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EFFECT OF PROHEXADIONE-CALCIUM APPLICATION AND IRRIGATION TECHNIQUE ON PLANT GROWTH, PHYSIOLOGICAL CHANGES AND POSTHARVEST QUALITY OF ROSELLE (Hibiscus sabdariffa L.)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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EFFECT OF PROHAXADIONE-CALCIUM APPLICATION AND IRRIGATION TECHNIQUE ON PLANT GROWTH, PHYSIOLOGICAL CHANGES AND POSTHARVEST QUALITY OF ROSELLE (*Hibiscus sabdariffa* L.*)*

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Hibiscus sabdariffa (roselle) belongs to the Malvaceae family and is commonly used to make healthful beverages and medicine. Roselle calyces are high in functional properties such as vitamin C, anthocyanin, and other phenolic compounds. They are beneficial to health since they are a powerful source of antioxidants as well as a natural food colourant. However, factors such as species, agronomic practices and environmental conditions are greatly influencing the contents of vitamin C and anthocyanin pigments in H. sabdariffa. Moreover, changes in temperature conditions during harvest and postharvest caused a greater loss of both vitamin C and anthocyanin, which eventually resulting in poor color and quality of calyces. In addition, the application of prohexadione-calcium (Proca) and partial rootzone drying (PRD) are reported can influence and regulate the content of vitamin C and anthocyanin pigment. Thus, a study was conducted to determine the effect of Proca application and PRD on growth, physiological changes and postharvest quality attributes of roselle. In 1st experiment, two different varieties of roselle (UMKL-1 and UKMR-2) were planted in a greenhouse and sprayed with four different 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 analysed. Plant height of roselle showed a reduction in growth with all Proca application with 300 mg L-1 gave the highest shoot reduction among other concentrations. Whereas calyx postharvest quality attributes showed that Proca application increased pH, C* value and anthocyanin content. However, the Proca treatment, had minimal significant interaction with the generation of vitamin C or generally known as ascorbic acid (AA) which was found in the highest concentration in the UMKL-1 line. As for roselle variety, UMKL-1 scientifically showed greater performance with Proca application on calyx postharvest quality attributes as compared to UKMR-2. As for the 2nd experiment, study was focused on Proca different frequency (0 = without spray, Once = 45 DAT, Twice = 45 and

52 DAT and Thrice = 45, 52 and 59 DAT) on selected variety of roselle (UMKL-1), sprayed with selected Proca concentration (100 mg L-1) from 1st study. Then, the PRD irrigation technique begins on the roselle plant after 30 DAT to compare full irrigation and PRD irrigation. The color attributes (L*, C*, h° value), physical (firmness, fresh weight of leaves, stem, total yield) and biochemical content [total anthocyanin content, ascorbic acid (AA) content, titratable acidity (TA), soluble solid concentrations (SSC)] were determined. The results demonstrated that 100 mg L-1 of Proca treatment accelerated AA and anthocyanin content. While calyx sprayed at 1x and 2x frequency increased anthocyanin content, Proca sprayed at 3x frequency increased the amounts of AA without affecting other postharvest quality parameters. These findings suggest that the application of the Proca and PRD technique presented in this study should be adopted for commercial production in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PENGGUNAAN PROHEXADIONE-KALSIUM DAN TEKNIK PENGAIRAN KEPADA PERTUMBUHAN, PERUBAHAN FISIOLOGI DAN KUALITI PASCATUAI ROSELLE (*Hibiscus sabdariffa* L.)

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Hibiscus sabdariffa (roselle) tergolong dalam keluarga Malvaceae dan biasanya digunakan untuk membuat minuman kesihatan dan ubat. Kelopak roselle tinggi ciri fungsinya seperti vitamin C, anthocyanin, dan sebatian fenolik yang lain. Bahan-bahan ini bermanfaat untuk kesihatan kerana ia merupakan sumber antioksidan yang kuat dan juga pewarna makanan semula jadi. Walau bagaimanapun, kepekatan vitamin C dan pigmen antosianin di H. sabdariffa sangat dipengaruhi oleh faktor seperti spesies, teknik agronomi, dan keadaan persekitaran. Lebih-lebih lagi, perubahan keadaan suhu semasa tuai dan pasca tuai menyebabkan kehilangan vitamin C dan anthocyanin yang lebih besar, yang akhirnya menghasilkan warna dan kualiti kelopak yang rendah. Selain itu, penggunaan prohexadione-calcium (Proca) dan pengeringan rootzone separa (PRD) dilaporkan dapat mempengaruhi dan mengatur kandungan vitamin C dan pigmen antosianin. Oleh itu, kajian dilakukan untuk mengetahui pengaruh aplikasi Proca dan PRD terhadap pertumbuhan, perubahan fisiologi dan sifat kualiti pasca tuai roselle. Dalam eksperimen pertama, dua jenis roselle yang berbeza (UMKL-1 dan UKMR-2) ditanam di rumah hijau dan disembur dengan empat kepekatan Proca yang berbeza (0, 100, 200 dan 300 mg L-1) pada 45 hari selepas pemindahan (DAT). Kelopak roselle dituai pada 75 DAT dan dianalisis. Ketinggian tanaman roselle menunjukkan penurunan pertumbuhan dengan semua aplikasi Proca dengan 300 mg L-1 memberikan penurunan tunas tertinggi di antara kepekatan yang lain. Sementara itu, sifat kualiti pasca tuai kelopak menunjukkan bahawa aplikasi Proca meningkatkan nilai pH, nilai C * dan kandungan antosianin. Walau bagaimanapun, rawatan Proca hampir tidak mempengaruhi pengeluaran vitamin C, juga dikenal sebagai asid askorbik (AA), yang dikenal pasti pada kepekatan tertinggi di varieti UMKL-1. Bagi varieti roselle, UMKL-1 menunjukkan prestasi yang lebih tinggi secara saintifik dengan

