DOI: 10.17138/TGFT(5)129-142

Research Paper

Screening of salt-tolerance potential of some native forage grasses from the eastern part of Terai-Duar grasslands in India

Evaluación de la tolerancia a la sal de algunas gramíneas forrajeras nativas de la parte oriental de los Terai-Duar Grasslands en la India

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Abstract

The salt tolerance of 12 native forage grasses from the eastern part of Terai-Duar grasslands was assessed using a rapid method of leaf disc senescence bioassay. Samples of these grasses were grown in untreated water as well as 100 and 200 mM NaCl solutions for periods of 3, 6 and 9 days. Discs of fresh leaf were then placed in untreated water as well as in 100 and 200 mM NaCl solutions for 96 hours. Quantitative effects were measured as the effects on chlorophyll concentration in leaves in response to exposure to the varying solutions. From these results, the salt sensitivity index (SSI) of the individual grasses was determined. The SSI values indicated that *Imperata cylindrica*, *Digitaria ciliaris* and *Cynodon dactylon* were most salt-tolerant of all grasses tested. Further characterization of the grasses was done by observing the changes in 6 biomarkers for salinity tolerance: relative water content, total sugar concentration, proline concentration, electrolyte leakage, membrane lipid peroxidation and H_2O_2 concentration following exposure to 100 and 200 mM NaCl concentrations for 3, 6 and 9 days. Finally, hierarchical cluster analysis using the software CLUSTER 3.0 was used to represent the inter-relations among the physiological parameters and to group the grasses on the basis of their salinity tolerance. The overall results indicated that *Imperata cylindrica*, *Eragrostis amabilis*, *Cynodon dactylon* and *Digitaria ciliaris* were potentially salt-tolerant grasses and should be planted on saline areas to verify our results. On the other hand, *Axonopus compressus*, *Chrysopogon aciculatus*, *Oplismenus burmanni* and *Thysanolaena latifolia* were found to be highly salt-sensitive and would be unsuitable for use in saline areas.

Keywords*:* Biomarkers, hierarchical cluster analysis, leaf disc senescence bioassay, salinity tolerance.

Resumen

En la University of North Bengal, Siliguri, India, utilizando a nivel de laboratorio un método rápido de bioensayo de senescencia de discos foliares, fue evaluada la tolerancia a salinidad de 12 gramíneas forrajeras nativas de la parte oriental de los Terai-Duar Grasslands en la India nororiental. Las gramíneas fueron cultivadas tanto en agua no tratada como en soluciones de 100 y 200 mM NaCl durante 3, 6 y 9 días. Después se colocaron discos de hoja fresca tanto en agua no tratada como en soluciones de 100 y 200 mM NaCl durante 96 horas. Los efectos cuantitativos se midieron como la

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concentración de clorofila en las hojas en respuesta a la exposición a las diversas soluciones. Los resultados, con base en un índice de sensibilidad a la sal, mostraron que *Imperata cylindrica*, *Digitaria ciliaris* y *Cynodon dactylon* fueron las gramíneas más tolerantes a la salinidad. Además se realizó una caracterización de las gramíneas mediante la determinación de los cambios en 6 biomarcadores para la tolerancia a la salinidad: contenido relativo de agua; concentración de azúcar total; concentración de prolina; pérdida de electrolitos; peroxidación lipídica de membrana; y concentración de H_2O_2 después de la exposición a concentraciones de 100 y 200 mM NaCl durante 3, 6 y 9 días. El análisis de conglomerados jerárquicos utilizando el software CLUSTER 3.0 para representar las interrelaciones entre los parámetros fisiológicos y agrupar las gramíneas sobre la base de su tolerancia a la salinidad mostró que, en general, que *Imperata cylindrica*, *Eragrostis amabilis*, *Cynodon dactylon* y *Digitaria ciliaris* fueron gramíneas potencialmente tolerantes a la sal que deberían ser cultivadas en suelos salinos para verificar nuestros resultados. Por otra parte, *Axonopus compressus*, *Chrysopogon aciculatus*, *Oplismenus burmanni* y *Thysanolaena latifolia* resultaron ser altamente sensibles a la sal y no son especies apropiadas para uso en áreas salinas.

Palabras clave*:* Análisis de conglomerados jerárquicos, bioensayo de senescencia de discos foliares, biomarcadores, tolerancia a la salinidad.

Introduction

In India, available fodder for stock is estimated to be 40–50% below requirements, and this scenario is gradually worsening due to the concomitant decrease in grass coverage and increase in livestock population (Indian Council of Agricultural Research 2009). Global climate change in the last decade has been correlated with changes in the productivity of forage grasses and is likely to have a detrimental effect on the overall grass coverage in the long term (Abberton et al. 2008). A huge proportion of land in the country is classified as wasteland due to the problems of soil salinity, alkalinity and waterlogging. The selection of grass germplasm for salinity tolerance is critical for more efficient utilization of these degraded lands by establishing stress-tolerant grasses in non-arable marginal areas (Ashraf 2006). Species that are relatively salt-tolerant show greater endurance and adaptability among the native species (Squires 2015). Therefore there is an urgent need to: identify salt-tolerant traits in wild forage grasses; evaluate their potential for enhancing the productivity of grasslands in their native habitats; and utilize them for the rejuvenation of grasslands and croplands with reduced or lost productivity.

Abiotic stresses, in particular water and salinity stress, play a major role in disrupting the growth and development of grasses including cereals (Tester and Bacic 2005). Salinity limits plant growth and productivity through the toxic effects of Na^+ and Cl^- ions, which leads to ionic imbalances, osmotic and oxidative stress (Munns and Tester 2008). Native grasses, however, show variable degrees of NaCl tolerance, especially those belonging to the subfamilies Panicoideae and Chloridoideae (Bromham and Bennett 2014; Roy and Chakraborty 2014). Salinity tolerance is a complex trait, governed by several physiological and biochemical parameters and these parameters greatly influence the normal growth and development of plants (Zhu 2000). Salt tolerance of any individual species is demonstrated as the ability to maintain an optimal physiological and biochemical equilibrium under NaCl treatment (Sairam and Tyagi 2004). Ashraf and Harris (2004) suggested different biomarkers as indicators of salinity tolerance, including soluble sugars, proteins, amino acids, ammonium compounds, polyamines, polyols, antioxidants and ATPases.

In the present study however, 6 biochemical markers, viz. relative water content (RWC), proline and soluble sugar concentrations, membrane lipid peroxidation (malondialdehyde, MDA), electrolyte leakage (EL) and H_2O_2 concentration were selected for use in screening for salinity tolerance of the selected grasses. Increase in leaf RWC in the halophyte *Atriplex nummularia* with increasing salinity indicated an efficient mechanism to adjust cell cytosol osmotically (Araújo et al. 2006). Accumulation of osmolytes like proline, soluble sugars and glycine betaine and elevated levels of antioxidative enzymes play a vital role in conferring salt tolerance in grasses (Roy and Chakraborty 2014). Accumulation of glycine betaine in *Cynodon* and *Spartina*, proline in *Paspalum* and myo-inositol in *Porteresia* has been found to confer salinity tolerance (Wyn Jones and Storey 1981; Marcum and Murdoch 1994; Sengupta et al. 2008). Accumulation of proline, fructans and soluble carbohydrates was also correlated with salinity tolerance in salt-tolerant cultivars of wheat (Kafi et al. 2003). MDA concentration has been proposed as an indicator of oxidative damage and a lesser accumulation of the same in root tissues was employed for screening the salt-

tolerant genotypes of *Cenchrus ciliaris* (Castelli et al. 2009). Electrolyte leakage as an indicator of cell membrane stability of durum wheat cultivars under osmotic stress was demonstrated, with level of electrolyte leakage being inversely related to degree of salt tolerance of cultivars (Bajji et al. 2002).

