EFFECTS OF DIFFERENT CONCENTRATIONS OF AUXIN AND GIBBERELLIN IN DEVELOPING SEEDLESS ROSELLE (*Hibiscus sabdariffa* L.) FRUIT AND POSTHARVEST QUALITY

Wan Zaliha, W.S. and Norsyuhada, J.
School of Food Science and Technology, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu, Malaysia.
Tel: 6(09)-6684981; Fax: 6(09)-6684949; Email: wzaliha@umt.edu.my

ABSTRACT

Parthenocarpy is the formation of fruit without pollination or fertilization which resulted in seedless fruit. Seedless fruit has higher commercial value than seeded fruit. The present study aimed to investigate the development of seedless roselle (*Hibiscus sabdariffa* L.) fruit by applying various concentrations of plant growth regulators (PGRs) such as auxin (IAA) and gibberellin (GA_3). Improvement of roselle crop through conventional hybridization is very difficult to be conducted as it has cleistogamous flower. In addition, roselle is very perishable and non-climacteric produce that exposed to rapid postharvest physiological deterioration following harvest. Thus, producing seedless fruit may reduce time and labour cost during decoring process. The experimental treatments were arranged in randomized complete block design and the treatments were control (without PGRs), GA_3 at 600 mg L^{-1}, GA_3 at 800 mg L^{-1}, GA_3 at 1000 mg L^{-1}, IAA at 800 mg L^{-1}, IAA at 1000 mg L^{-1}, and IAA at 1200 mg L^{-1}. The PGRs were sprayed on roselle flower buds at 40, 50, 60 and 70 day after transplanting (DAT). Among all PGRs treatments, plant treated with GA_3 at 800 mg L^{-1} had developed seedless roselle fruit as shown by the smallest capsule diameter, capsule volume and low number of seeds. Meanwhile, various concentrations of PGRs did not significantly affect the postharvest quality attributes of roselle fruit. As a conclusion, the application of 800 mg L^{-1} GA_3 was the effective concentration to produce seedless roselle fruit and maintain its postharvest quality.

Keywords: seedless fruit, capsule, parthenocarphy, auxin, gibberellin, decoring, anthocyanin

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an annual herb that belongs to the family of Malvaceae and cultivated mainly for its leaves, stem, seed and fruits (Fasoyiro et al. 2005). Roselle is categorized as a vegetable crop that widely grown in tropical Asia, Australia, West and Central Africa and in Central America. However, the origin of roselle is believed to be from West Africa (Mohamad et al. 2011). The brilliant red and fleshy cup-shaped fruits are the most important part of roselle plants that can be processed into food and beverages, pharmaceuticals and cosmetic products. In Malaysia, this crop is mainly used to produce pro-health juice, due to its high content of vitamin C and anthocyanins which are found in the fruits (Mohamad et al. 2011). Ministry of Agriculture and Agro-based Industry (2015) reported that the increasing of roselle production from 358 metric tons (mt) in year 2010 to 536 mt in 2014. The tremendous increased of roselle production could be due to the increased demand from various industries as aforementioned. Thus, this phenomenon has encouraged local farmers to cultivate roselle in a large scale.

Roselle fruit can be categorized as non-climacteric based on its ethylene dan respiration production. Being a non-climacteric, roselle fruits expose to rapid deterioration process and also has a short shelf life. Thus, the fruits need to be processed as soon as possible immediately after harvested. However, delays in processing roselle fruits cannot be avoided as it requires the removing of velvety capsule for further process. The process of removing of the capsule is known as decoring, it is time consuming and labour intensive. Therefore, inducing seedless roselle fruit by using plant growth regulators (PGRs) such as

auxin (indole acetic acid, IAA) and gibberellin (GA_3) could be better option to overcome the problems. There is little information available in developing seedless roselle fruit by using IAA, GA_3 and other hormones, and also through genetic modification.