aplikasi Proca pada sifat kualiti kelopak pasca tuai dibandingkan dengan UKMR-2. Bagi eksperimen ke-2, kajian difokuskan pada frekuensi berbeza Proca (0 = tanpa semburan, 1 × = 45 DAT, 2 × = 45 dan 52 DAT dan 3 × = 45, 52 dan 59 DAT) pada pelbagai jenis roselle (UMKL -1), disembur dengan kepekatan Proca terpilih (100 mg L-1) dari kajian pertama. Kemudian, teknik pengairan PRD bermula pada tanaman roselle selepas 30 DAT untuk membandingkan pengairan penuh dan pengairan PRD. Sifat warna (L *, C *, nilai h °), fizikal (ketegasan, berat daun segar, batang, jumlah hasil) dan kandungan biokimia [kandungan antosianin total (TAC), kandungan asid askorbik (AA), keasidan yang dapat dititratkan (TA), kepekatan pepejal larut (SSC)] ditentukan. Hasil kajian menunjukkan bahawa 100 mg L-1 rawatan Proca mempercepat kandungan AA dan antosianin. Walaupun calyx disembur pada frekuensi 1x dan 2x meningkatkan kandungan anthocyanin, Proca yang disembur pada frekuensi 3x meningkatkan jumlah AA tanpa mempengaruhi parameter kualiti pasca tuai yang lain. Penemuan ini menunjukkan bahawa penerapan teknik Proca dan PRD yang di tunjukkan di dalam kajian ini harus diadaptasikan untuk pengeluaran komersial pada masa akan datang.

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×	Multiply/interaction between
±	Plus-minus
−	Minus
>	Greater than
<	Less than
≥	Greater than or equal
≤	Less than or equal
/	Divide
°	Degree
°C	Degree Celsius
%	Per cent
µg	Microgram
µL	Microlitre
µM	Micromolar
µm	Micrometre
µmol	Micromole
AA	Ascorbic acid
ABA	Abscisic acid
ANOVA	Analysis of variance
cm	Centrimetre
CO_2	Carbon dioxide
DAT	Days after transplant
DPPH	2,2-diphenyl-1-picryl-hydrazyl

GA	Gibberellic acid
Ha	Hectare
HCL	Hydrochloric acid
H_2O	Water
Kg	Kilogram
L	Litre
LDW	Leaf dry weight
LFW	Leaf fresh weight
M	Molar
m	Metre
mg	Milligram
mL	Millilitre
mm	Millimetre
mmol	Millimole
NaCl	Sodium chloride
NaOH	Sodium hydroxide
O_2	Oxygen
PGR	Plant growth regulator
pH	Symbol denoting hydrogen ion in solution
PRD	Partial rootzone drying
Proca	Prohexadione calcium
RDW	Root dry weight
RFW	Root fresh weight
SDW	Stem dry weight

SFW	Stem fresh weight
SSC	Soluble solids concentration
TA	Titratable acid
WP	Water potential

CHAPTER 1

INTRODUCTION

Hibiscus sabdariffa or commonly known as roselle in Malaysia is an annual tropical herbaceous shrub that belongs to the Malvaceae family. The plant was originally from Africa, and it was cultivated since the 20th century in Asia countries such as Malaysia, India, Sri Lanka, and Thailand (Eslaminejad and Zakaria, 2011). Roselle is well-known all across the world due to the market demand. In the English-speaking region, roselle is known as rozelle, sorrel, red sorrel, sour-sour, guinea sorrel, lemon bush, Jelly okra, and Florida cranberry. In Europe, roselle is known as karkade or carcade in the pharmaceutical and food flavoring industries (Mahadevan et al., 2009). In Malaysia, roselle is categorized as one of the industrial crops along with coconut, oil palm, coffee, etc. There were approximately 59.3 ha of roselle planted area in 2016, with 276.4 tonnes of yield. In the same year, Johor had the largest roselle production with 148.1 tonnes per year (Table 1.1).

