Journal of Phytology 2009, 1(3): 177–184
© *Journal of Phytology, 2009*
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

EFFECT OF ALKALINITY ON GROWTH PERFORMANCE OF *JATROPHA CURCAS* INOCULATED WITH PGPR AND AM FUNGI

Ashwani Kumar[a], Satyawati Sharma[*a], Saroj Mishra[b]

[a]*Centre for Rural Development and Technology, Indian Institute of Technology Delhi, Hauz Khas New Delhi-110016, India*
[b]*Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas New Delhi-110016. India*

SUMMARY

A pot experiment was conducted to assess the effect of soil alkalinity on emergence, growth, leaf relative water content, total soluble sugar and soluble protein of seedlings of *Jatropha curcas* L. Na_2CO_3 was added to the soil and alkalinity was maintained at 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%. In general increased alkalinity caused reduction in growth. Hence we designed the experiment to test the efficacy of beneficial microbes (*Azotobacter, Microfoss* and arbuscular mycorrhizal fungi) individually and in combinations to alleviate the stressful effect of alkaline soil. The data pertaining to the effect of bioinoculants on different parameters of *Jatropha curcas* under alkaline stress were collected and statistically analyzed. The effect of bioinoculants on percentage seed germination and survival at 0.4% of Na_2CO_3 was found to be in order of; *Azotobacter*+AMF> AMF>*Azotobacter*+ *Microfoss*>*Microfoss* > *Azotobacter* >control (no germination) while at 0.5 % Na_2CO_3 germination was almost nil with all treatments. The survival percentages with respect to all treatments were found to be significant at 0.4%, Na_2CO_3 level over control. The combination of AM fungi and *Azotobacter* increased plant height, shoot diameter, shoot dry weight, leaf relative water content and soluble sugar content and decreased level of soluble protein at 0.4 % of Na_2CO_3 over other treatments. We conclude that the combinations of *Azotobacter* and AMF performed well up to 0.4 % of Na_2CO_3 in soil.

Keywords: alkalinity stress, bioinoculants, *Jatropha curcas*, leaf relative water content, wastelands.

Ashwani Kumar et. al. Effect of alkalinity on growth performance of *Jatropha curcas* inoculated with PGPR and AM fungi. J Phytol 1 (2009) 177-184
***Corresponding Author**

1. Introduction

Salt stress is a widespread environmental problem limiting crop productivity worldwide [1]. Although salinity and sodicity are common phenomena for arid and semiarid regions of the world, salt-affected soils have been recorded in practically all the climatic regions, and in a wide

range of altitudes. The world's land surface occupies about 13.2 × 109 ha, no more than 7 × 109 ha are potentially arable, and only 1.5 × 109 ha are currently cultivated. Of the cultivated area, about 0.34 × 109 ha (23%) are saline and another 0.56 × 109 ha (37%) are sodic [2]. Actually, the problem of soil alkalinization due to $NaHCO_3$ and Na_2CO_3, may be more severe than the problem of soil salinization caused by the neutral salts, such as NaCl and Na_2SO_4 [3-5]. These soils present a highly inhospitable environment to the plants. However, there have been some studies about alkaline soil [6], alkaline salt stress [7], and mixed salt stress on crops [8]. Now a day's interest of growing multipurpose crops for various biomass products is gaining importance to meet the mounting demand of expanding population.

Jatropha is a multipurpose species with many attributes and considerable potential. Nearly 40% of the land area in India is wasteland. Importance is given on the plantation of *Jatropha* species on wastelands, for the protection of the environment and fulfilling future energy requirements. Although this plant can grow on wastelands but its growth is limited. Inoculation of beneficial microbes to these lands may improve plant growth by enhancing plant resistance to adverse environmental stresses, e.g. water and nutrient deficiency and heavy metal contamination [9].

