Malays. Appl. Biol. (2022) 51(1): 29–36
https://doi.org/10.55230/mabjournal.v51i1.2006

THE RESPONSES OF ROSELLE (*Hibiscus sabdariffa* L.) TO THE APPLICATION OF PLANT GROWTH REGULATORS IN DEVELOPING SEEDLESS FRUIT AND MAINTAINING ITS QUALITY

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Accepted 31 December 2021, Published online 31 March 2022

ABSTRACT

Seedless fruit is a fruit developed to possess no mature seeds. Today lifestyle, consumers and/or manufacturers prefer fruit without seeds as removing it is time-consuming and troublesome. Indeed, seedless fruits have better organoleptic attributes than seeded fruits. To produce seedless roselle and maintain its postharvest quality, various spray applications of synthetic and naturally occurring plant growth regulators were investigated. Roselle is perishable and non-climacteric fruit that is exposed to rapid postharvest physiological deterioration following harvest. Thus, producing seedless fruit may reduce time and labor costs during the removing seed process. The experimental treatments were arranged in randomized complete block design with different types of plant growth regulators and spray applications. The PGRs treatments at varying frequencies were sprayed on roselle flower buds at 35, 45, 55, and 65 days after transplanting (DAT). Among all PGRs treatments, the plant treated with 800 mg/L GA_3 + seaweed extract (4 sprays) had developed seedless roselle fruit by exhibiting the smallest capsule diameter, capsule volume, and a low number of seeds. In a conclusion, the application of 800 mg/L GA_3 + seaweed extract (4 sprays) was the effective concentration to produce seedless roselle fruit and maintain its postharvest quality.

Key words: Natural occurring hormone, parthenocarpic, postharvest, seaweed, seedless

INTRODUCTION

Roselle (*Hibiscus sabdarifa* L.) is an annual or perennial plant under the Malvaceae family. It is widely cultivated in tropical and subtropical regions for its stem, fibers, fruits, leaves, and seeds (Osman *et al*., 2011). The plant is categorized as a vegetable crop that is thought to be originated from West Africa (Osman *et al*., 2011). Superb red and fleshy cup-shaped fruits are the most vital 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, owing to its high content of vitamin C and anthocyanins which are found in the fruits (Osman *et al*., 2011). Roselle was first introduced in the state of Terengganu in 1993 for commercial purposes. It is also planted in the states of Kelantan, Pahang, Selangor, and Johor. In 2015, the production of roselle was 536 metric tonnes. However, a tremendous decrease, about 17-fold can be seen in 2019 (Ministry of Agriculture & Agro-based Industry, 2019). Possibly, this might be attributed to the cumbersome process after harvest which is time-consuming and cost-effective. Thus, the production of seedless roselle fruit could be the alternative approach to revival its cultivation on large scale.

From a postharvest perspective, roselle fruit is categorized as non-climacteric based on its rapid deterioration behavior just after harvest. Hence, delays during processing roselle fruits should be avoided. However, roselle requires the removing of velvety capsule for the further process which is known as decoring and causes tedious tasks. Therefore, this study was conducted to evaluate the development of seedless roselle fruit by applying PGRs either synthetic [auxin (Naphthalene acetic acid, NAA) and gibberellin (GA_3)] alone or combine with naturally occurring hormone (seaweed extract) at varying frequencies as well as maintaining its postharvest quality.

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MATERIALS AND METHODS

Experimental location

Forty-eight roselle plants were grown at the greenhouse, Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu (UMT). Roselle variety UMKL-1 was used and obtained from Kompleks Pertanian Ajil, Kuala Berang. Meanwhile, seaweed (*Gracilaria fisheri*) was purchased from a local supplier in Kuala Terengganu.

Plant and material preparation

The roselle seedlings were first seeded in a peat moss medium before being transplanted into individual polybags. A total of 48 roselle seedlings about two-week-old were transplanted into polybags (20 cm × 24 cm) containing 30 kg of soil mixture (3 topsoils :1 sand:1/2 chicken manure). The plants were planted at a distance of 0.5 m × 0.5 m. Meanwhile, seaweed extract was collected by filtering the extraction solution using muslin cloth with the ratio of 1 portion of seaweed and 5 portions of water. The duration of the experiment was four months (from January 2019 to April 2019).

