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# Na and k content in leaves, shoots and roots of tomato seedling varied under iso- Osmotic stresses of NACL and PEG

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**Abstract**
A plug tray experiment for identification of early stage salt stress responses in tomato seedling under iso-osmotic stress was conducted under net house condition. An experiment was laid out in completely randomized design with factorial concept having ten treatment combinations comprising of two levels of sources of stress: PEG-8000 and NaCl and five levels of water potential *viz*., -0.2MPa, -0.4MPa, -0.6MPa, -0.8MPa, and -1.0MPa beside one control treatment. The treatments were replicate thrice and the effects of these treatments on $Na^+$ and $K^+$ content in leaves, shoot and root were studied. From the experimental results it was indicated that NaCl induced stress had significant effect on leaves, shoot and root $Na^+$ and $K^+$ content. $Na^+$ content was recorded higher in leaves followed by shoot and root at -1.0MPa and $K^+$ content was higher in leaves followed by shoot and root under lowest water potential of -0.2 MPa. However, PEG-8000 had non-significant effect on both Na+ and $K^+$ content in leaves, shoot and root accumulation.

**Keywords:** salinity, sodium chloride, $Na^+$, $k^+$

## 1. Introduction
Soil salinization is one of the major factors of soil degradation. It has reached 19.5 % of the irrigated land and 2.1 % of the dry-land agriculture existing on the globe (FAO, 2013) [4]. Salinity effects are more conspicuous in arid and semiarid areas where 25 % of the irrigated land is affected by salts. The major inhibitory effect of salinity on plant growth has been attributed to: 1) osmotic effect 2) ion toxicity 3) nutritional imbalance leading to reduction in photosynthetic activities and other physiological disorders (Ali *et al*., 2004) [1]. Reduction of growth response to salinity is usually attributed to either ion cytotoxicity (mainly because of $Na^+$, $Cl^-$ and) and/or low external osmotic potential (Munns and Termeat 1986) [7]. Ion cytotoxicity is caused by the replacement of $K^+$ by $Na^+$ in biochemical reactions and conformational changes, and by the loss of function of proteins as $Na^+$ and $Cl^-$ penetrates the hydration shells and interferes with the noncovalent interaction between their amino acids (Xu *et al*. 2000) [16]. Salinity also affects nutrient balance in plant tissues.
The constituent cations of total soluble salts in soils are usually sodium ($Na^+$), calcium ($Ca_2^+$), and magnesium ($Mg_2^+$) and the anions are chloride ($Cl^-$) and carbonate However, $Na^+$ dominates the cations and $Cl^-$ the anions in the majority of saline soils to the extent that NaCl comprises from 50–80% of the total soluble salts (Rengasamy, 2010) [10]. Salt stress has 3-fold effects on plant growth: it reduces soil water potential leading to osmotic stress, it induces ion imbalance in cells, especially lower concentrations of $K^+$ $Ca^{2+}$, and $NO_3^-$, and it causes ion ($Na^+$ and/or $Cl^-$) toxicity. Since salt stress involves both osmotic and ionic stresses, growth suppression is directly related to the total concentration of soluble salts and osmotic potential of the soil solution. The detrimental effect is observed at the whole-plant level as the death of plants or a decrease in productivity (Munns and Tester, 2008) [8] $Na^+$ and $Cl^-$ derived from NaCl are well known as the toxic ions to damage the plant cells in both ionic and osmotic levels. Plant growth and development are directly inhibited, leading to low yield prior to plant death (Mansour and Salama, 2004; Chinnusamy *et al*., 2005) [6, 3]. In normal conditions, the $Na^+$ concentration in the cytoplasm of plant cells was low in comparison to the $K^+$ content, frequently $10^{-2}$ versus $10^{-1}$ and even in conditions of toxicity, most of the cellular $Na^+$ content was confined into the vacuole (Apse *et al*., 1999) [2].
For most plants to tolerate salinity, $Na^+$ and $Cl^-$ uptake must be restricted while maintaining

the uptake of macronutrients such as $K^+$, $NO_3$ and $Ca_2^+$. The mechanisms of $Na^+$ and K+ transport in plants under salt stress have been extensively researched and reviewed (Shabala and Cuin, 2008) [11]. Salinity has combined effect of ionic and water stress and there is very little information is available on influence of osmotic stress on $Na^+$ distribution in plants under salinity stress. Reduced $Na^+$ loading into the xylem is one of the main mechanisms of salinity tolerance and it is often considered one of the most crucial features of restricting $Na^+$ accumulation in plant tissues (Tester and Davenport, 2003; Munns and Tester, 2008) [15, 8] Thus our research experiment aimed to know about effect of osmotic stress of salinity on $Na^+$ distribution in plant system.

