



Improvement of Fruit Quality and Marketability of “Washington Navel” Orange Fruit by Cytokinin and Gibberellin



Hayam M. Elmenofy¹, Abdelfattah M.A. Kheder² and Ahmed H.A. Mansour¹

¹Fruit Handling Research Department, Horticultural Research Institute, Agricultural Research Centre, Cairo, Egypt.

²Citrus Research Department, Horticultural Research Institute, Agricultural Research Centre, Cairo, Egypt.

THE current investigation aimed to maximize the productivity of “Washington Navel” orange by reducing fruit splitting %, increase fruit weight and size, quality, in addition to improve marketability. So, in the 2016/2017 and 2017/2018 seasons a field experiment was carried out on a private orchard in Kafr El-Sheik governorate, Egypt. Trees were treated with Benzyladenin (BA) at 50ppm, Sytofix (CPPU) at 10ppm, GA₃ at 50ppm, and GA₃ combined with BA or CPPU. Plant growth regulators (PGRs) were applied three times after the full bloom stage by 15, 30 and 45 days as foliar application. Results revealed that PGRs increased quality parameters, productivity, and chlorophyll content, as well as reduced fruit splitting % and carotene content. GA₃ plus CPPU gave the highest fruit fresh weight, fruit length, fruit yield, and SSC%. However, GA₃+BA registered the greatest fruit diameter, chlorophyll content, TA%, vitamin C, as well as decreased fruit splitting % and carotene content. Regarding the marketing period, GA₃ plus BA obtained the highest reduction % in weight loss, decayed fruit percentage, however, recorded the greatest vitamin C and chlorophyll contents. ABA results clarified that application of PGRs tended to decrease significantly its content in peel fruit, in particular, GA₃ alone or combined with BA showed the lowest content of ABA in both seasons. These results will not only help researchers to understand the roles of PGRs in navel orange fruit growth, development, and productivity but can also be applied to large-scale fruit production and prolong the marketing period.

Keywords: Benzyladenin, GA₃, Sytofix, Navel orange yield, Splitting, Quality.

Introduction

“Navel” orange is the main citrus cultivars in Egypt and is an important export crop for foreign markets. According to a recent report on orange export in Egypt, during the 2018/2019 season, Egypt exported orange to 98 countries with an estimated total quantity of 1.7 million metric tons. The top 10 export countries were Russia, Saudi Arabia, Netherland, China, United Arab Emirates, United Kingdom, Bangladesh, Ukraine, India, and Malaysia (Anonymous, 2019).

Fruit productivity, quality and marketability

are greatly affected by fruit development stages, which are considered as the main contributing factors. Fruit growth includes cell division, elongation phenomena and their combination leading to changes in fruit size (Ozga and Reinecke, 2003, Bons et al., 2015, Canli & Pektas, 2015 and Ferrer et al., 2017). Fruit development is controlled and signaled by combined plant growth regulators (PGRs) activity of the different levels of these substances and PGRs have been used in two phenological stages: cell division and expansion (Ginzberg et al., 2014 and Kumar et al., 2014). Miñana et al. (1989) pointed out

that several cytokinins in the ovary have been identified in citrus species and showed a direct link between these PGRs and the initial stage of fruit development.

Moreover, among plant hormones involved in plant development, stress response and fruit maturation processes is abscisic acid (ABA) as reported by Leng *et al.* (2014), Jia *et al.* (2016), Zhang *et al.* (2019). The concentration of ABA increased before the maturation stage and then declined toward harvest (Karppinen *et al.*, 2013, Kammappana *et al.*, 2014). During natural maturity, the peel of citrus fruit accumulates a significant amount of oxygenated coloured carotenoids. Genetic and biochemical analysis showed evidence indicates that the oxidative cleavage of cis-epoxycarotenoids by 9-cis-epoxycarotenoid dioxygenase (NCED) in the synthesis of abscisic acid (ABA) in the higher plants represents a crucial stage, as well as it is proposed that the predominant substrate in the peel of citrus fruits for NCED could be 9-cis-violaxanthin (Rodrigo *et al.*, 2006).

Cytokinins play a significant role in cell division stimulation and promoting cell differentiation (Del Pozo *et al.*, 2005, Schaller *et al.*, 2014). Benzyladenines (BA) and Sitofex (CPPU) are considered as the most important cytokinins (Bubán, 2000). BA reported previously that can stimulate cell division in apple (Schröder *et al.*, 2013), pear (Canli and Pektas, 2015) and tangor (Ferrer *et al.*, 2017). In addition, CPPU can improve fruit enlargement by promoting cell division and/or through cell expansion in many fruit varieties i.e. blueberry (Retamales *et al.*, 2014), grapes (Jáuregui-Riquelme *et al.*, 2017, Senthilkumar *et al.*, 2018), kiwifruit (Luo *et al.*, 2017), and pear (Zhang *et al.*, 2020). Several studies conducted on different fruit crops mentioned that combined cytokinins and gibberellins has a significant impact on different aspects of fruit growth, for example, apple (Milošević *et al.*, 2014, Fogelman *et al.*, 2015), pear (Canli and Pektas, 2015), blueberry (Milić *et al.*, 2018). However, so far, available information regarding the impact of PGRs on the growth, productivity, quality and marketability of navel orange is very limited.

With respect to the specific morphology of citrus fruit, splitting fruit is varied comparing to other crops, which results in the development of flavedo microcracks and initiation of the split fruit at the styler end of the citrus fruit by applying a pressure of the rapid expansion pulp during fruit

growing as reported previously by Cronjé *et al.* (2013). The splitting in citrus is usually started at the end of fruit blossom, which is the weakest part of the skin (Garcia-Luis *et al.* 1994). PGRs in the fruit epidermal layer may increase the flexibility of the peel due to enhance cell division and fruit cell density, thus reducing the susceptibility of splitting at the development stage of fruit expansion (Stern *et al.*, 2013).

Consequently, the current investigation aimed to evaluate the performance of two types of cytokinins (BA and CPPU) and GA₃ applied after full bloom in the form of foliar application on orange trees, to improve the productivity and fruit quality, especially fruit size as well as reduction of splitting fruit and the maintenance of marketability, and to estimate the level of abscisic acid during maturity stage in addition its relation with the splitting ratio.

Materials and Methods

Plant material

The present research was conducted during 2016/2017 and 2017/2018 seasons at a private orchard located in Dacult area, Kafrelsheikh governorate, Egypt. Healthy and uniform trees of “Washington Navel” orange (*Citrus sinensis* L.) budded on sour orange rootstock (*Citrus aurantium*) with approximately 16-years old trees were selected in the current study. Trees were planted at 5x5 m apart and growing in clay soil. The trees were subjected to the same agriculture practices as usually done in the same orchard.

Experimental design

The present study was carried out as a randomized complete block design with three replicates per treatment and one tree per replication. The plant growth regulators (PGRs) were applied in the form of foliar application, which were zero (control), Benzyladenin (BA, 50ppm), Sytofix (CPPU, 10ppm), GA₃ (50ppm), GA₃ (50ppm) plus BA (50ppm) and GA₃ (50ppm) plus CPPU (10ppm). The trees were sprayed till runoff by using a back-gun sprayer at the rate of 6 L per tree. Treatments were applied three times after the full bloom stage by 15, 30 and 45 days.