Parthenocarpy is the development of a fruit without the formation of seeds in the absence of pollinations, fertilization, or embryo development (Mezzetti 2004). It naturally occurs in a number of plant species and could be induced artificially. As reported by Mohamad et al. (2009) roselle is a self-pollinating crop species, and pollination occurs in bud stage before the flower blooms since it is a cleistogamous flower which stay enclosed and fertilized themselves. Thus, crop improvement through hybridization is difficult to be conducted. In line to this condition, the application of PGRs could be the best alternative in improving roselle plant as well as in developing seedless fruit. According to Acciarri et al. (2002), developing seedless eggplant (*Solanum melogena* L.) fruit by applying synthetic hormone was practical as it resulted in high quality fruits. In China, recent study succeeded in developing seedless loquat (*Eriobotrya japonica* Lindl.) fruit by exogenous spray of GA_3 at 100 mg L^{-1} (Mesejo et al. 2010). However, parthenocarpy can improve fruit quality as compared to the production of triploid cultivars that may resulted in poor quality fruits (Mesejo et al. 2010). Hence, this study aimed to determine the effects and the best concentration of IAA and GA_3 in developing seedless roselle fruit and at the same time maintaining its postharvest quality.

MATERIALS AND METHODS

Plant materials and experimental location

The experiment was conducted in the greenhouse at the School of Food Science and Technology, Universiti Malaysia Terengganu (5.4053° N, 103.0878° E). Roselle variety Terengganu (UMKL-1) seeds were purchased from Department of Agriculture, Ajil, Terengganu.

Experimental design and application of plant growth regulators

A total of 21 roselle seedlings about two-week-old were transplanted into polybags (20cm × 24cm) containing 30 kg of soil mixture (3 topsoil :1 sand :1 chicken manure). The roselle seedlings were initially sown in a peat moss medium before transplating into individual polybag as aforementioned. The duration of the experiment was five months (from November 2013 to May 2014). Roselle is a plant with indeterminate flowers and unlimited growth, meaning that it continue to produce flowers and fruits throughout its life. Roselle flower bud growth begins at day 20 after transplanting as reported by Wan Zaliha et al. (2014). However, in this study, the spray applications of PGRs were done on day 40, 50, 60 and 70 after transplanting. The visual assessment for seed and fruit development of roselle was made from flower bud to fully developed fruit from day 40 to 75 after transplanting (DAT). Meanwhile, harvesting of mature roselle fruits were done on 75, 85 and 95 DAT. The experiment was laid out as according to a randomized complete block design (RCBD), comprising of different concentrations of exogenous IAA and GA_3 sprays with three replications. The treatments were control (without PGRs), 600 mg L^{-1} GA_3, 800 mg L^{-1} GA_3, 1000 mg L^{-1} GA_3, 800 mg L^{-1} IAA, 1000 mg L^{-1} IAA, and 1200 mg L^{-1} IAA. All experimental plants received similar cultural practices including fertilization (350 kg ha^{-1} NPK 15:15:15 and 1200 kg/ha NPK 12:12:12:17:2), pesticides (imidacloprid) and fungicides (carbendazim and Mancozeb) sprays during the experiment. Manual watering was done twice a day at 0800 hours and 1700 hours.

Parameters measurement

Postharvest quality parameters of roselle fruit measured were fresh weight, number of fruit, capsule volume and size, number of seeds, fruit colour [(lightness (L*), chromacity value a* and b*, chroma C*

and hue angle (h^o)], soluble solids concentration (SSC), total anthocyanins concentration, titratable acidity (TA), and fruit firmness.

Fresh weight, number of fruits and seeds

Cumulative fresh weight of roselle fruit was weighed by using an electronic balance. The number of roselle fruits and seeds were counted manually. Irregular size and shape of roselle seeds was discarded as well as dead seeds.

