Journal of Sustainability Science and Management
Volume 17 Number 3, March 2022: 100-113
eISSN: 2672-7226

EFFECT OF DIFFERENT PROHEXADIONE-CALCIUM CONCENTRATION IN REGULATING GROWTH, PHYSIOLOGICAL CHANGES AND POSTHARVEST QUALITY OF DIFFERENT ROSELLE VARIETY UNDER GREENHOUSES

AZMI, M. N. A.[1], ZAHARAH, S. S.[1,2]* AND AHMAD, S. H.[1]

[1]Department of Crop Science, Faculty of Agriculture [2]Institute of Tropical Agriculture & Food Security (ITAFoS), Universiti Putra Malaysia, 43400 Seri Kembangan UPM Serdang, Selangor, Malaysia.

**Corresponding author: szaharah@upm.edu.my*
Submitted final draft: 4 August 2021 *Accepted: 18 September 2021* http://doi.org/10.46754/jssm.2022.03.009

Abstract: The brilliant red color of roselle is because of its anthocyanin content. The pigment has the potential to be commercialized as a natural color in the food industry. Anthocyanin content in roselle is influenced by several factors such as plant species, agronomic practices and environmental conditions. The application of plant growth regulators, such as prohexadione-calcium (Proca), has been reported to regulate color development, such as anthocyanin. Thus, this study was conducted to determine the effect of Proca application on growth and postharvest quality attributes of two varieties (UMKL-1 and UKMR-2) of roselle in greenhouse conditions. The plants were sprayed with four concentrations of Proca (0, 100, 200 and 300 mg L^{-1}) at 45 days after transplant (DAT). Roselle calyx was harvested at 75 DAT and analyzed. A reduction in height growth was observed with all Proca application and 300 mg L^{-1} gave the highest shoot reduction. Calyx postharvest quality showed that Proca application increased pH, C* value and anthocyanin content. As for roselle variety, UMKL-1 showed better response to Proca application, with improved calyx postharvest quality compared to UKMR-2. Therefore, the application of Proca can enhance anthocyanin regulation in roselle without adverse effect on its postharvest quality.

Keywords: Prohexadione-calcium, Proca, growth, quality, calyx, anthocyanin.

Introduction

Roselle (*Hibiscus sabdariffa)* is known worldwide for its high market demand. In Malaysia, roselle is an industrial crop along with coconut, oil palm, and coffee among others. Cultivation of roselle is usually for its leaves, seeds, stems and calyx. Its calyx can be eaten raw and or stored/dried to be processed into other products. Purbowati *et al.* (2019) said that the roselle calyx is in high demand due to its rich vitamin C and anthocyanin content. Vitamin C in roselle is three to nine times higher than blackcurrants, grapes and citrus (Musa *et al.,* 2006). The high anthocyanin content provides roselle calyx with a vivid red color, increasing its importance in culinary and medicinal applications. However, in roselle, similar to other plants, anthocyanins are unstable and easily hydrolyzed mainly due to factors such as plant species, light intensity, plant age and management practices like fertilization, irrigation and application of plant growth regulators (Aishah *et al.,* 2013; Souri *et al.,* 2019; Aghaye Noroozlo *et al.,* 2019) as well as the occurrence of environmental stresses (Ahmadi & Souri, 2018; Ebrahimi *et al.,* 2021). Aside of that, the discoloration of calyx can occur due to the bushy nature of the roselle plant, which results in the low amount of light reaching the calyx.

Several studies have shown that various factors are effective on the plant pigmentation including anthocyanins. Apart of plant species, environmental factors and agricultural practices are known to change the anthocyanin and carotenoids of plant tissues (Ghanbari *et al.,* 2017; Souri *et al.,* 2019). The use of plant growth regulators not only regulate plant growth and manipulate fruit development and quality, but also can modify the expression of genes in

anthocyanin biosynthesis (He *et al.,* 2010). The efficacy of Proca as a tool for enhancement of color development and anthocyanin formation in roselle calyx depends on the suitable concentration in foliar application and plant growth. The concentration of Proca in ranges of 50 m L^{-1} to 700 m L^{-1} has been reported to increase the red pigmentation of 'Fuji' and 'Cripps Pink' apple without affecting other postharvest quality attributes (Mata *et al.,* 2006; Medjdoub *et al.*, 2005; Wan Sembok, 2009). On the other hand, a high concentration of Proca has been reported to inhibit the flavanone-3-hydroxylase, which plays a key role in flavonoid biosynthesis, which can lead to the reduction of anthocyanin formation (Wilhelm Rademacher, 2000). Therefore, the objective of this study was to investigate the best concentration of exogenous Proca application on growth, physiology and postharvest quality of two roselle varieties, UMKL-1 and UKMR-2.

Materials and Methods

Location of Study

The experiment was carried out at a greenhouse in Agriculture University Park, Universiti Putra Malaysia, Malaysia. The average temperatures and relative humidities of the greenhouse were 39 ± 2°C/ 70 ± 2 (daytime) and 29 ± 2°C/ 80 ± 2 (nighttime).

