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Screening of citrus rootstocks for salt tolerance at early seedling stage

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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 nucellar citrus genotypes namely Rough lemon 8779, CRH-12, Gajanimma, Rangapur lime-Tirupati strain, Rangapur lime-Texas strain, Sour dig, Sour orange 8751, Emme kaipuli, Chinnado sour orange, Carrizo citrange, Balaji acid lime, Japanese summer sour orange and Australian sour orange subjected to salinity stress by NaCl, CaCl2 and NaCl + CaCl2 (1:1 w/w) at 0 mM, 50 mM, 100 mM and 150 mM concentrations in irrigation water. Hence the findings stated that the salinity caused reduction in seedling growth, biomass content. From the research findings it could be concluded that the Maximum reduction in plant height, stem diameter and number of leaves were noticed in the seedlings of Carrizo citrange, Chinnado 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 Carrizo citrange followed by Chinnado sour orange and CRH-12.

Keywords: Citrus rootstocks, growth and development and salinity tolerance

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) ^[10]. The deleterious effect of high salinity damages is noticed at 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) ^[11].

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. 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 (Ladaniya, 2008)^[3]. 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. 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) ^[9]. 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.

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, Emme kaipuli, Chinnado Sour Orange, Carrizo 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.

To prepare different levels of salinity i.e., 50 mm, 100 mm and 150 mm atomic mass of NaCl and $CaCl_2$ were multiplied with different salinity levels then divided with thousand and results were obtained in grams. i.e., 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.

The height of randomly selected plants from each treatment was measured using the measuring tape and their average was calculated. Number of leaves plant and 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 (cm²) 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 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 $^{\circ}$ C for 48 hours for measuring the dry weight.

Results and Discursion Plant height (cm)

Significant data (Table 1) was recorded among the genotypes. the plant height of thirteen genotypes before imposing the treatments were ranging from 14.64 cm to 22.99 cm and it varied from 19.34 cm to 32.10 cm at the termination of experiment, while, the difference between the above two were in the range of 4.34 cm to 8.65 cm. The genotypes G10, G2 and G3 depicted the maximum decrease in plant of 62.43%, 53.06% and 43.19%, respectively over control at higher NaCl levels whereas minimum decrease was noticed in the genotypes G13 (20.36%), G6 (27.92%) and G8 (32.27%). The genotypes G9 (47.46%), G2 (45.36%), G10 (42.32%) and G3 (40.42%) recorded maximum reduction at CaCl₂ 150 mm. The seedlings of G9 (51.24%), G10 (48.32%), G2 (47.73%) and G3 (42.25%) noted highest reduction in 150 mM NaCl + CaCl₂.

Stem diameter (cm)

Significant data was recorded among the genotypes. The data of table no1 showing that difference values between before imposition salinity and at termination seedling. maximum stem diameter was recorded in G13 (0.86 cm). Among the salinity levels maximum stem diameter was recorded in 50mM NaCl (0.76 cm), followed by 50 mm NaCl + CaCl₂ (0.70) and 50 mm CaCl₂ (0.68 cm) against the control with 0.94 cm. The minimum stem diameter was observed in 150 mM NaCl + CaCl₂ (0.27 cm), followed by 150 mm CaCl₂ (0.28). Among the interactions the maximum stem diameter was recorded in the combination of G13 x 50 mm NaCl (1.11 cm).

Number of leaves

The genotypes had strong variation (8.00 to 15.00) in number of leaves produced before imposition of salinity treatments that subsequently caused defoliation due to scorching effect of salt stress. At the termination of experiment, the number of leaves was in the range of 0 to 14.55 per seedling among all the genotypes. Increasing level of salinity reduced the number of leaves as the duration of salinity stress increased up to 40 days (Table 2 & 2a). At the highest salt concentration (150 mM) the maximum reduction was noticed in G8 (NaCl) 670.21%, G2 (CaCl2) 550.25% and G10 (NaCl + CaCl2) 628.20% while the minimum was in G13, G6, G7 & G4 at salinity levels.

Table 1: Effect of salinity on Plant height and stem diameter of nucellar citrus seedlings under varying levels of salinity

Destates			Diff			ght (c ween .		B						S [.] Differ			ter (cı een A		В			
Rootstock seedlings	Control		NaC	l		CaCl	2	NaC	Cl + C	aCl ₂		Control		NaCl			CaCl	2	NaC	l + C		
seedings	0	50	100	150	50	100	150	50	100	150	Mean	Control 0 mm	50	100	150	50	100	150	50	100	150	Mean
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	wiean	U IIIII	mm	mm	mm	mm	mm	mm	mm	mm	mm	wiean
G1	8.65	6.65	5.64	4.33	7.66	6.66	5.65	7.65	6.90	5.42	6.52	0.96	0.82	0.41	0.33	0.76	0.36	0.25	0.78	0.38	0.23	0.53
G2	6.36	5.42	4.79	3.44	5.25	4.45	3.45	5.34	4.63	3.33	4.65	0.73	0.44	0.22	0.07	0.36	0.17	0.05	0.35	0.18	0.05	0.26
G3	8.85	6.08	5.88	4.12	7.15	6.42	5.05	7.48	6.15	5.12	6.23	0.75	0.62	0.43	0.28	0.54	0.35	0.16	0.54	0.37	0.16	0.42
G4	9.45	7.09	6.99	6.88	8.42	7.08	7.05	8.41	7.14	7.05	7.56	1.06	0.82	0.63	0.42	0.78	0.58	0.35	0.78	0.56	0.34	0.63
G5	8.28	6.37	5.47	5.28	7.99	6.36	5.46	7.29	6.28	5.85	6.46	0.94	0.81	0.48	0.44	0.76	0.43	0.24	0.76	0.48	0.27	0.56
G6	9.08	7.46	6.75	5.11	8.45	7.42	6.36	8.85	7.34	6.65	7.35	1.19	0.90	0.69	0.50	0.82	0.65	0.42	0.87	0.66	0.48	0.72
G7	9.19	7.34	6.69	5.25	8.36	7.85	6.44	8.74	7.10	6.42	7.34	0.88	0.75	0.59	0.48	0.67	0.54	0.33	0.69	0.58	0.36	0.59

