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Evaluation of Citrus Rootstocks to Salinity Tolerance

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ABSTRACT: Citrus fruit being a major horticultural crop consumed globally, is severely affected by issues related to biotic and abiotic stresses. Following stress effects, a research study was carried out to evaluate the morphological and physiological responses of citrus rootstocks to different levels of salinity stress. The use of salt tolerant genotypes as rootstock to mitigate the adverse effects of salinity could be helpful for commercial citrus production in salt affected areas. The present investigation was carried at the Horticulture Research Station, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneshwar, during the years 2018-20, wherein the germination percentage nucellar citrus genotypes namely Rough lemon 8779, CRH-12, Gajanimma, Rangapur lime –Tirupati strain, Rangapur lime - Texas strain, Sour dig, Sour orange 8751, Emmekaipuli, Chinnato sour orange, *Carizo citrange*, Balaji acid lime, Japanese summer sour orange and Australian sour orange subjected to salinity stress by NaCl, $CaCl_2$, NaCl + $CaCl_2$ (1:1 w/w) at 0 mM, 25 mM, 50 mM and 75 mM concentrations in irrigation water. For germination studies the Coco peat was used as growing media. Among these genotypes, Rangapur lime – Tirupatistrain, Rangapur lime - Texas strain and Balaji acid lime showed early and maximum germination and it is confirmed that the citrus seeds can germinate under 75 mM salinity stress with congenial environment and growing media. Hence the findings stated that the salinity caused reduction in seedling growth, biomass content. From the research findings it could be concluded that the germination percentage, days taken for first germination, days taken for 50% flowering were unaffected by salinity level studied and Maximum reduction in plant height, stem diameter and number of leaves were noticed in the seedlings of *Carizo citrange*, Chinnato sour orange and CRH-12 whilst the minimum was recorded in Australian sour orange, Sour dig, Sour orange 8751, rough lemon and Rangpur lime seedlings and the least reduction of leaf area and root length, was in the seedlings of Australian sour orange, Sour dig and Sour orange 8751. The genotypes Australian sour orange, Sour dig, Sour orange 8751, Rough lemon and Rangpur lime depicted the lowest decrease in biomass content (fresh and dry weight of shoot and root) while the maximum reduction was noticed in *Carizo citrange* followed by Chinnato sour orange and CRH-12.

Keywords: Citrus rootstocks, Germination, Growth and development and Salinity stress.

INTRODUCTION

Biotic and abiotic stresses have become a serious issue all over the world, affecting plant growth and productivity. Abiotic stress causes a serious crop loss worldwide, contributing to the production decline of major crops by 50%. Moreover, soil salinity has become one of the major environmental factors affecting many crop plants' growth and productivity. The reduction in arable land due to salinization is in direct relation with the needs of the increasing population which is at an increasing rate (Sudhir and Murthy 2004). The deleterious effect of high salinity damages is noticed at germination, seedling stage, and other stages of plants life that lead to a significant decrease in growth, yield, and finally death of the plants. About 19.5% of total irrigated lands and 2.1% of total cultivated drylands are salt-affected throughout the world (FAO, 2016).

Citrus is one of the most important members of the Rutaceae family considered a major household item in the world of the fruit juice industry. The genus citrus consists of different species like mandarin, oranges, grapefruit, lemon, and lime with small categories as tangerine, pummelos, and tangelos, widely grown in the subtropical and tropical regions of the world (Chaudhary *et al*., 1989). It is one of the well-known fruits for their refreshing fragrance, providing an adequate amount of Vitamin C and phytochemicals like carotenoids, limonoids, flavanones, and Vitamin B complex that greatly pays off against cardiovascular and degenerative diseases, obesity, cancer, thrombosis, and atherosclerosis (Iglesias *et al*., 2007). For a particular area, while selecting fruit plants, rootstocks should be given careful consideration on which scion varieties are to be grafted or budded. Rootstocks affect the vigor, productivity, longevity, quality, and resistance to different diseases, insects, and pests of a scion variety. Rootstock should be adaptable to various soil and climatic conditions and resistant to different diseases and insect pests. Citrus is considered the top-ranked fruit of world production and is produced commercially in more than 50 countries. Citrus plants are considered to be sensitive to saline condition (Al-Yassin, 2005) due to the specific toxicity of Cl^- and/or Na^+ and to the osmotic effect caused by the high concentration of salts (Garcia-Sanchez *et al*., 2000) and plants face physiological disturbances and reduction in growth even at low to moderate exposure of salts. Semi-arid areas are preferable to citrus cultivation and in these areas soils and water contain many soluble salts like chlorides and sulfates which disturb the nutritional balance of plants resulting reduction in the growth and yield of citrus crops. The exposure of citrus to salinity causes serious physiological dysfunctions such as reduced leaf area, chlorotic or necrotic patches on leaves, delayed development, growth inhibition, and a limitation in development (Khoshbakht *et al*., 2018). Although Citrus species are classified as salt-sensitive (Mass1993), there is great variation in the ability of citrus plants to tolerate salinity depending upon rootstock (Zekri and Parsons 1992) and scion (Lloyd *et al*., 1990). The tolerance of the different species of Citrus can be determined by their capacity to exclude the potentially toxic Na^+ and Cl^- ions (Storey, 1995). Several approaches are used to mitigate the adverse effects of soil and irrigation water salinity but, a more permanent solution to this problem keeping in view the increasing utmost food demand of the world would be the use of salt-tolerant rootstocks. This study was aimed to investigate the performance of citrus rootstocks in terms of salinity tolerance; to find out the minimum level of salinity for better growth of citrus rootstock; to evaluate minimum toxicity symptoms of salinity stress on different citrus rootstocks.

