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Influence of NaCl induced salt stress on the seedling growth of citrus species

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Abstract
Salinity is a commonly occurring abiotic stress and poses as a major impediment in citrus production as the crop has less tolerance ability to this stress. It causes severe osmotic stress restraining overall performance of the plant. One month old seedlings of Rangpur lime, Rough lemon and Indian wild orange were established in sand media and later exposed to stress for 48 hrs in a low cost polyhouse. The different levels of sodium chloride (0 mM, 75 mM and 100 mM) was used for simulating salt stress. The morpho-physiological parameters *viz*., shoot and root length, fresh and dry weight of shoot and root and Relative water content (RWC) were reduced on exposing to stress with severity being in 100 mM NaCl. On comparing citrus species, Rangpur lime performed better at both stress levels than Rough lemon and Indian wild orange exhibiting its tolerance towards salinity.

Keywords: Citrus, salinity, sodium chloride, RWC, Rangpur lime

Introduction
Abiotic stresses *viz*. salinity, flooding, drought, metal toxicity and extreme in temperatures have adverse effect on growth and yield of the plants. Among all, soil salinization is considered as a major threat to sustaining global food security. It is a major problem in areas where irrigation is required (Flowers, 2004) [9] and in regions with hot dry condition (Ben-Asher, 1993) [3]. Salinity has affected one third of the world's irrigated lands and has led to abandoning of 10 m ha of irrigated lands every year (Abrol *et al*., 1988) [1]. In India, salinity has affected over 10.1 m ha of land (Sehgal and Abrol, 1994) [20]. Genus Citrus has less tolerance to stresses and hence considered to be salt sensitive (Maas, 1992) [14]. Salinity has reduced worldwide production of Citrus. Worldwide Citrus is grown as irrigated crop (Shalhevet and Levy, 1990) [21] and often water quality is deteriorated by improper soil drainage or run off, leading to salt accumulation with poor quality fruits (Levy and Syvertsen, 2004) [13]. Citrus rootstocks differ in their tolerance level to water stress and salinization based on their management practice, soil, climate and the scion cultivar used.

Soil salinity is a great threat to agriculture as it affects crop productivity by inducing osmotic stress and toxicity of ions, mostly sodium, chloride, calcium, magnesium, sulfate, potassium, bicarbonate, carbonate, nitrate, and occasionally borate ions (Bernstein and Hayward, 1958; Peck, 1975) [4, 16]. However, because of the abundance of NaCl in salt affected soils and irrigation water it is an ideal salt for simulating salt stress. Salinity affects all stages of plant, from germination to fruiting. It is known to reduce growth, germination (Sharma *et al*., 2013) [22], RWC (Sairam *et al*., 2005) [19] and yield (Zekri, 1993) [25] in various crops, including citrus. Citrus is an important horticulture commodity and its production is hampered largely by soil salinity in various parts of the world. The present study was carried out to screen best salt tolerant citrus rootstock at the initial stage of seedling development and to study the morpho-physiological changes of citrus seedlings under salinization.

Materials and Methods
Fully matured and ripened fruits of three citrus species *viz*., Rangpur lime (*Citrus limonia*), Rough lemon (*Citrus jambhiri*), and Indian wild orange (*Citrus indica*) were collected from Pasighat, Bodak and Yagrung regions of Arunachal Pradesh. Seeds extracted from the collected fruits were washed thoroughly and treated with fungicide. Seeds were then germinated in a hydroponical condition at a temperature of 25±2 °C. Once the seedlings were

uniformly developed they were transferred to clean plastic pots filled with priorly sterilized river sand. From the time of transplanting till establishment of seedlings [11], Hoagland solution (Hoagland and Arnon, 1950) was given in a uniform quantity to each pot. After establishment, seedlings were treated with different concentration of sodium chloride (0 mM, 75 mM and 100 mM) mixed along with Hoagland solution. The treated root and shoot samples were collected at 48 hrs after treatment application for further analysis.

Growth parameters
Shoot and root length from ten randomly collected seedlings were measured using a measuring scale. Fresh weight (FW) for ten randomly selected root and shoot was measured immediately after uprooting while dry weight (DW) from the same root and shoot samples were measured after oven drying for 48 h at 70°C till they attained stable weight.