Data collection

Maturity stage

At the stage of fruit maturity (from 210 to 230 days from full bloom), the abscisic acid was measured according to the method described by Hubick and Reid (1980) on 15 October and 15 November during two seasons.

Harvest stage

At harvest time (mid of december), a total of 20 fruits were randomly selected from each replicate to measure the following characteristics, fruit fresh weight (g), fruit length (cm), fruit diameter (cm), fruit shape index (%), fruit volume (ml), number of fruits/tree, fruit yield (kg/tree), fruit peel thickness (mm) and fruit splitting (%) per tree, which calculated by the following equation:

Fruit splitting (%) = (Weight of splitting fruits per tree / Total fruit yield per tree) x 100.

Chlorophyll and carotenoid contents in the peel of Washington navel orange were spectrophotometrically determined according to the method of Wellburn (1994). The absorbance of the extract was measured at a spectrum of 663 nm for chlorophyll a, 646 nm for chlorophyll b, and 470 for carotene by using a spectrophotometer (UV1901PC spectrophotometer). Pigment contents were calculated by the following equations:

Chlorophyll a ($\mu\text{g/ml}$) = $12.21 E_{663} - 2.81 E_{646}$

Chlorophyll b ($\mu\text{g/ml}$) = $20.13 E_{646} - 5.03 E_{663}$

Total chlorophyll ($\mu\text{g/ml}$) = chlorophyll a + chlorophyll b

Total carotenoids ($\mu\text{g/ml}$) = $[(1000 E_{470}) - (3.27 \times \text{chlorophyll a} + 104 \times \text{chlorophyll b})] / 198$

Where:

E = Optical density at the indicated spectrum length.

Results were expressed as mg/100 g of fresh weight (FW) as follows: $[(\text{Resulting value from each equation} \times \text{volume extract}) / (1000 \times \text{FW})] \times 100$.

Soluble solids content (SSC as Brix) was determined by hand refractometer and titratable acidity (TA) as g citric acid/100 ml juice (A.O.A.C., 2000). Vitamin C as mg ascorbic acid/100 ml juice was estimated by using 2, 6 dichlorophenol indophenol, according to A.O.A.C. (2000).

Fruit splitting/tree (%) = (Weight of splitting fruits per tree / Total fruit yield per tree) x 100.

Marketing period evaluation

After fruit harvesting, 15 fruits were collected from each replicate and stored at room temperature ($19 \pm 1^\circ\text{C}$ and relative humidity 60-65%) for 15 days. Then, the following parameters were measured three times every 5 days (5, 10,

15 days): weight loss (%), decayed fruits (%), chlorophyll content (mg/100g FW), carotene content (mg/100g FW), soluble solids content (SSC as Brix), TA (g citric acid/100ml juice) and vitamin C (mg ascorbic acid/100 ml juice).

Where:

Weight loss (%) = $[(\text{Initial fruit weight} - \text{Final fruit weight}) / \text{Initial Fruit weight}] \times 100$.

Decayed fruit (%) = (Weight of decay fruit / Initial fruit weight) x 100.

Statistical analysis

The obtained data of both seasons were subjected to analysis of variance using Statistix 8.1 software (Analytical Software, 2005). Means were compared for significant differences using Tukey test at $P = 0.05$. Data were presented as the means \pm standard deviations.

Results

Fruit fresh weight (g)

The fruit fresh weight of navel orange in response to plant growth regulators (PGRs) was investigated. There was a significant difference ($P < 0.05$) among various PGRs (Fig.1). Treated navel orange trees with GA_3 plus CPPU had a significantly greater average fruit weight (299.03 g and 321.04 g during 1st and 2nd seasons, respectively) compared to other treatments. The lowest fruit weight was recorded in the control, which was 238.67g and 242.98g in the first and second seasons, respectively.

Fruit length and diameter (cm)

Different PGRs significantly affected the fruit length and diameter during two seasons (Fig.2). Comparing to control, all PGRs treatments recorded the greatest value of fruit length and diameter. Navel orange trees subjected to combined treatment of GA_3 and CPPU showed the highest value of fruit length (8.15cm and 8.40cm in the 1st and 2nd seasons, respectively). However, combined treatments of GA_3 plus BA recorded the biggest fruit diameter in both seasons (7.13cm and 7.28cm, respectively). In contrast, untreated trees give the lowest fruit length and diameter during two experiment seasons.

Fruit shape index and fruit volume (cm^3)

The statistical analysis revealed that there were significant differences in the fruit shape and volume among all experiment treatments (Table 1). Application of CPPU alone or with GA_3 recorded the highest value of fruit shape

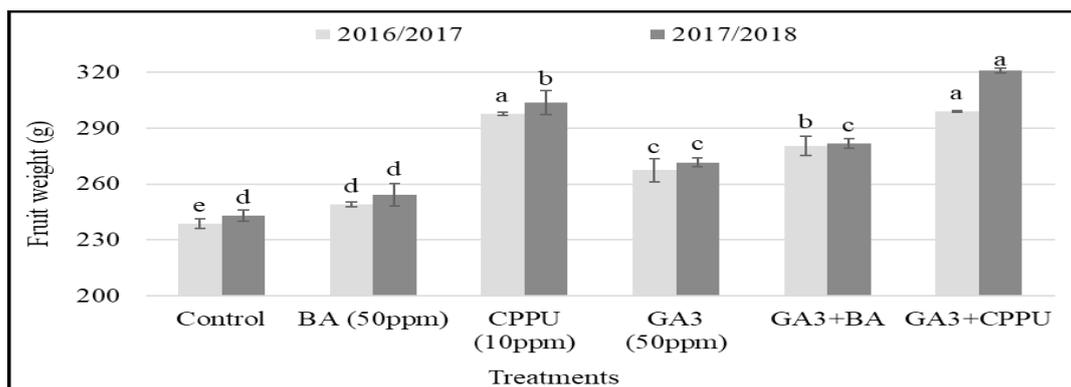


Fig. 1. Influence of benzyladenin (BA, Sytofix (CPPU), gibberellins (GA_3), GA_3 plus BA, and GA_3 plus CPPU on fruit fresh weight (g) of navel orange in 2016/17 and 2017/18 seasons. Data were presented as means \pm SDs and the different upper letters indicate significant differences at $P < 0.05$ level according to Tukey test.

