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'Pink' Waxapple Response to Salinity: Growth and Nutrient Uptake

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***Abstract*—**Waxapple (*Syzygium samarngense* Merr.et Perry) is an important tropical fruit in Taiwan. The famous producing area is located on the coast in Pingtung County. Land subsidence and climate change will tend to soil alkalization more seriously. This study was to evaluate the effects of NaCl in waxapple seedlings. NaCl salinity reduced waxapple shoot growth; it may due to reducing relative water content in leaf and new shoot. Leaf Cl and Na concentration were increased but K, Ca, and Mg content had no significant difference after irrigated with NaCl for six weeks. In roots, Na and Cl content increase significantly with 90 mM NaCl treatment, but K, Ca, and Mg content was reduced. 30-90mM Nacl treatment do not effect K/Na, Ca/Na and Mg/Na ratio, but decrease significantly in 90mM treatment in roots. The leaf and root electrolyte leakage were significantly affected by 90 mM NaCl treatment. Suggesting 90mM was optimum concentration for sieve out other tolerance waxapples verities.

***Keywords*—**Growth, NaCl stress, Nutrient, Waxapple.

I. Introduction

WAXAPPLE is one of the important tropical fruits which is mainly cultured in Pingtung, Taiwan. According to the statistics yearbook in 2012, around 77% of the waxapple are planted in Pingtung. What is more, 'Black pearl' is the most popular produce which was only grown around the coast of Pingtung county. Due to the land subsidence and climate change is the significant fact which may affect the environment; these two phenomena will be illustrated as below.

Climate change is a problem which makes the climate unstable. In Taiwan, rainfall increase year by year at north, but decrease year by year in south [19]. This phenomenon maybe reduces the ability of soil leaching the salt. Moreover, global warming will rises up the sea level [17]. Therefore, both of these problems will lead to the loss of cultivating area in the future. Temperature raises and more evaporation happened rapidly will accelerate the salt accumulation on ground. As [27] suggested, increased evaporative tend to increase transpiration volume of flow which will lead to the soil alkalization. Land subsidence is another problem to the farmland of west Taiwan which is close to the coast. Base on the announcement of Water Resource Agency (Taiwan), Pingtung County is one of the most serious area with land subsidence. The land subsidence will cause the groundwater alkalization, when it combines with climate change will make the excessive accumulation of salt in soil. That maybe is the reason why [24] found that the soil and groundwater where at some coast of Pingtung county contains salt, especially sodium and chloride.

All of these problems will threaten to waxapple industry which around the coast. Therefore, this study was carried to evaluate the effects of NaCl on seedlings growth, electrolyte leakagein, and nutrition content in waxapple.

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II. Materials and Method

A. Experimental Material and Salinity Treatments

Air layering 'Pink' waxapple seedlings (*Syzygium samarangense* Merr et. Perry) were used in this experiment. Thirty-two uniform seedlings were washed with distilled water and planted in the pot. Pruning shoot at 20 cm from pot top for growing new shoot, and preserve on new shoot for measuring. Transplanted individually into containers filled with perlites and transferred them into net house at Tropical Orchard of National Pingtung University of Science and Technology. Plants were irrigated every two days with 300 ml which contains 3000 fold commercial water-soluble fertilizer containing macro-and-micro-nutrients (Peter’s 20-20-20 N-P-K, Peters Corp., St. Louis MO, USA) [14] for one month. After that, three salinity treatments and control were used. Sodium chloride was applied in irrigation water with 0, 30, 60 or 90 mM. Treatments were maintained for 6 weeks.

B. Growth Measurements

Shoot relative growth rate: recorded primary and final shoot length. The difference of length at primary length percentage was calculated.

Relative water content (RWC): at the end of experiment, the plant was cleaned with water and collected separately for root, shoot, stem and leaf. The fresh weight was determined before oven-dried (70°C for 2-3 days) (W1). Then dry weight was weighted each part (W2).

$$RWC\ (\%) = (W2-W1) / W1 \times 100$$

Electrolyte leakage: Electrolyte leakage was measured with an electrical conductivity meter as described by [29]. Leaves were washed with deionized water, 10 leaf disks of diameter 0.6 cm were cut, and then immersed in 10 ml deionized water and incubated at room temperature (25˚C) on a shaker (100 rpm) for 24 hours. Electrical conductivity of samples (EC1) was measured. Samples were then put in boiling water for 30 min to completely release all electrolytes. When cooled it down to 25°C and the final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was expressed as:

$$EL = EC1 / EC2 \times 100$$

C. Leaf and Root Nutrient Determination

Ground the dry tissue. Calcium, potassium, magnesium and sodium concentration was determined with an inductively coupled plasma atomic emission spectrometer (ICPES). Ash would be used for determined Ca, K, Mg and Na. The method as follows: the powder sample weighted 0.2 g and add 2 ml concentrate H_2SO_4 and heated it up to 360°C to dissociate the sample into dark brown. After cooling, substance which was decolorized with 3 drops of $HClO_4$ and heated it again. After cooling, it was filtered and diluted to 50ml by deionized water for analysis. Chloride concentration was measured by SAT-500 Automatic Salt Analyzer (TOA-DKK, JAPAN).