G8	7.09	6.64	5.36	5.14	6.42	5.44	5.99	6.85	5.91	5.66	6.05	0.83	0.75	0.52	0.35	0.66	0.47	0.28	0.66	0.42	0.24	0.52
G9	6.66	5.82	4.66	4.36	5.28	4.69	3.47	5.96	4.28	3.25	4.84	0.79	0.52	0.33	0.24	0.45	0.28	0.16	0.48	0.23	0.12	0.36
G10	6.45	4.19	3.42	2.42	5.44	4.75	3.72	5.26	4.37	3.34	4.34	0.75	0.55	0.22	0.08	0.48	0.16	0.05	0.49	0.14	0.05	0.30
G11	7.98	6.38	4.55	4.47	6.10	5.35	5.61	6.15	5.46	5.25	5.73			0.55								
G12	8.45	6.19	6.28	5.84	7.65	6.05	5.64	7.68	6.88	5.62	6.53	1.08	0.85	0.68	0.55	0.77	0.58	0.46	0.73	0.57	0.40	0.67
G13	10.4	9.71	8.34	8.29	9.41	8.45	7.14	9.48	8.10	7.15	8.65	1.26	1.11	0.85	0.66	1.03	0.76	0.54	1.09	0.76	0.55	0.86
Mean	8.22	6.56	5.68	4.99	7.20	6.23	5.46	7.32	6.20	5.39		0.94	0.76	0.51	0.37	0.68	0.44	0.28	0.70	0.45	0.27	
	G	S	$G \!\!\times\!\! S$									G	S	$G\!\!\times\!\!S$								
CD (5%)	0.21	0.19	0.67									0.02	0.01	0.05								
Se(m)±	0.08	0.07	0.24									0.01	0.01	0.08								
Note: G1-R	ough le	mon	8779,	, G2-0	CRH-	12, G	3-Gaj	animr	na, G	4-Ran	gapur	lime-Tiru	pati st	rain, (G5-Ra	ngapı	ır lim	e-Tex	as str	ain, C	36-Soi	ur dig,
G7-Sour or	ange 87	51, G	8-En	nme k	caipul	i, G9-	Chinr	nado s	sour o	orange	, G10-	Carizocitr	ange,	G11-	Balaji	acid	lime,	G12-	Japar	iese s	umme	r sour
orange, G13	G7-Sour orange 8751, G8-Emme kaipuli, G9-Chinnado 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 1a: Effect of salinity on Plant height of nucelland	r citrus seedlings under varying levels of salinity
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								Pl	ant he	ight (c	m)									
			Befor	e imp	osition	of tre	atmen	ts (B)					At the	termi	nation	of exp	perime	ent (A)		
Rootstock		Na	Cl			CaCl ₂		Na	Cl + Ca	aCl ₂		Na	Cl			CaCl ₂		Na	Cl + Ca	aCl ₂
seedlings	0	50	100	150	50	100	150	50	100	150	0	50	100	150	50	100	150	50	100	150
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
G1	20.00											23.64					24.09		22.89	
G2												28.27								
G3																				
G4	15.50 21.42 21.43 22.48 20.42 17.83 17.90 24.63 22.50 23.78 24.53 27.50 27.83 20.01 33.57 24.27 25.01 32.53 26.53 16.00 20.28 18.84 23.77 25.42 19.14 16.47 22.35 21.98 21.85 27.37 25.83 30.65 33.84 26.22 23.52 30.76 29.1 16.75 24.39 17.62 24.11 29.42 22.69 18.63 23.25 18.75 19.96 25.03 30.76 23.09 29.39 37.41 29.05 24.09 30.54 25.03															29.12	29.30			
G5	16.75	24.39	17.62	24.11	29.42	22.69	18.63	23.25	18.75	19.96	25.03	30.76	23.09	29.39	37.41	29.05	24.09	30.54	25.03	25.81
G6																28.83	31.59	24.50		
G7	16.50	17.74	19.84	21.17	26.35	24.63	20.16	18.74	14.69	18.46	25.69	25.08	26.53	26.42	34.71	32.48	26.6	27.48	21.79	24.88
G8	13.70	16.85	20.94	26.69	21.48	21.14	18.28	22.94	16.86	22.84	20.79	23.49	26.30	31.83	27.9	26.58	24.27	29.79	22.77	28.50
G9	20.50	17.35	22.76	22.17	23.25	18.85	19.46	24.16	18.80	21.15	27.16	23.17	27.42	26.53	28.53	23.54	22.93	30.12	23.08	24.40
G10	12.00	18.85	24.72	20.18	24.36	19.52	26.36	14.84	16.20	19.08	18.45	23.04	28.14	22.60	29.8	24.27	30.08	20.10	20.57	22.42
G11	17.00	20.44	25.43	21.16	20.96	22.08	22.52	15.26	22.33	16.75	24.98	26.82	29.98	25.63	27.06	27.43	28.13	21.41	27.79	22.00
G12	17.55	19.44	22.10	24.06	22.28	20.04	21.85	18.95	21.74	14.64	26.00	25.63	27.38	29.90	29.93	26.09	27.49	26.63	28.62	20.26
G13	20.52	22.36	21.00	22.39	19.17	21.44	20.98	25.45	20.19	18.10	30.94	32.07	29.34	30.68	28.58	29.89	28.12	34.93	28.29	25.25
Note: G1-R	ough l	emon 8	8779, 0	G2-CR	H-12,	G3-Ga	janimr	na, G4	-Rang	apur li	me-Tir	upati s	train, (G5-Ra	ngapur	lime-	Fexas :	strain,	G6-So	ur dig,
G7-Sour ora	inge 8'	751, G	8-Emn	ne kaij	puli, G	9-Chir	nnado s	sour or	ange,	G10-C	arizoci	itrange	, G11-	Balaji	acid li	ime, G	12-Jap	anese	summe	er sour
orange, G13	-Austr	alian so	our ora	nge. E	ach va	lue rep	resents	the me	ean val	ue of t	hree sa	mples.								
						_														

