



## SCREENING THE VARIABILITY IN SALT TOLERANCE OF *SORGHUM BICOLOR* L. BY NUTRIENTS UPTAKE AND GROWTH ANALYSIS OF FOUR GENOTYPES

Munazza Gull<sup>1\*</sup>, Abida Kausar<sup>2</sup>

1. *Biochemistry department, Faculty of Science, King Abdulaziz University, 42805, Jeddah, Saudi Arabia.*
2. *Botany Department, Faculty of Science, Government College Women University, Faisalabad, Pakistan.*

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### ABSTRACT

The main objective of the present study was evaluation of genotypic potential among four sorghum genotypes through the analysis of different growth and nutrients uptake in salt stress. The four sorghum genotypes, JS-2002, Sandalbar, Noor and FJ-115 were utilized in the experiments to study the mechanism of salt tolerance using 0 or 100 mM NaCl stress. The analysis was carried out on growth and different ion concentrations e.g., sodium, potassium, calcium and chlorides of both shoots and roots. The differences among means were calculated and analysis of variance technique was used for comparison of salt tolerance between cultivars. Results showed that salinity adversely affected growth of all four sorghum genotypes. The fresh weight of plants was reduced by salt stress in sorghum. The uptake of toxic compounds was increased in the presence of salt stress as compared with non-stressed plants, which reduced the uptake of essential nutrients for plants. The highest fresh weight of plants and nutrient uptake potential was recorded in Sandalbar closely followed by JS-2002, which indicated these sorghum cultivars as salt tolerant. It is further concluded that the induction of the salt stress in the growth medium cause adverse effect on the growth and uptake of essential nutrients. It was observed that salt stress cause ion toxicity in sorghum plants. These results could be used in sorghum breeding programs to select the most tolerant cultivars.

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### Introduction

Salinity, the major problem of agricultural areas, caused by increasing concentrations of soluble salts in the root zone largely affects the crop production [1] resulting in shortage of food, feed and industrial raw material all over the world [2]. Various reports indicate that 7% of the agricultural soils throughout the world are salt-affected and this number may increase up to 20% due to salinity [2]. The soil degradation due to salt stress, water logging and therefore the depletion of natural resources in the world is creating problems for scientists to meet the agricultural needs of the increasing population [3]. Owing to population pressure and other natural resources, efficient use of saline areas for cultivation of different crops is direly needed [4]. The strong limitations of salt stress in the productivity of crops are more evident in arid and semi-arid lands, where low rainfall and high temperatures are the main contributing factors [2]. These salts in the roots' medium induces imbalance in water potential and result in the cellular dehydration in plants and inhibition of intracellular enzymes activity [2]. Moreover, existing agricultural practices have increased the extent of salinity by moving salts from deeper soil layers to the rhizosphere [5]. Irrigation systems in all over the world are also under the risk of salinity stress and about half of them are directly affected by salt stress [6].

Salt stress adversely effects seedling growth and development by altering certain metabolic processes in various plants [7, 8]. The salt deposition in plant leaves alters metabolism, reduces plant growth [9], declines the yield of different photosynthates and negatively effects the development of young leaves in plants. It affects the plants by lowering the water potential and creating ion toxicity and hindering the uptake of essential nutrients [8]. Ultimately, it causes cell death in salt sensitive genotypes and death of whole plant [10].

Sorghum (*Sorghum bicolor* L.) is cultivated for 3000 years in tropical and subtropical regions [11] in the world. It is a significant industrial crop, which is moderately tolerant salinity and can be grown in arid and semi-arid lands [12, 13]. It is the important and major crop among the cereals, which has potential to produce a yield and biomass under water scarcity conditions. It has significant grain crop that is the source of food, feed, fodder and biofuels in the global agro-ecosystem. It is a tropical C4 photosynthetic grass, which has higher carbon assimilation rate at high temperatures. Unfortunately, its breeding program has been less investigated in the last many decades, even though its genome has advantageous tolerant genes conferring resistance to both abiotic and biotic environmental stresses. Sorghum is a potential crop and has intraspecific variability for salinity tolerance [14]. Many genotypic variations for salinity and temperature tolerance in sorghum have been reported [15]. Hence, the development of salt and temperature tolerant sorghum genotypes is the best strategy to increase the crop production on saline soils and high temperature zones [14].