D. Data Analyses

The data were analyzed by using analysis of variance (ANOVA) procedures, with the SAS software package and means were separated by LSD test at $p \leqq 0.05$.

III. RESULTS

A. Shoot Relative Growth Rate

Salinity resulted in decreased shoot length in all of the treatment (Fig. 1). The shoot length growth decrease significantly at 30 mM NaCl treatment (37.9%) and same situation at 60 and 90 mM treatment. Although no significantly differences at higher salt concentrations but had less shoot growth rate at 60 and 90 mM treatments, 14.3 and 12.8%, respectively.

A. Relative Water Content (RWC)

Waxapple seedlings were treated with 90 mM would reduce RWC 1.25% in leaf. Although RWC was reduced in 30 and 60 mM treatments, they had no significant difference. Salinity will reduce RWC of new shoot in all NaCl concentration treatments. On the other hand, salinity had not caused the decreasing of RWC in stem and root (Table I)

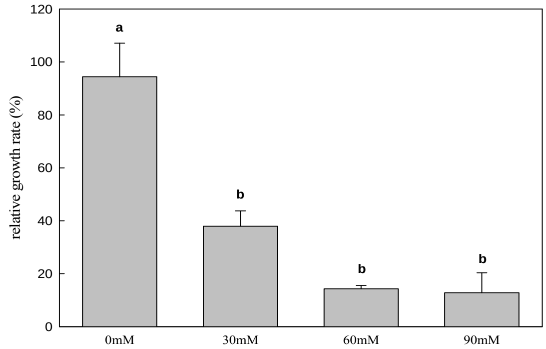

Fig. 1 Effect of irrigate with different NaCl concentration to shoot relative growth rate of 'Pink' waxapple seedlings after 6 weeks

TABLE I
EFFECT OF IRRIGATE WITH DIFFERENT NACL TO LEAF, NEW SHOOT, STEM, AND ROOT RELATIVE WATER CONTENT (%) OF 'PINK' WAXAPPLE SEEDLINGS AFTER 6 WEEKS

	Leaf		New Shoot		Stem		Root	
0mM	74.11	a	76.37	a	64.84	a	78.55	a
30mM	73.13	ab	74.41	b	64.32	a	77.31	a
60mM	72.89	ab	74.02	b	64.21	a	77.52	a
90mM	72.86	b	73.73	b	64.17	a	77.32	a

C. Leaf and Root Nutrient Content

In Table II, the Na content increase with enhanced concentration of NaCl treatment in leaf and root. Cl increase with 60 mM and 90 mM treatment in leaf, but increase significantly with 90 mM in root. K content was no significant difference in all treatment in leaf, but in root, it was decreased with enhance concentration of NaCl. Ca and Mg content have similar trend with K content: there was no difference in leaf but it was decrease significantly in root while treated with 90 mM NaCl, 3538.6 ppm and 2964.2 ppm respectively.

TABLE II
EFFECT OF IRRIGATE WITH DIFFERENT NACL CONCENTRATION TO MINERAL CONCENTRATION IN LEAF AND ROOT OF 'PINK' WAXAPPLE SEEDLINGS AFTER 6 WEEKS

	Leaf					Root				
	Na	K	Ca	Mg	Cl	Na	K	Ca	Mg	Cl
	(ppm)	(ppm)	(ppm)	(ppm)	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(%)
0mM	488.9^{c}	16361.6^{a}	5993.9^{a}	3018.2^{a}	0.86^{b}	1303.0^{d}	9056.6^{a}	5531.7^{a}	4056.9^{a}	0.61^{b}
30mM	743.8^{bc}	14025.8^{a}	5506.0^{a}	2503.0^{a}	0.68^{c}	5757.8^{c}	7695.1^{ab}	4011.3^{bc}	3435.3^{ab}	0.66^{b}
60mM	1007.9^{ab}	17342.4^{a}	7337.5^{a}	3363.0^{a}	1.01^{a}	8433.1^{b}	6726.1^{bc}	4704.9^{ab}	3758.6^{a}	0.67^{b}
90mM	1186.7^{a}	18945.6^{a}	8481.3^{a}	4056.4^{a}	1.03^{a}	14444.9^{a}	5393.2^{c}	3538.6^{c}	2964.2^{b}	1.57^{a}