In recent years the use of biological tools like AM fungi and PGPR has received increased attention as a practical way to alleviate soil stresses on plant growth. Relevance of using bioinoculants (N-fixers and P-solubilizers) individually as well as in consortia, lies in their ability to enhance biomass yield by increasing stress tolerance, nutrient recycling, uptake of nutrients, and synthesis of growth hormones, vitamins, antibiotics, and by improving soil conditions. It has been observed that mycorrhizal fungi also improve plant survival and growth under stress conditions [10]-[11]. Some successful examples of inoculation with plant growth-promoting rhizobacteria (PGPR) have been achieved both in laboratory and field trials [12]-[13].

The objective of this study was to improve *Jatropha* biomass on soil affected by alkalinity.

2. Material and Methods

The bioinoculants used in study were procured from Microbiology Division, Indian Agriculture Research Institute, New Delhi (*Azotobacter, Microfoss* and AM fungi) and seeds of *Jatropha* were procured from Regional Research Center, Bawal (C.C.S. Hissar Agriculture University), India.

The experiment was conducted at Micromodel (an experimental site), IIT Delhi, India (28.38 N, 77.12 E) during the month of March-May of 2006 with sterilized soil and gradually increasing alkalinity (0.1%, 0.2%, 0.3%, 0.4%, and 0.5% Na_2CO_3, created by adding 1 to 5 gm/kg of Na_2CO_3 in soil). Six treatment groups were set under each concentration and labeled as T1 (control), T2 (*Azotobacter*), T3 (*Microfoss*), T4 (*Azotobacter* + *Microfoss*), T5 (AM fungi) and T6 (*Azotobacter* + AM fungi). Seed treatment was applied for *Azotobacter* and *Microfoss*, and in case of AM fungi, soil based root inocula with ~100 spores/50g of soil was used. These bioinoculants were used separately as well as in combination.

Two seeds/pot (ten pots) of *Jatropha* were sown at 3 cm depth in pots filled with soil and vermicompost in the ratio of 3:1 per treatments. Once seedlings became established, pots were thinned to one plant per pot.

The soil used in the experiment was analyzed 45 days after planting and have following properties: Loamy soil, EC 0.18 dS m^{-1}, pH 7.26, organic carbon (C, %) 1.73, total

nitrogen (N, %) 0.51, 11.3mg/kg available phosphorus (P) and 54.3 mg/kg potassium (K). Soil electrical conductivity (EC) and pH were determined with a digital pH and EC meter (Scientific Systems, New Delhi, India). Total organic C and N were determined by combustion using a CHN analyzer Vario Max CN (Elementar, Hanau, Germany), available P was assessed using Olsen's method [14] and K was determined using ammonium acetate [15]. Soil pH values reached 10.79, 10.93, 11 and 11.14 respectively at 0.1%, 0.2%, 0.3%, and 0.4% Na_2CO_3 treatments.

The observation on physical parameters i.e., germination (%), survival (%), shoot length, stem girth (above 5 cm soil level) and shoot dry weight were recorded from three replicate from each treatment after 45 days of sowing the seeds.

Fresh weight (FW) and dry weight (DW) of shoots of each plant were determined after counting the leaf number. Leaf relative water content (RWC) was measured in the second or third youngest fully expanded leaf from top of the plant at the end of experiment to assess the relative tolerance of mycorrhizal and non-mycorrhizal plants using the following equations [16]:

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

where, FW is leaf fresh weight, DW is leaf dry weight after 24 h drying at 70°C, and TW is leaf turgid weight after submergence in distilled H_2O for 4 h. Plant dry weight were determined after oven drying at 70°C until they reached constant weight.

The total buffer-soluble proteins in the stressed plants were estimated by following the standard method [17]. Absorbance was recorded photometrically at 595 nm using bovine serum albumin as standard. The amount of total soluble sugar was estimated using Anthrone method [18]. Absorbance was recorded photometrically at 630 nm using glucose as standard.

The data collected in triplicate were analyzed by analysis of variance (ANOVA) using SPSS for Windows (version 10.0). The significance of difference was determined according to Duncan's multiple range test (DMRT). P values < 0.05 are considered to be significant. Vertical bars in figures indicate value of mean ± SE.

