



Effect of Salt Stress on Na⁺ and K⁺ uptake at Seedling Stage in Sorghum Cultivars

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Abstract

The presence of salts in soil or in irrigation water adversely affects plant growth and soil properties. This study focuses on the inhibitory effect of salinity on uptake of two macro nutrients in shoot of sorghum. Four sorghum (Sorghum bicolor (L.) Moench) cultivars viz. Pant Chari-1, Pant Chari-2, CSV-15 and HC-171 were studied in laboratory for sodium and potassium uptake under varying salt stresses. Seedlings were exposed to 0, 3, 6, 7.2, 10 and 12 EC and after ten days of starting the salt treatment, growth of shoot system and uptake of Na⁺ and K⁺ were determined. It was found that concentration of sodium ion increased while concentration of potassium ions decreased invariably from 3 to 12 EC salinities in all four genotypes. The tolerant genotype CSV-15 and HC-171 recorded minimum concentration of Na⁺ as compared to cultivar Pant Chari-1 and Pant Chari-2 at all level of salinity. Salinity stress negatively affected uptake of K⁺ in all cultivars of sorghum. Potassium ion content was highest in CSV-15 and lowest in sensitive genotype Pant Chari-2. Present study suggests that tolerance to salt stress in sorghum genotypes is related to maintain the concentrations of Na and K ions at varying salinity levels.

Keywords: Sorghum, Electrical conductivity (EC), Salt stress, Cultivars, Seedling growth.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), commonly known as jowar is a C4 grass cultivated for its grain used for food, both for animals and humans. It was originated in Africa, and now is cultivated widely in semi-arid and arid tropical regions. It is characterized as moderately salt tolerant¹. Excess salt concentration in tropic and semi-tropic regions is one of the major problems that adversely affect the plant growth and productivity²⁻³. In order to ensure agricultural sustainability and crop production in arid and semi-arid regions the main issue is to overcome salinity⁴. For a number of crops, under saline conditions it has been found that one stage is significantly different from the other stages. Therefore, the sensitivity or salt tolerance of a crop should be evaluated in three different crop stages, i.e. germination, vegetative growth and reproductive growth⁵.

Soil salinity is also a major problem in India which covered very large areas mainly in Uttar Pradesh (1.3 Mha), Gujrat (1.21Mha), Haryana (0.99Mha) and Maharashtra (0.6Mha). Salinity effect on plant growth is a complex phenomena which involves osmotic stress, ion toxicity and mineral deficiencies⁶⁻⁷. It has been reported that the reduced germination in salt affected soil is a consequence of either the direct toxic effects of salts or caused by osmotic stress which delay the germination process¹. The response of plant growth, ion concentrations of roots, shoots, leaf blades, and sheaths of two sorghum varieties has

been studied in relation to NaCl salinity⁸. They found that the increasing NaCl concentration significantly reduced the relative shoot growth rate and shoot dry weight. Some findings also suggested that the salinity reduced the dry matter yield and length of the shoot and root of sorghum genotypes⁹.

The objective of the present work was to evaluate the effect of salt stress on sodium and potassium ions uptake in four sorghum cultivars at seedling stage.

Materials and Methods

This research work was carried out to determine the uptake of sodium and potassium ions in four sorghum cultivars collected from sorghum breeder of G B Pant Agriculture and Technology University, Pantnagar, Utrakhnad, India under varying salt stress. An experiment was performed in Plant Physiology Laboratory of Department of Botany, Hindu College, Moradabad. The experiment followed a factorial arrangement with three replications with randomized block design. For this purpose, Petri plates of 3" diameter sized were washed thoroughly by sodium hypochlorite 2% and were sterilized in hot air oven at 70°C for 36 hours. These Petri plates were lined with sterilized filter paper and each Petri plate was moistened with 10 ml of saline water (3, 6, 7.2, 10 and 12 dS m⁻¹). By mixing salts of NaCl, Na₂SO₄, NaHCO₃ and CaCl₂, as described in the U. S. Saline Laboratory Staff, saline solutions of 3, 6, 7.2, 10 and 12 electrical conductivity (EC) were prepared¹⁰. The