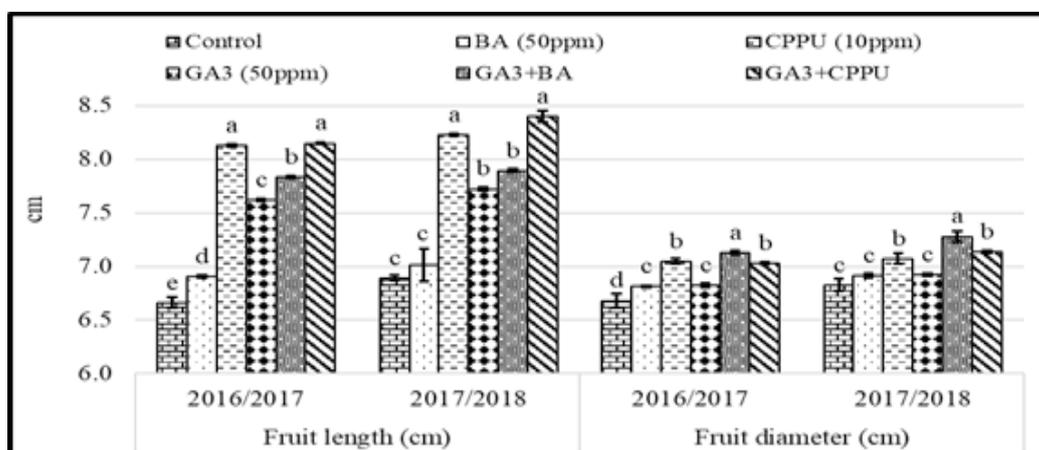


Fig. 2. Influence of benzyladenin (BA, Sytofix (CPPU), gibberellins (GA_3), GA_3 plus BA, and GA_3 plus CPPU on (a) fruit length (cm) and (b) fruit diameter (cm) in 2016/2017 and 2017/2018 seasons. Data were presented as means \pm SDs and the different upper letters indicate significant differences at $P < 0.05$ level according to Tukey test.

and volume compared to control and other PGRs treatments. The highest increase percentage of fruit volume compared to control was observed when navel orange trees were treated with GA_3 plus CPPU, which were 38.43 and 38.15% in the first and second seasons, respectively.

Number of fruits/tree:

Results illustrated in Table 1 indicated that the number of fruits per tree was significantly affected by PGRs treatments at the level of $P < 0.05$ during studied seasons. Spray application with GA_3 or GA_3 plus BA produced a higher number of navel orange fruits/tree, which were 326 and 324, respectively, in the first season and 314 and 316,

respectively, in the second season. Followed by CPPU in the first season and with no significant difference in the second season. However, the lowest fruit number per tree was observed in control treatment in both seasons.

Fruit yield (kg/tree)

The analysis variance of navel orange fruit yield per tree (kg/tree) confirmed that the effect of PGRs treatments was found to be significant for fruit yield ($P < 0.05$) during two seasons (Table 1). In general, all PGRs treatments give the greatest fruit yield per tree compared to control trees. During the two seasons, spraying orange trees with CPPU or CPPU plus GA_3 produced the

highest fruit yield of navel orange per tree. On other hand, the results showed that control trees recorded 22.40 and 25.40 reduction percentage in navel orange fruit yield per tree compared to CPPU or CPPU plus GA₃ treatments.

Fruit peel thickness (mm)

Exogenous application of solo BA, GA₃ and CPPU or in combination decreased the fruit peel thickness significantly compared to untreated trees (Table 2). The smallest fruit peel thickness was observed when navel orange trees sprayed with CPPU (0.33mm and 0.29mm during the 1st and 2nd seasons, respectively). The maximum reduction in fruit peel thickness compared to control was 36.54% and 42% during 2016/17 and 2017/18, respectively. This was followed by combined treatment of CPPU plus GA₃, which were 0.39mm and 0.34mm in the first and second seasons, respectively.

Fruit splitting (%)

A foliar application of plant growth regulators had a significant effect on the splitting of navel orange fruit in two seasons (Table 2). Treated navel orange trees with GA₃ plus BA led to a decrease in the fruit splitting significantly (1.65 and 3.38% in 2016/17 and 2017/18 seasons, respectively) with 84.64 and 68.02% reduction percentage compared to control in both seasons, respectively. This was followed by the application of GA₃, which were 5.47 and 4.68% in the 1st and 2nd seasons, respectively.

Chlorophyll content (mg/100g FW)

The application of GA₃ plus BA increased

chlorophyll content in the peel of navel orange fruits as presented in Table 2 (23.29mg/100g FW and 18.43mg/100g FW in first and second seasons, respectively). This was followed by foliar application by GA₃ (18.65 and 15.24mg/100g FW, during 2016/17 and 2017/18, respectively). However, untreated trees (control) recorded the lowest value of chlorophyll content with a reduction percentage of 56.93% in the 1st season and 41.67% in the 2nd season.

Carotene content (mg/100g FW)

As shown in Table 2, the results of carotene content were in contrast with chlorophyll content during both seasons. Trees subjected to GA₃ plus BA produced the lowest carotene content in fruit peel, which recorded 0.25 and 0.33 mg/100g FW in the first and second seasons, respectively. Moreover, the reduction percentage of GA₃+BA treatment compared to control trees was 84.07% and 83.66% in 1st and 2nd seasons, respectively.

Soluble solids content (SSC as Brix)

Among plant growth regulators, foliar application with single CPPU (11.93 and 12.43%) or in combination with GA₃ (12.13 and 12.73%) showed a significantly higher percentage of SSC in the navel orange fruit than those in the GA₃+BA (10.85 and 10.90%) as shown in Table 3.

Total acidity content (TA as g citric acid/100 ml juice)

Data illustrated in Table 3 clarified that obtained results of TA were in contrast with a soluble solids content in 2016/2017 and 2017/2018. Foliar application of GA₃ plus BA

TABLE 1. Influence of benzyladenin (BA), Sytofix (CPPU), gibberellins (GA₃), GA₃ plus BA and GA₃ plus CPPU on Fruit shape index, fruit volume (ml), number of fruit/tree and fruit yield (kg/tree) in 2016/2017 and 2017/2018 seasons.

Treatments	2016/17				2017/18			
	Fruit shape index	Fruit volume (ml)	Number of fruits/tree	Fruit yield (kg/tree)	Fruit shape index	Fruit volume (ml)	Number of fruits/tree	Fruit yield (kg/tree)
Control	1.00±0.00 ^c	170.00±5.00 ^c	303±3.23 ^d	72.38±1.53 ^d	1.01±0.01 ^c	173.00±6.24 ^d	296±3.52 ^d	72.05±1.76 ^d
BA (50ppm)	1.01±0.00 ^d	181.00±3.61 ^{bc}	307±1.63 ^c	76.74±0.29 ^c	1.01±0.03 ^c	184.00±4.58 ^d	306±1.38 ^b	77.93±1.71 ^c
CPPU (10ppm)	1.15±0.01 ^a	232.33±2.52 ^a	313±1.43 ^b	93.27±0.58 ^a	1.16±0.01 ^a	235.33±3.06 ^{ab}	317±2.01 ^a	96.56±2.64 ^a
GA ₃ (50ppm)	1.12±0.00 ^b	191.67±7.64 ^b	326±1.89 ^a	87.39±2.38 ^b	1.11±0.00 ^b	199.00±4.58 ^c	314±1.79 ^a	85.46±0.87 ^b
GA ₃ +BA	1.10±0.00 ^c	224.33±4.04 ^a	324±1.46 ^a	91.06±1.98 ^a	1.08±0.01 ^b	226.67±4.16 ^b	316±2.29 ^a	89.32±1.40 ^b
GA ₃ +CPPU	1.16±0.00 ^a	235.33±0.58 ^a	307±2.11 ^c	91.93±0.73 ^a	1.18±0.01 ^a	239.00±1.00 ^a	300±2.50 ^{bc}	96.58±0.57 ^a

Data were presented as means ± SDs and the different upper letters indicate significant differences at P < 0.05 level according to Tukey test.