MATERIALS AND METHODS

The HRS, Bhubaneswar is located at latitude of 20°15' N and longitude of 85°52' E. It is about 60 km away from the Bay of Bengal and at an altitude of 25.5 meters higher than mean sea level (MSL), with an average rainfall of about 1628 mm. Meteorological data during the investigations collected from the Meteorological Observatory of the OUAT, Bhubaneswar. The experiment was conducted in Factorial Completely Randomized Design (FCRD) with six plants in each genotype. The matured fruits of 13 nucellar citrus genotypes namely Rough lemon 8779, CRH-12, Gajanimma, Rangapur lime –Tirupati strain, Rangapur lime - Texas strain, Sour dig, Sour orange 8751, Emmekaipuli, Chinnato Sour Orange, *Carizo citrange*, Balaji acid lime, Japanese Summer Sour Orange and Australian Sour Orange were collected from the trees of respective genotypes growing at AICRP on Citrus, Tirupati. The seeds from ripened fruits were extracted and washed thoroughly in running water and shade dried for five days. 100 g of healthy seed were collected and were used for sowing.

Preparations of NaCl solution. To prepare different levels of salinity *i.e*., 25 mM, 50mMand 75 mM atomic mass of NaCl were multiplied with different salinity levels then divided with thousand and results were obtained in grams. *i.e*., 1.47 g, 2.94 g and 4.41 g then each level was dissolved in one liter of water. The electric conductivity (E.C.) of the media was determined before treatment application by taking random samples from the seedling transplantation media.

Growth measurements:

Plant height. The height of randomly selected plants from each treatment was measured using the measuring tape and their average was calculated. Number of leaves plant 1. The number of leaves per plant was counted carefully after application of treatment and their mean were taken. Stem girth (mm) Stem thickness of randomly selected plants from each treatment in every replication was measured by using digital Vernier caliper and the average was computed. Single leaf area (cm^2) of four leaves were randomly selected from all treatments of all replications and their areas were found through the graph paper method, then average leaf area per single leaf was obtained and recorded. Toxicity symptoms Toxicity symptoms like leaf tip burning, defoliation, yellowing, etc., particularly in the leaves were observed visually. Fresh weight and dry weight of shoots. All the shoots were detached and were weighed with the help of a digital electronic balance. The same shoot was then oven-dried at 80°C for 48 hours for measuring the dry weight. Fresh weight and dry weight of roots The roots were detached, then washed with tap water and weighed with the help of digital electronic balance. The same roots were then oven-dried at 80°C for 48 hours for measuring the dry weight.

RESULTS AND DISCUSSION

Days to emergence of first seedling. Days to emergence were recorded during two seasons, 2018-2019 and 2019-2020 and the following results were obtained.During, 2018-2019, days to emergence of first seedling depicted significant data (Table 1) among the genotypes ranging from 21.44 to 16.23. The maximum number of days for emergence was taken by G13 (21.44), followed by G6 (19.08). In contrast, least days were taken by G4 (16.23), followed by G9 (16.83). Among the salinity treatment levels, maximum number of days for germination was taken by the treatment, 75 mM NaCl + $CaCl_2$ (19.15) against the control with 15.85. Least number of days for germination was taken by 25mM NaCl + $CaCl_2$ (17.33). Pertaining to the interactions, maximum days were taken by G13 × 75mM NaCl (22.22), while least days were taken by G8 × 50 mM NaCl (15.40). In the following season (2019-2020), maximum days for emergence was taken by the genotype G13 (20.19) (Table 1), while minimum days by G4 (16.02), G8 (16.07) and G9 (16.74). Among the treatments, maximum days were taken by 75mM NaCl (18.78), followed by 75mM NaCl + $CaCl_2$ (22.04) and minimum was taken by 25mM NaCl + $CaCl_2$ (16.75). Regarding the interactions, G13 × 75mM $CaCl_2$ (22.14), has taken maximum days for emergence, while least was tan by G4 × 50 mM $CaCl_2$ (14.05).

Days to 50% emergence. Significant data was recorded among the genotypes for days to 50% emergence in both the seasons. During 2018-19, the genotype G13 showed (Table 1) the maximum days (24) followed by G5 (23.06) and G9 (22.98) for 50% emergence, while the minimum days by G7 (19.30). Among the salinity levels 75MM $CaCl_2$ recorded the maximum days (23.43) for 50% emergence, followed by 50mM NaCl + $CaCl_2$ (22.77), while least percentage was recorded by 25mM NaCl (21.15) against the control with 19.34 percentage. Among the interactions the maximum days were occupied by G13 × 75 mM NaCl + $CaCl_2$ (25.36), while minimum duration was observed in G7 × 50 mM NaCl (18.79), followed by G7 × NaCl (19.02). During 2019-20, in the Table 2 the genotype G8 showed maximum days (23.02), while minimum days for 50% emergence was showed by G7 (20.02), followed by G2 (20.70). Among the treatments, 50mM $CaCl_2$ (23.43) occupied the maximum no of days. In contrast, least days were taken by 25 mM $CaCl_2$ (20.92). Pertaining to the interactions, maximum days was seen in G12 × 50 mM $CaCl_2$ (26.21), while least was seen in G11 × 25 mM $CaCl_2$ (18.32).

Seed germination is a crucial stage for survivability of any crop, mainly in those crops which are propagated by means of sexual reproduction. The existence and distribution of any plant species mainly depend on their ability to complete germination and the ability of seedling to survive in unfavorable situations (Zivkovic *et al.*, 2007). Our results indicated that in the table number 1.1 Elucidates that, the maximum number of days for emergence was taken by the genotype (G13) Australian sour orange followed by (G6) Sour dig and (G5) Rangpur lime Texas strain which differs significantly from control.

