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IMPACT OF VARIOUS CONCENTRATIONS OF NACL ON MORPHOLOGICAL ATTRIBUTES OF DIFFERENT CITRUS ROOTSTOCKS UNDER FIELD CONDITIONS

KHAN F[1], SHAFIQ M[1], HAIDER MZ[2*], SAMI A[2], ARSHAD A[1], ANEES MM[1]

[1]*Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, P.O BOX. 54590, Lahore, Pakistan*
[2]*Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, P.O BOX. 54590, Lahore, Pakistan*
**Correspondence author email address:* zeech280@gmail.com

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Abstract *Citrus (Poncirus trifoliata), a widely cultivated fruit crop, is known for its salt sensitivity. Salinity poses a significant challenge to citrus production across various regions worldwide, where it acts as a limiting factor. When citrus trees are irrigated with saline water, their yields are significantly reduced. The accumulation of excessive chloride ions (Cl-) and sodium ions (Na+) can lead to specific ion toxicities, further exacerbating salinity's negative impact on citrus plants. However, this issue can be minimized by selecting proper rootstocks. Six treatments were administered to the plants in a study to assess the effects of different stress levels on citrus plants. These treatments involved varying concentrations of salt stress: 0mM, 10mM, 20mM, 30mM, 60mM, and 80mM. By subjecting the plants to these different stress levels, researchers aimed to understand the response of citrus plants to increasing salinity. Water salinity presents a significant issue for citrus cultivation due to its detrimental influence on crop yields. The high salt content in irrigation water negatively affects the growth of citrus trees and induces physiological disorders. In addition to impeding growth, salinity harms plant height and root development. These combined effects of salinity contribute to the overall decline in citrus productivity. Among the various citrus rootstocks tested in the study, C-35 exhibited the most significant susceptibility to salinity stress. As the salt concentrations increased, C-35 experienced the most severe adverse effects on growth and productivity. On the other hand, Poncirus trifoliata, a commonly used rootstock, demonstrated a higher degree of tolerance to salinity. Poncirus trifoliata remained relatively unaffected even at higher salt concentrations, making it a more suitable choice for citrus cultivation in saline environments.*

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Keywords: *Citrus, Salt sensitivity, Salinity, Yield reduction, Rootstocks, Ion toxicities*

Introduction

Citrus is a genus of flowering trees and shrubs in the rue family, Rutaceae. Its origin is in Southeast Asia. Citrus fruit has been cultivated in an ever-widening area since ancient times. They are unique fruits that contain healthy nutritional content. These fruits include lemons, oranges, grapefruits, mandarins, leeches, etc. Citrus fruits contain large amounts of vitamin C and are rich sources of antioxidants, vitamin A and potassium. They are the best and healthier substitute for sugary sweets, as most citrus fruits are sweet to taste (Garcia-Lor et al., 2013). Soil salinity is considered one of the major consequences of global climate change, which negatively affects agricultural yields worldwide (Almas et al., 2023a). This situation has boosted research on the mechanism's plants activate to respond to different abiotic stresses, which may provide better material for studying the stress tolerance mechanisms in plants. Moreover, salinization from irrigation sources is a growing problem in commercial agriculture (Garcia-Lor et al., 2013). Citrus is planted primarily in semiarid regions where irrigation is required for the best output. Many soils and streams in these places contain salts that can impede the growth and yields of citrus crops. Although Citrus species are classified as salt-sensitive, there is great variation in the ability of citrus trees to tolerate salinity depending on rootstock. This build-up causes adverse morphological, physiological and biochemical effects in different organs of citrus plants through an increased concentration of sodium and chloride (Balal et al., 2012). In citrus, canopy growth decreases, whereas the root system sustains less damage. Excess amounts of these salts enhance the soil matrix's osmotic potential (yS), restricting the plant's water intake. A high concentration of salts in the root zone substantially decreases leaf water potential (Garcia-Sanchez and Syvertsen, 2009).

