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Saline irrigation induced changes in growth, physiology and ionic relations in Kinnow budded on *Jatti khatti* and *Sohsarkar*

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ABSTRACT

A pot culture experiment was conducted to examine the comparative performance of Kinnow budded on two seedling rootstocks, *i.e.*, *Jatti khatti* and *Sohsarkar* under saline irrigation. One-year-old Kinnow plants budded on these rootstocks were irrigated with water containing 50, 75 and 100 mM NaCl or tap water (control, 0.0 mM NaCl). Results indicated pronounced effect of salt stress on different physiological parameters and ionic relations. The effect was more pronounced for both rootstocks at 100 mM NaCl stress with respect to leaf number, RWC and chlorophyll fractions. At 75 and 100 mM NaCl, higher reductions in the total chlorophyll content (12.43 and 15.85%) were recorded in *Jatti khatti* as compared to *Sohsarkar* rootstock. Kinnow scions on *Jatti khatti* rootstock accumulated 13.63 and 11.85% more Na^+ and Cl^- at 75 and 100 mM NaCl stress than Kinnow scions on *Sohsarkar* rootstock. The increasing levels of NaCl concentrations inhibited the accumulation of N, P, K, and Mg in Kinnow leaves budded on *Jatti khatti* rootstock, while reduction of K and Ca was more in Kinnow budded on *Sohsarkar* rootstock at higher NaCl concentrations. We conclude that both rootstocks show potential for growing Kinnow mandarin where irrigation water salinity is ≤ 50 mM NaCl; *Sohsarkar* rootstock may be more suitable for use where NaCl concentration of irrigation water is ≥ 50 mM but less than (≤ 75 mM).

Key words: Chlorophyll, Kinnow, nutrient, rootstock, salinity stress.

INTRODUCTION

Citrus is one of the world's major fruit and is the third most important fruit crop in India after banana and mango. Among the citrus fruits, Kinnow mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora) is commercially grown in many parts of India due to its higher productivity, superior fruit quality and good returns to the growers. In particular, commercial Kinnow cultivation has emerged as an attractive option for the farmers of arid and semi-arid regions of northern India. Nevertheless, in recent past, the profitability and sustainability of Kinnow orchards in many traditional growing regions have been reduced, threatened by emerging constraints such as secondary salinity and increasing scarcity of irrigation water. As citrus is a salt sensitive crop and as saline irrigation reduces tree growth and fruit yield relatively more as compared to other crops, there is an urgent need to identify salt tolerant rootstocks by screening the diverse germplasm (Zekri and Parsons, 16; Dubey *et al*., 5). The salinity induced changes limit tree growth, flowering, fruit set and fruiting. Physiological and nutritional imbalances manifested as toxic accumulation of Na^+ and Cl^- ions, impaired water relations and osmotic stress account for inadequate tree vigour, low cropping and poor fruit quality in salt affected soils. Of late, the need to evaluate indigenous citrus species for salt tolerance and their future use as parents in citrus breeding programme as well as rootstocks on budding/ grafting has been highlighted (Singh *et al*., 14).

Although, *Jatti khatti* is widely used as rootstock for Kinnow mandarin in India, it is categorized as sensitive to salinity. Similarly, *Sohsarkar* has been recommended as vigorous rootstock for Kinnow. Since salinity is posing a threat for Kinnow cultivation due to secondary salinization from irrigation sources, the present experiment was undertaken to evaluate the comparative performance of Kinnow plants budded on *Jatti khatti* (*Citrus jambhiri* Lush.) and *Sohsarkar* (*Citrus karna* Raf.) rootstocks under graded levels of NaCl stress. This study assumes significance as an understanding of underlying physiological mechanisms recounting for salt tolerance may enable refinements in agronomic interventions to productively utilize saline water in Kinnow orchards.

MATERIALS AND METHODS

The pot experiment was conducted by applying four levels of NaCl [0.0 mM (control), 50, 75 and 100 mM] to Kinnow plants budded onto seedlings of *Jatti Khatti* and *Sohsarkar* rootstocks during December 2013 to March 2014 at the Division of Fruits and Horticultural Technology, IARI, New Delhi.