Fruit diameter, length and volume

The diameter and length of roselle fruits were measured using a digital vernier caliper. For capsul volume or size measurement, the roselle fruit was assumed as spherical by using non-destructive estimation according to Mitchell (1986). The formula to measure roselle volume was based on bell pepper fruit volume below:

$$V_F = KD^2L\pi/6$$

V_F is fruit volume, K is shape factor that varies with fruit type, D and L are diameter and length, respectively. The value of K is 1.1 (Mathieu et al., 2003). It was assumed that the shape of roselle fruit was similar to bell peppers (Mathieu et al. 2003).

Fruit firmness, soluble solid concentration (SSC), titratable acidity (TA) and fruit colour

An electronic pressure tester, Texture Analyzer TA. TXplus (Stable Macro System, UK) was used to measure the fruit firmness and was equipped with P2 probe. The pre- and post-test speed of the probe was 1 mm/s, the test speed was 1 mm/s, target distance was 5 mm and penetration distance was 0.75 mm. The roselle calyx was first cut into 2.0 cm x 1.5 cm size and the calyx or fruit firmness was measured at two equatorial opposite sides. The firmness values were expressed in Newton (N).

A hand-held refractometer was used to measure SSC. A potentiometric method was used to determine TA and expressed as percent malic acid. Ten gram fresh weight of roselle fruit was homogenized with 40 ml of distilled water by using a hand-held blender. Next, the homogenate fruit was filtered using muslin cloth. An aliquot of 5 ml was used to titrate against 0.1N sodium hydroxide (NaOH). The end point colour changed to pink by reaction of phenolphthalein as indicator. The TA was calculated using the formula below:

$$\% \text{ Malic acid} = \frac{\text{Titrate} \times \text{normality of alkali} \times \text{volume made up} \times \text{equal weight} \times 100}{\text{volume taken for estimation} \times \text{weight of sample} \times 1000}$$

Where, equivalent weight of malic acid = 134 mg

Skin colour of roselle fruit was recorded using a Chroma Meter (Model CR400, Konica Minolta Inc. Japan). The colour data were expressed in L*, a* and b* values. L* represent the lightness coefficient which ranges from 0 (black) to 100 (white). a* ranges from -60 to +60, which indicates red (+60) and green (-60) colours. Meanwhile, b* ranges from-60 to +60, which indicates as yellow (+60) and blue (-60) colours. a* and b* were further used to calculate hue angle ($h° = \tan^{-1} b^*/a^*$) for colour interpretation. Hue angle (h°) represented red-purple (0°), yellow (90°), bluish green (180°) and blue (270°) (McGuire 1992).

Total anthocyanin concentration

Anthocyanin was extracted from the fruitcalyx and quantified following the method described by Wan Zaliha (2009). Total anthocyanins were calculated by using 2.74 x 10^4 L for idaein chloride (Giusti and Wrosltad 2001) and were expressed in mg $100g^{-1}$ of fresh weight.

Statistical analysis

The experimental data were subjected to an analysis of variance (ANOVA) using General Linear Models (GLM) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

As explained earlier, roselle has a cleistogamous flower where the flowers stayed enclosed and fertilized themselves. Based on the macro and microscopic observations, the self-pollination occured between day 1 and 10 after roselle flower bud appearance (Fig. 1) similar to the report by Wan Zaliha et al. (2014). The seed formation can be seen clearly after day 10 of the appearance of roselle flower bud in fruit without PGRs (Fig. 2).

Based on the visual assessment, on 75 DAT, roselle plants treated with different concentrations of GA_3 had the potential to develop seedless fruit compared to control and IAA treated plants (Fig. 3). Application of GA_3 at 800 mg L^{-1} had the ability to develop seedless roselle fruit by producing smaller capsule and lower number of seeds as compared to control and IAA-treated fruits.

