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Growth and Malondialdehyde Content of Salt-tolerant Grafted Rockmelon as Affected by Salinity Sources

Muhamad Hassan, Muhamad Hafiz[1*], Nor Danial, Nor Dalila[2], Ghazali, Noor Faimah[1] & Hamid, Muhammad Hanam[1]

[1]Agronomy and Production System Department, Horticulture Research Centre, Malaysian Agricultural Research and Development Institute, Sintok, 06050, Kedah, MALAYSIA

[2]Pest and Disease Management Department, Horticulture Research Centre, Malaysian Agricultural Research and Development Institute, Sintok, 06050, Kedah, MALAYSIA

*Corresponding author: harfieyz@mardi.gov.my

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Abstract: Supplementation of additional salt is one of the feasible approaches to increase fruit quality in rockmelon. However, continuous supply through fertigation could lead to salinity development and deleteriously affects rockmelon growth. In order to mitigate the salinity stress incidence at vegetative stage, the use of salt-tolerant grafted rockmelon enable to utilize the beneficial impact of salinity sources for growth optimization. Thus, an experiment was conducted to evaluate the growth performance and malondialdehyde (MDA) content of salt-tolerant grafted rockmelon under varying salinity sources; and further to select the most suitable salinity sources that can be used at vegetative stage. All grafted plants were arranged in Randomized Complete Block Design (RCBD) with 4 replications. Grafted rockmelon was then subjected to four types of salinity sources; basic nutrient solution (BNS) (EC=2.5 dS m-1) as control, NaCl (50 mM)+BNS (EC=7.1 dS m-1), KNO_3+BNS (EC=8.4 dS m-1), and high strength nutrient solution (NS) (EC=7.1 dS m-1) for 28 days. Data on the plant growth and samples for MDA content in leaves were collected at 30 days after transplanting (DAT). Salinity induced using KNO_3+BNS sustained the growth variables (stem diameter and total leaf area) and MDAcontent. Application of NaCl+BNS reduce significantly stem diameter accompanied with the highest MDA concentration in the leaves as compared to BNS. Salinity induced by KNO_3+BNS showed comparable results with BNS in overall growth measurements and MDA concentrations. In conclusion, incorporation of KNO_3+BNS is the most suitable salinity source to be used to sustain growth in salt-tolerant grafted rockmelon at vegetative stage.

Keywords: Grafted rockmelon, Salinity sources, potassium nitrate (KNO_3), NaCl, High strength nutrient solution

1. Introduction

In Malaysia, rockmelon is commercially grown to fulfill the demand for local and export markets in the form of fresh fruit and processed products. It is a significant fruit crop of cucumis with a sweet, savory taste, pleasant flavour and contains high nutritional value (Norrizah et al., 2012). Generally, rockmelon production has been cultivated through a fertigation system by application of nutrient solution (Ab Rauf & Shahruddin, 2022). Supplementation of nutrient solution through fertigation will further enhance the growth, yield and fruit quality of rockmelon (Monteiro et al., 2014). According to APCO Worldwide (2017), Malaysia is one of the top exporters, with output of high fruit quality rockmelon growing by 12% in the five years leading up to 2014. One of the feasible approaches to increase fruit quality in rockmelon is through the addition of soluble salts.

The addition of soluble salt as Na^+, K^+, Mg^{+2}, Ca^{+2}, Cl^-, SO_4^{-2}, HCO_3^-, CO_3^{-2} and NO_3^- into a nutrient solution could contribute to the high electrical conductivity or salinity levels (Hasegawa et al., 2000). This has been considered as a simple procedure in many plants to improve fruit quality and was demonstrated in several horticultural crops, such as tomatoes (Azarmi et al., 2010) and watermelon (Costa et al., 2013).