But among the saline treatments 75 mM NaCl + $CaCl_2$ took 19.15 days to germinate, which differs significantly from control (15.85). Days to 50% emergence the genotype G13 showed maximum days 24 followed by G5 (23.06 days) for 50% emergence, while the minimum days by G7 (19.30 days). Among the salinity levels 75mM $CaCl_2$ recorded the maximum days (23.43) for 50% emergence.

Table 1: Effect of salinity on Days to emergence of first seedling and Days to 50% emergence of nucellar citrus seedlings under varying levels of salinity (2018-20).

Rootstock seedlings	Days to emergence of first seedling										
		NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	Mean
G1	17.00	19.49	20.98	20.12	18.40	19.91	19.60	18.13	20.06	21.23	**17.61**
G2	17.00	18.83	20.43	20.61	18.35	19.23	20.00	18.77	20.08	19.48	**17.67**
G3	17.00	18.08	20.12	18.73	20.18	20.61	21.70	18.88	20.97	21.21	**17.31**
G4	17.00	19.49	19.66	19.92	21.21	19.22	20.99	18.24	20.23	20.80	**16.23**
G5	18.00	19.38	20.74	21.12	20.74	20.84	21.62	21.63	21.88	22.57	**18.64**
G6	19.00	20.13	20.10	20.17	19.78	20.13	19.83	19.70	20.47	21.80	**19.08**
G7	17.63	18.46	18.60	18.66	18.40	19.24	19.48	18.15	19.32	20.43	**18.37**
G8	16.50	18.22	19.23	20.91	19.13	19.71	21.63	19.23	19.80	21.47	**16.87**
G9	16.50	19.04	19.73	20.59	19.21	20.68	20.66	20.64	20.80	21.21	**16.83**
G10	18.00	20.58	20.30	19.23	19.32	20.30	21.60	20.10	19.50	19.50	**17.35**
G11	16.75	19.43	21.30	22.00	17.99	18.62	22.68	19.01	20.66	19.25	**18.33**
G12	16.30	21.72	22.15	20.87	18.23	22.78	19.57	18.73	19.73	20.00	**17.81**
G13	22.10	21.93	22.66	23.71	21.94	22.44	23.33	23.01	22.67	23.41	**21.44**
Mean	15.85	18.04	18.68	18.93	17.66	18.07	18.52	17.33	18.18	19.15	
	G	S	G×S								
CD (5%)	0.49	0.43	1.54								
Se(m)±	0.18	0.15	0.54								

Note: G1- Rough lemon 8779, G2- CRH-12, G3- Gajanimma, G4- Rangapur lime –Tirupatistrain, G5- Rangapur lime - Texas strain, G6- Sour dig, G7- Sour orange 8751, G8- Emmekaipuli, G9- Chinnato sour orange, G10- Carizocitrange, G11- Balaji acid lime, G12- Japanese summer sour orange, G13- Australian sour orange. Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes.

Table 2: Effect of salinity on Days to emergence of first seedling and Days to 50% emergence of nucellar citrus seedlings under varying levels of salinity (2019-20).

Rootstock seedlings	Days to emergence of first seedling											Days to 50% emergence										
		NaCl			$CaCl_2$			NaCl + $CaCl_2$					NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	Mean	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	Mean
G1	14.75	19.00	19.95	18.28	15.00	17.88	17.80	15.45	18.32	19.06	**17.55**	18.56	21.20	22.20	23.52	19.85	21.10	21.20	21.30	21.32	22.30	**21.26**
G2	15.35	18.65	19.85	18.21	16.89	15.49	18.00	16.54	17.15	17.90	**17.40**	18.00	18.74	21.10	23.20	19.22	20.10	22.32	21.08	22.51	20.74	**20.70**
G3	15.55	16.27	17.24	16.45	18.36	19.22	17.40	16.84	16.94	18.33	**17.26**	19.00	19.80	22.98	21.32	22.00	22.12	25.44	21.01	24.20	23.52	**22.14**
G4	13.85	16.52	18.44	19.63	18.41	14.05	17.98	14.20	18.46	19.64	**16.02**	20.00	21.55	20.55	21.32	24.00	22.98	24.21	21.50	22.30	23.52	**22.19**
G5	16.05	19.20	16.98	17.65	18.47	17.68	18.24	18.25	19.76	19.14	**18.14**	19.00	20.11	21.84	25.33	22.00	24.20	25.10	24.71	25.10	26.21	**23.36**
G6	19.00	18.74	19.19	18.55	17.04	16.55	18.66	18.33	19.46	22.09	**18.76**	20.33	21.60	21.30	21.65	21.30	20.10	21.52	22.10	22.41	22.20	**21.45**
G7	17.60	17.89	18.41	18.07	17.56	16.12	19.45	17.39	19.08	20.69	**18.23**	18.25	19.52	19.20	20.30	21.66	20.55	20.11	19.52	20.10	21.00	**20.02**
G8	14.00	15.44	15.40	17.14	15.66	16.41	17.22	15.46	16.00	17.94	**16.07**	19.00	21.10	23.55	25.10	21.60	23.21	25.17	23.32	24.10	24.00	**23.02**
G9	12.00	17.36	16.45	20.17	16.35	16.05	17.32	16.28	16.60	18.82	**16.74**	20.00	21.00	22.10	21.00	22.06	25.00	24.30	25.10	24.36	24.30	**22.92**
G10	16.00	18.75	19.60	15.46	16.39	17.45	18.62	17.19	17.00	18.30	**17.48**	20.10	22.51	20.15	23.10	22.25	23.15	24.57	23.00	22.14	22.10	**22.31**
G11	16.50	16.85	17.60	21.33	16.98	18.00	21.35	17.02	20.31	15.00	**18.09**	17.10	22.30	23.41	21.91	18.32	19.22	24.00	21.00	21.00	23.10	**21.14**
G12	15.60	18.44	19.20	21.74	17.45	19.55	16.14	16.46	16.46	16.50	**17.75**	18.00	25.20	25.41	20.09	18.55	26.21	23.00	21.00	23.00	23.20	**22.37**
G13	22.10	19.64	21.00	21.52	21.25	22.04	22.14	18.33	20.55	20.50	**20.91**	22.32	23.21	23.08	23.20	19.10	22.32	23.65	24.32	23.63	24.20	**22.90**
Mean	**16.03**	**17.90**	**18.41**	**18.78**	**17.37**	**17.42**	**18.49**	**16.75**	**18.16**	**18.76**		**19.20**	**21.37**	**22.07**	**22.39**	**20.92**	**22.33**	**23.43**	**22.23**	**22.78**	**23.11**	
	G	**S**	**G×S**									**G**	**S**	**G×S**								
CD (5%)	0.47	0.415	1.485									0.52	0.46	1.66								
Se(m)±	0.17	0.15	0.53									0.19	0.17	0.59								

