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ORIGINAL ARTICLE

Effect of $MgCl_2$ stress on germination, plant growth, chlorophyll content, proline content and lipid peroxidation in sorghum cultivars

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Present report deals with the effect of increasing levels (0-300 mM) of $MgCl_2$ salt on sorghum cultivars, Phule Vasudha and Phule Revati. Although $MgCl_2$ stress did not show considerable adverse effects on germination, however, at higher (>200 mM) concentrations, the seedlings turned brown and did not showed any further growth, with comparably higher magnitude of negative effects on Phule Revati than Phule Vasudha. Overall, increasing $MgCl_2$ stress reduced plant growth and biomass production significantly in both the cultivars, though with lesser extent in Phule Vsudha as compared to Phule Rvati. Contrasting behavior was evidenced in both the cultivars in terms of protein content under varying levels of $MgCl_2$ concentration at vegetative growth level, where salinity induced reduction in protein content was higher in Phule Revati than Phule Vasudha. The genotype Phule Vasudha showed higher proline content under non-saline condition. $MgCl_2$ stress-induced proline accumulation was observed in both the sorghum cultivars, however, interestingly, Phule Revati (439% of control plants) showed comparably higher proline content than Phule Vasudha (324% of control plants) at the highest (300 mM) level of stress. Even though, malondialdehyde (MDA: lipid peroxidation indicator) content was on higher side under non-saline conditions in cultivar Phule Vasudha as compared to cultivar Phule Revati, however the rate of increase in MDA with increasing salt stress was much higher in the latter cultivar, indicating the comparably higher level of lipid peroxidation under the influence of $MgCl_2$ stress. The salt tolerance nature of Phule Vasudha was positively correlated with its better performance in terms of physiological and biochemical parameters.

Key words: Sorghum, MgCl2 stress, proline, lipid peroxidation, protein content

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Soil salinity is one of the most severe factors limiting the agricultural crop productivity greatly with adverse effects on physiological and biochemical parameters both at whole-plant as well as cellular levels (Cuartero *et al*., 2006; Munns and Tester, 2008). Around 953 million ha land is under salinity covering about 8% of the total land area (Singh, 2009). Salt stress in soil or irrigation water is one of the major stresses especially in arid and semi-arid regions and limit plant growth and

productivity drastically (Allakhverdiev *et al.,* 2000; Koca *et al.,* 2007). Salinity stress has been reported to cause adverse effects on germination, growth and vigor, metabolism, flowering and fruiting processes and physiology, ultimately causing diminished economic yield and also quality of produce (Sairam and Tyagi, 2004).

Despite the fact that saline soils contain several types of soluble salts dominated by chlorides and sulfates of sodium, magnesium and calcium (mainly NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$) and to a lesser extent their carbonates with each salt having a different effect on plant growth (Tobe *et al*., 2004; Munns and Tester, 2008), NaCl has received major attention and much of the research has been historically focused on NaCl- induced salt stress in the plants and the mechanisms adapted by them to alleviate its deleterious effects (Munns and Tester, 2008; Niu and Rodriguez, 2008; Kumar *et al*., 2009). Salt stress related effects on plants are both quantitative as well as qualitative and depends upon variables such as plant species, intra-specific genotypes, specific salt type, its composition, concentration and duration of exposure.

Literature survey indicated that only a few reports deal with other soil salts as compared to NaCl. In spite of being an important component in saline soils and irrigation water the investigations involving the effects of $MgCl_2$ on various physiological and biochemical parameters, is very meager. Additionally, $MgCl_2$ is heavily applied as deicer in many countries especially to road surfaces and is of significant environmental concern, as small increase in the concentration of $MgCl_2$ in soil and water could have a significant impact on the plants of affected and nearby areas (Dougherty and Smith, 2006; Brandenburg and Kleier, 2011).

Although chloride (Cl^-) and magnesium (Mg^{2+}) are both essential nutrients required for normal plant growth, high concentration of $MgCl_2$ in soil may be toxic or may change water relationship and the accumulation of water and nutrients by the plants become difficult. However higher concentrations of $MgCl_2$ may be harmful to plant growth and viability, as the balanced magnesium ion concentration is particularly important in higher plant physiology (Shabala and Hariadi, 2005). High cytoplasmic Mg^{2+} concentrations may block a K^+ channel in the inner envelope membrane of the chloroplast, in-turn inhibiting the removal of H^+ ions from the chloroplast stroma. This leads to an acidification of the stroma that inactivates key enzymes in carbon fixation, which all leads to the production of oxygen free radicals in the chloroplast that can cause oxidative damage **(**Wu *et al*., 1991).