Experimental design

The experiment was laid out according to a randomized complete block design (RCBD), comprising of two factors viz. i) different sources of PGRs [seaweed extract, NAA, and GA3]] and ii) different spray applications [2 and 4 sprays] with four replications. The treatments were control (without PGRs: 0 mg/L (2 spray)), control (without PGRs: 0 mg/L (4 spray)), seaweed extract (2 sprays), seaweed extract (4 sprays), 400 mg/L NAA (2 sprays), 400 mg/L NAA (4 sprays), 400 mg/L NAA + seaweed extract (2 sprays), 400 mg/L NAA + seaweed extract (4 sprays), 800 mg/L GA_3 (2 sprays), 800 mg/L GA3 (4 sprays), 800 mg/L GA3 + seaweed extract (2 sprays), 800 mg/L GA_3 + seaweed extract (4 sprays). All experimental plants were 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 h and 1700 h. The spray applications of PGRs were done on days 35, 45, 55, and 65 after transplant. The visual assessment for seed and fruit development of roselle was made from flower bud to fully developed fruit from day 30 to 75 after transplanting (DAT). Meanwhile, harvesting of mature roselle fruits was done on 75, 85, and 95 DAT.

Postharvest parameters evaluation

The postharvest quality parameter of roselle fruits measured was fresh weight, number of fruits, diameter capsule, number of seeds, fruit firmness, total anthocyanin concentration, fruit color, fruit volume, and soluble solids concentration (SSC). The cumulative fresh weight and number of roselle fruits were recorded for every 10 days intervals (75, 85, & 95 days after harvest). The fresh weight was weighed by using an electronic balance while the number of roselle fruits and seeds was counted manually. Irregular size and shape of roselle seeds were discarded as well as dead seeds. Fruit diameter was measured using a digital vernier caliper. Fruit color was assessed by using a Minolta Chroma Meter (Model R200 Trimulus Colour Analyser, Minolta camera Co. Ltd., Japan). Colour data were expressed in L*, a*, and b* values. L* represented the lightness coefficient which ranges from 0 (black) to 100 (white). a* ranged from -60 to +60, which indicates red (+60) and green (-60) colours. Meanwhile, b* ranged from -60 to +60, which indicates yellow (+60) and blue (-60) colors. a* and b* were further used to calculate hue angle (hº= tan-1 b*/a*) for color interpretation. Hue angle (hº) represented red-purple (0º), yellow (90º), bluish-green (180º) and blue (270º) (McGuire, 1992). Fruit firmness was determined using Texture Analyser (Model TA-XTPlus). The opposite sides for each fruit were sliced into 2 × 2 cm size and punctured using a probe set of P2 over a metal plate. Fruit firmness was recorded and expressed in Newton (N). Soluble solids concentration (SSC) was evaluated using an infrared digital refractometer (Atago-Pallette PR 101, Atago Co. Ltd, Itabashi-Ku, Tokyo, Japan). Total anthocyanin concentration was determined using a modified method by Wan Zaliha (2009).

Statistical analysis

The data were subjected to two-way analysis of variance (ANOVA) using GLM (General Linear Models) procedures with SAS 9.3 software package, SAS Institute Inc, Cary, NC, USA. Treatments means were further separated by the Tukey test (HSD) for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Roselle starts to bear flowers after a flower bud appears. Roselle has a cleistogamous flower in which the flowers undergo self-fertilization before the flower bloom. Self-pollination occurred based on macro and microscopic observations between day 1 and 10 following the appearance of the roselle flower bud as reported by Wan Zaliha *et al*. (2013). The seed formation can be seen clearly in fruit after day 15. Based on the observation, the roselle fruit exhibit a sigmoid growth pattern (Figure 1).