seeds of sorghum cultivars were surface sterilized by 1% of HgCl₂ solution for 60 second and then repeatedly washed and air dried at 25⁰C. Successively, 20 seeds of each cultivar were placed on the moistened filter paper in the Petri plates and were kept at 30⁰C in BOD incubator. For the estimation of Na⁺ and K⁺ ions, samples were collected at 10 days after sowing from each salinity treatment including control in triplicate. The collected samples were washed in tap water, kept on filter paper and separated into shoot and root and dried separately at 60⁰C for 24 hours in hot air oven. Shoot samples were grind into fine powder. The extraction of sodium and potassium was conducted by following the procedure of Trolson¹¹. One gm of finely ground shoot dry matter was digested in beaker with 5 ml of concentrated HNO₃ and 1 ml of concentrated H₂SO₄. They were mixed well and kept overnight at room temperature followed by digestion on hot plate (70-80⁰C) until the volume of sample is reduced to 1ml. To this, 3 ml of double acid mixture of HNO₃ and HClO₄ in 3:1 ratio was added and digested till white fumes came out from the sample. Now samples were diluted with 2 ml of triple distilled water and filtered through Whatman filter paper no. 1. Repeated washings of digestion beaker and filter paper were done by taking 5 ml of distilled water and finally it was made to 50 ml. Sample analysis was done by computer attached with Inductively Coupled Plasma Spectrometer (ICP-OES) and the concentration of mineral sample was expressed in ppm (parts per million).

Statistical Analysis: Statistical analysis for analysis of variance (ANOVA) was performed using GLM procedure of statistical computer software package Minitab-14 (Minitab Inc., State College, PA). Means were separated using Least Significant Difference (LSD) test at 0.05 probability level. At P = 0.05 probability level, critical difference between means was constructed using the method described by Bruning and Kintz¹².

Results and Discussion

The results obtained on the uptake of Na⁺ ions under salt stress in four selected cultivars at seedling stage have been presented in Table-1 and Figure-1A. The concentration of Na⁺ increased significantly (Table-2a) from 3 to 12 EC salt concentrations in all the genotypes. Maximum concentration was noted in cultivar Pant Chari-1 and Pant Chari-2 while CSV-15 and HC-171 recorded minimum at different salt concentrations. However, at 10 dS m⁻¹ low Na⁺ ions accumulation was recorded in CSV-15 and HC-171 as compared to Pant Chari-1 and Pant Chari-2. Cultivars had different responses to Na⁺ uptake and highest value (1.29 to 1.63 ppm) was observed at 12 dS m⁻¹. Under non-saline conditions Na⁺ content differed from cultivars to cultivars.

Salinity stress negatively affected K⁺ content *i.e.* concentration of potassium ions increasing salinity levels (Table-2b). Accumulation of K⁺ ions varied from 5.1 to 6.46 ppm in different genotypes in control sets. Exposure to 3 and 6 EC salt stress had very little effect on K⁺ uptake which recorded 8 to

19% reductions. At 7.2 EC, more than 24% reductions were observed in four genotypes. At 10 EC, the reduction in K⁺ was 35% in CSV-15, 32% in HC-171 and 39% in Pant Chari-1 and Pant Chari-2 as compared to their respective controls. At 12 EC salinity level, CSV-15 and HC-171 registered 43 and 45% reductions as compared to 50 and 56% reductions in Pant chari-1 and Pant chari-2 respectively. Potassium ion concentration in all varieties showed similar pattern of reductions. The reduction was higher in sensitive varieties than tolerant ones though the varietal difference existed.