In addition to the characterization of 12 forage grasses that are widely grazed by and fed to livestock in the eastern parts of the Terai-Duar grasslands by observing the changes in 6 biomarkers for salinity tolerance, the objective of our study was to evaluate the salt-tolerance potential of those grasses by using a rapid screening technique where the inherent tolerance of saline conditions was assessed as a precursor to selective propagation in varied environmentally challenged wastelands.

Materials and Methods

Study area and plant materials

Twelve native grasses were collected from the different regions of the eastern part of the Terai-Duar grasslands (88.22–89.66° E, 26.45–26.86° N; Figure 1). These grasses are widely grazed by livestock and harvested by local people for feeding to domestic animals, viz. *Arundo donax* L. of the subfamily Arundinoideae; *Axonopus compressus* (Sw.) P. Beauv., *Capillipedium assimile* (Steud.) A. Camus, *Chrysopogon aciculatus* (Retz.) Trin., *Digitaria ciliaris* (Retz.) Koeler, *Arundinella bengalensis* (Spreng.) Druce, *Imperata cylindrica* (L.) Raeusch., *Oplismenus burmanni* (Retz.) P. Beauv., *Setaria pumila* (Poir.) Roem. & Schult. and *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda of the subfamily Panicoideae; and *Cynodon dactylon* (L.) Pers. and *Eragrostis amabilis* (L.) Wight & Arn. of the subfamily Chloridoideae. In the subsequent text only the generic names are used.

Experimental design and NaCl treatment

A rapid screening protocol was implemented for the differentiation of salt-tolerance potential of the forage grasses. The grasses were collected from their natural habitats and placed in small flasks containing 0.1X Hoagland solution with their roots intact, before being transferred to the plant growth chamber in the laboratory of the Department of Botany, University of North Bengal, Siliguri. Before NaCl treatment, the roots were gently washed with sterile dH_2O to remove any mud and then again transferred to conical flasks containing 0.1X Hoagland solution. The plants were then allowed to acclimatize for 48 hours in the growth chamber, with a standard temperature of 20–25 °C, RH 65–70% and 16 h photoperiod. Following acclimatization, 2 groups of plants were grown in NaCl treatments of 100 and 200 mM for 9 days, while the third group remained as control and the effects of NaCl on the plants in terms of several biomarkers after 3, 6 and 9 days of treatment were analyzed.

Three individual samplings from 3 different locations (Figure 1) were completed for each grass and the results were expressed as mean ± SD for all parameters analyzed. For grasses with broad leaves like *Thysanolaena* and *Arundo*, 3 plants were taken per sampling site, whereas for grasses with small narrow leaves, 5–6 plants were taken per sampling site.

Salt sensitivity index (SSI)

The youngest healthy fully expanded leaves from the plants were briefly washed in deionized water and 1 cm diameter leaf discs were finely cut and floated in a 5 ml solution of NaCl (100 and 200 mM) for 96 hours. Leaf discs floated in sterile dH_2O served as the experimental control for the bioassay (Fan et al. 1997). The effects of salt treatment on leaf discs were assessed by observing the phenotypic changes and the extent of NaCl effect in terms of SSI, which was quantified by estimating the chlorophyll concentration in NaCl-treated and control sets. Briefly, the leaf discs were crushed in 80% acetone and the absorbance was recorded in a UV-VIS spectrophotometer at 645 and 663 nm and the chlorophyll concentration was calculated using Arnon's formulae (Arnon 1949). SSI values were then calculated at 100 and 200 mM NaCl as the percent decrease in chlorophyll concentration of the NaCl treatment in comparison with the untreated leaf discs using the following formula:

$$\text{SSI} = \frac{\text{Chlorophyll conc. of NaCl-treated leaf discs}}{\text{Chlorophyll conc. of untreated leaf discs}} \text{ x } 100$$

Biochemical markers for assessment of NaCl tolerance

For an alternative screening of grasses for their salt-tolerant attributes, 6 different biochemical parameters were chosen, viz. relative water content (RWC), proline and soluble sugar concentrations, membrane lipid peroxidation (malondialdehyde, MDA), electrolyte leakage (EL) and H_2O_2 concentration. For these experiments, the first 3 fully expanded leaves from the top of each grass subjected to the various growth solutions were collected.

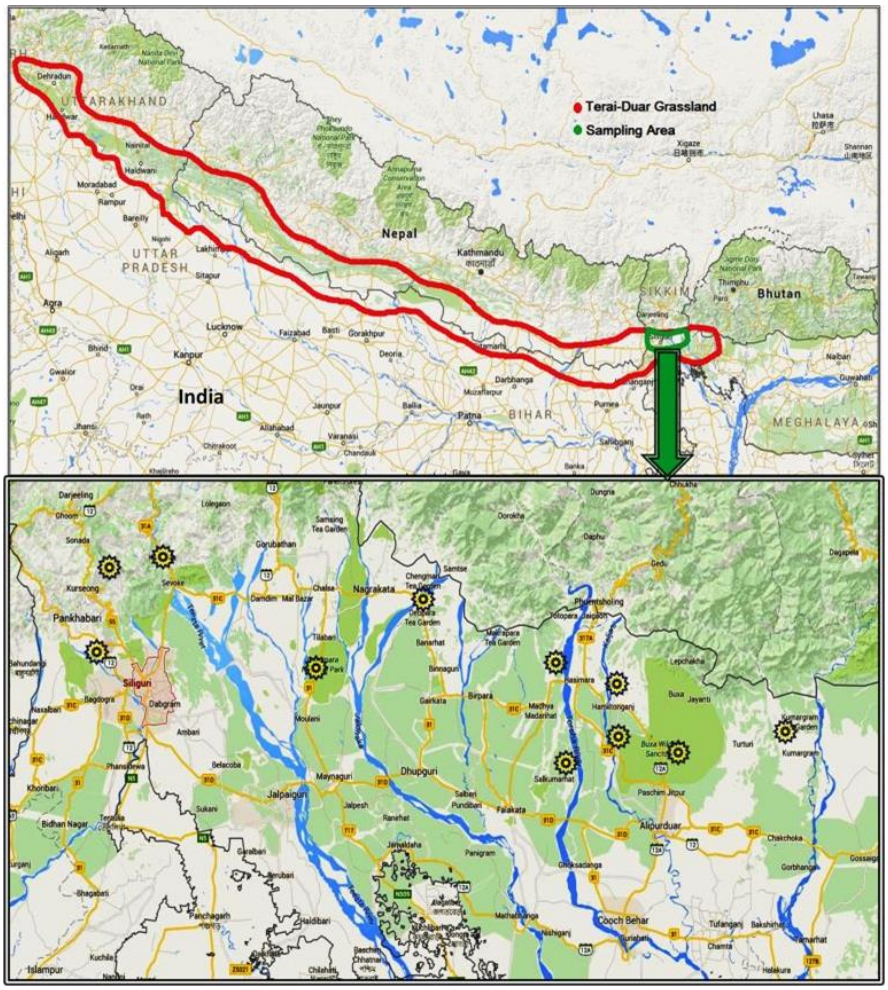

Figure 1. Geographical location of the Terai-Duar grasslands and the sampling area. Sampling area (enlarged view) with major locations from which the forage grasses were collected.

