Effect of salinity stress on growth and nutrient uptake in polyembryonic mango rootstocks

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ABSTRACT

Six-month-old, uniform-sized seedlings of seven mango (*Mangifera indica* **L.) rootstocks namely Moovandan, Bappakai, Nekkare, Kurukkan, Olour, Terpentine and Chandrakaran were irrigated to 70% of field capacity with water containing 0, 50, 100, and 150 mM NaCl for 90 days. Growth in terms of plant height, number of leaves and leaf area per plant decreased with increasing levels of salinity in all rootstocks. The decrease in growth was greatest in the salt-susceptible Chandrakaran rootstock (32.02%) at higher levels of salinity. However, in the salt-tolerant Olour and Nekkare, NaCl caused only a slight decrease in numbers of leaves and leaf area per plant. A declining trend was observed in fresh and dry weight of plant with increasing concentration of NaCl in all rootstocks and maximum decrease was found in Chandrakaran (73.43% in shoot and 57.20% in root) whereas minimum was in Olour. The concentration of Na+ ions in leaf tissues increased to a maximum (123.53%) in Chandrakaran and in root tissues Bappakai had the highest Na+ ions (77.27%) content. The maximum increase (109.09%) in leaf Cl- ions level occurred in Moovandan and Chandrakaran had the highest (139.29%) root Clions content at 150 mM NaCl. These data suggest that lower levels of Cl- and Na+ accumulation could be used as indicators for screening mango rootstocks for resistance to NaCl stress. Olour, Terpentine and Nekkare can exclude Cl- ions; however, Kurukkan, Bappakai, and Moovandan rootstocks seems to be Na+ excluder upto lower level of salt concentrations. Based on overall performance and leaf scorching, it could be said that salinity tolerance increased in the following order Chandrakaran < Moovandan < Bappakai < Nekkare < Kurukkan < Terpentine < Olour.**

Key words: Mango, rootstocks, NaCl tolerance, salinity stress.

INTRODUCTION

Salinity reduces the ability of plants to absorb water, causing rapid reductions in growth rate, ion imbalance and toxicity. In India, 4.10 mha lands have been reported to be saline soil (Anon, 3). Salt affected soils are spread widely covering the Indo-Gangetic plains, arid regions and coastal areas. The malady continues to increase due to the mismanagement of canal irrigation as well as due to brackish groundwater irrigation. Gupta and Sen (10) reported that soil was the major factor for decreasing plant growth in mango. Soil having pH 7.86, EC 0.49 mmhos/cm, ESP 11.9 and water having EC 0.50 mmhos/cm, pH 7.46 were the most suitable for initial establishment of mango seedlings. Hence establishment of mango orchards in areas having soil and/or water EC beyond this limit is difficult. Several studies have shown that salt stress severely affects seedling growth (Dubey *et al*., 7; Srivastav *et al*., 15), chlorophyll contents, $CO₂$ assimilation and nutrient uptake and ion homeostasis.

Mango is considered very sensitive to saline conditions particularly at early stage of growth, leading to scorched leaf tips and margins, leaf curling, and in severe cases reduced growth, abscission of leaves, and death of trees resulted inhibition of seedling growth (Dubey *et al*., 7; Srivastav *et al*., 15). However, salt-tolerance mechanisms are complex and include osmotic adjustment through the accumulation of compatible solutes, lowering the concentrations of toxic ions in the cytoplasm by restriction the influx of Na⁺, its sequestration in the vacuole, and/or Na⁺ ion extrusion and scavenging of reactive oxygen species (Mittler, 12). Although the problem of salinity is increasing, the genetic mechanism(s) of salt-tolerance are still largely unknown. In the present study, attempts were made to study the effects of high salinity on seven common mango rootstocks. We also studied the possible basis for salt-tolerance and developed criteria for screening mango rootstocks for their salttolerance.

