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Charaterization of salinity tolerance level in grafted rockmelon as affected by cucurbit rootstocks and NaCl levels

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Abstract: Supplementation of salt in normal fertigation system is a cheap and easy method for fruit quality improvement but it is not yet proven in rockmelon. However, supply of salt may detrimentally affect rockmelon growth and yield since it is classified as sensitive to salt stress. Grafting using salt-tolerant cucurbit rootstocks is a cheap and easy method to increase the salt tolerance level thus producing salt-tolerant rockmelon planting materials suited for salt supplementation. Rockmelon (*Cucumis melo* L.) var. Glamour scion was grafted onto three cucurbit rootstocks; rockmelon as control, bottle gourd (*Lagenaria siceraria*) var Mutiara and bitter gourd (*Momordica charantia*) var Wira. Grafted rockmelon seedlings were planted into polyethylene bags and subjected to four levels of NaCl at 0, 25, 50 and 75 mM. Two factorial experiment was done and arranged in Randomized Complete Block Design (RCBD) with three replications. Data on growth, sodium and chloride ion concentrations in leaf and root were collected at 18 days after transplanting (DAT). Self-grafted rockmelon showed significantly high performance in most of the growth parameters recorded as compared to others due to high graft compatibility followed by rockmelon/ bottle gourd. However, graft combination of rockmelon/ bottle gourd showed significantly lowest Na and Cl ions accumulation in the leaves as compared to others. This treatment graft combination also indicated highest salinity tolerance level due to the toxic ion exclusion mechanism available in bottle gourd rootstock. In conclusion, rockmelon/ bottle gourd can be classified as the least salt-sensitive among the graft combinations making it is suitable to be cultivated under saline environment for fruit quality enhancement.

Keywords: Salinity, Salt addition, Grafted rockmelon, Salt-tolerant rootstock, Cucurbit rootstock.
Abbreviations: NaCl_Sodium chloride, RCBD_Randomized complete block design, DAT_Days after transplanting, DAG_Days after grafting, DAS_Days after sowing, ANOVA_Analysis of variance, GLM_General linear model, HSD_Tukey's Honest Significant, SAS_Statistical analysis software, RH_Relative humidity, RM/BG_Grafted rockmelon/ bottle gourd, RM/BTG_Rockmelon/ Bitter gourd, S.D_Standard deviation, S.E_Standard error mean.

Introduction

Rockmelon is a high value crop that is widely grown in Malaysia. Nowadays, there is an increase in demand for high fruit quality rockmelon for the local market. This catalyse the increase of rockmelon production in Malaysia up to 45.6% between 2012-2018 (Department of Agriculture, 2018). To increase fruit quality, supplementation of salt into nutrient solution through fertigation is a feasible approach and fairly easy to be adapted. This application has been successfully demonstrated in various horticultural crop such as tomato and watermelon (Machado and Serralheiro, 2017). Salt supplementation can increase the dry matter components which ultimately enhanced the fruit quality attributes including total soluble solid, total titratable acidity and sugar acid ratio (Dias et al., 2018). Continuous supply of nutrient solution through fertigation without adequate leaching can lead to salinity development (Shrivastava and Kumar, 2015). This situation can further worsen with the salt supplementation in rockmelon cultivation.

Rockmelons has been reported as moderately sensitive to salt-stress (Pessarakli, 2016). This species can be deleteriously affected with several forms of salt damages including growth suppression, metabolic inhibition, physiological reduction and yield losses (Ulas et al., 2019). Under saline environment, the high salinity in the root zone will exert osmotic stress to the plants thus reducing the water influx into the roots resulting in plant water deficit (Munns and Tester, 2008). Furthermore, high concentration of certain types of ions under saline environment may induce ionic imbalance resulting in excessive absorption of the ions into the plant's tissues (Ghani et al., 2018). Previous study showed that, salt stress had reduced the shoot growth and negatively affected the physiological process in melon production. These changes are associated with excessive Na^+ and Cl^- ions with K^+ ion reduction (Ulas et al., 2020). Therefore, rockmelon variety that is tolerant to salinity is needed

Figure 1. Effects of NaCl levels on growth of self-grafted (A), rockmelon/ bottle gourd (B) and rockmelon/ bitter gourd (C) at 17 DAT. Increasing NaCl levels from left to right (0, 25, 50, 75 Mm.

to enable the use of salinity sources for growth and yield improvements without interfering the physiological process.