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One-year-old uniform sized budded plants of Kinnow were selected and transplanted from nursery to plastic pots (12 inches size) containing 8.0 kg of a 1:1:1 mixture of soil, sand and well decomposed farm yard manure. Each plant was given 15 g urea, 20 g single superphosphate and 10 g potassium sulphate fifteen days after transplanting. The mixture used in the pots had $EC_{(1:2)}$ of 0.20 dSm^{-1}, a pH of 7.2, a cation exchange capacity (CEC) of 10.65 cmol kg^{-1}, and an organic carbon content of 0.46%. Plants were irrigated for 30 days with normal water to enable initial establishment. The plants were then irrigated with different concentrations of NaCl, while the control plants were irrigated with tap water (pH, 7.2, EC = 0.42 dSm^{-1}) at weekly interval up to 90 days. The irrigation volumes were calculated by considering the loss of moisture from each pot, measured by directly weighing each pot.

Plant height and number of leaves per plant were recorded at 30 day intervals. After 90 days of the treatment, the leaf samples were analysed for determining the physiological changes and nutrient composition. The membrane injury index (MII) was estimated by the method of Blum and Ebercon (4), whereas, the method suggested by Barrs and Wheatherly (3) was employed to estimate the relative water content (RWC). Chlorophyll fractions (chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll) were estimated using the method of Hiscox and Israelstam (8). Leaf samples for mineral analysis were obtained by collecting composite sample of thirty leaves from all the direction from terminal 2 to 4 position of the plant. The collected leaves were thoroughly washed and oven dried at 65°C. The dried samples were then milled and subjected to analysis for N, P, K, Ca, Mg, Na and Cl. The samples were analysed for N using auto analyser (Isaac and Johnson, 9) and for P by vandate phosphomolybdate method (Jackson, 10). The Na and K contents of leaves were estimated using diacid [9:4 (v/v) HNO_3:$HClO_4$] digested samples by flame photometer following the method of Jackson (10). Ca and Mg were determined by atomic absorption spectrophotometer. The Cl^- ion content of leaves were quantified using mercuric (II) thiocynate (Adriano and Doner, 1). The experiment was conducted in Factorial Complete Randomised Design (FCRD) with three replications. Each replication consisted of three plants. Data were analysed using the SAS package 9.3 to calculate the F values. P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

Although rootstocks did not affect plant height, increasing NaCl concentration in irrigation water decreased plant height and the maximum decrease (18.94%) was recorded with the use of 100 mM NaCl water as compared to control (Table 1). Interaction between rootstock and salinity clearly indicated that significant decrease in plant height in Kinnow on *Jatti khatti* was recorded when the plants were treated with 100 mm NaCl water. Kinnow on Sohsarkar, however, did not exhibit any significant difference in plant height at different NaCl levels. Compared to control Kinnow on *Jatti khatti*, plant height was reduced by 23.15%. Number of leaves per plant significantly differed between the rootstocks and the highest number of leaves (85.53) were counted on Kinnow budded on Sohsarkar. Regardless of rootstocks, significant reduction in leaves/plant was noted when the plants were irrigated with water containing either 75 or 100 mM NaCl. From the interaction, it was evident that NaCl stress did not affect number of leaves (up to 75 mM) on either of the rootstock. However, significantly

Table 1. Effect of saline water stress on plant height, number of leaves, relative water content and membrane injury index in citrus rootstocks.

Treatment	Plant height (m)	No. of leaves $plant^{-1}$	RWC (%)	Membrane injury index
Rootstock				
Jatti khatti	0.84^{a}	81.83^{b}	91.97^{b}	0.19^{a}
Sohsarkar	0.89^{a}	85.83^{a}	92.74^{a}	0.18^{a}
Salinity				
Control	0.95^{a}	88.66^{a}	94.51^{a}	0.15^{c}
50 mM	0.90^{ba}	87.83^{a}	93.93^{a}	0.19^{b}
75 mM	0.86^{b}	83.66^{b}	91.28^{b}	0.20^{b}
100 mM	0.77^{c}	75.16^{c}	89.69^{c}	0.21^{a}
Rootstock × Salinity				
Jatti khatti				
Control	0.95^{a}	86.33^{ba}	94.85^{a}	0.14^{d}
50 mM	0.88^{ba}	85.66^{ba}	93.81^{a}	0.19^{ba}
75 mM	0.83^{ba}	82.00^{bc}	90.91^{b}	0.20^{ba}
100 mM	0.73^{b}	73.33^{d}	88.33^{c}	0.22^{a}
Sohsarkar				
Control	0.96^{a}	91.00^{a}	94.19^{a}	0.16^{dc}
50 mM	0.92^{a}	90.00^{a}	94.07^{a}	0.18^{bc}
75 mM	0.89^{ba}	85.33^{ba}	91.66^{b}	0.19^{ba}
100 mM	0.82^{ba}	77.00^{dc}	91.05^{b}	0.20^{ba}
LSD (P ≤ 0.05)				
Rootstock (R)	0.05	1.94	0.52	0.00
Salinity (S)	0.07	2.75	0.74	0.01
R × S	0.17	6.39	1.72	0.02