To cope with the problem of salinity, citrus respond by overproducing compatible osmolytes such as proline and absorbing ions from the substrate, decreasing the water potential in leaf tissue and thus creating a negative pump effect to maintain the water flux through xylem sap (Zeshan Haider et al.). This mechanism to avoid water deficit harms plant physiology because there is an increase in Cl^- concentration in leaf tissue, which is toxic for plant metabolism. However, turgor potential in leaves of citrus trees grown under salt stress remains at levels similar to those in non-salinized plants. The osmotic adjustment is achieved by accumulating Na^+ and Cl^- ions and compatible solutes (Aida et al., 2001; Sami et al., 2023a). This effective approach to avoiding water stress has long-term severe consequences since citrus trees are chloride-sensitive, and the cumulative accumulation of these ions in plant tissues causes well-documented detrimental effects. (Arbona et al., 2003). It has been reported that salinity also promotes particular gene expression in citrus (Aida et al., 2001). Citrus rootstock refers to selected plants within the genus citrus that furnish rootstock for other citrus plants. A rootstock plant must be compatible with a specific scion variety of choice and resistant to common threats, such as drought, frost, and citrus diseases. A rootstock primarily provides a reduction in juvenility (time to bearing) and tree vigor when compared with seedling trees; thus, citrus trees propagated with a rootstock combined with a pathogen-free scion bring a much-improved degree of uniformity and consistency to an orchard. Moreover, rootstocks have many specific characteristics that positively contribute to the performance of a citrus tree. They influence various horticultural traits and provide tolerance to pests and diseases and certain soil and site conditions that contribute significantly to orchard profit (Castle, 2010). Trifoliate orange and its hybrids, such as Carrizo citrange, are considered salt-sensitive. Citrus salinity depends on several factors: rootstock-scion combinations, irrigation system, soil type, and climate. Changing one or more of these factors (with the same irrigation water) could produce different results (Al-Yassin, 2004).

Salinity is an increasing risk to agriculture around the world. This is particularly important for cultivating salt-sensitive crops such as citrus. To develop crops tolerant to salinity, it is essential to understand the underlying physiological, molecular, and biochemical mechanisms and identify related genes and gene networks. Using brackish and saline water could help alleviate the world's water problem, this option is only possible with the development of salt-tolerant crops or management practices that alleviate salt stress. Increased infestation by pests will often accompany the increased salt and heavy metal contamination of soils, and pathogens are expected to take a major toll on crop yields. The susceptibility to salinity varies widely between citrus cultivars, pointing to the extensive genetic diversity that can be exploited to identify genes and their corresponding proteins essential for citrus salt tolerance. The process of protein degradation is therefore proposed as a significant target for improving salt tolerance in citrus (Acharya et al., 2018). This study aimed to screen out different citrus rootstocks against NaCl stress under field conditions. Moreover, morphological attributes of citrus rootstocks were also assessed as affected by salinity stress.

Materials and methods The experiment was conducted at National Agricultural Research Center, Islamabad from January to April (2019). The experiment was conducted to evaluate salinity's effect on different citrus rootstocks. There were 9 rootstocks of citrus, which we were going to check for salinity stress. Out of which 7 rootstocks were exotic, and 2 were local, which include Carrizo citrange, Cleopatra mandarin, Benton citrange, C-35, Sour orange, Rough lemon, *Poncirus trifoliate*, Swingle citrumelo, and Tryor citrange. The first step was the seed extraction of the rootstocks. We took the rootstocks of Carrizo citrange or any other rootstock. Then, gently cut them with a knife without damaging the citrus's seeds. Then, gently squeeze them into another container. Repeated the process. It was good to take as many seeds as possible so they can germinate in large amounts. After that put the seeds in the water. The rotten seeds got on top of the water, and the plum seeds were underneath the water. After that spread them on the paper sheet and applied a fungicide named "Ridomil". Then left them in the sun to dry and tagged them. Repeated the same process with other rootstocks. Then filled 450 pots with the soil and farm yard manure. Added some peat and moss. It gave more fertility. Sown the seeds of each variety in 50 pots. Put the seed in the center and then add some sand on top of it. Then irrigated it with water by applying the fungicide in the water named "Ridomil". Date of sowing seeds was 13-02-2019. Seedlings were grown in a greenhouse under natural photoperiods. After that applied the concentrations of NaCl in 10, 20, 30, 60 and 80 mM. There was total 6 treatments for 6 replications of each rootstock. The date of salinity stress was 22-03-2019. Another stress with same concentrations was given on 01-04-2019.