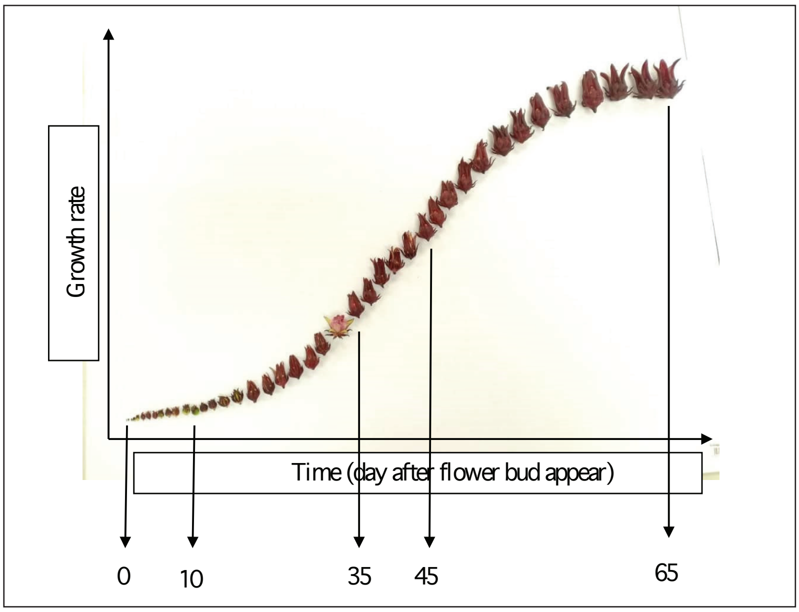

Fig. 1. Observation of growth and development of roselle fruit

In the present study, roselle plants treated with 800 mg/L GA_3 + seaweed extract had the potential to develop seedless fruit as compared to control plants and 400 mg/L NAA treated plants (Figure 2). The application of GA_3 at 800 mg/L + seaweed extract exhibits the smaller size of capsules and a smaller number of seeds than controlled and NAA-treated fruits. Possibly, NAA and GA_3 application induced the morphology, histology, and sugar metabolism of treated fruit as claimed by Tiwari (2011). A non-significant interaction ($P \geq 0.05$) between the two factors was recorded in diameter capsule, number of seeds, and fruit volume. However, a pronounced effect was observed in both factors (Table 1). Roselle fruits treated with NAA at 400 mg/L had the biggest diameter capsule and the smallest volume of roselle fruit. The smallest size of the capsule was obtained by the 800 mg/L GA_3 + seaweed extract that induces seedless roselle fruit, producing the smallest number of seeds (Table 1). Similarly, Siti Aisyah and Wan Zaliha (2020) reported that 400 mg/L of IAA combined with 400 mg/L GA_3 had developed seedless Melon Manis Terengganu (MMT) fruits with a smaller number of seeds. Possibly, IAA and GA_3 had discarded the occurrence of the zygote from developing into seeds during double fertilization (Russell, 1992).

Table 1. Effects of different types of PGRs and spray frequency on capsule diameter, number of seeds, number of fruits, fresh weight, and fruit volume of roselle fruits.

Treatment/Parameter		Capsule diameter (mm)	Number of seed	Number of fruits	Fresh weight (g)	Fruit volume
PGRs	CTRL	18.82^{a}	17.50^{a}	131^{ab}	0.805^{ab}	1784^{b}
	SW	18.15^{ab}	11.75^{ab}	139^{a}	0.896^{a}	1781^{b}
	NAA	17.01^{bc}	9.67^{ab}	43^{d}	0.185^{d}	1504^{bc}
	NAASW	18.68^{ab}	10.25^{ab}	66^{cd}	0.397^{cd}	2041^{ab}
	GA_3	15.29^{bc}	5.62^{b}	78^{c}	0.507^{c}	2090^{a}
	GA_3SW	12.95^{c}	2.50^{b}	98b^{c}	0.625^{bc}	1849^{ab}
Spray Application (SA)	2×	17.32^{a}	8.95^{a}	95^{a}	0.567^{a}	1901^{a}
	4×	16.19^{a}	10.20^{a}	91^{a}	0.572^{a}	1857^{a}
HSD0.05						
PGRs		8.40 ***	4.72 **	22.39 ***	22.08 ***	7.67 **
SA		2.74 ^{ns}	0.40 ^{ns}	0.36 ^{ns}	0.01 ^{ns}	3.35 ^{ns}
PGRs × SA		0.65 ^{ns}	0.47 ^{ns}	1.49 ^{ns}	0.98 ^{ns}	1.83 ^{ns}