Planting Materials

Two varieties of roselle (UMKL-1 and UKMR-2) were used in this study. Both variety seeds were obtained from the Department of Agriculture, Serdang, Selangor, Malaysia. The seeds were sown in peat-moss for 14 days and transplanted into 16" × 16" (21.5 cm long × 19 cm height × 29.4 cm width) polybags. Soil mixture with ratio 3:2:1 (topsoil: organic matter: sand) was used as media. Twenty-four plants of each variety were used in this experiment.

Treatments

Three concentrations of Proca (100, 200 and 300 m L^{-1}) were exogenously applied to the roselle plants. Proca chemical (250.26 molecular weight) was purchased from Sigma Aldrich, manufactured from the United States of America. One to two drops of Tween®20 were added to each Proca concentration solution before being sprayed onto the plant. Selected calyx that bloomed 45 days after transplanting (DAT) was tagged for further analysis. Unsprayed trees (sprayed with distilled water) served as a control. Roselle calyx was harvested at the commercial harvest stage approximately four weeks after being sprayed (75 DAT) for laboratory analysis. The experiment was carried out using a randomized complete block design (RCBD) consisted of three replications with two plants per replication per treatment.

Cultural Practices

Fertilizer application, irrigation, weeding management, and pest and disease control were conducted when needed. Two types of fertilizers, NPK green (15:15:15) and NPK blue (12:12:17:2) were used during the experimental period. NPK green was applied during the vegetative stage at one week after transplant. A weed mat was used and laid out at the planting sites to control weed growth. Pests and diseases were controlled by applying the recommended type of pesticides and insecticides only when necessary. Plants were irrigated twice daily using an irrigation system for ten minutes every day.

Data Collections

Plant Height

Plant height of roselle was recorded from the soil surface to the top of shoot at 15, 30, 45, 60, 75, 90 and 105 DAT. The mean data for plant height was expressed as centimeters (cm).

Fruit Weight

Fruit weight was defined as the fresh weight of roselle calyx and was determined by weighing 10 uniform sizes of the calyx with seeds for each treatment using a digital balance (Mettler Toledo Balance, Switzerland). Mean data for the fresh weight of calyx was expressed in grams (g).

Physiological Changes

Data for photosynthesis, stomatal conductance and transpiration rate were recorded at every replication for each treatment using Portable Photosynthesis System (LI-6400XT, LI-COR Ltd., United Kingdom) a day before harvest. The data was recorded around 11.00 am to 1.00 pm, with mean of light 1000 uml and temperature in the range of 35±2°C to 40±2°C. The mean data for photosynthesis, stomatal conductance and transpiration rate were expressed as µmol CO2 m^{-2} s^{-1}, mol H_2O m^{-2} s^{-1} and mmol H_2O m^{-2} s^{-1}, respectively.

Postharvest Quality

Calyx Color

The color of roselle calyx was recorded at commercial harvest time (75 DAT) by using chromameter (Konica Minolta CR-400 Chroma meter, Minolta Corp., Japan). The result was expressed as lightness (L*), chroma value (C*) and hue angle (h°). The L* value represented the lightness coefficient, which ranges from 0 (black) to 100 (white). The C* value corresponded to the intensity or color saturation. Low values represent dull color while high values represent vivid color. The H° angle represented the color tone red-purple (0° - 89°), yellow (90° - 179°), bluish-green (180° - 269°) and blue (270° - 360°).

Firmness

The firmness of fresh calyx has been determined by Instron (Instron Model 5543 Load Frames, Instron Corp., Massachusetts, USA) at the final harvest. The seed of calyx was removed first before firmness was determined. The firmness was expressed by the maximum force required to compress the calyx. The data were expressed in the unit of newton (N).

Soluble Solids Concentration

For the initial step, 10 g of calyx was weighed and blended with 40 mL distilled water to determine the soluble solids concentration (SSC). The mixture was filtered using cotton and 5 mL of aliquots were used for analysis. One drop of aliquot was used to determine SSC using a digital refractometer. The readings were corrected to a standard temperature of 20°C by adding 0.28% to obtain percentage SSC at 27°C.

pH

Five mL of aliquot prepared from SSC determination was used for pH measurement using Digital pH meter (GLP 21, Crison, Barcelona, Spain).

Titratable Acidity

Titratable acidity (TA) was determined using the method described by (Ding & Wahab, 2007): 10 g of calyx was blended with 40 mL of distilled water. The mixture was filtered through cotton into the vial. 5 mL of aliquot was taken and titrated with 0.1 N NaOH until it reached pH 8.1 for TA analysis. The titration was done with a pH meter due to the difficulty of determining the endpoint of by color (indicated by pink) as the aliquot was in red colour.

Anthocyanin Content

Anthocyanin extraction was done based on Wan Sembok's (2009) method with some modifications. One gram of calyx was soaked in 10 mL of methanol (96%): concentrated hydrochloric acid in the ratio (97:3 v/v). The extract was decanted and centrifuged at 5,000 rpm for 20 minutes at 4°C. Then, the supernatant was analyzed using a UV-VIS spectrophotometer. Total anthocyanins were calculated as cyanidin chloride equivalents using the standard curve equation obtained and expressed in mg g^{-1} as described by Humadi (2009).