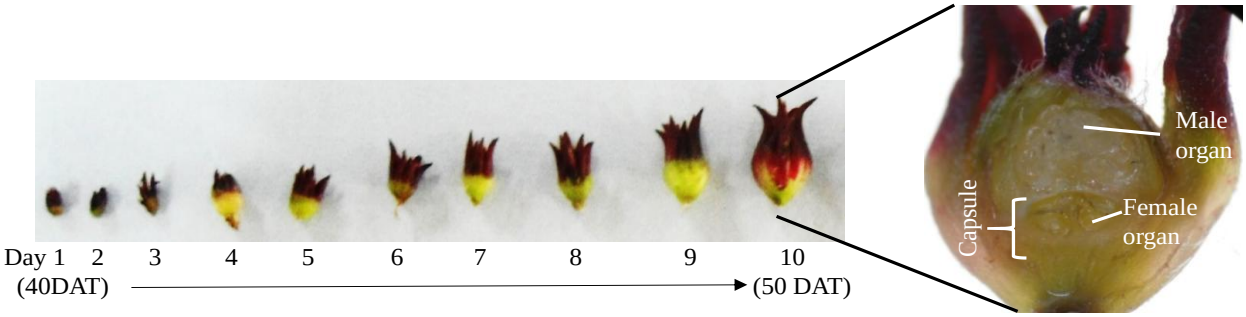

Figure 1. The development of roselle flower bud from 40 days after transplanting (DAT)

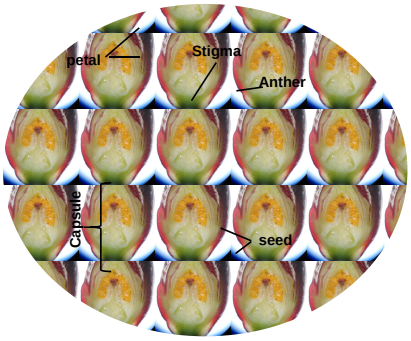

Figure 2. Seed formation before flower bloom on day 15 (55 DAT)

Figure 3. Visual assessment on diameter of capsule on 75 DAT. A: Control, B: 600 mg L^{-1} GA_3, C: 800 mg L^{-1} of GA_3, D: 1000 mg L^{-1} GA_3, E: 800 mg L^{-1} IAA, F:1000 mg L^{-1} IAA, G:1200 mg L^{-1} IAA

In this study, the volume and diameter of roselle capsule, and number of seeds were significantly affected by various concentrations of PGRs spray on 75 DAT (Figs 4, 5 and 6). Similar results were also observed by Wan Zaliha et al. (2014) where roselle fruits harvested on 70 DAT showed a significant effect on capsule diameter, volume and seeds number after sprayed with higher concentrations of GA_3 and IAA (600 and 800 mg L^{-1} repectively). However, in this present study, roselle fruits treated with IAA at high concentrations (800 to 1200 mg L^{-1}) did not show any changes in diameter and volume of roselle capsules. The smallest capsule size was obtained by the 800 mg L^{-1} GA_3 that induces seedless roselle fruits i.e. it produced the smallest number of seeds. As claimed by Wan Zaliha et al. (2014) the response of PGRs on target cell (roselle calyx) might be reduced as its effectiveness also reduced. In addition, hormones are produce in tiny amount in plants. They also suggested that the spray applications of PGRs should be applied continuous and more frequent to the target cell for better results. Meanwhile, Mesejo et al. (2010) reported that exogenous sprays of GA_3 at 100 mg L^{-1} had the ability to induce seedless loquat (*Eriobotrya japonica*). Tiwari (2011) found that external application of GA_3 induced seedless in chilli (*Capsicum annuum*) fruits by enhancing fruit set. Larger roselle capsule size was recorded in roselle fruit treated with IAA. The applications of IAA and GA_3 had induced different morphology, histology and sugar metabolism during fruit development as reported by Tiwari (2011). In addition, Serrani et al. (2007) and Vivian-Smith and Kultunow (1999) reported that auxin-induced tomato (*Lycopersicon esculentum*) fruits had bigger size than GA_3-treated-fruit.

