

Under NaCl stress, the morphology and photosystem of the sweet orange (*Citrus sinensis*) scion cv. Pusa Sharad were altered by rootstock.

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ABSTRACT

Research on the rootstock that gives the citrus scion cultivar [*Citrus sinensis* (L.) Osbeck] resistance to saline stress is scarce. At the ICAR-Indian Agricultural Research Institute's nursery unit in New Delhi, the effects of sodium chloride on sweet orange cv. Pusa Sharad (PS) grafted on eleven distinct rootstocks—Jatti khatti (JK), X-639 (X9), CRH-12 (C12), NRCC-1 (N1), NRCC-2 (N2), NRCC-3 (N3), NRCC-4 (N4), and Cleopatra mandarin (CM). Scion/rootstock pairs were treated with irrigation water containing 30 and 60 mM sodium chloride (NaCl) as opposed to the control (no NaCl) until the start of salt damage symptoms, or 42 days. In comparison to PS scions grafted onto JK, C12, N2, N4, N5, and TC rootstocks under 60 mM NaCl stress, the PS scion grafted onto CM, X9, C47, N1, and N3 rootstocks showed the least reduction in scion height, leaf area ratio, root to shoot ratio, total chlorophyll content, total carotenoid content, transpiration rate, photosynthesis rate, internal CO₂ concentration, and stomatal conductance under salinity stress. The results demonstrated that by boosting pigment content and fortifying the photosystem, a particular rootstock may improve salt-tolerance capability. PS scions grafted onto CM, C47, X9, N1, and N3 showed higher NaCl tolerance than those grafted onto JK, C12, N2, N4, N5, and TC; as a result, they are advised for regions with salt levels up to 60 mM.

Keywords: PCA, NaCl, correlation, gas exchange parameters, and salt damage index

Introduction

Grown all throughout the world, citrus (*Citrus sinensis* (L.) Osbeck) is prized for its nutritious content. In 2022–2023, 143.75 million tons of citrus were produced worldwide (Anonymous 2023). Oranges have the highest production percentage of any citrus fruit (50.56%) (Anonymous 2023). Due to the careless usage of saline water, the frequency of salinity stress has significantly grown in recent years (Chen et al. 2017). According to a comparative research, the total area of sweet oranges in India increased by 16% between 2018–2019 and 2019–2020, whereas the percentage rise between 2020–2021 and 2021–2022 was 7.83%, suggesting a sharp decrease in area (Anonymous 2023). Citrus plants' water and nutritional balance is upset by high soil salinity because too many ions build up in their tissues (Awasthi et al. 2015, Alam et al. 2020). These circumstances impede ICAR-Indian Agricultural Research Institute, New Delhi; 2School of Biological Sciences, The University of Western Australia, Perth, Australia, by causing cellular damage (Wu et al. 2019). *Corresponding author email: awasthiciah@yahoo.com; decreased photosynthetic rate, internal CO₂ concentration, pigment content, and transpiration rate (Kumar et al. 2021); stomata closure (Lopez-Climent et al. 2008).

It has been shown that grafting citrus plants greatly increases their resistance to salt chloride stress as compared to ungrafted plants. Since sour orange rootstock is a superior Cl[−] accumulator and transports more of it in the scion leaf tissue than Na⁺, grafted plants exhibit enhanced growth characteristics (Sykes 2011). On the other hand, it has been found that the tetrazyg rootstock prevents Cl[−] and Na⁺ from entering the Valencia scion leaf (Grosser et al. 2012). According to many studies, citrus species' capacity to withstand salt is either rootstock-dependent or genotypic (Alam et al. 2020, Vives-Peris et al. 2023). Because of this, the relationship between scion and rootstock is intricate and requires a deep comprehension of the transmission of salt tolerance from rootstock to scion and vice versa. The current study was designed to evaluate the performance of sweet orange cultivar because rootstocks vary in their capacity to withstand different levels of saline stress. Pusa Sharad planted onto eleven different rootstocks in terms of morpho-physiological and photosynthetic characteristics.

