

## WATER RELATIONS AND IONIC COMPOSITION IN THE SEEDLINGS OF SOME NEWLY DEVELOPED AND CANDIDATE CULTIVARS OF WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALINE CONDITIONS

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### Abstract

Influence of salt stress on water relation parameters and pattern of ion accumulation was observed in 10 spring wheat cultivars, S-24, Lasani, Fsd-2008, Saher-2006, Inqlab-91, AARI-10, P.B-18, S.H-20, M.P-65, and G.A-20 at the seedling stage. The wheat cultivars were grown under saline (150 mM) and non-saline regimes (0 mM). Salt stress markedly reduced different water relation attributes such as leaf osmotic potential ( $\Psi_w$ ), water potential ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) of the seedlings of all wheat cultivars. However, relatively less decline in leaf  $\Psi_p$  recorded in cvs. S-24 and G.A-20 could be attributed to their higher degree of salt tolerance. Of different ions, root and leaf  $K^+$  and  $Ca^{2+}$  concentrations reduced, while  $Na^+$  concentration increased in all wheat cultivars under saline regime.  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios in both root and leaf of all cultivars also decreased under stress conditions. However, maintenance of higher  $Ca^{2+}/Na^+$  and  $K^+/Na^+$  ratios in cv. S-24, Inqlab-91 and G.A-20 in their roots and leaves compared to the other cultivars could also be related to their higher degree of salt tolerance. Of all wheat cultivars, S-24, Inqlab-91 and G.A-20 could be categorized as salt tolerant on the basis of water relation attributes and pattern of ion accumulation.

### Introduction

Salinity of soil is contemplated as a major problem which negatively affects a multitude of metabolic processes of plants resulting in reduced growth and yield of most crops (Ahmad *et al.*, 2007; Ashraf, 2009; Abro *et al.*, 2009; Ahmad *et al.*, 2011). The main metabolic processes hindered by salinity include ion uptake, osmotic adjustment, photosynthesis, protein/nucleic acid synthesis, enzymatic activities and hormonal balance (Gad & Kandil, 2011; Akram *et al.*, 2011; Ahmad *et al.*, 2012). Plant species respond differentially to salt stress because of various factors which include salt level of growth medium, exposure period of the stress, nature of plant genotype, plant's developmental stage and surrounding environment (Ashraf *et al.*, 1987; Gorai *et al.*, 2011; Bahrani & Joo, 2011; Ashraf & Ashraf, 2012).

Water relation characteristics including osmotic potential, water potential and turgor potential are markedly affected due to saline stress. Reduced solute potential due to saline conditions significantly affects water uptake ability of plants (Munns, 2002) thereby causing water potential more negative which results in reduced growth of most plants (Khan, 2001; Cha-um *et al.*, 2010; Eisa *et al.*, 2012). To cope with these effects, different plant species adopt different mechanisms. Of these, osmotic adjustment is considered to be one of the key physiological strategies of plants to cope with salt stress (Farouk, 2011; Gorai *et al.*, 2011). For osmotic adjustment, the cells may accumulate inorganic ions ( $K^+$ ,  $Na^+$  and  $Cl^-$ ) and organic solutes (most commonly proline and glycinebetaine) to balance water potential of the cells and maintain their turgor. The osmotic adjustment occurs in both root and shoot when plants are under saline stress (Sanchez *et al.*, 2004). Osmotic adjustment particularly in leaf is important for plant survival, because it facilitates leaf expansion by maintaining higher stomatal conductance (Westgate & Boyer, 1985; Cha-um *et al.*, 2010; Eisa *et al.*, 2011), which is considered necessary to sustain photosynthesis in the leaves under salt stress.

Higher accumulation of toxic ions such as  $Na^+$  and  $Cl^-$  occurs in plants under saline conditions (Ashraf & Ahmad, 2000; Gorai *et al.*, 2011; Heidari *et al.*, 2011; Shahbaz & Zia, 2011). A number of studies on different plant species have shown that  $K^+$  uptake is hindered by saline stress which results in decreased  $K^+/Na^+$  ratio (Cha-um *et al.*, 2010; Gandonou *et al.*, 2011; Lenis *et al.*, 2011) which is harmful for plants, because  $K^+$  is very important for the maintenance of ionic homeostasis in the cytosol (Szczerba *et al.*, 2009), and turgor, osmotic adjustment and stomatal regulation (Chartzoulakis *et al.*, 2006; Najafian *et al.*, 2009; Kusvuran, 2012). The maintenance of high ratio of  $K^+/Na^+$  in plant tissues can be used as an effective selection criterion for salt tolerance in plants (Reynolds *et al.*, 2005).