ns= not significant (p>0.05), **= very significant (p<0.05) and * = highly significant (p<0.01). CTRL-Control, SW-Seaweed extract, NAA-Naphthaleneacetic acid, NAASW- Naphthaleneacetic acid + Seaweed extract, GA3-Gibberellin, and GA3SW-Gibberellin+Seaweed extract. The spray frequency : 2× (day 35 & 55 after transplanting); 4× (day 35, 45, 55 & 65 after transplanting).

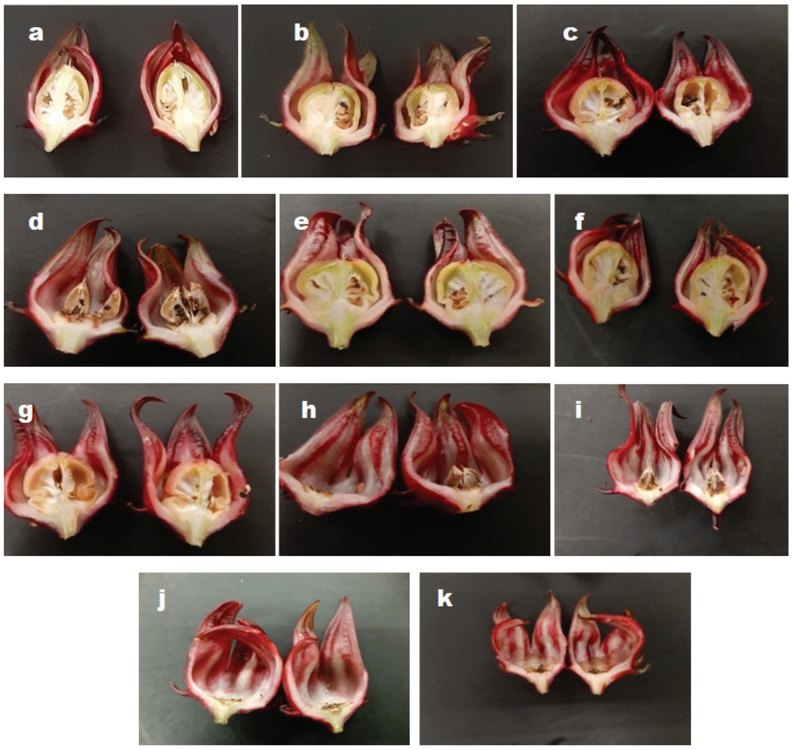

Fig. 2. Visual assessment on diameter of capsule on 75 DAT. (a) Control (without PGRs: 0 mg/L (2 sprays)), (b) Control (without PGRs: 0 mg/L (4 sprays)), (c) Seaweed extract (2 sprays), (d) Seaweed extract (4 sprays), (e) 400 mg/L NAA (2 sprays), (f) 400 mg/L NAA + Seaweed extract (2 sprays), (g) 400 mg/L NAA + Seaweed extract (4 sprays), (h) 800 mg/L GA3 (2 spray), (i) 800 mg/L GA3 (4 sprays), (j) 800 mg/L GA3 + Seaweed extract (2 sprays), (k) 800 mg/L GA3 + Seaweed extract (4 sprays) (photo: no scale).