METHODS AND MATERIALS

Plant materials and remedies: The current study was carried out from 2019 to 2022 at the ICAR-Indian

Agricultural Research Institute's nursery section in New Delhi. The 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, provided the fruits from the modified rootstock. In December 2019, these fruits were gathered especially for the purpose of growing the rootstocks. In July 2021, sweet orange scion cultivar Pusa Sharad was grafted onto 11 rootstocks (18 months old) (Table 1). After seven months, the grafted plants were acclimated to clay pots with a top diameter of 35 cm, height of 35 cm, and bottom diameter of 23 cm for thirty-five days before stress was applied. The 33 rootstock/scion combinations were set up as a factorial experiment with three replications using a fully randomized design (CRD). From February to April 2022, the experiment was carried out in the shade net house of the ICAR-IARI nursery unit in New Delhi. The plants were irrigated with water containing either 30 mM or 60 mM sodium chloride (NaCl) to produce salt stress. By dissolving 1753.2 mg/L for 30 mM and 3506.4 mg/L for 60 mM of NaCl in tap water, the NaCl solution was created based on molecular weight. Plants in the control group received tap water with a pH of 6.8 and an EC of 0.29 dS/m without NaCl. In order to keep the plants at field capacity, the watering schedule was spaced out by four days. Data were recorded and samples were gathered to record the findings at the experiment's conclusion, which was 42 days after it began.

Morphological parameters: the scion height (cm) was measured from the graft union to the tip of the apical meristem; the leaf area ratio (LAR) (cm²/g of dry weight) was measured in accordance with Radford (1967); and each plant's root to shoot ratio was computed by dividing the dry weight of the root by the shoot. The SDI, or salt damage index, was computed as

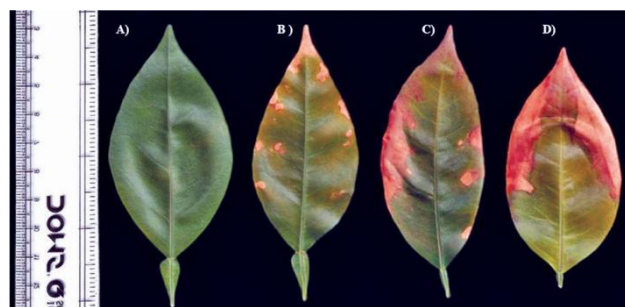
Table 1 List of rootstocks on which sweet orange cv Pusa Sharad was grafted

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.

according to Zhong et al. (2019) with certain adjustments (Fig. 1) as acute damage (level 4:3-4), medium damage (level 2:1-2), heavy damage (level 3:2-3), and no harm (level 1:0-1).

The formula for calculating the salt damage index was:

SDI is equal to (level value × number of leaves)/total number of leaves.



Sweet orange scion Pusa Sharad's rootstock-induced SDI grade scale is shown in Figure 1 as A. No damage, B. Medium damage, C. High damage, and D. Acute damage.

Physiological parameters: The methods described by Sairam et al. (1997) were used to assess the membrane stability index (MSI). The technique described by Hiscox and Israelstam (1979) was used to estimate the carotenoid and total chlorophyll.

Photosystem parameters: Using an infrared gas analyzer (IRGA), five fully expanded mature leaf measurements were taken between 09:30 and 11:30 hours on various rootstock/scion combinations of sweet orange cv. Pusa Sharad in ambient light. Using LCi-SD and ADC, the photo system variables were calculated as follows: photosynthetic rate (A) ($\mu\text{mole CO}_2/\text{m}^2/\text{sec}$), transpiration rate (E) ($\text{m. mole H}_2\text{O}/\text{m}^2/\text{sec}$), stomatal conductance (gs) ($\text{mole H}_2\text{O}/\text{m}^2/\text{sec}$), and intercellular CO_2 concentration (Ci) ($\mu\text{mole CO}_2/\text{mol}$) (Pandey et al. 2017).

Analysis of statistics: To compare the data between the rootstock/scion combinations of the stressed plants and their corresponding controls, data analysis was done using the SAS program. For statistical significance, a significance threshold of $P \leq 0.001$ was used. Additionally, Python tools such as sklearn, bioinfokit, seaborn, scipy, numpy, pandas, and matplotlib were used to perform principal component analysis (PCA) and Pearson correlation matrix.