## 2. Material and Methods

The experiments were conducted under net house condition in October-2015 at ASPEE College of Horticulture and Forestry, Navsari Agricultural University (NAU), Navsari-Gujarat, India (20° 55 ′ 38″ N, 72° 53 ′ 54″ E longitudes). The weather data recorded from meteorological observatory, (NAU). Average temperature was 290C, relative humidity 67% and full sunny days during growing season. Seeds of tomato variety GT-2 were obtained from the Regional Horticulture Research Station (RHRS), NAU, Navsari and seeds were treated with 1% sodium hypochlorite by immersing for 10 minutes, before sowing to prevent any seed-born disease incidence. Plug trays which contain 40 and 20 cavities were allotted for every factor of NaCl and PEG- 8000 treatments. Before starting the experiment, all trays were washed by detergent solution and put under sunlight for one day and the holes of trays pluged by filter paper to prevent loss of growing media. Mixture of cocopit, vermiculite and perlite were used as growing media in ratio of 3:1:1. Before mixing of these components, cocopit soaked in calcium nitrate for one night to remove salinity. The growing media and ratio was standardized for tomato seedlings raised by RHRS, Navsari. Photosynthetic parameters of tomato were studied for control and five different osmotic potentials (-0.2,-0.4, -0.6,-0.8 and -1.0MPa) of both NaCl and PEG-8000, which were prepared by adding various concentrations of NaCl and PEG-8000 to distilled water. Desired osmotic potential of NaCl (HiMedia Laboratories Pvt. Ltd) was prepared from the molal concentration of solutions using Van't Hoff's equation (1967) $\psi w = -miRt$ where m= molarity of NaCl, i= reaction quotient, R= the gas constant = 8.314 J/mol·K, t = temperature was used to calculate the osmotic potential of NaCl and different water potentials of PEG-8000 (HiMedia Laboratories Pvt. Ltd.) were calculated by using equation derived for PEG-8000. OP= 1.29xC2xT − 140xC2 − 4.0xC. where C= PEG concentrations and T= temperature. For both the treatments, daily temperature was used for calculation and fresh solutions were applied. The required amounts of solution were calculated on the basis of field capacity (1liter water in 10 kg pot/day). Treatments were applied after 5th Days after sowing.

### $Na^+$ /$K^+$ Analysis from Leaves, shoot and root

For analysing $Na^+$ /$K^+$ content from leaves, shoot and root five random plants were selected from each factor per repition. Afterwards plant sample were dried in hot air oven at 65-70$^0$C temperature and grind in grinder. Accurately weigh of 0.5 gram grind plant samples of leaves, root and shoot were prepared, add 10 ml $H_2SO_4$ and kept it for overnight. In next morning, 10-15 ml diacid ($HNO_3$:$HClO_4$) added and samples were remain stand for overnight. Afterwards samplings were kept on hot pelt till boil solution become transparently clear and volume was reduced up to 3-5 ml. After cooling the sample solution were transferred to volumetric flask and make up 100 ml volume with distilled water and filter through Whatman No. 1 filter paper, retained these solutions and recorded the reading by use of flame photometer (John and Styn, 1973) [5].

### Statistical analysis

The data recorded for during the investigation were statistically analysed by a procedure appropriate to design of the experiment as described by Panse and Sukahtme (1978) [9] and the significance of differences among means was tested by F" test at five per cent level. The critical differences was calculated whenever the difference among treatment found significant.

## 3. Result

### 3.1 $Na^+$ content (g/g dry weight) in leaves shoot and root

The mean data pertaining to stress induced by PEG-8000 and NaCl on $Na^+$ content in leaves, shoot and root of tomato seedlings are presented in table 1 Results of analysis of variance for $Na^+$ content in leaves, shoot and root were indicated significant differences between water potential (WP), source of stress (S) and their interaction (SxWP), as well as control vs. rest means at P=0.05 at 21st DAS. Significant differences were observed for $Na^+$ content of leaves shoot and root due to NaCl stress while PEG-8000 had non-significant differences on $Na^+$ content in leaves, shoot and root. The $Na^+$ content in leaves, shoot and root was increasing progressively with decreasing osmotic potential of NaCl. Mean data indicated that higher $Na^+$ content found in leaves followed by shoot and root. Increasing concentration of NaCl also increased Na+ content in leaves, shoot and root. Lowest amount of $Na^+$ content was recorded under leaves (3.33 g/g dry weight), shoot (1.17 g/g dry weight), and root (1.00 g/g dry weight) at -0.2MPa NaCl.