TABLE 2. Influence of benzyladenin (BA), Sytofix (CPPU), gibberellins (GA3), GA3 plus BA and GA3 plus CPPU on peel thickness (mm), splitting (%), chlorophyll content (mg/100g FW) and carotene content (mg/100g FW) in 2016/2017 and 2017/2018 seasons.

Treatments	2016/2017				2017/2018			
	Peel thick-ness (mm)	Splitting (%)	Chlorophyll (mg/100g FW)	Carotene (mg/100g FW)	Peel thick-ness (mm)	Splitting (%)	Chlorophyll (mg/100g FW)	Carotene (mg/100g FW)
Control	0.52±0.03 ^a	10.74±0.96 ^{ab}	10.03±0.39 ^c	1.57±0.20 ^a	0.50±0.03 ^a	10.57±0.50 ^{ab}	10.75±0.25 ^c	2.02±0.08 ^a
BA (50ppm)	0.49±0.02 ^{ab}	6.65±0.35 ^c	10.95±0.24 ^c	1.61±0.09 ^a	0.47±0.02 ^a	6.86±0.24 ^c	12.78±0.22 ^d	1.94±0.06 ^a
CPPU (10ppm)	0.33±0.01 ^d	11.78±0.89 ^a	11.99±0.21 ^d	1.64±0.04 ^a	0.29±0.02 ^b	11.16±0.16 ^a	12.32±0.22 ^d	1.94±0.10 ^a
GA ₃ (50ppm)	0.46±0.01 ^b	5.47±0.36 ^c	18.65±0.35 ^b	0.47±0.02 ^{bc}	0.45±0.02 ^a	4.68±0.18 ^d	15.24±0.24 ^b	0.65±0.05 ^b
GA ₃ +BA	0.49±0.02 ^{ab}	1.65±0.30 ^d	23.29±0.29 ^a	0.25±0.05 ^c	0.45±0.03 ^a	3.38±0.38 ^c	18.43±0.17 ^a	0.33±0.04 ^c
GA ₃ +CPPU	0.39±0.02 ^c	9.78±0.22 ^b	13.44±0.44 ^c	0.73±0.01 ^b	0.34±0.02 ^b	9.52±0.48 ^b	13.56±0.34 ^c	0.82±0.10 ^b

Data were presented as means ± SDs and the different upper letters indicate significant differences at $P < 0.05$ level according to Tukey test.

showed the highest TA content, which were 1.56 and 1.53 mg/100/ml juice during the first and second seasons, respectively. Meanwhile, the control treatment recorded a reduction percentage (19.23 and 16.99%, respectively in the two seasons) compared to combined treatment GA₃+BA.

Vitamin C content (mg ascorbic acid/100 ml juice)

The effect of PGRs treatment on vitamin C content of navel orange fruit was presented in Table 3. The greatest value of vitamin C was obtained from spraying navel orange trees with GA₃ plus BA (54.99 and 49.52 mg/100 ml juice during 1st and 2nd seasons, respectively), which was not significantly different from sole GA₃ treatment (54.34 and 49.93 mg/100 ml juice in both seasons). On other hand, vitamin C content in control trees was 48.28 and 45.44 mg/100 ml juice in 2016/17 and 2017/18 seasons, respectively.

Abscisic acid content (ABA, µgg-1 FW)

The endogenous hormone (ABA) content was measured two times during the maturity stage at 15 October and 15 November during both seasons. Data presented in Fig 3 showed an increase in fruit peel content of ABA at 15-November in both seasons compared to 15-October. In two sampling times (15-Oct. and 15-Nov.) control trees recorded the highest ABA content (0.35 and 0.56 µgg-1 FW in 1st and 2nd season and 0.35 and 0.45 µgg-1 FW in 2nd season at 15-Oct. and 15-Nov., respectively). In general, foliar application of PGRs tended to decrease significantly ABA content in peel navel orange fruits in two seasons. Sprayed trees with GA3 alone or combined with BA showed the lowest content of ABA in both

seasons. The reduction percentage compared to control trees in the 2018 and 2019 seasons were 51.43% and 50% at 15-Oct. and 15-Nov., respectively during the first season and were 44.44 and 51.11% in the second season at 15-Oct. and 15-Nov., respectively.

Marketing period evaluation

Weight loss (%)

Data presented in Fig. 4 show a continued increase in weight loss percentage as the marketing period increased. As a result, the highest percentage of fruit weight loss was obtained from the control treatment compared with other treatments, which were 3.92 and 3.72% at five days, 7.96 and 7.52% at 10 days, however, at 15 days were 11.54 and 10.24% in both seasons, respectively. In contrast, foliar application with GA₃ plus BA was lower in fruit weight loss percentage than all other treatments during all different samples time, which recorded 2.85% at 5 days, 5.12% at 10 days and 6.17% at 15 days in 2016/17. In 2017/18 season, GA₃ plus BA recorded 3.27, 5.18 and 6.84 at 5, 10 and 15 days, respectively.

Decayed fruits (%)

The effect of PGRs application on the percentage of decayed fruit at different time sampling during marketing was presented in Table 4. In general, at 5 days no decayed fruits were detected during two seasons. However, at 10 days, decayed fruits were observed only in control, BA and CPPU treatments, but no decayed fruits were observed in foliar application with solo GA₃ or in combination with BA or CPPU. Control treatment

TABLE 3. Influence of benzyladenin (BA), Sytofix (CPPU), gibberellins (GA₃), GA₃ plus BA and GA₃ plus CPPU on Soluble solids content (SSC as Brix), TA (g citric acid/100 ml juice) and Vitamin C (mg ascorbic acid/100 ml juice) in 2016/17 and 2017/18 seasons.

Treatments	2016/17			2017/18		
	Soluble solids content (SSC as Brix)	TA (g citric acid/100 ml juice)	Vitamin C (mg ascorbic acid/100 ml juice)	Soluble solids content (SSC as Brix)	TA (g citric acid/100 ml juice)	Vitamin C (mg ascorbic acid/100 ml juice)
Control	11.30±0.10 ^b	1.26±0.06 ^b	48.28±0.72 ^c	11.95±0.18 ^{ab}	1.27±0.09 ^c	45.44±0.44 ^b
BA (50ppm)	11.30±0.10 ^b	1.33±0.07 ^b	48.23±1.77 ^c	11.90±0.17 ^{ab}	1.30±0.07 ^c	45.49±1.00 ^b
CPPU (10ppm)	11.93±0.06 ^a	1.22±0.11 ^b	49.54±1.04 ^c	12.43±0.21 ^a	1.33±0.03 ^{bc}	45.52±0.98 ^b
GA ₃ (50ppm)	10.90±0.10 ^b	1.52±0.10 ^a	54.34±0.34 ^{ab}	11.10±0.50 ^{bc}	1.50±0.05 ^{ab}	49.93±1.02 ^a
GA ₃ +BA	10.85±0.15 ^b	1.56±0.04 ^a	54.99±1.00 ^a	10.90±0.30 ^c	1.53±0.06 ^a	49.52±1.01 ^a
GA ₃ +CPPU	12.13±0.32 ^a	1.19±0.04 ^b	51.22±0.78 ^{bc}	12.73±0.35 ^a	1.31±0.01 ^c	46.66±0.67 ^b

Data were presented as means ± SDs and the different upper letters indicate significant differences at P < 0.05 level according to Tukey test.

