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Rootstock mediated alteration in morphology and photosystem in sweet orange (*Citrus sinensis*) scion cv. Pusa Sharad under NaCl stress

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ABSTRACT

There is a dearth of rootstock studies on how salinity stress imparts tolerance to the scion cultivar in citrus [*Citrus sinensis* (L.) Osbeck]. The impact of sodium chloride on sweet orange cv. Pusa Sharad (PS) grafted on 11 different rootstocks i.e. Jatti khatti (JK), X–639 (X9), CRH–12 (C12), NRCC–1 (N1), NRCC–2 (N2), NRCC–3 (N3), NRCC–4 (N4), NRCC–5 (N5), Troyer citrange (TC), CRH–47 (C47) and Cleopatra mandarin (CM) was evaluated at the nursery unit of ICAR-Indian Agricultural Research Institute, New Delhi, during 2019–22. Irrigation water containing 30 and 60 mM of sodium chloride (NaCl) was applied to scion/rootstock combinations in comparison to control (without NaCl) till the onset of salt injury symptoms i.e. 42 days. Under salinity stress, the PS scion grafted onto CM, X9, C47, N1, and N3 rootstocks exhibited minimum reduction in the scion height, leaf area ratio, root to shoot ratio, total chlorophyll content, total carotenoid content, transpiration rate, photosynthesis rate, internal CO_2 concentration and stomatal conductance as compared to PS scions grafted onto JK, C12, N2, N4, N5, and TC rootstocks under 60 mM NaCl stress. Results showed that specific rootstock can enhance salt-tolerance potential by increasing pigment content and strengthening the photosystem. PS scions grafted onto CM, C47, X9, N1, and N3 demonstrated greater NaCl tolerance compared to those grafted onto JK, C12, N2, N4, N5, and TC and hence recommended for areas having salinity level up to 60 mM.

Keywords: Correlation, Gas exchange parameters, NaCl, PCA, Salt damage index

Citrus [*Citrus sinensis* (L.) Osbeck] is grown globally and known for its nutritional value. The total global production of citrus during the year 2022–23 was 143.75 million tonnes (Anonymous 2023). Among citrus fruits, oranges share maximum production (50.56%) (Anonymous 2023). In recent years, the frequency of salinity stress has increased considerably due to indiscriminate use of saline water (Chen *et al*. 2017). Comparative analysis of the increase in the total area of sweet oranges in India between 2018–2019 to 2019–2020 was 16%, whereas the per cent increase between 2020–2021 to 2021–2022 was 7.83%, thus indicating a rapid decline in area (Anonymous 2023). High soil salinity disturbs the water and nutrient balance of citrus plants by accumulation of excess ions in their tissues (Awasthi *et al*. 2015, Alam *et al*. 2020). Such conditions cause cellular damage (Wu *et al*. 2019) thus impairing stomata closure (Lopez-Climent *et al*. 2008), reduced photosynthetic rate, internal CO_2 concentration, pigment content and transpiration rate (Kumar *et al*. 2021).

Grafting citrus plants has been found to significantly enhance their tolerance to sodium chloride stress in comparison to ungrafted plants. Grafted plants demonstrate improved growth characteristics as sour oranges rootstock is better Cl^- accumulator and transport the same in higher amount in the scion leaf tissue than Na^+ (Sykes 2011). Conversely, the tetrazyg rootstock is reported to restrict Cl^- and Na^+ from the scion leaf of Valencia (Grosser *et al*. 2012). Several researchers have reported that the ability of citrus species to tolerate salt is either genotypic or rootstock dependent (Alam *et al*. 2020, Vives-Peris *et al*. 2023). Hence, the interaction between scion/rootstock is complex and needs a thorough understanding on how salinity tolerance is transmitted from the rootstock to scion and *vice versa*. Based on the fact that rootstocks differ in their ability to varying degree of salinity stress, the present investigation was planned to test the performance of sweet orange cv. Pusa Sharad with respect to morpho-physiological and photosynthetic parameters grafted onto 11 different rootstocks.

