



## SALINE WATER APPLICATION AT VARIOUS GROWTH STAGES OF WHEAT: EFFECT ON IONS CONTENT

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### ABSTRACT

Adverse effect of saline water on crops growth and yield may be associated to surplus amount of particular ion(s) and nutrient imbalances. Therefore, this pot study was carried out to quantify the effect of saline irrigation waters on ions concentration in sap and tissues of bread wheat (*Triticum aestivum* L. cv. Sarsabz). The crop was irrigated with saline waters (EC 2.0, 3.0, 4.0 and 5.0  $\text{dS m}^{-1}$ ; prepared from NaCl and  $\text{CaCl}_2$  salts) at three growth stages, i.e. early growth stage (emergence and tillering), later growth stage (booting and grain formation) and all growth stages (emergence, tillering, booting and grain formation). Saline waters were applied at these growth stages either alone and/or in cycles with tap water (EC 0.6  $\text{dS m}^{-1}$ ). Ions ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ ) concentration was determined in the flag leaf sap, straw and grains of wheat. Results showed that the  $\text{Na}^+$  concentration in flag leaf sap, straw and grains was significantly increased as a function of saline waters ( $P < 0.05$ ). There was a low concentration of  $\text{K}^+$  in flag leaf sap and wheat grains, and low  $\text{K}^+/\text{Na}^+$  ratio in flag leaf sap, straw and grains. Saline water having EC 5.0  $\text{dS m}^{-1}$  had more effect on  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{K}^+/\text{Na}^+$  ratio than other treatments. Among various growth stages, the plants irrigated with saline water at later stages of growth had less  $\text{Na}^+$ , more  $\text{K}^+$  and  $\text{K}^+/\text{Na}^+$  ratio in sap, straw and grains of wheat, when compared with plants irrigated at all growth stages with saline water treatments. The findings of this study specify that the saline water having  $\text{EC} \leq 5.0 \text{ dS m}^{-1}$  may be applied at later growth stages (booting and grain formation) of wheat cultivar Sarsabz with a minimum risk of  $\text{Na}^+$  buildup in plant tissues.

**Keywords:** ions content, saline irrigation water, wheat, growth stages

### INTRODUCTION

The identification of salt-sensitive or tolerant stage of a crop/genotype is prerequisite for attaining profitable yield in a saline environment. Nonetheless, there are limited studies which identify the sensitive growth stage of food crops including wheat (*Triticum aestivum* L.) to salinity. Most studies report that wheat is salt-sensitive at early growth stage. Ranjbar (2010) found that wheat was more sensitive during emergence and tillering stage, sensitive during stem elongation and relatively tolerant during grain filling period when the crop was exposed to

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saline nutrient solution ( $EC = 20 \text{ dS m}^{-1}$ ). Francois *et al.* (1994) suggested that the full yields of wheat can be obtained if moderately saline water is applied after terminal spikelet differentiation (TSD) period. In contrast to these studies, recently Mojid *et al.* (2013) found booting stage to be most sensitive growth stage in wheat when the crop was irrigated with  $EC 12 \text{ dS m}^{-1}$  saline water. In this study, the order of salt-tolerance was: tillering stage > grain filling stage > booting stage. The difference of result in these studies may be attributed to the salinity levels of water, composition of salts, crop cultivars, growth media and environmental factors. Despite providing management option for wheat cultivation in saline environment, above studies do not describe the reason of adverse effect of saline water at a specific growth stage. In a saline environment, the plant yield is reduced because of low osmotic potential, toxicity of specific ions and nutritional imbalances (Grattan and Grieve, 1998; Brady and Weil, 2002). In our earlier study (Bhatti *et al.*, 2015), we observed that the saline water application to wheat crop significantly decreased growth and yield components. The straw and grain yield was reduced to nearly 50% in saline water having  $EC 5.0 \text{ dS m}^{-1}$  in comparison to control treatment ( $EC 0.6 \text{ dS m}^{-1}$ ). The adverse effect was more pronounced when the wheat crop was irrigated with saline water at initial growth stage (emergence and tillering), and all growth stages (emergence, tillering, booting and grain formation) than later growth stages (booting and grain formation). This work is continuity to our earlier study, to determine the reason of this adverse effect on wheat growth and yield. We hypothesize that the observed reduction in growth and yield in wheat may be associated to high content of  $\text{Na}^+$  and  $\text{Cl}^-$ , low  $\text{K}^+$  content, and low  $\text{K}^+/\text{Na}^+$  ratio in plant tissues. The specific objective of present study was to describe the effect of saline waters on ions content of wheat in the scenario where such waters are applied at various growth stages. The findings of this study will be helpful to understand and correlate the negative effect of saline water on wheat growth and yield with ions content.