Note: G1- Rough lemon 8779, G2- CRH-12, G3- Gajanimma, G4- Rangapur lime –Tirupatistrain, G5- Rangapur lime - Texas strain, G6- Sour dig, G7- Sour orange 8751, G8- Emmekaipuli, G9- Chinnato sour orange, G10- Carizocitrange, G11- Balaji acid lime, G12- Japanese summer sour orange, G13- Australian sour orange. Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes.

Germination percentage. In the season 2018-2019, max germination percent (Table 3) was observed in the genotype G4 with 94.16%, followed by G5 (86.69%), while least was observed in G2 with 45.32 %, followed by G7 (48.94%). Among the treatments, 25mM NaCl + $CaCl_2$, depicted maximum germination percentage (79.39%), while least was observed in the treatment 75 mM NaCl + $CaCl_2$ (31.44), followed by 75 mM NaCl (31.55%). The interaction, G4 × 25 mM NaCl and G5 × 25 mM NaCl + $CaCl_2$ depicted maximum germination percentage (100 %) and minimum germination percent was seen in the interaction, G7 75 mM NaCl (8.90 %), followed by G7 × 75 mM NaCl + $CaCl_2$ (11.05 %). During the season 2019-2020, maximum germination percent (Table 3) was observed in the genotype, G4 (91.39%). Minimum germination percent was observed in the genotype G2 (48.55%), followed by G10 (48.79%). Among the treatments, maximum germination percent was seen in 25 mM NaCl + $CaCl_2$ (82.31%), while least was observed in 25 mM NaCl (21.20%). Among the interactions, G4 × 25 mM NaCl showed maximum percentage (100), while minimum germination percentage was obtained in the interaction G10 × 75 mM NaCl + $CaCl_2$ (12.65) and G7 × 75 mM NaCl + $CaCl_2$ (12.66). While, the maximum germination percent was observed in the genotype G4 with 94.16%, while least was observed in G2 with 45.32 %, followed by G7 (48.94). Among the treatments, 25mM NaCl + $CaCl_2$, depicted maximum germination percentage (79.39), followed by 25 mM $CaCl_2$ (77.40), while least was observed in the treatment 75 mM NaCl + $CaCl_2$ (31.44). This may be due to the salt susceptibility variation among genotypes. This indicates the determinate effect of salt solution at higher concentration which delays the process of germination.

Table 3: Effect of salinity on Germination percentage of nucellar citrus seedlings under varying levels of salinity Germination (%) (2018-20)

		NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	Control 0 mM	**25 mM**	**50 mM**	**75 mM**	**25 mM**	**50 mM**	**75 mM**	**25 mM**	**50 mM**	**75 mM**	**mean**
G1	99.00	84.75	62.23	39.75	86.73	79.45	45.78	89.48	76.34	37.85	**70.14**
G2	61.92	54.96	37.65	27.22	53.91	38.45	29.50	55.49	45.44	33.62	**43.82**
G3	77.39	61.64	52.96	33.66	66.49	53.45	40.64	34.58	26.21	26.62	**47.36**
G4	98.79	100.00	92.43	90.47	93.38	88.45	86.10	96.32	94.80	86.99	**92.77**
G5	100.00	95.74	86.82	79.63	96.71	81.78	42.65	94.50	88.07	92.64	**85.85**
G6	80.63	74.12	51.65	23.41	78.96	55.55	22.40	76.85	34.43	13.26	**51.13**
G7	80.95	75.00	56.50	13.90	77.40	53.00	15.00	70.26	43.60	11.86	**49.75**
G8	86.00	79.87	48.00	19.08	80.45	48.78	22.00	81.98	52.70	21.33	**54.02**
G9	98.32	97.12	60.73	34.00	89.77	46.78	17.44	96.92	49.61	13.80	**60.45**
G10	59.66	67.74	49.00	23.20	65.45	46.89	37.00	69.78	60.66	23.03	**50.24**
G11	100.00	98.14	79.65	30.45	88.45	69.47	35.10	97.96	75.37	40.06	**71.46**
G12	81.38	79.80	59.31	22.40	80.14	62.78	14.12	83.63	72.91	29.98	**58.64**
G13	95.33	88.45	46.67	22.74	92.12	68.16	33.13	96.65	69.51	26.16	**63.89**
mean	86.10	81.33	60.28	35.38	80.77	61.00	33.91	80.34	60.74	35.17	**61.50**
	G	S	G×S								
CD (5%)	1.88	1.65	5.94								
Se(m)±	0.67	0.59	2.13								

Note: G1- Rough lemon 8779, G2- CRH-12, G3- Gajanimma, G4- Rangapur lime –Tirupatistrain, G5- Rangapur lime - Texas strain, G6- Sour dig, G7- Sour orange 8751, G8- Emmekaipuli, G9- Chinnato sour orange, G10- Carizocitrange, G11- Balaji acid lime, G12- Japanese summer sour orange, G13- Australian sour orange. Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes.