Ascorbic Acid Content

Five grams of calyx was homogenized with 25 ml 6% metaphosphoric acid with 0.18 g EDTA (ethylenediamine tetra-acetic disodium salt). The homogenate was centrifuged at 5,000 rpm for 20 minutes before 500 µL of aliquot was mixed with 200 µL (3%) metaphosphoric acid,

1,400 µL distilled water and 200 µL diluted 1:5 v/v Folin reagent and left for 10 minutes at room temperature. The sample's absorbance reading was determined at 760 nm using UV-VIS spectrophotometer. The ascorbic acid concentration was quantified from the standard curve of L-ascorbic acid and expressed in mg 100 g^{-1} of fresh weight following Zargar Shooshtari *et al.* (2020).

Determination of Total Phenolic Content, Total Flavonoid Content and Total Antioxidant Activity of Calyx

Plant Extraction

Five grams of calyx were macerated using 70% ethanol in a conical flask for 72 hours in the dark, filtered and evaporated by a rotary evaporator at 40°C. The plant extraction was defined as crude and was used for further analysis to determine total phenolic content, total flavonoid content and antioxidant activity.

Determination of Total Phenolic Content

Total phenolic content of roselle calyx was determined with Folin Ciocalteau reagent following the method described by Sirag *et al.* (2014). 50 milligrams of crude extract were mixed with 1 mL of Folin Ciocalteau reagent and 7.5 mL of deionized water. The mixture was left at room temperature for 5 minutes before 10 mL of 7% sodium carbonate was added. The mixture was then incubated for 90 minutes at room temperature and the absorbance was determined against the reagent as blank at 760 nm using UV-VIS spectrophotometer. Total phenolic content was expressed as mg g^{-1} Gallic acid equivalent.

Determination of Total Flavonoid Content

The flavonoid content of roselle calyx was measured following a spectrophotometric method by Dewanto *et al*. (2002). 1 mL of 100 µg mL^{-1} plant extract was diluted with 4 mL distilled water in a 10 mL volumetric flask before 0.3 ml of 5% $NaNO_2$ was added. Then, 0.3 mL of 10% $AICl_3$ was added at 5 minutes and 2 mL of 1.0M NaOH was added at 6 minutes. Then 2.4 mL distilled water was added and the absorbance was read at 510 nm using a UV-VIS spectrophotometer. Total flavonoid content was determined as quercetin equivalents (mg g^{-1} of fresh weight).

Determination of Antioxidant Activity

In order to determine the antioxidant activity, the ethanolic extract of roselle calyx was prepared by diluting the crude extract (1 mg mL^{-1}) to final concentrations 250, 125, 50, 10 and 5 µg mL^{-1} in ethanol. Then, 1 mL of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in ethanol solution was added to a 2.5 mL solution of the different concentrations of the ethanolic extract and allowed to react at room temperature for 30 minutes. The absorbance of the mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula by Sirag *et al.* (2014): AA% = (Abs. of control – Abs. of the sample) × 100 Abs. of control.

Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. One (1) ml of DPPH solution (0.3 mM) plus methanol (2.5 ml) was used as control. Stock solution (1 mg ml^{-1}) of quercetin was prepared by diluting ed to final concentrations of 250, 125, 50, 10 and 5 µg ml^{-1} in ethanol used as a positive control (Mensor*et al*., 2001). All experiments were done in triplicate. A freshly prepared DPPH solution exhibits a deep purple color with a maximum absorbance at 518 nm. The purple color disappears when an antioxidant is present. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of the test compounds to act as a free-radical scavenger.

Statistical Analysis

Data were analysed with analysis of variance (ANOVA) using SAS 9.2 software package, SAS Institute Inc., Cary, NC, USA. The least significant difference (LSD) at $P \leq 0.05$ was conducted for separation of treatments means.

Results

Plant Height

Application of Proca significantly reduced effect on plant height of UMKL-1 and UKMR-2 cultivars compared to untreated trees (Figure 1A). All the trees were within a certain height range from 15 DAT to 45 DAT o (Figure 1A). UMKL-1 trees untreated with Proca grew rapidly from 45 DAT to 60 DAT, with a 30.2% increase in height, while treated trees grew less rapidly, with 15.3% for 100 mg L^{-1}, 14.8% for 200 mg L^{-1} and 5.3% for 300 mg L^{-1} within 14 DAT. For UKMR-2, untreated trees (0 mg L^{-1}) grew by 24.5%, whereas the Proca-treated trees increased slowly with 16.8%, 8.1%, 15.2% for 100 mg L^{-1}, 200 mg L^{-1} and 300 mg L^{-1}, respectively from 45 to 60 DAT.

15 days after Proca spray (60 DAT), seedlings started to show height differences with untreated seedlings. The highest plant height was in untreated control plants followed by trees treated with 100, 200 and 300 mg L^{-1} of Proca. The highest reduction of plant height was observed in tree treated with 300 mg L^{-1} Proca, at 46.5% difference with the control. Application of 100 mg L^{-1} resulted in lowest plant height difference at 14.2% at 60 DAT. At the end of the study (105 DAT), the plant height of the Proca-treated roselle eventually was significantly shorter than untreated trees which are around 120 to 140 cm while untreated trees were up to more than 160 cm height.