In general, the application of PGRs resulted in different effects in developing parthenocarpic fruit such as apple fruit, 'Gefner' custard apple, Rosa Canina, and Diospyros lotus (Kadioglu & Atalay, 1999; Watanabe *et al*., 2008; dos Santos *et al*., 2016). Parthenocarpy is the development of fruit without the formation of seeds in the absence of pollinations, fertilization, or embryo development (Mezzetti *et al*., 2004). It naturally occurs in several plant species and could be induced artificially. Parthenocarpy can be induced in many crops such as cucumber, tomato, bottle gourd, and eggplant by applying exogenous auxins, gibberellins, and cytokinins as claimed by Kim *et al*. (1992), Ikeda *et al*. (1999), Rasul *et al*. (2008) and Chen *et al*. (2017). However, the application of PGRs integrated with seaweed extract in producing seedless roselle fruit has not yet been sufficiently investigated or reported. As claimed by Khan *et al*. (2009) seaweed extracts demonstrate growth-stimulating activities from gibberellic acid, cytokinin, indole acetic acid, abscisic acid, and polyamine. Possibly, these phytohormones play a significant role in enhancing the production of seedless roselle fruit. On the other hand, Mesejo *et al*. (2010) claimed that seedless loquat (*Eriobotrya japonica*) induced by exogenous GA_3 sprays at 100 mg/L. Meanwhile, Tiwari (2011) found that by enhancing the fruit set, the external application of GA_3 induced seedless fruits in chili (*Capsicum annuum*).

In the present study, yield parameters which include several fruits and total fresh weight resulted in a significant effect ($P \leq 0.05$) of different sources of PGRs (Table 1). Regardless of spray frequency, NAA-treated plants had the lowest number of fruits and fresh weight, 43 fruit and 0.18 g respectively as compared to other treatments. However, plants that were treated with seaweed showed the highest fresh weight and number of fruits, 0.89 g and 139 fruits respectively (Table 1). Meanwhile, NAA had the lowest number of fruits (Table 1). Similarly, Wan Zaliha *et al*. (2015) claimed that NAA at a higher dose (1000 mg/L) resulted in high fruit drops thereby reducing cumulative yield. This could be the reason NAA-treated fruit had the least fruit number and fresh weight. In addition, NAA does not strengthen up the fruit attachment but only prevents further loosening from the fruit stem (Kvikliene *et al*., 2010) and can cause excessive fruit drops if fruits are not harvested (Curry, 2005).

Although the number of fruits treated with control and seaweed was similar, roselle treated with GA_3 and seaweed extract tends to have a slightly higher number of fruits than NAA. Talon *et al*., (1992) and Tiwari (2011) reported that GA_3 applications resulted in increased fruit production compared to IAA applications in mandarins (*Citrus reticulata*) and chili (*Capsicum annuum*). Besides, the plant treated with PGRs which is 800 mg/L GA_3 had a larger size of roselle fruit as compared to other treatments. Similarly, Davies (1995) reported that PGRs are extensively used in horticulture crops to enhance plant growth and improve yield by increasing fruit number, fruit set, and size.

There was no significant interaction between the two factors in all postharvest quality parameters evaluated except total anthocyanins concentration (Figure 3 & Table 2). However, a pronounced effect was observed in both factors. Previously, Wan Zaliha *et al*. (2013) reported that PGRs maintained roselle fruit quality and exhibited similar color attributes. Huang and Jiang (2012) also claimed that GA_3-treated broccoli (*Brassica oleracea var. Italica*) turned completely after three days into yellow color. As reported by Mesejo *et al*. (2010), fruits treated with PGRs tend to increase in size and total soluble solids. Meanwhile, Wan Zaliha *et al*. (2015) reported that GA_3 at 800 mg/L was effective in inducing roselle fruit parthenocarpy and maintaining other attributes of post-harvest quality.

Table 2. Effects of different types of PGRs and spray frequency on fruit firmness lightness (L*), soluble solids concentration, lightness, chromaticity value a* and b* and hue angle (h*) of roselle fruits.