**Corresponding author:* harfieyz@mardi.gov.my

Sodium chloride (NaCl), usually known as salt, is one of the most plentiful minerals on earth, which exists abundantly in the environment. It is the most common salt accumulated in salt-related soils (Padder et al., 2012) and contributes to most of the soluble salts in saline soil (Chinnusamy et al., 2006). Based on previous literature, increasing NaCl salinity on the nutrient solution from 1.25 to 4.86 dS m^{-1} increases total soluble solid (TSS) and total titratable acidity (TTA) of the fruits resulted in 63% and 78% respectively (Dias et al., 2018). The chemical compound of potassium nitrate (KNO_3) is an ionized salt comprising of potassium (K^+) and nitrate (NO_3^-) ions. Usherwood (1985) referred KNO_3 as a quality factor in plant production that had a beneficial impact on the quality criteria in fruits such as size, appearance, colour, soluble solids, acidity, vitamin content, flavour and shelf life. Previous findings in plum observed that, foliar application of KNO_3 had increased TSS content and was found the most effective to improve the yield and fruit quality (Jawandha et al., 2017). However, increasing salinity by salt addition may reduce plant growth and fruit yield due to salt-stress impairment (Zhang et al., 2016). While, members of the Cucurbitaceae including rockmelons has been reported as moderately sensitive to salt stress as the salinity threshold of 1.0 dS m^{-1} would contribute for 8.4% yield losses (Pessarakli et al., 2016). According to Rouphael et al. (2012), melon exposed to high salt concentrations in the nutrient solution causes the reduction in vegetative growth, fruit size and yield. Therefore, the addition of salinity sources under saline condition negatively impacts rockmelon performance.

In order to overcome salinity problems, application of grafting in rockmelon with salt-tolerant cucurbit rootstock like bottle gourd can potentially increase the salt-tolerant level. This approach could be the feasible approach to alleviate salt stress incidence in plants (Colla et al., 2010). Recent research stated that, bottle gourd has been classified as most salt-tolerant plant among *Cucurbitaceae* species. It has previously been demonstrated to be a potential salt-tolerant rootstock for cucumber (Huang et al., 2009) and watermelon (Yetisir & Uygur, 2010). Considering the ability of rockmelon/bottle gourd graft combination to mitigate salinity stress, the beneficial effects of salinity sources towards fruit quality is hypothesized to be utilized for sustaining the growth under saline condition. Thus, this research was conducted to evaluate the growth performances and MDA content of salt-tolerant grafted rockmelon under varying salinity sources; and further to select the most suitable salinity sources that can be used to sustain the growth at vegetative stage.

2. Materials and Methods

2.1 Experimental Site and Preparation of Planting Materials

This experiment was conducted in a rainshelter structure at MARDI Sintok, Kedah from May till July 2022 (Figure 1b). The planting materials used in this study were rockmelon cv. Glamour as scion and bottle gourd cv. Mutiara. Both cucurbits were germinated using 100% peatmoss in the germination tray and placed under 50% shading in rainshelter structure. At 10 days after germination process, the germinated plants were selected and transplanted into 400 ml pots filled with 100% cocopeat for grafting purpose. The 14-days old rockmelon and bottle gourd were preceded for grafting using Tongue Approach (TAG) technique as previously describe by Lee et al. (2010). Curing and hardening were followed as procedure describe by Lee and Oda, (2003). Bottle gourd as rootstock were transversely cut at 30-40° downward, while rockmelon scions were transversely cut at 30-40° upward to be grafted and cluttered together using a grafting clip. Then, grafted plants were closed in individual transparent cup and put under shelter with 100% shading as day/night temperatures of 27 (±5) °C, and RH 65 (±10) % under natural photoperiod conditions (12 hours light / 12 hours dark). At 4-5 DAG, all the transparent cups were removed for 12 hours from 12.00 pm until 12.00 am, and the plants were mist-sprayed with water once a day. At 6 days after grafting (DAG), transparent cups were removed for 6 hours from 12.00 pm until 6.00 pm. Transparent cups were fully removed at 7 DAG and all the plants were hardened under shelter with 25% shading, while mist-sprayed with water twice a day until 10 DAG (Figure 1a). At 14 DAG, uniform sizes of grafted plants were transplanted into the 12 litres white polyethylene bags filled with 100% cocopeat.

2.2 Treatments and Experimental Design

This experiment consisted of four treatments of salinity sources which were arranged in RCBD with four replications; ten plants per replication totalling to 160 grafted plants. Based on the previous literature, salinity level at 70 mM was negatively affected rockmelon growth and yield production (Colla et al., 2010). Therefore, salinity level at 50 mM was selected to be used in this experiment. The plants were treated with four salinity sources treatments with their respective concentrations as shown in Table 1.0.

Table 1.0: The salinity sources treatments with respective concentrations used in this experiment.