At 105 DAT, Proca spray significantly reduced the plant height of UKMR-2 roselle (Figure 1B). The untreated trees (control) exhibited the highest plant height, while the highest concentration of Proca (300 mg L^{-1}) resulted the highest reduction in plant height with a 44.1% difference compared to control. Treatment with 300 mg L^{-1} also resulted in the lowest plant height amongst other Proca treated trees with a 22.5% difference compared to 100 mg L^{-1} and 17.5% difference compared to 200 mg L^{-1}. The lowest concentration of Proca application resulted (100 mg L^{-1}) in the lowest reduction in plant height with a 21% difference compared to control, followed by 200 mg L^{-1} with a 24.8% difference.

Fresh Weight

The different concentrations of Proca had no significant effect on the calyx fresh weight (Table 1). However, the fruit weight of calyx was significantly ($P \leq 0.05$) affected on the roselle varieties used in this study. The fruit weight of UKMR-2 calyx was significantly lower ($P \leq 0.05$) as compared to UMKL-1 with a 21.5% difference.

Physiological Changes

Table 1 shows no significant effect the different concentrations of Proca had on physiological changes (photosynthetic rate, stomatal conductance and transpiration rate). The rate

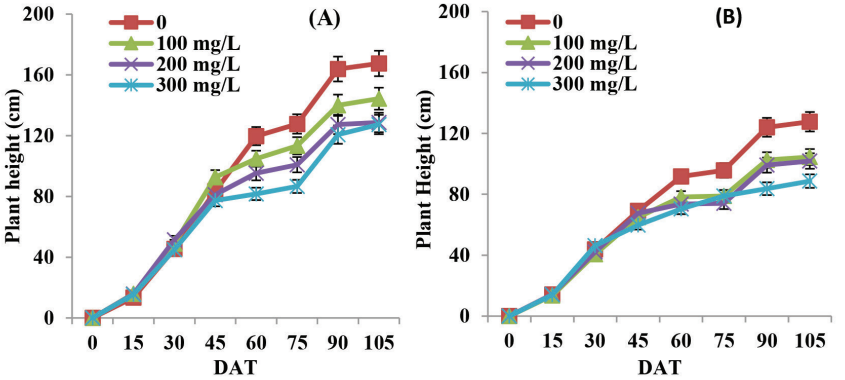

Figure 1: Effect of different concentration of Proca spray on plant height of (A) UMKL-1 and (B) UKMR-2. Error of each point is presenting standard error at $P \leq 0.05$

Table 1: Effects of different Proca concentration and variety on fruit weight (FW, g), photosynthetic rate (Ps, μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (gs, mol H_2O $m^{-2}s^{-1}$) and transpiration rate (Ts, mmol H2O $m^{-2}s^{-1}$) of roselle

Conc. (mg L^{-1})	Variety	FW	Ps	gs	Ts
0	UMKL-1	120.85 a	32.51 a	0.79 a	9.15 a
100		136.77 a	51.07 a	1.55 a	13.60 a
200		142.82 a	39.80 a	2.18 a	18.54 a
300		129.69 a	21.08 a	1.36 a	11.44 a
	Means	132.54a	36.11 a	1.47 a	13.18 a
0	UKMR-2	89.22 a	17.27 a	0.32 a	4.36 a
100		118.83 a	27.42 a	1.50 a	13.23 a
200		107.98 a	13.00 a	0.71 a	8.07 a
300		100.17 a	23.16 a	0.28 a	3.97 a
	Means	104.05b	20.21 a	0.70 a	7.41 a
LSD means at P ≤ 0.05 level and levels of significance for a two factor of ANOVA					
Conc. (C)		NS	NS	NS	NS
Var. (V)		**	NS	NS	NS
C x V		NS	NS	NS	NS

Means with similar letter and NS indicates non-significant different at P > 0.05

of photosynthetic, stomatal conductance and transpiration were also not significantly affected by the different Proca concentrations and roselle varieties tested in this study. The rate of photosynthetic, stomatal conductance and transpiration rate exhibited 1.79-, 2.10 – and 1.78-fold higher rates in UMKL-1 treated with Proca as compared with UKMR-2, respectively.

Calyx Color

The results showed that Proca levels and roselle variety significantly influenced titratable acidity and lightness (L*) value, while it has no significant effect on chroma value (C*), hue angle (H°), firmness, pH value, and soluble solids concentration (Table 2). There was a significant difference of L* value in UKMR-2 and UMKL-1 when applied with 300 mg L^{-1} Proca spray. After treatment with 300 mg L^{-1} of Proca, UKMR-2 had the highest L* value (37.95) while UMKL-1 had the lowest L* value (34.37) among other treatments. There was a 10.4% significant difference between the highest and lowest L* value. No significant influence was observed on L* value with 0, 100 and 200 mg L^{-1} in both varieties as all these treatments exhibited no significant difference among each other (Figure 1A).