Salinity resulted in decreasing K/Na, Ca/Na and Mg/Na ratio in all of the treatment and no significant difference among 30-90 mM (Table III). About decreasing 51%, 57%, and 53% compared with the control in K/Na, Ca/Na and Mg/Na ratio in leaves, respectively. In term of root, K/Na ratio will decrease when NaCl concentration increase. Ca/Na and Mg/Na had no significant difference between 30 and 60 mM NaCl; decrease about 15% and 16% compared with the control, respectively. K/Na, Ca/Na, and Mg/Na ratio in 90 mM NaCl treatment has most dramatic decrease, 0.4, 0.2, and 0.2, respectively.

D. Electrolyte Leakage

After culturing for 6 weeks, the electrolyte leakages of leaves were significantly affected by 90 mM NaCl treatment (Fig. 2). With respect to the electrolyte leakage of roots were increased at 30 mM NaCl, but no significant difference between 30 and 60 mM NaCl.

IV. DISCUSSION

The present experiment was conducted to evaluate the effects of increasing NaCl concentration in waxapple seedlings. These results suggested that NaCl salinity reduced waxapple shoot growth (Fig. 1); the serious influence of salt stress is growth suppression of plants [25]. NaCl treatments caused inhibition in plant growth due to decreasing of proliferation ratio, fresh weight, shoot length, and numbers of leaves [30], [20]. Aazami et al. [1] and Forner-Giner et al. [13] also assumed that salinization can inhibit both cell division and cell expansion in growing tissue of roots, stems and leaves, thereby, affecting shoot growth. Salinity reduced RWC in leaf and new shoot. Yeo et al. [26] suggested that initial growth reduction in rice caused by salinization is due to a limitation of water supply. Osmotic adjustment in the growing zone of leaves was correlated with the recovery of leaf elongation rate after plant exposure to salinity [8]. According to [4], the growth inhibition due to salinity has been explained by suppression of nutrient ions absorption due to higher uptake of Na and Cl ions, although different fruit trees differing in their ability to control absorption and transport of Na and Cl have widely difference salinity tolerances [25]. Leaf Cl and Na concentration were increased under saline treatments (Table II). A portion of Na was probably transported from the leaves through the phloem to root [16], and stem xylem transports about 90% of Cl from root to shoot [18]. Therefore, waxapples seedlings, which irrigated with NaCl, high concentration of Cl and Na in leafs. These seedlings absorbed NaCl from root to shoot, may caused the decrease of RWC in new shoot or growing tissue, this may lead to the inhibition of the cell division in this study.

TABLE III
EFFECT OF IRRIGATE WITH DIFFERENT NACL CONCENTRATION TO K/NA, CA/NA, AND MG/NA RATIO IN LEAF AND ROOT OF 'PINK' WAXAPPLE SEEDLINGS AFTER 6 WEEKS

	Leaf			Root		
	K/Na	Ca/Na	Mg/Na	K/Na	Ca/Na	Mg/Na
0mM	34.2^a	12.7^a	6.3^a	6.9^a	4.2^a	3.1^a
30mM	19.1^b	7.5^b	3.4^b	1.4^b	0.7^b	0.6^b
60mM	17.3^b	7.2^b	3.2^b	0.8^c	0.6^b	0.4^b
90mM	16.1^b	7.2^b	3.5^b	0.4^d	0.2^c	0.2^c

Saline-induced changes in the concentrations of the other elements analyzed varied with plant organ and element [22]. Although K, Ca, and Mg content no significant difference in leaves after irrigating with NaCl for six weeks, K, Ca, and Mg content decrease as treated with 90 mM NaCl. Chelli-Chaabouni et al. [7] also found that the K content in the roots showed a significant decrease but the K content in shoots and leaves were not significantly affected by salt at all NaCl concentrations for Pistachio (*P. atlantica*). K reduced in citrus leaves under salinity has not always been observed [2]. Ruiz et al. [22] found salinity lowered K concentrations in roots of all rootstocks, and in leaves of the 'Cleopatra' mandarin, sour orange and 'Carrizo' citrange rootstocks, whereas K concentrations increased in leaves and roots of *C. macrophylla wester* rootstocks. The decrease of K recorded in root, which resulted in an increase in the Na/K ration, may also provide a mechanism by which 'Sultana' vines achieve ionic balance following uptake of high Na concentrations in root [12], [25]. At high levels (100 mM) salinity led to increase in K concentration of laminae, which balanced the increase of Cl ions [12].

