Indian Journal of Experimental Biology
Vol. 57, September 2019, pp. 672-679

Stress induced changes in osmoprotectants, ionic relations, antioxidants activities and protein profiling characterize *Sporobolus marginatus* Hochst. ex A. Rich. salt tolerance mechanism

Anita Mann*, Ashwani Kumar, Mohana Saha, Charu Lata & Arvind Kumar
ICAR-Central Soil Salinity Research Institute, Karnal-132 001, Haryana, India

Received 17 October 2017; *revised 09 October 2018*

Soil salinization and alkalinization frequently co-occur in nature, but very few studies focus on the interactive effects of mixed salt and alkali stresses on plants. *Sporobolus marginatus* Hochst. ex A. Rich. (perennial halophytic grass) collected from extreme saline sodic Kachchh plains, Bhuj, Gujarat was analyzed to evaluate the stress specific responses in osmoprotectants, antioxidants, ionic relations and protein profiling under saline, sodic and mixed saline-sodic soils. Osmotic adjustments in terms of total soluble sugars (TSS), glycine betaine, proline content and protein concentration exhibited differential responses to variable stress conditions. Proline content increased 4.8 folds at pH_2 10.0, 5.2 folds at ECe 35 dSm^{-1} and 5 folds at pH_2 9.0 + ECe 20 dSm^{-1}. The greater accumulation of proline in *Sporobolus*, may presumably be one of the factor for tolerance to higher salt. At the same time, superoxide dismutase (SOD) activity in leaves increased with increasing sodicity i.e. 30.73 and 33.55 units g^{-1} FW at pH 9.5 and pH 10.0, respectively. Gradual increase in peroxidase enzyme (POX) activity was observed under all the stresses. Under control condition, POX activity was 21.67 units g^{-1} FW in *Sporobolus*, which increased to 26.56 units g^{-1} FW at pH 10.0, 27.89 units g^{-1} FW at ECe 35 dSm^{-1} and 27.44 units g^{-1} FW at pH 9.0 + ECe 20 dSm^{-1}. The basal activity of APX increased with increasing stress conditions and was maximum (43.91 units g^{-1} FW) at pH 10.0. On the other hand, 2 times higher glutathione reductase (GR) activity was obtained under sodic stress of pH 9.5 and pH 10.0. SDS-PAGE revealed that five new polypeptide bands of MW 58.5, 53.7, 39.7, 31.8 and 28.3 kDa were expressed at higher saline level while one polypeptide band of 39.7 kDa disappeared at higher salinity level of 35 dSm^{-1} which may be due to its degradation at higher salt concentration. Synthesis of common polypeptides of MW 98.1, 69.3, 35.45, 24.89 and 23.3 kDa under all the stress conditions need special mention. Furthermore, the enhanced expression of these proteins, which also existed in the control plants, were specifically increased under stress condition which revealed that these proteins were up-regulated in specific regions of *Sporobolus* adapted to salt stress. Therefore, further exploration is needed to test the contribution of salt stress related proteins/genes or regulatory factors from the salt tolerant grasses (STGs) for possible utilization in cereal crop improvement.

Keywords: Salinity, Proline, Glycine betaine, Halophyte, Peroxidase, Superoxide dismutase, Total soluble sugars

Salt affected soils (SAS) are widespread in irrigated arid and semi-arid regions of the world where irrigation is essential to increase agricultural production to satisfy food requirements. Soil salinization and alkalinization frequently co-occur in nature, so the conditions in natural saline–alkali soil are very complex. Some salt–alkali soils have high salinity but low pH, while some have low salinity but high pH. Neutral (NaCl and Na_2SO_4) and alkaline ($NaHCO_3$ and Na_2CO_3) salts are two distinct stresses for plants, and should be defined as salt and alkali stress, respectively[1,2]. When saline soil contains CO_3^{2-} and/or HCO_3^-, it causes injury to plants not only through salt stress but also through alkali stress[3,4]. Soil salinity is an increasing problem for agriculture, affecting the most productive crop areas of the world, cultivated under irrigation in arid and semiarid regions representing less than 15% of global arable land, but producing more than 40% of world food[5]. Globally more than 900 million hectares of land, approx. 20% of the total agricultural land are affected by salt, accounting for more than 6% of the world's total land area. In India, SAS occupy an area of about 6.73 million ha, of which saline and sodic soils constitute roughly 40% and 60%, respectively[6].

Salinity is one of the major abiotic stresses that hinder the performance of the crop plants all over the world. In most crop plants, the main toxic component of salinity is Na^+ and Cl^-, which interferes with the normal physiological processes, such as enzyme activities and protein synthesis, as well as causing osmotic imbalances[7,8]. Salinity stress leads to a series of changes in basic