Geographical Location

The experiment was conducted at the Fruit Crop Research Program, Horticultural Research Institute, National Agricultural Research Center (NARC) Islamabad from January-April. The experimental site is located at a longitude of 73.08(degrees) east and a latitude 33.42(degrees) on the global scale. The elevation of the site was 683 MSL (Mean Sea Level). The annual rainfall in this area is about 1000 mm.

Experimental Material

The materials used in this experiment were citrus rootstocks, pencils, tags, sand, pots, knife, growth regulators, fungicides, containers, and paper sheets.

Data to be recorded

The data were recorded on the base of salinity stress on rootstocks of citrus.

Results and discussion

Plant Height (cm)

The data regarding plant height is illustrated in Table 1, which elaborates significant differences among different concentrations of NaCl with citrus rootstocks at $p<0.05$, with that the least affected was the 80mM plants by salinity of C-35. Their plants can tolerate the salinity stress. The increased levels of NaCl in the watering solution promoted different levels of damage in plants. Firstly, growth was impaired by salinity. During the experimental period, control plants produced new stems that were 3-cm long on average. In 10, 20, and 30 mM NaCl-treated plants, newly formed stems were slightly shorter (2.5 cm on average). Growth declined in both 60 and 80 mM NaCl-treated plants, and new tissues were not observed. Significant interaction for days to plant height was observed with C-35 (30.5a) in 0mM then to 20mM (27.2ab) and 80mM (24.5ab). The least affected were *poncirus trifliata* in 80mM (2.25qr). This study reveals that salinity affects plant height, leaves, and roots. The number of stomata and epidermal cells generally declined with increased NaCl stress. The reductions in growth attributes could have resulted from a decline in the water potential in response to salt stress, which could reduce cell elongation and cell division.

Table 1: Effect of different concentrations of NaCl (0mM, 10mM, 20mM, 30mM, 60mM and 80mM) on Plant height (cm) of Cox mandarin, C-35, *Poncirus trifoliate*, Carrizo citrange, Try or citrange and Rough lemon

Rootstocks	Control	10mM	20mM	30mM	60mM	80mM
Cox mandarin	7.5^{kl}	6^{mn}	13.25^{fg}	12.25^{gh}	6.75^{lm}	9.5^{jk}
C-35	30.5^{a}	24^{ab}	27.2^{ab}	22.75^{bc}	23^{bc}	24.5^{ab}
Poncirus trifoliata	5.25^{op}	5.5^{no}	7.25^{lm}	3.75^{pq}	3.5^{r}	2.25^{qr}
Carrizo citrange	24.7^{ab}	19.5^{cd}	17.5^{de}	22.75^{bc}	18.75^{cd}	22.75^{bc}
Tryor citrange	21.25^{bc}	14.25^{fg}	16.25^{ef}	16.75^{ef}	12.5^{gh}	10.75^{hi}
Rough lemon	8.25^{kl}	9^{kl}	10^{ij}	8^{kl}	9.75^{jk}	8^{kl}

LSD (0.05) Rootstocks = 0.89 Treatment = 0.89 Interaction = 2.1

Data presented in columns elucidate different treatments followed by various alphabets indicating substantial differences according to LSD test at $P<0.05$.