In view of the facts described above, present investigation was aimed to facilitate better understanding of sorghum genotypes' response under $MgCl_2$ stress and ultimately the underlying $MgCl_2$ salt stress tolerance mechanisms. Present report deals with the effects of magnesium chloride salinity on plant germination, seedling growth, biomass production and biochemical parameters including chlorophyll content, total protein content, proline content and lipid peroxidation levels.

MATERIALS AND METHODS

Plant Material

Certified seeds of both the cultivars viz. Phule Vasudha and Phule Revati of sorghum (*Sorghum bicolor*) used for this study were procured from Mahatma Phule Agricultural University, Rahuri (Ahmednagar), India.

Salinity Treatment and Growth Conditions

Two separate experiments were designed to

study the physiological (Petri dish experiment) and biochemical parameters (Pot experiment) respectively under the influence of varying concentrations (0-300 mM) of $MgCl_2$.

Petri dish Experiment

This experiment was carried out to study the effect of $MgCl_2$ on germination and early seedling growth, fresh weight and dry weight and relative water content Seeds of both the cultivars were surface sterilized with 0.1% mercuric chloride for 4 min and then washed several times with sterile distilled water. Ten seeds of each cultivar were sown in a Petri dish (10 cm diameter) containing germination paper and the experiments were carried out in triplicate. Every day 5 ml Yoshida's nutrient solution (with or without varying levels of salts, i.e. 50, 100, 200 and 300 mM of $MgCl_2$) was applied per Petri dish and all the observations were recorded on 14thday after sowing (DAS).

Pot Experiment

Twenty seeds of each cultivar were sown in the insulated plastic pots (15 cm × 15 cm×12 cm) to ensure the required level of salinity. The pots were filled with garden soil mixed with vermiculite and sand (1:1:1) after passing through a 10-mm mesh screen, in the Greenhouse, Department of Biotechnology, Modern College, Ganeshkhind, Pune, India. Plants were irrigated to meet evapo-transpiration demand, and an equal amount of water was applied in all the pots. Plants were irrigated with salt-free water for first 45 days before introducing the salt treatments. Four salinity treatments along with non-saline water (control) were then applied using distilled water containing 50, 100, 200 and 300 mM of $MgCl_2$ and were maintained for 7 days and all the observations were recorded on 8th day after treatment.

Plant Growth Analysis:

The growth of plants was analyzed by determining the root length, shoot length, root length/ shoot length ratio, fresh weight (FW) and dry weight (DW) of plant tissues. The DW was observed by heating the samples in a hot air oven at 60°C for 48 h. Relative water content (RWC) as percentage of fresh weight was calculated by following the method described by Sumithra *et al*. (2006) by using the formula:

$$RWC\ (\%) = [(FW - DW)/FW] \times 100$$

Determination of chlorophyll content

Chlorophyll was extracted using 80% acetone, the absorbance of chlorophyll extract was recorded at 663 and 645 nm and chlorophyll content was calculated according to Arnon (1949). Leaf tissue (0.1 g) was homogenized in 80% acetone using mortar and pestle. Homogenate was centrifuged at 10,000 x *g* for 10 min. Pellet was re-homogenized in 80% acetone. The process was repeated till colorless pellet was obtained. Absorbance of supernatant was recorded at 663 and 645 nm on a UV-visible spectrophotometer (Chemito Spectrascan, UV 2600).

Protein estimation

Protein content was estimated using Lowry *et al*. (1951) method. Approximately 0.1 g of fresh leaf sample was homogenized in 0.2M (KH_2PO_4/K_2HPO_4) buffer with pH 7.0. The homogenate was centrifuged at 15,000 x *g* for 10 min. To 1 ml aliquot of supernatant, 5ml of alkaline $CuSO_4$ was added, followed by 10 min incubation at room temperature. Mixture was allowed to react with 0.5 ml of Folin Ciocalteu Reagent for 30 min in dark at room temperature. Absorbance was measured at 660 nm on a UV-visible spectrophotometer (Chemito Spectrascan, UV 2600)

spectrophotometer and protein concentration was measured using bovine serum albumin as standard.

Determination of Proline Content

Free proline content was estimated by following the method of Bates *et al*. (1973). Fresh samples (0.5 g each) were homogenized in 5 ml of 3% (w/v) sulphosalicylic acid using a mortar and pestle. About 2 ml of extract was taken in test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100°C for 30 min. After cooling the reaction mixture, 4 ml of toluene was added. After thorough mixing, the chromophore containing toluene was separated and absorbance of red color developed was read at 520 nm against toluene blank on UV-visible spectrophotometer (Chemito Spectrascan, UV 2600).