Recently physiological markers associated with salt tolerance have been investigated [16] and genetic maps indicating molecular basis of salt induced gene products have been constructed [1, 3]. The salt tolerant genotypes can be identified by screening the variations in morphological and physiological attributes of plants. The research work was planned to investigate the effects of NaCl on various growth and nutrient uptake potential of various sorghum cultivars to identify their salt tolerance ability.

## Material and Methods

The present research project was aimed to study yield and uptake of different nutrients of sorghum germplasm under salinity stress. The seeds of four sorghum genotypes were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. The four sorghum genotypes (JS-2002, Sandalbar, Noor and FJ-115) were utilized in the experiments to study the mechanism of salt tolerance using 0 or 100 mM NaCl stress.

The experiment was carried out under natural conditions. Washed river sand, after rinsing three times with distilled water, was used in the experiment. Ten seeds from each of the four genotypes were sown in earthen glazed pots measuring 35 cm in diameter, filled with 12 kg of air dried washed river sand and 0 and 100 mM NaCl solution and three replicates of each treatment were maintained. The pots were arranged in a randomized manner (completely randomized block design) and re-positioned randomly at various times during the course of study. After germination all the pots were irrigated with 1/5 Hoagland's nutrient solution (Table 1). During the course of study after every one week, the solution of the pots was changed. The pH of the solution was regularly monitored and maintained at 7.00 with H<sub>2</sub>SO<sub>4</sub> by using pH meter (WTW PH 530). Pots were weighed on alternate days and the water lost through evaporation was maintained by the addition of distilled water in all pots. The plants were allowed to grow for 140 days under 0 and 100 mM concentrations of NaCl in Hoagland's nutrient solution.

**Table 1:** Hoagland's nutrient solution composition used for plant growth.

Compound	Stock	mg/g	Concentration
KNO <sub>3</sub>	1 M	5	0.005 M
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	1 M	5	0.005 M
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1 M	2	0.002 M
KH <sub>2</sub> PO <sub>4</sub>	1 M	1	0.001 M
H <sub>3</sub> BO <sub>3</sub>	3.54 g L <sup>-1</sup>	1	0.5 mg L <sup>-1</sup>
MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.80 g L <sup>-1</sup>	1	0.5 mg L <sup>-1</sup>
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22 g L <sup>-1</sup>	1	0.05 mg L <sup>-1</sup>
CuSO <sub>4</sub> ·7H <sub>2</sub> O	0.078 g L <sup>-1</sup>	1	0.02 mg L <sup>-1</sup>
(NH <sub>4</sub> ) <sub>2</sub> MO <sub>4</sub> O <sub>24</sub>	0.18 g L <sup>-1</sup>	1	0.01 mg L <sup>-1</sup>
Ferri citrate	27.7 g L <sup>-1</sup>	1	5.00 mg L <sup>-1</sup>

After 140 days, the experiments were terminated and data was recorded for growth and different nutrients analysis. Three plants from each replication were harvested, their fresh weights were determined and their means were calculated; then plants were dried in oven for two days and cations (Na<sup>+</sup> and K<sup>+</sup>), calcium (Ca<sup>2+</sup>) and chloride (Cl<sup>-</sup>) nutrients were analyzed. Uptake of nutrients (Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>) by sorghum plant was recorded according to the method of Jackson [17].

## Digestion analysis

Wet digestion of dried plant material was carried out according to the method of Wolf [18]. Dried ground plant material (0.5 g) was taken in digestion tubes and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added to each tube. All the tubes were placed overnight at room temperature for thawing. Then 0.5 mL of H<sub>2</sub>O<sub>2</sub> (35%) was poured along the sides of the digestion tubes; we ported them in a digestion block and heated at 350 °C until fumes were produced. After continuous heating for another 30 minutes, the digestion tubes were deported and cooled for 5 minutes. Then 0.5 mL of H<sub>2</sub>O<sub>2</sub> was added slowly, and the tubes were heated by placing them back into the digestion block. The above step was repeated until the digested material became colorless. The extract was volumed up to 50 mL in volumetric flasks. This extract was filtered and used for determining sodium, potassium and calcium contents. The determination of chlorides was done by chloride meter. Dried ground plant material (1 g) was heated in 20 mL distilled water in 50 mL conical flask by incubating it in an oven at 65 °C overnight. The

mixture was filtered through Whatmann-40 filter paper and extract was used for the determination of chloride using chloride analyzer (Corning-920, Germany). Potassium ( $K^+$ ), Sodium ( $Na^+$ ) and Calcium ions' concentrations were analyzed by Flame-photometer (Jenway PFP 7).

