effect between plant spacing, biostimulant application and storage periods on SSC (Fig. 2), TA (Fig. 3) and TSS (Table 2) of fruits. Also, there was an isolated effect of the storage periods on the SSC/TA

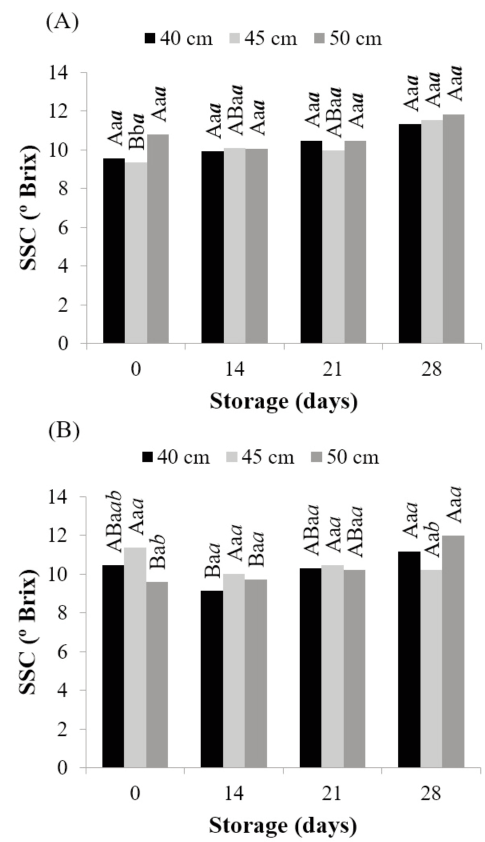

Fig. 2 - Means followed by the same letter do not differ by the Tukey test (p≤0.05). Uppercase letters compare the storage periods within the biostimulant x spacing combination; lowercase letters compare the presence (A) or absence (B) of the Crop Set® application within the storage period x biostimulant combination; italic lowercase letters compare the means of the plant spacings within storage periods x biostimulant combination. DMS for storage periods= 1.811; DMS for biostimulant application= 1.374; DMS for plant spacing= 1.649.

Table 1 - Average mass, number of fruits per plant and yield of 'Quetzali' watermelon depending on Crop Set ® application

Production attribute	Application of crop set	Plant spacing		
		40 cm	45 cm	50 cm
Average mass (kg)	With	3.43 Aa	3.51 Aa	3.92 Aa
	Without	3.41 Ba	3.47 Ba	4.02 Aa
Number of fruits per plant	With	1.16 Aa	1.06 Aa	1.43 Aa
	Without	0.97 Aa	1.39 Aa	1.58 Aa
Yield (t ha^{-1})	With	11.77 Aa	10.20 Aa	13.90 Aa
	Without	10.19 Aa	13.38 Aa	14.29 Aa

Means followed by the same letter do not differ by the Tukey test (p≤0.05). Uppercase letters compare plant spacings and lowercase letters compare application of biostimulant.

ratio, flesh firmness and pH of fruits (Fig. 4).

Fruits without application of biostimulant had the highest average mass in the larger plant spacing (50 cm) (Table 1).

The SSC increased, from 0 to 28 days, in fruit from plants on 45 cm spacing, with Crop Set® application, and non-spayed plants in growth on 40 and 50 cm spacings (Fig. 2). The biostimulant application only influenced on this physicochemical parameter at the day of the harvest, when fruits produced on 45 cm