Estimation of lipid peroxidation

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) contents as given by Heath and Packer (1968). Fresh samples (500 mg each) were homogenized in 10 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 5 min, then 2 ml aliquot of supernatant was taken and 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added into it. The mixture was heated at 95°C for 30 min, and then quickly cooled in an ice bath. After centrifugation at 10,000g for 10 min to remove suspended turbidity, the absorbance of supernatant was recorded at 532 nm absorbance on UV-visible spectrophotometer (Chemito Spectrascan, UV 2600). The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 $mmol^{-1}$ cm^{-1}.

Statistical analysis

The experiments were repeated three times for checking the reproducibility of results before conducting the statistical analyses. Means differing significantly were compared using Duncan's Multiple Range test (DMRT) at $P \leq 0.05$. All the statistical analyses were done using MSTAT-C statistical software package.

RESULTS AND DISCUSSION

Usually understanding the responses of plants at germination, early seedling and vegetative growth stages are considered as most important in terms of elucidating the salt sensitivity or its tolerance mechanisms and ultimately their survival under these harsh conditions (Danai-Tambhale *et al*., 2011). Amongst these stages, particularly seed germination is considered as the most critical in seedling establishment which consequently determines the successful crop production (Almansouri *et al*., 2001; Kumar *et al*., 2007). Therefore, present investigation was aimed to investigate the physiological and biochemical behavior of sorghum cultivars under varying concentrations of $MgCl_2$ stress. The findings of our group (unpublished data) suggested that these cultivars behave differently under NaCl-induced salt stress and in terms of their NaCl stress tolerance. Accordingly, the present investigation was also a step towards observing whether these cultivars behave similarly under NaCl and $MgCl_2$ induced salt stress.

Both the cultivars showed no significant effect on germination rate with increasing salinity levels as 100% seeds germinated at all the levels of $MgCl_2$ concentrations used, however, the seedlings turned brown after 2-3 days of germination and were not seen healthy in both the cultivars at 200 mM $MgCl_2$

and above concentration. (Table 1)

$MgCl_2$ induced salinity stress resulted in significant reduction of fresh weight and dry weight of seedlings, irrespective of the cultivar. However, the rate of decrease was more pronounced in the cultivar Phule Revati as compared to Phule Vasudha. Similar patterns were observed in terms of relative water content (Table 1). Therefore, cultivar Phule Vasudha showed slightly more tolerant nature against $MgCl_2$ salinity in terms of seedling germination and growth irrespective of the concentrations used. Similar results were reported by Brandenburg and Kleier (2011), where investigators observed reduced growth of Radish plants under increasing $MgCl_2$ concentrations (up to 150mM). The results were further supported by shoot length, root length and their ratios in the present study and Phule Vasudha showed comparably slightly lesser reduction in shoot growth than Phule Revati (Table 2).

Biochemical parameters were clearly affected under the influence of $MgCl_2$ stress. The differential behavior of intra-specific genotypes towards magnesium chloride stress was evident in present investigation and the results obtained may be proved to helpful in understanding the complexities hidden in the responses and tolerance mechanisms towards $MgCl_2$ induced salinity in the sorghum rhizosphere. Chlorophyll pigments were drastically reduced with progression in the concentration of $MgCl_2$, however, contradictory to the other parameters, the degree of reduction in chlorophyll content was higher in genotype Phule Vasudha as compared to Phule Revati (Table 3) and this might indicate the differential behavior and probable tolerance mechanisms adopted by the plants to lower the deleterious effects of salt stress.

Total soluble proteins were gradually decreased under the rising concentrations of $MgCl_2$ (Table 4). Once again, the cultivar Phule Vasudha showed more salt tolerant behavior than Phule Revati as comparably lower antagonistic effects of salinity was observed in terms of protein content in the earlier genotype.

Another important biochemical marker proline, which is considered as a major osmoregulator in plants under various stresses and very much sought after compatible osmolyte, which help plants to counteract and recovery from salt stress (Kumar *et al*., 2010) was also studied in the present investigation under magnesium chloride induced salinity stresses in two selected genotypes of sorghum. There was a steep increase in proline content in both the cultivars with increasing salinity levels from 0 mM to 300 mM. The genotype Phule Vasudha showed higher proline content under non-saline condition. However, amongst two genotypes, Phule Revati showed higher proline content under maximum salinity level (300 mM). The extent of accumulation was higher in the cultivar Phule Revati, where 439% increase in proline content as compared to 324% increase in Phule Vasudha was recorded at 300 mM $MgCl_2$ against the control plants of both the cultivars (Table 4), which might indicate higher stress state as high proline accumulation is considered a typical phenomenon under stress conditions.