Plant height (cm). The pooled data over two years of study presented in Table 4. Significant data was recorded among the genotypes. The maximum plant height was recorded in in the genotype G4 (5.09cm) followed by G12 (5cm) and G3 (4.92cm). The minimum plant height was recorded in G6 (3.79 cm). Among the salinity levels, 25 mM NaCl (4.64 cm) has recorded the maximum plant height followed by 25 mM $CaCl_2$ (4.53 cm), whereas minimum plant height was recorded in 75 mM NaCl + $CaCl_2$ (3.86 cm) against the control with 6 cm. Among the interactions the maximum plant height was recorded in the combination of G5 × 50 mM $CaCl_2$ (5.67 cm), followed by G4 × 25 mM NaCl (5.65 cm) and G12 × 25 mM NaCl + $CaCl_2$ (5.65 cm), while minimum was recorded in G2 × 75 mM NaCl + $CaCl_2$ (2.12 cm), G11 × 75 mM NaCl + $CaCl_2$ (3.10 cm) and G6 × 75 mM $CaCl_2$ (3.20 cm).

Number of leaves. Number of leaves was in the range of 2.18 to 3.51 among citrus germplasm during 2018-19 and 2019-20 (Table 4). Significant data was recorded among the genotypes for number of leaves. The maximum number of leaves was recorded in G4 (3.51) followed by G5 (3.12). The minimum number of leaves was recorded in G2 (2.18).

Table 4: Effect of salinity on Plant height and Number of leaves of nucellar citrus seedlings under varying levels of salinity.

Rootstock seedlings	Plant height (cm)											Number of leaves										
		NaCl			$CaCl_2$			NaCl + $CaCl_2$					NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	mean	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	mean
G1	6.12	5.02	5.00	4.71	4.22	4.00	3.80	4.85	4.22	3.52	**4.65**	3.80	3.45	3.55	2.80	3.20	2.80	2.20	3.02	2.89	1.56	**2.93**
G2	5.23	4.12	3.88	3.65	3.70	4.10	3.40	4.33	3.78	2.12	**3.83**	2.60	2.35	1.90	1.60	2.50	2.60	2.30	2.31	2.22	1.45	**2.18**
G3	6.89	5.20	5.12	5.00	4.15	4.56	4.10	5.32	4.23	4.65	**4.92**	2.95	3.10	3.74	2.60	2.80	3.10	2.50	3.26	2.56	2.32	**2.89**
G4	7.05	5.65	5.45	5.12	5.32	4.65	4.30	4.87	4.44	4.02	**5.09**	4.20	4.20	4.12	3.20	4.10	4.20	3.20	2.92	2.65	2.33	**3.51**
G5	6.25	4.88	4.00	3.85	5.44	5.67	5.23	3.50	3.45	4.36	**4.66**	3.99	3.20	3.50	1.86	3.90	3.80	3.50	3.10	2.75	1.56	**3.12**
G6	5.30	3.50	3.40	3.20	4.09	4.00	3.56	3.88	3.70	3.23	**3.79**	3.67	1.66	2.60	2.20	3.55	3.50	2.10	3.56	2.56	1.40	**2.68**
G7	5.88	5.12	5.10	4.90	5.33	5.20	4.83	4.22	4.02	3.66	**4.83**	4.52	3.50	3.61	2.41	4.16	3.70	2.65	2.24	1.98	1.78	**3.06**
G8	5.66	4.52	4.35	4.22	4.61	4.55	4.35	4.22	3.85	3.25	**4.36**	3.10	3.10	2.31	2.21	2.60	2.60	3.10	3.10	2.31	1.98	**2.64**
G9	6.22	5.12	5.08	4.90	4.85	4.69	4.20	4.82	4.28	4.10	**4.83**	3.20	3.20	2.80	2.10	3.02	2.90	1.77	3.20	1.77	1.22	**2.52**
G10	5.55	3.65	3.70	3.18	3.88	3.75	3.20	5.12	5.03	4.66	**4.17**	3.16	1.90	2.03	1.86	3.02	2.80	1.56	3.40	2.10	2.00	**2.38**
G11	6.08	5.36	5.15	4.88	4.16	4.16	4.01	3.25	3.15	3.10	**4.33**	4.12	2.60	3.88	1.90	3.85	3.40	2.50	2.10	2.00	1.57	**2.79**
G12	5.66	4.78	4.85	4.56	5.12	4.56	4.25	5.65	5.44	5.10	**5.00**	4.51	3.60	2.69	2.12	4.12	3.60	3.10	3.06	1.40	1.45	**2.97**
G13	6.10	5.35	3.25	3.72	4.10	3.75	3.60	4.08	3.75	3.45	**4.10**	4.22	2.10	2.30	1.88	3.50	3.10	2.85	2.33	1.80	1.54	**2.56**
mean	**5.94**	**4.64**	**4.49**	**4.30**	**4.53**	**4.43**	**4.06**	**4.47**	**4.10**	**3.86**		**3.70**	**2.92**	**3.00**	**2.21**	**3.41**	**3.24**	**2.56**	**2.89**	**2.23**	**1.70**	
	G	**S**	**G×S**									**G**	**S**	**G×S**								
CD (5%)	0.12	0.10	0.38									0.08	0.07	0.26								
Se(m)±	0.04	0.03	0.13									0.03	0.02	0.09								

Note: G1- Rough lemon 8779, G2- CRH-12, G3- Gajanimma, G4- Rangapur lime –Tirupatistrain, G5- Rangapur lime - Texas strain, G6- Sour dig, G7- Sour orange 8751, G8- Emmekaipuli, G9- Chinnato sour orange, G10- Carizocitrange, G11- Balaji acid lime, G12- Japanese summer sour orange, G13- Australian sour orange. Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes.