*Corresponding author
E-mail:anitadgr13@gmail.com; Anita.Mann@icar.gov.in

biosynthetic functions, including photosynthesis and photorespiration, amino acid and carbohydrate synthesis. To avoid the cellular damage due to reactive oxygen species (ROS) generation, plants produce a number of antioxidant enzymes that are induced and provide secondary protection against oxidative stress. Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic defense system. When ROS increases, chain reactions start in which superoxide dismutase (SOD), a metalloenzyme catalyzes the dismutation of O_2^- radical to molecular O_2 and H_2O_2. The H_2O_2 is then detoxified either by catalase/peroxidase or in ascorbate glutathione cycle which involves oxidation and reduction of ascorbate and glutathione through ascorbate peroxidase (APX) and glutathione reductase (GR)[9-11]. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. Specific polypeptides could be potentially used as a marker to decipher the differential expression of genes during stress to identify adaptation response towards stresses[12]. It is also well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Stresses induce quantitative and qualitative changes in protein content of the plant cells. So, the present experiment was designed to invade into salinity, sodicity and mixed salinity-sodicity stress tolerance in terms of scavenging system and protein profiles of salt tolerant grass halophyte, *Sporobolus marginatus*. *Sporobolus* is a variable tufted or stoloniferous perennial grass with well-grown stem leaves and basal glabrous leaf-sheaths reaching upto 15-90 cm in height. The roots are very thick and are covered with a soft felt of root-hairs. *Sporobolus* is distributed throughout Northwest India, Baluchistan and tropical East Africa. Its drought tolerance is excellent and it inhabits saline soils (user lands) in India and the Kafue flood plain in Zambia. It is seen on pure salt crust. Its natural habitat is dry grassland, and often on alkaline soils. It is adapted to very dry conditions, at a rainfall of around 300 mm per year and at 125-375 mm in India. It grows on a wide range of soils, from loose sandy loams to loams and alluvial silts. It covers saline soil well and helps minimize upward capillary movement of salts. Therefore, the studies were conducted on *Sporobolus* to explore its potential under abiotic stress of salinity and sodicity.

Materials and Methods

Experimental details (Plant material and growth conditions)

The present study was conducted to evaluate the responses of antioxidant enzymes and protein profiling of *Sporobolus marginatus* Hochst. ex A. Rich. (halophytic grass) in salt affected soils (alkaline/saline). Seeds as well as root slips of *Sporobolus marginatus* were collected from extreme saline sodic Kachchh plains, Bhuj, Gujrat and established in pots under controlled conditions. After establishment, these grasses were transferred to micro-plots (2.5×1.5×0.5 m) of Crop Improvement Division, Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, India. Initially, soils were sandy loam with 0.6 dSm^{-1} ECe, 7.1 pH, 0.33% organic carbon with 14% clay. Different treatments of alkalinity/salinity were imposed in these micro-plots separately (pH 9.5 and 10.0 and EC_e 15, 25, 35 dSm^{-1}) and in combination (pH 9.0 with ECe 10, 15, 20 dSm^{-1}) with 3 replications. The net house was covered with a high quality polythene sheet to maintain the desired salt stress in the micro-plots. Soil was made saline by passing 3 pore volumes (700 L) of respective saline water with equal proportions of Cl^- and SO_4^{-2} of Na^+, Ca^{2+} and Mg^{2+} and alkalinity was developed by mixing required amount of $NaHCO_3$ on the basis of sodicity curve drawn earlier for these soils[13]. Irrigation was supplied at IW/CPE ratio of 0.8 with constant irrigation water depth of 6 cm. The samples were harvested from 5 cm above the soil surface after 120 days of transplanting at vegetative stage of plants. At harvest, soil samples were taken at 15-30 cm depth for analysis of ECe and pH.

Osmotic adjustments and Ionic relations

Total soluble sugars[14], proline content[15], glycine betaine[16] and protein content[17] were analyzed from fresh leaves. Na^+ and K^+ content were measured with flame photometer (PFP7, Jenway, Bibby Scientific, UK) after di-acid [HNO_3: $HClO_4$ (3:1)] digestion of oven dried plant material.

Antioxidant enzyme analysis

Extraction medium for SOD, APX and GR was 0.1 M phosphate buffer (pH 7.5) containing 5% (w/v) polyvinyl polypyrrolidone (PVPP), 1 mM EDTA, and 10 mM β-mercapto-ethanol as described by Chawla *et al.*[18]. For peroxidase, extraction was done in 0.01 M phosphate buffer (pH 7.5) containing 3% (w/v) PVPP. One g of leaf tissue was homogenized in 5 mL of ice cold extraction medium in pre-cooled

mortar and pestle, centrifuged at 10000 ×g for 15 min at 4°C and the supernatant was used for the crude enzyme preparation.

Superoxide dismutase activity was determined by quantifying the ability of the enzyme to inhibit light induced conversion of nitroblue tetrazolium (NBT) to formazan[19]. One enzyme unit was defined as the amount of enzyme which could cause 50% inhibition of the photochemical reaction. Method of Nakano and Asada[20] was employed to assay APX. One unit of APX corresponded to 1.0 O.D. change per min. Glutathione reductase activity was determined by the method of Halliwell & Foyer[21]. One enzyme unit was defined as 100 μmole of NADPH oxidized per minute. Peroxidase activity was assayed at 37°C as described by Shannon *et al*.[22]. The unit of POX activity was defined as 1.0 μM of H_2O_2 utilized per min.