## **MATERIALS AND METHODS**

A detailed description of materials and methods has been reported in our earlier study (Bhatti *et al.*, 2015). In brief, a pot experiment was conducted where a non-saline soil was placed in 5 kg plastic containers. Wheat (cv. Sarsabz) was irrigated with saline waters having  $EC 2.0, 3.0, 4.0,$  and  $5.0 \text{ dS m}^{-1}$ , and tap water having  $EC 0.6 \text{ dS m}^{-1}$ . Saline waters were prepared from  $\text{NaCl}$  and  $\text{CaCl}_2$  salts at 20:1. Tap and saline waters were applied to wheat crop at four growth stages, namely emergence, tillering, booting and grain formation stage. These growth stages are reported early (emergence and tillering), late (booting and grain formation) and all (emergence, tillering, booting and grain formation). Saline waters and tap water were applied either alone or in cycles to wheat crop. This trend formulated thirteen treatments, which were replicated thrice. A detailed description of treatments, preparation of saline waters, soil properties and agronomic parameters recorded can be referred in our earlier study (Bhatti *et al.*, 2015).

### **Plant analysis**

The concentrations of various ions in plant tissues were analyzed in flag leaf sap, straw and grain samples.

#### **a) Flag leaf sampling, sap extraction and chemical analysis**

From each replication, flag leaves of the plants were detached. These leaves were stuffed in Eppendorf tubes and stored at  $-10^{\circ}\text{C}$ . Before analysis, the lamina of the flag leaves was removed and the sap was extracted as proposed by Gorham *et al.* (1997). Sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) were assessed by flame photometer. The  $\text{K}^+/\text{Na}^+$  ratio was calculated from the values of  $\text{Na}^+$  and  $\text{K}^+$ .

#### **b) Preparation of straw and grain samples for chemical analysis**

Straw and grain samples of each replication of a treatment were prepared for the analysis of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ . Samples for  $\text{Na}^+$  and  $\text{K}^+$  were digested by acid wet digestion technique, and analyzed by flame photometer. The samples for  $\text{Cl}^-$  analysis were digested by dry ash method, and analyzed by titration with silver nitrate ( $\text{AgNO}_3$ ) solution. The  $\text{K}^+/\text{Na}^+$  ratio was calculated from the values of  $\text{Na}^+$  and  $\text{K}^+$ .