Among the salinity levels, 25 mM $CaCl_2$, recorded the maximum (3.41), followed by 50 mM $CaCl_2$ (3.24), whereas minimum number of leaves was recorded in 75 mM NaCl + $CaCl_2$ (1.70) followed by 75mM NaCl (2.21). Among the interactions the maximum number of leaves was recorded in the combination G4 × 25 mM NaCl and G4 × 50 mM $CaCl_2$ (4.20), followed by G7 × 25 mM $CaCl_2$ (4.16) and minimum was recorded in the interaction G9 × 75 mM NaCl + $CaCl_2$ (1.22).

Root length. Pooled data (Table 5) of root length showed significant data among the genotypes and the maximum root length was recorded in G4 (5.50cm), while minimum was recorded in G2 (3.61cm). Among the salinity levels, 25mM $CaCl_2$ (4.89 cm) has recorded the maximum root length. Whereas, minimum root length was recorded in 75 mM $CaCl_2$ (3.02). Among the interactions the minimum root length was recorded in the combination of G2 × 75 mM $CaCl_2$ (2.25 cm), while maximum was recorded in G4 × 25 mM $CaCl_2$ (6.10).

Plant fresh weight. The genotypes showed significant data for plant fresh weight (Table 5). The maximum plant fresh weight was recorded in G6 (1.34g) followed by G1 (1.32g). The minimum plant fresh weight was recorded in G10 (0.80g).Among the salinity levels 75 mM $CaCl_2$ (1.26 g) recorded the maximum plant fresh weight followed by 25 mM $CaCl_2$ (1.23g) whereas, minimum plant fresh weight was recorded in 75 mM NaCl (0.84g). Coming to the interactions the maximum plant fresh weight was recorded in the combination of G6 x 25 mM NaCl + $CaCl_2$ (1.44g), while least plant fresh weight was observed in the combination G2 × 75 mM NaCl (0.36g), G10 × 75 mM NaCl (0.50g) and 75 mM NaCl + $CaCl_2$ (0.55g).

Table 5: Effect of salinity on Root length and Plant fresh weight of nucellar citrus seedlings under varying levels of salinity.

Rootstock seedlings	Root length (cm)										
	control	NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	mean
G1	5.52	4.68	4.74	4.78	5.75	4.25	3.32	4.33	4.66	4.65	**4.67**
G2	4.36	3.86	3.63	3.66	4.45	3.36	2.25	3.65	3.45	3.42	**3.61**
G3	5.22	4.75	4.23	4.65	5.25	4.88	3.85	4.42	4.00	4.52	**4.58**
G4	6.84	5.56	5.20	5.36	6.10	5.42	4.41	5.25	5.80	5.10	**5.50**
G5	4.96	4.23	3.36	3.78	4.65	3.20	2.98	3.98	3.18	3.66	**3.80**
G6	4.41	3.65	3.24	3.94	4.77	3.15	3.75	3.45	3.10	3.88	**3.73**
G7	4.44	3.65	3.36	3.86	4.14	3.99	2.89	3.20	3.03	3.74	**3.63**
G8	4.10	3.98	3.48	3.52	4.20	3.42	2.44	3.88	3.34	3.14	**3.55**
G9	4.44	3.74	3.35	3.96	4.64	3.10	2.69	3.42	3.20	3.66	**3.62**
G10	4.42	4.08	3.60	4.02	4.28	3.88	2.64	3.98	3.66	3.87	**3.84**
G11	5.53	4.63	4.20	4.54	5.62	4.74	2.44	4.45	4.00	4.42	**4.46**
G12	4.25	4.12	3.82	4.14	4.80	3.36	2.90	3.98	3.66	3.96	**3.90**
G13	4.65	3.88	3.65	3.78	4.97	3.25	2.74	3.44	3.45	3.42	**4.72**
mean	**4.86**	**4.22**	**3.84**	**4.15**	**4.89**	**3.85**	**3.02**	**3.96**	**3.73**	**3.96**	
	G	**S**	**G×S**								
CD (5%)	0.15	0.13	0.48								
Se(m)±	0.05	0.04	0.17								