OUTCOMES AND TALK

Morphological characteristics: The findings from the non-salinized treatment (control) may indicate that the plants' morphological traits are influenced by the rootstock selection. 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), and N2 (14.70–12.30 cm; 15.9–14.4) under both 30 mM and 60 mM NaCl stress. However, when grafted onto X9 (13.6–13.2 cm; 18.5–18 cm²/g), N1 (17.1–16.9 cm; 21.3–18.4 cm²/g), C47 (13.2–13.1 cm; 26.1–21.4 cm²/g), and CM (13.4–13.2 cm; 12.8–12.5 cm²/g), the effect of NaCl was comparatively minimal at 60 mM. The impact of interaction

The leaf area ratio was significantly affected by the ratio of salinity to rootstock; CM had the lowest values (12.5 cm²/g), followed by N5 at 60 mM NaCl. In terms of the salinity and rootstock interaction impact on scion height, C12 had the lowest height (8.9 cm) at 60 mM NaCl. The investigated rootstocks reacted differentially under control and at various salinity stress levels, such as 30 mM and 60 mM NaCl stress. Perez-Tornero et al. (2009) have also shown the detrimental effects of NaCl stress on development metrics in citrus. The study's findings unequivocally indicate that the effects of NaCl on scion leaf area differ depending on the rootstock, with tolerant rootstocks showing more leaf area, less carbon famine, and higher survival rates and scion height. It was discovered that C47 had the lowest root to shoot ratio (0.77), comparable to N4 and X9. On the other hand, C12 had the greatest root to shoot ratio (1.01). The root to shoot ratio dramatically rose in C12 (1.12), TC (1.02), JK (0.97), and N5 (0.94) at increasing NaCl concentrations of 60 mM. In the control group, C47 (0.75), N4 (0.75), and X9 (0.76) showed the least amount of the NaCl interaction impact on the root to shoot ratio, while C12 (1.12) showed the greatest interaction effect. The roots of citrus plants are crucial for taking up water and nutrients from the ground. Because of the damage to the root system, the osmotic potential of the soil solution rises under salty soil conditions, making it more difficult for the roots to collect water. The allocation of additional resources to shoot growth in the case of PS grafted onto CM, C47, and X9 must have improved photosynthetic capability and increased water absorption via transpiration. The results are consistent with those of Karimi and Roosta (2014) in sour oranges at 40 mM NaCl. The maximum damage was seen in C12, TC, and JK under 60 mM NaCl stress, according to the SDI recorded for PS on 11 distinct citrus genotypes. It peaked in

TC at 30 mM NaCl. Nevertheless, PS grafted onto X9, N1, N3, C47, and CM at different levels of NaCl stress showed no visible damage. At 60 mM NaCl, C12 showed the strongest relationship between salinity and rootstock, followed by JK. Increased Cl-accumulation in the leaf tissues of the rootstocks JK, C12, and TC must have limited the intake of water and nutrients and eventually increased the generation of reactive oxygen species (ROS), which resulted in the degradation of chlorophyll and a reduction in ATP synthesis (Yang et al. 2011).

Physiological characteristics: JK (0.76), N4 (0.76), C12 (0.72), and C47 (0.15) showed the highest and lowest electrolyte leakage, respectively. According to our research, a rise in NaCl stress increases electrolyte leakage, which may be caused by lower stomatal resistance, osmotic potential, membrane damage, protein degradation, and lipid breakdown (Rolny et al. 2011, Ashraf and Harris 2004). Between control and salt-stressed plants, there were notable variations in the amount of carotenoid and total chlorophyll carotenoid in the leaf. PS grafted onto X9 (1.60 mg/g) had the highest chlorophyll content and the lowest N2 concentration (0.64 mg/g), which was followed by