MATERIALS AND METHODS

The pot experiment was conducted with four levels of NaCl, *i.e.*, 0.0 mM NaCl (Tap water), 50, 100 and 150 mM NaCl on seven different mango rootstocks, namely, Moovandan, Bappakai, Nekkare,

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Kurukkan, Olour, Terpentine and Chandrakaran during 2010-2012. Stones of these seven rootstocks were collected from the Mango Germplasm blocks of the Division of F&HT, IARI, New Delhi and Indian Institute of Horticultural Research, Bengaluru. Stones were thoroughly washed with running tap water and sown in the nursery beds immediately after extraction from the fruits. Six-month-old uniform seedlings were selected on the basis of vigour, leaf size and shape. These seedlings were then transplanted in plastic pots (30 cm size) each containing 8.0 kg of a 1:1:1 (w/w) mixture of soil, sand and well rotted farm yard manure (FYM). The mixture used in pots had $EC_{(1:2)}$ of 0.15 dS m⁻¹, a pH $_{(1:2)}$ of 7.12, a cation exchange capacity (CEC) 10.71 cmol kg-1, and organic carbon 0.46%. One month after transplanting into pots, 20 g mixture of urea, single super phosphate and potassium sulphate in the ratio of 1:2:1 was applied to each pot. Seedlings were irrigated for 30 days with tap water until the beginning of the experiments with NaCl treatment. At the beginning of experiment, a single shoot per plant was left. These seedlings were then irrigated with different concentrations of NaCl, *i.e.*, 50, 100 and 150 mM NaCl and control plants were irrigated with tap water (EC 0.20 dS $m⁻¹$) at three days interval considering the loss of moisture measured by direct weighing of pots. The treatments were maintained for three months.

Plant height and No. of leaves per plant were measured at 30-day intervals and leaf area was measured by using a standard leaf area meter (Model LiCOR 3100). The shoot and root dry weights were taken after drying them in hot air oven at 70°C for 48 hours. The total Na and K contents of plant leaves and roots were estimated using 9:4 (v/v) $\mathsf{HNO}_{_3}$:HClO $_4$ diacid-digested samples and a microprocessorbased flame photometer (Flame Photometer-128; Systronics, New Delhi) following the method of Jackson (11). The CI⁻ ion contents of plant leaves and roots were quantified using mercuric (II) thiocyanate, as suggested by Adriano and Doner (1). However, Cl- ion extraction from leaves and roots was done using 0.1 M sodium nitrate at a (w/v) ratio of 1:100. The experiment was conducted in Factorial Complete Randomized Design (FCRD) with four replications. Each replication consisted of three plants. Data were analysed using the SPSS package (SPSS 16.0; SPSS Inc., Chicago, IL, USA) to calculate F values. Statistically significant differences were identified using analysis of variance (ANOVA); used Duncan's Multiple Range Test for determining significant mean differences and terms were considered significant at LSD (*P ≤ 0.05*).

RESULTS AND DISCUSSION

Plant height significantly influenced by both salinity and rootstocks (Table 1). The maximum reduction in plant height (32.02%) was measured in Chandrakaran when seedlings were irrigated with highest concentration of NaCl salt followed by Bappakai (29.78%) as compared to respective controls, whereas minimum inhibition in plant height was recorded in Moovandan (15.78%) at 150 mM. However, at lower and moderate concentration of NaCl salt (50-100 mM), maximum reduction was found in Bappakai followed by Chandrakaran. It is worthwhile to mention that at lower salinity level, minimum reduction in plant height (2.12%) was recorded in Moovandan. Differences in salt tolerance ability of different mango rootstocks were also reported by Dubey *et al*. (7) and Srivastav *et al*. (15). Reductions in plant height in a saline habitat may be due to presence of NaCl that alters the nutritional balance of plants, resulting in high ratios of Na+/Ca++, Na⁺/K⁺, Na⁺/Mg⁺⁺, Cl⁻/NO₃⁻, and Cl⁻/ H₂PO₄⁻ (Grattam and Grieve, 9), which may cause reductions in growth. Major saline ions can affect nutrient uptake through competitive interaction or by affecting the ion selectivity of membrane. In our results, reduction in plant height in Chandrakaran rootstock appeared to be due to increase in foliar concentration of Cl- and decrease in Ca2+ accumulation. Sodium accumulation seems to play an important role in reduction in plant height in Moovandan. In Bappakai, higher amount of Na+, Cland reduced uptake of Ca^{2+} and Mg²⁺ caused reduction in shoot growth.