Rootstock seedlings	Plant fresh weight (g)										
	Control	NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	mean
G1	**1.53**	**1.42**	**1.37**	**1.10**	**1.42**	**1.34**	**1.17**	**1.41**	**1.30**	**1.11**	**1.32**
G2	1.15	0.92	0.68	0.36	1.22	1.10	0.98	1.20	1.01	0.56	**0.92**
G3	1.38	1.22	1.01	0.88	1.23	1.12	0.89	1.20	1.03	0.88	**1.08**
G4	1.49	1.42	1.23	1.01	1.40	1.32	1.20	1.45	1.22	0.95	**1.27**
G5	1.38	1.32	1.05	0.89	1.32	1.21	1.13	1.33	1.29	1.04	**1.20**
G6	1.55	1.40	1.32	1.20	1.42	1.31	1.22	1.44	1.32	1.20	**1.34**
G7	1.35	1.10	0.95	0.85	1.31	1.10	1.20	1.35	1.21	1.01	**1.14**
G8	1.12	0.98	0.88	0.70	1.12	0.87	0.80	1.17	1.11	0.80	**0.96**
G9	1.24	1.22	0.96	0.90	1.18	1.02	0.93	1.23	1.05	0.93	**1.07**
G10	1.12	0.92	0.82	0.50	0.92	0.85	0.71	0.96	0.65	0.55	**0.80**
G11	1.16	1.10	0.99	0.74	1.12	1.01	0.95	1.16	0.99	0.81	**1.00**
G12	1.32	1.24	0.93	0.70	1.24	1.12	0.91	1.23	1.10	0.89	**1.07**
G13	1.25	1.20	1.10	1.05	1.14	1.04	0.98	1.22	1.07	0.85	**1.19**
mean	**1.31**	**1.19**	**1.02**	**0.84**	**1.23**	**1.11**	**1.01**	**1.26**	**1.10**	**0.89**	
	G	**S**	**G×S**								
CD (5%)	2.06	1.80	6.52								
Se(m)±	0.74	0.64	2.34								

Note: G1- Rough lemon 8779, G2- CRH-12, G3- Gajanimma, G4- Rangapur lime –Tirupatistrain, G5- Rangapur lime - Texas strain, G6- Sour dig, G7- Sour orange 8751, G8- Emmekaipuli, G9- Chinnato sour orange, G10- Carizocitrange, G11- Balaji acid lime, G12- Japanese summer sour orange, G13- Australian sour orange. Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes.

Dry shoot weight. Significant data was recorded (Table 6) among the genotypes. The maximum shoot dry weight was recorded in G1 (0.33g) followed by G13 (0.30g) and G11 and G12 (0.30), while the minimum shoot dry weight was recorded in G10 (0.16g) followed by G2 (0.18g) and G8 (0.20g). Among the salinity levels, maximum dry shoot weight was observed in 25 mM NaCl (0. 30g), followed by 25 mM NaCl + $CaCl_2$ (0.29g) and 50 mM NaCl (0.26g), whereas minimum was recorded by 75 mM NaCl + $CaCl_2$ (0.17g). Among the interactions the maximum shoot dry weight was recorded in the combination of G1 × 25 mM NaCl + $CaCl_2$, G1 × 25 mM NaCl (0.42g), while minimum was observed in the interaction, G2 × 75 mM $CaCl_2$ (0.10g).

Dry root weight (g). Significant data was recorded among the genotypes. The data presented in Table 6 maximum root dry weight was recorded in G1 (0.26g). The minimum root dry weight was recorded in G10 (0.14g). Among the salinity levels 25 mM NaCl and 25 mM NaCl + $CaCl_2$ (0.25) recorded the maximum root dry weight, whereas minimum root dry weight was recorded in 75 mM $CaCl_2$ (0.13).

Table 6: Effect of salinity on Dry Shoot weight and Dry Root weight of nucellar citrus seedlings under varying levels of salinity.

Rootstock seedlings	Dry Shoot weight (g)										
	Control	NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	mean
G1	0.40	0.42	0.38	0.27	0.37	0.28	0.25	0.42	0.32	0.21	**0.33**
G2	0.23	0.25	0.21	0.14	0.18	0.13	0.10	0.24	0.18	0.14	**0.18**
G3	0.36	0.32	0.28	0.22	0.32	0.26	0.19	0.30	0.25	0.18	**0.27**
G4	0.33	0.32	0.29	0.20	0.29	0.24	0.20	0.30	0.24	0.18	**0.26**
G5	0.28	0.26	0.23	0.21	0.25	0.20	0.16	0.22	0.18	0.14	**0.21**
G6	0.39	0.32	0.28	0.19	0.33	0.24	0.21	0.32	0.26	0.18	**0.27**
G7	0.36	0.32	0.26	0.21	0.32	0.27	0.22	0.30	0.22	0.18	**0.27**
G8	0.26	0.22	0.18	0.12	0.22	0.18	0.16	0.25	0.21	0.15	**0.20**
G9	0.28	0.30	0.25	0.20	0.25	0.21	0.13	0.22	0.18	0.14	**0.22**
G10	0.19	0.18	0.16	0.11	0.17	0.15	0.12	0.18	0.16	0.13	**0.16**
G11	0.35	0.36	0.28	0.18	0.32	0.28	0.21	0.33	0.26	0.18	**0.28**
G12	0.38	0.30	0.24	0.16	0.35	0.31	0.23	0.33	0.28	0.21	**0.28**
G13	0.34	0.36	0.28	0.20	0.31	0.26	0.21	0.35	0.28	0.22	**0.30**
mean	**0.32**	**0.30**	**0.26**	**0.18**	**0.28**	**0.23**	**0.18**	**0.29**	**0.23**	**0.17**	
	G	**S**	**G×S**								
CD (5%)	0.00	0.00	0.01								
Se(m)±	0.00	0.00	0.01								

