

### **GROWTH OF WHEAT AND Na<sup>+</sup>/ K<sup>+</sup> RATIO UNDER NEUTRAL AND ALKALINE SALTS STRESS**

#### **ABSTRACT**

Saline soils contain multiple types of soluble salts. Role of alkaline salts (AS) may be different than neutral salts (NS) for crop growth. Usually these types of salinity frequently co-exist. Neutral salts generally induce osmotic stress and ion-induced injury to growing crop plants. Behavior of AS may be different due to high pH. This study was conducted to see the response of wheat growth and Na<sup>+</sup>/ K<sup>+</sup> ratio under AS and NS application as nutrient solution study. Germinated disinfected seeds of wheat (Cv. Pak-13) and raised in sand. Ten days seedlings were transferred to pots containing standard nutrients solution, with the application of 30 and 60 mM of NS (NaCl, Na<sub>2</sub>SO<sub>4</sub>) and AS (NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>) separately in 1:1 ratio using complete randomize design. The seedling growth period in salt solution comprised 33 days. The growth parameters were decreased significantly ( $p < 0.01$ ) under salt stress. The magnitude of loss of bio mass was 11 percent higher in AS than that of NS application. Phosphorus and sulphur concentration were lower 10 and 7 percent with AS than NS respectively besides high Na<sup>+</sup>/K<sup>+</sup> ratio and pH. Further such studies on salt tolerant crop varieties can be carried out to differentiate response under different types of salts.

*Keywords: Wheat; types of salt stress; bio-mass; phosphorus, sulphur; Na<sup>+</sup>/K<sup>+</sup> ratio*

#### **1. INTRODUCTION**

On earth, salts are 'the life line' of living organisms. When salts are dissolved in water, their function is dependent on the nature of the salt and the living recipient. Out of all living organisms, the plants categorized as glycophytes can only survive when salts in soils are available to the root system in a limited amount (usually  $EC_e < 4.0 \text{ dSm}^{-1}$ ). Low  $EC_e$  is a 'salvation' for crop plants. The composition of salts determines most of the chemical processes surrounding the roots of a crop plant. When soil nutrient solution crosses a concentration threshold of salts, then it gives birth to soil salinity problems. Saline soils contain multiple types of soluble salt components and the compositions of soluble salts in saline soils are quite different among locations [1] (Tobe *et al.*, 2004). Saline soils are heterogenic in salt types. Soil salinization is an important agriculture-related problem, which frequently co-exists with salt and alkali stresses [2] (Wang *et al.*, 2011). Soil salinity is composed of different types of salts,

mainly neutral and alkaline salts. Neutral salts (NS) compose NaCl and Na<sub>2</sub>SO<sub>4</sub>. Alkaline salts (AS) are consisted of NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>. Salts in soil cause osmotic stress, nutritional effects, ion toxicity and physiological disorders [3] (Mudgal *et al.*, 2010), for growing crops. Salt stress exerts distinct effects on growth, ion balance, compatible solutes, and metabolism in old and young leaves [4] (Hajlaoui *et al.*, 2010). Increasing salinity stress is antagonistic to fresh mass of plant [5] (Badr *et al.*, 2006). Alkaline salts are understood as more destructive to plants at different growth stages than neutral salts [6] (Yang *et al.*, 2008). This type of salt stress causes high pH stress [7] (Shi *et al.*, 2002), and can seriously affect mineral nutrition in the form of ion imbalances. This may also affect the oxygen supply capacity of the roots besides structure and function of root cells. Many saline soils are also alkaline due to the presence of sodium carbonates [8] (Rengasamy, 2010). Alkaline salt stress is linked with high pH [9] (Ghoulam *et al.*, 2002; [10] De-Lacerda *et al.*, 2003). Neutral salts generally induce osmotic stress and ion-induced injury to crop plants [11] (Shi and Wang, 2005). Such salts cause harm to the plant mainly through osmotic stress and ion toxicity.

Neutral and alkaline salt stress may affect growth of wheat differentially and Na<sup>+</sup>/K<sup>+</sup> ratio may be higher under neutral salt stress. Alkaline salt stress may cause stronger destructive effects on plants than neutral salt stress and the response of wheat may be variable.