SDS-PAGE analysis

Fifty μg of protein extract was resolved through electrophoresis by the method of Laemmli[23]. The proteins were separated at 15 mA in the 4% stacking gel and at 25 mA in 10% resolving gel. After electrophoresis, gels were fixed and stained with 0.25% (w/v) CBB R-250 in 40% methanol with 7% glacial acetic acid and then destained in 10% methanol with 7.5% glacial acetic acid. After destaining, the gels were stored in 7% glacial acetic acid.

Statistical analysis

All the data were subjected to analysis of variance using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Least significant difference test was applied at 5% probability level to compare the mean differences.

Results and Discussion

The status of soil EC_e and pH was quantified in the microplots after harvesting of halophytic grass, *Sporobolus*. A significant reduction of 3.6% in soil EC_e and 4% reduction in soil pH was observed. This proves that the roots of halophytic grass absorb the toxic ions from the soil to improve the soil pH and ECe. Boyko[24] was one of the pioneers who first suggested that halophytic plants can be used to desalinate the soil and water. It was found that *S. salsa* produces biomass of about 20 t dry weight ha^{-1} containing 3-4 t of salt[25]. Native flora of saline waste lands in Pakistan was made productive by growing salt tolerant plants, namely, *Suaeda fruticosa*, *Kochia indica*, *Atriplex crassifolia*, *Sporobolus arabicus*, *Cynodon dactylon*, *Desmostachya bipinnata*, *Polypogon monspeliensis*, *S. nudiflora*, *Salsola baryosma*, *Haloxylon recurvum* and *Atriplex lentiformis*[26]. Eslamzadh[27] observed high (30%) Na^+ accumulation corresponding to 31500 μg g^{-1} dry weight in *Salicorni aeuropia*. Cultivation of *S. portulacastrum* accumulated NaCl and reduced electrical conductivity of saturation extract (ECe) from 4.9 to 2.5 dS m^{-1} in 120 days by accumulating NaCl. Farah Al-Nasir[28] reported that different halophytic species showed decrease in soil salinity to various levels at the end of the experiment with salinity decreasing from an average ECe of 84 to 5.46, 5.04 and 6.3 mS cm^{-1} in the 0-30-cm depth and from 49.6 to 5.46, 13.45 and 7.14 mS cm^{-1} for the lower soil depth (30–60 cm depth) for *Atriplex hallimus* L., *Atriplex numularia* L., and *Tamarix aphylla* L., respectively.

Osmolyte concentration and Ionic relations

Osmotic adjustments in terms of total soluble sugars (TSS), glycine betaine, proline content and protein concentration exhibited differential responses to variable stress conditions (Table 1). Total soluble sugars significantly increased with the increase in stress conditions regardless of the sodic or saline treatments in comparison to unstressed plants (control). In *S. marginatus*, TSS content was 3.62 mg g^{-1} dry wt. in control which decreased with sodic conditions i.e. 3.19 mg g^{-1} dry wt. at pH 9.5 and 3.08 mg g^{-1} dry wt. at pH 10.0 (Table 1). But under saline conditions alone and in combination, the sugar content significantly increased to 5.5 mg g^{-1} dry wt. at EC_e 35 dSm^{-1} and 4.2 mg g^{-1} at pH 9.0 + EC_e 20 dSm^{-1}.

Proline accumulation is an important physiological index for plant response to abiotic stresses. An increased accumulation of proline content has been observed as depicted in Table 1, which might counteract the adverse effects of toxic salt ions in cell vacuoles[29]. Under control conditions proline content was 0.98 mg g^{-1} F.W. which increased 4.8 times at pH 10.0, 5.2 folds at EC_e 35 dSm^{-1} and 5 folds at pH 9.0 + EC_e 20 dSm^{-1} over the respective control treatment (Table 1). The greater accumulation of proline in *Sporobolus*, may presumably be one of the factors for its tolerance to higher salt content in soil. A possible reason for this increased level of proline during salt stress could be an alteration in the activities of the enzymes involved in the biosynthesis and degradation of proline. Proline is thought to play a multifunctional

Table 1 — Salt stress induced changes in osmolyte concentration and ionic relations in *Sporobolus marginatus*