C12 (0.82) and N5 (0.81). JK (0.61 mg/g) showed the lowest chlorophyll content at 60 mM in the interaction effect between salinity and rootstock, while CM (1.63 mg/g) was similar to X9 in the control group. Higher saline levels cause the scion grafted onto vulnerable rootstocks to experience nutritional deprivation and chlorosis, which results in a deterioration in the photosystem (Hussain et al. 2012). Carotenoid content varied significantly between control and salt-stressed plants; it was highest 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). However, there was no significant difference for the interaction effect. It has been hypothesized that changes in the ratio of chlorophyll composition have a major impact on how plants absorb light and transmit energy, which changes the amount of chlorophyll and carotenoid in the scion. Similar findings were made by Ashraf and Harris (2013), who hypothesized that in high NaCl circumstances, sensitive plants often exhibit a drop in these pigments. Reduced pigment production results from oxidative stress and ion imbalances brought on by salt, which disrupt essential enzymes involved in pigment synthesis pathways (Abdelmageed et al. 2018).

Photosystem: under comparison to the stressed treatments, all PS grafted onto various rootstocks showed greater values of stomatal conductance (gs), transpiration (E), leaf net photosynthesis (A), and intercellular CO₂ concentration (Ci) under the control condition. The gas exchange characteristics in PS grafted onto JK, C12, N2, N4, N5, TC, and N3 under 30 mM and 60 mM NaCl stress were significantly reduced after 42 days of NaCl stress (Fig 2). Toxic ion damage to leaf stomatal control and interference with osmotic regulation are responsible for the decrease in photosystem parameters with increasing salinity levels (Simpson et al. 2015). Our results show that 30 mM and 60 mM NaCl stress did not substantially damage PS grafted onto CM, C47, X9, N1, and N3, indicating less Cl-ion intoxication and improved photosynthetic apparatus performance. The current study's results are consistent with those of Vives-Peris et al. (2023), who found no discernible impact on photosynthetic metrics in Oronules mandarin grafted onto Cleopatra mandarin up to 90 mM NaCl stress.

Analysis of correlation: Leaf area ratio (LAR), root: shoot ratio (RSR), chlorophyll content (CHL), photosynthesis (PHO), stomatal conductance (SC), intercellular CO₂ concentration (ICC), and transpiration (T) all exhibited positive associations with shoot height (SH). However, it showed unfavorable relationships with carotenoid content (CAR), MSI, and SDI. It was discovered that LAR had negative connections with SDI and MSI and positive associations with RSR, carotenoid content (CAR), leaf net photosynthesis (LNP), stomatal conductance (SC), ICC, and T. While SDI exhibited negative relationships with other variables, it was positively connected with MSI, suggesting a relationship between salt damage and membrane stability. CHL