#### Statistical Analysis:

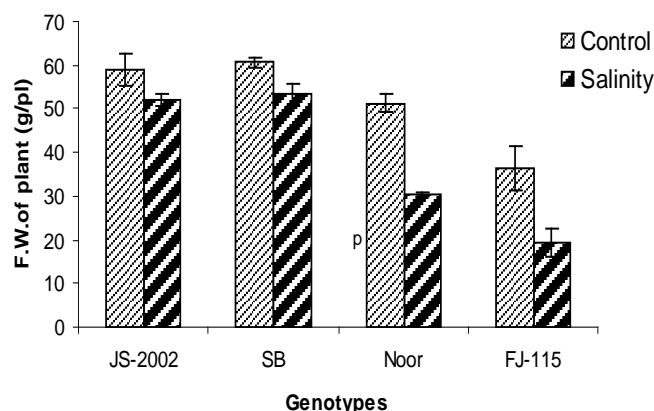
The data collected for growth and different nutrients were statistically analyzed by using STATISTICA Computer Program and analysis of variance technique. The differences among significant means were assessed by Least Significant Difference (LSD) test at the probability level of 5% [19].

## RESULTS

The main objective of the present study was to determine the effect of salt stress on nutrients uptake and growth of four sorghum cultivars.

#### Fresh biomass production under salinity stress

Fresh weights of plants were adversely affected by salinity in all sorghum genotypes (Figure 1, Table 2). All genotypes responded differently for plant fresh weight and variations among them were significant. The highest fresh weight of plants was recorded in Sandalbar closely followed by JS-2002. However, the lowest plant biomass was maintained by FJ-115, while Noor was medium in growth performance. The interaction between salinity and genotypes was significant and under non-saline conditions, Sandalbar and JS-2002 maintained the highest plant fresh weights followed by Noor, while the lowest was in FJ-115. Under saline environments, maximum reduction in shoot fresh weights was determined in FJ-115 followed by Noor, JS-2002 and Sandalbar. Data clearly indicated that plant biomass was significantly reduced by salinity and genotypes JS-2002 and Sandalbar showed the best performance than others (Table 2). Differences among the genotypes in response to salinity were significant. The maximum fresh biomass was obtained in Sandalbar followed by JS-2002, Noor and FJ-115. The results showed that salinity and genotypes' interaction was significant. The Sandalbar and JS-2002 maintained the highest biomass of plants in salt stress conditions than others. Under salinity, maximum decrease in fresh weights was estimated in FJ-115 followed by Noor, JS-2002 and Sandalbar in salt stress. The results showed that salt stress significantly reduced the fresh weights of plants (Figure 1) of all genotypes at 100 mM NaCl concentration.



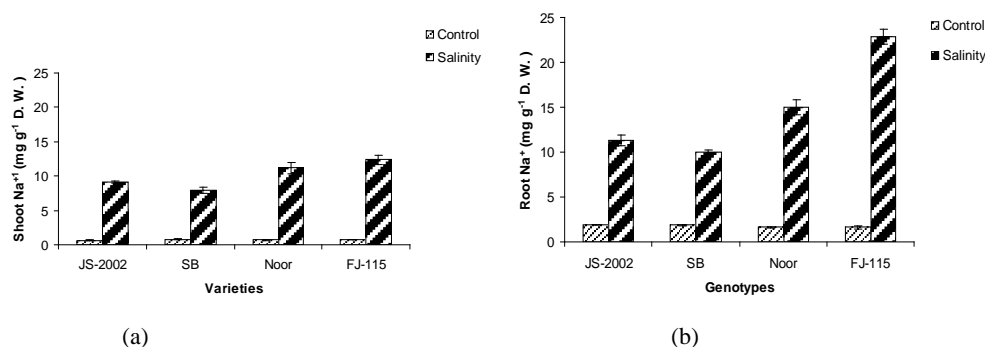
**Figure 1:** Fresh weights of four sorghum genotypes subjected to NaCl (0, 100 mM) salinity stress.