### 3.2 $K^+$ content (g/g dry weight) in leaves, shoot and root

The mean data pertaining to stress induced by PEG-8000 and NaCl on $K^+$ content in leaves, shoot and root of tomato seedlings are presented in table Result of analysis of variance for $K^+$ content in leaves, shoot and root was indicated significant differences between water potential (WP), source of stress (S)and their interaction (SxWP) as well as control vs. rest mean at P=0.05. Significant difference was observed for $K^+$ content in leaves, shoot and root under NaCl induced stress. The $K^+$ content in leaves, shoot and root was decreased progressively with decreasing osmotic potential of NaCl. There was no significant effect of PEG-8000 on $K^+$ content in leaves, shoot and root and remain at par with control at all water potential. Mean data indicated that higher $K^+$ content found in leaves followed by shoot and root at -0.2MPa, afterward decreasing water potential, decreased $K^+$ content in leaves, shoot and root in all treatments. Maximum $K^+$ content was recorded in leaves (5.43 g/g dry weight), shoot (4.00 g/g dry weight), and root (3.60 g/g dry weight) at -0.2MPa of NaCl while, lowest amount of $K^+$ content was recorded leaves (1.10 g/g dry weight), shoot (1.13 g/g dry weight), and root (1.00 g/g dry weight) at -1.0 MPa NaCl.

The data on $Na^+$ /$K^+$ are presented in Table 1and 2 and indicated that treatment of NaCl, increased $Na^+$ content in leaves, shoot and root. However, NaCl increasing more $Na^+$ concentration in leaves followed by shoot and root. A higher $Na^+$ concentration in root or shoot increases the osmotic

potential and decreases water uptake, while $K^+$ concentration in root or shoot of tomato plants, changes little under saline environment. The accumulation of $Na^+$ interferes with $K^+$ selective ion channels in the root plasma membrane and thus reduced intake of $K^+$ and $Na^+$ transport from root to shoot is unidirectional and the resultant build-up of $Na^+$ in leaves more as compared to root (Flowers *et al*., 2000). PEG-8000 had a non-significant effect on $Na^+$ and $K^+$ content in leaves, shoot and root. These results are similar with Shiyab *et al*. (2013) [13], Shaheen *et al*. (2013) [12] and Sing *et al*. (2012) [14].

**Table 1:** Effects of PEG -8000 and NaCl induced stress on $Na^+$ content (g/g dry weight) in leaves, shoot and root of tomato seedling at 21st day after sowing (DAS)

| Na +content in leaves,shoot and root (g/g dry weight) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Water potential(WP)** | **PEG-8000($S_1$)** | **NaCl ($S_2$)** | **Mean** | **ANOVA** | | | | |
| | -0.2MPa | 0.15 | 3.33 | 1.74 | | | | | |
| | -0.4 MPa | 0.15 | 3.70 | 1.92 | | **S** | **WP** | **SxWP** | **Control vs. Rest** |
| | -0.6 MPa | 0.16 | 4.32 | 2.24 | SEM± | 0.05 | 0.07 | 0.10 | 0.07 |
| | -0.8 MPa | 0.18 | 4.93 | 2.55 | CD at 5% | 0.13 | 0.21 | 0.29 | 0.21 |
| **Leaves** | -1.0 MPa | 0.18 | 6.20 | 3.19 | CV% | 8.1 | | | |
| | Mean | 0.16 | 4.49 | 2.33 | | | | | |
| | Control mean | 0.15 | | | | | | | |
| | Rest mean | 2.33 | | | | | | | |
| | **Water potential(WP)** | **PEG-8000($S_1$)** | **NaCl ($S_2$)** | **Mean** | **ANOVA** | | | | |
| | -0.2MPa | 0.13 | 1.17 | 0.65 | | | | | |
| | -0.4 MPa | 0.13 | 2.47 | 1.30 | | **S** | **WP** | **SxWP** | **Control vs. Rest** |
| | -0.6 MPa | 0.14 | 3.17 | 1.66 | SEM± | 0.05 | 0.7 | 0.11 | 0.08 |
| **Shoot** | -0.8 MPa | 0.15 | 3.8 | 1.98 | CD at 5% | 0.14 | 0.22 | 0.31 | 0.23 |
| | -1.0 MPa | 0.17 | 4.37 | 2.27 | CV% | 12.62 | | | |
| | Mean | 0.14 | 2.99 | 1.57 | | | | | |
| | Control mean | 0.13 | | | | | | | |
| | Rest mean | 1.57 | | | | | | | |
| | **Water potential(WP)** | **PEG-8000($S_1$)** | **NaCl ($S_2$)** | **Mean** | **ANOVA** | | | | |
| | -0.2MPa | 0.06 | 1.00 | 0.53 | | | | | |
| | -0.4 MPa | 0.05 | 1.30 | 0.67 | | **S** | **WP** | **SxWP** | **Control vs. Rest** |
| | -0.6 MPa | 0.05 | 1.73 | 0.89 | SEM± | 0.04 | 0.06 | 0.08 | 0.06 |
| **Root** | -0.8 MPa | 0.07 | 2.40 | 1.23 | CD at 5% | 0.11 | 0.17 | 0.24 | 0.18 |
| | -1.0 MPa | 0.10 | 3.03 | 1.56 | CV% | 13.39 | | | |
| | Mean | 0.06 | 1.89 | 0.98 | | | | | |
| | Control mean | 0.05 | | | | | | | |
| | Rest mean | 1.13 | | | | | | | |