higher defoliation was noticed under 100 mM NaCl irrigation on both the rootstocks. As compared to non-salinised plants, significant reductions of 15.06 and 15.38% in leaf number were recorded in Kinnow on *Jatti khatti* and *Sohsarkar*, respectively at 100 mM NaCl stress. Visual symptoms of leaf injury characterized by tip burn, marginal scorch and withered appearance of the plant were also observed at 100 mM NaCl stress on both rootstocks. Reduction in plant height with increasing salinity, particularly in Kinnow on *Jatti khatti* seem to be due to the osmotic stress and increase in foliar concentration of Na^+ and Cl^- and reduced uptake of Mg^{2+}, which might have affected the chlorophyll pigments resulting in reduced photosynthesis and restricted growth in salinized Kinnow plants. Our findings support the view of Walker *et al*. (15) who proposed that osmotic effects and nutrients imbalances such as increase in foliar concentration of Na^+ and Cl^- and reduced uptake of Ca^{2+} and Mg^{2+} could play an important role in plant growth reduction under salt stress. Differences in salt tolerance of different rootstock have also been reported by (Kakade *et al*., 12; Singh *et al*., 14). Higher leaf abscission in Kinnow plants budded on *Jatti khatti* and *Sohsarkar* rootstocks at 100 mM NaCl stress indicates cellular damage which may be due to excessive absorption of Na^+ and Cl^- ions by the roots and subsequential accumulation in leaves. Salinity induced leaf abscission in citrus plants has also been reported by Zekri and Parsons (16).

The main effect of salinity and interaction effect between rootstock and salinity did not show any significant variation in the relative water content (RWC%) up to 50 mM NaCl stress but significant reductions were observed at 75 and 100 mM NaCl concentration. As compared to control, significant reductions of 3.41 and 5.09% were observed at 75 mM and 100 mM salinity levels respectively. The interaction effect between rootstock and salinity showed similar reduction in Kinnow budded on *Jatti khatti* rootstock exhibiting reductions of 4.15 and 6.87% at 75 and 100 mM NaCl stress, respectively. Reduction was also noticed in Kinnow budded on *Sohsarkar* rootstock but the values obtained at 75 and 100 mM NaCl stress were statistically at par with those obtained at 75 mM NaCl stress in Kinnow budded on *Jatti khatti*. The decrease in RWC seems related to high salt concentration of the external solution, which causes osmotic stress and dehydration at the cellular level (Greenway and Munns, 7). Earlier research on citrus (Patel *et al*., 13; Singh *et al*., 14) have also revealed progressive reductions in leaf RWC with increasing salinity.

The lowest MII was found in the control plants in both the stock-scion combinations with non significant difference. Interaction effect between rootstock and salinity levels as compared to control, exhibited increase in MII with increasing salinity but the differences were not significant. However, the data showed that the per cent increase in the MII at 50, 75 and 100 mM NaCl concentrations was to the tune of 35.71, 42.85 and 57.14% in Kinnow budded on *Jatti khatti* rootstock, while an increase of 12.5, 18.75 and 25.0% was recorded in Kinnow budded on *Sohsarkar*. These observations on differential membrane injuries in Kinnow plants budded on the two different rootstocks with more pronounced effects in *Jatti khatti* might be due to the difference in plasma membrane composition of the citrus rootstocks. The findings of the present study is in accordance with earlier findings of Dubey *et al*. (5) who reported that salinity induced membrane damage varies with the rootstock genotype.

Observations on chlorophyll fractions revealed significantly higher leaf chlorophyll *a* and *b* in Kinnow

Table 2. Effect of saline water stress on chlorophyll fractions in Kinnow budded on *Jatti khatti* and *Sohsarkar* rootstocks.