Selection in conventional breeding program is promising to develop salt-tolerant variety but it is time consuming with low commercial success. Instead, the use of salt-tolerant rootstock in grafting technique can be a technically and financially feasible approach to alleviate salt stress in rockmelon. Grafting of salt sensitive scion on salt resistant rootstocks within other species in *Cucurbitaceae* family has been extensively to ameliorate salt induced damages to the shoot (He et al., 2009). The use of resistant genotypes as rootstock is considered a simple but effective method for improving crop tolerance to salt stress (Gong et al., 2014). According to Kusvuran et al. (2021), lower Na^+ and Cl^- accumulations in the shoot of grafted melon compared to ungrafted plants had shown that the application of salt-tolerant rootstock to plays a significant role in mitigating Na toxicity thus increasing the salt tolerance levels.

The extent to which salinity reduces growth varies substantially across species within the same families. Among the cucurbit species, bottle gourd and bitter gourd were known to be a highly potential rootstock for increasing salt-tolerant level in grafted plant of rockmelon. Within the cucurbit family, bottle gourd has been proven to be a promising salt-tolerant rootstock for watermelon (Yetisir and Uygur, 2010) and cucumber (Huang et al., 2009) as higher growth performance was recorded under salt stress compared to the self-grafted plants. Comprehensive screening conducted among four cucurbit species under varying NaCl levels had revealed that bitter gourd is the least salt-sensitive plants based on lower Na^+ and Cl^- accumulation in the leaves with low relative water content reduction (Ghani et al., 2018). In theory, grafting rockmelon with other potential cucurbits species can significantly improve the salt tolerance levels of rockmelon thus enabling quality improvement via salt supplementation. Thus, to verify this hypothesis, this study was conducted to determine the salt tolerance level of grafted rockmelon scion with different cucurbit rootstocks based on the plant growth, physiological responses and Na and Cl ion contents to increasing NaCl levels.

Results

Vegetative growth

Table 1 shows the effect of different cucurbit rootstocks and NaCl levels on growth of grafted rockmelon. Generally, all growth measurements of plant height, leaf number and total leaf area were significantly affected by cucurbit rootstocks at $P \leq 0.01$ while scion diameter at $P \leq 0.05$. Plant height, scion diameter and total leaf area were also significantly affected by NaCl levels at $P \leq 0.01$ while leaf number at $P \leq 0.05$. There were no significant interactions recorded between the main factors for all growth measurement. In terms of cucurbit rootstock effect, self-grafted rockmelon had a significantly higher in plant height and leaf number with 53.23% and 30.95% increments compared to rockmelon/ bitter gourd. Scion diameter

Table 1. Effects of different cucurbit rootstocks and NaCl levels on plant height, stem diameter, leaf number, and total leaf area of grafted rockmelon.

Factors	Levels	Plant height (cm)	Scion diameter (mm)	Leaf number	Total leaf area (cm)
Rootstock	Rockmelon	102.2^{a}±19.58	5.61^{a}±0.29	21.0^{a}±1.58	1608.4^{a}±488.7
	Bottle gourd	82.8^{b}±11.92	5.20^{b}±0.41	18.2^{b}±1.64	1403.6^{a}±386.9
	Bitter gourd	47.8^{c}±12.18	5.30^{ab}±0.69	14.5^{c}±1.38	558.6^{b}±253.6
NaCl (mM)	0	88.6^{a}±26.86	5.80^{a}±0.48	18.6^{a}±3.16	1584.5^{a}±635.86
	25	83.2^{ab}±27.29	5.43^{ab}±0.42	18.7^{a}±2.95	1292.9^{ab}±553.05
	50	71.9^{bc}±27.04	5.28^{b}±0.30	17.3^{ab}±3.0	1021.0^{bc}±496.51
	75	66.6^{c}±25.79	4.98^{b}±0.47	16.8^{b}±3.27	862.3^{c}±505.02
		F-test (Significant level)			
Rootstock		**	*	**	**
NaCl		**	**	*	**
Rootstock X NaCl		ns	ns	ns	ns

**Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means with the different letters in each column within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean ± S.D; n=3).

Table 2. Effects of different cucurbit rootstocks and NaCl levels on leaf, stem, root dry weight and relative chlorophyll content of grafted rockmelon.

Factors	Levels	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)
Rootstock	Rockmelon	6.391^{a}±1.57	4.185^{a}±1.01	1.283^{a}±0.35
	Bottle gourd	3.990^{b}±1.14	2.772^{b}±0.72	0.96^{a}±0.5
	Bitter gourd	2.011^{c}±0.75	1.396^{c}±0.50	0.311^{b}±0.11
NaCl (mM)	0	5.197^{a}±2.48	3.385^{a}±1.53	1.018^{a}±0.51
	25	4.539^{ab}±2.28	3.066^{a}±1.40	0.873^{a}±0.53
	50	3.776^{bc}±1.92	2.562^{ab}±1.31	0.913^{a}±0.69
	75	2.996^{c}±1.55	2.124^{b}±1.11	0.602^{a}±0.37
		F-test (Significant level)		
Rootstock		**	**	**
NaCl		**	**	ns
Rootstock X NaCl		ns	ns	ns

**Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means with the different letters in each column within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean ± S.D; n=3).