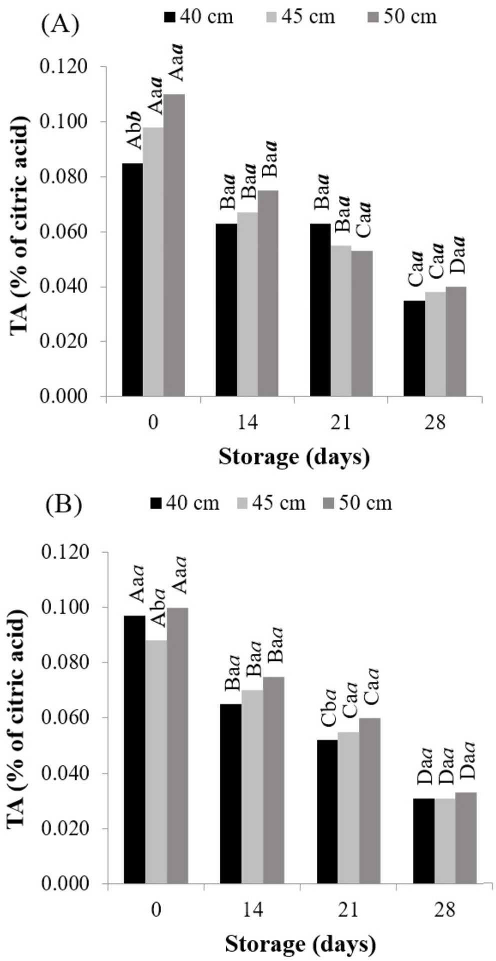

Fig. 3 - Means followed by the same letter do not differ by the Tukey test (p≤0.05). Uppercase letters compare the storage periods within the combination of biostimulant x spacing; lowercase letters compare the presence (A) or absence (B) of the Crop Set® application within the storage period x biostimulant combination; italic lowercase letters compare the means of the plant spacings within storage periods x biostimulant combination. DMS for storage periods= 0.012; DMS for biostimulant application= 0.009; DMS for plant spacing= 0.011.

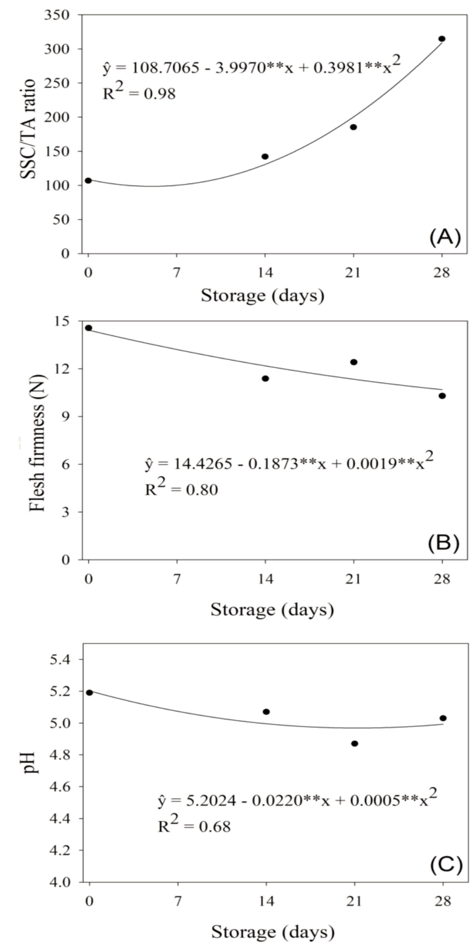

Fig. 4 - SSC/TA ratio (A), flesh firmness (B) and pH (C) of ‘Quetzali’ watermelon depending on storage periods.

Table 2 - SSC/TA ratio (A), flesh firmness (B) and pH (C) of ‘Quetzali’ watermelon depending on storage periods

Storage period (days)	Application of Crop Set®			No application of Crop Set®		
	40 cm	45 cm	50 cm	40 cm	45 cm	50 cm
0	7.66 Aa*a*	5.23 Bb*b*	7.80 ABa*a*	6.53 ABa*a*	7.67 Aa*a*	7.66 Aa*a*
14	7.74 Aa*a*	8.30 Aa*a*	8.55 Aa*a*	8.74 Aa*a*	6.91 Aa*ab*	6.26 ABb*b*
21	4.52 Ba*a*	4.80 Ba*a*	4.48 Ca*a*	5.14 Ba*a*	4.36 Ba*a*	4.94 Ba*a*
28	4.18 Ca*a*	4.48 Ba*a*	5.49 BCa*a*	4.43 Ba*a*	5.73 ABa*a*	5.06 Ba*a*