Salinity sources
Basic nutrient solution (BNS) = 2.50 dS m^{-1}
NaCl (50 mM)+BNS (2.50 dS m^{-1}) = 7.13 dS m^{-1}
KNO_3 (50 mM)+BNS (2.50 dS m^{-1}) = 8.55 dS m^{-1}
High strength nutrient solution (NS) = 7.13 dS m^{-1}

The concentration of the BNS used in this study is in accordance to MARDI's formulation that is specifically recommended for rockmelon (Shahid et al., 2009). While, the commercial NaCl salt from groceries and KNO_3 as commercial soluble fertilizer grade (13-0-46) for fruit crops were used in this study. The solution of the treatments was manually drenched every day for 30 days in a sufficient volume with drainage. The frequency of the nutrient solution given was increased gradually according with the growing stages as employed in commercial rockmelon cultivation. The EC of the growing media was determined using pour-through method (Cavins et al., 2000) at 15 and 30 DAT from 1.00 to 2.00 pm. The EC of four treatments solutions were 2.73, 8.62, 9.25 and 9.05 for BNS, NaCl+BNS, KNO_3+BNS and high strength NS respectively.

a

b

Fig. 1: (a) Successful grafted rockmelon at 10 DAG (b) Grafted rockmelon planted in the polybags under rainshelter structure.

2.3 Plant Maintenance

The maintenance of grafted rockmelon follows the same standard operating procedure recommended by MARDI (Shahid et al., 2009). This was done for vegetative stage for 30 days started with transplanting date until early flowering time. This comprises of proper agronomic planning including pest and disease management. As the plant grew, excess water shoots were removed to increase the growth of the main shoot. The growing shoots were attached to a rope to support the structure of the plant as well as to facilitate the maintenance procedure.

2.4 Data Collection

2.4.1 Growth measurements

The growth parameters were taken at 30 days after transplanting (DAT). Plants were sampled at random from each treatment plot for determination of plant height, stem diameter, leaf number, total leaf area and dry weight matter such as leaf, stem and root. Measurement of scion height was taken from the marked level at 0.2 cm on top of the graft union to the highest shoot tip using a ruler. Scion diameter was measured at the same marked level of scion height using electronic digital solar caliper (Model Mitutoyo Series No. 500, Japan). Leaf number was manually counted and recorded based on fully expanded leaves. The whole plants were then harvested and separated into leaves, stems and roots for total leaf area measurement and dry weight determination. Total leaf area was measured using automatic leaf area meter (LI-3100C, LI-COR, Lincoln, Nebraska, USA). While dry weight of each plant parts was determined using digital analytical balance (Mettler Toledo EL 204, Switzerland) after drying in an oven at 70 °C for 72 hours.

2.4.2 Malondialdehyde content (MDA) determination

To estimate lipid peroxidation, the concentration of MDA was assessed by the thiobarbituric acid (TBA) test according to the procedure of Wang et al. (2009). One gram of fresh leaf samples was homogenized in 5 mL 0.6% TBA in 10% trichloroacetic acid (TCA). The mixture was heated at 100 °C for 15 minutes in a water bath. After cooling in ice, the mixtures were centrifuged at 5000 rpm for 10 minutes. The absorbance of supernatants were read at 450, 532 and 600 nm and MDA content was calculated on a fresh weight basis using the following formula:

$$\text{MDA (nmol g/FW)} = 6.45\,(\text{OD532}-\text{OD600}) - 0.56\,(\text{OD450}) \times 1000.$$

2.5 Data Analysis

All the data taken was computed using statistical analysis software (SAS) version 9.4 (SAS Institute Inc., Cary, NC). GLM procedure was used to do analysis of variance and mean comparisons were calculated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$. Relationships among the variables for all salinity sources treatments were pooled and determined using Pearson correlation coefficients (r) at $P \leq 0.05$ by CORR procedure.

3. Results and Discussions

Table 1 showed the effect of salinity sources on plant height and stem diameter of grafted rockmelon at 30 DAT. Stem diameter was significantly affected ($P \leq 0.01$) by salinity sources at 30 DAT whereas no significant effect ($P \leq 0.05$) was observed in plant height and leaf number of grafted rockmelon. Salinity induced by NaCl+BNS and high strength NS significantly reduced stem diameter as compared to control resulted in 6.50% and 7.97%.

Table 1: Effect of salinity sources on plant height, stem diameter and leaf number of grafted rockmelon at 30 DAT (Mean ± S.D; n=4)

Factor	Treatments	Plant height (cm)	Stem diameter (mm)	Leaf number
Salinity sources	BNS	$206.1^{a}\pm22.24$	$9.54^{a}\pm0.14$	$35.7^{a}\pm1.0$
	NaCl+BNS	$176.4^{a}\pm18.25$	$8.92^{b}\pm0.34$	$33.2^{a}\pm1.71$
	KNO_3+BNS	$190.6^{a}\pm10.33$	$9.73^{a}\pm0.21$	$37.02^{a}\pm2.16$
	High strength NS	$196.7^{a}\pm12.46$	$8.78^{b}\pm0.38$	$35.9^{a}\pm0.96$
		F-test (Significant level)		
Salinity sources		ns	**	ns

Means in each column with different letters indicate significant differences at P<0.05 according to DMRT

Table 2 showed the effect of salinity sources on plant height and stem diameter of grafted rockmelon at 30 DAT. Total leaf area was significantly affected ($P \leq 0.01$) by salinity sources whereas no significant effect ($P \leq 0.05$) was observed for the rest of the growth parameters measurement. No significant differences were found between BNS and the rest of the salinity sources tested. Meanwhile, salinity induced by KNO_3+BNS and high strength NS significantly increased stem diameter as compared to NaCl+BNS resulted in 45.65% and 49.84%.