Source of variation	Degree of freedom	Analysis of variance among determined traits (Means sum of squares) Osmolyte concentration and Ionic relations							
		TSS	Proline content	Glycine betaine	Protein content	Root Na^+ (% DW)	Shoot Na^+ (% DW)	Root K^+ (% DW)	Shoot K^+ (% DW)
Replication	1	0.0748	0.0572	0.2004	0.0005	0.0008	0.0035	0.0001	0.0003
Treatments	8	1.591**	4.406**	51.645**	0.3802**	0.105**	1.999**	0.118**	0.129**
Error	8	0.0441	0.0027	0.9947	0.0091	0.0001	0.0147	0.0014	0.0035
Treatments		TSS	Proline content	Glycine betaine	Protein content	Root Na^+ (% DW)	Shoot Na^+ (% DW)	Root K^+ (% DW)	Shoot K^+ (% DW)
Control		3.62^{de}	0.98^{h}	11.09^{g}	15.80^{c}	0.73^{g}	1.28^{e}	1.17^{a}	1.59^{a}
pH9.5		3.19^{ef}	2.95^{de}	17.19^{ef}	15.93^{bc}	0.55^{h}	2.33^{d}	0.80^{c}	1.03^{bc}
pH10.0		3.08^{f}	4.70^{c}	20.96^{cd}	15.95^{bc}	0.30^{i}	3.10^{c}	0.35^{e}	0.98^{bcde}
EC_e 15 dSm^{-1}		2.71^{f}	1.58^{g}	15.99^{f}	15.40^{d}	0.80^{f}	2.58^{d}	0.99^{b}	1.00^{bcd}
EC_e 25 dSm^{-1}		4.07^{cd}	2.88^{e}	19.48^{de}	15.84^{c}	0.89^{d}	3.20^{c}	0.83^{c}	0.85^{e}
EC_e 35 dSm^{-1}		5.50^{a}	5.10^{a}	28.50^{a}	15.83^{c}	0.95^{b}	4.28^{a}	0.70^{d}	0.65^{f}
pH9.0 + EC_e 10 dSm^{-1}		4.69^{b}	2.13^{f}	16.55^{f}	15.89^{bc}	0.84^{e}	2.60^{d}	1.10^{a}	1.08^{b}
pH9.0 + EC_e 15 dSm^{-1}		4.55^{bc}	3.00^{d}	22.04^{bc}	16.10^{b}	0.91^{c}	3.88^{b}	0.94^{b}	0.93^{cde}
pH9.0 + EC_e 20 dSm^{-1}		4.20^{c}	4.90^{b}	23.96^{b}	17.02^{a}	1.04^{a}	4.35^{a}	0.80^{c}	0.88^{de}
General Mean		3.96	3.13	19.53	15.97	0.78	3.06	0.85	1.00
CV(%)		5.31	1.64	5.11	0.60	1.13	3.96	4.32	5.91
SE(m)		0.210	0.052	0.997	0.096	0.009	0.121	0.037	0.059
LSD at 5%		0.4843	0.1188	2.2999	0.2203	0.0202	0.2798	0.085	0.1359

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. TSS, total soluble sugars; DW, dry weight]

role in the defense mechanism. It acts as a mediator of osmotic adjustment, a stabilizer of sub-cellular structure, a scavenger of free radicals and stress related signal[30, 31]. Glycine betaine was extracted from fresh leaves, with water and quantified using a colorimetric method. Increasing trend was observed for glycine betaine in all the treatments compared to control. In *S. marginatus*, the content was 11.09 mg g^{-1} under control conditions while an increase in betaine content was observed under the saline stress (28.5 mg g^{-1} at EC_e 35 dSm^{-1}) than the sodicity stress alone (20.96 mg g^{-1} at pH 10.0) and mixed salinity-sodicity stress (23.96 mg g^{-1} at pH 9.0 + EC_e 20.0 dSm^{-1}). Protein accumulation were particularly important for cell survival under stress conditions and causes membrane stabilization[29]. The relative contribution of protein towards the osmoprotection depends strongly on stress levels. Maximum protein accumulation of 17.02 mg g^{-1} F.W. was observed at pH 9.0 + EC_e 20 dSm^{-1} (Table 1). At the same time, the absolute concentration of total soluble proteins increased with increased sodicity/salinity.

The data presented in Table 1, revealed that sodicity/salinity stresses significantly influenced the ionic concentration. Nevertheless, Na^+ distribution in roots and shoots indicated a tendency for restricted sodium uptake with increasing stress conditions[32]. *S. marginatus* exhibited significantly lower ($P \leq 0.01$) root Na^+ concentrations (0.73% DW) as compared to shoot Na^+ concentrations (1.28% DW). In *S. marginatus*, roots restrict the uptake of Na^+ under sodic conditions (0.55% at pH 9.5 and 0.3% at pH 10.0), while under salinity and combined conditions, root Na^+ concentrations increased. Shoot Na^+ concentrations was 3.1% at pH 10.0, 4.28% at EC_e 35 dSm^{-1} and 4.35% at pH + EC_e 20 dSm^{-1}. In the present study, the level of K^+ gradually decreased while that of Na^+ dramatically increased. Mean root K^+ concentration was 0.85% in *S. marginatus*. Under sodic conditions alone, root K^+ concentration decreased to 70.08% as compared to control (1.17%). *S. marginatus* accumulated sufficient amount of K^+ in the shoots to protect from the injuries of salt stress. Combined saline-alkaline stress significantly reduced shoot K^+ concentration (Table 1). Ion accumulation in the shoot parts is possibly attributable to an enhanced selective ion uptake in favour of K^+ over Na^+ at the root level on one hand and a high transport capacity in favour of Na^+ versus K^+ from the root to the shoot on the other[31].