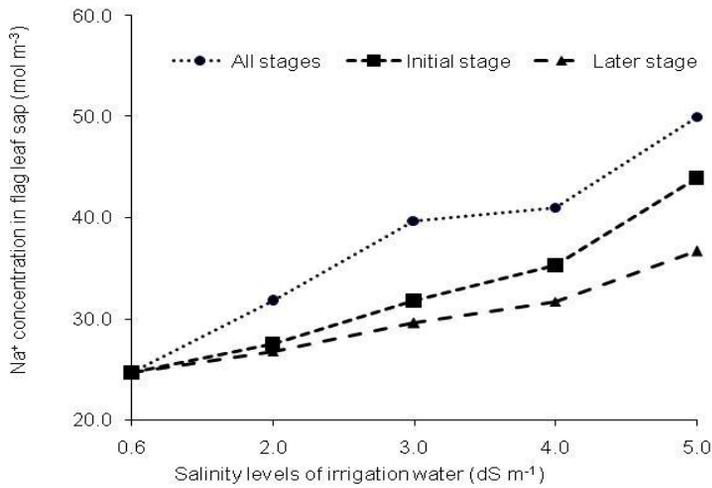
### **Statistical analysis**

The data for ions concentration in flag leaf sap, straw and grain samples were subjected to ANOVA (Balanced ANOVA) using statistical software Minitab (10). The difference among treatment means was determined by LSD test using a  $P$  value of 0.05.

## **RESULTS AND DISCUSSION**

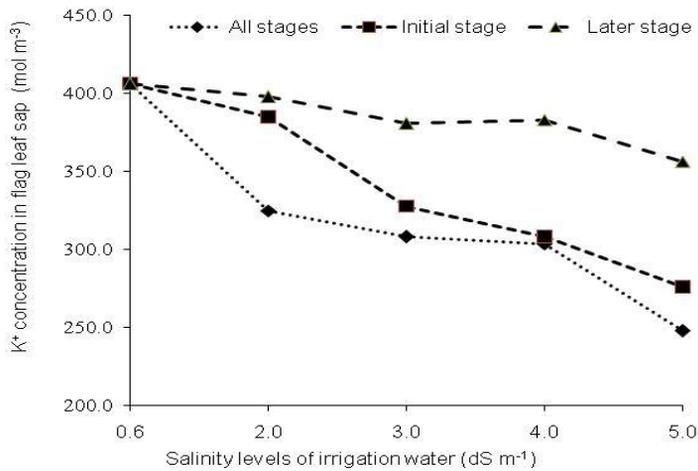
### **Sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) concentrations, and $\text{K}^+/\text{Na}^+$ ratio in flag leaf sap**

The effect of saline waters on  $\text{Na}^+$  ( $\text{mol m}^{-3}$ ) and  $\text{K}^+$  ( $\text{mol m}^{-3}$ ) concentration, and  $\text{K}^+/\text{Na}^+$  ratio in flag leaf sap is shown in Figure 1, 2 and 3, respectively. A highly significant ( $P < 0.05$ ) effect of saline water on  $\text{Na}^+$  and  $\text{K}^+$  concentrations and  $\text{K}^+/\text{Na}^+$  ratio in flag leaf sap was observed. The plants irrigated with EC 5.0 dS  $\text{m}^{-1}$  accumulated higher concentration of  $\text{Na}^+$ , while lower concentration of  $\text{K}^+$  and lower  $\text{K}^+/\text{Na}^+$  ratio than other treatments. A decreased concentration of  $\text{K}^+$  in plant tissues is because high levels of  $\text{Na}^+$  interferes with  $\text{K}^+$  accumulation by the roots, disturb the root membranes integrity and alter root selectivity (Grattan and Grieve, 1998). An increase in the concentration of  $\text{Na}^+$ , a decrease in  $\text{K}^+$  concentration and  $\text{K}^+/\text{Na}^+$  ratio in flag leaf sap of wheat with the increase in salt stress has also been described by many investigators (Rajpar and Wright, 2000; Saqib *et al.*, 2004; Rajpar *et al.*, 2006).