Table 1b: Effect of salinity on Stem diameter of nucellar citrus seedlings under varying levels of salinity

								Stem	ı diam	eter (c	m)									
			Befor	e imp	ositior	n of tre	eatmer	nts (B)					At the	termi	natior	n of ex	perim	ent (A))	
Rootstock			NaCl			CaCl ₂		Na(Cl + Ca	aCl ₂			NaCl			CaCl ₂		Na	Cl + Ca	aCl ₂
seedlings	0	50	100	150	50	100	150	50	100	150	0	50	100	150	50	100	150	50	100	150
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
G1	3.26	3.49	1.86	1.83	3.32	2.88	3.12	2.83	1.86	1.80	4.22	4.31	2.27	2.16	4.08	3.24	3.35	3.61	2.24	2.03
G2	3.48	2.18	1.85	1.92	3.07	2.93	2.85	2.60	2.25	1.99	4.21	2.62	2.07	1.99	3.43	3.1	2.85	2.95	2.43	2.04
G3 1.86 2.82 1.86 2.88 2.88 2.44 2.47 2.76 2.08 2.00 2.61 3.44 2.29 3.16 3.42 2.79 2.56 3.3 2.45 2.16 G4 1.98 2.95 1.89 3.23 2.42 2.66 2.38 1.98 2.04 2.02 3.04 3.77 2.52 3.65 3.2 2.76 2.66 2.36															2.16					
G5 2.22 2.44 1.90 1.66 1.95 1.65 2.76 1.92 1.95 2.88 3.16 3.25 2.38 2.10 2.71 2.08 2.94 2.68 2.43 3.15																				
G6	G5 2.22 2.44 1.90 1.66 1.95 1.65 2.76 1.92 1.95 2.88 3.16 3.25 2.38 2.10 2.71 2.08 2.94 2.68 2.43 3.15 G6 2.87 2.66 2.07 3.04 1.82 1.56 1.95 1.64 3.02 2.74 4.06 3.56 2.76 3.54 2.64 2.21 2.32 2.51 3.68 3.22														3.22					
G7	1.80	2.58	2.12	1.98	1.64	1.99	1.40	1.70	1.82	3.05	2.68	3.33	2.71	2.46	2.31	2.53	1.73	2.39	2.40	3.41
G8	1.69	2.17	2.40	2.72	1.90	2.48	1.80	2.41	2.64	3.26	2.52	2.92	2.92	3.07	2.56	2.95	2.08	3.07	3.06	3.50
G9	1.77	1.82	2.79	1.66	1.88	2.66	1.90	2.36	2.58	1.84	2.56	2.34	3.12	1.90	2.33	2.94	2.06	2.84	2.81	1.96
G10	1.85	1.90	2.70	1.64	2.62	1.60	1.77	2.38	2.56	1.97	2.6	2.45	2.92	1.72	3.1	1.76	1.75	2.87	2.7	2.02
G11	1.64	1.80	1.82	2.85	2.50	1.82	2.16	2.25	1.83	2.28	2.68	2.7	2.37	3.27	3.32	2.26	2.43	3.09	2.31	2.6
G12	1.98	2.15	2.72	2.75	2.40	1.75	2.32	1.74	1.80	1.75	3.06	3.00	3.4	3.3	3.17	2.33	2.76	2.47	2.37	2.15
G13	1.66	2.22	1.68	2.78	1.80	1.64	2.10	2.29	1.99	2.88	2.92	3.33	2.53	3.44	2.83	2.4	2.64	3.38	2.75	3.43
Note: G1-Roug																				
G7-Sour orang	e 8751	l, G8-1	Emme	kaipu	li, G9-	Chinn	ado so	our ora	nge, G	10-Ca	rizocit	range,	G11-I	Balaji :	acid li	me, G	12-Jap	anese	summe	er sour
orange, G13-A	ustralia	an sour	orang	ge. Eac	h valu	e repre	sents t	he mea	ın valu	e of the	ree san	nples.								

genotypes.