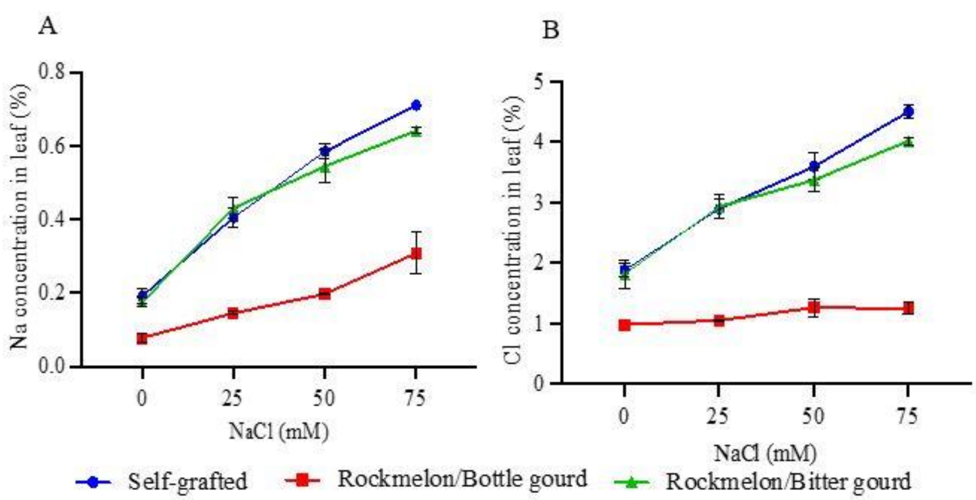

Figure 2. Effect of NaCl levels on Na (A) and Cl (B) concentration in leaf of each graft combinations (Mean ± S.E.; n=3).

of self-grafted rockmelon was significantly highest with 7.31% larger in size compared to rockmelon/ bottle gourd. In addition, self-grafted and rockmelon/ bottle gourd total leaf area was significantly highest with 65.27% and 52.54% higher amount compared to rockmelon/ bitter gourd. Regardless of cucurbit rootstocks, the plant height was significantly reduced at 50 mM salinity and increased in salinity up to 75 mM resulted in 24.83% reduction compared to control. Similar reduction pattern was also observed in stem diameter and total leaf area resulting in 14.14% and 45.58% decrease respectively. For leaf number data, significant reduction was recorded at 75 mM of salinity resulted in 9.68% decrease. Figure 1 showed the effect of NaCl levels on the growth appearance of different graft combinations. The appearances of each graft combination clearly corroborated with the plant height

Table 3. Pearson's linear correlation coefficients (r) between growth parameters and nutrient contents in leaf of self-grafted rockmelon (A), rockmelon/ bottle gourd (B) and rockmelon/ bitter gourd (C).

A	NaCl	HT	LN	SD	TLA	LDW	SDW	Na	Cl
NaCl	1	-0.68*	-0.76**	-0.67*	-0.86**	-0.88**	-0.76**	0.98**	0.96**
HT		1	0.93**	0.68*	0.92**	0.84**	0.93**	-0.70*	-0.69*
LN			1	0.65*	0.89**	0.90**	0.95**	-0.77**	-0.73**
SD				1	0.80**	0.78**	0.74**	-0.66*	-0.70*
TLA					1	0.95**	0.97**	-0.85**	-0.83**
LDW						1	0.95**	-0.88**	-0.81**
SDW							1	-0.77**	-0.72**
NA								1	0.97**
Cl									1

NaCl: NaCl salinity, HT: Plant Height, LN: Leaf Number, SD: Stem diameter, TLA: Total leaf area, LDW: Leaf dry weight, SDW: Stem dry weight, Na: Leaf sodium, Cl: Leaf chloride, **Significant at P≤0.01, *Significant at P≤0.05, ns: not significant.