3. Results and Discussion

Soils pH increased from 7.5 to 11 with increase in Na_2CO_3 concentration (from 0 to 0.5%) whereas the E.C. of soil remained unaffected (0.117). Results revealed Na_2CO_3 posed adverse effects on percentage germination, survival, shoot length, stem girth and shoot dry weight. The percentage seed germination got decreased significantly at increased level of Na_2CO_3 (from 0.1% to 0.4%) with all treatments, however soil amended with bioinoculants increased percentage germination as compared to control. At high concentration (0.4%, Na_2CO_3), the effect of different treatments was found to be in order of; *Azotobacter* + AMF > AMF > *Azotobacter* + *Microfoss* > *Microfoss* > *Azotobacter* and no germination with control (Table 1). No germination could be observed in any treatments at 0.5 % of Na_2CO_3 concentration. The decreased germination at higher alkalinity level might have been due to reduced imbibitions of water by seeds needed for germination.

The plant survival with different pH was recorded with the interval of 25 days till complete mortality was observed at all the salt levels in all treatments. However, same trends were seen in percentage survival of seedlings with respects to all treatments at 0.4% alkalinity as observed in germination. Interestingly, the combination of consortia (*Azotobacter* and AM fungi) of bioinoculants enhanced germination

and seedling establishment significantly ($P<0.05$) upto certain extent at high salt concentration (0.4%, Na_2CO_3). Similarly, increased alkalinity affected shoot length and shoot diameter, however it was observed that treatments T6 and T5 improved shoot length over T2 at 0.4%, Na_2CO_3 concentration and same trend were also seen with shoot diameter (Table 1).

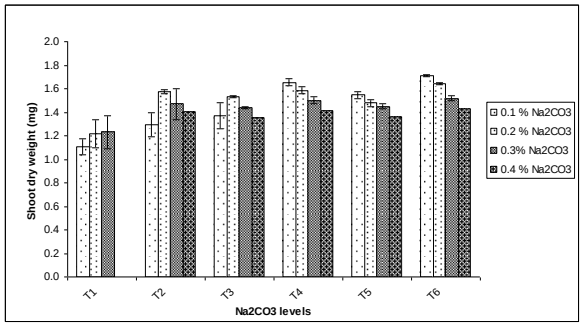
Table 1. Effect of various treatments and different levels of Na_2CO_3 on germination (%), survival (%), shoot length and shoot diameter.

Trt	Salt (%)	Germination (%)	Survival (%)	Shoot length (cm)	Shoot diameter (cm)
T1 (Control)	0.1	73.67 bc	90.33 abc	20.96 cde	0.66 bcd
	0.2	58.67 e	80.57 de	15.88 fg	0.62 d
	0.3	38.67 ghi	53.67 j	11.22 h	0.42 gh
	0.4	15.33 k	Nil	Nil	Nil
T2 (*Azotobacter*)	0.1	75.33 b	92 abc	23.08 cd	0.66 bcd
	0.2	65.33 d	82 de	17.83 ef	0.63 cd
	0.3	40.33 gh	67 gh	15.88 fg	0.49 f
	0.4	32.00 j	60.33 hi	10.80 h	0.38 hi
T3 (*Microfoss*)	0.1	77.00 b	92 abc	26.89 ab	0.67 bc
	0.2	68.67 cd	83.67 cde	19.94 de	0.63 cd
	0.3	43.67 g	70.33 fg	18.00 ef	0.53 ef
	0.4	34.67 ij	62 h	11.65 h	0.41 gh
T4 (T2+T3)	0.1	78.67 ab	93.67 ab	28.49 a	0.68 b
	0.2	73.67 bc	87 bcd	22.74 cd	0.63 cd
	0.3	50.33 f	72 fg	18.67 ef	0.53 ef
	0.4	35.33 ij	65.33 gh	12.24 h	0.37 i
T5 (AM)	0.1	80.33 ab	92 abc	26.46 ab	0.72 a
	0.2	75.33 b	87 bcd	19.01 ef	0.65 bcd
	0.3	50.33 f	72 fg	16.30 fg	0.53 ef
	0.4	37.00 ij	67 gh	11.89 h	0.42 gh
T6 (Azo+AM)	0.1	83.67 a	97 a	28.96 a	0.75 a
	0.2	77.00 b	92 abc	23.75 bc	0.67 bc
	0.3	53.67 ef	77 ef	20.28 cde	0.55 e
	0.4	38.67 ghi	72 fg	13.25 gh	0.45 g

Different letters in each column indicate significant differences for means from three replicates at $p<0.05$ according to DMRT.