In this present study, IAA at high concentrations of 1000 mg L^{-1} and 1200 mg L^{-1} caused the roselle fruits to drop prematured before harvested. This also reduced the number and fresh weight of IAA treated-fruit. The decrease in capsule size of GA_3-treated-roselle resulted in similar reduction of fresh weight on IAA-treated-roselle (Fig. 7). Even though the number of GA_3- and IAA-treated-fruits were similar, roselle treated with GA_3 tend to had a slightly higher number of fruits than IAA (Fig. 8). Talon et al. (1992) and Tiwari (2011) reported that the applications of GA_3 resulted in high production of fruit set in mandarins (*Citrus reticulata*) and Chilli (*Capsicum annuum*) as compared to IAA applications.

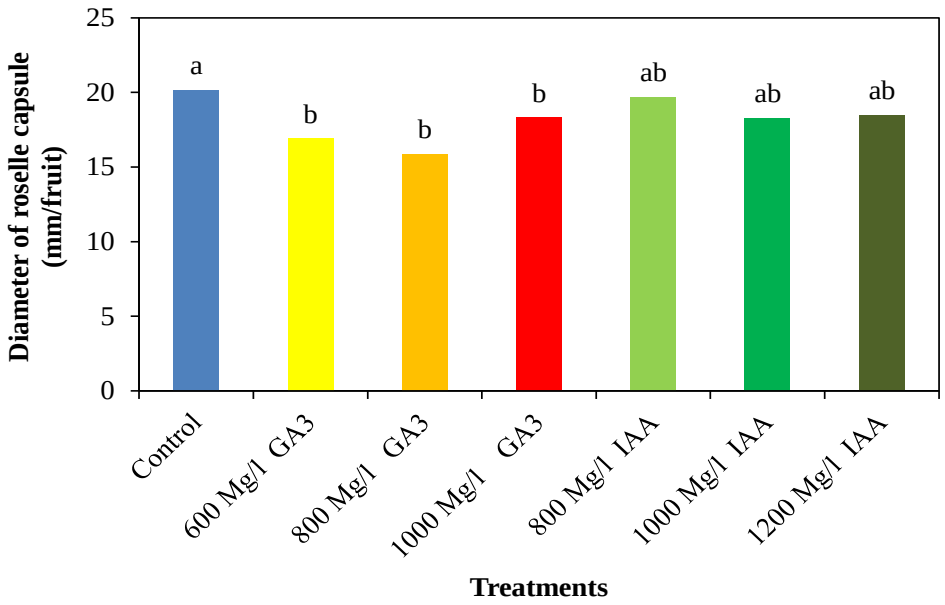

Figure 4. Effects of different concentrations of PGRs on volume of roselle capsule. Means with different letters are significantly different at the 5% level according to LSD test.