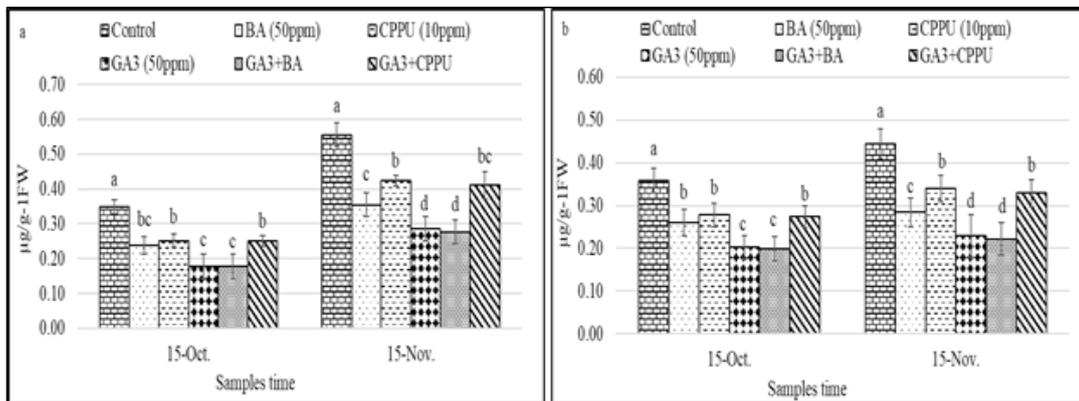


Fig. 3. Influence of benzyladenin (BA, Sytofix (CPPU), gibberellins (GA₃), GA₃ plus BA, and GA₃ plus CPPU on abscisic acid (ABA) content in fruit peel of navel orange during (a) 2016/17 and (b) 2017/18 seasons. Data were presented as means ± SDs and the different upper letters indicate significant differences at P < 0.05 level according to Tukey test.

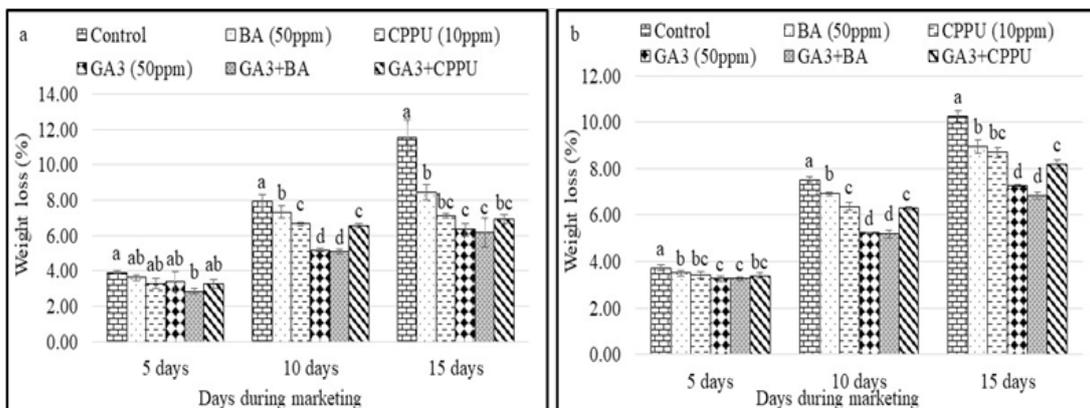


Fig. 4. Influence of benzyladenin (BA, Sytofix (CPPU), gibberellins (GA₃), GA₃ plus BA and GA₃ plus CPPU on weight loss% during (a) 2016/17 and (b) 2017/18 seasons. Data were presented as means ± SDs and the different upper letters indicate significant differences at P < 0.05 level according to Tukey test.

recorded the maximum percentage of decayed fruits, which were 8.62 and 10.75% during the 2016/17 and 2017/18 seasons, respectively, at 10 days. Moreover, a similar trend was observed at 15 days, where control treatment showed the highest decayed fruit percentage in both seasons (21.33 and 20.13%). Combined treatment of GA₃ plus BA showed the highest reduction percentage in decayed fruit percentage over other PGRs treatments relative to the control (83.92 and 69.30% in 1st and 2nd seasons, respectively).

Chlorophyll content (mg/100g FW)

Data illustrated in Table 4 demonstrate that chlorophyll content was decreased by increasing the marketing period in all treatments. Treated navel orange trees by GA₃ plus BA gave the maximum value of chlorophyll content in fruit peel at 5 days (15.24 and 14.38 mg/100g FW during first and second seasons, respectively). Moreover, in the same treatment, chlorophyll content was gradually decreased by marketing period progress and reached to the maximum reduction at 15 days, which were 53.54 and 62.17% in 2016/17 and 2017/18 seasons, respectively, comparing 5 days. In general, control treatment showed the lowest chlorophyll content during marketing at all sampling times (4.54, 1.66 and 1.48 mg/100g FW in 2016/17 and 5.24, 1.56, and 1.33 mg/100g FW in 2017/18 at 5, 10 and 15 days, respectively).

Carotene content (mg/100g FW)

The results of PGRs application presented in Table 4 confirmed that carotene content in fruit peel was in contrast with chlorophyll content. Whereas, carotene content was increased gradually by extending the marketing period. Overall sampling time, control treatment showed the highest carotene content (5.10, 9.32 and 11.22 mg/100g FW at 5, 10 and 15 days, respectively, during the first season and 9.37, 12.32, and 14.32 mg/100g FW at 5, 10 and 15 days, respectively, during the second season). However, GA₃ plus BA showed the lowest value of carotene content at 5, 10 and 15 days in both seasons, which were 0.73, 1.73 and 3.04 mg/100g FW in 2016/17 and 1.16, 2.24 and 2.37 mg/100g FW in 2017/18.

Soluble solids content (SSC as Brix)

The influence of foliar application with various PGRs on the content of the soluble solids during marketing, the percentage of SSC at 5, 10 and 15 days during marketing was measured (Table 5). Obtained data revealed that SSC was gradually increased with the advancing of marketing duration in 2016/17 and 2017/18 seasons. The

minimum value of SSC was recorded in fruit juice of navel orange trees treated with GA₃ alone or in combination with BA. However, control treatment showed the highest SSC content, which was 12.64, 12.97, and 13.34 % in 1st seasons and 12.86, 12.97 and 13.13 in 2nd seasons at 5, 10 and 15 days, respectively.