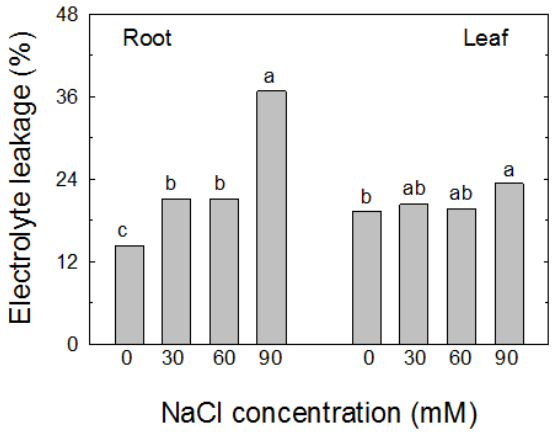

Fig. 2 Effect of irrigate with different NaCl concentration to electrolyte leakage (%) of 'Pink' waxapple seedlings after 6 weeks

Calcium is known to play key roles in maintaining the integrity of the plasma membrane in the root cells, thus limiting the toxic effect of Na [21], [15]. Cramer et al. [9] demonstrated that Ca protects membranes from the adverse effects of Na, thereby; it maintains membrane integrity and minimizes leakage of cytosolic K. Although calcium content without statistically significant differences (Table II) in this study, Ca content slightly increased in 60 and 90 mM NaCl concentration in leaves, 22% and 41% higher than the control separately. The results indicated that 'Pink' waxapple seedlings uptake more Ca to maintaining membrane integrity in order to alleviate NaCl toxicity at 60 mM NaCl.

NaCl salinity reduced Mg concentrations of leaf in citrus [22]. But, in our results, Mg content in leaf was no significantly decreased in 90 mM NaCl treatment. NaCl treatment to 'sunburst' mandarin grafted on different rootstocks, salinity reduced root Mg and Ca concentration but no significantly difference in 30 to 90 mM treatments [14], which is similar to our results. Bernstein et al. [5] also found that increases in salinity ($NaCl+CaCl_2$) only reduced Mg concentration of leaves in beet and had little or no effect in leaves from five other vegetable crops that they examined. Al-Yassin [2]suggested that the increasing of Ca has been shown to decrease Mg concentration, primarily due to the displacement of Mg from the soil complex, and the high levels of Mg must had hindered the absorption of Ca by the plant due to the action of Ca-Mg antagonistic effect. Mg application (2 mM) to Pistachio reduced biomass in non-saline and saline conditions, as well as reduced K and Ca concentration in shoot [28]. It may due to antagonistic effect among K, Ca and Mg; indeed, application Mg to enhance crop tolerance toward saline is not available. In this study, suggested that Ca precedence over Mg absorbed to waxapple when NaCl stress. Ebert [10] also indicated the ability of plants to maintain the selectivity for Ca and K in a saline environment is often associated with salt tolerance.

Positive correlations have been demonstrated between sensitivity to salinity and membrane damage in foxtail millet (*Setaria italica*) seedlings [23]. Increase in membrane permeability or loss of membrane integrity, as evidenced by the increase in solute leakage [11]. Sodium ion and relative electrolyte leakage in oil palm seedlings in response to salt stress increased, leading to damage to photosynthetic pigments, diminished the photosynthetic abilities and reduced the growth performance [6]. Our result (Fig. 2) also demonstrated that the increase electrolyte leakage as NaCl concentration increased in waxapples, and relative growth rate decreased may associated with membrane integrity be destroyed. Ashraf and Haris [3] also indicated that the improving stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions.

In the waxapple seedlings research in this study, 90mM NaCl induced significant decreases of shoot relative growth, leaves and shoot relative water content, and increase Na, Cl and electrolyte leakage in leaves and roots, while leave necrosis did not appear. This indicates that 'Pink' waxapple is moderately salt tolerant. The range of salt concentrations tolerated by crops varies greatly from species to species [2]. According to [20], after *in vitro* culture with 60 mM NaCl for 8 weeks; visible symptoms of salt injury observed in the shoots, for example, necrosis and fallen leaves. As a result, we suggesting waxapple have ability to modulating threaten within 30-60 mM NaCl, therefore, 90 mM was optimum concentration for sieve out other tolerance waxapples verities.

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