The degree of salt tolerance in wheat differs at different growth stages, and seedling growth stage is reported as more sensitive than the other stages (Ashraf, 2009; Jenkins *et al.*, 2010; Shahbaz & Ashraf, 2013). Therefore, the present study was performed to examine the salt stress effect on physiological attributes at the seedling growth stage of 10 wheat genotypes including some candidate cultivars (AARI-10, P.B-18, M.P-65, G.A-20 and S.H-20) yet to be registered and some newly developed cultivars (S-24, Lasani, Saher-2006, Inqlab-91, Fsd-2008).

### Materials and Methods

An experiment was conducted to examine the salt tolerance in 10 wheat cultivars, five newly developed (S-24, Lasani, Inqlab-91, Fsd-2008 and Saher-2006) and five candidate (AARI-10, S.H-20, P.B-18, G.A-20 and M.P-65) using ionic composition and leaf water relation attributes, osmotic potential, water potential and turgor potential, as selection criteria. Of these 10 cultivars, the seed of nine (Inqlab-91, Fsd-2008, Lasani, Saher-2006, P.B-18, AARI-10, M.P-65, G.A-20 and S.H-20) was collected from the Ayub Agricultural Research Institute,

Faisalabad, Pakistan and that of cv. S-24 from the Department of Botany, University of Agriculture, Faisalabad. The experiment was performed in the research area of the Botanical Garden, of the University of Agriculture, Faisalabad, Pakistan. All wheat cultivars were grown in plastic pots, each pot having diameter of 30 cm filled with 11 kg well washed river sand. Two salt (NaCl) levels (0 mM and 150 mM) prepared in full strength Hoagland's nutrient solution were applied. All pots were arranged in a completely randomized design with four replicates. The seedlings were thinned to four in each pot after germination and salt stress applied after 15 days of germination. Data for contents of different ions and water relation attributes were recorded when the seedlings were 45-day old.

**Leaf water potential ( $\Psi_w$ ):** Early in the morning at 6:00 a.m. leaf water potential was measured using the 3<sup>rd</sup> leaf from each plant with a Scholander type water potential apparatus (Arimad-2-Japan).

**Osmotic potential ( $\Psi_s$ ):** The same leaf used for the measurement of  $\Psi_w$  was kept in a freezer for one week at -21°C. After extracting the sap from the frozen leaf material, osmotic potential determined with an osmometer (VAPRO, Model 5520, USA).

**Turgor potential:** As described by Nobel (1991) leaf turgor potential was calculated using the following equation:

$$\Psi_p = \Psi_w - \Psi_s$$

**Determination of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  in the leaf and root tissues:** The root and leaf material was oven-dried at 65°C and ground it to form a powder. A sample (0.1 g) of each tissue was digested in 2 ml sulfuric acid-hydrogen peroxide mixture as described by Wolf (1982). The amounts of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  in the digested samples were appraised with a flame photometer (PFP-7, Jenway Ltd).

**Statistical analysis:** Bartlett's test was performed on data of each variable by using the MSTAT computer package (MSTAT Development Team, 1989) to work out analysis of variance. All means within a variable were compared using the LSD test at 5% probability.

## Results

In the present study, 10 newly developed and candidate wheat (*Triticum aestivum* L.) cultivars were assessed for salt tolerance on the basis of plant water relations and ionic contents at the seedling stage. Leaf water relation parameters including water potential ( $\Psi_w$ ), osmotic potential ( $\Psi_s$ ) and turgor potential ( $\Psi_p$ ) were markedly affected due to saline stress. Leaf water potential ( $\Psi_w$ ) and  $\Psi_s$  decreased (more -ve) significantly in all wheat cultivars at the seedling stage under salt stress (150 mM). Higher reduction in leaf  $\Psi_w$  was observed in cvs. Lasani and AARI-10, while less in cvs. S-24 and Fsd-2008 under salt stress. Cultivars M.P-65, AARI-10 and G.A-20 showed a maximum decrease in  $\Psi_s$  under saline conditions, and less reduction in  $\Psi_s$  was recorded in