Total acidity (TA g citric acid/100 ml juice)

Plant growth regulators had various effects on TA content during marketing at different sampling times (5, 10 and 15 days) in the 1st and 2nd seasons (Table 5). The greatest TA value was recorded when navel orange trees sprayed with GA₃ plus BA (1.32, 1.23 and 1.21 g/100ml juice at 5, 10 and 15 days during 2016/17 and 1.28, 1.18 and 1.16 g/100ml juice at 5, 10 and 15 days during 2017/18). However, solo CPPU or in combination with GA₃ obtained the lowest values of TA during both seasons at 5, 10 and 15 days.

Vitamin C content (mg ascorbic acid/100 ml juice)

Results in Table 5 clear that vitamin C content was gradually decreased with the marketing period extending in all experimental treatments. The lowest content of vitamin C was observed at control treatment overall sampling time (41.98, 40.11 and 27.24 mg/100ml juice during 2016/17 and 35.94, 32.56 and 29.99 mg/100ml juice during 2017/18 at 5, 10 and 15 days, respectively). In contrast, the greatest vitamin C content was obtained with the application of solo GA₃ or in combination with BA. The maximum increase percentage compared to the control treatment of two seasons were 19.97, 19.87 and 34.01% at 5, 10 and 15 days during marketing, respectively.

Discussion

Pre- and post-harvest practices significantly influence fruit losses in citrus. Application of exogenous of different plant growth regulators has become an efficient approach for most plants, especially for fruit due to their important use in agriculture (Ashebre, 2015). Although many studies have reported the economic application of PGRs on flowers, fruits, and shoots (Eman *et al.*, 2007, Whiting, 2007 and Hanafy *et al.*, 2012). Knowledge of the mechanisms underlying plant growth regulators on fruit productivity and quality, and fruit splitting as well as prolong marketing period is very limited in navel orange. Therefore, foliar application with solo or combined various PGRs was carried out in the current investigation.

The obtained results corroborated previous findings that plant growth regulators used

TABLE 4. Influence of benzyladenin (BA), Sytofix (CPPU), gibberellins (GA₃), GA₃ plus BA and GA₃ plus CPPU on decayed fruit (%), chlorophyll content (mg/100g FW) and carotene content (mg/100g FW) at three time sampling during marketing period in 2016/17 and 2017/18 seasons

Treatments	2016/17								
	Decayed fruits (%)			Chlorophyll (mg/100g FW)			Carotene (mg/100g FW)		
	5 days	10 days	15 days	5 days	10 days	15 days	5 days	10 days	15 days
Control	0.00	8.62±0.62 ^a	21.33±0.67 ^a	4.54±0.26 ^d	1.66±0.06 ^d	1.48±0.11 ^c	5.10±0.40 ^a	9.32±0.32 ^a	11.22±0.24 ^a
BA (50ppm)	0.00	2.99±0.49 ^b	11.13±0.87 ^b	4.59±0.20 ^d	1.79±0.09 ^d	1.61±0.11 ^c	4.05±0.20 ^b	7.38±0.38 ^b	11.09±0.30 ^a
CPPU (10ppm)	0.00	3.44±0.22 ^b	10.23±0.23 ^b	5.88±0.09 ^c	3.65±0.15 ^c	1.82±0.12 ^c	3.86±0.20 ^b	7.46±0.46 ^b	11.16±0.16 ^a
GA ₃ (50ppm)	0.00	0.00±0.00 ^c	3.45±0.25 ^d	10.51±0.49 ^b	7.52±0.15 ^b	3.72±0.12 ^b	1.15±0.15 ^d	1.97±0.01 ^d	4.01±0.27 ^c
GA ₃ +BA	0.00	0.00±0.00 ^c	3.43±0.43 ^d	15.24±0.24 ^a	8.96±0.68 ^a	7.08±0.29 ^a	0.73±0.13 ^d	1.73±0.17 ^d	3.04±0.24 ^d
GA ₃ +CPPU	0.00	0.00±0.00 ^c	5.98±0.30 ^c	5.65±0.44 ^c	4.45±0.45 ^c	1.80±0.20 ^c	2.29±0.29 ^c	3.70±0.20 ^c	6.73±0.37 ^b
2017/18									
Control	0.00	10.75±0.75 ^a	20.13±0.40 ^a	5.24±0.24 ^c	1.56±0.16 ^c	1.33±0.17 ^c	9.37±0.37 ^a	12.32±0.32 ^b	14.32±0.32 ^a
BA (50ppm)	0.00	3.84±0.10 ^b	10.88±0.40 ^b	6.88±0.20 ^d	4.54±0.46 ^b	1.54±0.12 ^c	9.28±0.28 ^a	12.44±0.44 ^b	15.13±0.13 ^a
CPPU (10ppm)	0.00	3.88±0.12 ^b	10.74±0.30 ^b	6.58±0.10 ^d	4.25±0.25 ^b	1.54±0.14 ^c	9.16±0.16 ^a	13.43±0.43 ^a	14.62±0.32 ^a
GA ₃ (50ppm)	0.00	0.00±0.00 ^c	6.24±0.76 ^c	12.22±0.22 ^b	7.88±0.12 ^a	5.14±0.14 ^a	1.75±0.25 ^b	2.16±0.09 ^d	4.97±0.42 ^c
GA ₃ +BA	0.00	0.00±0.00 ^c	6.18±0.18 ^c	14.38±0.38 ^a	8.35±0.35 ^a	5.44±0.16 ^a	1.60±0.10 ^b	2.24±0.24 ^d	2.37±0.17 ^d
GA ₃ +CPPU	0.00	0.00±0.00 ^c	7.22±0.22 ^c	9.54±0.16 ^c	4.23±0.23 ^b	2.99±0.11 ^b	2.13±0.04 ^b	3.81±0.19 ^c	6.71±0.27 ^b

Data were presented as means ± SDs and the different upper letters indicate significant differences at P < 0.05 level according to Tukey test.