B	NaCl	HT	LN	SD	TLA	LDW	SDW	Na	Cl
NaCl	1	-0.38ns	0.04ns	-0.50ns	-0.62*	-0.51ns	-0.48ns	0.91**	0.66*
HT		1	0.57ns	0.61*	0.81**	0.63*	0.69*	-0.05ns	0.10ns
LN			1	0.41ns	0.25ns	0.21ns	0.35ns	0.20ns	0.15ns
SD				1	0.66*	0.42ns	0.61*	-0.26ns	-0.09ns
TLA					1	0.69*	0.75**	0.32ns	-0.16ns
RWC						0.03ns	-0.14ns	-0.37ns	-0.60*
LDW						1	0.90**	0.45ns	-0.26ns
SDW							1	-0.31ns	-0.04ns
Na								1	0.71**
Cl									1

NaCl: NaCl salinity, HT: Plant Height, LN: Leaf Number, SD: Stem diameter, TLA: Total leaf area, LDW: Leaf dry weight, SDW: Stem dry weight, Na: Leaf sodium, Cl: Leaf chloride **Significant at P≤0.01, *Significant at P≤0.05, ns: not significant.

C	NaCl	HT	LN	SD	TLA	LDW	SDW	NA	Cl
NaCl	1	-0.76**	-0.89**	-0.76**	-0.77**	-0.81**	-0.77**	0.94**	0.91**
HT		1	0.85**	0.89**	0.98**	0.91**	0.94**	-0.71**	-0.66*
LN			1	0.85**	0.87**	0.87**	0.84**	-0.83**	-0.75**
SD				1	0.93**	0.92**	0.85**	-0.76**	-0.73**
TLA					1	0.93**	0.89**	-0.75**	-0.73**
LDW						1	0.92**	-0.82**	-0.73**
SDW							1	-0.75**	-0.60*
NA								1	0.90**
Cl									1

NaCl: NaCl salinity, HT: Plant Height, LN: Leaf Number, SD: Stem diameter, TLA: Total leaf area, LDW: Leaf dry weight, SDW: Stem dry weight, Na: Leaf sodium, Cl: Leaf chloride, **Significant at P≤0.01, *Significant at P≤0.05, ns: not significant.

parameter that was shown in Table 1. The plant height reductions were obviously seen at 50 mM of NaCl level when compared to the control.

Table 2 shows the effects of different cucurbit rootstocks and NaCl levels on leaf, stem and root dry weight of grafted rockmelon. Leaf, stem and root dry weights parameters were significantly affected (P≤0.01) by cucurbit rootstocks while only leaf and stem dry weight were significantly affected (P≤0.01) by NaCl levels. Leaf dry weight was significantly highest in self-grafted rockmelon compared to rockmelon/ bottle gourd and rockmelon/ bitter gourd with the increments of 37.57% and 68.71% respectively. Similar pattern was also observed in stem dry weight as self-grafted rockmelon was significantly higher compared to rockmelon/ bottle gourd and rockmelon/ bitter gourd with the increments of 33.76% and 66.64% respectively. In addition, self-grafted rockmelon and rockmelon/ bottle gourd were significantly higher compared to rockmelon/ bitter gourd in the root dry weight with the increments of 75.76% and 67.6% respectively. Leaf dry weight was significantly reduced at 50 mM NaCl salinity and further increased in salinity to 75 mM resulted in 42.3% reduction compared to control. While, stem dry weight was significantly reduced at 75 mM resulted at 37.25% decrement as compared to control.

Based on correlation analysis in Table 3, growth parameters include plant height, leaf number, stem diameter, total leaf area, leaf and stem dry weight were negatively correlated with NaCl levels in graft combination of self-grafted rockmelon (Table 3A) and rockmelon/ bitter gourd (Table 3C). Among all growth parameters, the strongest correlations observed in self-grafted rockmelon is total leaf area (r=-0.86; P≤0.01) (Table 3A), whereas the strongest correlation in rockmelon/ bitter gourd is leaf number (r=-0.89; P≤0.01) (Table 3C). Among all growth parameters, only total leaf area was negatively correlated (r=-0.62; P≤0.05) with NaCl levels in rockmelon/ bottle gourd (Table 3B). This relationship also shown the lowest correlation compared to other graft combinations. It primarily showed that, total leaf area measurements are the most significant variables that negatively impacted by the increments of NaCl concentrations in all graft combinations.

Increasing salinity levels regardless of rootstocks used (Table 1) had reduced the scion growth based on the plant height, stem diameter, leaf number, total leaf area, leaf and stem dry weight. Most of the growth parameters showed severe reduction starting at 50 mM NaCl salinity. These results were supported by the correlation analysis, where total leaf area of all graft combinations of self-grafted rockmelon (r=-0.86; P≤0.01) (Table 3A), rockmelon/ bottle gourd (r=-0.62; P≤0.05) (Table 3B) and rockmelon/ bitter gourd (r=-0.77; P≤0.01) (Table 3C) were significantly negatively correlated with NaCl levels.