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MATERIALS AND METHODS

Plant material and treatments: Present experiment was conducted at nursery unit of ICAR-Indian Agricultural Research Institute, New Delhi, during 2019–22. The fruits from the improved rootstock were collected from Central Citrus Research Institute (CCRI), Nagpur and the germplasm repository of the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi. These fruits were collected specifically for raising the rootstocks during December 2019. A total of 11 rootstocks (18 months old) (Table 1) were grafted with sweet orange scion cultivar Pusa Sharad during July 2021. After a period of 7 months, the grafted plants were acclimated to terracotta pots having top diameter 35 cm, height 35 cm, and bottom diameter 23 cm for 35 days prior to the imposition of stress. The 33 rootstock/scion combinations were arranged in a complete randomized design (CRD) as a factorial experiment with 3 replications. The experiment was conducted in the shade net house of the nursery unit of ICAR–IARI, New Delhi, from February to April 2022. Salt stress was induced by irrigating the plants with water containing either 30 mM or 60 mM of sodium chloride (NaCl). The NaCl solution was prepared on the basis of molecular weight by dissolving 1753.2 mg/L for 30 mM and 3506.4 mg/L for 60 mM of NaCl in tap water. For the control group, plants were watered (tap water) without NaCl having a *p*H of 6.8 and EC 0.29 dS/m. The watering schedule followed a 4-day interval, ensuring that the plants were maintained at field capacity. At the termination of the experiment i.e. after 42 days of initiation of the experiment, data were recorded and samples were collected for recording the observations.

Morphological parameters: The scion height (cm) was taken from graft union to the tip of the apical meristem; leaf area ratio (LAR) (cm^2/g of dry weight) was measured according to Radford (1967); the ratio of root to shoot of each plant was calculated by dividing the dry weight of root to the shoot. The salt damage index (SDI) was calculated as per Zhong *et al*. (2019) with certain modifications (Fig 1) as no damage (level 1:0-1), medium damage (level 2:1-2), high damage (level 3:2-3) and acute damage (level 4:3-4).

The salt damage index was calculated as:

SDI = (level value × no. of leaves)/total no. of leaves

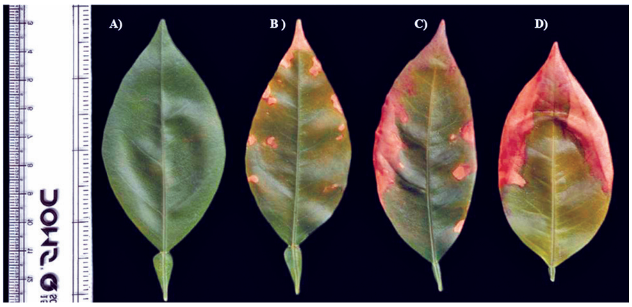

Fig 1 Rootstock induced grade scale of SDI of sweet orange scion Pusa Sharad as A. No damage; B. Medium damage; C. High damage; D. Acute damage.

Table 1 List of rootstocks on which sweet orange cv Pusa Sharad was grafted

Genotype	Parentage
JK	*Citrus jambhiri* Lush.
X9	*C. reshni* Hort. Ex Tanaka × *Poncirus trifoliata* (L.) Raf.
N1	*C. jambhiri* Lush. × [*C. sinensis* (L.) Osbeck × *P. trifoliata* (L.) Raf.]
N2	*C. jambhiri* Lush. × [*C. sinensis* (L.) Osbeck × *P. trifoliata* (L.) Raf.]
N3	*C. jambhiri* Lush. × *P. trifoliata* (L.) Raf.
N4	*C. jambhiri* Lush. × *P. trifoliata* (L.) Raf.
N5	*C. jambhiri* Lush. × [*C. sinensis* (L.) Osbeck × *P. trifoliata* (L.) Raf.].
C12	*C. limonia* (L.) Osbeck × *P. trifoliata* (L.) Raf.
TC	*C. sinensis* (L.) Osbeck × *P. trifoliata* (L.) Raf.
C47	*C. reshni* Hort. ex Tan. × *P. trifoliata* (L.) Raf.
CM	*C. reshni* Hort. ex Tan.

Physiological parameters: The membrane stability index (MSI) was measured as per the procedures outlined by Sairam *et al*. (1997). The carotenoid and total chlorophyll were estimated according to the method given by Hiscox and Israelstam (1979).