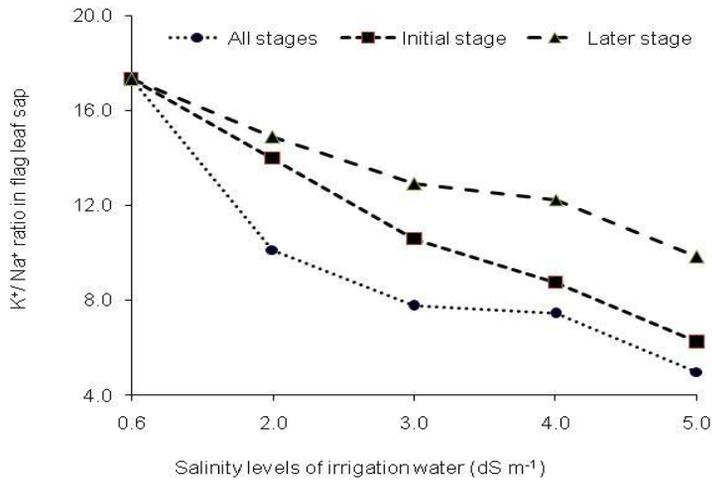
Source of variation	S.E.D.	L.S.D.
Salinity levels	2.014	5.591***
Growth stages	1.560	6.713***
Salinity levels x Growth stages	3.489	NS

**Figure 1.** Sodium (Na<sup>+</sup>) concentration (mol m<sup>-3</sup>) in flag leaf sap of wheat as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 *P* level; NS = Non significant



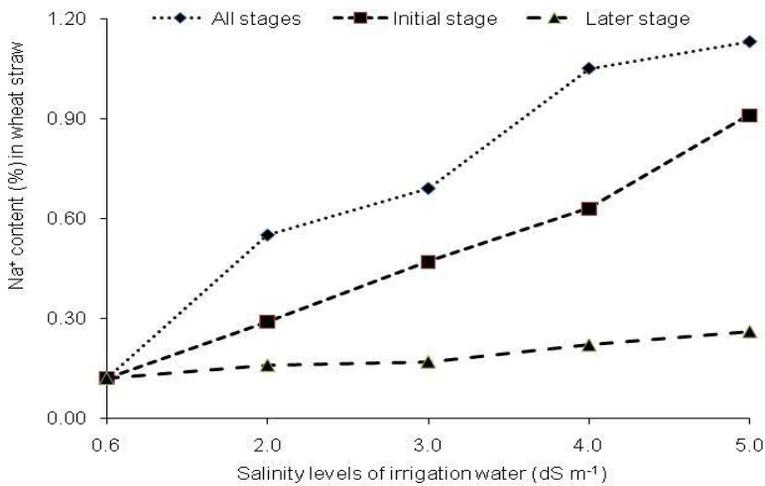
Source of variation	S.E.D.	L.S.D.
Salinity levels	21.216	58.896***
Growth stages	16.436	NS
Salinity levels x Growth stages	36.751	NS

**Figure 2.** Potassium (K<sup>+</sup>) concentration (mol m<sup>-3</sup>) in flag leaf sap of wheat as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 *P* level; NS = Non significant



Source of variation	S.E.D.	L.S.D.
Salinity levels	1.218	3.381***
Growth stages	0.943	NS
Salinity levels x Growth stages	2.109	NS

**Figure 3.** The K<sup>+</sup> / Na<sup>+</sup> ratio in flag leaf sap of wheat as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level; NS = Non significant



Source of variation	S.E.D.	L.S.D.
Salinity levels	0.100	0.278***
Growth stages	0.077	0.331***
Salinity levels x Growth stages	0.173	0.245*

**Figure 4.** Sodium (Na<sup>+</sup>) content (%) in wheat straw as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level