## 2. MATERIALS AND METHODS

Treated seeds of *Triticum aestivum* (cv. Pak-13) with sodium hypochlorite (1% w/v) for 15 minutes [12] (Britto and Kronzucker, 2002) and then germinated in quartz sand, moist with distilled water. Foam-plugged the seedlings in lids of plastic pots containing 2.5 L of continuously aerated nutrient solution as constituted by [13] Hoagland and Arnon (1950). After 12 days of sowing, applied neutral salts solution (NaCl: Na<sub>2</sub>SO<sub>4</sub>=1:1) @ 30 and 60mM. Added separately alkaline salts solution (NaHCO<sub>3</sub>:Na<sub>2</sub>CO<sub>3</sub>=1:1) @ 30 and 60mM. Replaced the nutrient solution weekly. Light intensity remained at 450 μmol m<sup>-2</sup> S<sup>-1</sup>. Adjusted photoperiod at 16 hd<sup>-1</sup> light period and maintained temperature at 25±2°C. Unplugged the whole plants on the 32<sup>nd</sup> day of transplantation to pots. Rinsed the plants with distilled water, blotted with tissue paper and recorded their fresh mass. Dried the plant samples at 65°C to constant mass. Recorded dry mass of each sample and digested samples in 2% nitric acid solution along with a few drops of

perchloric acid. Determined phosphorus in the plant digested material as described by [14] Jackson (1962). Analyzed sulphur in the digested material as given by [15] Verma *et al.* (1977). By flame photometry determined the concentrations of Na<sup>+</sup> and K<sup>+</sup> ions. Analyzed the data according to two factors complete randomized design and compared treatment means using LSD test by using the statistical software, [16] Statistix 8.1 (2005).

### 3. RESULTS AND DISCUSSIONS

Seedlings of wheat (cv. Pak-13) responded significantly ( $p \leq 0.01$ ) for growth and ion relations on the application of neutral and alkaline salts.

#### 3.1 Effect of types of salt stress on bio mass

Shoot fresh mass (SFM) decreased 4 and 7 percent on the application of neutral salts (NS) solution (NaCl: Na<sub>2</sub>SO<sub>4</sub>=1:1) @ 30 and 60mM respectively. On the application of alkaline salts (AS) solution (NaHCO<sub>3</sub>:Na<sub>2</sub>CO<sub>3</sub>=1:1) @ 30 and 60mM, SFM decreased 12 and 20 percent respectively. Root fresh mass (RFM) decreased 5 and 10 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM RFM decreased 15 and 18 percent respectively. Shoot dry mass (SDM) decreased 9 and 12 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM, SDM decreased 18 and 21 percent respectively. Root dry mass (RDM) decreased 5 and 8 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM, RDM decreased 13 and 16 percent respectively (Table 1).

Table 1 Impact of neutral salts (NS) and alkaline salts (AS) stresses on the bio mass (mg plant<sup>-1</sup>) of wheat.

Salinity (mM)	Fresh mass		Dry mass	
	Shoot	Root	Shoot	Root
Control	826.5 a	461.5 a	41.3 a	23.1 a
NS (30)	793.4 b	438.4 b	37.6 b	21.9 b
NS (60)	760.4 c	415.4 c	36.4 c	21.2 c
AS (30)	727.3 d	392.3 d	33.9 d	20.1 d
AS (60)	661.1 e	378.5 e	32.7 e	19.4 e

Means sharing similar letter(s) for a parameter in a column do not differ significantly at  $p < 0.01$

CV ( $p < 0.01$ ) = 6.98 percent

Shoot height (SH) decreased 5 and 10 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60 mM, SH decreased 15 and 20 percent respectively. Root length (RL) decreased 8 and 13 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM, RL decreased 18 and 22 percent respectively (Fig.1).