Means followed by the same letter do not differ by the Tukey test (p≤0.05). Uppercase letters compare the storage periods within the combination of biostimulant x spacing; lowercase letters compare the presence (A) or absence (B) of the Crop Set® application within the storage period x biostimulant combination; italic lowercase letters compare the means of the plant spacings within storage periods x biostimulant combination. DMS for storage time= 2.499; DMS for biostimulant application= 1.896; DMS for plant spacing= 2.275.

spacing with Crop Set® application had lower SSC than fruits by plants without application (Fig. 2). The reduction in spacing from 50 to 45 cm possibly increased the competition for water, mineral and luminous resources, leading to a reduction in the size of the fruits and, consequently, to an increase in the SSC due to the concentration effect. Besides that, fruits by non-sprayed plants in 45 and 50 cm spacings showed the highest SSC at 0 and 28 days, respectively. It is important to highlight that fruits produced on 40 cm spacing did not differed from SSC values of higher plant spacing, and therefore is the plant spacing with the best effect on SSC of fruits (Fig. 2).

During the storage, the TA of the fruits decreased as a function of storage periods (Fig. 3). When Crop Set® was applied, there was an effect of plant spacings only at the harvest day, in which fruits of 40 cm spacing shown a lowest TA than fruits of 45 and 50 cm spacings (Fig. 3A). No-application of Crop Set® did not affect the TA of fruits under different spacings (Fig. 3B).

When comparing fruits with and without application, we observed Crop Set® effect at zero and 21 days of storage. At the harvest day, fruits of plants cultivated in 40 cm spacing had lower TA with Crop Set® application, while in 45 cm plant spacing, the fruits had higher TA values with application of plant biostimulant. At 21 days of storage, in fruits by 40 cm plant spacing, Crop Set® application influenced the TA of fruits, increasing this value compared to the fruits without application of the biostimulant (Fig. 3).

With Crop Set application, fruits in 40 cm plant spacing showed a highest sugar content at the first 14 days of storage. On 45 and 50 cm spacing, fruits had the highest sugar content at 14 days, with further reduction of these values (Table 2). In all plant spacings, without Crop Set application, fruits shown high sugar content until 14 days of storage, followed by the decrease of these values. When comparing fruits with and without Crop Set® application, we observed that the plant biostimulant reduced sugar content of 45 cm spacing fruits at harvest. In contrast, this product shown a positive effect on 50 cm plant spacing fruits at 14 days of storage, elevating their sugar content (Table 2).

Comparing the different spatial arrangements in Crop Set® sprayed plants, we observed a lowest sugar content in fruits in 45 cm plant spacing, only at harvest. In plants without application, the 40 cm spacing favored sugar accumulation on the fruits, differing of the highest plant spacing at 14 days of storage (Table 2).

During storage, SSC/TA ratio had an increase of 65% from zero (106.81) to 28 days (314.61) (Fig. 4A). Despite the reduction in both parameters, the more pronounced reduction in TA, compared to SSC, increased the SSC/TA ratio.

The flesh firmness of fruits varied from 14.6 N to 10.9 N during storage, decreasing 25% (Fig. 4B).

During storage, we observed a variation of the pH in the fruits, starting in 5.19 at the harvest and ending in 5.02 at 28 days (Fig. 4C).

4. Discussion and Conclusions

The highest average mass of fruits by non-sprayed plants produced in the larger plant spacing (50 cm) can be attributed to the lower competition among the plants for soil nutrients, water and solar radiation, due to the lower density, which can lead to greater fruit development. Furthermore, a larger plant spacing reduce incidence of diseases, improving the mass of fruits (Bastos *et al*., 2008; Ban *et al*., 2011; Jafari *et al*., 2016).

The use of plant growth regulators can affect crop growth and development, stimulating cell division and increasing nutrient and water uptake (Castro and Vieira, 2001). In this study, the application of the biostimulant may have caused this effect, which reflects the absence of difference between plant spacings in relation to the average mass of fruits by sprayed plants.