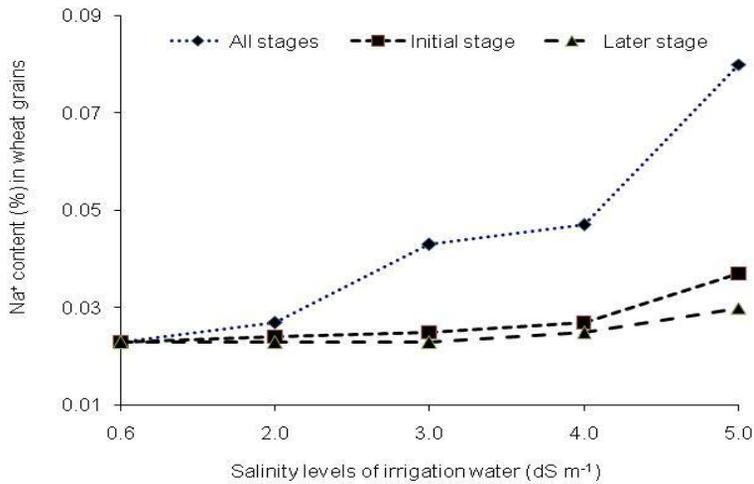
Among various growth stages, the effect of saline water was only significant for  $\text{Na}^+$  concentration in flag leaf sap ( $P < 0.05$ ). The plants irrigated at later growth stages significantly accumulated less  $\text{Na}^+$  than the plants irrigated at all growth stages. A higher concentration of  $\text{Na}^+$  in plants irrigated at all growth stages is associated with continuous supply of  $\text{Na}^+$  with each irrigation event. A difference of  $\text{Na}^+$  concentration in wheat leaves as a function of growth stages has also been reported (Maas and Poss, 1989).

The effect of interaction of salinity levels x growth stages for  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{K}^+/\text{Na}^+$  ratio in flag leaf sap was found non-significant ( $P > 0.05$ ). However, the plants irrigated with  $\text{EC } 5.0 \text{ dS m}^{-1}$  at all and early growth stages had more  $\text{Na}^+$ , less  $\text{K}^+$  and  $\text{K}^+/\text{Na}^+$  ratio compared to the plants in other treatments.

#### **$\text{Na}^+$ , $\text{K}^+$ and $\text{Cl}^-$ contents and $\text{K}^+/\text{Na}^+$ ratio in straw and grains of wheat**

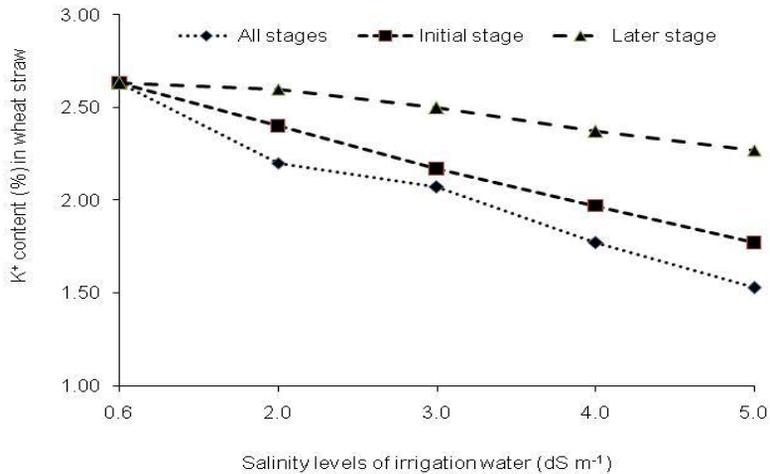
The data regarding the effect of saline irrigation water on  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{K}^+/\text{Na}^+$  ratio and  $\text{Cl}^-$  content in straw and grains of wheat as a function of various growth stages are presented in Figures 4 to 11. These ions responded differently to different salinity levels of irrigation water. Significant ( $P < 0.05$ ) effect of saline water was observed for  $\text{Na}^+$  and  $\text{K}^+/\text{Na}^+$  ratio in straw and grains of wheat. There was a higher concentration of  $\text{Na}^+$  in wheat straw and grains when plants were irrigated with  $\text{EC } 5.0 \text{ dS m}^{-1}$  than all other treatments. For  $\text{K}^+/\text{Na}^+$  ratio in wheat straw and grains, the plants irrigated with  $\text{EC } 5.0 \text{ dS m}^{-1}$  had significantly lower  $\text{K}^+/\text{Na}^+$  ratio than the plants irrigated with  $\text{EC } 2.0 \text{ dS m}^{-1}$  and tap water. The effect of saline water was only significant ( $P < 0.05$ ) for  $\text{K}^+$  content in grains and  $\text{Cl}^-$  content in straw. The  $\text{K}^+$  was significantly lower, while  $\text{Cl}^-$  was significantly higher in plants irrigated with  $\text{EC } 5.0 \text{ dS m}^{-1}$  when compared with plants irrigated with  $\text{EC } 2.0 \text{ dS m}^{-1}$  and control treatment. An increase in  $\text{Na}^+$  and  $\text{Cl}^-$  content in wheat tissues and a decrease in  $\text{K}^+$  and  $\text{K}^+/\text{Na}^+$  ratio may be attributed to application of  $\text{NaCl}$  enriched saline water. An increase and/or decrease in these ions ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ) and  $\text{K}^+/\text{Na}^+$  ratio in wheat tissues as a function of salinity levels is well documented (Chachar *et al.*, 1990; Astarai and Chauhan, 1994; Sharma, 1995).