The control plants (without treatment) showed very poor growth this may be attributed to nutrient deficiency i.e., the lack of available P and N in the unfertilized soil. In addition, sterilization of the soil might have killed the native microflora which assists nutrient uptake and plant growth. At higher Na_2CO_3 concentration shoot dry and fresh weight

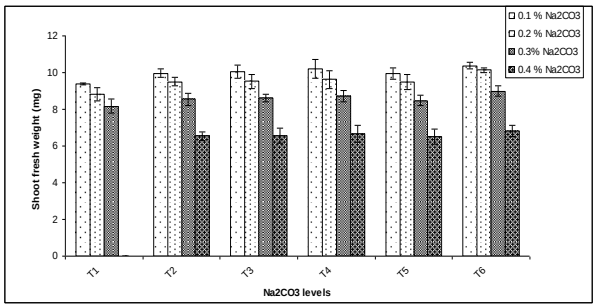
decreased (0.4%, Fig. 1 & 2) and treatment T6 performed best at Na_2CO_3 concentration from 0.1% to 0.4%.



Vertical bars indicate Mean ± S.E.

Fig. 1 Effect of various treatments on shoot dry weight of *Jatropha* at different levels of Na2CO3.

Reduction in plant growth as a result of salt stress has also been reported in several other plant species [19-20]. It could have been due to a number of reasons i.e., reduction of the photosynthesizing leaf area and a remarkable decrease of plant dry matter accumulation [21-22], high pH and ion imbalance around rhizosphere caused by alkaline salt stress [8]. The results shown in fig. 3 indicated reduction of leaf relative water content (LRWC) with increased alkalinity levels, but inoculation of bioinoculants particularly AM fungi individually as well in combination maintained high leaf relative water content status over other treatments.

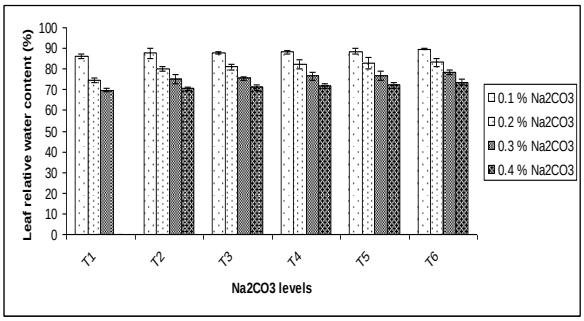


Vertical bars indicate Mean ± S.E.

Fig. 2. Effect of various treatments on fresh weight of shoot of *Jatropha* at different levels of Na_2CO_3

The RWC affects many metabolic activities due to reduced hydration under osmotic stress conditions [20]. It is generally considered that salt stress inhibits plant growth by water deficiency and ion toxicity besides other factors [23-25].

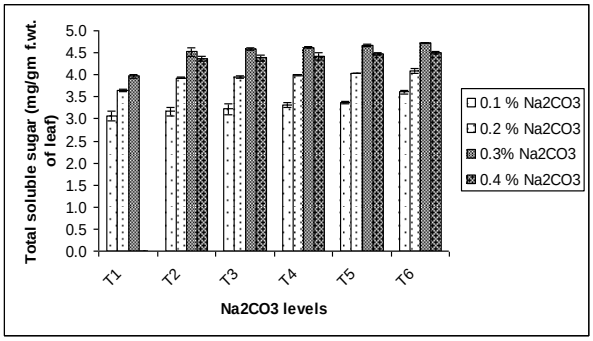
Soluble sugar content helps the plant to maintain osmotic adjustment to overcome the effect of salt stress and it was increased in leaf with increased alkalinity from 0.1 to 0.4%. Some researchers agree that salt stress and water deficit induce accumulation of carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch [26] for maintaining the osmotic balance [27].



Vertical bars indicate Mean ± S.E.