There was a significant difference for TA between UMKL-1 and UKMR-2. Table 2 showed that Proca-free (0 mg L^{-1}) UMKL-1 had a lower percentage of TA than UKMR-2 with a 54.3% difference. The TA of Proca-fee UKMR-2 also had the highest TA (8.97%) while UMKL-1 with 0 mg L^{-1} and 100 mg L^{-1} of Proca had the lowest TA (4.1% and 4.75%), respectively. The application of different Proca concentrations on UMKL-1 showed that only 200 mg L^{-1} had a significant effect as compared to control with a 46% difference. Meanwhile, UKMR-2 treated with 100 mg L^{-1} and 200 mg L^{-1} of Proca significantly reduced the TA of roselle with a 36% difference than the control (0 mg L^{-1}) (Figure 2B).

Table 2: Effect of different Proca concentration and roselle variety on lightness value (L*), chroma value (C*), hue angle (H°), firmness (firm), pH value, titratable acid (TA) and soluble solids (SSC) concentration

Conc. (mg L^{-1})	Variety	L*	C*	H°	Firm (N)	SSC (%)	pH	TA (%)
0		35.60 ab	11.11 b	4.72 a	2.18 b	4.85 a	2. 44b	4.10 b
100		35.9 ab	14.01 a	6.46 a	2.93 a	4.99 a	2.76 a	4.47 b
200	UMKL-1	37.06 a	12.44 ab	3.33 a	2.86 a	4.45 a	2.82 a	7.59 a
300		34.37 b	12.25 ab	4.84 a	2.92 a	4.45 a	2.72 a	6.02 ab
	Means	35.56b	12.45a	4.84a	2.72b	4.69b	2.69a	5.55a
0		35.65 b	11.44 a	3.63 a	3.18 a	5.39 a	2.32 b	8.97 a
100		36.40 ab	12.42 a	3.27 a	3.34 a	4.85 b	2.68 a	5.75 b
200	UKMR-2	36.38 ab	13.54 a	4.63 a	3.30 a	4.99 b	2.72 a	5.76 b
300		37.95 a	11.66 a	2.09 a	2.75 a	5.12 ab	2.68 a	7.56 a
	Means	36.59a	12.26a	3.40a	3.14a	5.09a	2.60b	7.01a
Conc. (C)		NS	**	NS	NS	NS	**	NS
Var. (V)		**	NS	NS	**	**	**	NS
C x V		**	NS	NS	NS	NS	NS	**

Means with different letter and ** indicates significantly difference at P ≤0.01, while means with a similar letters and NS indicate a non-significant difference at P > 0.01

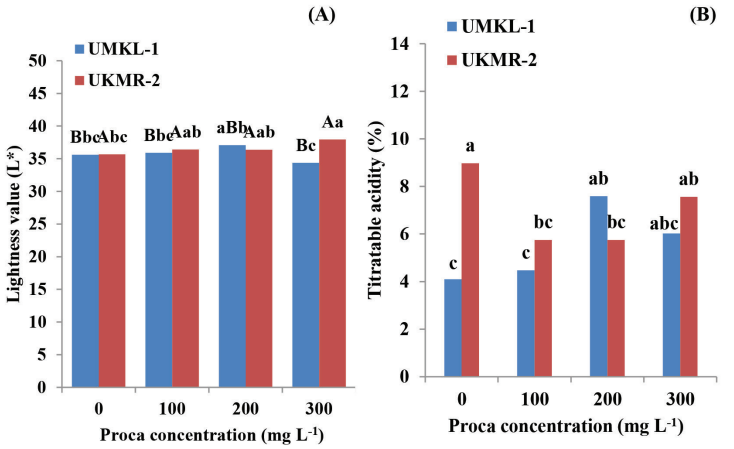

Figure 2: (A) Lightness and (B) Titratable acidity as influenced by the interaction between the different concentrations of Proca and roselle variety. Mean with a different letter indicates there was a significant difference at P ≤ 0.01. Capital letter indicates significant difference between variety, small letter indicates significant difference between Proca concentration

Proca levels had significant effect on the chroma (C*) value and pH value of roselle (Figure 3). In general, the application of Proca increased the C* value. However, 100 and 200 mg L^{-1} of Proca concentration significantly increased the C* value, with 17.1% and 15.2% difference as compared to control, respectively. Meanwhile, no significant difference was observed between 300 mg L^{-1} of Proca application with untreated trees (0 mg L^{-1}). Amongst Proca-treated trees, no significant difference was observed regarding C* value (Figure 3A).

A similar result was observed on the effect of different concentrations of Proca on the pH value of roselle calyx. In general, Proca applications of 100, 200 and 300 mg L^{-1} significantly increased the pH value of roselle calyx as compared to the control treatment (0 mg L^{-1}) with 14.3%, 16.4% and 13.4%, respectively. No significantly different pH value of the calyx was observed amongst the different Proca treatments (Figure 3B).

There was significant difference among the varieties on certain postharvest qualities of the firmness, pH and soluble solids concentration (SSC) (Figure 4A-C). UKMR-2 had a higher firmness value and SSC as compared to UMKL-1 with a 15.4% and 7.9% difference. Whereas UMKL-1 had the higher pH value with a 3.3% difference as compared to UKMR-2.

