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Salinity tolerance potential of Citrus (*Citrus* spp.) rootstock genotypes

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ABSTRACT

In semi-arid ecosystem, salinity is major stress which influences the performance of citrus plants. An experiment was conducted at ICAR-Central Citrus Research Institute, Nagpur, Maharashtra during 2015–18 in containerized nursery to screen 4 hybrids (NRCC 2, NRCC 3, NRCC 6, CRH 12) as well as promising citrus (*Citrus* spp.) rootstock genotypes (Alemow, Volkamer lemon, Shekwasha mandarin, commercially used Rangpur lime and rough lemon) against salinity stress. Salinity gradient 0-80 mili mole (mM) was developed using salt mixture and found inversely proportional to plant growth parameters and leaf content of most of the nutrients. At highest salinity, commercially used rootstocks were unable to survive. At 80 mM salinity level, decrease in plant height (48.9%) and stock girth (14.8%) was lowest in Shekwasha, while lowest defoliation was observed in Alemow (9.7%) rootstock. Lowest reduction in leaf N and P content was observed in Alemow; Ca in NRCC-6 and Mg in Shekwasha. Substantial reduction in leaf K content (2.6–64.0%) was observed in most of the rootstocks with increasing salinity levels except in Alemow, NRCC-6 and Shekwasha. It was found that the parentage of trifoliate orange in hybrids imparted further sensitivity for salt tolerance. In general, it was observed that Alemow and NRCC-6 rootstocks were least affected by salinity treatments as compared to other citrus rootstocks.

Keywords: Citrus hybrid rootstocks, Leaf chlorophyll, Leaf nutrient content, Plant growth, Salinity tolerance

In world, only 10% of the total productive area is conducive for crop production, while about 90% is under stress. Salt affected soils occupy about 2.5×10^6 ha in India (Singh and Bandopadhyay 1996). Accumulation of salt especially NaCl in the soil is one of the most important factors limiting plant growth and yields. The inhibition of plant growth under saline conditions may be due to water and osmotic stresses, specific ion toxicity and/or ionic imbalance, acting on biophysical and metabolic processes of growth (Greenway and Munns 1980). Globally, India ranks 3rd (12.5 million tons) in citrus (*Citrus* spp.) production after China (35.47 million tons) and Brazil (19.07 million tons) (FAO 2017). In India, citrus is grown under varied climatic and geographical zones covering an area of 1 million ha with annual fruit production of 12.5 million tonnes with the productivity of 9.7 tonness/ha (NHB 2017). A salt sensitive, citrus is being grown in all the states of India and experiencing growth and yield reduction (Bowman 2004, Murkute *et al.* 2005). However, differences in relative tolerance of citrus rootstocks to salt stress have been previously reported (Murkute *et al.* 2005, 2006, 2009). Patil and Bhambota (1978) also reported that Cleopatra mandarin and Rangpur lime were found to be relatively salt tolerant rootstocks, while trifoliate orange and its hybrids, Carrizo and Troyer citranges were very sensitive to salinity.

In citrus '*Phytophthora* root rot' was observed to be usually severe in the areas where salinity is a serious problem (Grieves and Walker 1983). Although a salt tolerant rootstock is required on priority, the tolerance of selected salt tolerant rootstock to *Phytophthora* is also highly demanded. Hence, an attempt has been made to screen some hybrid rootstocks having trifoliate orange parentage and some selected citrus genotypes for salinity tolerance under central Indian conditions.

MATERIALS AND METHODS

Experiments were conducted during 2015–18 at containerized nursery of ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, consisted of 9 rootstock; 5 salinity levels, replicated thrice under factorial randomized block design. Nine rootstocks, viz. rough lemon (*Citrus taitensis* Risso), Rangpur lime [*Citrus limon* (L.) Burm. fil.], Alemow (*Citrus macrophylla*), NRCC 2 [Rough lemon × Troyer citrange (*Citrus aurantium* (L.) × *Citrus trifoliata* (L.)], NRCC 3 [Rough lemon × Troyer (*Citrus aurantium*

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(L.) × *Citrus trifoliata* (L.)], NRCC 6 [Rough lemon × trifoliate orange (*Citrus trifoliata* (L.)], CRH 12 [Rough lemon × trifoliate orange (*Citrus trifoliata* (L.)], Volkamer lemon (*Citrus volkameriana* Ten.) and Shekwasha (*Citrus reticulata* Blancoac) were used for study. Salinity levels 0, 20, 40, 60 and 80 mM were developed using salt combination [3 NaCl: 1 (1 $CaCl_2$: $1MgCl_2$)]. Plastic trays of 0.40 m × 0.30 m × 0.10 m (length × breadth × depth) size were used for growing the seedlings under primary nursery.