Table-1
Sodium and potassium ion uptake in four cultivars of jowar (*Sorghum bicolor* (L.) Moench) at seedling stage under varying salt stress

Cultivars	EC of saline water (dS m ⁻¹)	10 Days after germination		
		Na ⁺	K ⁺	Na ⁺ /K ⁺
Pant Chari-1	Control	0.27	5.6	0.04
	3	0.42	4.8	0.08
	6	0.84	4.5	0.18
	7.2	1.22	3.9	0.31
	10	1.32	3.4	0.38
	12	1.58	2.8	0.56
Pant Chari-2	Control	0.29	5.1	0.05
	3	0.72	4.6	0.16
	6	1.02	4.3	0.23
	7.2	1.16	3.8	0.30
	10	1.41	3.1	0.45
	12	1.62	2.2	0.73
CSV-15	Control	0.19	6.46	0.02
	3	0.25	5.88	0.04
	6	0.67	5.56	0.12
	7.2	1.08	4.92	0.21
	10	1.16	4.19	0.27
	12	1.29	3.62	0.35
HC-171	Control	0.23	6.1	0.03
	3	0.46	5.4	0.08
	6	0.79	5.2	0.15
	7.2	1.13	4.6	0.24
	10	1.22	4.1	0.29
	12	1.35	3.3	0.40
CD at 5%	Pant Chari-1	Pant Chari-2	CSV-15	HC-171
For Na ⁺	0.032	0.04	0.038	0.038
For K ⁺	0.384	0.377	0.036	0.348
For Na ⁺ /K ⁺	0.038	0.035	0.042	0.078

Non-significant for salinity

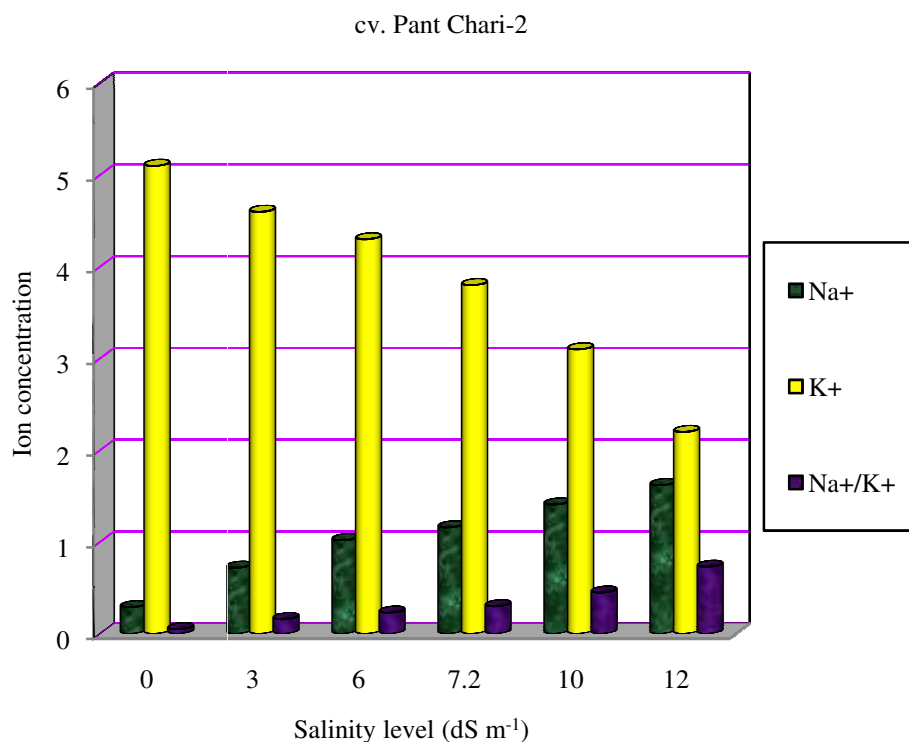
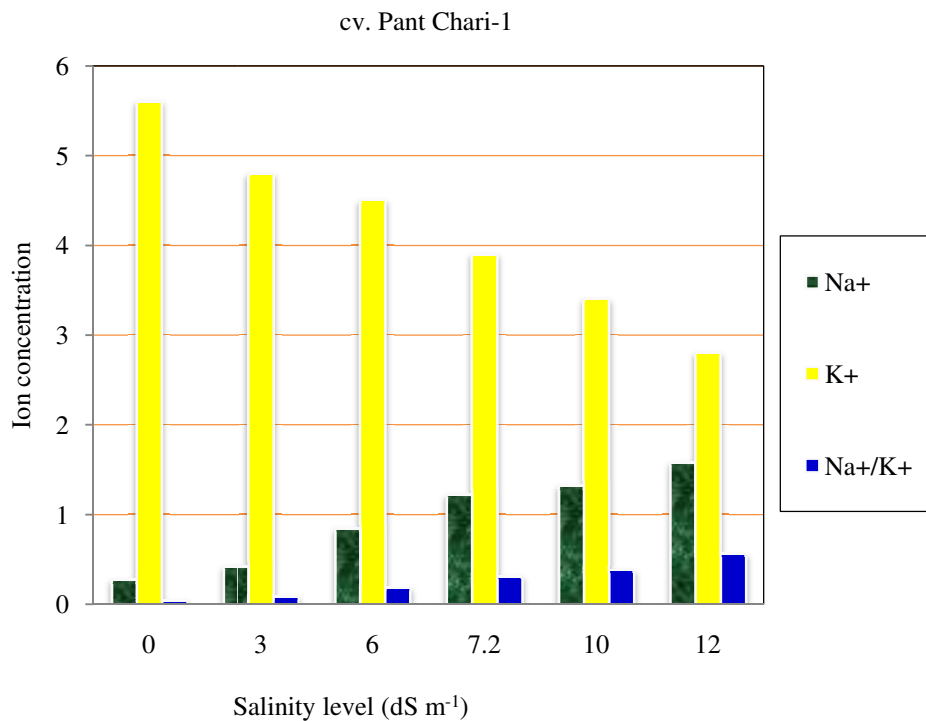