Salinity had significant variation with respect to number of leaves per plant (Table 1). A perusal of interaction effect showed significant variation in number of leaves in different treatments. The maximum reduction in number of leaves (70.59%) was found in Chandrakaran when irrigated with 150 mM NaCl followed by Terpentine (61.93%) and Moovandan (53.99%), while minimum reduction (30.58%) was recorded in Nekkare. At lower salinity level, the minimum reduction in leaf count (13.05%) was recorded in Bappakai followed by Nekkare (13.93%) and Kurukkan (20.00%). However, different trend was observed with 100 mM NaCl stress. Nekkare reduced the minimum number of leaves (22.25%) when seedlings were irrigation with 100 mM NaCl followed by Olour (29.41%) and Kurukkan (30.00%). Munns (14) indicated that salt in plants reduces growth by causing premature senescence of old leaves and hence reduced supply of assimilates to growing regions. Sensitive cultivars accumulate ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and progressively death of the plant.

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Treatment		Plant height	No. of leaves plant ⁻¹	Leaf area plant ¹
Rootstock	NaCl (mM)	(cm)		(cm ²)
Moovandan	Control	44.37	16.67 ^m	139.28 ⁱ
	50	43.43	13.67kl	135.08fgh
	100	40.30 ^q	9.67efgh	133.11 ^{efg}
	150	37.37 ^p	7.67cde	131.24 ^e
Bappakai	Control	23.07k	15.33^{lm}	185.58 ^r
	50	19.33 ^{gh}	13.33kl	183.12 ^{qr}
	100	18.13fg	10.33ghi	180.34 ^{pq}
	150	16.20 ^{de}	7.33bcd	177.67 ^{nop}
Nekkare	Control	34.10°	12.00 ijk	183.23 ^{qr}
	50	31.00 ⁿ	10.33ghi	180.02°P
	100	29.07m	9.33defgh	179.37°P
	150	28.30 ^m	8.33cdefg	177.22 ^{mno}
Kurukkan	Control	15.10 ^{cd}	10.00fghi	137.83hi
	50	14.23bc	8.00 _{cdef}	135.61 ^{gh}
	100	13.27ab	7.00 abc	133.37 ^{efg}
	150	12.13^{a}	5.00 ^a	132.12 ^{ef}
Olour	Control	21.83^{jk}	17.00^m	175.69mm
	50	20.07hij	13.00 ^{jk}	174.61lm
	100	19.50fgh	12.00 ijk	174.60mm
	150	17.03ef	10.67hi	173.27
Chandrakaran	Control	25.30	17.00 ^m	126.66 ^d
	50	21.30ijk	12.00ijk	121.59c
	100	20.03 ^{hij}	8.00cdef	118.53 ^b
	150	17.20 ^{ef}	5.00 ^a	115.23 ^a
Terpentine	Control	37.90 ^p	14.00kl	152.34k
	50	33.93°	11.00hij	149.71k
	100	31.00 ⁿ	7.00 _{abc}	145.64
	150	29.00^m	5.33^{ab}	143.91 ^j
LSD $(P \le 0.05)$		1.73	1.89	2.73

Table 1. Effects of rootstock and NaCl stress on plant height, No. of leaves per plant and leaf area per plant in mango seedlings.