Fig. 3. Effect of various treatments on Leaf relative water content (%) of *Jatropha* at different levels of Na_2CO_3.

In our experiment, dual inoculation with AMF and *Azotobacter* seemed to be the most effective in improving total soluble sugar content in *Jatropha* leaf (Fig. 4). Results pertaining to total soluble protein content in *Jatropha* leaf showed linear increased in soluble protein with increased Na_2CO_3 concentration (from 0.1 % to 0.4%) as a mechanism to maintained the osmotic balance and leaf water status at high alkalinity (Fig. 5).



Vertical bars indicate Mean ± S.E.

Fig. 4 Effect of various treatments on total soluble sugar of *Jatropha* at different levels of Na_2CO_3

But inoculation of bioinoculants reduces the soluble protein content in *Jatropha* leaf by reducing the impact of alkalinity by maintaining the high water status over control. A higher content of soluble proteins has also been observed in salt tolerant than in salt sensitive cultivars of barley [28], sunflower [29], finger millet [30], and rice [31].

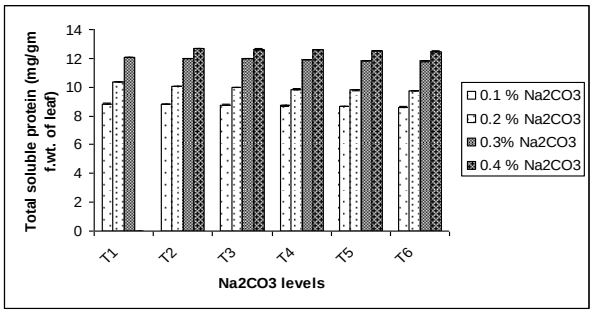


Fig. 5. Effect of various treatments on total soluble protein of Jatropha at different levels of Na2CO3

Conclusions

We conclude that the inoculation of biofertilizers individually as well as in combination enhanced seed germination, survival, height and biomass yield. However the response varied when these bioinoculants and AM fungi were used individually and in combination. Although the increased levels of alkalinity affected growth parameters and biomass yield, the bioinoculants protected plants from the alkalinity strokes up to certain levels i.e. 0.4% Na2CO3.

Acknowledgements

Authors wish to thank Indian Institute of Technology and Council of Scientific and Industrial Research, New Delhi, India for providing financial support.

References

1. Jain, P.K., K. Paliwal, R.K. Dixon and D.H. Gjerstad, 1989. Improving productivity of multipurpose tree on substandard soil in India. J. For., 87:38–42.
2. Tanji, K.K., 1990. Nature and extent of agricultural salinity. In: Tanji, K.K. (Ed.), Agricultural Salinity Assessment and Management. American Society of Civil Engineers, New York, pp. 1–18.
3. Shi, D.C. and L.J. Yin, 1993. Difference between salt (Na2CO3) and alkaline (Na2CO3) stresses on *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr. plants. Acta Botanica Sinica, 35: 144–149 (in Chinese with English abstract).
4. Tang, C. and N.C. Turner, 1999. The influence of alkalinity and water stress on the stomatal conductance, photosynthetic rate and growth of *Lupinus angustifolius* L. and *Lupinus pilosus* Murr. Australian Journal of Experimental Agriculture, 39: 457–464.
5. Shi, D. and Y. Sheng, 2005. Effect of various salt–alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. Environmental and Experimental Botany, 54: 8–21
6. Hartung, W., L. Leport, R.G. Ratcliffe, A. Sauter, R. Duda and N.C.Turner, 2002. Abscisic acid concentration, root pH and anatomy do not explain growth differences of chickpea (*Cicer arietinum* L.) and lupin (*Lupinus angustifolius* L.) on acid and alkaline soils. Plant Soil, 240: 191–199.
7. Campbell, S.A. and J.N. Nishio, 2000. Iron deficiency studies of sugar beet using an improved sodium bicarbonate-buffered hydroponics growth system. Journal of Plant Nutrition, 23: 741–757.
8. Shi, D.C., Y.M. Sheng and K.F. Zhao, 1998. Stress effects of mixed salts with various salinities on the seedlings of *Aneurolepidium*

chinense. Acta Botanica Sinica, 40:1136–1142 (in Chinese with English abstract).