Our results showed an increase of SSC in fruits on 45 cm spacing, with Crop Set® application, and non-spayed plants in growth on 40 and 50 cm spacings. According to Yau *et al*. (2010), the SSC of fruits usually decrease after a few days of storage due to the respiration process of the fruit, which is the oxidative breakdown of sugars into simpler molecules. In this case, the increase on the SSC of watermelon fruits observed during storage can be attributed to the solubilization of pectins. The sweetness is the most critical quality trait of watermelon, being mostly influenced by mono- and di-saccharides found in the fruit juice, and partly on other solutes, being all contributes to the juice SSC (Kyriacou *et al*., 2018).

In relation to the Crop Set® application, Martins *et al*. (2013) observed that this biostimulant raised the SSC of 'Quetzali' and 'Style' watermelons. Some studies did not find influence of plant spacing on SSC of watermelon fruits (Bastos *et al*., 2008; Gomes *et al*., 2017). The SSC in an important quality attribute of watermelon fruits, being desirable that they have values of SSC higher than 8°Brix (Tlili *et al.*, 2011), reached in this study.

Watermelon acidity is mainly attributed to the accumulation of malic acid (Özdemir *et al*., 2016) and its content tends to decrease during storage due to its use as a substrate in respiration (Silveira *et al*., 2013). Corroborating with the results of the present study, Silva *et al*. (2016) and Yau *et al*. (2010) also observed decreasing of the TA of fruits during the watermelon storage. Gomes *et al*. (2017) and Bastos *et al.* (2008) reported in their works that the plant spacing have not influence on TA of watermelon fruits. In its turn, Campagnol *et al*. (2012) found effect of plant spacing on fruit acidity, with the highest values in lowest plant spacing. In relation to Crop Set® application, Martins *et al.* (2013) did not observe an effect of this plant biostimulant on the TA of 'Quetzali' watermelons, differently of 'Style' fruits, which TA had reduction when this product was applied in the plants. Aroucha *et al.* (2018) emphasize that small variations in acidity levels of watermelon fruits are little significant, due to the low concentration of organic acids.

The reduction of sugar content of fruits observed at the last days of storage is related to the respiratory process of the fruits, that involves oxidative degradation of carbohydrates and organic acids.

The SSC/TA ratio of the fruits increased over storage. This parameter in an important indicator of flavor of fruits. Generally, highest SSC/TA ratio indicates a greater sweetness of fruits. This ratio is used to evaluate maturity and palatability of watermelon fruits. Values found in this work are much higher than those pointed by Campagnol *et al*. (2012) in 'Smile' watermelon, which showed SSC/TA ratio variation from 75.55 to 81.88.

The flesh firmness of the fruits decreased 25% over storage. This attribute is important to detect ripeness of watermelon fruits, being associated with the pectin solubilization and depolymerisation (Kyriacou *et al*., 2018). The same way that this work, Martins *et al*. (2013) did not observe effect of Crop Set® application on flesh firmness of watermelons of cultivars Quetzali and Style. Besides that, Campagnol *et al.* (2012) also did not appoint influence of plant spacing on flesh firmness of 'Smile' watermelon fruits.

A small variation on pH was observed on the fruits, and it is explained by the buffer capacity of some fruits, which stabilizes pH even when the decrease of TA is high (Paulson and Stevens, 1974).

In conclusion, the pre-harvest application of plant biostimulant Crop Set® negatively influenced some quality characteristics of 'Quetzali' watermelon, including the decrease of SSC of fruits, depending on the plant spacing and storage periods. In relation to titratable acidity and total sugar content, the effect of this product varies with plant spacing and storage days. The 40 cm plant spacing provided higher total sugar content to the fruits. Still, the 50 cm spacing increased mass of fruit without alter the yield, besides increasing acidity and soluble solids content at the end of storage, being the recommended plant spacing for 'Quetzali' watermelon plants.

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