Antioxidant enzyme analysis

Plants exposed to salinity are prone to oxidative stress because of the formation of ROS such as O_2^-, H_2O_2 and OH. These ROS can affect the integrity of cellular membranes, enzymes activities and the plant photosynthetic apparatus[33]. Plant cells contain

an array of antioxidant enzymes that scavenge or prevent the formation of the aggressive ROS, which protects cells from oxidative damage[34]. The result presented in Figure 1 and 2 demonstrates that the activity of SOD, POX, APX and GR increased due to salt stress. Superoxide dismutase (SOD) could catalyze dis-proportionation reaction of two superoxide radicals to generate O_2 and H_2O_2, and then H_2O_2 was catalyzed and removed by peroxidase(POD) and catalase (CAT), which was the response started first in the resistance to oxidative stress of plants[34]. The increase in SOD activity was significantly higher under sodic and mixed saline-sodic stress. SOD activity in leaves of *Sporobolus* was 20.39 units g^{-1} FW in control which increased with increasing sodicity i.e. 30.73 and 33.55 units g^{-1} FW at pH 9.5 and pH 10.0, respectively. Although SOD activity increased under salt stress but the per cent increase was less than the sodic and mixed saline-sodic stress. SOD is one of several important antioxidant enzymes with the ability to repair oxidation damage caused by ROS. Thus, SOD is considered as a key enzyme for maintaining normal physiological conditions and coping up with oxidative stress in the regulation of intracellular levels of ROS[35]. Maximum SOD activity of 36.35 units g^{-1} FW was observed under the stress condition of pH 9.0 + EC_e 20 dSm^{-1}. SOD is the major scavenger of superoxide ($O_2^{\cdot -}$) to form H_2O_2 and O_2, and plays an important role in defense activity against the cellular damage caused by environmental stress[36]. Gradual increase in peroxidase enzyme activity was observed under all the stresses. Under control condition, POX activity was 21.67 units g^{-1} FW in *Sporobolus*, which increased to 26.56 units g^{-1} FW at pH 10.0, 27.89 units g^{-1} FW at EC_e 35 dSm^{-1} and 27.44 units g^{-1} FW at pH 9.0 + EC_e 20 dSm^{-1}. Peroxidase (POD) could remove H_2O_2 in plants to prevent the cell membrane from oxidation by H_2O_2. Therefore, the enhancement of POD activity could effectively resist the oxidative stress caused by salt stress, thereby improving the salt tolerance of plants[37]. Nair & Dagla[38] have studied the adaptive role of peroxidase in stem tissue of desert shrub, *Leptadenia pyrotechnica* collected from Thar desert and observed the adaptive modification of the plant within pH range of 6-8.

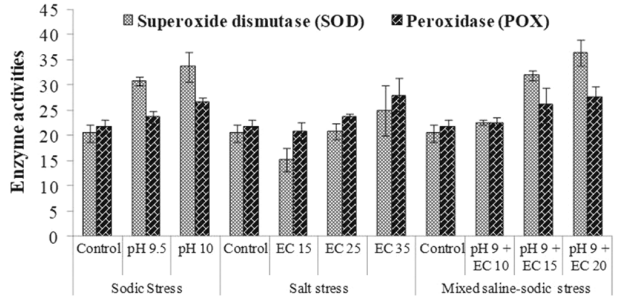

Fig. 1 — Salt stress modulation in superoxide dismutase (SOD) and peroxidase (POX) activity of *Sporobolus marginatus*

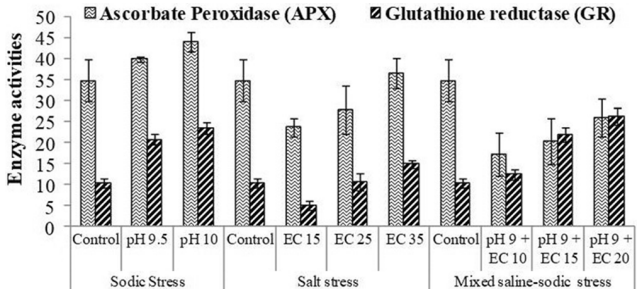

Fig. 2 — Salt stress modulation in ascorbate peroxidase (APX) and glutathione reductase (GR) activity of *Sporobolus marginatus*

APX and GR are, respectively, responsible for H_2O_2 detoxification in green leaves. APX is considered to be a key antioxidant enzyme in plants and GR has a central role in maintaining the reduced glutathione (GSH) pool during stress[39]. The basal activity of APX was 34.72 units g^{-1} FW under control conditions, which increased with increasing stress conditions and was maximum (43.91 units g^{-1} FW) at pH 10.0 in *Sporobolus*. While under saline stress, the APX activity was low as compared to their respective control but increased simultaneously with increased stress. Similar results were obtained under mixed saline-sodic stress conditions. On the other hand, two times higher GR activity was obtained under sodic stress of pH 9.5 and pH 10.0 as compared to GR activity under control conditions (10.39 units g^{-1} FW). Under saline stress, GR activity showed similar trend as that of APX. But the stress condition of mixed salinity-sodicity enhanced the GR activity in leaf tissue and maximum value of 26.39 units g^{-1} FW was obtained at pH 9.0 + EC_e 20 dSm^{-1}. Salinity stress is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O_2^- and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses[10,11,40]. Mann *et al*.[8] have reported upregulation of transcripts for antioxidant enzymes peroxidase, ascorbate peroxidase, catalase etc under salt stress of EC 30 dS/m in grass halophyte *Dichanthium annulatum* scavenging the toxicity of salt stress. Singh and Bhardwaj[41] have reported that seed pre-treatment with

proline improved drought tolerance through increased accumulation of proline, glycine betaine and upregulated activities of antioxidant enzymes SOD, POX, APX and GR in wheat seedlings. The potential of APX to metabolize H_2O_2 depends on the redox state of such compounds. APX and GR are believed to act in conjunction for H_2O_2 scavenging during environmental stresses[42]. It has been suggested that the coordinated activity of the different H_2O_2-scavenging enzymes play a part in the plant redox homeostasis[43].