Number of Leaves

The data regarding the number of leaves is illustrated in Table 2, which elaborates on significant differences among concentrations regarding their impact on citrus rootstocks. The most affected leaves by the salinity were cox mandarin, followed by tryor citrange. The leaves were wilted, and leaf abscission occurred in them. Some leaves showed small areas of necrotic tissue 14 d after the onset of salt treatment (4% and 9% of the leaves in 60 and 80 mM NaCl-treated plants, respectively). Finally, preliminary experiments indicated that after 14 days of salt stress, leaf abscission occurred in the two groups of plants grown under higher salinity (15% for 60 mM and 32% for 80 mM NaCl-treated plants) (Arbona et al., 2003; Sami et al., 2023b). Significant interaction for days to no. of leaves was observed with Cox mandarin (2.25ab) in 30mM (1.75bc) and 30mM (3.25a) in tryor citrange. The least affected were *poncirus trifoliata* in 10mM (1d). The results regarding growth attributes and plant biomass reported in the current study follow the findings of Ashraf and Ahmad (2000) and Ltaief *et al.*, (2007). Some leaves were wilted, and some plants didn't have roots. Leaf Cl^- accumulated with water use because there were positive relationships between leaf Cl^- and water use in all the concentrations. Some variations in leaf area growth of rootstock seedlings could have been attributable to species differences, as the larger Carr seedlings also have larger entire leaves than the smaller trifoliate Pon seedlings. Leaf Cl– accumulation was positively related to leaf water use (Almas et al., 2023b; Syvertsen et al., 2010).

Table 2: Effect of different concentrations of NaCl (0mM, 10mM, 20mM, 30mM, 60mM and 80mM) on Number of Leaves of Cox mandarin, C-35, *Poncirus trifoliate*, Carrizo citrange, Tryor citrange and Rough lemon

Rootstocks	Control	10mM	20mM	30mM	60mM	80mM
Cox mandrin	2^{ab}	1.5^{cd}	2.25^{ab}	1.75^{bc}	1.75^{bc}	2.25^{ab}
C-35	1.25^{d}	2^{ab}	1^{d}	1.5^{cd}	2.25^{ab}	1.75^{bc}
Poncirus trifoliata	1.75^{bc}	1^{d}	1.25^{d}	2.75^{ab}	2^{ab}	2^{ab}
Carrizo citrange	1.25^{d}	1.75^{bc}	1.5^{cd}	1.75^{bc}	1.75^{bc}	1.5^{cd}
Tryor citrange	2^{ab}	2^{ab}	3^{ab}	3.25^{a}	1.5^{cd}	2^{ab}
Rough lemon	1.25^{d}	1.75^{bc}	2.75^{ab}	2^{ab}	1.25^{d}	2^{ab}

LSD (0.05) Rootstocks = 2.8 Treatments = 2.8 Interaction = 3.4

Data presented in columns elucidate different treatments followed by various alphabets indicating substantial differences according to LSD test at $P<0.05$.

Root Length (cm)

The data regarding root length is illustrated in Table 3, which elaborates on significant differences among the concentrations provided. C-35 was the most affected by salt, followed by tryor citrange, and the least affected was *Poncirus trifoliate*. Some of the rootstocks were without any roots because they were badly affected. Significant interaction for days to root length was observed with C-35 (9.25a) in 10mM then *Poncirus* (8ab) in 80mM, then tryor citrange (8.75ab) in 60mM. The least affected were cox mandarin in 10mM (1m). Hydraulic conductivity of roots is reduced by salinity. In a study with Cox mandarin and *Poncirus trifoliate* rootstocks, decreased hydraulic conductivity of roots when grown at 30-60 mM NaCl saline conditions (Zekri, 1991) reported that a decrease in root conductivity caused by NaCl at -0.20 and -0.35 MPa for some citrus rootstocks. These diminished for Cox mandarin and *Poncirus trifoliate* 19-30 %. Furthermore, a positive linear relationship occurs between root hydraulic conductivity and stomatal conductance (Maas, 1993; Sami et al., 2023c). Citrus develops a relatively shallow root system compared with some deciduous fruit trees. The maximum rooting depth in deep, well-drained soils is 1.2 to 1.5 m, with the main root system spreading to a depth of 0.6 to 0.9m. However, the roots can be much deeper in well-drained sandy soils. On the other hand, citrus trees can survive in shallow soils but may develop smaller trees (Shalhevet and Levy, 1990; Almas et al., 2023b).