The effect of saline water at different growth stages of wheat was significant ( $P < 0.05$ ) only for  $\text{Na}^+$  in straw and grains, and  $\text{K}^+/\text{Na}^+$  ratio in straw and grains. The plants received saline water at later stages significantly accumulated less  $\text{Na}^+$  and had more  $\text{K}^+/\text{Na}^+$  ratio than the plants irrigated with saline water continuously (all growth stages). A high content of  $\text{Na}^+$  in the plants irrigated with saline water at all growth stages was possibly because these plants were exposed to more  $\text{Na}^+$  with each irrigation event (irrigated four times with saline water) than the plants irrigated with saline water at later stages (irrigated with saline water two times). Ranjbar (2010) suggested that the saline irrigation water cannot be used for entire growing period of wheat crop. The effect of interaction of salinity levels x growth stages was found significant ( $P < 0.05$ ) only for  $\text{Na}^+$  in straw and grains. The plants irrigated with saline water having  $\text{EC } 5.0 \text{ dS m}^{-1}$  at all growth stages accumulated more  $\text{Na}^+$  in straw and grains than all other plants.



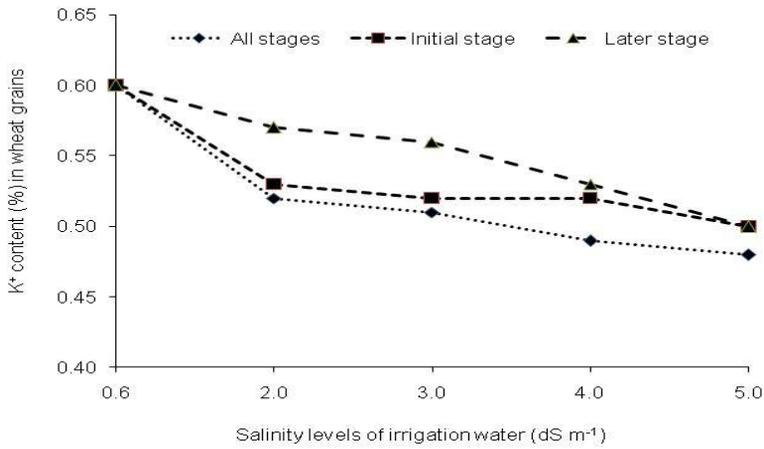
Source of variation	S.E.D.	L.S.D.
Salinity levels	0.005	0.014***
Growth stages	0.004	0.017***
Salinity levels x Growth stages	0.009	0.021*

**Figure 5.** Sodium (Na<sup>+</sup>) content (%) in wheat grains as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level