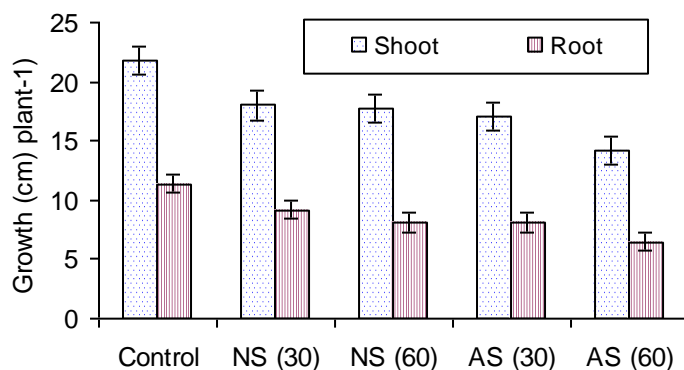


Fig.1 Effect of neutral salts (NS) and alkaline salts (AS) on shoot height and root length of wheat

Under the influence of both types of stresses, the magnitude of bio mass of shoot and root of wheat (cv. Pak-13) decreased. Alkaline salts affect pH of the nutrients supply to the roots. Such salts keep pH high, following restricted availability to macronutrients. Under osmotic effect of dissolved ions, the availability of potassium ion also remains limited especially in the presence of sodium ion. In this study the type of salts, from lower to higher concentrations, affected biomass of shoot and root system. Higher concentration of sodium ion caused deficiency of water in plant tissue as found by [5] Badr-uz-Zaman *et al.*, 2006). Sodium ion stress interferes biochemical processes [17] (Tavakkoli *et al.* 2010). Also, lesser availability of water caused lesser flow of nutrients to the shoot system from root [18] (Badr-uz-Zaman *et al.* 2008). Fresh mass of plant material consists of tissue besides water and other chemicals. As water maintains physico-chemical properties of a cell or an organ, therefore in fresh mass consideration water contents of an organ cannot be ignored [19] (Badr-uz-Zaman *et al.*, 2010a). Dry mass of plant material consists of tissue and chemicals indicating the net out come of the resultant metabolic activities. Dry mass of shoot and root decreased under both types of applied

salts. When the relations and metabolic activities of macro and micro nutrients are on positive side, then tissue developments in growing plants continues and this positive effect is reflected as dry mass of a plant organ.

### Effect of types of salt stress on s and p concentration in wheat

Sulphur in shoot decreased 5 and 10 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM, sulphur decreased in shoot 15 and 20 percent respectively. Sulphur in root decreased 6 and 14 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM, sulphur decreased in root 21 and 24 percent respectively. Phosphorus in shoot decreased 6 and 8 percent on the application of NS solution: @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM phosphorus decreased in shoot 13 and 15 percent respectively. Phosphorus in root decreased 6 and 11 percent on the application of NS solution @ 30 and 60mM respectively. On the application of AS solution @ 30 and 60mM phosphorus decreased in root 13 and 15 percent respectively (Table 2).

Table 2 Effect of neutral salts (NS) and alkaline salts (AS) stresses on concentrations of sulphur and phosphorus in wheat.

Salinity (mM)	Sulphur (%)		Phosphorus (%)	
	Shoot	Root	Shoot	Root
Control	0.30 a	0.29 a	0.48 a	0.46 a
NS (30)	0.29 a	0.27 b	0.45 b	0.43 b
NS (60)	0.26 b	0.25 c	0.44 b	0.41 c
AS (30)	0.24 c	0.23 d	0.42 c	0.40 c d
AS (60)	0.23 c	0.22 d	0.41 c	0.39 d

Means sharing similar letter(s) for a parameter in a column do not differ significantly at  $p < 0.01$

CV ( $p < 0.01$ ) = 7.52.98 percent

Sulphur and phosphorus were not applied as supplementary nutrients, but as per standard recipe. In both types of salt stress, the concentration of sulphur and phosphorus in shoot and root decreased. The influential parameters of neutral and alkaline salts affected sulphur and

phosphorus metabolism in the growing plants. For a healthy growth of a plant it is pertinent that root and shoot system must be in co-ordination. The higher the intake of nutrients by a root system according to the growth requirements of a plant and higher is the nutrients transport and the metabolic activities. Uptake and translocation of nutrients play essential roles in plant for signal transduction, growth, and development [20] (Wang *et al.*, 2017). In this study, it might be that types of salts for stress affected sulphur and phosphorus acquisition by interfering with  $K^+$  uptake by carriers and channels as revealed by [21] Maathuis and Amtmann (1999).