cvs. S-24, Saher-2006, Inqlab-91, Fsd-2008, Lasani and P.B-18. Leaf  $\Psi_p$  also decreased significantly in all wheat cultivars under salt stress. Of all wheat cultivars, the maximum reduction in leaf  $\Psi_p$  was recorded in Lasani and AARI-10, while cvs. S-24 and G.A-20 showed minimum leaf  $\Psi_p$  under saline conditions (Fig. 1; Table 1).

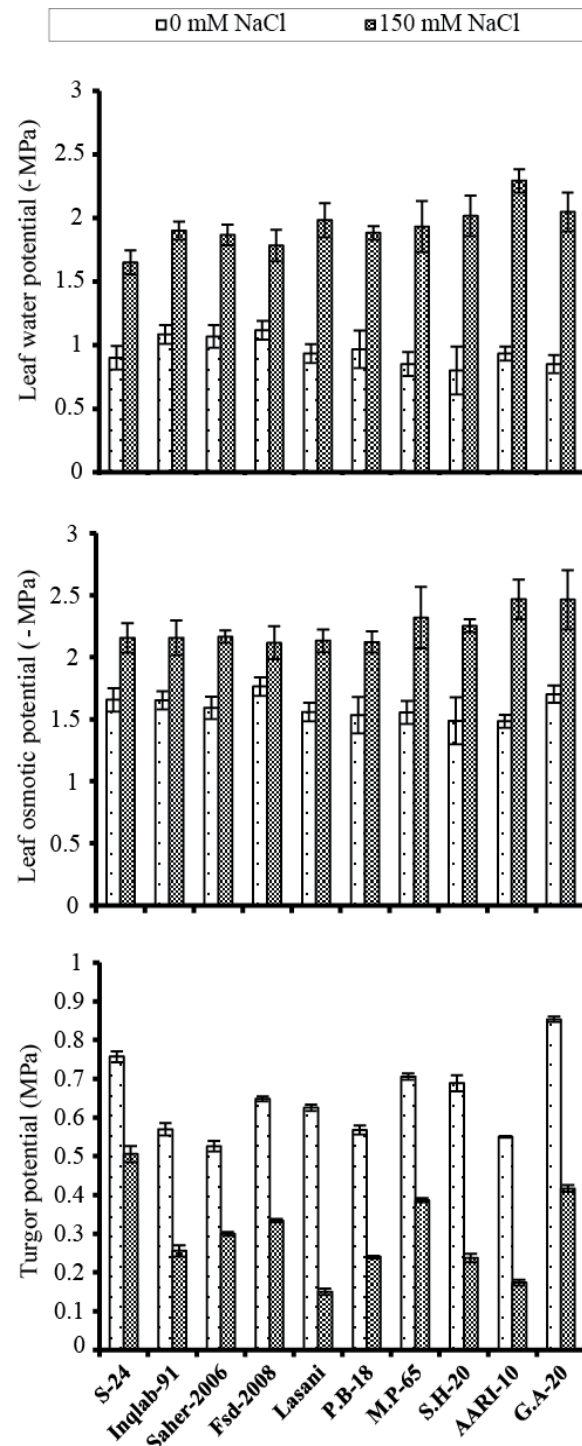


Fig. 1. Leaf water relation parameters of different cultivars of wheat (*Triticum aestivum* L.) as influenced by salt stress (150 mM) applied at the seedling stage (mean  $\pm$  S.E.).