Na and Cl ion concentrations

Table 4 shows the effects of different cucurbit rootstocks and NaCl levels on Na and Cl concentrations in leaf and root of grafted rockmelon. Significant interaction was detected between the two factors in term of the Na and Cl concentrations in leaf (P≤0.01). In addition, the Cl concentration in root was also significantly affected by rootstocks (P≤0.01). Cl concentration in root of rockmelon/ bottle gourd was significantly highest with 33.38% and 41.15% greater concentrations than self-grafted and rockmelon/ bitter gourd respectively.

Figure 2A showed the effect of NaCl levels on Na concentration in leaf of each graft combinations. Generally, with the increase of NaCl level from 0 to 75 mM resulted in significant increase of Na concentration in leaf for all graft combinations with the increment was more pronounced in self-grafted plant at 73.0%, followed by rockmelon/ bitter gourd and lastly rockmelon/ bottle gourd resulted in 72.59% and 66.67% respectively. At highest NaCl level (75 mM), Na concentrations in leaf of self-grafted and rockmelon/ bitter gourd was significantly higher compared to rockmelon/ bottle gourd resulted in 56.52% and 51.84% increase respectively.

Figure 2B shows the effect of NaCl levels on Cl concentration in the leaves of each graft combinations. In general, increased of NaCl levels from 0 to 75 mM was seen to significantly increase Cl concentration in leaf of self-grafted rockmelon and rockmelon/ bitter gourd resulted in 58.16% and 54.69% increase respectively. In contrast, there was no significantly difference observed in rockmelon/ bottle gourd. At the highest NaCl levels (75 mM), Cl concentrations in self-grafted rockmelon and rockmelon/ bitter gourd were significantly higher compared to rockmelon/ bottle gourd resulted in 72.15% and 10.74% increase respectively.

Discussion

Generally, the lowest plant height, leaf number, total leaf area (Table 1), leaf and stem dry weight (Table 2) recorded in this study was from graft combination of rockmelon/ bitter gourd. The findings reveal that, there are variation in compatibility levels of rockmelon and bitter gourd, as both species are from distinct genera and were classified as intrafamilial graft. As reported by Mudge et al. (2009), intrafamilial grafts are rarely compatible or has incompatibility problems. In this case, water and nutrient uptake could be decreased in rockmelon scion when grafted onto bitter gourd as a result of reduced in scion vigour by insufficient rootstock-scion interactions. This hypothesis was validated by Davis et al. (2008), that stated graft incompatibility can cause undergrowth, which reduces the amount of water and nutrients passing through the graft union and eventually causing the plant to wilt. Additionally, incompatibility between both species was seen after reaching a particular age and development stage. This is in agreement with Aloni et al. (2008) findings where an effective graft in cucurbits at early growth stage were shown to be incompatible after 25 days of grafting. Characterization of incompatibility is difficult since graft combinations may initially combine successfully but later acquire incompatibility symptoms as a result of either failure at the union or the emergence of aberrant growth patterns (Tamilselvi and Pugalendhi, 2017).

Incompatibility found in rockmelon/ bitter gourd was expected related with the low phloem and xylem segmentation between graft union. This is supported by Melnyk (2017), as usual incompatibility had presented the cell division and callus formation, but the attachment strength is low and there may be no phloem and xylem differentiation. Moreover, the size of bitter gourd stem was naturally smaller compared to rockmelon scion, and this causes large differences in sizes between rootstock-scion combinations which presumably impaired the vascular bundle formation at the graft union. It is generally understood that the connection of vascular cambium is required for grafting. Thus, the disconnection between the vascular cambiums is closely associated with localized incompatibility. Localized incompatibility causes disruption of vascular cambium, vascular discontinuity, lower rate of tissue differentiation and lignification which further can causes graft union to rupture (Pina et al., 2017). Our results in Table 1 and 2 are in aggreement with Noor et al. (2019), who demonstrated that the growth parameters such as leaf number and total leaf area of grafted cucumber (cv. Kalaam) with bitter gourd srootstock was significantly lower as compared to bottle gourd rootstock. In general, larger differences in taxonomic proximity between bitter gourd and rockmelon stem size can significantly affect the compatibility level which can reduce the leaf expansion and growth rate. Commonly observed symptoms that are related with scion growth termination due to incompatibility such as shrivelling of leaves and early leaf drop (Rasool et al., 2020) was also observed in this study.

As recorded in Table 1 and 2, growth reduction in high salinity condition is mainly associated with the osmotic stress and ion toxicity that generate a repression in plant growth. Munns and Tester (2008) discovered that, once the salt concentration surrounding the roots rises to a critical level during the first osmotic phase, the rate of shoot growth decreases dramatically. Later, the rate at which developing leaves expand is reduced, new leaves emerge more slowly and lateral buds develop slowly or remain dormant, resulting in fewer lateral shoots development. Furthermore, increases of salt concentrations thus resulting in premature ageing and reduction in nutrients supply to plant parts. This consequently inhibits plant development as a whole (Maksimovic and Ilin, 2012). Growth reduction found in our study is similar with several *Cucurbitaceae* species such as in bottle gourd, squash (Naseer et al., 2022) and watermelon (Yanyan et al., 2018) which were grown under high salinity levels.