#### Salinity and nutrients imbalance

##### Sodium contents in shoots and roots

Sodium contents in shoots and roots significantly increased with the application of NaCl stress in all genotypes (Table 2). However, sodium ( $Na^+$ ) contents in roots were higher than in shoots. The variations among different sorghum genotypes were significant in both shoots and roots. Under saline conditions, maximum  $Na^+$  contents in roots were recorded in sorghum line FJ-115 followed by Noor (Figure 2). However, differences between FJ-115 and Noor were significant, while Sandalbar and JS-2002 genotypes contained lower amount of  $Na^+$  concentration. In case of shoots, although the trend was similar but variation between Noor and FJ-115 were non-significant. Similarly, JS-2002 and Sandalbar maintained lower  $Na^+$  contents in shoots but differences between both of these genotypes were non-significant. Interactions between salt stress and genotypes were also significant in both the cases (Table 2) and clearly indicated that the genotypes Noor and FJ-115 maintained higher concentration of  $Na^+$  in case of both shoots and roots, while minimum amount of  $Na^+$  contents were recorded in Sandalbar which was closely followed by JS-2002 in both the cases of shoots and roots. The pattern of all the genotypes under control conditions for  $Na^+$  contents was similar in both roots and shoots. From these results, it was very

clear that salinity enhanced the  $\text{Na}^+$  content in both roots and shoots and the genotypes Sandalbar and JS-2002 successfully maintained lower amounts of  $\text{Na}^+$  contents.



**Figure 2:** Sodium concentration of shoots and roots of four sorghum genotypes subjected to NaCl (0, 100 mM) NaCl salinity stress.

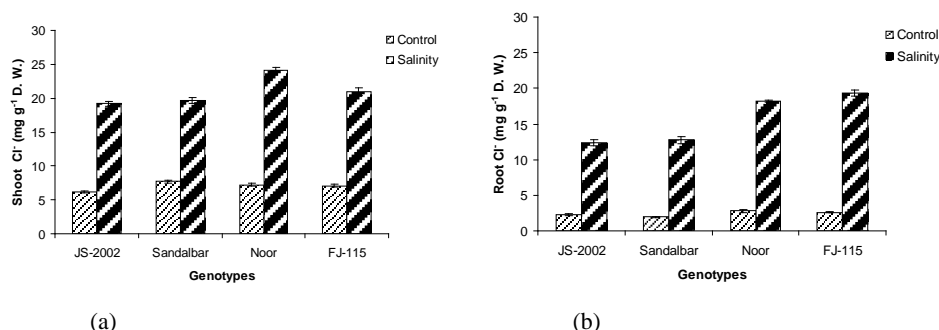
### Chloride contents in shoots and roots

Chloride contents in shoots and roots were significantly enhanced by salinity in all genotypes and their differences were found significant both in cases of shoots and roots (Table 2).

In shoots, the pronounced effect of salinity with respect to chloride accumulation was recorded in Noor followed by FJ-115, Sandalbar, and JS-2002. However, in case of roots, maximum chloride contents were accumulated in FJ-115 closely followed by Noor, and minimum amount was determined in JS-2002 and Sandalbar (Figure 3).

Interaction between salinity and genotypes was also significant in both cases of shoots and roots. Maximum chloride was determined in Sandalbar followed by Noor, FJ-115 and JS-2002 genotypes in shoots growing under normal conditions. However, in case of roots, the pronounced amount of chloride was measured in Noor followed by FJ-115, JS-2002 and Sandalbar in non-saline environments.

In contrast, in saline medium, maximum increase in chloride was determined in Noor followed by FJ-115, and minimum increase was found in JS-2002 followed by Sandalbar in the shoots of sorghum genotypes in salinity. Similarly, higher increase was calculated in Noor followed by FJ-115 and minimum increase was recorded in Sandalbar followed by JS-2002, in case of roots in salinity stress.



**Figure 3:** Chloride concentration of shoots and roots of four sorghum genotypes subjected to NaCl (0, 100 mM) salinity stress.

**Table 2:** Mean squares values from analyses of variance of data for fresh weights and sodium and chloride concentrations ( $\text{mg g}^{-1}$  D. W.) of shoots and roots of four genotypes of sorghum subjected to NaCl (100 mM) salinity stress.