Relative water content. RWC was measured following the protocol of Barr and Weatherley (1962). Briefly, fresh leaf samples from control and different treatment sets were weighed to obtain fresh weight (FW). The samples were then immediately hydrated to full turgidity for 4 h, dried of surface moisture and weighed to obtain fully turgid weight (TW). Samples were then oven-dried at 80 °C for 24 h and weighed to determine dry weight (DW). RWC was calculated by the following equation:

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

Proline. Extraction and estimation of proline were done by the method of Bates et al. (1973). Leaf tissue was homogenized in 3% sulfosalicylic acid. Ninhydrin reagent was used for the estimation of proline in the extract, which was separated in a separating funnel using toluene, prior to recording the absorbance at 520 nm.

Total sugar. Soluble sugar in leaves was extracted in 95% ethanol following the method of Harborne (1973). Anthrone reagent was used to estimate total sugar following the method of Plummer (1978). Briefly, 4 ml of anthrone reagent was added to 1 ml test solution and kept over boiling water bath for 10 min, after which the absorbance was taken at 620 nm. Total sugar was finally calculated using a standard curve of D-glucose.

Membrane lipid peroxidation. Membrane lipid peroxidation was measured in terms of concentration of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction, following the method of Heath and Packer (1968). Leaves were homogenized in 0.1% (w/v) trichloroacetic acid (TCA) and estimation was done with 0.5% (w/v) TBA in 20% TCA. The absorbance of the reaction mixture was determined at 532 and 600 nm and the MDA content was calculated using an extinction coefficient of 155 mM/cm.

Electrolyte leakage. Electrolyte leakage (EL) was measured as described by Lutts et al. (1996). Leaves were washed thoroughly with deionized water and placed in culture tubes containing 10 ml of deionised water on a rotary shaker for 24 h. Subsequently, the electrical conductivity of the solution (L_t) was determined and the samples were then autoclaved at 120 °C for 20 min and cooled to room temperature before determining the final electrical conductivity (L_0). EL was calculated as follows:

$$\text{Electrolyte leakage (\%)} = (L_t / L_0) \times 100$$

H_2O_2 concentration. The extraction and estimation of H_2O_2 were done by the method given by Jana and Choudhuri (1981) with slight modification. Leaf tissue was homogenized in 50 mM phosphate buffer (pH 6.5) and mixed with 0.1% titanium sulphate in 20% (v/v) H_2SO_4 and centrifuged at 6,000 rpm for 15 min. Absorbance was measured at 410 nm and H_2O_2 concentration was measured using the extinction coefficient of 0.28 µmol/cm.

Hierarchical cluster analysis

For cluster analysis of the grasses for their NaCl tolerance, the data for fold change values of RWC, proline, soluble sugar, MDA, EL and H_2O_2 after NaCl treatments for 3, 6 and 9 days with respect to the control sets were taken. Hierarchical cluster analysis was performed using the CLUSTER 3.0 program by the uncentered matrix and complete linkage method following the protocol of de Hoon et al. (2004). The resulting tree figure was displayed using the software package, Java Treeview, as described by Chan et al. (2012).

Statistical analysis

All experiments were repeated with sampling from 3 different locations (n = 3) for each species. Species and treatment means were statistically analyzed using Least Significant Difference ($P \leq 0.05$) for a completely randomized design.

Results

Salt sensitivity index (SSI) of grasses

Chlorophyll concentration in fresh untreated leaves varied from 0.72 mg/g (*Capillipedium*) to 1.45 mg/g (*Oplismenus*). SSIs of grasses determined by leaf disc assay and represented in terms of % decrease in chlorophyll concentration in the leaf discs floated in 100 mM and 200 mM NaCl solutions relative to the control sets, i.e. leaf discs kept in sterile dH_2O, are shown in Table 1. At 100 mM NaCl, the senescence assay indicated that *Setaria, Thysanolaena, Imperata* and *Cynodon* were least affected with SSI values of 0.45–7.36. At the same time, *Capillipedium, Axonopus* and *Arundinella* were much more sensitive (SSI values of 24.20–18.37). However, at 200 mM NaCl, *Imperata, Digitaria* and *Cynodon* were least affected by salt concentration (SSI values of 6.59–15.00). Interestingly, *Thysanolaena* and *Setaria* were more affected by 200 mM NaCl, showing marked increases in SSI values (23.38 and 57.98, respectively). *Capillipedium* showed the highest sensitivity to both 100 and 200 mM NaCl with SSI values of 24.20 and 61.93, respectively. This result was also reciprocated by the phenotypical changes in the leaf discs floated in NaCl solutions, which can be clearly observed in Figure 2.

Effect of NaCl on biochemical markers for analysis of salinity tolerance

Relative water content. Leaf RWC values were found to decrease in all grasses with both increase in NaCl concentration and duration of treatment (Table 2). The fold change values of RWC in plants subjected to 100 and 200 mM NaCl in comparison with the control sets revealed the smallest changes in *Cynodon* and *Imperata* and the largest changes in *Chrysopogon* and *Digitaria* (Figure 3a).

Proline concentration. Proline concentration in fresh untreated leaves varied from 11.6 µg/g (*Chrysopogon*) and 12.4 µg/g (*Setaria*) to 63.1 µg/g (*Imperata*) and 64.5 µg/g (*Digitaria*). During the first 3 days of NaCl treatment (100 and 200 mM), proline concentration in fresh tissue increased with increase in NaCl concentration in all grasses except *Axonopus*, where levels of proline declined (Table 3; Figure 3b). The largest increases (on a percentage basis) were recorded in *Cynodon, Arundinella* and *Imperata*. Similarly after 6 and 9 days of treatment, proline concentrations increased as NaCl concentration increased in all grasses except *Axonopus*, *Chrysopogon*, *Thysanolaena* and *Oplismenus*, where concentrations declined with increasing NaCl concentration. The largest percentage increases in proline concentration were observed in *Cynodon* and *Arundinella* (1.8–3-fold increase).

Table 1. Chlorophyll concentration in detached leaf discs of grasses dipped in 0, 100 and 200 mM NaCl solutions and salt sensitivity index expressed as relative % decrease of chlorophyll concentration of detached leaves at 100 and 200 mM NaCl.

