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

Nesara Begane, Lobsang Wangchu, Siddhartha Singh, Lakidon Khonglah, Longing Langstieh and Amrita Thokchom

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) and in regions with hot dry condition (Ben-Asher, 1993). 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). In India, salinity has affected over 10.1 m ha of land (Sehgal and Abrol, 1994). Genus Citrus has less tolerance to stresses and hence considered to be salt sensitive (Maas, 1992). Salinity has reduced worldwide production of Citrus. Worldwide Citrus is grown as irrigated crop (Shalhevet and Levy, 1990) 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). 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). 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) and yield (Zekri, 1993) 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 morphophysiological changes of citrus seedlings under salinization.

Materials and Methods
Fully matured and ripened fruits of three citrus species viz., Rangpur lime (Citrus limoniana), Rough lemon (Citrus jambhiri), and Indian wild orange (Citrus indica) were collected from Pasighat, Bodak and Yagurong 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

Corresponding Author:
Nesara Begane
Department of Fruit Science, Central Agricultural University, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh, India
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 "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 "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 was found to be significant and it exhibited decline in RWC with 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].

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) [22]. 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.
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       | 11.440       | 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           |           |
| CD (p<0.05)| S           | V             | S X V            | 0.108     | CD @ 5%      | S           | V               | S X V     |
| 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           |           |
| CD (p<0.05)| S           | V             | S X V             | 0.021     | CD @ 5%      | S           | V               | S X V     |
| 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           |           |
| CD (p<0.05)| S           | V             | S X V             | 0.004     | CD @ 5%      | S           | V               | S X V     |
| 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 | 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             |         |
| CD (p<0.05)| S           | V           | S X V              |         |
| S.E(m)     | 0.680        | 0.680       | 3.501              | 1.178   |

C - Citrus species, S - Salinity levels

### References
1. Abrol IR, Yadav JSP, Massoud FI. Salt affected soils and their management. FAO Soils Bull. 39, United Nations, Rome, 1988.
2. Ackerson RC, Youngner VB. Responses of bermuda grass to salinity. Agron. J. 1975; 67:678-681.
3. Ben-Asher J. A simplified model of integrated water and solute uptake by salts and selenium accumulating plants. Soil Sci. Soc. Am. J. 1993; 58:1012-1016.
4. Bernstein L, Hayward HE. Physiology of salt tolerance. Ann. Rev. Plant Physiol. 1958; 9:25-46.
5. Craine JM. Reconciling plant strategy theories of Grime and Tilman. J. Ecol. 2005; 93:1041-1052.
6. Dong Y, Ji T, Dong S. Stress responses to rapid temperature changes of the juvenile sea cucumber (Apostichopus japonicus Selenka). J. Ocean Uni. Chin. 2007; 6:275-280.
7. Dudeck AE, Singh S, Giordana CE, Nell TA, Mc Connell DB. Effects of sodium chloride on Cynodon turf grasses. Agron. J. 1983; 75:927-930.
8. El-Desouky SA, Atawia AA. Growth performance of some citrus rootstocks under saline conditions. Alexandria J agric. Res. 1998; 42:231-254.
9. Flowers TJ. Improving crop salt tolerance. J. Exp. Bot. 2004; 55:307-319.
10. Gomez AK, Gomez AA. Statistical procedures for agricultural research. 2nd edn. Wiley India Private Limited, Ansari road, Daryaganj, New Delhi, 2010, 134-138p.
11. Hoagland DR, Arnon DI. The water culture method for growing plants without soil. Circular 347. California Agricultural Experiment Station, Berkeley, CA, 1950.
12. Houshmandfar A, Moraghebi F. Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower. Afr. J Agric. Res. 2011; 6:1463-1468.
13. Levy Y, Syvertsen J. Irrigation water quality and salinity effects in Citrus trees. Hortic. Rev. 2004; 30:37-82.
14. Maas EV. Salinity and citiculture. Proceedings of the International Society of Citriculture: Volume 3 Pests and their control, harvesting procedures, postharvest physiology and residues, processing biochemistry of citrus products, economics, marketing and trade, integrated control in citiculture, plenary symposium: 7th International Citrus Congress, 1992, 1290-1301p. Acireale, Italy.
15. Manske GGB, Vlek PLG. Root architecture of wheat as a model plant. In: Waisel Y., Eshel, A. and Kafkafi, U. (eds) Plant roots: the hidden half, 3rd edn. New York, NY:Marcel Dekker, 2002, 249-259p.
16. Peck AJ. Effects of land use on salt distribution in the soil. In: Poljakoff-Mayber, A. and Gale, J. (eds) Plants in Saline Environments. Springer-Verlag, Berlin and Heidelberg, 1975, 77-90p.
17. Rahneshan Z, Nasibi F, Moghadam AA. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (Pistacia vera L.) rootstocks. J Plant Interact. 2018; 13:73-82.
18. Romero-Aranda R, Moya JL, Tadeo FR, Legaz F, Primo-Millo E. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. Plant Cell Environ. 1998; 21:1243-1253.
19. Sairam RK, Srivastava GC, Agarwal S, Meena RC. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biol. Plant. 2005; 49:85-91.
20. Sehgal J, Abrol IP. Soil degradation in India: Status and impact. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 1994, 80p.
21. Shalhevet J, Levy Y. Citrus trees. In: Stewart, A.R. and Nielsen, D.R. (eds) Irrigation of agricultural crops. Madison, 1990, 951-986p.
22. Sharma LK, Kaushal M, Bali SK, Choudhary OP. Evaluation of rough lemon (Citrus jambhiri Lush.) as rootstock for salinity tolerance at seedling stage under in vitro conditions. African J. Biotech. 2013; 12:6267-6275.
23. Silva MA, Jifon JL, Silva JAG, Sharma V. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. Braz. J Plant Physiol. 2007; 19:193-201.
24. Weatherley PE. Studies in the water relation of cotton plant, I. The field measurement of water deficit in leaves. New Phytol. 1950; 49:81-97.
25. Zekri M. Salinity and calcium effects on emergence, growth and mineral composition of seedlings of eight citrus rootstocks. J Hortic. Sci. 1993; 68:53-62.