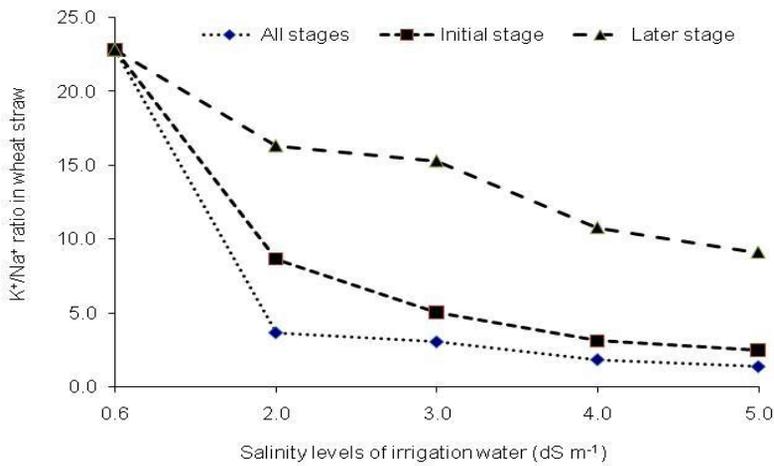
Source of variation	S.E.D.	L.S.D.
Salinity levels	0.380	NS
Growth stages	0.295	NS
Salinity levels x Growth stages	0.659	NS

**Figure 6.** Potassium (K<sup>+</sup>) content (%) in wheat straw as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; NS= Non significant



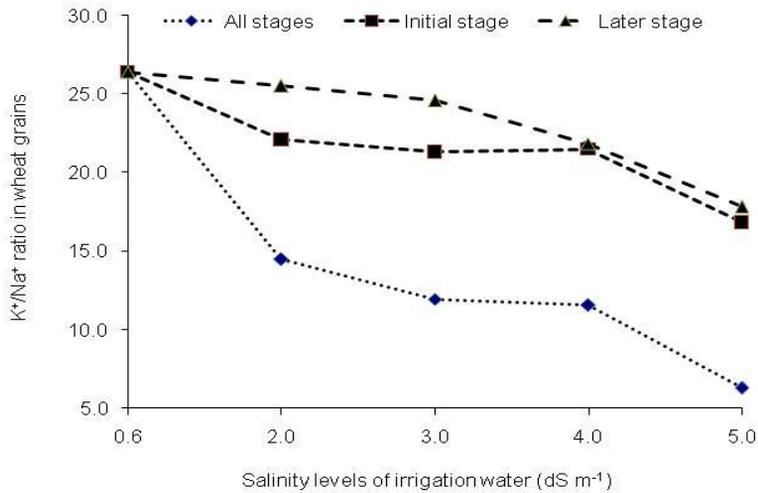
Source of variation	S.E.D.	L.S.D.
Salinity levels	0.018	0.050***
Growth stages	0.014	NS
Salinity levels x Growth stages	0.030	NS

**Figure 7.** Potassium (K<sup>+</sup>) content (%) in wheat grains as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level; NS = Non significant



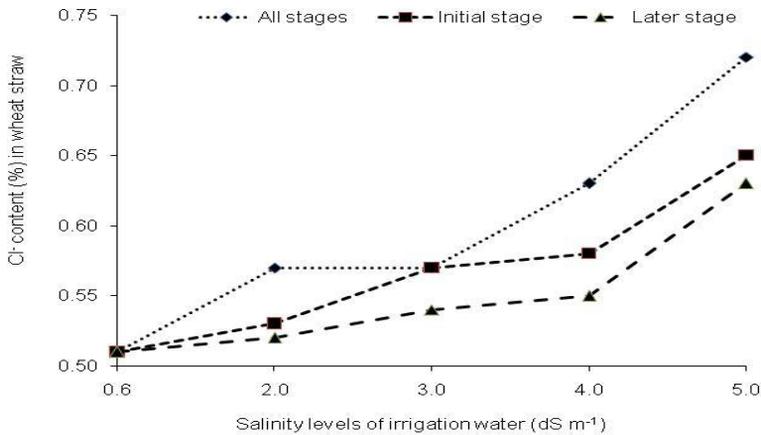
Source of variation	S.E.D.	L.S.D.
Salinity levels	1.645	4.566***
Growth stages	1.274	5.482***
Salinity levels x Growth stages	2.850	NS