When analyzing the distribution of Na concentration in leaf, rockmelon/ bottle gourd had the lowest overall Na concentrations with the NaCl levels increase as compared to other graft combinations (Figure 2A). Our findings had also corroborated this as the lowest significant correlation was observed between NaCl levels and Na concentrations in the leaves of rockmelon/ bottle gourd (r=0.90; P≤0.01) (Table 3B) as compared to others graft combinations. This suggested that, the use of bottle gourd rootstock presumably use its own capability to store relatively toxic

Table 4. Effects of different cucurbit rootstocks and NaCl levels on Na and Cl concentrations in leaf and root of grafted rockmelon.

Factors	Levels	Na (%) Leaf	Na (%) Root	Cl (%) Leaf	Cl (%) Root
Rootstock	Rockmelon	$0.473^{a}\pm0.03$	$0.069^{a}\pm0.015$	$3.218^{a}\pm0.257$	$0.497^{b}\pm0.135$
	Bottle gourd	$0.183^{b}\pm0.042$	$0.078^{a}\pm0.031$	$1.137^{b}\pm0.303$	$0.746^{a}\pm0.105$
	Bitter gourd	$0.448^{a}\pm0.033$	$0.058^{a}\pm0.016$	$3.034^{a}\pm0.125$	$0.439^{b}\pm0.061$
NaCl (mM)	0	$0.149^{d}\pm0.026$	$0.062^{a}\pm0.017$	$1.561^{d}\pm0.215$	$0.51^{a}\pm0.086$
	25	$0.327^{c}\pm0.033$	$0.068^{a}\pm0.017$	$2.293^{c}\pm0.215$	$0.56^{a}\pm0.083$
	50	$0.442^{b}\pm0.04$	$0.067^{a}\pm0.013$	$2.741^{b}\pm0.331$	$0.587^{a}\pm0.151$
	75	$0.553^{a}\pm0.041$	$0.077^{a}\pm0.035$	$3.257^{a}\pm0.151$	$0.586^{a}\pm0.082$
		F-test (Significant level)			
Rootstock		**	ns	**	**
NaCl		**	ns	**	ns
Rootstock X NaCl		**	ns	**	ns

**Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means with the different letters in each column within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean ± S.D; n=3).

ions in its root to prevent excessive transport to shoot (scion). Previous study had demonstrated that, the use of bottle gourd as a rootstock for cucumber, resulted in an alleviation of the deleterious effects of salt by reducing the accumulation of Na concentrations in the leaves (Huang et al., 2009). The highest Cl concentration in the root and lowest Cl concentrations in the leaf of rockmelon/ bottle gourd (Table 4) had shown the rootstock capacity to exclude and stored Cl in their root. This was also supported by the lowest Cl ion concentration increments recorded in the leaf of rockmelon/ bottle gourd (Figure 2B) and significant correlation observed between NaCl levels and Cl concentrations in the leaves of rockmelon/ bottle gourd (r=0.66; P≤0.01) (Table 3B). The outcomes regarding on beneficial impact of bottle gourd in alleviating salinity stress by Na and Cl ions restriction in the leaves has been broadly reported in previous studies. As reported by Colla et al. (2010), when bottle gourd was used as the rootstock for several cucurbit species under saline conditions, the accumulation of toxic ions in the shoot had considerably reduced. This has been proven with previous findings in grafted watermelon (Yetisir and Uygur, 2010) and grafted cucumber (cv. Jinchun No. 2) onto bottle gourd rootstock (Huang et al., 2009) which had low Na and Cl ions concentrations in the shoot. Bottle gourd rootstock presumably use its capability to store relatively toxic ions in its root to prevent excessive transport to shoot. This strategy adopted in alleviating salinity stress on grafted plants is described by the reduction of shoot toxic ion (Na^{+} and/ or Cl^{-}) through toxic ion exclusion and toxic ion retention within the rootstock (Edelstein et al., 2010). This finding was also in agreement with Al-Harbi et al. (2016) which stated that the beneficial benefits of grafting on salt tolerance in tomatoes can be attributed by the reduction of Cl^{-} ion entrance to the epigeous biomass.