The MDA content was monitored as an indicator of level of peroxidation of lipids, which is considered as an important sign for level of stress-induced deleterious effects on plant cells and tissues, under stress conditions (Kumar *et al*., 2010). Even though, MDA content was on higher side under non-saline conditions in cultivar Phule Vasudha as compared to cultivar Phule Revati, however the rate of increase with increase in salt

stress was much higher in the latter cultivar. At 300 mM $MgCl_2$ concentration, Phule Vasudha showed 191% increase in MDA content against control plants, whereas, at the same stress level, 273% increase against control plants of Phule Revati was evidenced (Table 4).

Table 1. Effects of different concentrations of $MgCl_2$ on germination, biomass production and relative water content in sorghum cultivars. Results are mean of three replicates ± standard error. Means within a column followed by different letters were significantly different from each other according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

$MgCl_2$ Stress (mM)	Sorghum cultivars							
	Phule Vasudha				Phule Revati			
	Ger (%)	FW (g)	DW (g)	RWC (%)	Ger (%)	FW (g)	DW (g)	RWC (%)
0	100	1.12 ± 0.2^{e}	0.13 ± 0.02^{d}	88.55 ± 3.2^{d}	100	1.03 ± 0.3^{e}	0.14 ± 0.04^{c}	86.54 ± 3.9^{e}
50	100	0.79 ± 0.1^{d}	$0.11 \pm 0.01^{c,d}$	$85.44 \pm 2.8^{b,c}$	100	0.77 ± 0.2^{d}	0.13 ± 0.03^{c}	82.46 ± 2.7^{c}
100	100	0.59 ± 0.1^{c}	0.09 ± 0.01^{c}	84.21 ± 2.1^{b}	100	0.49 ± 0.1^{c}	0.08 ± 0.01^{b}	84.01 ± 3.1^{d}
200	100	0.29 ± 0.07^{b}	0.04 ± 0.005^{b}	84.97 ± 3.3^{b}	100	0.10 ± 0.03^{b}	0.02 ± 0.005^{a}	80.23 ± 2.2^{b}
300	100	0.04 ± 0.001^{a}	0.01 ± 0.002^{a}	70.00 ± 2.9^{a}	100	0.02 ± 0.003^{a}	0.01 ± 0.003^{a}	66.67 ± 2.6^{a}

Ger: germination; FW: fresh weight; DW: dry weight; RWC: relative water content

Table 2. Effects of different concentrations of $MgCl_2$ on root length, shoot length and root/shoot ratio in sorghum cultivars. Results are mean of three replicates ± standard error. Means within a column followed by different letters were significantly different from each other according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

$MgCl_2$ Stress (mM)	Sorghum cultivars					
	Phule Vasudha			Phule Revati		
	Root Length (cm)	Shoot Length (cm)	Root/Shoot ratio	Root Length (cm)	Shoot Length (cm)	Root/Shoot ratio
0	14.7 ± 1.3^{e}	11.2 ± 0.8^{d}	0.76^{b}	11.9 ± 1.2^{d}	13.3 ± 1.4^{d}	1.12^{c}
50	11.3 ± 0.9^{d}	7.6 ± 0.4^{c}	0.67^{a}	11.1 ± 1.0^{d}	7.6 ± 0.7^{c}	0.68^{a}
100	7.7 ± 0.9^{c}	5.4 ± 0.4^{b}	$0.70^{a,b}$	5.7 ± 0.8^{c}	3.7 ± 0.4^{b}	0.65^{a}
200	1.2 ± 0.2^{b}	1.1 ± 0.1^{a}	0.92^{c}	2.1 ± 0.3^{b}	1.9 ± 0.2^{a}	0.95^{b}
300	0.2 ± 0.01^{a}	--	--	0.4 ± 0.1^{a}	--	--

Table 3. Effects of different concentrations of $MgCl_2$ on chlorophyll 'a' (Chl a), chlorophyll 'b' (Chl b) and total chlorophyll (Total Chl) in sorghum cultivars. Results are mean of three replicates ± standard error. Means within a column followed by different letters were significantly different from each other according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