### 3.3 Effect of types of salt stress on $Na^+/K^+$ in wheat

In shoot and root,  $Na^+/K^+$  ratio increased than the control on the application of both types of salt solutions. In addition,  $Na^+/K^+$  ratio was higher in case of AS than NS applied solutions. The magnitude of  $Na^+/K^+$  ratio was higher in shoot than the root (Fig.2).

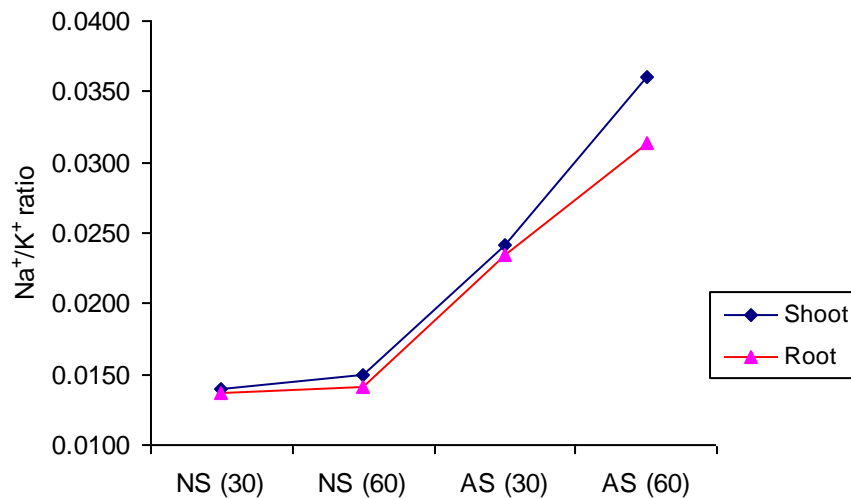


Fig. 2 Effect of neutral salts (NS) and alkaline salts (AS) on  $Na^+/K^+$  ratio in shoot and root of wheat.

Presence of sodium ion affected osmotic property and presence of carbonates and bicarbonates affected pH of the nutrients supply from the surroundings to the roots. To increase mass and volume, turgor pressure of cell is desirable, which is also dependent on tissue water contents. This increases expansion as well as longitudinal growth of a plant tissue [22] (Badr-uz-Zaman *et al.*, 2010b). Under salinity,  $Na^+/K^+$  ratio in wheat is increased [23] (Niazi *et al.*, 2007). Usually with application of NS, this ratio remains higher than the presence of AS. But in this study,  $Na^+/K^+$  ratio in seedlings was higher with AS application. pH trended to be higher in the

presence of AS, and may have influenced metabolic or biochemical processes of wheat. Under genetic, this wheat variety may have inclined to accumulate more sodium ion than potassium ion in the organs. In addition, the magnitude of  $\text{Na}^+/\text{K}^+$  ratio was higher in shoot than the root. Root system was genetically unable to accumulate more sodium ion than the shoot system. Wheat shows high genetic variability across the synthetic backcross lines and their parents at various growth stages under controlled as well as under salt stress field conditions [24] (Dadshani *et al.* 2019).

#### 4. CONCLUSIONS:

The magnitude of loss of bio mass was 11 percent higher in AS than that of NS application. This may be due to 7 percent lower P concentration in AS besides high  $\text{Na}^+/\text{K}^+$ .