Seedling emergence, survival and growth: The appearance of shoot at the surface of growing medium was considered as emergence. Plant height was measured with measuring tape while stock diameter was measured with Vernier caliper.

Estimation of leaf nutrient content and physiological parameters: Composite leaf samples were used for analyzing total macronutrients, viz. N, P, K, Ca, Mg as well as for K using standard methods (Chapman and Pratt 1961). Leaf chlorophyll contents as SPAD value was recorded with Chlorophyll Fluorometer (Make WALZ Junior Pam).

Statistical analysis: To detect significant differences among different treatments and interactions, statistical analysis for shortest significant range tests (LSD) was performed using OP Stat (Sheoran *et al.* 1998).

RESULTS AND DISCUSSION

Seedlings emergence, survival and growth: The data on seedling emergence on 30^{th} day revealed that increasing salinity levels caused a gradual and significant decrease in seedling emergence in all the rootstocks. Seedlings emergence amongst different rootstocks varied from 21.7–100% amongst various salinity levels (Table 1). At highest salinity level of 80 mM, maximum seedling emergence (39.1%) was observed in Alemow, closely followed by Shekwasha (38.5%) and NRCC 6 (33.3%) rootstock. The depression and delay of seedlings emergence of citrus rootstocks was also reported by El-Desouky and Atawia (1998). Increasing reverse osmotic pressure and concomitant salt toxicity could be the predisposal factors to reduce the physico-chemical processes in seed germination (Murkute *et al.* 2005, 2010). Salinity affects germination or emergence of citrus seeds either by decreasing the osmotic potential of soil solution to a point which will prevent the uptake of water or by being toxic to the embryo and seedlings (Zekri 1993, El-Shazly 2008). Surprisingly lowest emergence was observed in commonly used rough lemon (21.7%) followed by CRH 12 (17.4%) at 80 mM salinity level. Kaushal *et al.* (2013) reported significant decrease in seed germination and growth of rough lemon seedlings with increase in salt concentration and at NaCl 0.6% concentration, there was no seed germination.

The data on survival potential of seedlings revealed that regardless of rootstocks, increasing salinity levels had adverse effect on plant survival (Table 1). It significantly varied from 50.4–91.7, 11.1–58.3, 7.7–47.8 and 0.0–34.8% at 20, 40, 60 and 80 mM, respectively and lowest percentage was observed at 80 mM level. At 80 mM level, highest

Table 1 Effect of salinity (NaCl) on emergence (%), survival (%) of the seedlings and seedling height (cm)

Rootstock	Seedling emergence (%)					Seedlings survival (%)					Seedling height (cm)				
	Control	20 mM	40 mM	60 mM	80 mM	Control	20 mM	40 mM	60 mM	80 mM	Control	20 mM	40 mM	60 mM	80 mM
Rough lemon	100.0	30.8	23.1	15.4	7.7	100.0	15.4	15.4	7.7	0.0	10.84	8.17	6.30	4.50	0.00
Rangpur lime	100.0	80.0	50.0	40.0	30.0	90.0	77.8	11.1	11.1	0.0	8.42	7.18	6.40	6.00	0.00
Alemow	100.0	100.0	60.9	47.8	39.1	100.0	78.9	52.2	47.8	34.8	11.52	9.65	9.38	6.18	5.86
NRCC 2	100.0	60.0	50.0	33.3	33.3	96.7	51.7	44.8	24.1	20.7	13.77	12.92	8.83	7.19	6.10
NRCC3	100.0	68.2	45.5	40.9	22.7	86.4	57.9	42.1	36.8	10.5	13.17	11.29	8.73	7.90	6.45
NRCC 6	100.0	86.7	46.7	43.3	33.3	100.0	86.7	46.7	30.0	26.7	14.53	10.42	8.25	6.18	5.38
CRS 12	100.0	47.8	39.1	26.1	17.4	87.0	40.0	30.0	25.0	10.0	6.01	5.57	4.40	3.03	1.73
Volkameriana	100.0	68.2	36.4	31.8	31.8	95.5	38.1	23.8	19.0	9.5	12.39	11.97	10.17	9.50	5.60
Shekwasha	100.0	92.3	61.5	46.2	38.5	92.3	91.7	58.3	41.7	33.3	13.51	10.68	9.66	9.26	6.91
Tukey's HSD at 5%															
Main plot treatment (Horizontal) means					6.21					6.21					2.45
Sub plot (Vertical) treatment means					3.75					3.75					1.05
Sub plot treatment means at same level of main plot treatment					11.24					11.24					3.16
Main plot treatment means at same or different level of sub plot treatment					11.75					11.75					3.71