Table 2: Effect of salinit	n Number of leaves and Leaf area of nucellar citrus seedlings under v	arving levels of salinity
LUDIC M. Effect of Summe	in rumber of leaves and hear area of nuceriar endus seedings ander v	arying levels of summy

							'leave een A	~	R					Di	Le fferen		ea (cn tween		d R			
Rootstock			NaCl	Diffe				1	Cl + C	CaCl ₂				NaCl	neren				1	Cl + C	aCl ₂	
seedlings	0	50	100	150	50	100	150	50	100	150	Mean	0	50	100	150	50	100	150	50	100	150	Mean
	Mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	wream	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	Mean
G1	3.60	-3.80	-5.50	-7.00	-4.40	-7.10	-9.00	-3.10	-4.20	-7.74	-4.82	2.85	2.11	1.88	1.64	2.26	1.65	1.35	2.06	1.76	1.50	1.91
G2	2.10	-6.00	-8.00	-9.19	-7.00	-7.64	-8.78	-5.00	-8.00	-9.00	-6.65	2.45	1.93	1.76	1.42	2.10	1.55	1.25	1.86	1.66	1.31	1.73
G3	2.60	-4.00	-6.40	-8.00	-5.00	-4.54	-7.03	-3.00	-5.50	-10.09	-5.10	2.18	2.06	1.44	1.20	1.88	1.23	0.85	1.96	1.36	0.91	1.51
G4	2.90	-3.00	-5.40	-7.00	-4.00	-4.62	-5.58	-2.00	-4.50	-6.00	-3.92	3.54	3.29	2.84	2.33	3.06	2.68	2.10	3.12	2.76	2.22	2.79
G5	3.10	-3.00	-5.30	-8.00	-4.00	-5.90	-8.23	-2.00	-4.50	-7.00	-4.48	3.45	3.33	2.90	2.09	3.08	2.64	1.86	3.12	2.82	1.99	2.73
G6	3.50	-2.00	-4.50	-6.00	-3.00	-5.30	-6.50	-1.00	-3.50	-8.20	-3.65	3.85	3.55	2.94	2.32	3.36	2.70	2.11	3.42	2.80	2.21	2.93
G7	3.30	-2.00	-4.80	-6.00	-3.00	-5.20	-6.50	-1.00	-3.50	-9.05	-3.78	3.57	3.42	2.98	2.32	3.12	2.79	2.00	3.26	2.90	2.18	2.85
G8	2.50	-6.00	-8.50	-10.3	-6.52	-6.77	-6.00	-5.00	-4.34	-10.00	-6.10	2.77	2.49	1.84	1.35	2.32	1.47	1.10	2.44	1.62	1.19	1.86
G9	2.40	-7.09	-6.66	-10.7	-3.67	-5.55	-7.09	-7.00	-9.00	-6.55	-6.10	2.64	2.33	1.64	1.24	2.16	1.38	0.98	2.26	1.52	1.12	1.73
G10										-10.00					1.04	1.74	1.02	0.62	1.86	1.08	0.88	1.36
G11	3.10	-6.00	-8.80	-10.0	-7.00	-7.68	-9.28	-5.00	-7.00	-8.00	-6.57	3.12	2.77	2.06	1.64	2.86	1.88	1.22	2.66	1.98	1.42	2.16
G12	3.20	-4.00	-6.70	-8.00	-5.00	-3.85	-6.69	-3.00	-5.60	-7.00	-4.66	2.86	2.70	2.34	1.72	2.44	2.02	1.56	2.65	2.13	1.64	2.21
G13	3.50	-1.00	-3.50	-5.00	-2.00	-4.00	-6.00	-1.00	-2.60	-3.55	-2.52	3.98	3.46	3.24	2.41	3.30	2.87	2.06	3.38	3.02	2.34	3.01
Mean	2.86	-4.15	-6.21	-7.96	-4.66	-5.67	-7.26	-3.55	-5.48	-7.86		3.03	2.73	2.24	1.75	2.59	1.99	1.47	2.62	2.11	1.61	
	G	S	$G \!\!\times\!\! S$									G	S	$G \!\!\times\!\! S$								
CD (5%)	0.52	0.46	1.65									0.07	0.06	0.22								
Se(m)±	0.19	0.16	0.59									0.03	0.02	0.08								
Note: G1-R G7-Sour ora orange, G13	inge	8751,	G8-E	mme	kaipul	li, G9	-Chini	nado s	sour o	range,	G10-C	arizoc	citrang	ge, G1	1-Bala	aji aci	d lim	e, G12	2-Japa	nese	summ	er sour