**Table 2:** Effects of PEG -8000 and NaCl induced stress on $K^+$ content (g/ g dry weight) in leaves, shoot and root of tomato seedling at 21st day after sowing (DAS)

| K +content in leaves,shoot and root (g/ g dry weight) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Water potential(WP)** | **PEG-8000($S_1$)** | **NaCl ($S_2$)** | **Mean** | **ANOVA** | | | | |
| | -0.2MPa | 0.15 | 5.43 | 2.79 | | | | | |
| | -0.4 MPa | 0.15 | 3.87 | 2.01 | | **S** | **WP** | **SxWP** | **Control vs. Rest** |
| | -0.6 MPa | 0.16 | 2.50 | 1.33 | SEM± | 0.03 | 0.05 | 0.08 | 0.06 |
| | -0.8 MPa | 0.17 | 1.57 | 0.87 | CD at 5% | 0.10 | 0.16 | 0.22 | 0.17 |
| **Leaves** | -1.0 MPa | 0.17 | 1.10 | 0.63 | CV% | 9.49 | | | |
| | Mean | 0.16 | 2.89 | 1.52 | | | | | |
| | Control mean | 0.22 | | | | | | | |
| | Rest mean | 1.49 | | | | | | | |
| | **Water potential(WP)** | **PEG-8000($S_1$)** | **NaCl ($S_2$)** | **Mean** | **ANOVA** | | | | |
| | -0.2MPa | 0.16 | 4.00 | 2.08 | | | | | |
| | -0.4 MPa | 0.14 | 3.32 | 1.73 | | **S** | **WP** | **SxWP** | **Control vs. Rest** |
| | -0.6 MPa | 0.13 | 2.10 | 1.11 | SEM± | 0.03 | 0.05 | 0.07 | 0.05 |
| **Shoot** | -0.8 MPa | 0.11 | 1.17 | 0364 | CD at 5% | 0.09 | 0.15 | 0.211 | 0.16 |
| | -1.0 MPa | 0.10 | 1.30 | 0.70 | CV% | 9.01 | | | |
| | Mean | 0.13 | 2.37 | 1.25 | | | | | |
| | Control mean | 0.18 | | | | | | | |
| | Rest mean | 1.25 | | | | | | | |
| | **Water potential(WP)** | **PEG-8000($S_1$)** | **NaCl ($S_2$)** | **Mean** | **ANOVA** | | | | |
| | -0.2MPa | 0.17 | 3.60 | 1.88 | | | | | |
| | -0.4 MPa | 0.17 | 2.92 | 1.54 | | **S** | **WP** | **SxWP** | **Control vs. Rest** |
| | -0.6 MPa | 0.15 | 1.99 | 1.07 | SEM± | 0.04 | 0.05 | 0.06 | 0.06 |
| | -0.8 MPa | 0.15 | 1.43 | 0.79 | CD at 5% | 0.12 | 0.17 | 0.23 | 0.018 |
| **Root** | -1.0 MPa | 0.14 | 1.00 | 0.57 | CV% | 8.92 | | | |
| | Mean | 0.15 | 2.18 | 1.17 | | | | | |
| | Control mean | 0.15 | | | | | | | |
| | Rest mean | 1.16 | | | | | | | |

## 4. Conclusion

Na accumulated more in leaves as compared to shoot and root due to NaCl treatments and may be responsible for poor growth of cv. GT-2 in salinity condition. It is reflecting lack of ion exclusion mechanism of GT-2. Moreover, NaCl stress retard K translocation as K content was found statistically equal to control in roots while in leaves it was significantly lower than the control. Thus more Na content in leaves retard the early seedling growth in tomato is inhibitory as compared to PEG-8000 induced drought. PEG-8000 had no significant influence on Na and K content.

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