Treatment	Chlorophyll '*a*' (mg g^{-1} FW)	Chlorophyll '*b*' (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)
Rootstock			
Jatti khatti	1.45^{b}	0.27^{b}	1.73^{b}
Sohsarkar	1.58^{a}	0.30^{a}	1.88^{a}
Salinity			
Control	1.72^{a}	0.32^{a}	2.04^{a}
50 mM	1.60^{b}	0.32^{a}	1.93^{b}
75 mM	1.48^{c}	0.25^{b}	1.73^{c}
100 mM	1.28^{d}	0.23^{c}	1.51^{d}
Rootstock × Salinity			
Jatti khatti			
Control	1.67^{b}	0.31^{ba}	1.98^{b}
50 mM	1.58^{c}	0.33^{a}	1.91^{cd}
75 mM	1.39^{d}	0.23^{d}	1.62^{e}
100 mM	1.16^{e}	0.21^{d}	1.38^{f}
Sohsarkar			
Control	1.77^{a}	0.33^{a}	2.10^{a}
50 mM	1.62^{cb}	0.33^{a}	1.96^{cb}
75 mM	1.56^{c}	0.28^{bc}	1.85^{d}
100 mM	1.39^{d}	0.25^{dc}	1.64^{e}
LSD (P ≤ 0.05)			
Rootstock (R)	0.01	0.01	0.02
Salinity (S)	0.02	0.01	0.02
R × S	0.06	0.03	0.06

on *Sohsarkar* rootstock (Table 2). A general decrease in chlorophyll *a* and *b* concentration was noted with increasing NaCl concentrations and significantly lower contents of chlorophyll *a* and *b* were measured at 100 mM NaCl concentration. The interaction effects of rootstock and salinity level showed reduction in chlorophyll *a* and *b* contents for both the rootstocks but higher reduction was observed in Kinnow budded on *Jatti khatti* at each salinity level. As compared to non-salinised plants, reductions of 16.76% and 30.53% were recorded when the plants were irrigated with water containing 75 and 100 mM NaCl, whereas the corresponding values for *Sohsarkar* were 11.86 and 21.46%, respectively. The interaction effect of rootstock and salinity level on chlorophyll '*b*' contents were found to be non significant at 100 mM salinity levels in both stock-scion combinations. Total chlorophyll content in citrus rootstocks exhibited a trend similar to chlorophyll '*a*' with respect to rootstock and salinity levels. The mean effect of rootstock was found to be significant with the highest total chlorophyll content in Kinnow budded on *Sohsarkar* rootstock. The mean effect of salinity was also found to be significant. The minimum total chlorophyll content was recorded at 100 mM NaCl stress. The interaction effect of rootstock and salinity level revealed that salt stress induced a sharp reduction in total chlorophyll content. At 75 and 100 mM NaCl concentrations, reductions in total chlorophyll content in Kinnow budded on *Jatti khatti* rootstock were to the tune of 18.18 and 30.30%, respectively, while, in Kinnow budded on *Sohsarkar* corresponding values were 11.90 and 21.42% (Table 2). Chlorophyll is a membrane bound pigment and its integrity depends on membrane stability. As cell membranes are damaged under saline conditions, chlorophyll seldom remains intact. Reduction in chlorophyll could also be due to reduced activity of specific enzymes.

The mean effect of rootstock on leaf ionic relations (Table 3) revealed non-significant variations with respect to nitrogen content. Significant differences were, however, observed with respect to P, K, Ca, Mg, Na^+ and Cl^- concentrations. Increasing salinity reduced N, P, K, Ca and Mg but increased toxic Na^+ and Cl^- ions in leaf tissues. The increase was more apparent

Table 3. Effect of salinity levels on leaf ionic relations in Kinnow budded on *Jatti khatti* and *Sohsarkar* rootstocks.

Treatment	N	P	K	Ca	Mg	Na	Cl
Rootstock							
Jatti khatti	1.78^{a}	0.14^{b}	1.25^{a}	1.69^{a}	0.67^{a}	0.99^{a}	0.91^{a}
Sohsarkar	1.89^{a}	0.16^{a}	1.06^{b}	1.51^{b}	0.64^{b}	0.90^{b}	0.86^{b}
Salinity							
Control	2.03^{a}	0.18^{a}	1.89^{a}	2.28^{a}	0.91^{a}	0.36^{d}	0.70^{d}
50 mM	1.94^{b}	0.16^{b}	1.15^{b}	2.02^{b}	0.77^{b}	0.81^{c}	0.85^{c}
75 mM	1.75^{c}	0.15^{c}	0.92^{c}	1.32^{c}	0.62^{c}	1.17^{b}	0.96^{b}
100 mM	1.52^{d}	0.13^{d}	0.68^{d}	0.79^{d}	0.32^{d}	1.43^{a}	1.04^{a}
Rootstock × Salinity							
Jatti khatti							
Control	2.83^{a}	0.17^{ba}	1.95^{a}	2.36^{a}	0.94^{a}	0.34^{e}	0.72^{d}
50 mM	1.70^{c}	0.15^{c}	1.23^{c}	2.12^{b}	0.83^{b}	0.85^{d}	0.84^{c}
75 mM	1.34^{e}	0.14^{c}	0.97^{e}	1.45^{d}	0.65^{d}	1.25^{b}	0.98^{b}
100 mM	1.23^{f}	0.12^{d}	0.87^{f}	0.83^{f}	0.28^{f}	1.51^{a}	1.10^{a}
Sohsarkar							
Control	2.78^{a}	0.19^{a}	1.82^{b}	2.19^{b}	0.88^{ba}	0.38^{e}	0.68^{d}
50 mM	1.90^{b}	0.17^{b}	1.07^{d}	1.93^{c}	0.72^{c}	0.76^{d}	0.86^{c}
75 mM	1.45^{d}	0.16^{bc}	0.87^{f}	1.20^{e}	0.60^{d}	1.10^{c}	0.93^{b}
100 mM	1.34^{e}	0.14^{c}	0.49^{g}	0.74^{g}	0.36^{e}	1.35^{b}	0.98^{b}
LSD (P ≤ 0.05)							
Rootstock (R)	0.02	0.005	0.02	0.02	0.02	0.03	0.02
Salinity (S)	0.03	0.007	0.03	0.03	0.03	0.05	0.03
R × S	0.09	0.018	0.08	0.07	0.07	0.12	0.07