Table 2a: Effect of salinity on Number of leaves of nucellar citrus seedlings under varying levels of salinity

								N	umbe	r of lea	ves									
			Bef	ore imp	osition	1 of tre	eatmen	ts (A)					At th	e term	inatio	n of ex	perim	ent (B)		
Rootstock			NaCl			CaCl ₂	:	Na	Cl + C	aCl ₂			NaCl			CaCl ₂	:	Na	Cl + Ca	aCl ₂
Seedlings	0	50	75	100	50	75	100	50	75	100	0	50	100	150	50	100	150	50	100	150
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
G1	12.69	11.98	9.85	11.62	12.68	9.96	10.96	11.62	10.78	11.99	16.29	8.18	4.35	4.62	8.28	2.86	1.96	8.52	6.58	5.99
G2	8.84	10.52	8.65	9.3	8.84	8.84	9.63	12.12	11.66	10.04	10.94	4.52	0	0	1.84	0	0	7.12	3.66	1.04
G3	11.75	12.1	7.55	8.5	10.63	7.76	8.39	10.06	8.28	11.34	14.35	8.1	1.15	0.5	5.63	0.46	0	7.06	2.78	4.34
G4	12.85	10.85	9.2	9.42	12.85	10.85	7.14	12.25	8.98	8.09	15.75	7.85	3.8	2.42	8.85	4.45	0	10.25	4.48	2.09
G5	10.25	8.85	8.44	8.94	8.62	11.62	9.34	10.85	9.78	9.46	13.35	5.85	3.14	0.94	4.62	5.72	0.84	8.85	5.28	2.46
G6	11.08	9.45	6.98	7.76	9.54	11.2	8.28	12.62	10.08	12.85	14.58	7.45	2.48	1.76	6.54	5.9	1.78	11.62	6.58	7.85
G7	10.14	7.25	8.62	10.92	6.5	12.43	7.98	12.42	7.49	11.65	13.44	5.25	3.82	4.92	3.5	7.23	1.48	11.42	3.99	6.65
G8	8.54	8.65	9.46	10.71	8.84	8.62	6.78	10.84	6.9	10.42	11.04	2.65	0.96	0	1.84	0	0	5.84	0	0.42
G9	9.36	9.45	7.68	11.32	7.89	9.20	7.64	8.96	11.7	8.1	11.76	1.45	0	0	0	0	0	1.96	2.7	0
G10	9.95	8.05	7.84	8.45	9.64	7.1	8.26	8.84	10.64	9.52	11.35	0	0	0	0	0	0	0.84	1.64	-0.48
G11	8.41	9.94	8.84	11.52	8.98	8.63	9.84	7.43	12.18	7.98	11.51	3.94	0.04	1.52	1.98	0	0	2.43	5.18	-0.02
G12	10.52	7.42	10.28	11.54	10.82	9.52	11.24	6.84	8.8	9.15	13.72	3.42	3.58	3.54	5.82	2.52	2.24	3.84	3.2	2.15
G13	11.05	8.95	11.84	12.98	8.62	8.42	10.25	9.89	9.6	8.33	14.55	7.95	8.34	7.98	6.62	4.42	4.25	8.89	7	4.33

Table 2b: Effect of salinity on Leaf area of nucellar citrus seedlings under varying levels of salinity

								Ι	∠eaf ar	ea (cm	1 ²)									
			Befor	e imp	osition	of tre	atmen	ts (B)					At the	termi	nation	of exp	perime	ent (A)		
Rootstock			NaCl			CaCl ₂		NaC	Cl + Ca	aCl ₂			NaCl			CaCl ₂		Na	Cl + Ca	aCl ₂
seedlings	0	50	100	150	50	100	150	50	100	150	0	50	100	150	50	100	150	50	100	150
	Mm	Mm	Mm	Mm	Mm	Mm	Mm	Mm	Mm	Mm	mm	Mm	Mm	Mm	Mm	Mm	Mm	Mm	Mm	Mm
G1	25.62	22.12	30.66	26.66	22.66	23.63	23.65	20.62	22.00	22.41	28.47	24.23	32.54	28.3	24.92	25.28	25	22.68	23.76	23.91
G2	13.10	21.12	21.25	11.22	16.25	10.85	17.42	18.42	20.10	20.20	15.55	23.05	23.01	12.64	18.35	12.4	18.67	20.28	21.76	21.51
													26.86							
G4	24.10	26.42	24.05	19.68	26.00	29.52	24.63	23.15	19.38	21.40	27.64	29.71	26.89	22.01	29.06	32.2	26.73	26.27	22.14	23.62
G5	28.12	21.25	28.26	21.98	28.98	26.84	26.84	24.20	18.19	20.95	31.57	24.58	31.16	24.07	32.06	29.48	28.7	27.32	21.01	22.94
G6	16.28	20.15	28.34	24.74	24.76	23.42	25.42	25.10	26.64	22.84	20.13	23.7	31.28	27.06	28.12	26.12	27.53	28.52	29.44	25.05
G7	22.62	23.62	27.05	23.16	23.66	25.95	23.65	26.50	25.26	21.42	26.19	27.04	30.03	25.48	26.78	28.74	25.65	29.76	28.16	23.6
G8	20.42	28.05	22.84	24.46	22.98	24.55	20.42	22.60	24.25	25.95	23.19	30.54	24.68	25.81	25.3	26.02	21.52	25.04	25.87	27.14
G9	22.84	27.15	23.05	25.50	24.74	21.84	21.65	19.40	20.35	24.62	25.48	29.48	24.69	26.74	26.9	23.22	22.63	21.66	21.87	25.74
G10	17.26	19.66	21.65	21.58	19.41	26.75	25.98	18.68	21.05	23.42	19.4	21.68	22.86	22.62	21.15	27.77	26.6	20.54	22.13	24.3
																				29.62
G12	26.62	21.42	28.05	21.95	20.63	24.42	24.43	21.90	23.41	24.85	29.48	24.12	30.39	23.67	23.07	26.44	25.99	24.55	25.54	26.49
G13	28.52	25.33	19.62	22.45	22.84	31.62	28.13	22.70	25.88	22.40	32.5	28.79	22.86	24.86	26.14	34.49	30.19	26.08	28.9	24.74