Biochemical Content

There was significant interaction (P≤0.05) for ascorbic acid content (AAC) between roselle variety and Proca concentration sprayed (Table 3). In general, the application of Proca increased the AAC of UMKL-1 roselle as compared to the untreated plants (0 mg L-1). Whereas UKMR-2 react differently where the AAC was significantly decreased with Proca application as compared to the UKMR-2 control (0 mg L-1). Highest AAC (266.23 µg g^{-1}) was found on UMKL-1 roselle calyx sprayed with 100 mg L^{-1} of Proca, while the lowest (100.04 µg g^{-1}) was UKMR-2 with 300 mg L^{-1} of Proca spray with 20.2% difference. 100 mg L^{-1} of Proca increased the AAC of UMKL-1 with a 24.1% difference as compared to control, followed by 200 mg L^{-1} (19.5%) and 300 mg L^{-1} (4.9%) respectively. As

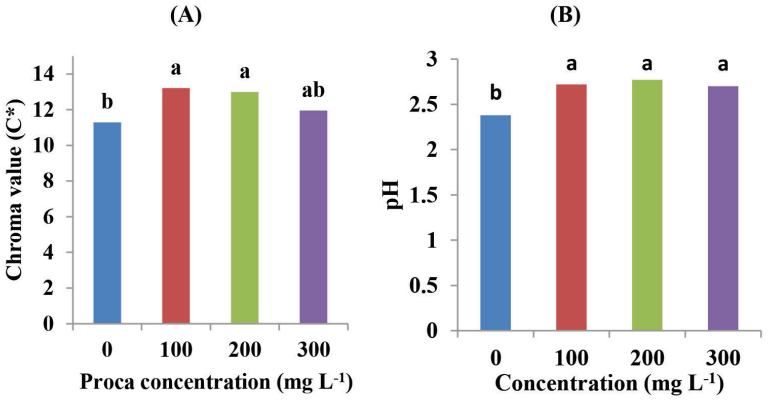

Figure 3: (A) Chroma value (C*) and (B) pH of roselle calyx as influenced by different concentrations. Mean with a different letter indicates there was a significant different at P ≤ 0.01

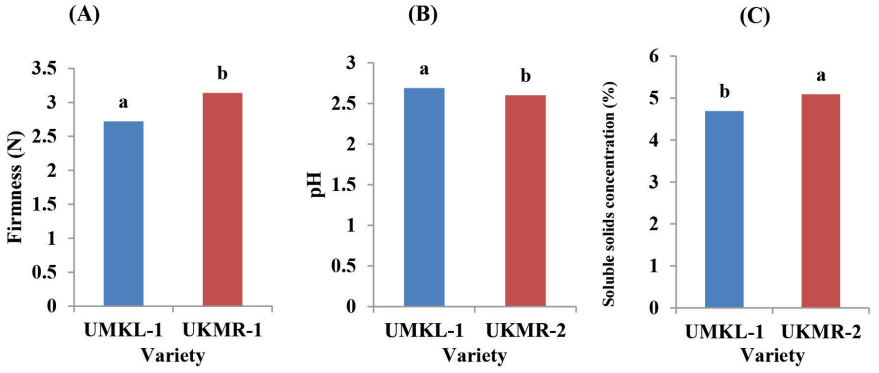

Figure 4: (A) Calyx firmness, (B) pH value and (C) SSC as influenced by different roselle varieties. Mean with a different letter indicates there was a significant difference at P ≤ 0.01

for UKMR-2, significant decreases were shown with 200 and 300 mg L^{-1} of Proca with 25.4% and 46% difference as compared to control (Figure 5A).

No significant interaction (P>0.05) was observed due to the different concentrations of Proca applied and roselle varieties on total anthocyanin content (TA), total phenolic (TPC), total flavonoid (TFC) and DPPH. As for TAC

Table 3: Effect of different Proca concentration and roselle variety on ascorbic acid content (AAC), total anthocyanin content (TAC), total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant activity (DPPH) of roselle calyx

Conc. (mg L^{-1})	Variety	AAC (µg g^{-1})	TAC (mg g^{-1})	TPC (mg g^{-1})	TFC (mg g^{-1})	DPPH (%)
0	UMKL-1	202.11 a	39.93 b	38.15 b	5.61 a	79.43 a
100		266.23 a	190.88 a	42.60 ab	2.64 a	79.71 a
200		212.54 a	148.56 a	31.78 b	2.96 a	82.89 a
300		150.97 a	105.61 ab	39.49 b	3.85 a	79.99 a
	Means	233.46 a	121.25 a	38.01a	3.76 a	80.51a
0	UKMR-2	186.33 a	60.81 a	53.31 a	6.94 a	74.00 b
100		183.02 a	103.34 a	40.47 b	1.50 b	81.01 a
200		100.04 c	97.17 a	40.04 b	1.14 b	81.34 a
300		138.25 b	83.01 a	39.42 b	1.75 b	81.10 a
	Means	151.91 b	86.08 b	43.31a	2.87a	79.36b
LSD of means at P ≤ 0.05 level and levels of significance for a two factor of ANOVA						
Concentration (C)		**	**	*	NS	NS
Variety (V)		**	**	NS	NS	*
C x V		**	NS	NS	NS	NS

Means with different letters, * and ** indicate significant differences at P≤0.05 and 0.01, while means with a similar letters and NS indicate non-significant differences at P>0.05