in case of Na^+ ion and the highest accumulation was noted in plants irrigated with 100 mm NaCl. Interaction effect between rootstock and salinity also exhibited significant differences. A general decrease in the nutrient content of leaf tissue was observed with increasing salinity and the decrease was more pronounced at 75 and 100 mM NaCl concentration in both rootstock-scion combinations. Comparative analysis of the data revealed that reduction in N, P, K, and Mg were more on Kinnow budded on *Jatti khatti*, while reductions in K and Ca contents were more in Kinnow budded on *Sohsarkar*. As regards Na^+ and Cl^- accumulation, it was also observed that significantly higher Na^+ accumulation was observed in the leaves of Kinnow budded on *Jatti khatti* rootstock which accumulated 13.63 and 11.85% more Na^+ than Kinnow on *Sohsarkar* at 75 and 100 mM NaCl stress, respectively. Although significant increase in chloride content was recorded with increasing salt concentration, the accumulative differences in both rootstock-scion combinations were statistically non significant at 0, 50 and 75 mM NaCl levels, but at 100 mM NaCl stress compared to Kinnow on *Sohsarkar* 12.24% more Cl^- accumulation was observed in Kinnow on *Jatti khatti* rootstock. Significantly higher reductions in leaf nutrients in Kinnow budded on *Jatti khatti* may be attributed to higher accumulation of Na^+ and Cl^- because *Jatti khatti* is reported to be a good accumulator of both Na^+ and Cl^- (Gonzalez *et al*., 6). Lower accumulation of Na^+ ions in tissue of Kinnow plants on *Sohsarkar* indicated that it could partially exclude Na^+ from leaves by accumulating Na^+ in roots. A similar mechanism has been observed in Cleoptera mandarin and sour orange by Jose *et al*. (11). Higher sodium and chloride accumulation in leaf tissues of Kinnow budded on *Jatti khatti* rootstock also showed a decrease in leaf nitrogen. A competition between Cl^- and NO_3^- N uptake may occur under salinity stress, which can decrease N concentration in leaves.

One of the primary plant responses to salinity is an influx of Na^+ and Cl^- and a decrease in K^+, Ca^{2+} and Mg^{2+} contents in plant tissues. In this study, the NaCl treated plants showed decrease in leaf K^+ in both the rootstock-scion combinations. Reductions in leaf K^+ concentration were however, more in Kinnow on *Sohsarkar* rootstock at 50 and 75 mM NaCl levels compared to Kinnow on *Jatti khatti* which may be due to direct effect of Na^+, displacing K^+ from the leaf tissues. Reduction in K^+ accumulation due to NaCl stress have also been reported by Anjum (2) on Cloeptera and Troyer rootstocks. Salt stress decreased Ca^{2+} and Mg^{2+} in the leaf tissue of both the rootstock-scion combinations with apparently higher reduction of Ca^{2+} in Kinnow budded on *Sohsarkar* rootstock, indicating that high Na^+ in the leaf tissue has displaced Ca^{2+} in the cell membrane altering their integrity. Based on these results, it is concluded that both the rootstocks can be utilized for growing Kinnow mandarin up to 50 mM NaCl, while, Kinnow on *Sohsarkar* can be grown up to 75 mM NaCl stress without much deleterious effects.

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