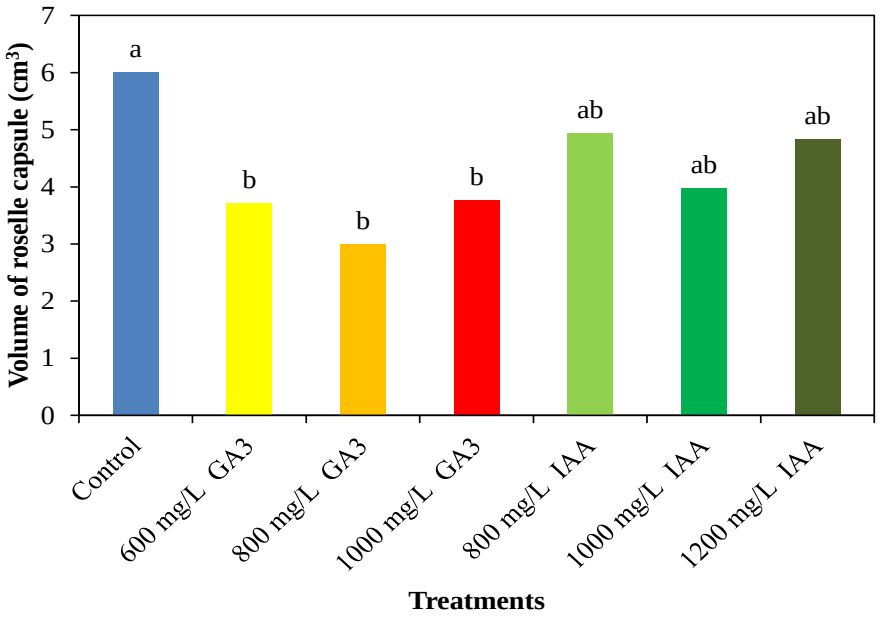

Figure 5. Effects of different concentrations of PGRs on diameter of roselle capsule. Means with different letters are significantly different at the 5% level according to LSD test.

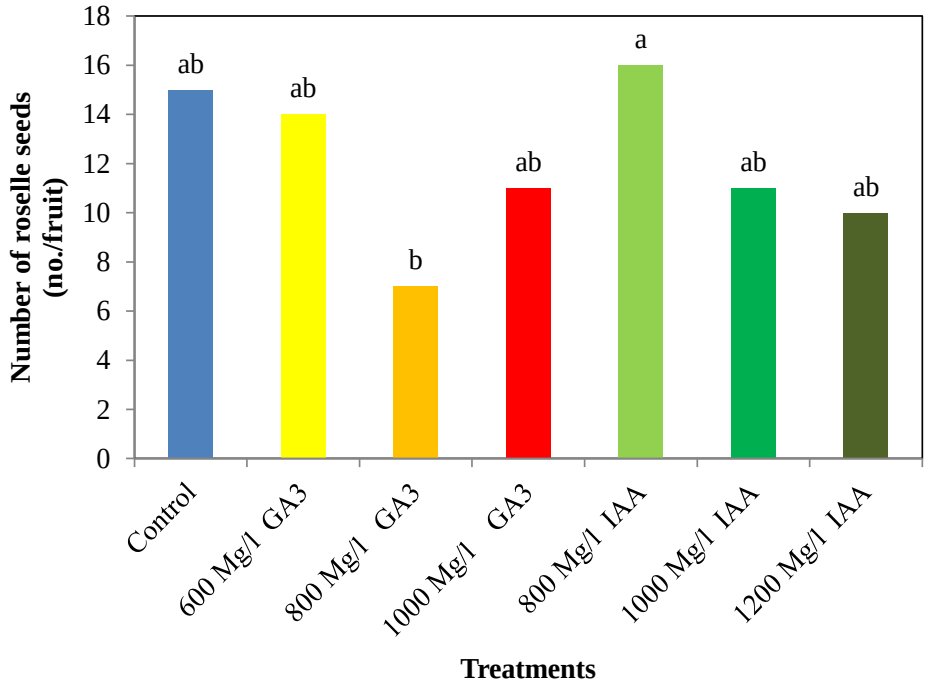

Figure 6. Effects of different concentrations of PGRs on number of roselle seeds. Means with different letters are significantly different at the 5% level according to LSD test.