$MgCl_2$ Stress (mM)	Sorghum cultivars					
	Phule Vasudha			Phule Revati		
	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)
0	1.27 ± 0.13^{e}	0.59 ± 0.05^{d}	1.86 ± 0.11^{e}	1.16 ± 0.09^{d}	0.37 ± 0.04^{e}	1.53 ± 0.10^{e}
50	0.99 ± 0.10^{d}	0.38 ± 0.06^{c}	1.37 ± 0.08^{d}	1.07 ± 0.08^{c}	0.32 ± 0.03^{d}	1.39 ± 0.09^{d}
100	0.91 ± 0.09^{c}	0.31 ± 0.04^{b}	1.22 ± 0.09^{c}	0.99 ± 0.03^{b}	0.29 ± 0.02^{c}	$1.28 \pm 0.08^{b,c}$
200	0.87 ± 0.08^{b}	0.26 ± 0.03^{a}	1.13 ± 0.10^{b}	0.99 ± 0.05^{b}	0.25 ± 0.02^{b}	1.24 ± 0.05^{b}
300	0.70 ± 0.08^{a}	0.27 ± 0.05^{a}	0.97 ± 0.08^{a}	0.85 ± 0.02^{a}	0.13 ± 0.02^{a}	0.97 ± 0.03^{a}

FW: fresh weight

Table 4. Effects of different concentrations of $MgCl_2$ on protein content, proline content and lipid peroxidation level (MDA content) in sorghum cultivars. Results are mean of three replicates ± standard error. Means within a column followed by different letters were significantly different from each other according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

$MgCl_2$ Stress (mM)	Sorghum cultivars					
	Phule Vasudha			Phule Revati		
	Protein content (mg/g FW)	Proline content (mg/g FW)	MDA content (n mol/g FW)	Protein content (mg/g FW)	Proline content (mg/g FW)	MDA content (n mol/g FW)
0	33.20 ± 1.8^{e}	21.24 ± 1.3^{a}	28.39 ± 1.2^{a}	29.84 ± 1.5^{d,e}	18.44 ± 0.9^{a}	19.35 ± 1.2^{a}
50	29.95 ± 1.9^{d}	29.49 ± 1.5^{b}	50.97 ± 2.0^{b}	28.06 ± 1.7^{d}	20.37 ± 1.1^{b}	43.87 ± 1.6^{b}
100	25.99 ± 1.5^{c}	34.03 ± 1.9^{c}	50.32 ± 1.8^{b}	22.41 ± 1.8^{c}	25.10 ± 1.7^{c}	58.71 ± 1.9^{c}
200	23.99 ± 1.1^{b}	57.85 ± 2.3^{d}	64.52 ± 2.7^{c}	17.52 ± 0.9^{b}	39.81 ± 1.9^{d}	61.29 ± 2.2^{d}
300	21.45 ± 1.2^{a}	68.88 ± 2.1^{e}	82.58 ± 3.1^{d}	14.27 ± 0.8^{a}	80.96 ± 2.1^{e}	72.26 ± 2.1^{e}

In conclusion, overall, Phule Vasudha showed better $MgCl_2$ salt stress tolerance at the germination, seedling and at vegetative growth stages than its counterpart cultivar Phule Revati. The results obtained in the present investigation are also in harmony of our groups findings, where Phule Vasudha exhibited better tolerance nature to NaCl and Na_2SO_4 stresses (unpublished data). Sorghum cultivar Phule Vasudha also showed lower antagonistic effects on biochemical parameter including Chlorophyll content and protein content. This cultivar also showed lower levels of salt stress-induced lipid peroxidation. All these biochemical parameters seem to have played an important role in its better $MgCl_2$ salt tolerance nature.

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REFERENCES

Allakhverdiev, S.I., Sakamoto, A., Nishiyama, Y., Inaba, M., Murata N. (2000) Ionic and Osmotic Effects of NaCl-Induced Inactivation of Photosystems I and II in *Synechococcus* sp. *Plant Physiol.*, **123**, 1047–1056.

Almansouri, M., Kinet, J. M., Lutts, S. (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant Soil.,* **23**,243-254.

Arnon, D.I. (1949) Copper enzymes in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., **24**, 1-15.

Bates, L.S., Waldran, R.P., Teare, I.D. (1973) Rapid determination of free proline for water stress studies. *Plant Soil*., **39**, 205-208.

Brandenburg, W., Kleier, C. (2011) Effect of $MgCl_2$ on Germination, Growth and Biomass Allocation of the Radish CV."Cherry Belle". *American J. Env. Sci*., **7**, 132-135.