to enhance fruit size and quality of many horticultural fruit species (Ozga and Reinecke, 2003). In this study, treating navel orange trees with solo CPPU or in combination with GA₃ increased fruit fresh weight, fruit length and diameter, fruit shape index, fruit yield and SSC value. This notion was supported by Kassem et al. (2010) who reported that application of GA₃ increased the weight of fruit, dimensions and crop yield. Furthermore, Khot et al. (2015) confirmed that CPPU with GA₃ enhanced berry length and diameter, bunch weight of seedless grapes as well as yield (Marvet et al., 2001). These results might be attributed to the effect of GA₃ and CPPU on enhancing cell division and elongation (Zhang and Whiting, 2011 and Khot et al., 2015). Moreover, several researchers mentioned the positive effect of CPPU plus GA₃ on SSC (Canli et al., 2015, Khot et al., 2015). Fruit fresh weight might be increased by application of CPPU due to its positively facilitates the movement of the nutrients towards feeding on other sections of the tree (Ainalidou et al., 2016, Barkule et al., 2018) or through cell expansion by increasing the absorption of osmotic water (Sánchez-Mde et al., 2005 and Nardozza et al., 2017). Furthermore, CPPU might increase fruit size by promoting cells and lateral growth (Moriyama et al., 1994 and Zhang & Whiting, 2011). Additionally, Eman

et al. (2007) mentioned that GA₃ treatment increased fruit size and quality of citrus fruit. In addition, Endo et al., (2016) and Liu et al. (2018) reported that some hormone-related genes i.e. auxin and GA₃, chromatin reshaping, and transcription regulations could be regulated to increase citrus fruit size and weight in the fruit growth and development process. Plant hormones can also regulate carbohydrate transportation and distribution plus control fruit growth and development (Zhang et al., 2016). In contrast, Abd El-Raheem et al. (2013) revealed that CPPU has a low impact on fruit size. Furthermore, Lorenzo et al. (2007) demonstrated that GA₃ with cytokinin could enhance fruit size but did not change fruit shape. Milić et al. (2018) indicated that GA₃ did not affect fruit size and yield, but the application of BA gradually increased the fruit weight of blueberry. As reported previously by Stylianidis et al. (2004), Stern et al. (2007), Milošević et al. (2014) and Canli and Pektas (2015) fruit size and shape can be increased by application of GAs plus BA. Under CPPU treatment in kiwifruit recorded the highest SSC as reported previously by Hayward (2013). On contrary, Kim et al. (2006) revealed that CPPU significantly reduced SSC in kiwifruit. However, Pujari et al. (2016) mentioned that no significant effects of CPPU on the fruit quality.

TABLE 5. Influence of benzyladenin (BA), Sytofix (CPPU), gibberellins (GA₃), GA₃ plus BA and GA₃ plus CPPU on Soluble solids content (SSC as Brix), TA (g citric acid/100 ml juice) and Vitamin C (mg ascorbic acid/100 ml juice) at three time sampling during marketing period in 2016/2017 and 2017/2018 seasons.

Treatments	2016/17								
	Soluble solids content (SSC as Brix)			TA (g citric acid/100 ml juice)			Vitamin C (mg ascorbic acid/100 ml juice)		
	5 days	10 days	15 days	5 days	10 days	15 days	5 days	10 days	15 days
Control	12.64±0.14 ^a	12.97±0.06 ^a	13.34±0.24 ^a	1.18±0.02 ^b	1.02±0.02 ^b	0.87±0.02 ^b	41.98±1.00 ^b	40.11±0.11 ^c	27.24±0.24 ^e
BA (50ppm)	12.35±0.10 ^{abc}	12.93±0.12 ^{ab}	12.97±0.06 ^{ab}	1.19±0.01 ^b	1.03±0.03 ^b	0.89±0.01 ^b	42.41±0.41 ^b	40.32±0.32 ^c	30.85±1.00 ^b
CPPU (10ppm)	12.55±0.15 ^a	12.70±0.10 ^{abc}	12.80±0.20 ^b	1.12±0.02 ^c	0.95±0.05 ^{bc}	0.87±0.03 ^b	43.98±0.98 ^b	41.22±0.22 ^b	31.22±0.22 ^b
GA ₃ (50ppm)	12.15±0.15 ^{bc}	12.46±0.20 ^c	12.74±0.00 ^b	1.22±0.02 ^b	0.98±0.02 ^{bc}	0.91±0.01 ^b	47.22±0.75 ^a	42.68±0.12 ^a	33.58±0.50 ^a
GA ₃ +BA	12.00±0.20 ^c	12.55±0.10 ^{bc}	12.65±0.15 ^b	1.32±0.03 ^a	1.23±0.02 ^a	1.21±0.01 ^a	47.56±1.00 ^a	42.81±0.31 ^a	33.85±0.15 ^a
GA ₃ +CPPU	12.42±0.12 ^{ab}	12.75±0.15 ^{abc}	12.86±0.10 ^b	1.12±0.00 ^c	0.91±0.01 ^c	0.88±0.02 ^b	44.41±0.41 ^b	41.53±0.50 ^b	30.99±0.60 ^b
2017/18									
Control	12.86±0.15 ^a	12.97±0.15 ^a	13.13±0.12 ^a	1.18±0.00 ^{bc}	0.98±0.02 ^{bc}	0.88±0.02 ^b	35.94±0.44 ^d	32.56±0.50 ^f	29.92±1.00 ^e
BA (50ppm)	12.55±0.11 ^{ab}	12.95±0.00 ^a	13.07±0.12 ^a	1.15±0.03 ^{cd}	0.99±0.02 ^b	0.88±0.02 ^b	41.67±1.00 ^e	37.68±0.30 ^e	33.05±0.40 ^d
CPPU (10ppm)	12.66±0.10 ^{ab}	12.90±0.10 ^a	13.00±0.17 ^{ab}	1.11±0.02 ^d	0.96±0.01 ^{bc}	0.84±0.01 ^{bc}	42.69±0.30 ^{bc}	38.95±0.05 ^d	33.38±0.22 ^d
GA ₃ (50ppm)	12.20±0.30 ^b	12.57±0.12 ^b	12.81±0.11 ^{ab}	1.23±0.03 ^{ab}	1.16±0.00 ^a	1.11±0.02 ^a	44.33±0.33 ^{ab}	42.75±0.25 ^b	38.95±0.50 ^b
GA ₃ +BA	12.20±0.20 ^b	12.47±0.15 ^b	12.68±0.10 ^b	1.28±0.02 ^a	1.18±0.02 ^a	1.16±0.02 ^a	45.91±0.30 ^a	44.31±0.31 ^a	42.75±0.25 ^a
GA ₃ +CPPU	12.60±0.10 ^{ab}	12.91±0.00 ^a	13.03±0.15 ^a	1.12±0.02 ^{cd}	0.94±0.03 ^c	0.81±0.02 ^c	42.95±1.00 ^{bc}	41.67±0.20 ^c	35.35±0.15 ^c

Data were presented as means ± SDs and the different upper letters indicate significant differences at P < 0.05 level according to Tukey test.

In addition, the obtained results showed that peel thickness was higher with a solo BA or with GA₃. In general, the cuticular membrane of PGRs treated fruit appeared to be much thicker than the fruit of control treatment (Fogelman et al., 2015). Biton et al. (2014) revealed that microscopic analysis tissue subjected to BA plus GA₃ treatment showed a high-density region of cells of a vertical alignment just below the cuticle. GA₃ treatment can increase citrus fruit peel thickness and quality (Eccher and Hajnajari, 2006, Eman et al., 2007). However, the result of splitting percentage was in contrast with peel thickness results, where GA₃+BA gave lowest splitting percentage. These results agreed with Ali et al. (2000) who observed a negative correlation between peel thickness and splitting rate of fruit. Where, if peel of citrus fruit is thicker, it can be misshapen less, cracks resistance will be greater. Observation done by Stern et al. (2013) revealed that spraying fruit with BA+GA₃ decreased significantly the incidence of splitting during fruit harvest, however, treatment with solo PGR is insufficient. This could happen due to increase in the plasticity of cell walls to promote cell growth and extension that could inhibit fruit splitting (Biton et al., 2014, Ginzberg et al., 2014). As well as, during the early stages of the fruit growth for most of the fruit growing period up to before harvest by one month, GA₃ plus BA induce inhibition expression of ACO2 gene (Biton et al., 2014). Comparing splitting percentage with fruit shape index, treatment increase fruit shape index recorded the highest percentage of splitting fruits (Li and Chen, 2017), it might be due to the response of peel fruit to the growth of the pulp (Petrus and Stander, 2013). Where, during the period of maximum increase in citrus fruit diameter, fruit transform from globose to oblate in shape and pressure exerted by the pulp forces the rind to enclose its expanding volume due to the predominant occurrence of the pulp and albedo's cell growth (Bain, 1958, García-Luis et al., 2001).