Grass	Chlorophyll concentration (mg/g fresh weight of tissue, fwt)			Salt sensitivity index (% decrease in chlorophyll conc.)	
	Concentration of NaCl (mM/L)			Concentration of NaCl (mM/L)	
	0	100	200	100	200
Arundo	1.22 ± 0.21	1.00 ± 0.07	0.75 ± 0.05	18.37	38.11
Axonopus	1.00 ± 0.12	0.78 ± 0.04	0.60 ± 0.01	21.72	39.94
Capillipedium	0.72 ± 0.09	0.55 ± 0.02	0.27 ± 0.01	24.20	61.93
Chrysopogon	0.78 ± 0.11	0.70 ± 0.04	0.53 ± 0.02	10.29	32.49
Cynodon	1.17 ± 0.22	1.09 ± 0.08	1.00 ± 0.03	7.36	15.00
Digitaria	0.91 ± 0.08	0.8 ± 0.04	0.78 ± 0.03	11.86	14.11
Arundinella	1.29 ± 0.08	1.02 ± 0.08	0.71 ± 0.05	21.33	44.58
Eragrostis	0.94 ± 0.07	0.82 ± 0.07	0.65 ± 0.01	12.61	30.33
Imperata	1.35 ± 0.14	1.28 ± 0.11	1.26 ± 0.08	5.67	6.59
Oplismenus	1.45 ± 0.17	1.22 ± 0.15	1.12 ± 0.12	15.82	22.35
Setaria	0.80 ± 0.11	0.80 ± 0.04	0.33 ± 0.01	0.45	57.98
Thysanolaena	0.81 ± 0.08	0.80 ± 0.02	0.62 ± 0.02	1.52	23.38

Values for chlorophyll concentration are mean ± SD (n = 3). Greater values of salt sensitivity index denote greater sensitivity or susceptibility to NaCl, whereas lower values denote lesser sensitivity.

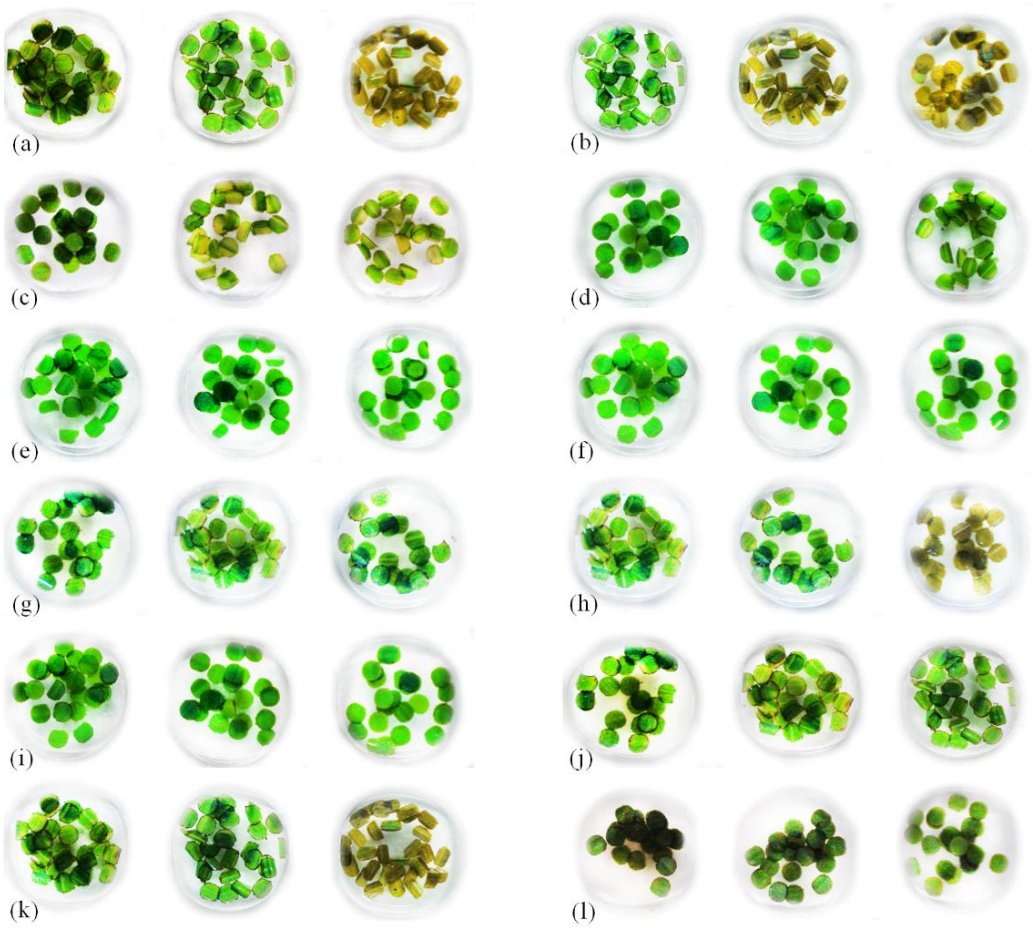

Figure 2. Leaf disc senescence bioassay: Phenotypic changes observed as chlorophyll bleaching occurs in response to 0, 100 and 200 mM NaCl treatment (left to right) after 96 h. (a) *Arundo*; (b) *Axonopus*; (c) *Capillipedium*; (d) *Chrysopogon*; (e) *Cynodon;* (f) *Digitaria*; (g) *Arundinella*; (h) *Eragrostis*; (i) *Imperata*; (j) *Oplismenus*; (k) *Setaria*; and (l) *Thysanolaena*.

Table 2. Relative water content (%) of grasses under treatment of 0, 100 and 200 mM NaCl solutions for 3, 6 and 9 days.

Grass	Concentration of NaCl (mM/L) and duration of treatment								
	3 days[1]			6 days[2]			9 days[3]		
	0	100	200	0	100	200	0	100	200
Arundo	85.2 ±1.1	80.6 ±2.1	78.5 ±1.1	84.1 ±0.9	77.5 ±0.6	71.2 ±1.4	84.6 ±1.2	70.2 ±0.8	66.5 ±2.2
Axonopus	84.5 ±1.2	78.6 ±2.3	74.1 ±1.7	83.2 ±1.5	76.5 ±0.7	74.2 ±0.9	83.2 ±1.3	73.1 ±1.1	68.6 ±0.4
Capillipedium	85.2 ±1.4	77.3 ±1.8	76.5 ±1.3	86.6 ±1.2	75.4 ±1.2	72.3 ±0.8	85.5 ±2.1	75.5 ±1.1	70.1 ±0.9
Chrysopogon	82.1 ±0.9	74.3 ±1.2	72.1 ±2.3	81.8 ±0.8	73.2 ±1.5	69.4 ±0.6	82.6 ±2.2	66.5 ±1.5	60.7 ±1.1
Cynodon	91.5 ±0.8	89.6 ±1.5	87.2 ±2.5	90.2 ±1.3	87.2 ±1.1	82.9 ±1.8	90.7 ±1.2	84.2 ±1.7	81.5 ±0.8
Digitaria	84.1 ±1.2	78.6 ±2.4	74.5 ±1.2	83.9 ±2.1	77.2 ±0.8	68.9 ±0.9	85.8 ±1.5	74.3 ±1.3	61.2 ±0.6
Arundinella	80.1 ±2.1	75.5 ±1.2	72.5 ±2.5	81.5 ±2.3	73.2 ±1.2	70.8 ±0.7	80.6 ±1.5	70.4 ±2.1	65.4 ±1.4
Eragrostis	85.1 ±1.9	81.2 ±1.1	79.6 ±2.6	83.2 ±1.8	76.7 ±1.6	72.1 ±1.2	84.1 ±1.8	71.2 ±2.4	63.1 ±0.7
Imperata	82.5 ±1.4	80.2 ±0.9	78.2 ±1.6	81.9 ±0.9	79.2 ±1.8	77.6 ±1.8	80.5 ±0.9	76.1 ±1.5	75.9 ±0.9
Oplismenus	87.3 ±0.8	80.5 ±0.9	77.6 ±1.4	86.5 ±1.4	78.2 ±0.8	74.6 ±1.9	85.9 ±1.1	76.1 ±0.8	72.3 ±1.1
Setaria	82.4 ±0.6	77.5 ±1.2	74.1 ±0.7	80.5 ±1.2	74.1 ±1.1	70.6 ±0.3	80.5 ±2.1	71.1 ±0.6	62.3 ±1.3
Thysanolaena	86.5 ±1.1	80.5 ±1.7	76.2 ±1.2	87.1 ±2.2	74.5 ±0.7	70.2 ±1.6	85.2 ±1.5	70.7 ±1.3	64.2 ±1.8