Means followed by the same letter (S) within treatment are not significantly different at $P \le 0.05$ according to the F test using Duncan's multiple range test (DMRT)

A perusal of data presented in Table 1 clearly indicated significant differences with regard to leaf area per plant at different salinity levels. The maximum reduction in leaf area was found by 9.02% in Chandrakaran when irrigated with 150 mM NaCl followed by Moovandan (5.77%). However, minimum reduction in leaf area per plant was recorded in Olour (0.61%) when irrigated with 50 mM NaCl followed by Bappakai (1.33%). Results showed significant decrease in leaf area of mango

leaves with application elevated salt treatment. Under saline condition as soon as new cell starts its elongation process, the excess of Na⁺, Cl⁻ and other ions modifies the metabolic activities of cell wall, which causes deposition of several materials on cell wall and limits the cell wall elasticity (Yasar *et al.*, 16). Cell walls become rigid and turgor pressure efficiency in cell enlargement is decreased with application of elevated salt treatment. The other anticipated cause of reduction in leaf area and dry

matter content could be the reduced development and differentiation of tissues, shrinkage of the cell contents, unbalanced nutrition, damage of membrane and disturbed avoidance mechanism (Akram *et al.*, 2). The growth inhibition of leaves sensitive to salt stress appears to be also a consequence of inhibition by salt of symplastic xylem loading of $Ca²⁺$ in the root. Shoot growth is more sensitive than root growth to salt**-**induced osmotic stress probably because a reduction in the leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent salt concentration in the soil (Munns and Tester, 13) as present investigation showed.

Fresh weight of shoot and root varied significantly by both salinity and rootstocks (Table 2). Peeps into interaction showed significant reduction in fresh weight of shoot and root in all rootstocks with increasing concentration of NaCl. The maximum reduction in fresh weight of shoot by 73.43% and in root by 57.20%

Table 2. Effects of rootstock and NaCl stress on fresh and dry weight of shoot and root of mango seedlings.

Means followed by the same letter(s) within treatment are not significantly different at *P* ≤ 0.05 according to the F test using Duncan's multiple range test (DMRT)

was recorded in Chandrakaran with application of 150 mM NaCl as compared to respective control, whereas, minimum reduction in fresh weight of shoot (12.89%) and root tissues (7.94%) was recorded in Olour. Dry weight of shoot and root influenced significantly by salinity and rootstocks (Table 2). At higher concentration of NaCl, maximum reduction in dry weight of shoot by 81.54% and root by 76.92% was found in Chandrakaran, while minimum reduction in dry weight of shoot and root was noticed in Olour by 16.98% and in Nekkare by 26.77% as compared to their respective controls. Salinity exerts detrimental effect on fresh and dry weights of shoot and root. Probably the negative effect of salinity on plant provoked osmotic potential by salt in growing medium; hence, root cells could not obtain required water from medium. Therefore in plants, the uptake of some mineral nutrients which dissolved in water was also restricted; thus, growth and development of plants are inhibited due to occurring defect in metabolism. As salt concentration increases besides nutrient imbalance, hyperosmotic stress and ion disequilibrium plays a pivotal role in disturbing the cellular functions of plant (Foolad, 8). Considering that leaf area was more affected than number of leaves, our results suggest that salinity also affected cell elongation ratio, therefore decreasing leaf size.

A perusal of data showed significant effect of interaction between salinity and rootstocks on tissue $K⁺$ content (Table 3). Leaf and root tissues $K⁺$ content decreased with increasing level of NaCl concentration in all rootstocks. In comparison to non salinised respective control, maximum reduction in leaf and root K+ content by 84.94 and 80.11% was recorded in Chandrakaran when seedlings were treated with 150 mM NaCl and minimum reduction by 31.13% in shoot and 28.99% in root was recorded in Olour rootstock. It is pertinent to mention that leaf K⁺ content was more affected as comparison to root K⁺ content in all rootstocks under NaCl stress. These outcomes suggest that there was a competition between Na+ and $K⁺$ regarding their uptake. The reduction in K uptake caused by Na is a well-know competitive process in plant roots. Similar findings were reported in canola cultivars (Bandeh-Hagh *et al.*, 5). It is well documented that a greater degree of salt tolerance in plants is associated with a more efficient system for the selective uptake of K^* over Na⁺ (Ashraf and O'Leary, 4).