seedling survival (34.8%) was observed in Alemow followed by Shekwasha (33.3%) and NRCC 6 (26.7%) rootstocks while seedlings of rough lemon and Rangpur lime were unable to survive at this salinity level. Earlier Arbona *et al.* (2003) also notified rough lemon sensitive to high salt tolerance. Such decrease in survival could be attributed to decreased photosynthesis rate and transpiration process. El-Desouky and Atawia (1998) stated that biological activities of endogenous phytohormones i.e. cytokinins, gibberellins and auxins in citrus plants, significantly decreases under salinity, reducing the plant survival. The data indicated that at 20 mM level, survival of Shekwasha (91.7%), NRCC 6 (86.7%), Alemow (78.9%) and Rangpur lime (77.8%) were least affected. While survival of CRH 12 (40.0%) and Volkamer lemon (38.1%) seedlings was less than 50%, so the critical salt level for these rootstocks can be considered as 20 mM. Earlier Volkamer lemon was reported as moderate in salt tolerance (Anjum *et al.* 2000). At 40 mM, only Shekwasha (58.3%) and Alemow (52.2%) rootstocks recorded survival of more than 50% seedlings and can be considered as critical limit for these rootstocks.

Effect of salinity on vegetative growth revealed a gradual decrease in growth parameters with increasing salinity levels. Plant height significantly varied from 6.01–14.53 cm, 5.57–12.92 cm, 4.40–10.17 cm, 3.03–9.50 cm and 0.0–6.91 cm at 0, 20, 40, 60 and 80 mM salinity levels, respectively (Table 1). With respect to rootstock effect, the data revealed that the maximum plant growth was observed in NRCC 6 (14.53 cm) at control, followed by Shekwasha (13.51 cm). Similarly, stock girth of the plants varied from 2.07–2.47 mm amongst different rootstock genotypes in control treatments and was highest under rough lemon rootstock (Table 2). The data on effect of salinity levels revealed that at 80 mM salinity level, decrease in plant height (48.9%) and stock girth (14.8%) was lowest in Shekwasha, closely followed by Alemow rootstock. Decreasing vegetative growth under saline conditions may be attributed to the effect of salinity in increasing osmotic pressure, thereby decreasing water and nutrient uptake which reflected in reducing plant growth (El-Shazy 2008).

Similarly, increasing salinity levels led to a significant increase in leaf defoliation amongst all the rootstocks. Number of leaves per plant varied from 10.5–15.8, 9.5–14.0, 8.33–14.8, 5.8–13.9 and 0.0–13.7 at 0, 20, 40, 60 and 80 mM, salinity levels, respectively (Table 2). Amongst the rootstock genotypes, number of leaves was maximum in Shekwasha. At 80 mM salinity level lowest defoliation was observed in Alemow (9.7%) followed by NRCC 6 (23.2%) rootstock. Decreasing leaf number due to salinity may be attributed to increased chloride contents in the leaves which defoliated later, as a way of eliminating Cl hazard (Garcia *et al.* 2002). It is also reported that one of the general effects of salinity is to reduce growth rate, resulting in smaller leaves and sometimes fewer leaves (Shannon and Grieve 2004).