Shoot relative water content
Relative water content (RWC) was measured in shoots of the seedlings using the method given by [24]Weatherley (1950) with slight modifications and calculated as: RWC (%) = [(FW−DW)/(TW−DW)]×100. Turgid weights (TW) for the shoots were measured after placing them in saturated bottles at 4 °C for 48 h.

Data analysis
Data obtained were subjected to statistical analysis of variance by completely randomized design (CRD) with two factors: salinity levels (0, 75 and 100 mM) and citrus species (Rangpur lime, Rough lemon and Indian wild orange) with three replicates. Significance and non-significance of the variance due to different treatments will be determined by calculating the respective 'F' values as the method described by [10] Gomez and Gomez (2010).

Results and Discussion
Shoot and Root length (cm)
Inhibition of shoot growth is a visible symptom of salinity stress. At seedling stage most of the plant species shows stress induced biochemical and morphological changes within 48-72 hrs of exposure. So we started our analysis by having the first uprooting after 48 hrs of treatment application.
The salt stress significantly suppressed shoot growth in the citrus species (Table 1). The treatment with 100 mM NaCl recorded lower shoot length (12.27 cm) while highest shoot length (12.76 cm) was noticed under control condition with 0 mM NaCl. Among citrus species highest shoot length (14.36 cm) was recorded in Indian wild orange whereas the lowest (11.45 cm) was recorded in Rangpur lime. The interaction effect of citrus species with salinity significantly inhibited shoot length. The inhibiting effect was more pronounced with the increasing concentration of salt. Maximum decrease was recorded in Indian wild orange treated with 100 mM NaCl (4.49%) and lowest decrease was recorded in Rangpur lime treated with 75 mM NaCl (1.18%) compared to their respective control.
The decline in root length was evident under stress condition (Table 1). Root length was maximum under control condition (15.02 cm) and it decreased with the concomitant increase in salt level. Lower root length was noticed in seedlings treated with 100 mM NaCl (14.47 cm). Higher root length among citrus species was recorded in Rough Lemon (15.69 cm) while lower root length in Indian wild orange (13.14 cm). Conversely, under the combined effect of citrus species with salt stress least decline in root length was noticed in Rangpur lime treated with 75 mM NaCl (0.45%) and maximum decline in Indian wild orange treated with 100 mM NaCl (8.09%).
Root is the primary organ to be exposed to osmotic stress (Manske and Vlek, 2002) [15]. Roots affected with stress reduce uptake of nutrients and water thereby altering activity of hydrolytic enzymes, consecutively inhibiting seedling growth (Houshmandfar and Moraghebi, 2011) [12]. Conversely, the inhibitory effect was more on shoot length compared to root length except for Indian wild orange treated with 100 mM NaCl. The osmotic stress induced by salt or stress inhibits cell elongation and expansion by lowering turgor pressure, closure of stomata, meager supply of assimilates and modification in phytohormones levels (El-Desouky and Atawia, 1998) [8]. In our experiment leaf injuries in salt treated seedlings were not noticed at 48 hrs. Conversely, growth inhibition was noticeable at 48 hrs uprooting. As reported by Ackerson and Youngner (1975) [2] and Dudeck *et al*. (1983) [7], salinity effects appear before buildup of ion in plants. Hence, the dwindling effects of salt stress at 48 hrs could be because of nutritional imbalance rather than toxicity of ions.

Fresh and dry weight of shoot and root (g)
Decrease in plant biomass is a common phenomenon under salt and drought stress, mainly because of the dwindling effects of stress on plants that eventually cause cell death. According to Romero-Aranda *et al*. (1998) [18] and Dong *et al*. (2007) [6], reduction in biomass might be because of reduced leaf area and number of leaves. This could be because of the disturbances in physiological and biochemical aspects of the seedlings affected with salt stress (Craine, 2005) [5]. In this experiment, compared to control, growth parameters like fresh and dry weight of shoots and roots gradually declined with the concomitant increase of treatment concentrations (Table 2 & 3). Citrus species exhibited varied response with regard to fresh and dry weight of shoots and roots. Fresh and dry weight of shoots was maximum in Indian wild orange while minimum in Rangpur lime. Whereas, fresh and dry weight of roots was maximum in Rough lemon while minimum in Indian wild orange. The interaction effect was found to be more inhibiting in Indian wild orange compared to other species.