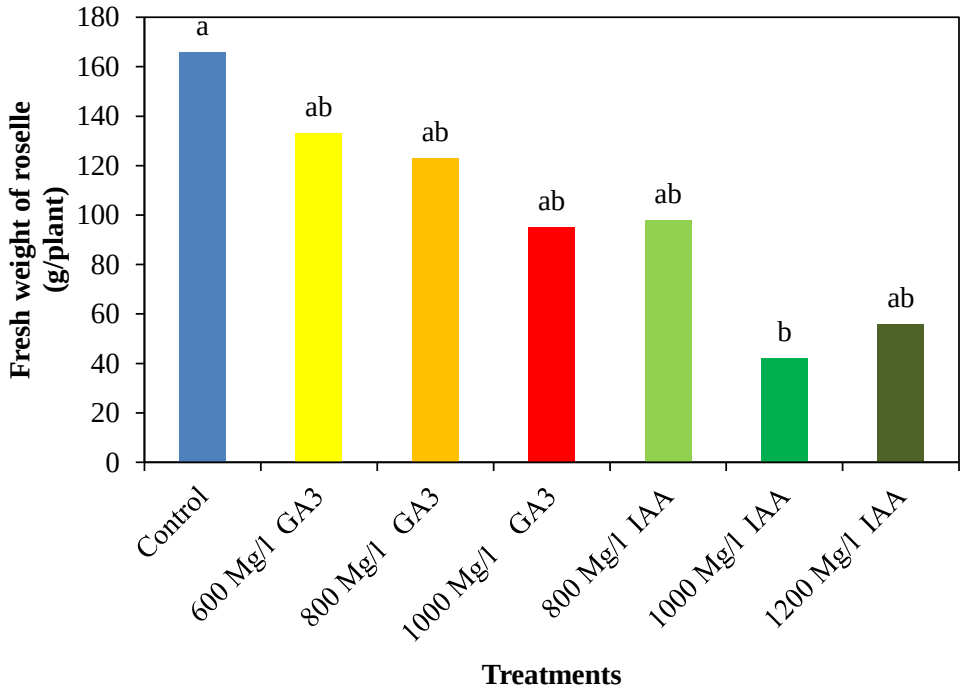

Figure 7. Effects of different concentrations of PGRs on fresh weight of roselle fruit. Means with different letters are significantly different at the 5% level according to LSD test.

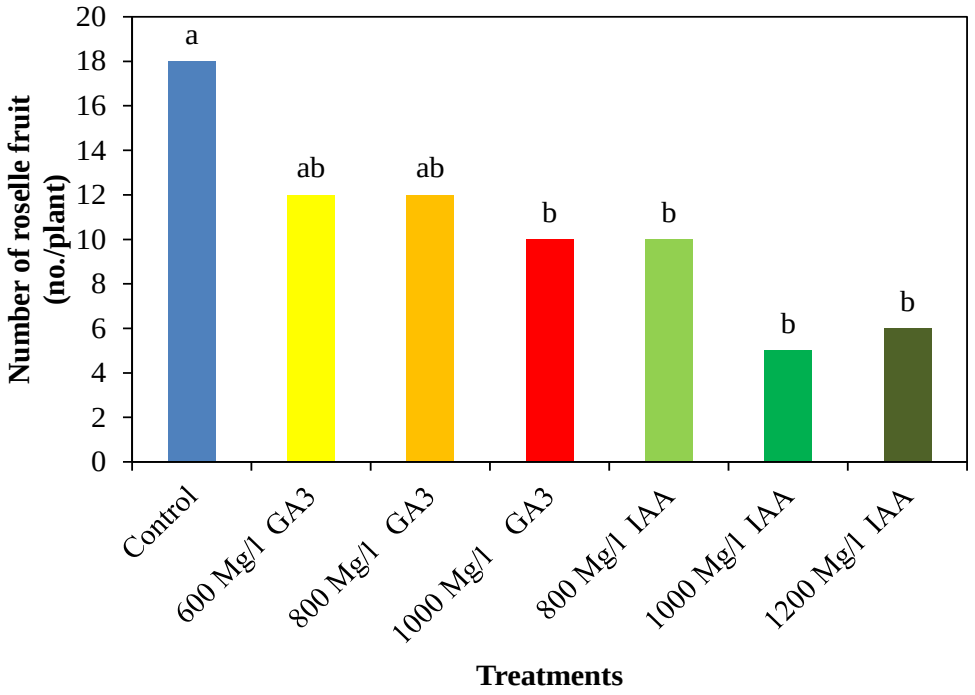

Figure 8. Effects of different concentrations of PGRs on number of roselle fruit. Means with different letters are significantly different at the 5% level according to LSD test.