Leaf chlorophyll content as indicated by SPAD values significantly varied from 0.469–0.739 amongst the treatments (Table 2). In rough lemon, Rangpur lime,

Table 2 Effect of salinity (NaCl) on stock diameter (mm), number of leaves and leaf chlorophyll content

Rootstock	Stock diameter (mm)					Number of leaves					Leaf chlorophyll content				
	Control	20 mM	40 mM	60 mM	80 mM	Control	20 mM	40 mM	60 mM	80 mM	Control	20 mM	40 mM	60 mM	80 mM
Rough lemon	2.47	2.20	2.15	2.05	0.00	13.48	11.00	10.00	9.00	0.00	0.697	0.674	0.632	0.632	0.572
Rangpur lime	2.25	2.22	2.10	1.99	0.00	12.67	11.00	8.33	8.00	0.00	0.710	0.704	0.679	0.679	0.638
Alemow	2.30	2.24	2.04	1.93	1.85	15.13	15.03	14.79	13.89	13.67	0.764	0.745	0.712	0.698	0.684
NRCC 2	2.34	2.12	2.02	1.81	1.70	12.33	10.63	10.11	9.11	7.67	0.725	0.703	0.691	0.622	0.592
NRCC3	2.20	2.03	1.90	1.82	1.76	14.12	11.11	10.67	9.56	9.33	0.726	0.722	0.601	0.581	0.469
NRCC 6	2.23	2.09	2.04	2.03	1.84	13.67	13.53	12.15	11.20	10.50	0.729	0.669	0.629	0.598	0.602
CRS 12	2.07	1.92	1.84	1.74	1.53	10.52	9.50	9.00	5.83	3.67	0.706	0.700	0.674	0.622	0.577
Volkameriana	2.30	2.25	2.15	1.94	1.81	14.25	14.00	13.33	12.83	10.33	0.739	0.663	0.652	0.600	0.558
Shekwasha	2.30	2.25	2.13	2.01	1.96	15.80	13.40	12.50	11.00	10.20	0.724	0.716	0.677	0.668	0.594
Tukey's HSD at 5%															
Main plot treatment (horizontal) means					0.24					2.56					0.07
Sub plot (vertical) treatment means					0.20					1.43					0.03
Sub plot treatment means at same level of main plot treatment					0.60					4.29					0.08
Main plot treatment means at same or different level of sub plot treatment					0.59					4.59					0.10

Alemow and Shekwasha rootstocks, influence of salinity levels on leaf chlorophyll content was comparatively less and recorded only 0.8–9.4% decrease at 20–60 mM salinity levels as compared to plants grown under control. At 80 mM salinity level, leaves of almost all the rootstocks looked yellowish having chlorophyll content in the range of 0.469–0.684 amongst the rootstock genotypes. Kaushal *et al.* (2013) reported severe injury symptoms of chlorosis as well as necrosis, which progressed rapidly in the tips along with the leaf margins and then advanced basipetally.

Leaf nutrient content: With respect to rootstock effect, the data revealed significant variation amongst the rootstock genotypes. Without salinity stress, maximum leaf content of N, P and Mg was observed in Shekwasha while K and Ca was in rough lemon rootstock (Table 3). The differential capacity of the rootstock for certain nutrient uptake was found related to inherent nutrient absorption characteristics of a particular rootstock under different agro-climatic conditions (Marathe *et al.* 2004) and to the physical differences among the root system (El-Shazly 2008).

There was a significant gradual decrease in leaf nutrient content and it was very significant at higher salinity levels. The data showed that leaf N and P content significantly varied from 1.32–2.46 and 0.024–0.106% respectively, amongst the treatments (Table 3). Decrease in leaf N content was comparatively less at 20 mM salinity level in most of the rootstocks except NRCC 3 and Volkamer lemon. At highest salinity level (80 mM) lowest reduction in leaf N content was observed in Alemow (25.0%) followed by NRCC 6 (27.7%) rootstock. Reduction in leaf P content was comparatively higher even at low salinity level. The highest reduction was observed in Volkamer lemon and NRCC 3 rootstocks at almost all the salinity levels. The reduction in leaf P was significantly low (46.4%) in Alemow followed by NRCC 6 (50.0%) rootstock.