Increasing of the growth measurements as affected by KNO_3+BNS application might be ascribed by the increased cell division and cell elongation, which are correlated with plant mineral ion compositions. In general, the nitrate and potassium found in KNO_3 are essential for plant growth and development. Potassium (K) is the most prevalent inorganic cation and is essential for proper plant development (White & Karley, 2010). K is also highly necessary for cell proliferation, which is a critical mechanism for plant function and development (Hepler et al., 2001). In terms of potassium's growth-promoting mechanism, it stimulates and regulates ATPase in the plasma membrane to provide acid stimulation, which then causes cell wall loosening and hydrolase activation, resulting in cell expansion (Oosterhuis et al., 2014). Furthermore, nitrate is a key component of KNO_3 that promotes plant growth by producing amino acids and proteins (Liu et al., 2014). It is absorbed by the roots, translocated to the shoot, stored in the vacuole, and assimilates into decreased N products (Sivasankar & Oaks, 1996). It is also reported that, nitrate absorption and transport appear to be salt sensitive, and changes in nutrient supply can mitigate the deleterious effects of salinity (Jabeen & Ahmad, 2011). Consequently, both nitrate and potassium ions in KNO_3 salts aided the grafted rockmelon in becoming bigger and heavier. Similar with finding by Kaya et al. (2007), the application of KNO_3 helped the melon plants to improve cell membrane stability with nitrogen uptake under salinity condition.

Table 2: Effect of salinity sources on total leaf area, leaf, stem and root dry weight of grafted rockmelon at 30 DAT (Mean ± S.D; n=4)

Factor	Treatments	Total leaf area (cm^2)	Leaf dry wight (g)	Stem dry weight (g)	Root dry weight (g)
Salinity sources	BNS	3600.1^{ab}±1136.5	15.218^{a}±5.78	10.240^{a}±3.93	1.788^{a}±0.30
	NaCl+BNS	2506.3^{b}±970.7	11.443^{a}±4.9	5.695^{a}±2.69	0.708^{a}±0.43
	KNO_3+BNS	4611.2^{a}±1256.7	16.530^{a}±4.96	11.165^{a}±3.34	1.585^{a}±0.99
	High strength NS	4997.1^{a}±1260.8	12.925^{a}±3.72	8.110^{a}±2.38	1.058^{a}±0.39
		F-test (Significant level)			
Salinity sources		*	ns	ns	ns

Means in each column with different letters indicate significant differences at P<0.05 according to DMRT

At vegetative stage (30 DAT), comparable growth measurement of stem diameter between plants treated under KNO_3+BNS and control is attributed by the increase of cell division and cell elongation which is related to mineral ion compositions of the plants. Generally, nitrogen and potassium existed in KNO_3 play an important role in plant growth and development. On the other hand, salinity induced by NaCl+BNS ultimately reduced the stem diameter and total leaf area of grafted rockmelon. Application of NaCl in 30 days progressively increase the salinity levels in the growing medium. This condition excessively depicted to the response of plant growth to salt stress. According to Munns et al. (1995), growth reduction is attributed to water deficiency development. This is also related with ion accumulation, particularly Na^+ in the leaf blade which accumulates after it is deposited in the transpiration stream, causing premature ageing and growth reduction in plant parts' (Munns, 2002). Gabrijel et al. (2009) have stated that, salt stress reduces plant biomass such as leaf, stem and root which is accompanied by the increase of Na^+ and Cl^-. Salt stress was observed to severely impact melon growth and yield. Our result was corroborated with findings by Kaya et al. (2007), the dry weight material for shoot, root and entire plant was reduced at 25.24%, 14.21% and 21.5%, respectively in a pot experiment of melon (cv. Tempo) in NaCl salt-treated plants (150 mM) as compared to control. In addition, growth reductions were observed for stem height diameter, total leaf area and leaf number of melon (cv. Citirex) as increased of salt stress at 8 dS m^{-1} (Ulas et al., 2019).