Shoot relative water content (%)
Relative water content is an important indicator in assessing tolerance ability of a plant under stress conditions. RWC shows the hydration level in plant cells and tissues which is essential for physiological metabolism (Silva *et al*., 2007) [23]. In this experiment, RWC decreased with the concomitant increase of treatment concentrations (Table 4). Salt treatment with 100 mM NaCl recorded lower level of RWC (81.50%) while control recorded maximum RWC (91.11%). However, citrus species showed no significant difference among each other. The interaction effect was found to be significant and it exhibited decline in RWC with concomitant increase of treatment concentrations. Rangpur lime recorded least reduction both at 75 mM (5.61%) and 100 mM NaCl (6.41%) while maximum reduction was noticed in Indian wild orange [75 mM NaCl: (10.73%) and 100 mM NaCl: (15.74%)]. Plants exposed to salt and stress show severe reduction in RWC and the results are in accordance with Rahneshan *et al*. (2018) [17].

In conclusion, results of the present study indicate that citrus species differed in their tolerance ability towards salt stress. Decline in shoot and root length, fresh and dry weight of shoot and root and RWC were more pronounced in salt level with 100 mM NaCl as compared to 75 mM NaCl. On comparing citrus species, Rangpur lime performed better with least reduction in morpho-physiological parameters under stress condition followed by Rough lemon. Whereas Indian wild orange which is said to be a hardy species exhibited poor growth on exposing to stress condition indicating its sensitivity towards salinity. In this experiment, seedlings on exposure to salinity showed no leaf injuries suggesting the dwindling effects of salt stress at 48 hrs could be because of nutritional imbalance rather than toxicity of ions.

Table 1: Effect of salt stress on shoot and root length of citrus species

Treatments	Shoot length (cm)				Root length (cm)			
	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)
0 mM	11.607	11.880	14.801	12.763	15.603	15.867	13.600	15.023
75 mM	11.470	11.500	14.154	12.375	15.533	15.661	13.333	14.842
100 mM	11.289	11.400	14.136	12.275	15.367	15.567	12.500	14.478
Mean (C)	11.455	11.593	14.364		15.501	15.698	13.145	
		S	V	S X V		S	V	S X V
CD (*p*<0.05)		0.108	0.108	0.187	CD @ 5%	0.157	0.157	0.272
S.E(m)		0.036	0.036	0.063	S.E(m)	0.053	0.053	0.091

C - Citrus species, S - Salinity levels

Table 2: Effect of salt stress on shoot and root fresh weight of citrus species

Treatments	Shoot fresh weight (g)				Root fresh weight (g)			
	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)
0 mM	0.564	0.698	0.883	0.715	0.266	0.443	0.260	0.323
75 mM	0.544	0.639	0.790	0.658	0.258	0.407	0.235	0.300
100 mM	0.499	0.612	0.733	0.615	0.241	0.391	0.217	0.283
Mean (C)	0.536	0.650	0.802		0.255	0.414	0.237	
		S	V	S X V		S	V	S X V
CD (*p*<0.05)		0.021	0.021	0.036	CD @ 5%	0.006	0.006	0.011
S.E(m)		0.007	0.007	0.012	S.E(m)	0.002	0.002	0.004

C- Citrus species, S- Salinity levels

Table 3: Effect of salt stress on shoot and root dry weight of citrus species

Treatments	Shoot dry weight (g)				Root dry weight (g)			
	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)
0 mM	0.157	0.180	0.185	0.174	0.079	0.071	0.061	0.070
75 mM	0.148	0.161	0.161	0.157	0.075	0.064	0.054	0.064
100 mM	0.134	0.151	0.148	0.144	0.071	0.061	0.049	0.060
Mean (C)	0.146	0.164	0.165		0.075	0.065	0.055	
		S	V	S X V		S	V	S X V
CD (*p*<0.05)		0.004	0.004	0.007	CD @ 5%	0.002	0.002	NS
S.E(m)		0.001	0.001	0.002	S.E(m)	0.001	0.001	0.001

C- Citrus species, S- Salinity levels

Table 4: Shoot relative water content in citrus species after 48 hours of treatment exposure

Treatments	RWC (%)			
	Rangpur lime	Rough lemon	Indian wild orange	Mean (S)
0 mM	89.451	90.087	93.817	91.118
75 mM	84.435	82.986	83.755	83.725
100 mM	83.716	81.738	79.054	81.503
Mean (C)	85.867	84.937	85.542	
		S	V	S X V
CD (*p*<0.05)		2.021	NS	3.501
S.E(m)		0.680	0.680	1.178

C- Citrus species, S- Salinity levels

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