[1]LSD (P≤0.05) Species = 2.23; Treatment = 1.12. [2]LSD (P≤0.05) Species = 3.41; Treatment = 1.7. [3]LSD (P≤0.05) Species = 5.19; Treatment = 2.59. Values represent mean ± SD, where n = 3.

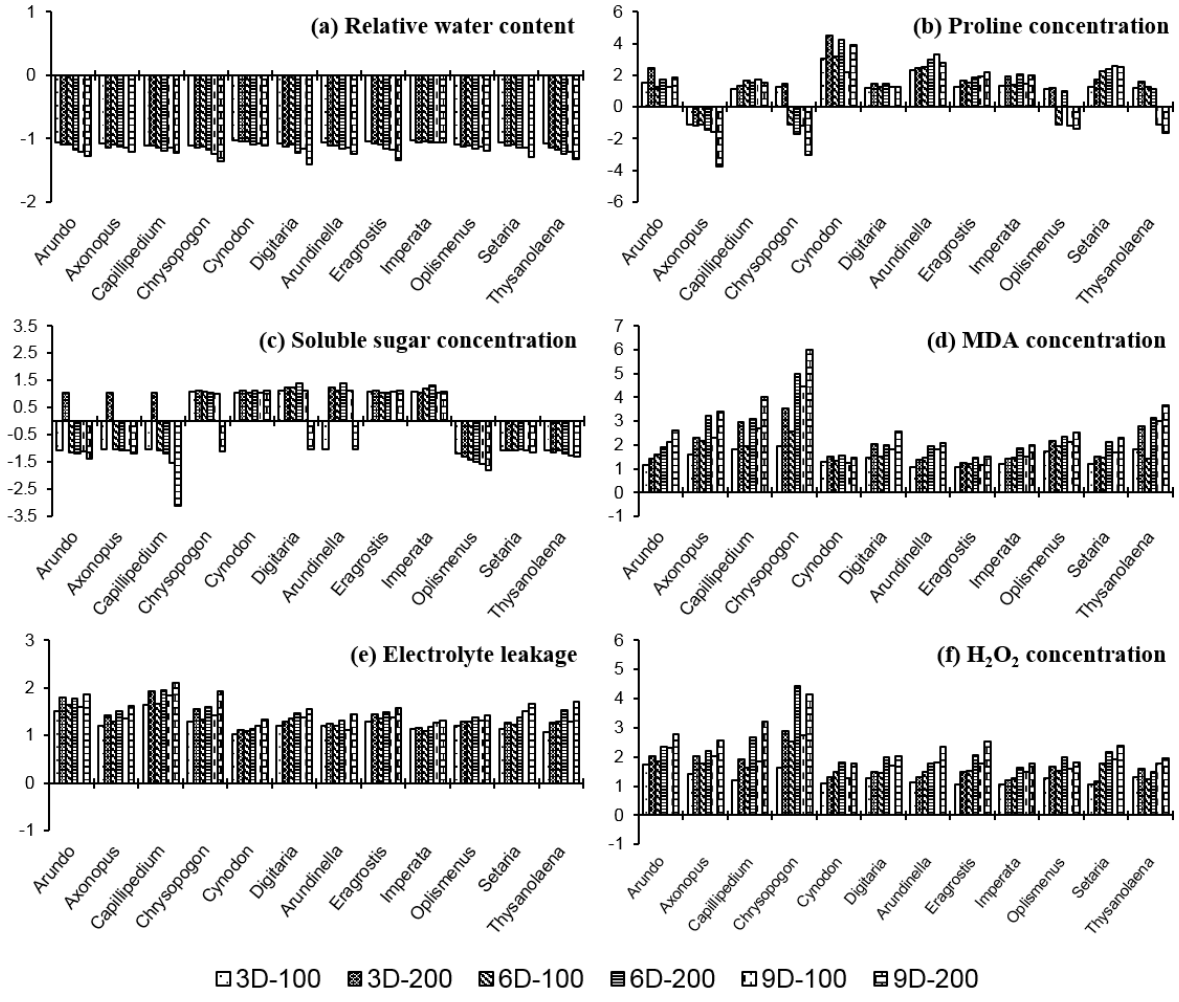

Figure 3. Fold change values of the biochemical markers in grasses subjected to NaCl stress. (a) Relative water content; (b) Proline concentration; (c) Soluble sugar concentration; (d) MDA concentration; (e) Electrolyte leakage; and (f) H_2O_2 concentration. 3D, 6D and 9D represent the duration of exposure to NaCl solutions (days) and 100 and 200 represent the concentrations of NaCl (mM/L).

Table 3. Proline concentration (µg/g fwt) in grasses under treatments of 0, 100 and 200 mM NaCl solutions for 3, 6 and 9 days.