**Table 1. Mean square values from analyses of variance of data for different ions and water relation parameters of 10 wheat cultivars under salt stress**

Source	df	$\Psi_w$	$\Psi_s$	$\Psi_p$	
Salt stress (S)	1	14.573***	6.0786***	1.828***	-
Cultivars (Cv)	9	0.043 ns	0.0324 ns	0.059*	-
S × Cv	9	0.079**	0.0493 ns	0.010 ns	-
Error	40	0.026	0.0303	0.025	-
	df	Leaf K <sup>+</sup>	Root K <sup>+</sup>	Leaf Na <sup>+</sup>	Root Na <sup>+</sup>
Salt stress (S)	1	183.75**	3.75 ns	1560.6***	228.15***
Cultivars (Cv)	9	351.66***	4.704***	20.67**	10.046***
S × Cv	9	185.83***	10.890***	26.058***	16.483***
Error	40	25.00	0.925	129.62	1.212
	df	Leaf Ca <sup>2+</sup>	Root Ca <sup>2+</sup>	Leaf K <sup>+</sup> /Na <sup>+</sup>	Root K <sup>+</sup> /Na <sup>+</sup>
Salt stress (S)	1	21.6***	202.58***	1046.43**	2.049***
Cultivars (Cv)	9	4.337***	18.63***	5.007 ns	0.170***
S × Cv	9	4.079**	11.376**	12.178*	0.147***
Error	40	5.4125	2.075	5.0415	0.014
	df	Leaf Ca <sup>2+</sup> /Na <sup>+</sup>	Root Ca <sup>2+</sup> /Na <sup>+</sup>	-	-
Salt stress (S)	1	49.054***	6.795***	-	-
Cultivars (Cv)	9	0.2736*	0.1988**	-	-
S × Cv	9	0.705***	0.1755***	-	-
Error	40	0.117	0.0374	-	-

\*, \*\*, \*\*\* = significant at 0.05, 0.01, and 0.001 level, respectively  
ns = non-significant

Data presented in Fig. 2 showed that leaf K<sup>+</sup> decreased significantly in all wheat cultivars under saline regimes. All cultivars showed differential behavior in response to salt stress. The maximum reduction in shoot K<sup>+</sup> concentration was recorded in cv. PB-18. A significant decrease in root K<sup>+</sup> concentration was observed in all wheat cultivars. Root K<sup>+</sup> concentration was higher in cvs. S-24, Saher-2006, Inqlab-91 and PB-18 as compared to that in the other cultivars. Overall, cv. PB-18, was the lowest in leaf and root K<sup>+</sup> accumulation. Root Na<sup>+</sup> increased in all wheat cultivars under saline conditions. However, increase in Na<sup>+</sup> concentration was greater in the leaves than that in the roots. Leaf Na<sup>+</sup> increased in all wheat cultivars under salt stress. Of all wheat cultivars, Inqlab-91, S.H-20 and G.A-20 showed higher values of leaf Na<sup>+</sup>. The maximum increase in root Na<sup>+</sup> was recorded in cvs. AARI-10 and M.P-65 and the minimum in cvs. Fsd-2008, Lasani and P.B-18 (Fig. 2). When salt stress applied at the seedling stage, root and shoot Ca<sup>2+</sup> concentrations decreased significantly in all wheat cultivars. Decrease in Ca<sup>2+</sup> was higher in the leaves than that in the roots. The maximum reduction in root Ca<sup>2+</sup> was observed in cvs. S-24, Inqlab-91 and G.A-20, while the minimum in cvs. Saher-2006, Fsd-2008 and S.H-20 (Fig. 2; Table 1).

Root and leaf K<sup>+</sup>/Na<sup>+</sup> ratios were markedly affected in all wheat cultivars under salt stress. Decrease in root K<sup>+</sup>/Na<sup>+</sup> ratio was observed in all cultivars under saline stress. However, values of leaf K<sup>+</sup>/Na<sup>+</sup> ratios were greater than those for root K<sup>+</sup>/Na<sup>+</sup> ratios. Root K<sup>+</sup>/Na<sup>+</sup> ratio was higher in cvs. S-24, M.P-65, S.H-20 and AARI-10 than those in the other wheat cultivars under saline environment. Leaf K<sup>+</sup>/Na<sup>+</sup> ratio in different wheat cultivars showed an inconsistent pattern. However, under salt stress, cvs. S-24, Inqlab-91 and G.A-20 showed higher leaf K<sup>+</sup>/Na<sup>+</sup> ratios. Although, leaf and root

Ca<sup>2+</sup>/Na<sup>+</sup> ratios decreased markedly under saline stress in all wheat cultivars, higher Ca<sup>2+</sup>/Na<sup>+</sup> ratios in the roots were recorded in wheat cvs. Saher-2006, M.P-65, S.H-20 and AARI-10, while the leaf Ca<sup>2+</sup>/Na<sup>+</sup> were higher in cvs. S-24, Inqlab-91 and G.A-20 (Fig. 3).