Figure 2 showed the effect of salinity sources on malondialdehyde content in grafted rockmelon. Salinity induced by NaCl+BNS significantly increased the malondialdehyde content as compared to control, KNO_3+BNS and high-strength NS.

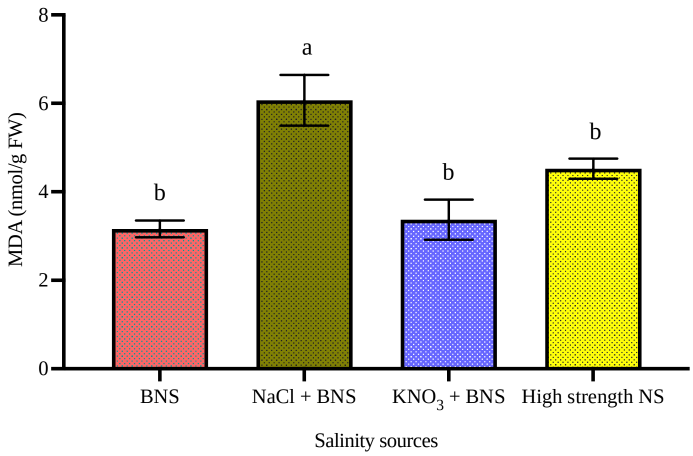

Fig. 2: Effect of salinity sources on malondialdehyde content (MDA) of grafted rockmelon at 30 DAT (Mean ± S.D; n=4)

Result in table 3 showed the relationships among the selected significant parameters including stem diameter, total leaf area and MDA of grafted rockmelon. Stem diameter was positively correlated (r=0.64; P≤0.01) with total leaf area measurement whereas no significant correlation was observed between stem diameter and MDA content in the leaf. On the other hand, total leaf area was negatively correlated (r=-0.65; P≤0.01) with MDA content.

Table 3: Pearson's linear correlation coefficients (r) among stem diameter, total leaf area and MDA of grafted rockmelon at 30 DAT

	Stem diameter	Total leaf area	MDA
Stem diameter	1	0.64**	-0.36ns
Total leaf area		1	-0.65**
MDA			1

**Significant at P≤0.01, *Significant at P≤0.05, ns: not significant

Results obtained from this study showed the application of NaCl+BNS resulted in an enhancement in MDA accumulation in the leaves of grafted rockmelon due to oxidative stress. The outcome of process in lipid peroxidation is the production of MDA and its concentration can be an essential biological parameter of oxidative stress (Yang et al., 2010). MDA has been utilised as an indication of the degradation of polyunsaturated fatty acids in the biomembrane, and tends to accumulate more under salt stress (Zhu et al., 2008). The hostile influence of NaCl stress in lipid peroxidation of MDA was associated with the sensitivity of the species, and was reported among cucurbitaceae family including pumpkin (Sevengor et al., 2011) and cucumber (Ahmad et al., 2017). Reduction of biochemical activity as MDA accumulation certainly has reduced the growth of grafted rockmelon at vegetative stages. This has been supported by the correlation analysis as MDA production in the leaves was negatively correlated (r=-0.65; P≤0.01) with total leaf area measurement. Our result was corroborated with previous finding in grafted citrus, an increment of MDA content in the leaves had reduced the scion length and total leaf area by NaCl application at 60 mM (Shafieizargar et al., 2015).

Nevertheless, comparable results between control and both treatments as KNO_3+BNS and high strength NS indicated the selective response of grafted rockmelon towards salinity sources. Under saline environment, both treatments implied a better protection from oxidative damage. Both applications had prevented the accumulation of MDA in plants, reflecting their influential role in controlling salinity stress. The chemical compound of potassium nitrate (KNO_3) is an ionized salt comprising of potassium (K^+) and nitrate (NO_3^-) ions. In plant growth and development, those elements play a crucial part in balanced water transmission and also for turgor and osmotic regulation in plants (Chen et al., 2014). Umar (2006) has also reported that, the application of KNO_3 could alleviate the severe effects of several number in biotic and abiotic stresses. Similar finding was found in *Aloe vera* L., the foliar application of KNO_3 had mitigated the damaging effect of salinity by the lowest MDA accumulation as compared to control (Ebrahimzadeh et al., 2022).

4. Conclusion

To conclude, application of KNO_3+BNS was observed as the most suitable salinity sources to be applied in salt-tolerant grafted rockmelon at vegetative stage. This is based on the comparable stem diameter, total leaf area and MDA concentration with BNS application. This practice can be adopted in salt-tolerant grafted rockmelon cultivation to enhance the early growth performances under saline environment.

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