#### REFERENCES.

1. Tobe K, Li XM, Omasa K. Effects of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (Chenopodiaceae). Seed Science Research. 2004; 14: 345–353.
2. Wang H, Ahan J, Wu ZH, Shi DC, Liu B, Yang CW. Alteration of nitrogen metabolism in rice variety ‘Nipponbare’ induced by alkali stress. Plant and Soil. 2011; 355:131–47.
3. Mudgal V, Madaan N, Mudgal A. Biochemical mechanisms of salt tolerance in plants: A Review. International Journal of Botany. 2010; 6: 136-143.
4. Hajlaoui H, El Ayebb N, Garrecc JP, Dendend M. Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of twosilage maize (*Zea mays* L.) varieties. Industrial Crops and Products. 2010; 31, 122–130.
5. Badr Z, Rehana A, Salim M, Safdar A, Niazi BH, Arshad A, Mahmood IA. . Growth and ionic relations of *Brassica campestris* and *B. juncea* (L.) Czern & Coss. under induced salt stress. Pakistan Journal of Agricultural Sciences. 2006; 43(3-4): 103-107.
6. Yang CW, Jianaer A, Li CY, Shi DC, Wang DL. Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali resistant halophyte *Chloris virgata*. Photosynthetica. 2008; 46:273–278.
7. Shi DC, Yin SJ, Yang GH, Zhao KF. Citric acid accumulation in an alkalitolerant plant *Puccinellia tenuiflora* under alkaline stress. Acta Botanica Sinica. 2002; 44, 537–540.

8. Rengasamy P. Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology*. 2010;( 37): 613–620.
9. Ghoulam C, Foursy A, Fares K. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany*. 2002; 7:39–50.
10. De Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, Prisco JT. Solute accumulation and distribution during shoot and leaf development in two sorghum varieties under salt stress. *Environmental and Experimental Botany*. 2003; 49(2): 107-120.
11. Shi DC, Wang DL. Effects of various salt–alkaline mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. *Plant and Soil*. 2005; 271: 15–26.
12. Britto DT, Kronzucker HJ.  $\text{NH}_4^+$  toxicity in higher plants: a critical review. *Journal of Plant Physiology*. 2002; 159: 567-584.
13. Hoagland DR, Arnon DI. The water culture method of growing plants without soil. Univ. California, Berkeley College Agriculture. Circ.1950; 344.
14. Jackson ML. Soil Chemical Analysis. Contable Co. Ltd. London. 1962; p62
15. Verma BC, Swaminathan KS, Sud KS. An improved turbidimetric method for sulphur determination in plants and soils. *Talanta*. 1977; 24:49-50.
16. Statistix 8.1. Statistical Software for Windows, Tallahassee, Florida. 2005.
17. Tavakkoli E, Rengasamy P, McDonald GK. High concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *Journal of Experimental Botany*. 2010; 61(15):4449–4459.
18. Badr Z, Salim M, Rehana A, Niazi BH, Mahmood IA, Ali A. Growth Responses and Ionic Relations in two *Brassica* species under water stress conditions. *Pakistan Journal of Scientific and Industrial Research*. 2008; 51(1): 31-35.
19. Badr Z, Salim M, Rehana A. Role of  $\text{Ca}^{2+}$  on Growth of *Brassica campestris* L. and *B. juncea* (L.) Czern& Coss under  $\text{Na}^+$  Stress. *Journal of Integrative Plant Biology*. 2010a; 52 (6): 549–555.
20. Wang D, Lv S, Jiang P, Li Y. Roles, regulation, and agricultural application of plant phosphate transporters. *Front. Plant Science*. 2017; 8(817): 1-14.
21. Maathuis FJM, Amtmann A.  $\text{K}^+$  nutrition and  $\text{Na}^+$  toxicity: the basis of cellular  $\text{K}^+/\text{Na}^+$  ratios. *Annals of Botany*. 1999; 84: 123–133.



22. Badr Z, Ali A, Mahmood IA, Arshadullah. M, Armaghan S, Adil MK. Potassium consumption by rice plant from different sources under salt stress. Pakistan Journal of Scientific and Industrial Research. 2010 b; 53(5): 271-277.
23. Niazi BH, Badr Z, Salim M, Athar M. Growth response, water relations and K/Na ratio in wheat under sodium and calcium interaction. Journal of Applied Sciences and Environmental Management. 2007; 11(1): 47-50.
24. Dadshani S, Sharma RC, Baum M., Ogbonnaya FC, Le'on J, Ballvora A. Multidimensional evaluation of response to salt stress in wheat. PLoS ONE. 2019; 14(9): 1-24.

UNDER PEER REVIEW