Under salinity stress, K as an osmotic regulator plays an important role in maintaining the plant's osmotic potential especially in roots and its presence is necessary for water transportation through xylem and preservation of water in plant (Grattan and Grieve 1998). Higher concentration of K in tissues was one of the characteristics of salinity tolerant cultivars (Ai-Yassin 2004, Murkute *et al.* 2005). With regard to salinity levels, substantial reduction in K content (2.6–64.0%) in the leaves was observed in most of the rootstocks with increasing salinity levels except Alemow, NRCC 6 and Shekwasha. Rather, as compared to control, increased leaf K content was observed at 20 to 60 mM salinity levels in Alemow and NRCC 6 rootstock while in Shekwasha it was increased at 20 mM and decreased at all other salinity treatments. However, at 80 mM salinity level, decreased K content (12.8–64.0%) was observed in all the rootstocks. At this highest salinity level, lowest decrease in K content was observed in Alemow followed by Shekwasha and NRCC 6 rootstock. In citrus, reduced K uptake under saline conditions is caused by Na is well known competition process (Cedra *et al.* 1990). Leaf Ca content significantly varied between 0.0–3.26% amongst the

Table 3 Effect of salinity (NaCl) on nitrogen, phosphorous and potassium content in the leaves

Rootstock	Leaf nitrogen content					Leaf phosphorous content					Leaf potassium content				
	Control	20 mM	40 mM	60 mM	80 mM	Control	20 mM	40 mM	60 mM	80 mM	Control	20 mM	40 mM	60 mM	80 mM
Rough lemon	2.34	2.20	2.15	1.90	0.00	0.098	0.087	0.075	0.065	0.000	1.54	1.50	1.36	1.26	0.00
Rangpur lime	2.30	2.25	2.01	1.90	0.00	0.090	0.083	0.072	0.066	0.000	1.36	1.30	1.20	1.08	0.00
Alemow	2.40	2.30	2.04	1.90	1.80	0.112	0.106	0.092	0.076	0.060	1.49	1.54	1.60	1.50	1.30
NRCC 2	2.34	2.14	1.84	1.69	1.60	0.088	0.074	0.060	0.046	0.031	1.55	1.02	0.98	0.84	0.78
NRCC3	2.18	1.87	1.64	1.53	1.43	0.072	0.064	0.051	0.034	0.024	1.40	1.14	1.02	0.80	0.67
NRCC 6	2.22	2.10	1.84	1.66	1.56	0.082	0.074	0.062	0.048	0.041	1.30	1.40	1.52	1.60	1.05
CRS 12	2.01	1.84	1.74	1.46	1.32	0.074	0.062	0.055	0.046	0.034	1.20	1.04	0.94	0.72	0.61
Volkameriana	2.34	2.04	1.88	1.74	1.64	0.096	0.084	0.061	0.046	0.032	1.50	1.04	0.94	0.67	0.54
Shekwasha	2.54	2.46	2.35	1.85	1.66	0.108	0.094	0.077	0.061	0.054	1.40	1.44	1.34	1.26	1.20
Tukey's HSD at 5%															
Main plot treatment (horizontal) means					0.19					0.02					0.17
Sub plot (vertical) treatment means					0.08					0.01					0.11
Sub plot treatment means at same level of main plot treatment					0.25					0.02					0.33
Main plot treatment means at same or different level of sub plot treatment					0.29					0.03					0.34

rootstocks at different salinity levels (Supplementary Table 1). Such decrement can be attributed to the effect of salinity in reducing plant growth and inducing imbalance between nutrients. Variation in leaf content of Mg (0.21–0.55%) was less as compared to other nutrients. However lowest reduction was observed in Shekwasha rootstock.

In general, it was found that at highest salinity level (80 mM), lowest reduction in leaf content of N and P was observed in Alemow; K and Ca in NRCC 6 while Mg content was in Shekwasha rootstock. It was interesting to note that at lower salinity levels (up to 60 mM), adverse effect on commonly used rough lemon and Rangpur lime rootstock of central India was low as compared to other rootstock genotypes.

As a conclusion, the data of seed germination proved that salt tolerance is not a constant character in citrus rootstocks but varies with environment and plant development. It can be reported that the studied citrus rootstock genotypes can be classified as follows: Alemow, Shekwasha and NRCC 6 as tolerant rootstocks at all salinity levels; rough lemon and Rangpur lime at moderate salinity levels; NRCC 2 and NRCC 3 as moderately tolerant rootstocks; and Volkamer lemon, CRS 12 and NRCC 3 as sensitive rootstocks under the circumstances of present study.

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