Treatment/Parameter		Fruit Firmness (N)	SSC (%)	Fruit Colour			
				L*	a*	b*	Hue angle (h°)
PGRs	CTRL	1.85^{a}	9.09^{a}	47.25^{a}	16.41^{a}	5.37^{a}	18.00^{a}
	SW	2.28^{a}	8.28^{b}	46.52^{a}	14.72^{a}	4.22^{a}	15.76^{a}
	NAA	1.27^{a}	8.83^{ab}	45.59^{ab}	15.91^{a}	5.17^{a}	17.85^{a}
	NAASW	1.73^{a}	9.12^{a}	42.68^{bc}	14.28^{a}	4.47^{a}	15.73^{a}
	GA_3	1.64^{a}	9.86^{a}	41.03^{c}	12.30^{a}	2.68^{a}	11.87^{b}
	GA_3SW	2.02^{a}	9.60^{a}	41.87^{bc}	12.82^{a}	2.11^{a}	8.98^{c}
Spray Application (SA)	2×	1.64^{a}	9.04^{a}	44.10^{a}	14.42^{a}	4.03^{a}	15.10^{a}
	4×	2.12^{a}	9.31^{a}	43.86^{a}	14.02^{a}	3.78^{a}	13.44^{b}
$HSD_{0.05}$							
PGRs		1.29 ^{ns}	8.40 ***	14.31***	6.91 ^{ns}	13.68 ^{ns}	35.83 ***
SA		4.58 ^{ns}	1.97 ^{ns}	0.00 ^{ns}	0.11 ^{ns}	0.06 ^{ns}	6.36 **
PGRs x SA		0.48 ^{ns}	0.45 ^{ns}	1.01 ^{ns}	0.93 ^{ns}	0.76 ^{ns}	2.37 ^{ns}

ns= not significant (p>0.05), **= very significant (p<0.05) and * = highly significant (p<0.01). CTRL-Control, SW-Seaweed extract, NAA-Naphthaleneacetic acid, NAASW- Naphthaleneacetic acid + Seaweed extract, GA3-Gibberellin, and GA3SW-Gibberellin+Seaweed extract. The spray frequency : 2× (day 35 & 55 after transplanting); 4× (day 35, 45, 55 & 65 after transplanting).

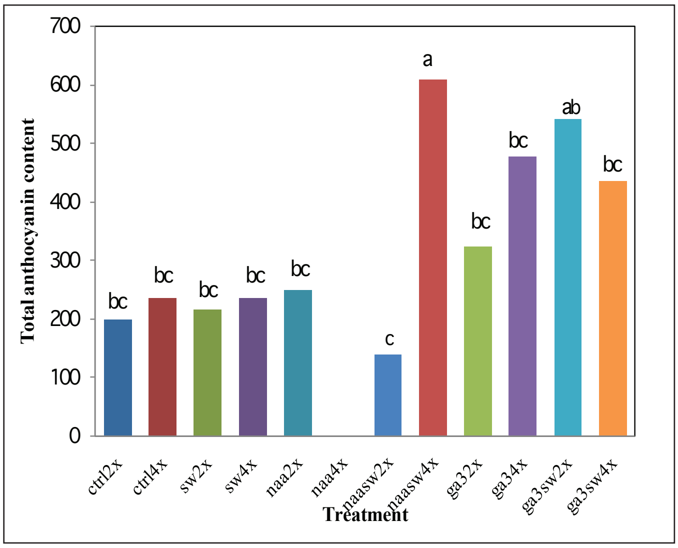

Fig. 3. Effect of different types of PGRs and spray frequency on total anthocyanins content. Means with different letters are significantly different at the 5% level according to the HSD test.

CONCLUSION

Among all PGRs treatments, the plant treated with 800 mg/L GA_3 + seaweed extract had developed seedless roselle fruit as shown by the smallest capsule diameter and the least number of seeds. However, this treatment was not able to obtain 100% capsules and seedless roselle fruits. Based on observation, the combination of 800 mg/L GA_3 + seaweed extract can produce 75% seedless fruit only. Other than that, this combination maintains other fruit quality parameters such as soluble solids concentration, titratable acidity, fruit firmness, and fruit color.

ACKNOWLEDGEMENTS

The authors wish to thank Universiti Malaysia Terengganu for the financial support (Grant TAPE:55180) and the staff of Postharvest Technology Laboratory, Faculty of Fisheries and Food Science for their kind help.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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