## Discussion

Soil water status is considered as the main factor which affects growth and development in plants subjected to saline stress (Rampino *et al.*, 2006; Dulai *et al.*, 2011; Kanwal *et al.*, 2011; Kausar & Shahbaz, 2013). Low water potential of the saline grown medium causes accumulation of solutes in the cells, which lowers the cell osmotic potential. The reduced cellular osmotic potential is necessary for osmoregulation i.e., maintenance of turgor pressure (Navarro *et al.*, 2003; Szczerba *et al.*, 2009; Cha-um *et al.*, 2010). Osmoregulation is considered as an important mechanism for the normal cell functioning (Taiz & Zeiger, 2010) because maintenance of water status in plant is an essential phenomenon for normal plant growth and development under stressful environments (Ali & Ashraf, 2011). In the present study, all wheat cultivars maintained  $\Psi_w$  to a more negative level under salt stress compared with that at non-saline conditions (Fig. 1). This is a common plant response to salinity which has been widely reported in different reports (Suárez & Medina, 2008; Gorai *et al.*, 2011; Kusvuran, 2012). In the present study, cvs. S-24, M.P-65 and G.A-20 showed less decrease in turgor potential under salt stress as compared to the other wheat cultivars. This indicates high tolerance of these cultivars to salinity as has been reported earlier in different plant species (Farouk, 2011; Heidari *et al.*, 2011; Jabeen & Ahmad, 2012; Eisa *et al.*, 2012).