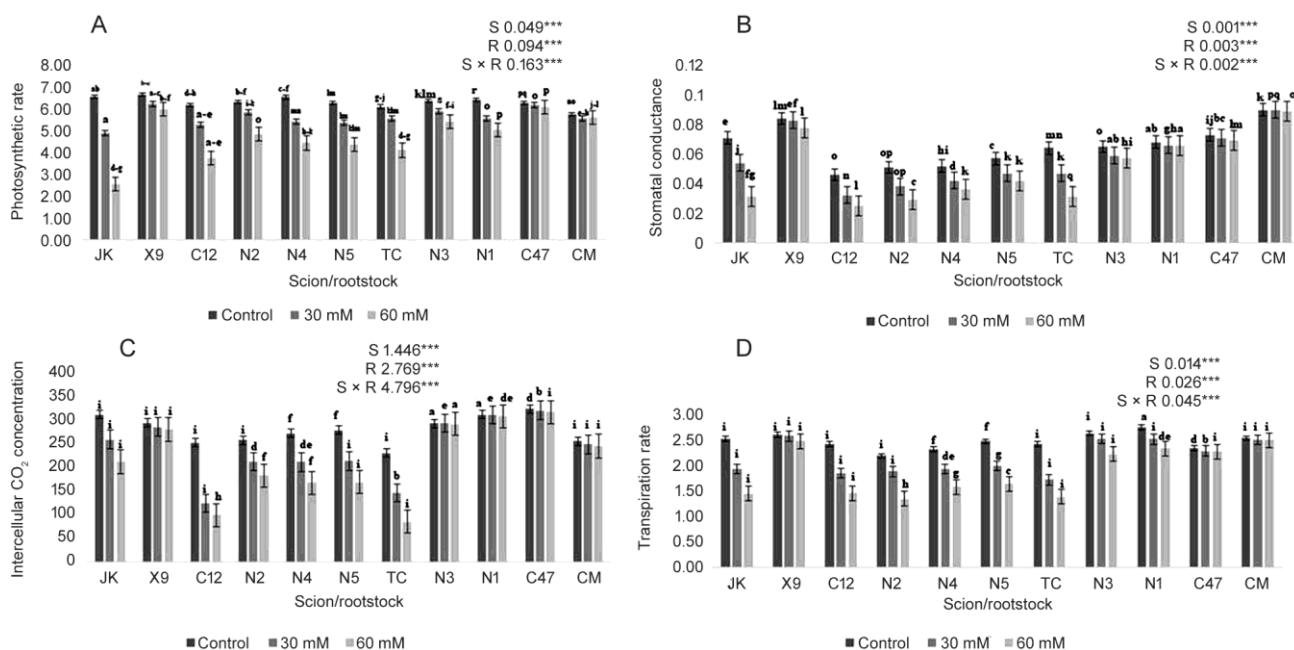


Figure 2 shows the photosynthetic rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$) (A), stomatal conductance ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$) (gs), intercellular CO_2 concentration ($\mu\text{mol CO}_2/\text{mol}$) (C_i), and transpiration rate ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$) (E) of sweet orange cv. Pusa Sharad (PS) grafted onto 11 distinct citrus genotypes.

Significant differences between scion-rootstock combinations are shown by letters, and LSD at $P \leq 0.001$ is indicated by ***.

ICC and T showed favorable relationships with CAR, LNP, and SC. These findings imply that under NaCl stress, the various scion/rootstock combinations cause stomatal closure as a consequence of ionic concentration imbalances, which lowers transpiration and photosynthesis rates (Lopez-Climent et al. 2008).

Principal component analysis (PCA): A PCA was carried out in order to comprehend the connection between essential factors and the tolerance of scion/rootstock combinations under 30 mM and 60 mM NaCl stress. 59.49% of the variance was explained by the first principal component 1 (PC1). While SH, LAR, ICC, RSR, and TR contributed negatively to PC1, MSI contributed positively. 13.65% of the variance was explained by the second component 2 (PC2), with SDI contributing positively and CAR, SCHL, and PHO contributing negatively. A clear differentiation depending on stress and genotype was shown by the PCA analysis. In order to differentiate the data from control and NaCl-stressed scion/rootstock combinations, the first component was essential. The location of the rootstock or scion