SDS-PAGE analysis

Under individual salt and sodic stresses and mixed salt-sodic stress, qualitative and quantitative differences were seen in polypeptide separation between stressed and control plants. SDS-PAGE protein profiling showed differential expression of polypeptides in *Sporobolus marginatus* (grass halophytes) under different stress conditions. Presence or absence of polypeptides could be potentially used as marker(s) to decipher the differential behavior of plants under different stresses. Total 14 polypeptides were resolved under control condition in *Sporobolus* and two new polypeptides were synthesized at pH 9.5 indicating *de novo* synthesis of new polypeptides of MW 78.6 and 19.97 kDa (Fig. 3), which also were observed at pH 10.0. The intensity of bands at 69.3 and 35.45 kDa decreased with increased sodic stress conditions. These proteins might be synthesized either *de novo* in response to sodicity stress or may be present constitutively at low concentration and get expressed when plants are exposed to different stresses[44]. At higher pH level of 10.0, pattern of polypeptide resolution remained unaffected. This suggested that polypeptide expression varied depending upon the different developmental stages and the differential gene expression of concerned structural or regulatory gene(s). The timing of the synthesis of particular polypeptides coincides with the protein content during different stresses associating their involvement in tolerance[45].

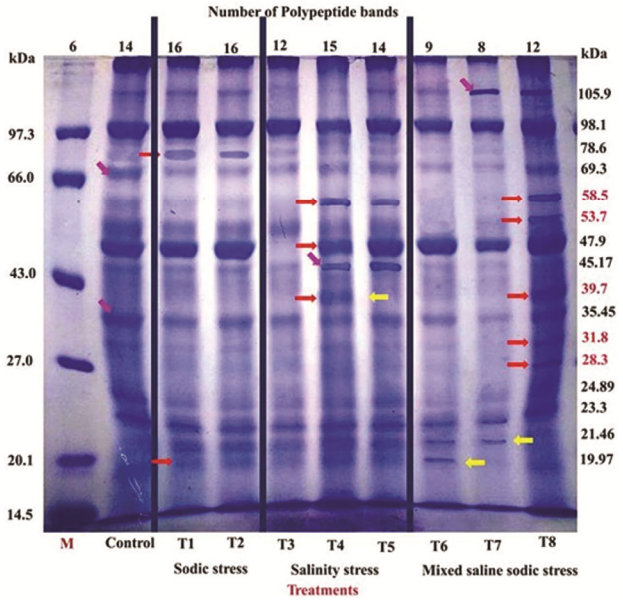

Fig. 3 — Salt stress modulation in protein profile resolutions of *Sporobolus marginatus* [M, Molecular weight marker; Control, pH 7.0 and EC 0.63 dSm^{-1}; T1, pH 9.5; T2, pH 10.0; T3, EC_e 15 dSm^{-1}; T4, EC_e 25 dSm^{-1}; T5, EC_e 35 dSm^{-1}; T6, pH 9.0 + EC_e 10 dSm^{-1}; T7, pH 9.0 + EC_e 15 dSm^{-1}; and T8, pH 9.0 + EC_e 20 dSm^{-1}]

Salt stress also causes considerable changes in the expression of soluble proteins or *de novo* synthesis of proteins of different molecular masses. Similarly, in present study also in *Sporobolus* under salt stress the number of polypeptide bands increased with intensified stress. At saline condition of EC_e 15 dSm^{-1}, total 12 polypeptide bands of molecular weight ranging from 105.9 to 19.97 kDa appeared. But, with increase in salt stress 3 new polypeptide bands of MW 58.5, 47.9 and 39.7 kDa were expressed. Also one polypeptide band of 39.7 kDa disappeared at higher salinity level of 35 dSm^{-1} which may be due to its degradation at higher salt concentration since depressed protein synthesis and accelerated degradation of proteins in plants in response to salt stress has been reported by number of workers[44,46].

On the other hand, 9 polypeptide bands were resolved at stress level of pH 9.0 + EC_e 10 dSm^{-1} in *Sporobolus*. Similar to salt stress, under the conditions of mixed salt-sodic stress, the number of polypeptide bands increased with intensified stress. Differences in polypeptide resolution indicate their cellular and molecular adaptive mechanism to osmotic stress[29,47]. With the increase in salinity level from 10 dSm^{-1} to 15 dSm^{-1} along with pH 9.0, polypeptide band of 19.97 kDa disintegrated. But, further increase towards higher saline level of 20 dSm^{-1} along with pH 9.0 was accompanied by appearance as well as disappearance of polypeptide bands. Five new polypeptide bands of MW 58.5, 53.7, 39.7, 31.8 and 28.3 kDa were expressed while one polypeptide of 21.46 kDa disappeared. This shows that with dissolution of low molecular weight proteins, higher molecular weight peptides were expressed at extreme higher salt stress which may find a role in salt tolerance.