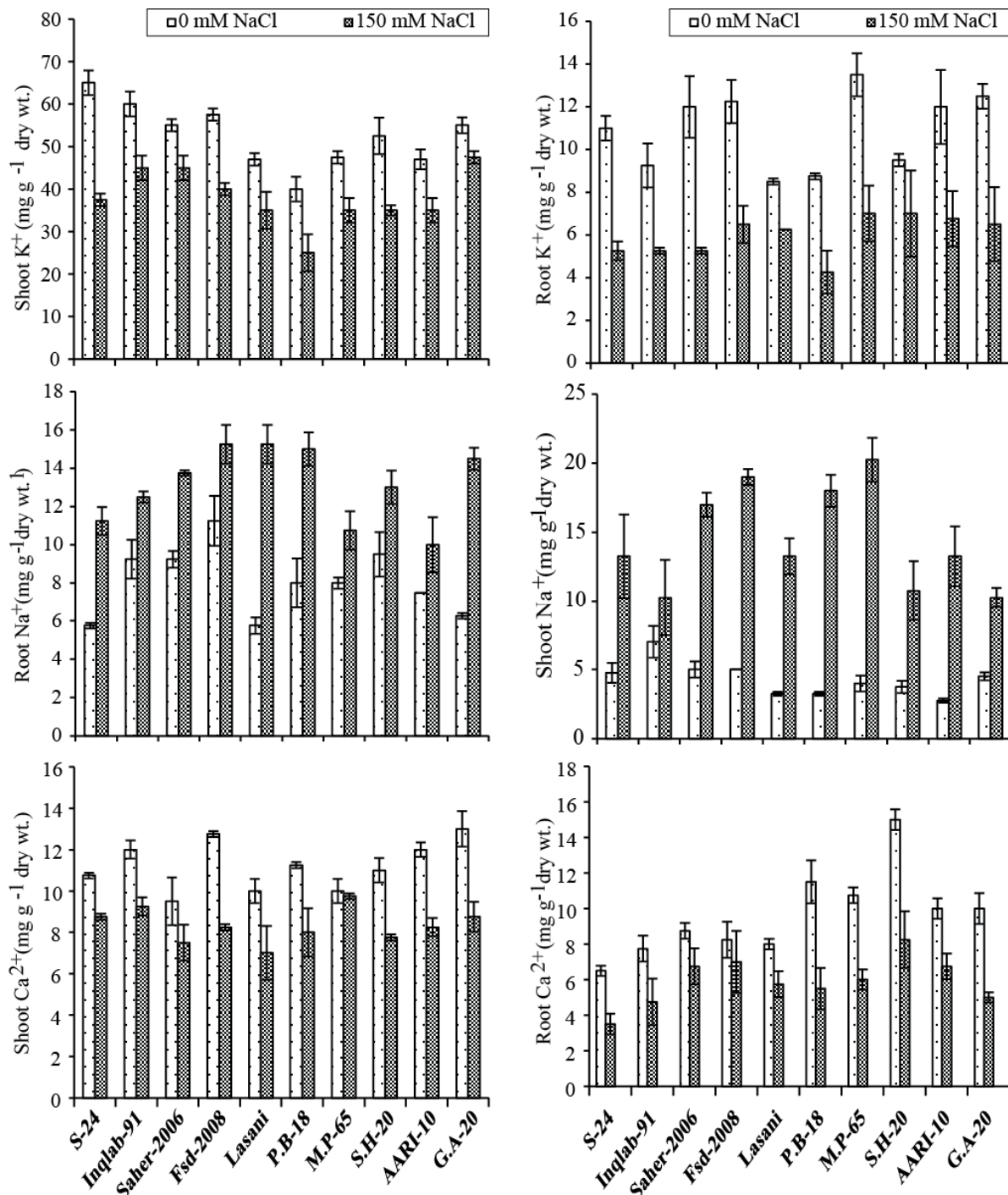


Fig. 2. Shoot and root K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup> of different cultivars of wheat (*Triticum aestivum* L.) as influenced by salt stress (150 mM) applied at the seedling stage (mean ± S.E.).

Pattern of ion accumulation, transport and their distribution in different plant parts and within the cell is a vital indicator for salt tolerance mechanism in most plants (Ashraf, 2004; Munns & Tester, 2008; Akram *et al.*, 2009; Akram & Ashraf, 2011). Excessive accumulation of ions in salt-stressed plants can decline leaf functions and ionic imbalance, and cause ion toxicity (Yildirim *et al.*, 2009). Exclusion of Na<sup>+</sup> and Cl<sup>-</sup> and maintenance of high K<sup>+</sup>/Na<sup>+</sup> ratios in the tissues are

among the important aspects of mechanisms of salt tolerance in most plants (Zheng *et al.*, 2008; Shahbaz *et al.*, 2011). In this study, salt stress caused a marked increase in Na<sup>+</sup> concentration in the roots and leaves of all wheat cultivars under salt stress. However, leaf Na<sup>+</sup> concentration was greater than root Na<sup>+</sup> in almost all wheat cultivars and cvs. AARI-10 and M.P-65 showed maximum concentration of root Na<sup>+</sup>. In contrast, leaf Na<sup>+</sup> concentration was higher in cvs. Inqlab-91, S.H-20

and G.A-20. Generally, it has been observed that when plants grow under saline environments, they absorb higher amount of  $\text{Na}^+$ , but antagonistically lower amount of  $\text{K}^+$  and  $\text{Ca}^{2+}$ . For the maintenance of cell membrane integrity and functioning, a reasonable amount of  $\text{K}^+$  and  $\text{Ca}^{2+}$  is required by plants grown under saline stress (Wenxue *et al.*, 2003; Kader & Lindbeg, 2010). Adequate maintenance of  $\text{K}^+$  in tissues is dependent upon the selective uptake of  $\text{K}^+$ , and cellular compartmentation and distribution of  $\text{K}^+$  and  $\text{Na}^+$  ions in shoots (Siringam *et al.*, 2011). However, maintenance of high  $\text{K}^+/\text{Na}^+$  ratio under saline environment is considered as an important salt tolerance selection criterion (Chinnusamy *et al.*, 2005; Siringam *et al.*,

2011). Both root and leaf  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  ratios decreased consistently in all wheat cultivars. However, these ionic ratios were greater in shoots than those in roots. High ratios of  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  in wheat cvs. S-24, Inqlab-91 and G.A-20 could have played an important role in their tolerance to salt (Ashraf & Khanum, 1997; Ashraf & Orooj, 2006).

Overall, salt tolerance in the 10 wheat cultivars was found to be linked to high leaf turgor potential and  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  ratios in plant tissues. So, newly developed wheat cultivars S-24, Inqlab-91 and G.A-20 were more salt tolerant than the other cultivars examined in this study on the basis of plant water relation parameters and ionic contents measured at the seedling stage.