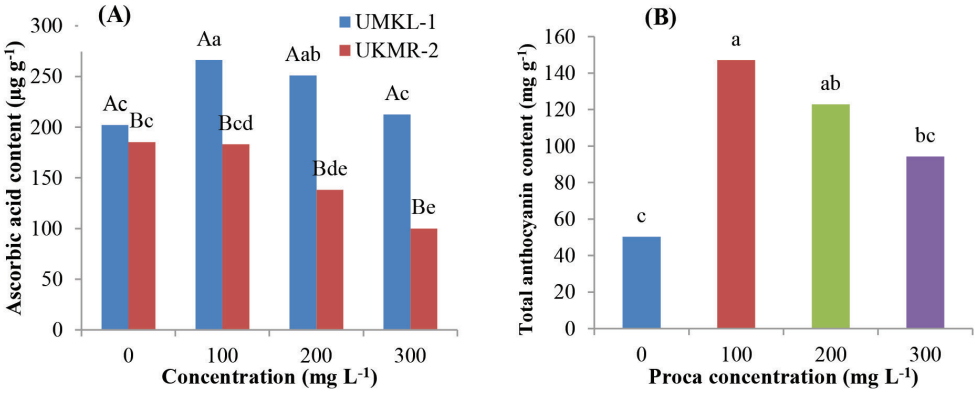

Figure 5: (A) Effect of different concentrations of Proca application on UMKL-1 and UKMR-2 on ascorbic acid content (AAC) and (B) total anthocyanin content as influenced by different concentration of Proca application of roselle varieties. A similar letter indicates there is no significant difference at P>0.05. Capital letter indicates significant difference between variety, small letter indicates significant difference between Proca concentration

in roselle calyx, there was a significant effect (P≤0.05) of Proca concentration and roselle variety used. In general, various concentrations of Proca significantly increased (P≤0.05) the TAC in roselle calyx as compared to the Proca-free trees (control) (Figure 5B). As for roselle variety, a significant difference was seen between UMKL-1 and UKMR-2, where UMKL-1 contains 29% more TAC as compared to UKMR-2 (Figure 6A).

The highest TAC (190.88 mg g^{-1}) was observed in UMKL-1 sprayed with 100 mg L^{-1} followed by 200 mg L^{-1} (148.56 mg g^{-1}) and 300 mg L^{-1} (105.61 mg g^{-1}) of Proca with 378.04%, 272.05% and 164.49% difference as compared to control (39.93 mg g-1). A similar trend was found when roselle variety UMKL-1 was sprayed with Proca, however, no significant effect was recorded between all of them (Table 3.3). The TPC was significantly reduced 21% with 300 mg L-1 of Proca application, whereas 100 and 200 mg L-1 did not have a significant effect as compared to the control (0 mg L-1) (Figure 6B).

Discussion

Different Proca concentrations were found to be significantly effective in regulating certain growth and postharvest quality aspects of two different roselle plant varieties. As expected, the application of Proca reduced the growth of the shoot and lowered the plant height of the roselle. The reduction in plant height with the application of Proca was probably due to the reduction in endogenous concentrations of GA_1 and increase of GA_{20} concentrations via interfering the 3-β hydroxylation of GA_{20} to GA_1 (Wan Sembok, 2009). GA biosynthesis inhibitors, when applied to plants at higher doses, could reduce the number of leaves. In the present investigation, the significant reduction of height of the roselle plants was pronounced after 60 and 15 DAT of Proca application at different concentrations ranged from 100 mg L^{-1} to 300 mg L^{-1}. Previous research had also discovered a reduction of plant height (Unrath, 1999; Wan Sembok, 2009). Proca's marked effect (i.e., 50%) on shoot length was accompanied by a reduction in leaf number in apple trees under orchard conditions (W. Rademacher & Kober, 2003). According to other researchers (Reekie *et al.,* 2003), Proca causes a proportional drop in shoot dry weight and an increase in root dry weight in strawberry plants. The variety has a greater influence than the application of Proca on leaf photosynthesis, stomatal conductance and transpiration rate. Strawberry plants in the field responded to Proca treatment with better photosynthetic rates (Reekie *et al.,* 2005). The positive effect of Proca on photosynthesis in roselle could be related to the lower specific leaf area (SLA) and higher chlorophyll content on a leaf area basis. Similar effects were observed

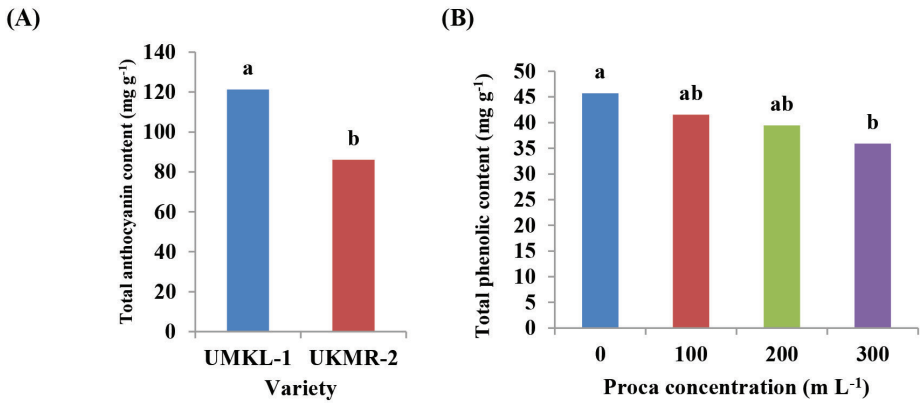

Figure 6: Total anthocyanin content (TAC) as influenced by (A) roselle varieties and (B) different concentrations of Proca application. A similar letter indicates it is no significant difference at P>0.05