S.V.	Degree of freedom	F. W. of plants (g/plant)	$\text{Na}^+$ in shoots ( $\text{mg g}^{-1}$ D. W.)	$\text{Na}^+$ in roots ( $\text{mg g}^{-1}$ D. W.)	$\text{Cl}^-$ in Shoots ( $\text{mg g}^{-1}$ D. W.)	$\text{Cl}^-$ in roots ( $\text{mg g}^{-1}$ D. W.)
Genotypes (G)	3	1122.4626***	533.708**	1019.937**	1163.434**	1052.845**
NaCl treatments(S)	1	1021.7041***	5.928**	48.458**	9.321**	23.407**
G x S	3	73.246655***	6.060**	52.895**	7.186**	16.400**
Error	16	7.8597285	0.495	0.656	0.369	0.296
Total	23					

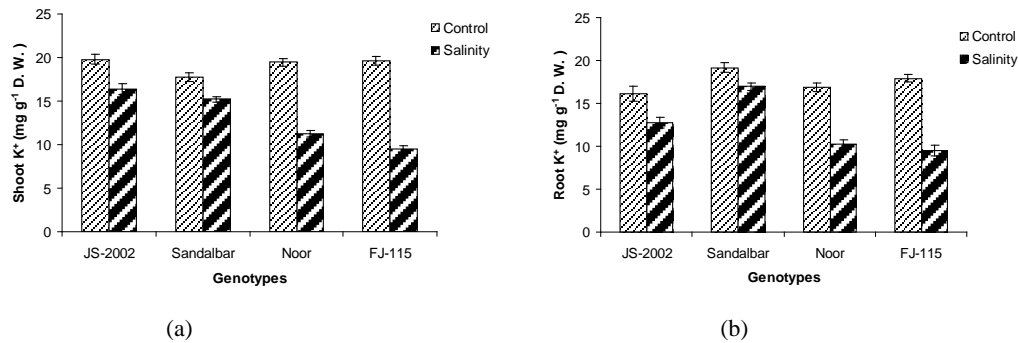
### Potassium contents in shoots and roots

The trend of potassium ( $\text{K}^+$ ) contents in shoots and roots was reverse to that of sodium and significantly, less concentration under saline medium as compared to control was observed in all genotypes. However, the variations among different genotypes were significant in both shoots and roots (Table 3). Under saline conditions maximum  $\text{K}^+$  contents in roots were recorded in the genotype Sandalbar followed by JS-2002. However, differences between Sandalbar and JS-2002 were

significant, while FJ-115 and Noor contained lower amount of  $K^+$  concentration. In case of shoots, the trend was different; the variety JS-2002 followed by Sandalbar contained higher amount of  $K^+$  than other genotypes but differences between both of these genotypes were significant. However, variation between Noor and FJ-115 were non-significant (Figure 4).

Interaction between salinity and genotypes were also significant in both cases and clearly indicated that variety Sandalbar and JS-2002 maintained higher concentration of  $K^+$  both in case of shoots and roots, while minimum amount of  $K^+$  contents was recorded in Noor which was closely followed by FJ-115 with regard to both shoots and roots (Table 3).

The pattern of all the genotypes under control conditions for  $K^+$  contents was similar in both shoots and roots. From these results, it is clearly indicated that salinity reduced the  $K^+$  contents in both roots and shoots and the genotypes Sandalbar and JS-2002 successfully maintained higher amounts of  $K^+$  contents.

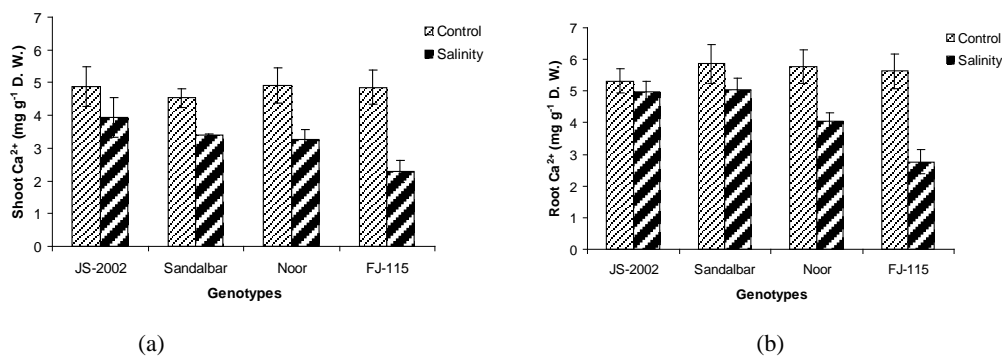


**Figure 4:** Potassium concentration of shoots and roots of four sorghum genotypes subjected to NaCl (0, 100 mM) salinity stress.