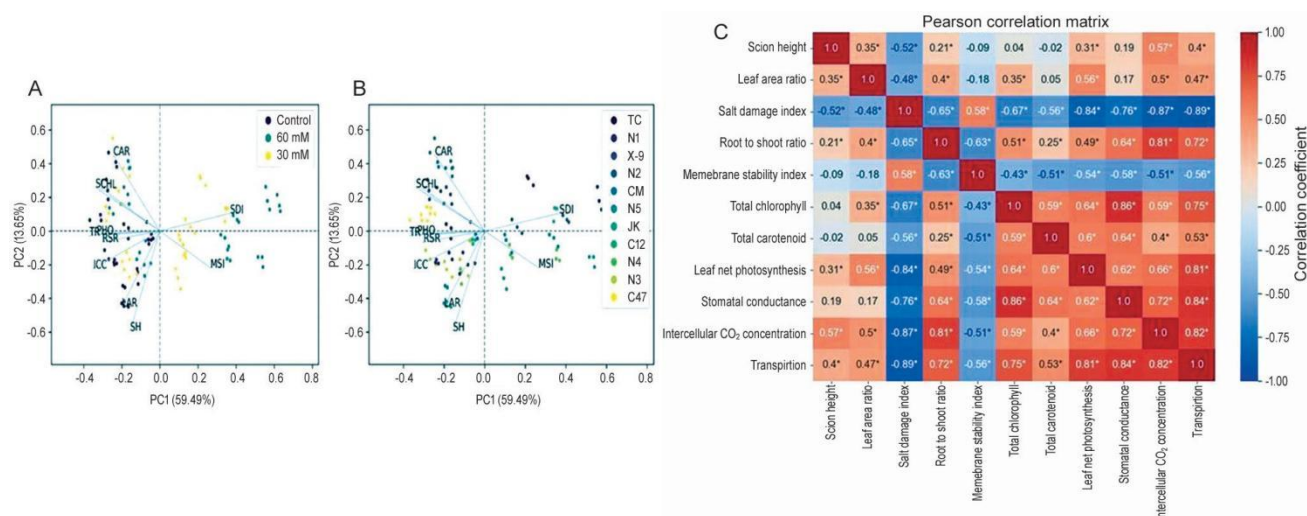


Figure 3 shows morphophysiological and photosynthetic characteristics together with Pearson's correlation

matrix (C) and principal component analysis (PCA).

Plot showing the control (purple), 30 mM (yellow), and 60 mM (blue) salinity levels; B. Plot showing the various scion/rootstock combinations.

SH stands for scion height; LAR for leaf area ratio; SDI for salt damage index; RSR for root to shoot ratio; CHL for total chlorophyll; CAR for total carotenoid; PHO for leaf net photosynthesis; SC for stomatal conductance; ICC for internal CO₂ concentration; and T for transpiration rate.

PS grafted onto JK demonstrated the most changes between the control and 60 mM NaCl stress conditions, according to combinations in the PCA graph (Fig. 3). PS grafted onto C47, on the other hand, just little changed. Eleven unique variable groups representing positive and negative factor space were examined in four planes. A positive association was shown by SDI's placement on both axes' positive planes. Conversely, at least one variable on the negative axis was negatively correlated with the other ten variables. Similar findings were also reported by Vives-Peris et al. (2023), who found that the susceptible Navelina orange grafted onto Carrizo citrange showed the biggest difference between stressed and control conditions, while the tolerant Navelina orange grafted onto Citrus macrophylla showed the least difference.

Our work indicates that PS grafted onto JK, N4, N5, and TC shown tolerance up to 30 mM NaCl stress. In every treatment combination, PS grafted onto CM, C47, X9, N1, and N3 rootstocks demonstrated flawless graft union, opulent growth, and a robust photosystem. These results highlight the importance of rootstock selection in improving salt stress tolerance and opening the door to more productive citrus production in areas with difficult salinity conditions. While CM, C47, X9, N1, and N3 may be used as rootstocks up to 60 mM NaCl stress for the scion cultivar Pusa Sharad, JK, N4, and N5—areas that are less susceptible to salinity—can be used as rootstocks. The underlying rootstock-dependent reactions on Pusa Sharad for fruit quality criteria for commercialization might be investigated further.

REFERENCES

- Ahn Y K, Ji K, Ksiksi T S, and Abdulmageed A H A. 2018. The accumulation of carotenoids and the expression patterns of important genes in orange peel are affected by salt stress. *Plant Growth Regulation Journal* 37(3): 1092–1102.
- Alam A, Datta A, Ullah H, and Attia A. 2020. Salinity stress's effects on three citrus rootstock seedlings' development, mineral nutrient accumulation, and metabolic indicators. *Fruit Science International Journal* 20(4): 786–804.
- Agricultural Statistics at a Glance 2022, Anonymous, 2023. Area and Horticultural Crop Production. The Indian government. Division of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, pages. 92.
- Harris P. J. C. and Ashraf M. 2004. Possible biochemical markers of plants' ability to withstand salt. *Plant Science* 166: 3–16.
- Harris, P. J., and Ashraf M. (2013). An summary of photosynthesis under difficult conditions. *Photosynthetica* 51: 163–90.
- In 2015, Awasthi O P, Dubey A K, Sharma V K, Prakash J, Goswami A K, and Sharma R M. Growth, physiology, and ionic relations in Kinnow budded on Jatti Khatti and Sohsarkar were altered by saline irrigation. 313–18 in *Indian Journal of Horticulture* 72(3).
- In 2017, Chen L, Li C, Feng Q, Wei Y, Zheng H, Zhao Y, and Li H. changes in community structures and soil microbial metabolic activity along an irrigation water salinity gradient in a typical dry area of China. *Science of the Whole Environment* 598: 64–70.
- Gmitter J A, Omar A A, and Grosser J W. 2012. Valencia orange trees with allotetraploid rootstocks are tolerant to salinity. (In)
- Florida State Horticultural Society Proceedings, Vol. 125, pp. 50–55.
- Israelstam G. F. and Hiscox J. D. 1979. Chlorophyll may be extracted from leaf tissue without the need for maceration. Hussain S, Luro F, Costantino G, Ollitrault P, and Morillon R. *Canadian Journal of Botany* 57(12): 1332–34.
2012. Low chloride buildup as a sign of salt tolerance: a physiological study of citrus species and genera' responses to salt stress. *Journal of Botany in South Africa* 81: 103–112.
- In 2014, Karimi H R and Roosta M K. *Journal of Stress Physiology and Biochemistry* 10(3): 5–17.
- Kumar S, Awasthi O P, Sharma R M, and Pradhan S 2021. Inter-specific hybrid of *P. atlantica* and *P. vera* l. cv. Badamiriz-e-zarand as pistachio rootstock to salinity stress according to some growth indices and echo-physiological and biochemical parameters. Kinnow mandarin (*Citrus nobilis* × *Citrus deliciosa*) physiological and biochemical reactions to mutagenesis caused by EMS. 1015–19 in *The Indian Journal of Agricultural Sciences*, 91(7). Perez-