Table 1.1: Planted area and production of industrial crops by the state in Malaysia (2016).

States	Roselle		
	Planted area (ha)	Harvested area (ha)	Production (Tonnes)
Johor	23.0	23.0	148.1
Kedah	1.7	1.7	6.2
Kelantan	-	-	-
Melaka	-	-	-
Negeri Sembilan	2.0	2.0	19.2
Pahang	3.7	3.7	10.4
Perak	3.3	1.1	9.8
Perlis	-	-	-
Pulau Pinang	8.6	4.6	35.8
Selangor	5.4	5.4	18.4
Terengganu	11.6	11.5	28.4
Peninsular Malaysia	59.3	53.0	276.4
Sabah	-	-	-
Sarawak	-	-	-
WP Labuan	-	-	-
Malaysia	59.3	53.0	276.4

Source: Statistik Tanaman Industri Malaysia (2016).

Roselle is commonly cultivated and harvested for its leaves, seeds, stalks, and calyx. Its calyx can be either freshly consumed or preserved and dried to be processed into other products. Fresh calyx usually is washed and chopped

before been added to fruit salads. The sour taste of the fresh calyx is better suited to making tea, syrup, jams, jellies, and beverages rather than consuming it directly (Eslaminejad and Zakaria, 2011). It also has been used as traditional medicine for a digestive agent, purgative, diuretic, and remedy for diseases such as cancer, obesity, and hypertension (Odigie et al., 2003; Osuntogun and Aboaba, 2004). In Sudan and Nigeria, the calyx is boiled with sugar to produce a drink called “Karkade” or “Zoborodo”, while Egyptians used it to make “cacody” and fermented drinks (Da-Costa-Rocha, 2014).

Purbowati et al. (2019) indicated that the roselle calyx is in high demand because of its high vitamin C and anthocyanin content. Because of its high nutritional value, particularly antioxidant properties, this brilliant red-colored crop is widely used in foods, beverages, and remedies. The Malaysian Agricultural Research and Development Institute (MARDI) has conducted numerous studies on roselle calyxes, which are a promising high-quality source of antioxidants and vitamin C (Ibrahim and Mazuki, 2013). According to Musa et al. (2006), the vitamin C content of roselle was 2.5, 3, and 9 times higher than that of blackcurrant, grapes, citrus, and grapes. Vitamin C is essential for both human and plant cellular metabolism. Vitamin C aids human physiological processes such as iron absorption, immunological activation, and collagen production, according to Paciolla et al. (2019). Vitamin C acts as a reducing agent, eliminating free radicals and damaging oxygen-derived species in the human biological system. According to a study by Devaki and Raveendran (2017), adequate vitamin C intake in a balanced diet could treat diseases like cancer and the common cold by acting as an antioxidant. Vitamin C is also abundant in plants, where it plays a variety of roles in physiological processes, including photosynthesis, vegetative development, and stress response.

Meanwhile, various pigments, including delphinidin-sambubioside, cyaniding-3-sambubioside, cyaniding-3-glucoside, and delphinidin-3-glucoside, contributed to the high pigment anthocyanin concentration (Juliani et al., 2009). All of these pigments contribute to the calyx's deep red color. As a result, many industries, such as the food and pharmaceutical industries, benefit from higher commercial value. Synthetic food colorants are being phased out of the food industry in favor of natural ones, which are healthier and more appealing to consumers. However, in Malaysia, synthetic food colorants are more widely available as there is no alternative substitute available to consumers in the market (Moshfeghi et al., 2013). Usage of roselle as a substitute for the source of natural food colorant has obtained attention lately due to its intense red color and high amount of anthocyanin. Thus, it can be fully utilized as a source of red dye in making food products.

According to Wong et al. (2002) anthocyanins of roselle are sensitive, unstable, and easily hydrolyzed. Aishah et al. (2013) added that anthocyanin pigment in the plant is influenced by several factors such as species, light intensity, fertilizer application, irrigation, and application of plant growth regulators. Discoloration of red calyx also can occur due to the crop load. Roselle plant possessed bushy characteristics resulted in a low amount of light penetration reached the calyx.

This resulted in less anthocyanin was synthesized in calyx during planting. Aside from that, changes in temperature conditions during harvest and postharvest handling of roselle also can cause anthocyanin to easily degraded and lost its color and quality. It is important to obtain good coloration and a good amount of anthocyanin during planting to harvest the high quality of calyx.