Photosystem parameters: Five fully expanded mature leaf measurements were recorded during 09:30–11:30 h on the different rootstock/scion combination of sweet orange cv. Pusa Sharad under ambient light with infrared gas analyser (IRGA). The photo system variables were determined as photosynthetic rate (*A*) (μmole CO_2/m^2/sec), transpiration rate (*E*) (m. mole H_2O/m^2/sec), stomatal conductance (g_s) (mole H_2O/m^2/sec) and intercellular CO_2 concentration (*Ci*) (μmole CO_2/mol) using LCi-SD, ADC (Pandey *et al*. 2017).

Statistical analysis: Data analysis was performed using the SAS software to compare the data between the stressed plants' rootstock/scion combinations and their respective controls. Significance level of $P \leq 0.001$ was used for statistical significance. In addition, Pearson correlation matrix and principal component analysis (PCA) were conducted using Python packages, including sklearn, bioinfokit, seaborn, scipy, numpy, pandas, and matplotlib.

RESULTS AND DISCUSSION

Morphological parameters: The data generated from the non-salinized treatment (control) could decipher that the choice of rootstock influence the morphological characteristics of the plants. Significant reductions were observed in the leaf area ratio (cm/g) and height of the sweet orange cv. Pusa Sharad when grafted onto JK (16.4 cm–14.5 cm; 23–14.4), C12 (10.8 cm–8.90 cm; 15.1–13.5), TC (11.10 cm–9.70 cm; 18.1–15.6), N2 (14.70–12.30 cm; 15.9–14.4) under both 30 mM and 60 mM NaCl stress as compared to control. However, the impact of NaCl on the growth of PS was relatively nominal at 60 mM when grafted onto X9 (13.6–13.2 cm; 18.5–18 cm^2/g), N1 (17.1–16.9 cm; 21.3–18.4 cm^2/g), C47 (13.2–13.1 cm; 26.1–21.4 cm^2/g) and CM (13.4–13.2 cm; 12.8–12.5 cm^2/g). The interaction effect between salinity vs rootstock had a significant impact on the leaf area ratio, with the lowest values observed in CM (12.5 cm^2/g), followed by N5 at 60 mM NaCl. The lowest height was documented in C12 (8.9 cm) at 60 mM NaCl for salinity and rootstock interaction effect on scion height. The rootstocks under investigation responded differently both under control and different levels of salinity stress i.e. 30 mM and 60 mM NaCl stress. The negative impact of NaCl stress on growth parameters as witnessed in the present study has also been reported in citrus by Perez-Tornero *et al*. (2009). The findings of the study clearly suggest that the impact of NaCl on scion leaf area varies with rootstocks whilst the tolerant rootstocks exhibiting larger leaf area, reduced carbon starvation, and increased survival rate and scion height. The root to shoot ratio was found to be the lowest in C47 (0.77) and at par with with N4 and X9. Conversely, the highest root to shoot ratio was observed in C12 (1.01). At ascending NaCl concentration of 60 mM, the root to shoot ratio increased significantly in C12 (1.12), TC (1.02), JK (0.97) and N5 (0.94). The interaction effect of NaCl on the root to shoot ratio was least pronounced in C47 (0.75) with N4 (0.75) and X9 (0.76) in the control group, while the maximum interaction effect was observed in C12 (1.12). Citrus trees' roots are essential for absorbing water and nutrients from the soil. In saline soil conditions, the osmotic potential of the soil solution increases, making it more challenging for the roots to extract water due to the damage in the root system. In case of PS grafted onto CM, C47, and X9, the allocation of more resources to shoot development must have enhanced photosynthetic capacity and increase water uptake through transpiration. The findings are in consonance with Karimi and Roosta (2014) in sour oranges at 40 mM NaCl. The SDI documented for PS on 11 different citrus genotypes, revealed the highest damage in C12, TC, and JK under 60 mM NaCl stress. At 30 mM NaCl, it was maximum in TC. However, no visual damage was observed in PS grafted onto X9, N1, N3, C47 and CM at varying level of NaCl stress. The interaction effect of salinity and rootstock was most pronounced in C12, followed by JK at 60 mM NaCl. The rootstocks JK, C12, and TC showed increased accumulation of Cl^- in the leaf tissues which must have restricted water and nutrient uptake and ultimately enhanced the production of reactive oxygen species (ROS) leading to chlorophyll degradation and decreased ATP synthesis (Yang *et al*. 2011).