#### Calcium contents in shoots and roots

Data regarding calcium contents revealed that these contents in shoots and roots were significantly reduced by the use of salinity in sorghum genotypes. The variations among the genotypes were non-significant (Table 3). However, all the genotypes showed similar behavior towards salinity both in cases of shoots and roots (Figure 5).

The salinity and genotypes interaction also showed non-significant results in both shoots and roots. Minimum decrease in saline conditions was observed in JS-2002 followed by Sandalbar in case of both roots and shoots. However, maximum decrease in calcium contents was calculated in FJ-115 followed by Noor in saline environments in both shoots and roots (Figure 5 a, b). It is evident from the results that Sandalbar and JS-2002 was successful in maintaining maximum amount of calcium in their shoots under saline conditions. Similar results were obtained in case of roots of sorghum genotypes (Figure 5 a).



**Figure 5:** Calcium concentration of shoots and roots of four sorghum genotypes subjected to NaCl (0, 100 mM) salinity stress.

**Table 3:** Mean squares values from analyses of variance of data for potassium and calcium concentrations (mg g<sup>-1</sup> D. W.) of shoots and roots of four genotypes of sorghum subjected to NaCl (100 mM) salinity stress.

S.V.	Degree of freedom	K <sup>+</sup> in Shoots (mg g <sup>-1</sup> D. W.)	K <sup>+</sup> in roots (mg g <sup>-1</sup> D. W.)	Ca <sup>2+</sup> in shoots (mg g <sup>-1</sup> D. W.)	Ca <sup>2+</sup> in roots (mg g <sup>-1</sup> D. W.)
Genotypes (G)	3	222.736**	239.882**	16.286**	12.298**
NaCl treatments (S)	1	14.215**	23.373**	0.782 <sup>NS</sup>	1.724 <sup>NS</sup>
G x S	3	20.458**	35.557**	0.688 <sup>NS</sup>	1.846 <sup>NS</sup>
Error	16	0.662	1.460	1.086	0.578
Total	23				

## DISCUSSION

The objective of the present study was to select the salt tolerant sorghum cultivars by investigating the variations in their growth and nutrient uptake potential.

It is apparent from the results that salinity greatly affects all growth parameters like fresh weight and ions uptake. This antagonistic effect in growth might be because of slow nutrient rate of transport like  $\text{NO}_3^-$  that cause reduction of N compounds and enhanced the accumulation of  $\text{Na}^+$  ions in plants under elevated salinity stress [20]. Uptake and accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  toxic ions rigorously hinder the transport of  $\text{NO}_3^-$  [21]. It was reported that plant growth and yield reduced with rising salinity stress in plants [22]. The growth and yield production and potential of salinity tolerance in plant are directly linked to supplementary absorption of  $\text{K}^+$  and poorer shoot  $\text{Na}^+$  concentrations which may certainly linked to  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  ions accumulation in plants [15]. The results of current study regarding growth reduction associations with salinity stress have been well documented in many previous investigations [2, 3, 23].

It has been reported that salinity had a pronounced effect on the development of sorghum genotypes by causing a significant reduction in plant height, fresh and dry weights of both shoots and roots. Similar findings were also reported by other researchers [24, 25] that salinity negatively affects growth, yield and productivity of plants throughout the world especially in irrigated areas. In the current study, fresh/dry weight of both shoot and root of FJ-115 were markedly reduced by salinity, while these parameters were moderately decreased in Noor, however, both JS- 2002 and Sandalbar genotypes were successful in maintaining these attributes very close to normal even at 100 mM NaCl, thus supported that they are salt tolerant. Under saline conditions, osmotic/water stress and ion-toxicity are the major barriers for the development of plants [26]. Investigations proved that seedling growth was inhibited by salinity due to slow mobilization of food reserve to the growing parts of seedlings [27]. Present investigations also confirmed that salinity affected root growth more severely than that of shoot. Limitations to plant development induced by salt stress cannot be explained by only a single physiological or biochemical process but it depends on many physiological and biochemical attributes such as photosynthesis, transpiration, leaf water, osmotic and turgor potentials, as well as stomatal conductance. The growth inhibition in many plants subjected to salinity is mostly associated with the slowdown of rate of photosynthesis [28].