Application of plant growth regulator is one of the agricultural practices that were commonly used for ages in crop production. Plant growth regulators (PGRs) are organic compounds that promote, inhibit, or otherwise modify physiological processes in plants. There are five well-known basic phytohormones, namely auxins, gibberellins, cytokinin, abscisic acid, and ethylene. Oligosaccharins, brassinosteroids, jasmonates, salicylates, and polyamines are other identified compounds that can also regulate various changes in plant growth and development. The list is likely to extend, with newer PGRs will be discovered. PGRs not only used to regulate the growth of plants, but it is also widely used as a manipulator for fruit development and storage quality. As for anthocyanin biosynthesis, it was reported that the application of exogenous plant growth regulators can be used as a tool to modify the expression of the genes involved in the anthocyanin biosynthesis, also increase production and accumulation of anthocyanins in fruits (He et al., 2010).

Prohexadione-calcium (Proca) is a plant growth regulator commonly used in agricultural and horticultural practices. Its roles as growth retardants were widely used to inhibit plant growth in crop production. There are numerous reports also studied on Proca's role as a growth inhibitor. Lately, its role as a tool to regulate color development and anthocyanin pigment and antioxidant was proved to be succeeded. It was reported that the 'fuji' apple red color was improved with the application of Proca (Mata et al., 2006a). A study conducted by Wan Zaliha (2009) also found a similar result which showed that the red color of the "Cripps Pink' apple was improved with the application of Proca. Since this plant bio-regulator is still new in Malaysia, there are still few studies conducted focusing on the efficiency of Proca on Malaysian crops. Thus, the efficacy of Proca on Malaysian crops mainly focused on vitamin C content and anthocyanin regulation of roselle warrants to be investigated.

Different concentrations of Proca application influence the physiological changes and postharvest attributes of roselle calyces, such as firmness, soluble solids concentration (SSC), titratable acidity (TA), and nutritional value. Amarante et al. (2020) studied that the application of Proca during post-bloom and preharvest affects the occurrence of calcium-related physiological disorders and decay in the 'Braeburn' apple trees. The study found that both modes of Proca treatment reduced the gene expression responsible for transporting calcium (Ca) into vacuoles, increased total water-soluble Ca, and reduced electrolyte leakage in the fruit at harvest. The effect of various concentrations and frequencies of Proca treatment on the antioxidant content of roselles, such as vitamin C and anthocyanin, was highlighted in this study. A study conducted by Wan Zaliha et al. (2016) showed that the exogenous spray of ProCa (100 and 150 mg L^{-1}) successfully reduced shoot growth, improved anthocyanin accumulation, and

fruit color of roselle cultivated on BRIS soil. Conversely, the Proca spray has no significant impact on other quality parameters such as fresh weight and the number of roselle fruits. However, the chromaticity value a*, chroma, and lower lightness on the fruit surface were higher in the Proca-sprayed roselles compared to the control.

Water plays an important role to obtain maximum and good quality crop yield. Application of water deficit technique was reported can influence the vitamin C and anthocyanin biosynthesis in plants due to the ABA production. The levels of ABA in plants usually increase with growth restriction caused by water stress. The ABA produced will result in the growth of the plant stunted. The stunted plant will help to improve the light penetration to the fruits for anthocyanin biosynthesis. Partial root-zone drying (PRD) is one of the well-known water deficit techniques where half part of plant roots is irrigated and switched alternately. This technique is used as a water-saving strategy and at the same time, it can maintain yield production. There are few studies conducted investigating the effect of PRD on the regulation of anthocyanin of plants. It is believed this technique also can influence the anthocyanin formation in fruits as ABA produced can improve light penetration to the fruits, thus it can enhance the color property and increase anthocyanin content without affecting other postharvest quality attributes. Thus, the PRD technique as a tool to obtain a good yield of anthocyanin content of roselle is crucial to be studied.

PRD implies the development of abiotic stress to the plant, which alters the physiology of the plant. According to Ghafari et al. (2020), PRD treatments on apple trees resulted in increased expression of abiotic stress-responsive transcription factors (TFs). As a result, crop yields were maintained while water was used efficiently. The use of PRD techniques at room temperature does not decrease the fruit quality of apple trees after harvest or after storage, according to Zegbe and Serna-Pérez (2011). However, after 3 years of evaluation, there was no significant change in fruit quality at harvest in terms of mean fruit weight, flesh hardness, and SSC concentration in PRD-treated apple trees compared to control irrigation. In general, the PRD technique must be studied as a tool for obtaining excellent postharvest qualities of roselle calyx and a high yield of beneficial antioxidants like vitamin C and anthocyanin content. Therefore, the objectives of this study are to investigate how Proca treatments affect roselle vegetative growth, physiological changes, and postharvest quality under different irrigation methods (control and PRD) and Proca application (concentration and frequencies).

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