Physiological parameters: The maximum and minimum electrolyte leakage was observed in JK (0.76), N4 (0.76), C12 (0.72) and C47 (0.15) respectively. Our findings indicate that an increase in NaCl stress enhances electrolyte leakage and may be attributed to membrane damage, protein degradation, lipid breakdown, reduced stomatal resistance and osmotic potential (Rolny *et al*. 2011, Ashraf and Harris 2004). Significant differences in carotenoid and total chlorophyll carotenoid content in leaf was observed between control and salt-stressed plants. PS grafted onto X9 (1.60 mg/g) recorded maximum chlorophyll content and was registered minimum in N2 (0.64 mg/g), followed by N5 (0.81) and C12 (0.82). In terms of the interaction effect between salinity and rootstock, JK (0.61 mg/g) exhibited the least chlorophyll content at 60 mM, while CM (1.63 mg/g) was comparable to X9 in the control group. The decrease in chlorophyll content at higher salinity levels indicates nutrient deficiency and chlorosis in the scion grafted onto susceptible rootstocks, leading to a decline in the photosystem (Hussain *et al*. 2012). Significant differences were observed in carotenoid content between control and salt stressed plants and was higher in PS grafted onto CM (0.21 mg/g), followed by X9 (0.21 mg/g) and C47 (0.20 mg/g) and lowest in JK (0.13 mg/g) whereas non-significant difference for interaction effect was observed for carotenoid content. It has been postulated that alteration in the ratio of chlorophyll composition has a significant effect on the light absorption and energy transfer processes in plants which alters the chlorophyll and carotenoid contents in the scion. Similar were the observations of Ashraf and Harris (2013) who speculated that susceptible plants tend to show a decrease in these pigments under high NaCl conditions. Reduced pigment production is caused by the disruption of vital enzymes involved in pathways for pigment synthesis caused by oxidative stress and ion imbalances brought on by salinity (Abdelmageed *et al*. 2018).

Photosystem: In the control condition, all PS grafted onto different rootstocks exhibited the higher values of stomatal conductance (*gs*), transpiration (*E*), leaf net photosynthesis (*A*) and intercellular CO_2 concentration (*Ci*) compared to the stressed treatments. After 42 days of NaCl stress, significant reduction was witnessed in the gas exchange parameters in PS grafted onto JK, C12, N2, N4, N5, TC, and N3 at 30 mM and 60 mM NaCl stress (Fig 2). The reduction in photosystem parameters at ascending level of salinity can be attributed to interference in osmotic regulation and damage to leaf stomatal regulation caused by toxic ions (Simpson *et al*. 2015). Our findings indicate that PS grafted onto CM, C47, X9, N1, and N3 were not significantly affected by 30 mM and 60 mM NaCl stress, thus suggesting lower Cl^- ion intoxication and a better functioning of photosynthetic machinery. The finding of the present study is in consonance with Vives-Peris *et al*. (2023) who observed no significant effect on photosynthetic parameters in Oronules mandarin grafted onto Cleopatra mandarin up to 90 mM NaCl stress.

Correlation analysis: The shoot height (SH) showed positive correlations with leaf area ratio (LAR), root: shoot ratio (RSR), chlorophyll content (CHL), photosynthesis (PHO), stomatal conductance (SC), intercellular CO_2 concentration (ICC) and transpiration (T). While, it exhibited negative correlations with SDI, MSI, and carotenoid content (CAR). LAR was found to have positive correlations with RSR, carotenoid content (CAR), leaf net photosynthesis (LNP), stomatal conductance (SC), ICC, and T, while it showed negative correlations with SDI and MSI. SDI was positively correlated with MSI, indicating an association between salt damage and membrane stability, while it showed negative correlations with other variables. CHL,