ABA results clarified that application of solo GA₃ or combined with BA reduced ABA content at 15-October to 15-November. This observation was similar to previous results of Sun et al. (2010) and Jia et al. (2011), who showed that ABA levels were low in the early stages and were higher in various stages of fruit maturation. Gambetta et al. (2012, 2014) revealed that ABA content increases in flavedo as fruit matures and color development simultaneously. The lower ABA content in the GA₃+BA treatment might be decreased the splitting percentage.

In this study, during the marketing period, decayed fruit and weight loss were decreased by the application of GA₃ plus BA. The obtained results were in the same trend with the result of ABA in the same treatment. Biton et al. (2014) reported that GAs in combination with BA can serve as a feasible and fast way to postpone the incidence of the disease effectively. Taniguchi et al. (2007) pointed that plant cells can react to cytokinin application and produce disease-resistance proteins through modulation of expression patterns of enzymes that metabolize cytokinin. This could contribute to the increased resistance of fruit subjected to GAs plus BA and have modified a large number of the host reactions that influence fungal production (Biton et al., 2014). However, the obtained results showed higher chlorophyll content and lower carotene content under GA₃+BA. These results reflect the lower ABA content for the same treatment in current investigation, where ABA derived from carotenoids. Similar results were reported previously by Biton et al. (2014). This result was also confirmed previously by Gambetta et al. (2012, 2014) who mentioned that BA plus GA₃ can preclude the transfer of chloroplast to chromoplast and associated with delayed chlorophyll degradation and carotenoids accumulation. Moreover, the gained results of this investigation were in contrast with Abd El-Raheem et al. (2013) who showed that CPPU research in orange fruit had an essential effect on color, internal maturity by delays and destruction of chlorophyll. Furthermore, obtained data revealed that no significant difference between CPPU and control in Vitamin C as reported previously by Ainalidou et al. (2016), who pointed that content of ascorbic acid did not significantly affected by CPPU, due to ascorbic acid oxidation to dehydroascorbic acid by CPPU-induced ascorbate peroxidase.

Conclusion

From the previous results, foliar application of various PGRs, sprayed three times after full bloom stage by 15, 30 and 45 days, on the studied parameters were extremely significant. All PGRs increased fruit fresh weight, length and diameter, number of fruits per tree, fruit yield and chlorophyll content compared to control. However, reduced of fruit splitting percentage and carotene content as compared to control treatment. It can be recommended to sprayed navel orange trees by GA₃ at 50 ppm plus Sytofix (CPPU) at 10 ppm or GA₃ at 50ppm plus BA at 50ppm to obtain the

highest yield, the lowest percentage of splitting fruits and the highest quality characteristics of the fruits, as well as, the lowest percentage of losses, and the highest quality during the marketing period. Finally, to gain valuable insights the relation between using PGRs and improvement of fruit quality and marketability of orange fruit, more studies are needed at the level of enzyme activity and gene expression.

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Conflict of interest

The authors declare no conflict of interest or personal relationships that could have appeared to affect the work reported in current study.

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تحسين جودة الثمار والقدرة التسويقية للبرتقال "ابوسره" بواسطة السيتوكينين والجبريللين

هيام مصطفى المنوفى^١ ، عبدالفتاح منتصر خضر^٢ و أحمد حسن أحمد منصور^١
^١قسم تداول الفاكهة - معهد بحوث البساتين - مركز البحوث الزراعية - القاهرة - مصر.
^٢قسم بحوث الموالح - معهد بحوث البساتين - مركز البحوث الزراعية - القاهرة - مصر.

كان الهدف من الدراسة الحالية هو زيادة إنتاجية أشجار البرتقال ابو سره وذلك عن طريق تقليل نسبة الثمار المتشقة، وزيادة وزن وحجم الثمار والمحصول وتحسين جودة الثمار وكذلك تحسين قدرتها التسويقية. لذلك أجريت هذه التجربة في موسمي ٢٠١٧/٢٠١٦ و ٢٠١٨/٢٠١٧ في بستان خاص، محافظة كفر الشيخ، جمهورية مصر العربية. حيث، تمت معاملة الأشجار باستخدام بنزيل ادنين (BA) بتركيز ٥٠ جزء في المليون ، والسيتوفيكس (CPPU) عند ١٠ جزء في المليون ، وحامض الجبريللين (GA_3) عند ٥٠ جزء في المليون و GA_3 مع BA و GA_3 مع CPPU. تتم المعاملة بمنظمات النمو النباتية ثلاث مرات بعد مرحلة الإزهار الكامل بحوالي ١٥، ٣٠، و ٤٥ يوماً عن طريق الرش الورقي. أوضحت النتائج أن منظمات النمو النباتية أدت إلى زيادة صفات الجودة والمحصول ومحتوى الكلوروفيل، وكذلك تقليل نسبة الثمار المتشقة ومحتوى قشره الثمار من الكاروتين. أعطى الرش بمعاملة $GA_3 + CPPU$ أعلى وزن للثمار، وطول الثمار والمحصول ومحتوى المواد الصلبة الذائبة. ومع ذلك، سجلت معاملة $GA_3 + BA$ أكبر قطر للثمرة، ومحتوى الكلوروفيل، ونسبة الاحماض القابلة للمعايرة، وفيتامين سي، وكذلك أقل نسبة ثمار متشقة ومحتوى كاروتين. فيما يتعلق بفترة التسويق، ادت معاملة الثمار بـ $GA_3 + BA$ على تقليل نسبة الفقد في وزن الثمار و تقليل نسبة الثمار التالفة بينما سجلت أعلى نسبة من فيتامين سي ومحتوى الكلوروفيل. أدى تطبيق منظمات النمو النباتية إلى تقليل محتوى حامض الأبسيسك (ABA) بشكل كبير في قشر الثمرة. أظهرت معاملة GA_3 منفردا أو مع BA أقل محتوى من ABA في كلا الموسمين. لن تساعد هذه النتائج الباحثين على فهم أدوار منظمات النمو النباتية في نمو ثمار برتقال ابوسره وتطويرها وإنتاجيتها فحسب، بل يمكن أيضاً تطبيقها على إنتاج الفاكهة على نطاق واسع وإطالة فترة التسويق.