Note: G1-Rough lemon 8779, G2-CRH-12, G3-Gajanimma, G4-Rangapur lime-Tirupati strain, G5-Rangapur lime-Texas strain, G6-Sour dig, G7-Sour orange 8751, G8-Emme kaipuli, G9-Chinnado 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 3: Content of Fresh weight of shoot and Dry weight of shoot of nucellar citrus seedlings under varying levels of salinity

			Fre	sh we	ight o	of sho	ot (g)							D	ry wei	ight o	f sho	ot (g)				
Rootstock	Control		NaCl			CaCl	2	NaC	$\mathbf{l} + \mathbf{C}$	aCl ₂		control		NaCl		(CaCl	2	NaC	Cl + C	aCl ₂	
Seedlings	0	50	100	150	50	100	150	50	100	150	Mean	0	50	100	150	50	100	150	50	100	150	mean
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	wiean	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mean
G1	8.36	7.02	5.56	4.47	7.56	5.98	4.63	7.42	5.98	4.63	6.16	2.63	2.02	1.41	0.85	2.36	1.86	1.25	2.22	1.56	1.06	1.72
G2	4.69	4.20	3.25	2.10	4.98	3.74	2.28	4.53	3.56	2.18	3.55	1.85	1.22	0.45	0.33	1.56	1.08	0.35	1.42	0.65	0.30	0.92
G3	6.69	6.20	5.68	4.10	6.36	5.96	4.45	6.24	5.86	4.35	5.59	2.66	2.25	2.10	0.70	2.86	2.45	0.85	2.35	2.15	0.75	1.91
G4	7.36	6.03	4.85	4.02	6.40	5.20	4.08	6.12	5.05	4.00	5.31	2.45	1.85	1.48	1.00	2.20	1.75	1.20	2.00	1.55	1.10	1.66
G5	6.55	6.14	5.26	4.35	6.85	5.63	4.63	6.33	5.55	4.48	5.58	2.82	2.14	1.25	1.08	2.30	1.68	1.14	2.25	1.58	1.04	1.73
G6	9.25	8.18	7.11	6.00	8.63	7.22	6.05	8.33	7.22	6.05	7.40	3.38	3.10	2.58	2.12	3.05	2.69	2.07	3.00	2.50	2.00	2.65
G7	8.22	7.06	6.00	5.28	7.08	6.04	5.41	7.23	6.04	5.41	6.38	3.19	2.85	2.00	2.00	2.96	2.12	2.08	2.85	2.04	2.00	2.41
G8	8.55	7.19	6.28	5.54	7.46	6.58	5.85	7.46	6.58	5.85	6.73	1.74	1.50	1.49	1.00	1.88	1.69	1.10	1.55	1.49	1.00	1.44
G9	7.32	6.56	5.17	4.22	6.80	5.36	4.34	6.80	5.36	4.34	5.63	1.33	1.41	1.02	0.70	1.69	1.22	0.87	1.51	1.10	0.78	1.16
G10	3.22	2.58	2.08	1.05	3.01	2.45	1.16	2.95	2.26	1.00	2.18	1.05	1.61	1.40	0.58	1.75	1.69	0.55	1.71	1.42	0.50	1.23
G11	7.25	6.21	6.00	5.25	6.45	6.12	5.86	6.45	6.12	5.86	6.16	2.80	2.25	2.00	1.32	2.36	2.04	1.52	2.30	2.00	1.41	2.00
G12	7.85	7.48	6.09	6.78	7.74	6.32	6.91	7.74	6.32	6.91	7.01	2.84	2.00	1.70	0.82	2.10	1.85	1.10	2.00	1.75	0.86	1.70
G13	8.56	7.89	7.16	7.10	8.12	7.78	7.20	8.00	7.50	7.06	7.64	3.79	3.12	2.21	2.15	3.26	2.52	2.20	3.20	2.35	2.05	2.69
	7.22	6.36	5.42	4.64	6.73	5.72	4.83	6.58	5.65	4.78		2.50	2.10	1.62	1.13	2.33	1.90	1.25	2.18	1.70	1.14	
	G	S	$G\!\!\times\!\!S$									G	S	$G\!\!\times\!\!S$								
CD (5%)	0.17	0.15	0.55									0.05	0.04	0.17								
Se(m)±	0.06	0.05	0.19									0.01	0.01	0.06				l	l		l	
Note: G1-R	ough len	10n 8'	779 (H-12	G3-	Gaian	imm	G4-	Rang	anur lii	ne-Tirur	ati st	rain (75-Ra	ngani	ır lim	e-Tex	as str	ain (ur dio