Cuartero, J., Boların, M.C., Asıns, M.J., Moreno, V. (2006) Increasing salt tolerance in the tomato. *J. Exp. Bot*., **57**, 1045-1058.

Curtin, D., Steppuhn, H., Selles, F. (1993) Plant responses to sulfate and chloride salinity: growth and ionic relations. *Soil Sci. Soc. Am. J*., **57**, 1304-1310.

Danai-Tambhale, S., Kumar, V., Shriram, V. (2011)

Differential Response of Two Scented Indica Rice (*Oryza sativa*) Cultivars under Salt Stress. *J. Stress Physiol. Biochem*., **7**, 387-397.

Dougherty, C.K., Smith, G.R. (2006) Acute effects of road de-icers on the tadpoles of three anurans. *Appl. Herpetol*., **3**, 87-93.

Heath, R.L., Packer, L. (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stiochiometry of fatty acid peroxidation. *Arch*. *Biochem*. *Biophys*., **125**,189-198.

Hela, M., Olfa, B., Imen, B.S., Nawel, N., Abidi, W., Huang, J., Hanen, Z., Abdelali, H., Mokhtar, L., Ouerghi, Z. (2012) Enhanced accumulation of root hydrogen peroxide is associated with reduced antioxidant enzymes under isoosmotic NaCl and Na_2SO_4 salinities. *Afr. J. Biotechnol*., **11**, 8500-8509.

Koca, M., Bor, M., Ozdemir, F., Turkan I. (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot*., **60**: 344–351.

Kumar, V., Shriram, V., Jawali, N., Shitole, M.G. (2007)Differential response of *indica* rice genotypes to NaCl stress in relation to physiological and biochemical parameters. *Arch. Agron. Soil Sci.*, **53**, 581-592.

Kumar, V., Shriram, V., KaviKishor, P.B., Jawali, N., Shitole, M.G. (2010) Enhanced proline accumulation and salt stress tolerance of transgenic *indica* rice by over expressing P5CSF129A gene. *Plant Biotech Rep.,* **4**, 37-48.

Kumar, V., Shriram, V., Nikam, T.D., Jawali, N., Shitole, M.G. (2009) Antioxidant enzyme activities and protein profiling under salt stress in *indica* rice genotypes differing in salt tolerance. *Arch. Agron. Soil Sci*., **55**, 379-394.

Kumar, V., Shriram, V., Nikam, T.D., Jawali, N. ,Shitole, M.G. (2008) Sodium chloride induced changes in mineral elements in *indica* rice cultivars differing in salt tolerance. *J. Plant Nutr*., **31**, 1999-2017.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem*., **193**, 265-75.

Munns, R., Tester, S. (2008) Mechnaism of salinity tolerance. *Annu. Rev. Plant Biol*., **59**, 651-681.

Niu, G., Rodriguez, D.S. (2008) Responses of growth and ion uptake of four rose rootstocks to chloride- or sulfate-dominated salinity. *J. Amer. Soc. Hort. Sci*., **133**, 663-669.

Sairam, R.K., Tyagi, A. (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Curr*. *Sci*., **86**, 407-421.

Shabala, S., Hariadi, Y. (2005) Effects of magnesium availability on the activity of plasma membrane ion transporters and light-induced responses from broad bean leaf mesophyll. *Planta*, **221**, 56-65.

Singh, G. (2009) Salinity-related desertification and management strategies: Indian experience. *Land Degrad*. *Develop*., **20**, 367-385.

Sumithra, K., Jutur,P..P, Carmel, B.D., Reddy, A.R. (2006) Salinity-induced changes in two cultivars of *Vignaradiata*: Responses of antioxidative and proline metabolism. *Plant Growth Regul*., **50**,11-22.

Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy, P., McDonald, G.K. (2011) Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. *J. Exp. Bot*., **62**, 2189-2203.

Tobe, K., Li, X., Omasa, K. (2004) Effects of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (*Chenopodiaceae*). *Seed Sci. Res*., **14**,345–353.

Turkan, I., Demiral, T. (2009) Recent development in understanding salinity tolerance. *Env*. *Exp*. *Bot*., **67**, 2-9.

Wu, W., Peters, J., Berkowitz, G.A. (1991) Surface Charge-Mediated Effects of Mg^{2+} on K^{+} Flux across the Chloroplast Envelope Are Associated with Regulation of Stromal pH and Photosynthesis. *Plant Physiol*., **97**, 580–587.