Figure-1A
 Effect of saline water irrigation on Na⁺, K⁺ and Na⁺/K⁺ concentration (ppm) in two cultivars of sorghum at seedling stage (10 DAG)

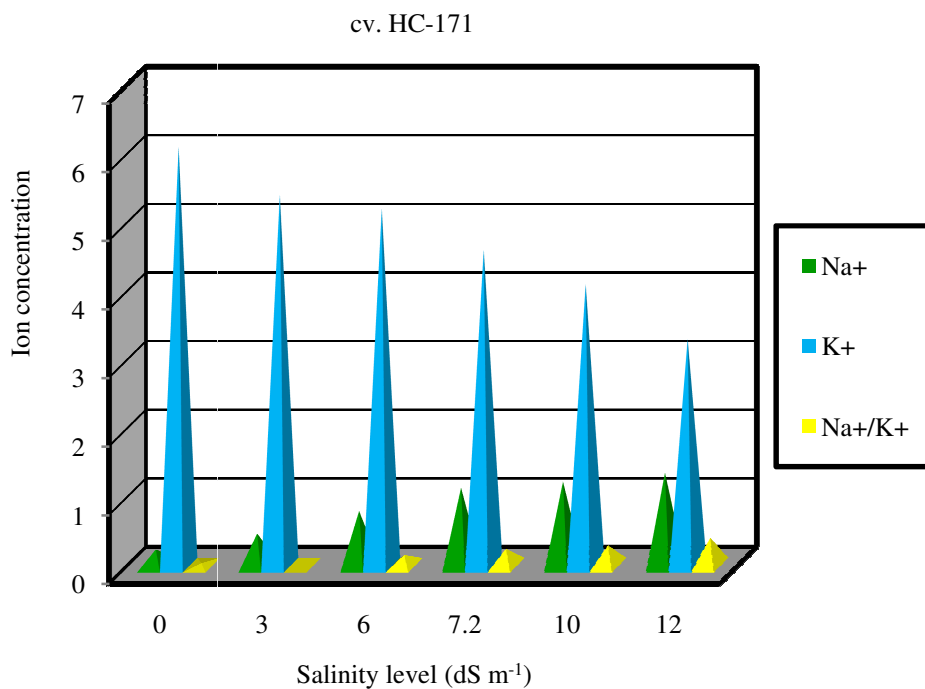
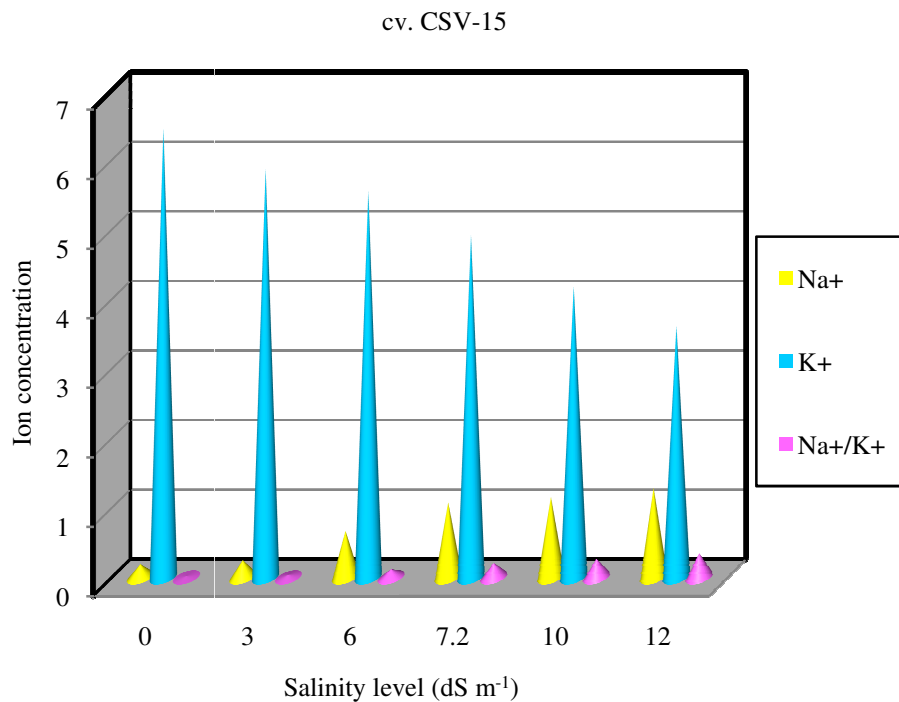