- Clemente R M, Gomez-Cadenas A, Lopez-Climent M F, and Arbona V. 2008. Citrus photosynthetic machinery performance and salt tolerance are related. 176–84 in *Environmental and Experimental Botany* 62(2).
- Singh M P, Paul V, and Pandey K. (2017). Manual of an ICAR-sponsored training course on physiological methods to assess how agricultural plants are affected by climate change, January 16–25, 2017. Plant Physiology Division, IARI, New Delhi.
- Perez-Tornero O, Navarro J M, Tallon C I, and Porras I. 2009. Salinity causes physiological and growth alterations in micropropagated Citrus macrophylla explants. *Plant Physiology Journal* 166(17): 1923–33.
- P. J. Radford, 1967. The use and misuse of growth analysis formulas. 171–75 in *Crop Science* 7(3).
- Carrion C, Rolny N, Costa L, and Guamet J J. 2011. Is the electrolyte leakage assay a clear indicator of membrane degradation during leaf senescence? *Plant Biochemistry and Physiology* 49: 1220–27.
- Saxena D C, Shukla D S, and Sairam R K. 1997. Wheat genotypes' resistance to drought is correlated with stress-induced damage and antioxidant enzymes. *Plantarum Biologia* 40: 357–64.
- In 2015, Simpson C R, Nelson S D, Melgar J C, Jifon J, Schuster G, and Volder A. Salinity's effects on citrus trees' physiological characteristics, both grafted and ungrafted. *Scientia Horticulturae* 197: 483-89.
- Sykes, S. R. (2011). Citrus rootstock germplasm brought to Australia from the People's Republic of China has the ability to exclude salt and chloride. 443–49 in *Scientia Horticulturae* 128(4).
- Lopez-Climent M F, Moliner-Sabater M, Gomez-Cadenas A, Vives-Peris V, and Perez-Clemente R M. 2023. Grafted citrus plants' ability to withstand salt stress depends on their morphological, physiological, and molecular scion characteristics. *Plant Science Frontiers* 14: 1145625.
- In 2019, Wu L, Chen J, Chen L, Wang J, Zhang H, Gao Q, and Deng X. effects of salt on citrus fruit quality, growth, physiological response, and yield. Yang J Y, Zheng W, Tian Y, Wu Y, and Zhou D W. *PLoS One* 14(1): e0210115. 2011. effects of different mixed salt-alkaline stressors on *Medicago ruthenica* seedlings' growth, photosynthetic pigment concentrations, and photosynthesis. 275–84 in *Photosynthetica* 49.
- In 2019, Zhong Y P, Qi X J, Chen J Y, Zhi L I, Bai D F, Wei C G, and Fang J B. Four kiwifruit genotypes' growth and physiological reactions to salt stress and resistance assessment. *Integrative Agriculture Journal*, 18(1), 83–95.