Note: G1-Rough lemon 8779, G2-CRH-12, G3-Gajanimma, G4-Rangapur lime-Tirupati strain, G5-Rangapur lime-Texas strain, G6-Sour dig, G7-Sour orange 8751, G8-Emme kaipuli, G9-Chinnado 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 4: Content of fresh weight of root and dry weight of root of nucellar citrus seedlings under varying levels of salinity

				Fresh	weig	ht of r	oot (g	()							Dry v	weigh	t of ra	ot (g)				
Rootstock			NaCl			CaCl	2	Na(Cl + C	aCl ₂				NaCl			CaCl	2	Na(Cl + C	aCl ₂	
Seedlings	0	50	100	150	50	100	150	50	100	150	Mean	0	50	100	150	50	100	150	50	100	150	Mean
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	Mean	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	Mean
G1	3.60	3.10	2.78	1.95	2.80	2.20	1.80	3.00	2.50	1.84	2.56	0.92	0.83	0.72	0.55	0.66	0.64	0.53	0.79	0.68	0.58	0.69
G2	2.58	2.18	1.78	0.96	1.98	1.21	0.78	2.10	1.60	0.84	1.60	0.63	0.58	0.45	0.30	0.50	0.40	0.24	0.55	0.41	0.28	0.43
G3	3.98	3.49	2.92	2.08	3.05	2.55	1.88	3.38	2.79	2.02	2.81	0.85	0.72	0.66	0.55	0.62	0.60	0.50	0.70	0.66	0.52	0.64
G4	3.25	3.04	2.54	1.85	2.78	2.30	1.66	2.96	2.44	1.80	2.46	0.76	0.72	0.61	0.54	0.62	0.55	0.50	0.68	0.61	0.54	0.61
G5	3.55	3.18	2.66	2.10	2.98	2.25	1.65	3.08	2.56	2.05	2.61	0.82	0.72	0.65	0.60	0.70	0.60	0.52	0.74	0.62	0.58	0.66
G6	3.54	3.22	3.02	2.26	2.86	2.66	1.88	3.06	2.86	2.16	2.75	0.95	0.90	0.85	0.70	0.80	0.78	0.65	0.88	0.85	0.68	0.80
G7	3.89	3.38	2.95	2.36	2.90	2.64	1.86	3.38	2.90	2.26	2.85	0.81	0.78	0.71	0.68	0.71	0.67	0.60	0.76	0.71	0.65	0.71
G8	3.98	3.59	2.86	2.22	2.06	2.41	1.58	3.29	2.77	2.09	2.69	0.73	0.66	0.61	0.53	0.61	0.58	0.50	0.65	0.61	0.55	0.60
G9	2.54	2.22	1.78	0.90	1.89	1.12	0.68	2.09	1.65	0.80	1.57	0.70	0.66	0.62	0.54	0.60	0.57	0.52	0.66	0.62	0.54	0.60
G10	2.14	1.88	1.38	1.07	1.76	0.92	0.78	1.79	1.29	1.00	1.40	0.75	0.70	0.63	0.57	0.72	0.59	0.54	0.78	0.65	0.59	0.65
G11	3.52	3.20	2.64	2.08	2.84	1.98	1.48	3.10	2.60	2.08	2.55	0.62	0.57	0.52	0.47	0.50	0.47	0.40	0.57	0.52	0.47	0.51
G12	3.66	3.28	2.72	1.85	2.03	2.08	1.25	3.28	2.72	1.85	2.47	0.73	0.68	0.62	0.53	0.64	0.59	0.47	0.68	0.62	0.53	0.61
G13	3.58	3.19	2.88	2.14	2.94	2.62	2.08	3.49	2.88	2.14	2.79	0.87	0.82	0.76	0.72	0.80	0.74	0.70	0.86	0.79	0.68	0.77
Mean	3.37	3.00	2.53	1.83	2.53	2.07	1.49	2.92	2.43	1.76		0.78	0.72	0.65	0.56	0.65	0.60	0.51	0.72	0.64	0.55	
	G	S	$G \!\!\times\!\! S$									G	S	$G \!\!\times\!\! S$								
CD (5%)	0.08	0.07	0.24									0.02	0.02	0.06								
Se(m)±	0.03	0.02	0.09									0.01	0.01	0.02								

Note: G1-Rough lemon 8779, G2-CRH-12, G3-Gajanimma, G4-Rangapur lime-Tirupati strain, G5-Rangapur lime-Texas strain, G6-Sour dig, G7-Sour orange 8751, G8-Emme kaipuli, G9-Chinnado 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.