Figure-1B
 Effect of saline water irrigation on Na⁺, K⁺ and Na⁺/K⁺ concentration (ppm) in two cultivars of sorghum at seedling stage (10 DAG)

Ratio of sodium and potassium ions (Na^+/K^+) was also affected by varying salt concentrations in different sorghum genotypes at 10 days old seedlings. Highly significant genotypic and salinity level interactions were observed (Table-2c). With increasing salinity stress from 3 to 12 EC, the ratio Na^+/K^+ was increased in all genotypes. Under non-saline conditions Na^+/K^+ was very low but it increased when seedlings were exposed to higher salinity levels (Figure-1 A and B).

Na^+/K^+ in the sensitive genotypes (Pant Chari-1 and Pant Chari-2) become higher than in the tolerant (CSV-15 and HC-171). The ionic analysis performed for sodium concentration at seedling stage showed that CSV-15 accumulated least amount of Na^+ suggesting that it is most tolerant to salinity. Conversely, Pant Chari-2 had the highest Na^+ uptake and was thus considered most susceptible cultivar. On the basis of sodium and potassium ion uptake at varying salt concentration order of salinity tolerance in four sorghum genotypes was CSV-15 > HC-171 > Pant Chari-1 > Pant Chari-2.

Table-2a
Analysis of Variance for macro nutrient (Na^+) at seedling stage

Pant Char-1						Pant Chari-2					
Source	SS	df	MS	F	P	Source	SS	df	MS	F	P
Total	3.4116	17	0.200682			Total	3.4116	17	0.200682		
Between	3.406	5	0.6812	1459.714		Between	3.406	5	0.6812	1459.714	
Within	0.0056	12	0.000467			Within	0.0056	12	0.000467		
CSV-15						HC-171					
Source	SS	df	MS	F	P	Source	SS	df	MS	F	P
Total	3.4116	17	0.200682			Total	3.4116	17	0.200682		
Between	3.406	5	0.6812	1459.714		Between	3.406	5	0.6812	1459.714	
Within	0.0056	12	0.000467			Within	0.0056	12	0.000467		