Grass	Concentration of NaCl (mM/L) and duration of treatment								
	3 days[1]			6 days[2]			9 days[3]		
	0	100	200	0	100	200	0	100	200
Arundo	40.5 ±0.8	60.8 ±0.3	98.3 ±0.1	45.2 ±0.4	57.9 ±0.5	78.2 ±0.9	42.5 ±0.2	55.2 ±0.4	78.4 ±1.6
Axonopus	32.3 ±0.7	29.8 ±0.1	27.4 ±0.7	30.2 ±0.3	26.5 ±0.2	20.7 ±0.1	30.5 ±0.3	19.7 ±0.1	8.2 ±0.1
Capillipedium	39.3 ±0.7	45.2 ±0.2	51.3 ±0.7	35.9 ±0.4	60.4 ±0.6	55.2 ±0.8	36.6 ±0.1	64.2 ±0.2	56.1 ±1.1
Chrysopogon	12.2 ±0.2	15.3 ±0.2	17.8 ±0.1	11.9 ±0.1	10.8 ±0.1	7.1 ±0.5	10.6 ±0.7	8.8 ±0.4	3.5 ±0.2
Cynodon	48.1 ±1.2	145.3 ±2.1	215.1 ±2.5	50.1 ±0.7	160.2 ±1.5	210.8 ±2.3	45.8 ±0.2	100.3 ±1.3	178.2 ±1.4
Digitaria	65.3 ±1.1	78.9 ±1.7	95.6 ±1.5	63.2 ±1.1	80.6 ±1.1	90.7 ±1.5	66.1 ±0.2	82.6 ±1.4	85.1 ±0.9
Arundinella	30.5 ±0.8	70.1 ±1.1	75.2 ±1.5	34.2 ±0.6	86.1 ±0.9	102.5 ±1.6	32.1 ±0.1	107.1 ±1.5	90.2 ±1.3
Eragrostis	40.5 ±0.7	51.2 ±0.9	68.7 ±1.1	42.5 ±0.6	65.4 ±0.8	79.8 ±0.9	44.4 ±0.2	86.5 ±1.5	97.3 ±1.5
Imperata	63.3 ±0.9	83.5 ±1.1	120.2 ±0.9	60.7 ±0.1	85.2 ±0.9	125.3 ±1.3	65.4 ±0.4	96.9 ±1.6	132.1 ±1.1
Oplismenus	23.1 ±0.5	25.6 ±0.7	27.1 ±0.5	22.7 ±0.3	20.1 ±0.5	23.5 ±0.3	20.9 ±0.1	17.6 ±0.4	15.2 ±0.5
Setaria	12.2 ±0.1	15.5 ±0.3	20.8 ±0.4	14.3 ±0.3	32.1 ±0.2	34.5 ±0.1	10.6 ±0.7	27.6 ±0.4	26.7 ±0.1
Thysanolaena	25.6 ±0.3	30.8 ±0.4	41.1 ±0.8	23.2 ±0.2	28.7 ±0.3	26.2 ±0.5	20.2 ±0.6	17.8 ±0.7	12.5 ±0.1

[1]LSD (P≤0.05) Species = 40.82; Treatment = 20.41. [2]LSD (P≤0.05) Species = 41.82; Treatment = 20.91. [3]LSD (P≤0.05) Species = 40.15; Treatment = 20.07. Values represent Mean ± SD, where n = 3.

Total sugar concentration. Concentration of sugars in untreated fresh leaves varied from 16.1 mg/g (*Capillipedium*) to 56.9 mg/g (*Eragrostis*). Changes in concentration followed no consistent pattern across the various grasses subjected to NaCl treatments (Table 4; Figure 3c), with some showing decreases while a few showed increases. Those showing greatest decreases were *Capillipedium* (69% decrease) and *Oplismenus* (45% decrease), with most of the grass species showing little change in sugar concentration over the 9 days, even at 200 mM NaCl.

Membrane lipid peroxidation. MDA concentration in untreated fresh leaves varied from 2.2 mM/g (*Chrysopogon*) to 11.9 mM/g (*Arundo*). Concentrations showed a consistent pattern, increasing across all concentrations and durations of NaCl treatment in all grasses with greater responses to increasing concentration than to increasing duration of exposure (Table 5; Figure 3d). After 9 days, greatest increases in MDA concentration occurred in *Chrysopogon* (5-fold), *Capillipedium* (3-fold) and *Axonopus* (2.4-fold).

Table 4. Soluble sugar concentration (mg/g fwt) in grasses under treatments of 0, 100 and 200 mM NaCl solutions for 3, 6 and 9 days.

Grass	Concentration of NaCl (mM/L) and duration of treatment								
	3 days[1]			6 days[2]			9 days[3]		
	0	100	200	0	100	200	0	100	200
Arundo	35.2 ±0.7	33.1 ±0.3	36.7 ±0.1	34.1 ±0.2	30.2 ±0.1	28.9 ±0.1	33.9 ±0.1	31.1 ±0.1	24.6 ±0.1
Axonopus	50.1 ±1.5	48.9 ±1.5	52.1 ±0.6	47.8 ±1.4	46.8 ±0.8	45.1 ±1.2	47.5 ±0.9	44.3 ±1.2	40.1 ±1.1
Capillipedium	15.6 ±0.2	14.9 ±0.1	16.5 ±0.2	16.1 ±0.1	15.1 ±0.1	13.4 ±0.3	16.7 ±0.1	10.9 ±0.1	5.4 ±0.1
Chrysopogon	32.1 ±0.1	34.4 ±0.4	36.7 ±0.3	30.5 ±0.2	33.1 ±0.2	31.6 ±0.2	30.9 ±0.2	31.5 ±0.2	27.8 ±0.1
Cynodon	40.1 ±0.1	42.1 ±1.4	45.3 ±0.2	41.8 ±0.5	43.2 ±0.8	46.3 ±1.4	40.5 ±0.9	42.6 ±1.1	44.9 ±1.2
Digitaria	35.4 ±0.2	40.1 ±0.6	44.3 ±1.4	34.6 ±0.3	43.2 ±1.3	47.6 ±1.6	36.1 ±0.2	40.5 ±1.3	35.5 ±0.2
Arundinella	29.8 ±0.1	28.6 ±0.1	36.5 ±0.5	27.6 ±0.1	31.5 ±0.2	38.7 ±0.2	30.5 ±0.2	34.2 ±0.3	29.9 ±0.1
Eragrostis	56.1 ±1.1	60.3 ±0.7	62.3 ±0.7	57.8 ±1.3	60.5 ±1.5	61.4 ±0.2	56.8 ±0.6	61.3 ±0.5	63.3 ±0.3
Imperata	33.2 ±0.9	36.1 ±0.5	35.3 ±0.2	30.8 ±0.2	36.6 ±0.4	40.9 ±1.5	33.3 ±0.3	34.5 ±0.6	35.7 ±0.7
Oplismenus	40.5 ±0.2	34.5 ±0.3	31.2 ±0.3	43.2 ±0.5	30.6 ±0.2	28.7 ±0.2	41.9 ±1.4	26.7 ±0.3	23.2 ±0.2
Setaria	49.2 ±1.1	46.5 ±1.2	45.5 ±1.5	47.8 ±1.2	44.4 ±1.1	46.5 ±0.3	47.7 ±0.5	44.3 ±0.3	41.1 ±1.2
Thysanolaena	36.5 ±0.5	34.2 ±0.3	31.3 ±0.1	35.5 ±0.2	33.3 ±0.2	29.8 ±0.3	37.7 ±0.2	30.1 ±0.2	28.9 ±0.3

[1]LSD (P≤0.05) Species = 4.96; Treatment = 2.48. [2]LSD (P≤0.05) Species = 7.24; Treatment = 3.62. [3]LSD (P≤0.05) Species = 6.92; Treatment = 3.46. Values represent Mean ± SD, where n = 3.

Table 5. MDA concentration (mM MDA/g fwt) of grasses under treatments of 0, 100 and 200 mM NaCl solutions for 3, 6 and 9 days.