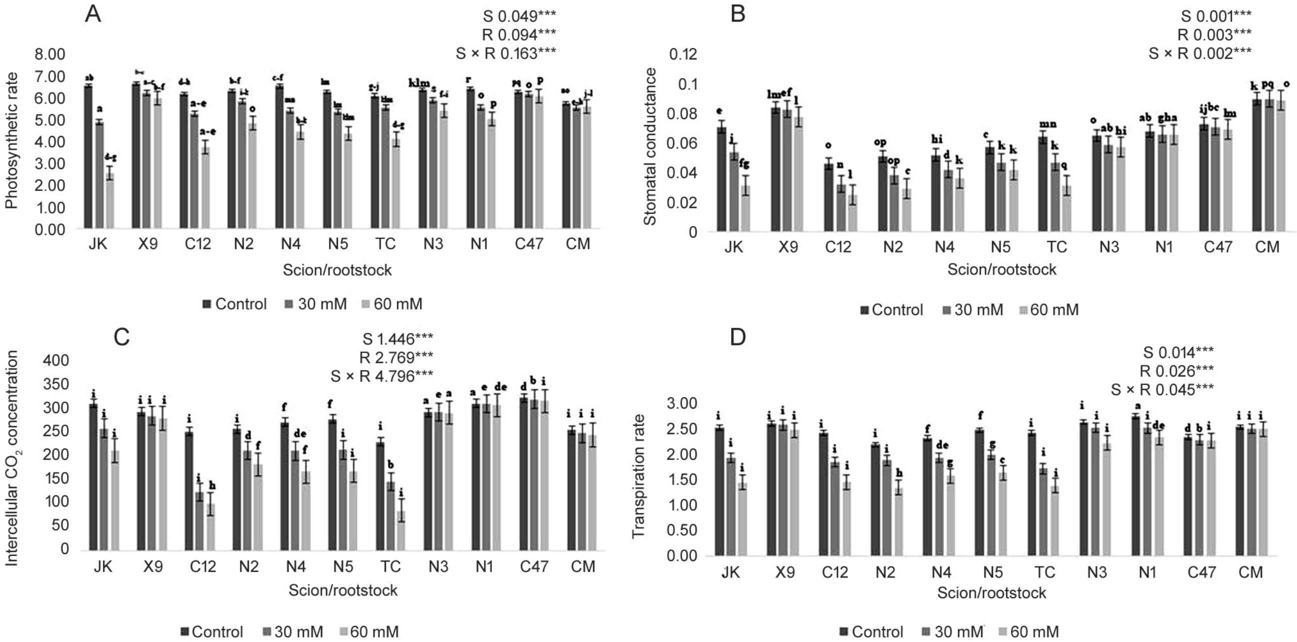

Fig 2 Photosystem: A. Photosynthetic rate (µmol $CO_2/m^2/s$) (*A*); B. Stomatal conductance (mol $H_2O/m^2/s$) (*gs*); C. Intercellular CO_2 concentration (µmol CO_2/mol) (*Ci*) and D. Transpiration rate (mmol $H_2O/m^2/s$) (*E*) of sweet orange cv Pusa Sharad (PS) grafted onto 11 different citrus genotypes.
Indication of letters are significant different among scion-rootstock combinations and *** indicate LSD at P≤0.001.

CAR, LNP and SC demonstrated positive correlations with ICC and T. These results suggest that under NaCl stress, the different scion/rootstock combinations lead to stomatal closure due to imbalances in ionic concentrations thus resulting in reduced rates of transpiration and photosynthesis (Lopez–Climent *et al*. 2008).

Principal component analysis (PCA): To understand the relationship between critical variables and the tolerance of scion/rootstock combinations at 30 mM and 60 mM NaCl stress, a PCA was performed. The first principal component 1 (PC1) accounted for 59.49% of the total variation. MSI had a positive contribution to PC1, while SH, LAR, ICC, RSR, and TR had negative contributions. The second component 2 (PC2) explained 13.65% of the variation, with SDI making a positive contribution, while CAR, SCHL and PHO had negative contributions. The PCA analysis clearly demonstrated a separation based on genotype and stress. The first component played a crucial role in distinguishing the data obtained from control and NaCl stressed scion/rootstock combinations. The position of the scion/rootstock