Rootstock seedlings	Dry Root weight (g)										
	Control	NaCl			$CaCl_2$			NaCl + $CaCl_2$			
	0 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	25 mM	50 mM	75 mM	mean
G1	0.33	0.31	0.25	0.19	0.29	0.26	0.18	0.33	0.24	0.2	**0.26**
G2	0.2	0.19	0.17	0.14	0.18	0.16	0.12	0.18	0.15	0.12	**0.16**
G3	0.3	0.22	0.2	0.15	0.26	0.22	0.15	0.31	0.24	0.18	**0.22**
G4	0.29	0.22	0.17	0.15	0.25	0.21	0.16	0.27	0.21	0.17	**0.21**
G5	0.26	0.21	0.18	0.14	0.21	0.18	0.13	0.25	0.19	0.16	**0.19**
G6	0.33	0.3	0.24	0.18	0.26	0.19	0.14	0.29	0.25	0.21	**0.24**
G7	0.31	0.32	0.23	0.21	0.28	0.17	0.15	0.28	0.24	0.19	**0.24**
G8	0.22	0.25	0.22	0.18	0.17	0.11	0.08	0.19	0.16	0.11	**0.17**
G9	0.22	0.26	0.28	0.21	0.19	0.13	0.08	0.22	0.16	0.11	**0.19**
G10	0.18	0.21	0.16	0.10	0.16	0.15	0.09	0.18	0.11	0.08	**0.14**
G11	0.26	0.25	0.18	0.15	0.20	0.14	0.10	0.25	0.17	0.13	**0.18**
G12	0.24	0.23	0.17	0.15	0.18	0.12	0.10	0.23	0.19	0.14	**0.18**
G13	0.26	0.26	0.19	0.17	0.21	0.17	0.16	0.26	0.22	0.20	**0.21**
mean	**0.26**	**0.25**	**0.20**	**0.16**	**0.22**	**0.17**	**0.13**	**0.25**	**0.19**	**0.15**	
	G	**S**	**G×S**								
CD (5%)	0.00	0.00	0.02								
Se(m)±	0.00	0.00	0.01								

Note: G1- Rough lemon 8779, G2- CRH-12, G3- Gajanimma, G4- Rangapur lime –Tirupatistrain, G5- Rangapur lime - Texas strain, G6- Sour dig, G7- Sour orange 8751, G8- Emmekaipuli, G9- Chinnato sour orange, G10- Carizocitrange, G11- Balaji acid lime, G12- Japanese summer sour orange, G13- Australian sour orange. Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes.

Pertaining to the interactions, the maximum root dry weight was recorded in the combination of G1 × 25 mM NaCl + $CaCl_2$ and G6 × 5 mM NaCl (0.33g) followed by G7 × 25 mM NaCl (0.32g). In contrast, least minimum was recorded in G10 × 75 mM NaCl + $CaCl_2$ and G8, G9 × 75 mM $CaCl_2$ (0.08g).

A plant undergoes different stages of growth and development during its entire life cycle and among these, seedling stage is the most vulnerable for its survival during adverse conditions. Citrus, being a salt sensitive crop (Abo-Rekab and Zeinab 2014), suffers severely during early stages of growth under salinity (Srivastav *et al*., 2007).

Our results on growth and development of citrus rootstock seedlings under varying levels of salinity showed that salinity stress caused negative impact on their growth and biomass content including plant height, number of leaves, leaf area, intermodal length, fresh weight of shoot, fresh weight of root, dry weight of shoot, dry weight of root and stem diameter, which are documented in Table 4. The genotypes Australian sour orange, Sour dig and Sour orange 8751 showed less reduction in growth and biomass content while maximum was noted in *Carizocitange*, Chinnato sour orange and CRH-12. The adverse effect of salinity on growth and development of plant is because of two reasons as explained by Munns *et al*. (2006). The first reason is osmotic stress that causes an immediate effect on the water uptake capacity of plants, slowing down the growth. Second one is the entry of salts into the different plant parts which causes cell injury in the transpiring leaves with simultaneous effect on photosynthesis and growth morphology (Netondo *et al*., 2004). In the present studies it was observed that salinity stress caused severe scorching of leaves which led to their senescence and defoliation, due to retardation of nutrients supply and photosynthesis, which ultimately affected the plant growth. Forner-Giner *et al*. (2011) also confirmed that inhibition in cell division and cell expansion in growing tissues of roots, stem and leaves under salinity stress were collectively responsible for growth reduction in citrus. Several hormones (Auxin, Cytokinin, Gibberellins and Brassinolides) play an important role in cell elongation and division. Under salinity stress and the reduced concentrations of this growth regulating hormones inhibits the cell expansion (Zhu, 2001). With support of the above reasons, Rhodes (1994) stated that reduction in cytoplasmic volume and the impaired cell turgor pressure under saline conditions resulted in plant growth inhibition. We also found a reduction in the number of leaves, leaf area, stem girth and internodal length under varying levels of salinity stress. Roy *et al*. (2014) also found that graded levels of NaCl salt affected the plant height, stem diameter, number of leaves, leaf area and survivability of mango. Significant reduction in growth parameters (shoot length, number of leaves, leaf area, fresh and dry weight etc.) under salinity stress was also observed by Perez Tornero *et al*. (2009); Tsai *et al*. (2015); Sharma *et al*. (2013) in citrus microphylla, Pink wax apple, Sour orange and Citrus jambhiri respectively. Zhu (2001) noted that higher salt stress disrupted the water and ion homeostasis at the cellular and whole plant level and that this imbalance led to molecular damage, growth inhibition or sometimes death of the plant.

CONCLUSIONS

In the light of above presented results, it was observed that different salinity levels show a detrimental influence on all the growth attributes of citrus rootstock. Increasing soil salinity levels from 0 mM to 75 mM NaCl attained reduction in vegetative growth (plant height, number of leaves, stem thickness, leaf area, root and shoot fresh weight, root, and shoot dry weight). The maximum value of growth attributes, less toxicity symptoms, were recorded in Australian sour orange rootstocks compared to other citrus rootstocks. Among the citrus rootstocks, sour orange give best results regarding growth performance under saline condition, while '*Carizo citrange*' was found the least tolerant rootstock.

FUTURE SCOPE

Further studies on stock-scion interactions with tolerant rootstocks for the problematic situation in commercial cultivars could be helpful to confirm our contention.

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