Grass	Concentration of NaCl (mM/L) and duration of treatment								
	3 days[1]			6 days[2]			9 days[3]		
	0	100	200	0	100	200	0	100	200
Arundo	12.1 ±0.1	14.2 ±0.8	17.3 ±0.4	11.3 ±0.2	18.2 ±0.8	21.6 ±0.3	12.3 ±0.1	26.1 ±0.6	32.3 ±0.2
Axonopus	10.1 ±0.1	16.2 ±0.2	23.1 ±0.2	10.6 ±0.4	23.1 ±0.4	34.2 ±0.3	11.1 ±0.3	25.6 ±0.4	37.6 ±0.4
Capillipedium	5.6 ±0.2	10.1 ±0.3	16.7 ±0.1	5.7 ±0.1	11.1 ±0.1	17.6 ±0.2	4.9 ±0.3	13.2 ±0.6	19.8 ±0.6
Chrysopogon	2.2 ±0.7	4.3 ±0.1	7.8 ±0.1	2.1 ±0.5	5.4 ±0.2	10.5 ±0.8	2.2 ±0.1	9.8 ±0.1	13.2 ±0.2
Cynodon	10.2 ±0.6	13.2 ±0.2	15.6 ±0.2	10.5 ±0.1	14.1 ±0.1	16.4 ±0.1	11.2 ±0.1	13.9 ±0.2	16.5 ±0.4
Digitaria	3.5 ±0.4	5.1 ±0.1	7.2 ±0.7	4.1 ±0.9	6.2 ±0.7	8.1 ±0.6	3.7 ±0.9	6.7 ±0.3	9.5 ±0.8
Arundinella	4.8 ±0.8	5.1 ±0.1	6.7 ±0.1	4.5 ±0.1	6.7 ±0.1	8.8 ±0.2	4.1 ±0.8	7.5 ±0.7	8.5 ±0.9
Eragrostis	8.6 ±0.6	9.1 ±0.1	10.7 ±0.6	8.1 ±0.4	9.7 ±0.3	11.8 ±0.4	8.8 ±0.5	10.1 ±0.1	13.4 ±0.6
Imperata	5.4 ±0.3	6.5 ±0.1	7.8 ±0.5	4.8 ±0.8	7.1 ±0.2	8.9 ±0.3	5.1 ±0.2	7.7 ±0.3	10.1 ±0.2
Oplismenus	9.8 ±0.5	17.1 ±0.2	21.3 ±0.7	9.5 ±0.1	18.6 ±0.1	22.5 ±0.1	10.1 ±0.2	21.3 ±0.3	25.4 ±0.4
Setaria	10.1 ±0.3	12.1 ±0.3	15.4 ±0.3	9.7 ±0.2	14.3 ±0.2	20.5 ±0.1	10.2 ±0.2	17.3 ±0.7	23.7 ±0.4
Thysanolaena	3.1 ±0.6	5.6 ±0.2	8.7 ±0.4	3.4 ±0.3	4.9 ±0.6	10.7 ±0.3	3.6 ±0.5	10.8 ±0.6	13.2 ±0.1

[1]LSD (P≤0.05) Species = 3.34; Treatment = 1.67. [2]LSD (P≤0.05) Species = 5.07; Treatment = 2.53. [3]LSD (P≤0.05) Species = 6.25; Treatment = 3.12. Values represent Mean ± SD, where n = 3.

Electrolyte leakage. Electrolyte leakage levels in untreated fresh leaves varied from 5.1% (*Arundinella*) to 15.5% (*Setaria*) and increased across all concentrations and durations of NaCl treatment in all grasses (Table 6; Figure 3e). *Arundo* and *Capillipedium* showed the greatest increases in electrolyte leakage with exposure to NaCl treatment with a much greater response to increasing concentration (80–90%) than to duration of exposure (10–24%). The lowest responses occurred with *Cynodon* and *Imperata*.

H_2O_2 concentration. Concentrations of H_2O_2 in untreated fresh leaves ranged from 2.4 µmol/g (*Chrysopogon*) to 11.8 µmol/g (*Digitaria* and *Thysanolaena*) and increased across all concentrations of and durations of exposure to NaCl solutions for all grasses (Table 7; Figure 3f). The most responsive grasses were *Chrysopogon*, *Capillipedium* and *Arundo*, while the least responsive were *Cynodon* and *Imperata*.

Hierarchical cluster analysis for the evaluation of NaCl tolerance

Based on the variable effects of NaCl treatment on biochemical parameters, the grasses were grouped according to their NaCl tolerance through hierarchical cluster analysis, where the fold change values of all parameters were taken into consideration (Figures 3a–3f).

Table 6. Electrolyte leakage (%) of grasses under treatments of 0, 100 and 200 mM NaCl solutions for 3, 6 and 9 days.

Grass	Concentration of NaCl (mM/L) and duration of treatment								
	3 days[1]			6 days[2]			9 days[3]		
	0	100	200	0	100	200	0	100	200
Arundo	14.1 ±0.5	21.2 ±0.1	25.4 ±0.5	14.1 ±0.6	23.1 ±0.3	24.9 ±0.2	14.3 ±0.2	22.9 ±0.4	26.7 ±0.3
Axonopus	10.1 ±0.7	12.2 ±0.3	14.3 ±0.3	10.3 ±0.3	13.4 ±0.2	15.6 ±0.3	10.6 ±0.3	14.3 ±0.6	17.2 ±0.3
Capillipedium	8.7 ±0.1	14.3 ±0.5	16.7 ±0.6	9.1 ±0.3	15.1 ±0.3	17.8 ±0.3	8.8 ±0.3	16.2 ±0.6	18.6 ±0.3
Chrysopogon	5.2 ±0.5	6.7 ±0.3	8.1 ±0.3	6.1 ±0.5	8.2 ±0.4	9.7 ±0.3	5.5 ±0.3	7.8 ±0.3	10.6 ±0.2
Cynodon	11.9 ±0.9	12.1 ±0.4	13.2 ±0.5	12.2 ±0.4	13.2 ±0.5	13.9 ±0.3	10.8 ±0.4	12.9 ±0.4	14.3 ±0.4
Digitaria	11.2 ±0.8	13.4 ±0.3	14.5 ±0.3	10.7 ±0.7	14.5 ±0.5	15.6 ±0.3	11.1 ±0.4	15.2 ±0.2	17.2 ±0.3
Arundinella	5.2 ±0.6	6.2 ±0.2	6.5 ±0.2	5.1 ±0.2	6.1 ±0.3	6.7 ±0.1	4.9 ±0.3	5.5 ±0.3	7.1 ±0.3
Eragrostis	10.1 ±0.9	13.1 ±0.2	14.5 ±0.3	10.4 ±0.3	14.2 ±0.4	15.4 ±0.2	10.6 ±0.3	14.5 ±0.2	16.7 ±0.4
Imperata	14.3 ±1.1	16.1 ±0.3	16.5 ±0.3	14.5 ±0.4	15.8 ±0.3	17.2 ±0.3	14.9 ±0.4	18.8 ±0.3	19.7 ±0.3
Oplismenus	13.4 ±0.7	16.1 ±0.4	17.2 ±0.4	13.1 ±0.3	16.8 ±0.2	18.1 ±0.4	13.4 ±0.2	17.5 ±0.2	19.2 ±0.6
Setaria	15.1 ±0.8	17.2 ±0.2	19.3 ±0.4	15.4 ±0.2	18.9 ±0.4	21.3 ±0.4	16.1 ±0.3	24.3 ±0.3	26.7 ±0.5
Thysanolaena	7.6 ±0.6	8.1 ±0.1	9.7 ±0.5	7.3 ±0.3	9.5 ±0.3	11.2 ±0.3	7.8 ±0.4	10.1 ±0.4	13.4 ±0.4

[1]LSD (P≤0.05) Species = 2.6; Treatment = 1.3. [2]LSD (P≤0.05) Species = 2.53; Treatment = 1.27. [3]LSD (P≤0.05) Species = 2.82; Treatment = 1.41. Values represent Mean ± SD, where n = 3.