Table 3: Effect of different concentrations of NaCl (0mM, 10mM, 20mM, 30mM, 60mM and 80mM) on Root Length (cm) of Cox mandarin, C-35, *Poncirus trifoliate*, Carrizo citrange, Tryor citrange and Rough lemon

Rootstocks	Control	10mM	20mM	30mM	60mM	80mM
Cox mandrin	1.5^{lm}	1^{m}	2.25^{kl}	2.25^{kl}	2.25^{kl}	3^{jk}
C-35	6.75^{bc}	9.25^{a}	9.25^{a}	6.25^{cd}	6^{cd}	4.5^{fg}
Poncirus trifoliata	4.5^{fg}	5.5^{de}	6.25^{cd}	6^{cd}	5.5^{de}	8^{ab}
Carrizo citrange	5^{ef}	4^{gh}	4.25^{fg}	5.75^{de}	3.25^{ij}	5.5^{de}
Tryor citrange	4.5^{fg}	5^{ef}	5.5^{de}	5.25^{de}	8.75^{ab}	7.25^{ab}
Rough lemon	5.25^{de}	4.5^{fg}	5.75^{de}	3.75^{hi}	5.5^{de}	4^{gh}

LSD (0.05) Rootstocks = 1.5 Treatment = 1.52 Interaction = 3.73

Data presented in columns elucidate different treatments followed by various alphabets indicating substantial differences according to LSD test at P<0.05.

Number of Roots

The data regarding the number of roots is illustrated in Table 4, which elaborates that tryor citrange has the lowest no. of roots, followed by *Poncirus trifoliate*. Some of the rootstocks had no leaves because they were severely damaged. Significant interaction for days to root length was observed with *Poncirus* (11ab) in 20mM then *Poncirus* (10.5ab) in 80mM, then tryor citrange (11.75a) in 60mM. The least affected were C-35 in 30mM (2.25n). Root growth is often less sensitive to salinity than shoot growth, so the root: shoot ratio increases at high salinity conditions. Root growth may not decrease at low salinity while shoot growth declines (Ackerson and Youngner, 1975; Dudeck et al., 1983). These effects are also clear in the short term (one or two days), before saline ions accumulation in the shoot would have built up to high levels.

Table 4: Effect of different concentrations of NaCl (0mM, 10mM, 20mM, 30mM, 60mM and 80mM) on number of roots of Cox mandarin, C-35, *Poncirus trifoliate*, Carrizo citrange, Tryor citrange and Rough lemon

Rootstocks	Control	10mM	20mM	30mM	60mM	80mM
Cox mandrin	3^{lm}	3.5^{kl}	7.25^{cd}	6.75^{ef}	5,25^{ij}	3^{lm}
C-35	9.25^{ab}	4.25^{ij}	5.25^{gh}	2.25^{n}	3.75^{jk}	7de
Poncirus trifoliata	8.25^{ab}	9^{ab}	11^{ab}	9.25^{ab}	8.25^{ab}	10.5^{ab}
Carrizo citrange	7.75^{bc}	6.5^{ef}	7.5^{bc}	7.5^{bc}	8.25^{ab}	9.25^{ab}
Tryor citrange	8.5^{ab}	5.5^{fg}	2.5^{mn}	6^{ef}	11.75^{a}	4.75^{hi}
Rough lemon	7.25^{cd}	9.5^{ab}	5^{gh}	4.5^{ij}	8.5^{ab}	10.75^{ab}

LSD (0.05) Rootstocks = 0.5 Treatments = 0.57 Interaction = 1.4

Data presented in columns elucidate different treatments followed by various alphabets indicating substantial differences according to LSD test at P<0.05.

Conclusion

Substantial interaction for plant height was observed with C-35 in 0mM, then to 20mM and 80mM. P*oncirus trifliata* was the least affected rootstock on 80mM. Regarding the number of leaves, notable interaction was observed by Tryor citrange with 30mM and 30mM. In the root length, a significant difference for days to root length was experienced with C-35 in 10mM than *Poncirus* in 80mM and Troyer citrange in 60mM. The least affected were cox mandarin in 10mM. For root number, a significant interaction was observed with *Poncirus* (11ab) in 20mM, then *Poncirus* (10.5ab) in 80mM than tryor citrange (11.75a) in 60mM. The least affected were C-35 in 30mM (2.25n). All plants were well-salinized.

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Declaration

Data Availability statement

All data generated or analyzed during the study have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this research.

Consent for publication

Not applicable

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Conflict of interest

The authors assure that there were no financial relationships involved that could be perceived as a conflict of interest.