NaCl stress and rootstocks interaction also has significant effect on leaf and root Na⁺ accumulation (Table 3). Sodium accumulation in leaf and root tissues increased with increasing level of NaCl concentration in all rootstocks. Chandrakaran had higher Na⁺ accumulation (123.53%) in leaf tissue

and Bappakai had higher Na+ accumulation (77.27%) in root tissues as compared to respective control at higher salinity level, whereas minimum accumulation in leaf and root was estimated in Olour (57.14 and 42.86% higher than respective control). Olour and Kurukkan recorded minimum reduction (21.43 and 19.05% lower than respective control) in leaf and root Na+ accumulation at lower salinity level. Data pertaining to Cl- accumulation in leaf and root tissues showed significant variation in NaCl stress and rootstocks (Table 3). In case of leaf CI-, maximum increase (109.09% more than respective control) was recorded in Moovandan followed by Bappakai (100.00%) at higher salinity level (150 mm NaCl) and minimum increase was found in Olour (43.48%); whereas, in case of root CI, maximum increase (139.29% more than respective control) was recorded in Chandrakaran followed by Bappakai (107.32%) and minimum increase was found in Olour (61.46%). Chloride accumulation in both leaf and root tissues was increased minimum in Olour at each salinity level as compared to other rootstocks. Salt tolerance has been associated with the ability to restrict the uptake and/or translocation of Na+ and Cl- ions from the root to shoot. Results showed Na+ and Cl- accumulation in leaf and root tissues increased with increasing level of NaCl concentration in all rootstocks. Chandrakaran and Moovandan had higher Na+ and Cl- accumulation in leaf tissues respectively at higher salinity level whereas minimum accumulation of Na+ and Cl- in leaf was estimated in Olour. In contrast to leaf tissues, Na⁺ and CI⁻ ion contents in root tissues increased most in Nekkare and Chandrakaran respectively at higher salinity levels, while minimum accumulation of Na+ and Cl- in root was estimated in Olour also. Almost similar trend was also recorded at lower salinity levels for both ions. These results revealed that Olour had a greater ability to restrict Na⁺ and Cl⁻ ions translocation to leaf tissues and Chandrakaran had ability to decrease Na⁺ ion translocation from roots. However, Moovandan had ability to decrease Cl- ion translocation from roots. Previously it was also reported in other crops that salt exclusion mechanism works effectively in olive (Chartzoulakis, 6). Based on the above findings, it was concluded that under high salinity stress, the seedlings of salt-tolerant mango rootstocks exhibited lower levels of Na+ and Cl- concentrations. Moreover, salt stress increased the Na+ and Cl- concentrations concomitant with a decrease in K⁺ accumulations in comparatively salt sensitive rootstocks. Thus, in addition to the toxic effects of high concentrations of Na+ and Cl- in plant tissue, the salinity induced changes in mineral nutrient uptake and utilization likely contributed to

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Means followed by the same letter(s) within treatment are not significantly different at *P* ≤ 0.05 according to the F test using Duncan's multiple range test (DMRT).

the reduction in plant growth. Furthermore, tolerant behavior of Olour, Turpentine, Kurukkan and Bappakai may be due to impeding the uptake of CI⁻ and Na⁺ ions accumulation. Olour, Terpentine and Nekkare can exclude Cl- ions; however, Kurukkan, Bappakai, and Moovandan roootstock seems to be Na⁺ excluder upto lower level of salt concentrations. Based on overall performance and leaf scorching, it could be said that salinity tolerance increased in the following order Chandrakaran < Moovandan < Bappakai < Nekkare < Kurukkan < Terpentine < Olour rootstocks.

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