The significantly highest Na and Cl ion concentrations detected in the leaves of self-grafted rockmelon (Table 4) showed that the rootstock has the lowest capability of excluding the toxic ion from moving to the shoot. This was supported with the strongest correlation observed between NaCl levels and both ion concentrations as Na (r=0.98; P≤0.01) and Cl (r=0.96; P≤0.01) (Table 3A) in self-grafted rockmelon. It clearly showed that rockmelon rootstocks can be categorized as the highest Na and Cl ion includer compared to others rootstocks tested. Our finding was supported with Pessarakli (2016), where rockmelon was categorized as moderately sensitive to salt-stress. Thus, its accumulated high amount of Na and Cl concentrations in their plant parts. Previous finding also showed that, Na and Cl ion concentrations mostly accumulated higher in leaves and shoot of ungrafted melon (cv. Galia) as compared to grafted plants (Yarsi et al., 2017).

In summary, self-grafted rockmelon had recorded the highest values in most of the growth parameters measured. It is followed by rockmelon/ bottle gourd and rockmelon/ bitter gourd. This result is due to the rootstock and scion of the control are from the same species thus having a good compatibility level. Eventhough the growth parameters can be a good early indicator of successful grafting, the Na and Cl ions concentration in the leaves is the main indicator for salt-tolerance ability in plants (Colla et al., 2013). The results had shown that the graft combination of rockmelon/ bottle gourd recorded the lowest Na and Cl ions concentration especially in the leaves, followed by rockmelon/ bitter gourd and lastly self-grafted rockmelon. Due to the high Na and Cl accumulation in the shoot, self-grafted rockmelon will eventually succumb to the toxicity as it is unable to tolerate the increase in salt levels. Therefore, rockmelon/ bottle gourd was selected as the least salt-sensitive grafting combination, although having the second best growth performance after self-grafted rockmelon.

Materials and Methods

Experimental design and treatments

Using randomized complete block design (RCBD), two factorial study was done with cucurbit rootstocks and NaCl levels as the different factors. The cucurbit rootstock treatments comprise of rockmelon (*Cucumis melo* L.) var. Glamour as control, bottle gourd (*Lagenaria siceraria*) var. Wira and bitter gourd (*Momordica charantia*) var Mutiara. The rockmelon seeds was produced by Sakata seed company while the bottle gourd and bitter gourd were produces by Leckat seed company. These rootstocks were grafted with rockmelon scion var. Glamour. Based on the previous studies regarding on salinity threshold levels in melon (Pereira et al., 2017), NaCl concentrations ranging from 0-75 mM was selected to be adapted in this study. Hence, four levels of NaCl concentration were used as 0, 25, 50 and 75 mM. Therefore, there were three cucurbit rootstocks and four levels of NaCl concentrations giving a total factorial treatment combinations of 12. Each treatment combination has 3 replications with 6 plants in each replication totaling to 216 grafted plants for the whole study.

Grafting and hardening process

The experiment was conducted at University's Agriculture Park nursery, Universiti Putra Malaysia (2.98675, 101.70932). During the experiment, average maximum temperature was recorded once a day at 2.00 pm as recorded 35.1 (±5) °C with average relative humidity (RH) at 44 (±10) % under natural photoperiod conditions (12 hours light/ 12 hours dark). Rockmelon, bottle gourd and bitter gourd seeds were sown in the germination tray filled with peatmoss media and the seedlings were watered twice a day. At 13 days after sowing (DAS), all uniformed sized of scion (rockmelon) and rootstock (rockmelon, bottle gourd and bitter gourd) were selected and grafted together using tongue approach grafting (TAG) technique as procedure described by Lee et al. (2010). The 14-days old cucurbit rootstocks were preceded for grafting with 12-days old rockmelon scion. Grafted plants were covered with individual transparent cup and placed under shelter structure of 100% shading with day/night temperatures of 27 (±5) °C, and RH 65 (±10) % under natural photoperiod conditions (12 hours light/ 12 hours dark). Hardening process was done according to procedures described by Hassan et al. (2022). Transparent cups were fully removed at 7 days after grafting (DAG) and all the plants were hardened under shelter with 25% shading and mist-sprayed with water twice a day at 9.00 am and 12.00 pm until 10 DAG. At 11 to 20 DAG, the grafted plants were mist-sprayed four times a day at 9.00 am, 12.00 pm, 3.00 pm and 6.00 pm.

Plant maintenances

At 14 DAG, uniformed sized of grafted plants were selected and transplanted into 4 litres black polyethylene bags filled with 100% cocopeat media. The cocopeat growing media used were added with 6 g/L N:P:K (15:15:15) compound fertilizer, 2 g/L ground magnesium limestone and 1 g/L mixture of micronutrients (3.2% Mg, 2.68% Fe, 1.36% Mn, 0.48% Zn, 1.36% Cu, 0.7% B, 0.02% Mo) (Ghani et al., 2018). Therefore, the amount of fertilizer given for each plants based on 4 litres of cocopeat media was 24 g of N:P:K (15:15:15) compound fertilizer, 8 g of ground magnesium limestone and 4 g of micronutrients.