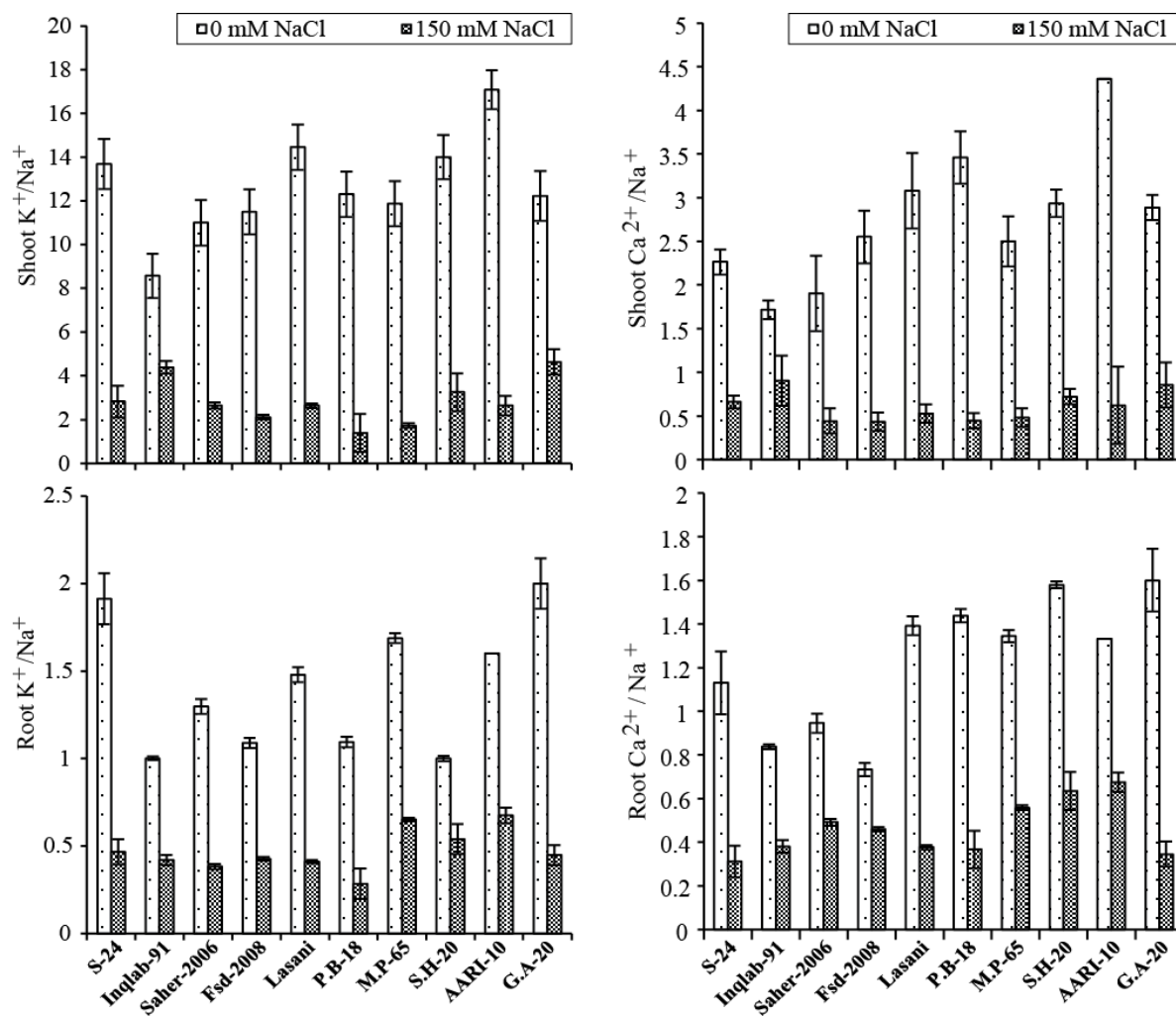


Fig. 3. Shoot and root  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  of different cultivars of wheat (*Triticum aestivum* L.) as influenced by salt stress (150 mM) applied at the seedling stage (mean  $\pm$  S.E.).

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