Specific expression of stress proteins is an important adaptive manifestation in maintaining the integrity of cellular membranes to ensure their normal functioning under salinity stress[48]. Synthesis of common polypeptides of MW 98.1, 69.3, 35.45, 24.89 and 23.3 kDa under all the stress conditions need special mention. Furthermore, the enhanced expression of these proteins, which also existed in the control plants, were specifically increased and clearly observed under stress condition which revealed that these proteins were up-regulated in specific regions of *Sporobolus* adapted to salt stress. Comparison of protein profiles of *Sporobolus* under different stresses indicated that there was synthesis of one specific prominent polypeptide band of 98.1 and 47.9 kDa with all treatments except at EC_e 15 dSm^{-1}. Changes in the gene activation, transcription, and translation (protein synthesis) often occur during the acclimatization process under stressful environments and thus are thought to be involved in the induction of tolerance to salts[49]. Mann et al[8] have reported differential expression of 448 transcripts including dehydration responsive element binding protein under salt stress of EC 30 dS/m in grass halophyte *Dichanthium annulatum* imparting tolerance. It is likely that the genes that are responsible for the superior salt tolerance in halophytes will serve as a subset of the genes for improvement in crops. Therefore, further exploration is needed to test the contribution of salt stress related genes or regulatory factors from the STGs for possible utilization in cereal crop improvement.

Conclusion

The increased proline content along with increased antioxidant enzyme activities in *Sporobolus* may presumably be one of the factors for tolerance to salt stress. The physiological and biochemical parameters studied at saline and alkaline stresses defined the optimum tolerance limit of *Sporobolus* upto ECe ~ 25 dSm^{-1} and pH ~9.5 and hence this halophyte is best suited for these salt affected areas.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgement

The authors acknowledge ICAR-National Agricultural Science Fund (NASF), New Delhi for funding this work.

References

1 Shi DC & Yin LJ, Difference between salt (NaCl) and alkaline (Na_2CO_3) stresses on *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr. plants. *Acta Bot Sin*, 35 (1993) 144.

2 Yang CW, Chong JN, Li CY, Kim CM, Shi DC & Wang DL, Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. *Plant Soil*, 294 (2007) 263.

3 Shi DC & Wang DL, Effects of various salt–alkaline mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. *Plant Soil*, 271 (2005) 15.

4 Kumar A, Kumar A, Kumar P, Lata C & Kumar S, Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of marvel grass. *Indian J Expt Biol*, 56 (2018a) 573.

5 Munns R & Tester M, Mechanisms of salinity tolerance. *Ann Rev Plant Biol*, 59 (2008) 651.

6 Sharma DK & Singh A, Salinity research in India-achievements, challenges and future prospects. *Water Energy Internat*, 58 (2015) 35.

7 Kumar A, Mann A, Kumar A, Devi S & Sharma PC, Potential and role of halophyte crops in saline environments. In: *Engineering practices for management of soil salinity*. (Eds. Gupta SK, Goyal MR & Singh A; Apple Academic Press Inc., Oakville), 2018b, 329.

8 Mann A, Kumar N, Lata C, Kumar A, Kumar A & Meena BL, Functional annotation of differentially expressed genes under salt stress in *Dichanthium annulatum*. *Plant Physiol Rep*, 24 (2019) 104.

9 Noctor G & Foyer CH, Ascorbate and glutathione: Keeping active oxygen under control. *Ann Rev Plant Physiol Plant Mol Biol*, 49 (1998) 249.

10 Mann A, Bishi SK, Mahatma MK & Kumar A, Metabolomics and salt stress tolerance in plants. In: *Managing salt tolerance in plants: molecular and genomic perspectives*. (Taylor and Francis Group/LLC), 2015, 251-266.

11 Kumar A, Mann A, Lata C, Kumar N & Sharma PC, Salinity induced physiological and molecular responses of halophytes. In: Research developments in saline agriculture. (Eds. Dagar JC; Springer Nature Singapore Pte Ltd.), 2019, 331-356.

12 Hieng B, Ugrinovich K, Sustar-Vozlich J & Kidric M, Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* L. cultivars differing in sensitivity. *Plant Physiol*, 161 (2004) 519.

13 Singh RB, Minhas PS, Chauhan CPS & Gupta RK, Effect of high salinity and SAR water on salinization, sodication and yield of pearl millet – wheat system. *Agric Water Manage*, 21 (1992) 93.

14 Yemn EW & Willis AJ, The estimation of carbohydrates in plant extracts by anthrone. *Biochem J*, 57 (1954) 508.

15 Bates LS, Waldren RP & Teare ID, Rapid determination of free proline for water-stress studies. *Plant Soil*, 39 (1973) 205.

16 Grieve CM & Grattan SR, Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70 (1983) 303.

17 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the

principle of protein dye binding. *Anal Biochem*, 7 (1976) 248.

18 Chawla S, Jain S & Jain V, Alkalinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). *J Plant Biochem Biotech*, 22 (2013) 27.

19 Nishikimi M, Rao NA & Yagi K, The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophy Res Comm*, 48 (1972) 849.

20 Nakano Y & Asada K, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*, 22 (1981) 867.

21 Halliwell B & Foyer CH, Properties and physiological functions of a glutathione reductase purified from spinach leaves by affinity chromatography. *Planta*, 139 (1978) 9.