Table-2b
Analysis of Variance for macro nutrient (K^+) at seedling stage

Pant Char-1						Pant Chari-2					
Source	SS	df	MS	F	P	Source	SS	df	MS	F	P
Total	3.4116	17	0.200682			Total	3.4116	17	0.200682		
Between	3.406	5	0.6812	1459.714		Between	3.406	5	0.6812	1459.714	
Within	0.0056	12	0.000467			Within	0.0056	12	0.000467		
CSV-15						HC-171					
Source	SS	df	MS	F	P	Source	SS	df	MS	F	P
Total	3.4116	17	0.200682			Total	3.4116	17	0.200682		
Between	3.406	5	0.6812	1459.714		Between	3.406	5	0.6812	1459.714	
Within	0.0056	12	0.000467			Within	0.0056	12	0.000467		

Table-2c
Analysis of Variance for macro nutrient (Na⁺/K⁺) at seedling stage

Pant Char-1						Pant Chari-2					
Source	SS	df	MS	F	P	Source	SS	df	MS	F	P
Total	3.4116	17	0.200682			Total	3.4116	17	0.200682		
Between	3.406	5	0.6812	1459.714		Between	3.406	5	0.6812	1459.714	
Within	0.0056	12	0.000467			Within	0.0056	12	0.000467		
CSV-15						HC-171					
Source	SS	df	MS	F	P	Source	SS	df	MS	F	P
Total	3.4116	17	0.200682			Total	3.4116	17	0.200682		
Between	3.406	5	0.6812	1459.714		Between	3.406	5	0.6812	1459.714	
Within	0.0056	12	0.000467			Within	0.0056	12	0.000467		

Discussion: Ionic concentrations in plants is generally affected by salinity and the uptake of Na⁺ and K⁺ can play an important role in salt tolerance of *Sorghum bicolor*. The mean differences were found statistically significant and cultivars differed in uptake and translocation of Na⁺ and K⁺. Macro nutrient uptake in some cultivars of sorghum was found proportional to the salt stress¹³. However at 12 dS m⁻¹, all genotypes accumulated higher (1.29 to 1.62 ppm) Na⁺ ion in shoot. At high salinity levels (7.2, 10 and 12 EC), the cultivars tried to accumulate ions and prevented translocation of ions at lower salt concentrations (3 and 6 EC). The high level of salinity (7.2, 10 and 12) reduced K⁺ content in tolerant varieties. Present results indicate that K⁺ and Na⁺ concentrations and their distribution in the shoot were important to determine the salt tolerance of selected sorghum cultivars however these results may not be applicable to all sorghum genotypes. Low Na⁺, high K⁺ uptake and low Na⁺/K⁺ showed positive relationship with salt tolerance¹⁴⁻¹⁶.

The tolerance to high salt concentration in sorghum seems to be related to the genotype ability to avoid accumulation of harmful levels of Na⁺ or to maintain adequate levels of K⁺ especially in the shoot. Under varying salt stress the physiological response of common bean seedlings was studied and found that the cultivar HRS 516 was most tolerant to salinity whereas RO 21 was the most susceptible¹⁷. A distinctive phenomenon of salt tolerance mechanism is the balance between Na⁺ and K⁺ uptake in plant tissues. It happens because of the selective distribution of Na⁺/K⁺ with partial exclusion of Na⁺ from growing tissues and transport of K⁺ from meristematic cells and mesophyll cells of leaf¹⁸.

The ratio Na⁺/K⁺, which was very small in control set, increased a lot after the seedlings were exposed to higher levels of salinity in four genotypes, particularly in the sensitive one. Similar

results were also observed in olive plant that Na⁺/K⁺ was always higher in salt sensitive Leccino than in salt tolerant Frontoio leaves, indicating a reduced selectivity of Leccino for K⁺ ions¹⁹.

The results suggest that the translocation rate of the ions (Na⁺ and K⁺) and the Na⁺/K⁺ might be evaluated as reliable criteria giving clues to salt tolerant (CSV-15 and HC-171) sorghum cultivars. In this respect, CSV-15 may be defined as more tolerant to salinity than Pant Chari-1 and Pant Chari-2. So these results suggest that tolerance to salt stress in four sorghum genotypes studied here may be related to plants ability to prevent accumulation of toxic ions like Na⁺ and K⁺.