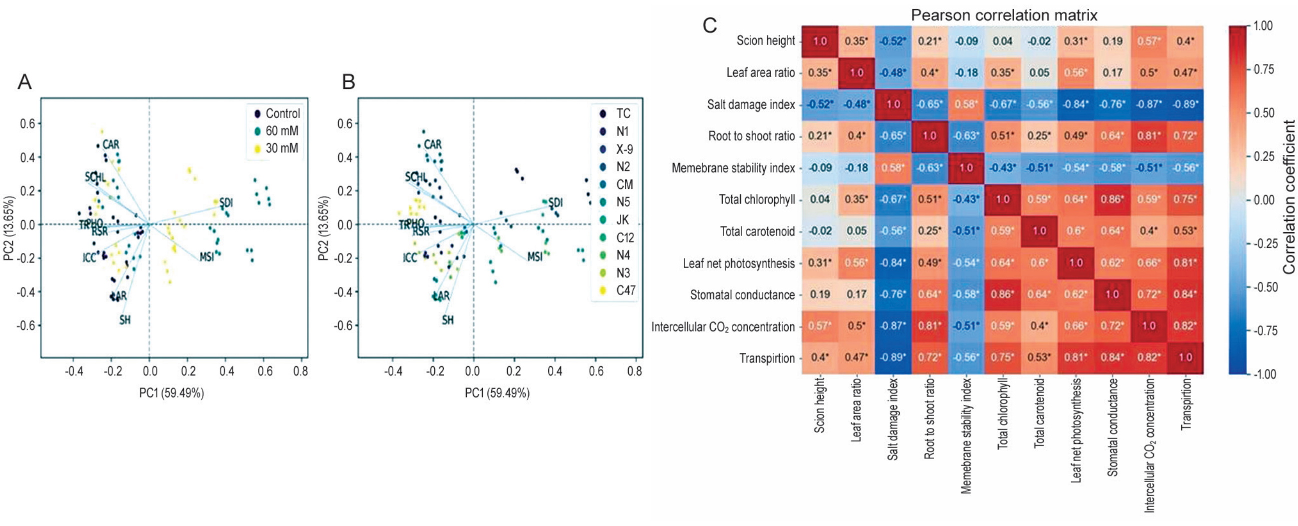

Fig 3 Principal component analysis (PCA) and Pearson's correlation matrix (C) with morphophysiological and photosynthetic parameters. A. Plot representing control (purple colour), 30 mM (yellow colour) and 60 mM (blue colour) different levels of salinity; B. Plot indicating different scion/rootstock combinations.
SH, Scion height; LAR, Leaf area ratio; SDI, Salt damage index; RSR, Root to shoot ratio; CHL, Total chlorophyll; CAR, Total carotenoid; PHO, Leaf net photosynthesis; SC, Stomatal conductance; ICC, Internal CO_2 concentration; T, Transpiration rate.

combinations in the PCA graph (Fig 3) showed that PS grafted onto JK exhibited the greatest differences between the control and 60 mM NaCl stress conditions. In contrast, PS grafted onto C47 showed only minor differences. We investigated eleven scion variable groups across four planes, representing positive and negative factor space. SDI was positioned on the positive plane on both axis, indicating a positive correlation. On the other hand, the other 10 variables showed a negative association with at least one variable on the negative axis. Vives-Peris *et al*. (2023) also reported similar results, where the greatest difference was observed between stressed and control conditions in the susceptible Navelina orange grafted onto Carrizo citrange, while the least difference was observed in the tolerant Navelina orange grafted onto *Citrus macrophylla*.

Based on our experiment, it is concluded that PS grafted onto JK, N4, N5 and TC showed tolerance up to 30 mM NaCl stress. PS grafted onto CM, C47, X9, N1, and N3 rootstocks exhibited perfect graft union, luxurious growth, and a strong photosystem in all the treatment combinations. These findings underscore the significance of rootstock selection in enhancing the salt stress resilience and paving the way for more efficient citrus cultivation in regions with challenging salinity conditions. It is recommended that areas which are less prone to salinity, JK, N4, and N5 can be used as rootstocks while CM, C47, X9, N1, and N3 can be utilized as rootstocks up to 60 mM NaCl stress for scion cultivar Pusa Sharad. Further research could delve into the underlying rootstock-dependent responses on Pusa Sharad for fruit quality parameters for commercialization.

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