Each of the NaCl solutions was prepared separately in a 200L drum container based on their respective concentration treatment. At DAT, all of the grafted plants was treated with four different levels of NaCl concentrations accordingly by manually drenching in a period of 15 days. Approximately, 300 ml of the NaCl solutions treatments was given once per day for the first seven days and twice per day for the following days as the plants grew. As the plants reached 5 and 10 DAT, compound fertilizer using N:P:K (15:15:15) was given for each plant approximately at 1.5 g of 4 litres cocopeat substrate to avoid nutrient deficiency incidence (Shafieizargar et al., 2015). The electrical conductivity (EC) of the media was checked using EC meter (5061 Pen SHSX) at 7 DAT from 9.00 am to 10.00 am. Determination of EC were done by following the procedure described in pour-through method (Cavins et al., 2005). Based on the NaCl level treatments which are 0, 25 50 and 75 mM NaCl, the EC recorded were 1.71, 3.65, 5.72, 7.98 dS m^{-1} respectively.

Data collection

Growth measurements

At 17 DAT, four plants in each replication was measured and sampled for determination of plant height, stem diameter, leaf number, total leaf area and dry weight matter of leaf, stem and root. Measurement of plant height was taken from the marked level of 0.2 cm on top of the graft union to the highest shoot tip using a measuring tape. 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 based on fully expanded leaves. The whole plants were then harvested and separated into leaf, stem and root to determine the leaf areas and dry matter weight. Total leaf area was measured using automatic leaf area meter (LI-3100C, LI-COR, Lincoln, Nebraska, USA). 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 (Ghani et al., 2018).

Na and Cl ion determinations

At 18 DAT, all fully expanded leaves and roots from four plants in each replication were harvested for Na ion determination following method described by Awang et al. (2009). The samples were washed with deionized water prior to drying at 70 °C for 72 hours before grounded using grinder. Briefly, 0.25 g of dry samples was transferred into the 100 ml digestion flask with 5 ml of concentrated H_2SO_4 were added for each sample. After that, the samples were left for 12 hours. Then, the flasks were heated in a digestion plate for 45 minutes at 285 °C prior addition of 2 ml of 50% H_2O_2. The procedure was repeated three times to complete the digestion process. The flask was removed from the digestion plate and cooled to room temperature before being made up with 100 ml of distilled water in the volumetric flask. Na ion concentration were quantified using an Atomic Absorption Spectrophotometer (Model 5100, Perkin Elmer, USA). Cl ion concentration was extracted from the precipitation and the titration was done according to the method described by Johnson and Ulrich (1959). The samples were extracted with distilled water, and were titrated with silver nitrate using potassium chromate as the indicator.

Statistical analysis

The data collected were computed using statistical analysis software version 9.4 (SAS Institute Inc., Cary, NC). General Linear Model (GLM) procedure was used to do analysis of variance (ANOVA) and the mean comparisons were calculated using Tukey's Honest Significant (HSD) difference at $P \leq 0.05$ and $P \leq 0.05$. Relationships among the variables for each cucurbit rootstocks were determined using Pearson correlation coefficients (r) at $P \leq 0.05$ by CORR procedure. Quantitave variables such as NaCl levels, growth parameters, Na and Cl ion concentrations in the leaves were computed in this analysis.

Conclusion

In conclusion, self-grafted rockmelon exhibited higher growth measurements due to good self-compatibility. It is followed by rockmelon/ bottle gourd and lastly rockmelon/ bitter gourd. However, graft combination of rockmelon/ bottle gourd had the lowest Na and Cl concentrations in the leaves. It is followed by rockmelon/ bitter gourd and self-grafted rockmelon. In order to classify the salinity tolerance levels among graft combinations, Na and Cl ions concentration in the leaves is considered to be the main indications instead of early growth performances parameters. This is because, continuous toxic ions (Na^+ and Cl^-) accumulation in the leaves will negatively affect overall shoot growth, flowering and fruiting performances at later stages. Conversely, low salt accumulation under saline condition will show better plant growth performances at later stages. In this case, graft combination of rockmelon/ bottle gourd is classified as the least salt-sensitive due to reduced accumulation of toxic ions in the leaf. Therefore, the level of salt-tolerant between graft combinations from least sensitive to most sensitive is rockmelon/ bottle gourd > rockmelon/ bitter gourd > self-grafted rockmelon. Graft combination of rockmelon/ bottle gourd is recommended to be cultivated under saline environment.

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