9. Shen, D., 1997. Microbial diversity and application of microbial products for agricultural purposes in China. Agriculture Ecosystem and Environment, 62: 237–245.
10. Rabie, G. H. and Almadini, A. M., 2005. Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. African Journal of Biotechnology, 4: 210-222.
11. Ghazi, N. and Al-Karaki, G.N., 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scientia Horticulturae, 109:1–7.
12. Hall, J.A., D. Pierson, S. Ghosh and Glick, B.R., 1996. Root elongation in various agronomic crops by the plant growth promoting rhizobacteria Pseudomonas putida GR12-2. Israelean Journal of Plant Science, 44: 37– 42.
13. Glick, B.R., L. Changping, G. Sibdas and Dumbroff, E.B., 1997. Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Soil Biology and Biochemistry, 29:1233-1239.
14. Olsen, S.R., C.V. Cole, F.S. Watanabe and Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. (Circular of the US Department of Agriculture 939) USDA, Washington, D.C.
15. Hanway, J.J. and Heidel, H., 1952. Soil analysis methods as used in Iowa state college soil testing laboratory. Iowa Agric, 57: 1–31.
16. Schonfeld, M.A, R.C. Johnson, B.F. Carver and Mornhinweg, D.W., 1988. Water relations in winter wheat as drought resistance indicator. Crop Science, 28: 526-531.
17. Bradford, M.M., 1976. A rapid and sensitive method for the quantiation of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry, 72: 248–54.
18. Thimmaiah, S.R., 2004. Standard Methods of Biochemical Analysis. New Delhi India: Kalyani Publishers, 545 pp.
19. C.A. Jaleel, R. Gopi, B. Sankar, P. Manivannan, A. Kishore, R. Sridharan and R. Panneerselvam, 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South African Journal of Botany, 73: 190–195.
20. Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety, 60: 324–349.
21. AliDinar, H.M., G. Ebert and P. Ludders, 1999. Growth, chlorophyll content, photosynthesis and water relations in guava (*Psidium guajava* L.) under salinity and different nitrogen supply. Gartenbauwissenschaft, 64: 54–59.
22. Chartzoulakis, K. and G. Klapaki, 2000. Response of two green house pepper hybrids to NaCl salinity during different growth stages. Scientia Horticulturae, 86: 247–260.
23. Bertamini, M., L. Zulini, K. Muthuchelian and N. Nedunchezhian, 2006. Effect of water deficit on photosynthetic and other physiological responses in grapevine (*Vitis vinifera* L. cv Riesling) plants. Photosynthetica, 44: 151–154.
24. Ghoulam, C., A. Foursy and K. Fares, 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five beet cultivars. Environment and Experimental Botany, 47:39–50.

25. Lacerda, C.F., J.Cambraia, M.A. Oliva, H.A. Ruiz, and J.T. Prisco, 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environment and Experimental Botany, 49: 107-120.
26. Parida, A.K., A.B. Das and P. Das, 2002. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. Journal of Plant Biology, 45: 28–36.
27. Ingram, J. and D. Bartels, 1996. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Molecular Biology, 47: 377–403.
28. Hurkman, W.J., C.S. Fornari and C.K. Tanaka, 1989. A comparison of the effect of salt on polypeptide and translatable mRNA in roots of a salt tolerant and salt sensitive cultivar of barley. Plant Physiology, 90: 1444–1456.
29. Ashraf, M. and Tufail, M. 1995. Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). Journal of Agronomy and Soil Science, 174: 351–362.
30. Uma, S., T.G. Prasad and M.U. Kumar, 1995. Genetic variability in recovery growth and synthesis of stress proteins in response to polyethylene glycol and salt stress in finger millet. Annals of Botany, 76: 43–49.
31. Pareek, A., S.L. Singla and A. Grover, 1997. Salt responsive proteins/genes in crop plants. in: Jaiwal, P.K. (Eds.), Strategies for Improving Salt Tolerance in Higher Plants. Oxford and IBH Publication Co., New Delhi, pp. 365–391.