in root or foliar application of ammonium (Dehnavard *et al.,* 2017; Aghaye Noroozlo *et al.,* 2019) or amino acids (Souri *et al.,* 2017) on different horticultural crops. As supported by previous reports, they noted that Proca decreases SLA (Guak *et al*., 2001) and increases chlorophyll per unit leaf area in apple leaves (Sabatini *et al*., 2003). According to a study by (Reekie *et al.,* 2005), the beneficial effects of Proca on photosynthesis were much greater in the field than in the control environment. Proca-induced reduction in SLA under the lower light conditions in the growth chambers resulted in a relatively small effect on net photosynthesis. However, the effect on leaf morphology was significantly more obvious in the field, where light levels are about five times higher than in the growth chambers. This research was conducted under a controled environment; therefore, more research is needed to determine the effectiveness of Proca treatment on roselle growth and postharvest quality in the open field. Variety UMKL-1 had significantly higher fresh weight of calyx than UKMR-2 (Table 3.1). This may be due to the higher number of branches for variety UKMR-2, which causes an enlargement in the canopy size (Osman *et al.,* 2011). By having lots of branches, the number of fruits also increases and the production of UKMR-2 calyx had a lower weight compared to UMKL-1.

In this study, we found that a single application of various Proca concentrations exhibited a significant effect on the calyx quality compared to the control. The intensity of calyx color might be due to the increased TAC concentrations. The effect of various Proca concentrations on AAC indicated that 100 mg L-1 spray was sufficient to increase TAC as it was the highest among other Proca concentrations applied. The increase of AAC in Proca-sprayed plants may be attributed to the reduction in shoot growth, which consequently helps improve light penetration to the calyx (Rehman *et al.,* 2018). The increased light penetration into calyx has been reported to up-regulate the activities of the enzyme involved in anthocyanin biosynthesis, such as phenylalanine ammonia-lyase (PAL) and UDP galactose:flavonoid 3-O-galactosyltransferase (UFGalT) (Wan Sembok, 2009).

However, total phenolic content (TPC) reacted differently to the various Proca applications, where it was found that under 300 mg L^{-1} of Proca concentration, TPC was lower than the control plants. A prior study on 'Florina and Jonagold' apples found a drop in TPC due to the influence of Proca spray (Mikulic-Petkovsek *et al.,* 2009). This could be due to the differences in environmental factors at both locations such as light and temperature (Poiroux-Gonord *et al.,* 2010), which contributed a major role in regulating polyphenolic compounds. The AAC was found to be higher with various Proca applications as compared to the control. It was found that a single spray of 100 mg L^{-1} was sufficient to increase the AAC. The increase of AAC might be due to improved light penetration during calyx development due to reduction of shoot growth. Although light is not essential for ascorbic acid synthesis, the amount and intensity of light during growing have great influence on the amount of ascorbic acid formed, as it is synthesized from sugars supplied during plant photosynthesis (Lee & Kader, 2000).

In comparison to the control treatment, the three concentrations of Proca applications produced slightly higher total antioxidant activities in the DPPH solution. The Proca treatment of 200 mg L-1 was shown to be the most effective in promoting antioxidant activities in plants. However, there was no significant impact of Proca application towards the antioxidant activities in the roselle calyx. The presence of free-radical scavenger and antioxidant activities may be correlated to the phenolic compound, ascorbic acid and anthocyanin presence in the roselle calyx (Sirag *et al.,* 2014). As demonstrated in Table 3, such natural compounds were detected in both varieties of roselle studied in this research.

The roselle variety used in this study had no significant interaction on most parameters. except for L* value, TA and ascorbic acid content. It was shown that no consistent interaction between various Proca concentrations and

roselle varieties (UMKL-1 and UKMR-2) was used except for AAC. The AAC of UMKl-1 was significantly increased with Proca spray, whereas consistently decreased in UKMR-2 calyx. Among these two varieties, it was found that UMKL-1 warrants to be investigated further for a source of natural food colourant due to its prominent AAC and TAC attributes as compared to UKMR-2. These greater qualities in UMKL-1 than UKMR-2 could be related to the plant structure of UKMR-2, which had bushy characteristics and received less light penetration to the calyx than UMKL-1 (Osman *et al.,* 2011).

Conclusion

In conclusion, due to its high TAC in the calyx and the efficacy of Proca in giving the highest amount in UMKL-1 roselle, a concentration of 100 mg L^{-1} Proca and the UMKL-1 variety is recommended due to its promising result in obtaining optimum content of vitamin C and anthocyanin.

Acknowledgements

This study was funded by the Fundamental Research Grant Scheme (FRGS) (Vot Number: 5524387). The authors would like to express her deepest gratitude and appreciation to the Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor for facility support during the research. The authors would like to thank all academic and support staff for their assistance throughout the study.

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