Conclusion

On the basis of the results obtained from this study, it is concluded that the salt stress caused a significant decreased in the plant growth as well as macro nutrients concentration. Four sorghum (*Sorghum bicolor* (L.) Moench) cultivars viz. Pant Chari-1, Pant Chari-2, CSV-15 and HC-171 were studied in laboratory for sodium and potassium uptake under varying salt stresses. It was found that concentration of sodium ion increased while concentration of potassium ions decreased invariably with salt stress in all cultivars. It seems that an increase in Na⁺/K⁺ ratio was due to the inhibitory effect of high Na⁺ on K⁺ ion concentration. Since cultivars CSV-15 and HC-171 have the mechanism to tolerate salinity at germination and seedling growth stages, they are highly recommended for planting in saline soils.

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References

1. Igartua E., Gracia M.P. and Lasa J.M. (1994). Characterization and genetic control of germination immergence responses of grain sorghum to salinity. *Euphytica*, 76, 185-193.
2. Eisa S.S. and Ali S.H. (2003). Biochemical, physiological and morphological responses of sugarbeet to stalinization. Departments of Agricultural Botany and Biochemistry. Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
3. Francois L.E., Donovan T.J. and Maas E.V. (1984). Salinity effects on seed yield, growth and germination of grain sorghum. *Agron. J.*, 76, 741 – 744.
4. Gossett D.R., Millhollon E.P. and Lucas M.C. (1994). Antioxidant response to NaCl stress in salt-tolerance and salt-sensitive cultivars of cotton. *Crop Sci.*, 34, 706 -714.
5. Koca M., Bor M., Ozdemir F. and Turkan I. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental Experimental Botany*, 60, 344-351.
6. Hasegawa P.M., Bressan R.A., Zhu J.K. and Bohnert H.J. (2000). Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol.*, 51, 463-485.
7. Roy P., Niyogi K., Sen Gupta D.N. and Ghosh B. (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H⁺-ATpase in salt tolerant and salt sensitive rice cultivars. *Plant Sci.*, 168, 583-591.
8. Netondo G.W., Onyango J.C. and Beck E. (2004). Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci.*, 44, 806-811.
9. Lacerda De C.F., Cambraia J., Oliva cano M.A. and Ruiz H.A. (2001). Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. *R. Bras. Fisiol. Veg.*, 13(3), 270-284.
10. Richards L.A. (1954). Diagnosis and Improvement of Saline and Alkali Soils. U. S. Salinity Laboratory Staff, U. S. Dept. of Agriculture, Washington, D. C., vii -160.
11. Trolson J.E. (1969). Outline for in vitro digestion of forage samples. Research Station Shift Current, Saskatchewan, Canada.
12. Bruning J.L. and Kintz B.L. (1968). Computational Handbook of Statistics. Scott Foresman and Company, Oakland.
13. Chartzoulakis K., Loupassaki M., Bertaki M. and Androulakis I. (2002). Effects of NaCl salinity on growth, ion content and CO₂ assimilation rate of six olive cultivars. *Scientia Horticulturae*, 96, 235-247.
14. Ashraf M., Aasiya K. and Khanum A. (1997). Relationship between ion accumulation and growth in two spring wheat lines differing in salt tolerance at different growth stages. *Journal of Agronomy and Crop Science*, 178, 39-51.
15. Sherif M.A., El-Beshbeshy T.R. and Richter C. (1998). Response of some Egyptian varieties of wheat (*Triticum aestivum* L.) to salt stress through potassium application. *Bulletin of Faculty of Agriculture*, University of Cairo, 49, 129-151.
16. Gorham L. (1990). Salt tolerance in the triticale: Ion discrimination in rye and triticale. *J. Exp. Botany.*, 4, 601-614.
17. Gama P.B.S., Inanaga S., Tanaka K. and Nakazawa R. (2007). Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *African J. Biotechnology*. 6 (2), 79-88.
18. Ashraf M. (2001). Relationships between growth and gas exchange characteristics in some salt-tolerant amphidiploid Brassica species in relation to their diploid parents. *Environ. Exp. Bot.*, 45, 155-163.
19. Tattini M., Bertoni P. and Caselli S. (1992). Genotypic responses of olive plants to sodium chloride. *J. Plant Nutr.*, 15, 1467-1485.