Leaf area (cm)

The maximum leaf area was recorded in G13 (3.01 cm^2) followed by G6 (2.93 cm^2) and G7 (2.85 cm^2), while minimum area was observed in G10 (1.36 cm^2), followed by G3 (1.51 cm^2) and G9 (1.73 cm^2). Among the salinity levels, 50 mM NaCl (2.73) has recorded the maximum leaf area, against the control with 3.03. Minimum leaf area was

observed in the treatment 150 mm CaCl₂ (1.47). Pertaining to the interactions the maximum leaf area was recorded in the combination of G6 x 50 mm NaCl (3.55 cm²), while minimum was recorded in the combination, G10 x 150 mm CaCl₂ (0.62 cm²).

Shoot fresh weight

Significant data was recorded among the genotypes. The maximum shoot fresh weight was recorded in (Table 3) G13 (7.64 g). The minimum shoot fresh weight was recorded in G10 (2.18 g). Among the salinity levels 50 mm NaCl (4.64 g) has recorded the minimum shoot fresh weight. Whereas maximum was recorded in 50 mm NaCl (6.73 g), followed by 50 mm NaCl + CaCl₂ (6.58 g), against the control with 7.22 g. Among the interactions the maximum shoot fresh weight was recorded in the combination of G6 x 50 mm CaCl₂ (8.63 g), while minimum was recorded in G10 x 150 mm NaCl + CaCl₂ (1.00 g).

Shoot dry weight

Significant data was recorded among the (Table 3) genotypes. The maximum shoot dry weight was recorded in G13 (2.69 g), while the minimum values were observed in G2 (0.92). Among the salinity levels, 50 mm CaCl₂ (2.33 g) recorded the maximum shoot dry weight, while control recorded 2.50 g shoot dry weight. In contrast, minimum shoot dry weight was observed in 150 mm NaCl (1.13), followed by 150 mm NaCl + CaCl₂ (1.14). Among the interactions, the minimum shoot dry weight was recorded in the combination of G2 x 150 mm NaCl + CaCl₂ (0.30), whereas maximum was recorded in G13 x 50 mm CaCl₂ (3.26).

Root fresh weight

Significant data (Table 4) was recorded among the genotypes. The maximum root fresh weight was recorded in G7 (2.85 g). The minimum root fresh weight was recorded in G10 (1.40 g). Among the salinity levels minimum root fresh weight was observed in 150 mm CaCl₂ 1.49 g), while the maximum root fresh weight was recorded in 50 mm NaCl (3.00) followed by 50 mm NaCl + CaCl₂ 2.92 g), against the control with 3.37 g. Among the interactions the maximum root fresh weight was recorded in the combination of G8 x 50 mm NaCl (3.49 g), while minimum was recorded in G10, G2 x 150 mm CaCl₂ (0.78 g).

Root dry weight

Significant data (Table 4) was recorded among the genotypes. The maximum root dry weight was recorded in G6 (0.80 g). The minimum root dry weight was recorded in G2 (0.43 g). Among the salinity levels 50 mm NaCl and 50 mm NaCl + CaCl₂ (0.72 g) recorded the maximum root dry weight, against the control with 0.78 g. In contrast, minimum values were observed in 150 mm CaCl₂ (0.51), followed by 150 mm NaCl + CaCl₂ (0.55). Among the interactions the maximum root dry weight was recorded in the combination of G6 x 50 mm NaCl (0.90 g), while minimum was recorded in G2 x 150 mm CaCl₂ (0.24 g).

Discursion

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)^[1], suffers severely during early stages of growth under salinity (Srivastav *et al.*, 2007)^[8]. 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. 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)^[2] 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)^[12]. With support of the above reasons, Rhodes (1994)^[6] 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)^[7] also found that graded levels of NaCl salt affected the plant height, stem diameter, number of leaves, leaf area and survivability of mango. He also stated that salinity stress slowed down the photosynthesis, caused stomatal closure, and thereby caused growth reduction. The excessive accumulation of toxic ions under high load of salt in the leaves depend on the capacity of plant to compartmentalize the salt in the vacuoles and build up in the toxic levels in the cytoplasm as per Munns et al., (2006)^[5]. Excessive concentration of Na⁺ and Cl⁻ under salinity stress caused the necrosis symptoms which were observed during our experiment. Our results indicated that the salinity affects above ground portions more than the root portions. We observed the reduction in root length after uprooting of the seedlings from saline medium due to increased osmotic stress. Foolad (2004)^[13] related the negative effect of salinity to the osmotic potential of the solution wherein root cells of the plant could not get enough water from the growing medium due to high osmotic potential. It indirectly affected the uptake of essential mineral nutrients dissolved in the water and collectively, led to hyperosmotic stress and disequilibrium of ions, disturbing the plant metabolic functions at cellular level. The adverse effect of salinity stress on plant growth and development is multi directional causing induction in water deficit, nutritional imbalance, oxidative stress, physiobiochemical changes and alterations at morphological, cellular, and molecular levels. The findings of our study regarding the negative effect of salinity has also been observed in different crops like citrus (Zekri and Parsons, 1990b)^[14].

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 150 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 and rootstocks compared to other citrus rootstocks. Among the citrus rootstocks, sour orange give best results regarding growth performance under saline condition, while '*Carrizo citrange*' was found the least tolerant rootstock.

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