All the postharvest quality parameters were not affected with the application of both PGRs except for TA and total anthocyanins concentration (Tables 1 and 2). Similarly, Wan Zaliha et al. (2014) also reported that application of PGRs did not enhance the quality of roselle fruit. All PGR treated plants had colour indexes similar to control (Table 1). Huang and Jiang (2012) claimed that broccoli (*Brassica oleracea* var. Italica) treated with GA_3 turn into yellow colour completely after three days. Shaheen et al. (1988) reported that most seedless date palm (*Phoenix dactylifera* L.) fruits maintained and improve their colour characteristics after treated with PGRs. The roselle fruit treated with 600 mg L^{-1} GA_3 had the highest TA and the lowest value when treated with 1200 mg L^{-1} IAA (Table 2). The mechanism involved in the increased and decreased in TA values by GA_3 treatment is still unknown. Meanwhile, SSC and fruit firmness, both showed no apparent effect with the application of PGRs (Table 2). As reported by Mesejo et al. (2010), fruits treated with PGRs tend to increase in size thus increasing the total soluble solids. Although no significant difference was observed, roselle fruits treated with GA_3 tend to have firmer fruits compared to IAA treatment. For total anthocyanins concentration, no specific trend can be deduced in relation to the application of PGRs (Table 2). The amount of anthocyanins in roselle fruits either treated on untreated with PGRs ranged between 536.50 and 673.54 mg $100g^{-1}$ fresh weight.

Table 1. Effects of different concentrations of GA_3 and IAA on roselle fruit colour

Treatment	Lightness (L*)	Chromaticity a*	Chromaticity b*	Hue angle (h°)	Chroma (C*)
Control	25.29[a]	18.96[a]	4.71[a]	15.51[a]	19.71[a]
600 $Mg \cdot l^{-1}$ GA_3	24.06[a]	17.91[a]	1.57[a]	5.01[a]	17.98[a]
800 $Mg \cdot l^{-1}$ GA_3	24.19[a]	17.70[a]	1.62[a]	5.01[a]	17.78[a]
1000 $Mg \cdot l^{-1}$ GA_3	24.74[a]	16.16[a]	3.30[a]	12.93[a]	16.76[a]
800 $Mg \cdot l^{-1}$ IAA	26.38[a]	23.34[a]	3.71[a]	8.82[a]	23.65[a]
1000 $Mg \cdot l^{-1}$ IAA	26.24[a]	19.47[a]	3.14[a]	8.96[a]	19.73[a]
1200 $Mg \cdot l^{-1}$ IAA	25.22[a]	18.76[a]	0.64[a]	1.95[a]	18.77[a]

Means with different letters are significantly different at the 5% level according to LSD test.

Table 2. Effects of different concentrations of GA_3 and IAA on SSC, TA, fruit firmness and total anthocyanins concentration of roselle fruit

Treatment	SSC (%)	TA (% malic acid)	Fruit firmness (N)	Total anthocyanins (mg/100g fresh weight)
Control	6.04[a]	0.89[ab]	1.21[a]	550.35[b]
600 $Mg \cdot l^{-1}$ GA_3	6.26[a]	1.33[a]	2.04[a]	595.06[b]
800 $Mg \cdot l^{-1}$ GA_3	6.31[a]	0.69[ab]	1.47[a]	542.82[b]
1000 $Mg \cdot l^{-1}$ GA_3	7.01[a]	1.03[ab]	1.97[a]	692.98[a]
800 $Mg \cdot l^{-1}$ IAA	7.58[a]	0.77[ab]	0.83[a]	610.49[b]
1000 $Mg \cdot l^{-1}$ IAA	6.40[a]	0.82[ab]	1.10[a]	673.54[a]
1200 $Mg \cdot l^{-1}$ IAA	6.77[a]	0.53[b]	1.58[a]	536.50[b]

Means with different letters are significantly different at the 5% level according to LSD test.

CONCLUSION

The application of GA_3 at 800 mg L^{-1} was effective to induce parthenocarpy in roselle fruits and maintain other postharvest quality attributes such as SSC, fruit colour and fruit firmness. Further study is needed to understand the mechanism involved.

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