22 Shannon LM, Key E & Law JY, Peroxidase isoenzymes from horse reddish roots: isolation and physical properties. *J Biol Chem*, 241 (1966) 2166.

23 Laemmli UK, Cleavage of structural proteins during the assembly of the head of the bacteriophage Ty. *Nature*, 277 (1970) 680.

24 Boyko H, Basic Ecological Principles of Plant Growing by Irrigation with High Saline Seawater. In: *Salinity and Aridity* (Eds. Boyko H & Junk DW, The Hague), 1966, 131.

25 Ke-Fu Z, Desalinization of Saline Soils by *Suaeda salsa*. *Plant Soil,* 135 (1991) 303.

26 Mahmood H, Malik K A, Lodhi M A K& Sheikh K H, Seed Germination and Salinity Tolerance in Plant Species Growing on Saline Wastelands. *Biol Plant*, 38 (1996) 309.

27 Eslamzadh T,*Salicorni aeuropea*, a Bioaccumulator in Maharloo Salt Lake Region. *Int J Soil Sci*, 1 (2006) 75.

28 Al-Nasir F, Bio-reclamation of a Saline Sodic Soil in a Semi-arid Region/Jordan. *Am Eurasian J Agric. Environ Sci,* 5 (2009) 701.

29 Lata C, Kumar A, Sharma SK, Singh J, Sheokand S, Pooja, Mann A & Rani B, Tolerance to combined boron and salt stress in wheat varieties: Biochemical and molecular characterization. *Indian J Expt Biol*, 55 (2017) 321

30 Nanjo T, Fujita M, Seki M, Kato T, Tabata S & Shinozaki K, Toxicity of free proline revealed in an Arabidopsis T-DNA-tagged mutant deficient in proline dehydrogenase. *Plant Cell Physiol*, 44 (2003) 541.

31 Kumar A, Kumar A, Lata C & Kumar S, Eco-physiological responses of *Aeluropus lagopoides* (grass halophyte) and *Suaeda nudiflora* (non-grass halophyte) under individual and interactive sodic and salt stress. *South Afr J Bot*, 105 (2016) 36.

32 Mangalassery S, Dayal D, Kumar A, Bhatt K, Nakar R, Kumar A, Singh JP & Misra AK, Pattern of salt accumulation and its impact on salinity tolerance in two halophyte grasses in extreme saline desert in India. *Indian J Expt Biol*, 55 (2017) 542.

33 Parida A & Das AB, Salt tolerance and salinity effects on plants. *Ecotox Env Safety*, 60 (2005) 324.

34 Rani B, Kumari N, Pooja, Jain V, Dhawan K, Monika, Avtar R, Kumar A & Sheoran P, Antioxidative System as Influenced by High Temperature stress in *Brassica juncea* (L) Czern & Coss. *Curr Trend Biotech Pharm*, 10 (2016) 118.

35 Mittler R, Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*, 7 (2002) 405.

36 Meloni DA, Oliva MA, Martinez CA & Cambrai J, Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ Exp Bot*, 49 (2003) 69.

37 Mandhania S, Madan S & Sawhney V, Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol Plant*, 227 (2006) 227.

38 Nair S & Dagla HR, Thermostability assessment, profiling and localization of peroxidase activity in stem tissues of *Leptadenia pyrotechnica*: a defensive enzyme for survival in high temperature conditions. *Indian J Expt Biol*, 56 (2018) 694.

39 Pastori G, Foyer CH & Mullineaux P, Low temperature-induced changes in the distribution of H_2O_2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. *J Exp Bot*, 51 (2000) 107.

40 Foyer C & Noctor G, Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol Planta*, 119 (2003) 355.

41 Singh N & Bhardwaj RD, Proline treatment ameliorates water deficit induced oxidative damage in wheat seedlings. *Indian J Expt Biol*, 57 (2019) 399.

42 Sairam RK & Saxena DC, Oxidative stress and Antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. *J Agric Crop Sci*, 184 (2000) 55.

43 Foyer CH & Noctor G, Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. *Plant Cell*, 17 (2005) 1866.

44 Lata C, Soni S, Kumar N, Kumar A, Pooja, Mann A & Rani S, Adaptive mechanism of stress tolerance in *Urochondra* (grass halophyte) using roots study. *Indian J Agric Sci*, 89 (2019) 1050.

45 Kumar A, Sharma SK, Lata C, Sheokand S & Kulshreshta N, Combined effect of boron and salt on polypeptide resolutions in wheat varieties differing in their tolerance. *Indian J Agric Sci*, 85 (12) (2015) 1626.

46 Chandershekhar V, Kar M & Chavan PD, Influence of salt stress on biochemical processes in chickpea. *Plant Soil*, 96 (1986) 439.

47 Sadasivam S & Manickam A, Biochemical methods. 2nd Edn., (New Age International (P) Publishers, Delhi), 1996, 107-109.

48 Wahid A, Perveen M, Gelani S & Basra SMA, Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J Plant Physiol*, 164 (2007) 283.

49 Moran PJ, Cheng Y, Cassell JL & Thompson GA, Gene expression profiling of *Arabidopsis thaliana* in compatible plant-aphid interactions. *Arch Insect Biochem Physiol*, 51 (2002) 182.