The increase in  $\text{Na}^+$  may be due to the lack of sodium ATP-ases in plant cell membranes. So the presence of high concentrations of  $\text{Na}^+$  in the plant growth medium enhanced the entry of  $\text{Na}^+$  into the plant cells and its exclusion from the cells is inhibited due to lack of ATP-ases in plant cell membranes. So due to this reason  $\text{Na}^+$  accumulated more in the plant cells under saline environments which disrupt many metabolic processes in plants [29]. Salinity increased accumulation of  $\text{Na}^+$  in leaves, which ultimately decreased dry biomass of plants. However, accumulation of  $\text{Na}^+$  was always higher in root as compared to shoot that is due to the retention mechanism of  $\text{Na}^+$  in roots [30].

The finding of present study indicated that under salinity stress  $\text{Cl}^-$  contents were higher in sensitive genotypes than tolerant genotypes. Leaves contained more  $\text{Cl}^-$  than roots. These investigations are in parallel to the results of many researcher [31] who also supported that  $\text{Cl}^-$  contents were increased in leaves due the mobile nature of  $\text{Cl}^-$ . Higher  $\text{Cl}^-$  contents adversely affected the plant growth either by increasing osmotic potential of plant or by specific ions toxicity [32]. However, literature confirmed that in tolerant crop genotypes cytotoxic ions under salt stress conditions compartmentalized in the vacuole and is used as osmotic solutes and helpful in osmotic adjustments [33].

The growth and cell membrane permeability are negatively linked with concentrations of sodium ions, which adversely affects divisions, and elongation of the cell [34].  $\text{K}^+/\text{Na}^+$  ratio and  $\text{Ca}^{2+}$  concentrations in plant shoot were inversely related with increasing salinity levels [29]. As the ratio of  $\text{Na}^+/\text{Ca}^{2+}$  increased in plant, it increased the cell membrane permeability which ultimately enhanced absorption of  $\text{Na}^+$  and  $\text{Cl}^-$  in plant and caused decrease in the uptake of  $\text{K}^+$ . It was observed that higher values of  $\text{Cl}^-$  and  $\text{Na}^+$  reduced the uptake of  $\text{Ca}^{2+}$  and  $\text{K}^+$  in plants due to their mutual competition between ions on a carrier protein for a transport site [34]. The presence of  $\text{K}^+$  plays an important role in several metabolic processes like turgor mediated stomatal movements, osmotic adjustments, translocation, and accumulation of carbohydrates [34]. Potassium plays as an important cofactor for many enzymatic activities and used as necessary index of salinity tolerance in plants [3, 34]. However, concerning this fact, it was suggested that photosynthesis rate was directly related with decreased levels of  $\text{K}^+$  in leaf. The sensitive genotypes exhibit more ions accumulation and less production in saline environments [35]. Several workers have investigated that plant tolerance in the rooting medium was under genetic control and genetic variations are the basic tools for crop improvements [3, 10]. Sorghum has showed great potential of genetic variability [22, 23]. These genetic variations provide a great deal of information about sorghum genotypes that could be grown in salt-affected areas to enhance its productivity, and also for determining degree of salinity tolerance in sorghum for further utilization in breeding programs.

## CONCLUSION

According to the results of this study, it was noted that saline soils reduce the uptake of essential nutrients by antagonistic effects of ions with sodium and chloride which are more accumulated and as a result disrupt many physiological and biochemical pathways in sorghum plants. The saline soils also reduce the fresh weight of plant tissues and increase the ions toxicity. Data analysis indicated that Sandalbar and JS-2002 sorghum cultivars appeared as strong salt tolerant under both

saline and non-saline conditions as compared to Noor and FJ-115. These results could be used in sorghum breeding programs to select the salt tolerant cultivars to obtain better growth and yield.

#### Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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