**Figure 8.** The K<sup>+</sup> / Na<sup>+</sup> ratio in wheat straw as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level; NS = Non significant



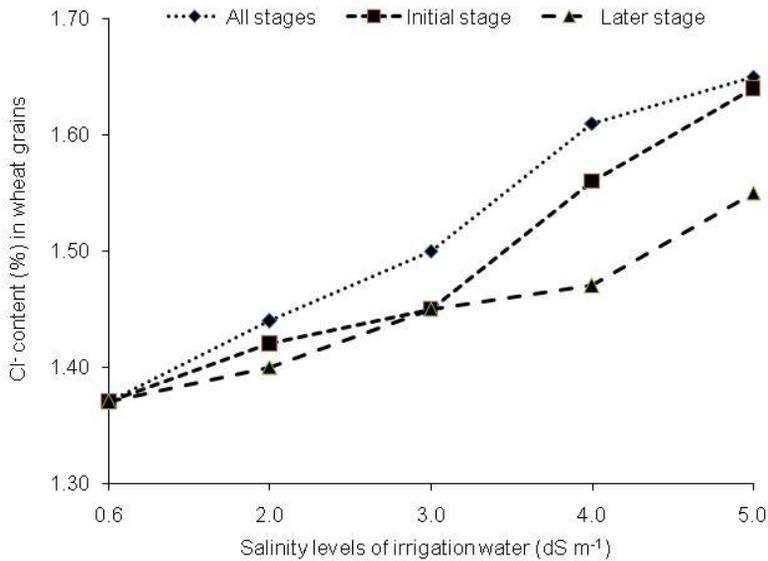
Source of variation	S.E.D.	L.S.D.
Salinity levels	2.324	6.451***
Growth stages	1.800	7.745***
Salinity levels x Growth stages	4.025	NS

**Figure 9.** The K<sup>+</sup> / Na<sup>+</sup> ratio in wheat grains as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level; NS = Non significant



Source of variation	S.E.D.	L.S.D.
Salinity levels	0.043	0.119*
Growth stages	0.033	NS
Salinity levels x Growth stages	0.075	NS

**Figure 10.** Chloride (Cl<sup>-</sup>) content (%) in wheat straw as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; \* Significant at 0.05 P level; NS = Non significant



Source of variation	S.E.D.	L.S.D.
Salinity levels	0.144	NS
Growth stages	0.112	NS
Salinity levels x Growth stages	0.250	NS

**Figure 11.** Chloride (Cl<sup>-</sup>) content (%) in wheat grains as influenced by saline irrigation water and growth stages; each marker point is a mean of three replications; NS = Non significant.

## CONCLUSION

Increasing salinity of irrigation water significantly increased Na<sup>+</sup> concentration in flag leaf sap, straw and grains, and decreased K<sup>+</sup> concentration in flag leaf sap and grains of wheat. Consequently, there was a decrease in K<sup>+</sup>/Na<sup>+</sup> ratio in flag leaf sap, straw and grains of wheat. The effect of saline water having EC 5.0 dS m<sup>-1</sup> was more profound on the concentration of these ions in leaf sap and plant tissues. The application of saline waters on wheat at various growth stages indicates that the Na<sup>+</sup> concentration can be minimized in wheat sap and tissues by applying saline waters at later stages of growth (booting and grain formation). The findings of present study further elaborates that the adverse effect of saline waters on growth and yield of wheat (cv. Sarsabz) in our earlier study (Bhatti *et al.*, 2015) may be associated to the higher Na<sup>+</sup> and lower K<sup>+</sup> in leaf sap, straw and grains. We suggest that the wheat growers may irrigate the crop with saline waters (up to 5.0 dS m<sup>-1</sup>) at later growth stages to attain minimum yield loss and to anticipate low Na<sup>+</sup> concentration in plant tissues.

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