Table 7. H_2O_2 concentration (µmol/g fwt) in grasses under treatment with 0, 100 and 200 mM NaCl solutions for 3, 6 and 9 days.

Grass	Concentration of NaCl (mM/L) and duration of treatment								
	3 days[1]			6 days[2]			9 days[3]		
	0	100	200	0	100	200	0	100	200
Arundo	6.5 ±0.1	11.2 ±0.2	13.1 ±0.3	6.6 ±0.4	12.3 ±0.2	15.6 ±0.1	6.7 ±0.3	15.4 ±0.3	18.7 ±0.5
Axonopus	7.2 ±0.3	10.2 ±0.3	14.5 ±0.2	8.1 ±0.1	14.3 ±0.2	17.8 ±0.2	8.3 ±0.1	16.7 ±0.1	21.3 ±0.3
Capillipedium	4.5 ±0.2	5.4 ±0.4	8.7 ±0.2	4.1 ±0.2	6.7 ±0.1	10.9 ±0.3	4.8 ±0.3	8.8 ±0.1	15.4 ±0.5
Chrysopogon	2.5 ±0.8	4.1 ±0.2	7.2 ±0.4	2.1 ±0.3	5.3 ±0.2	9.3 ±0.4	2.7 ±0.1	7.4 ±0.5	11.2 ±0.2
Cynodon	10.1 ±0.7	11.2 ±0.2	13.2 ±0.5	9.7 ±0.4	14.5 ±0.3	17.6 ±0.3	10.3 ±0.4	13.2 ±0.5	18.1 ±0.3
Digitaria	12.1 ±0.8	15.4 ±0.3	17.8 ±0.3	11.7 ±0.5	17.1 ±0.4	23.1 ±0.5	11.9 ±0.1	20.1 ±0.4	24.3 ±0.2
Arundinella	6.8 ±0.5	7.6 ±0.5	8.9 ±0.2	6.6 ±0.6	9.9 ±0.4	11.7 ±0.4	6.5 ±0.4	11.7 ±0.4	15.3 ±0.5
Eragrostis	4.5 ±0.4	4.7 ±0.2	6.7 ±0.2	4.1 ±0.3	6.2 ±0.5	8.4 ±0.1	4.3 ±0.2	7.6 ±0.3	10.9 ±0.2
Imperata	8.7 ±0.3	9.1 ±0.7	10.3 ±0.3	8.2 ±0.3	10.3 ±0.3	13.4 ±0.3	8.6 ±0.4	12.9 ±0.5	15.2 ±0.3
Oplismenus	11.3 ±0.4	14.3 ±0.2	18.7 ±0.3	10.9 ±0.2	16.5 ±0.2	21.8 ±0.1	11.1 ±0.1	17.6 ±0.3	20.1 ±0.5
Setaria	8.5 ±0.5	9.1 ±0.3	9.8 ±0.2	8.1 ±0.3	14.3 ±0.2	17.6 ±0.2	7.9 ±0.8	15.1 ±0.2	18.9 ±0.3
Thysanolaena	11.1 ±0.9	14.5 ±0.2	17.6 ±0.1	12.2 ±0.6	15.2 ±0.3	18.1 ±0.3	12.1 ±0.7	21.5 ±0.3	23.8 ±0.1

[1]LSD (P≤0.05) Species = 2.1; Treatment = 1.05. [2]LSD (P≤0.05) Species = 2.19; Treatment = 1.09. [3]LSD (P≤0.05) Species = 2.42; Treatment = 1.21. Values represent Mean ± SD, where n = 3.

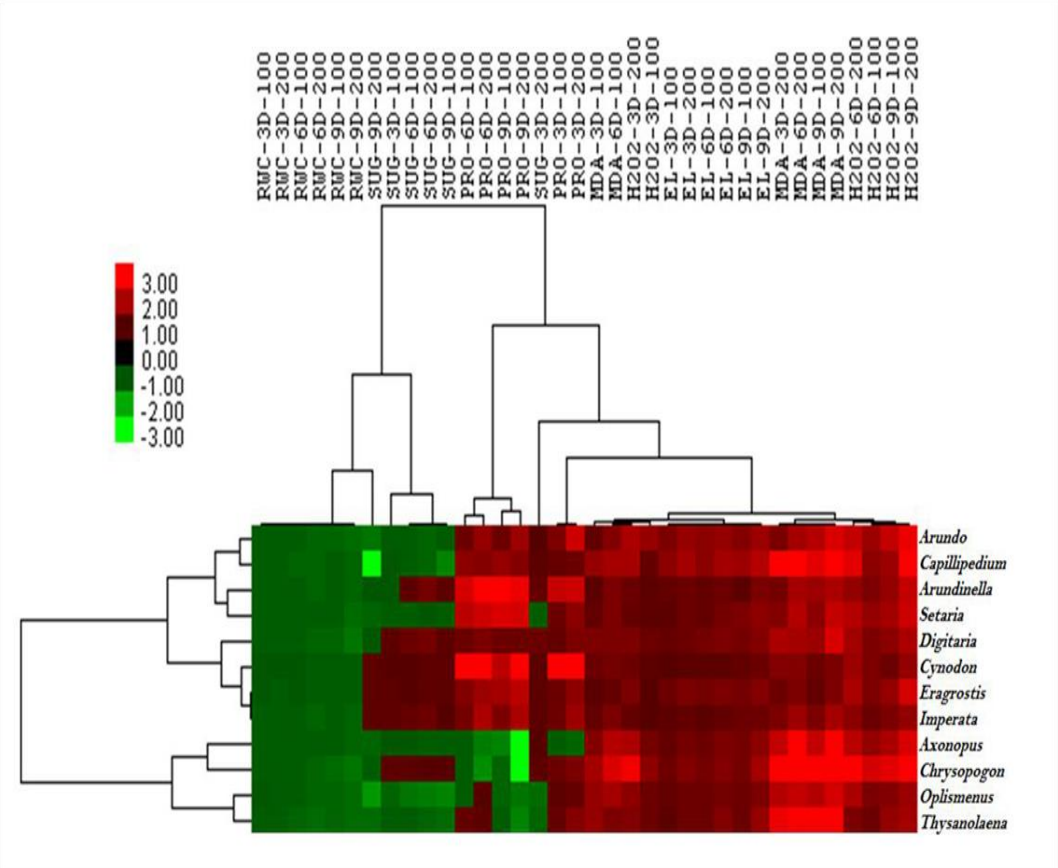

Figure 4. Hierarchical cluster analysis of the grasses using the fold change values of relative water content (RWC); proline concentration (PRO); soluble sugar concentration (SUG); membrane lipid peroxidation (malondialdehyde, MDA); electrolyte leakage (EL); and H_2O_2 concentration after NaCl treatments (100 mM and 200 mM) for 3, 6 and 9 days. Resulting tree figure was displayed using Java Treeview after hierarchical cluster analysis through CLUSTER 3.0. The color grids in the cluster analysis represent the relative fold change values (-3 to +3 shown by different colors) of the specific biochemical markers for each of the individual grasses. For the analysis of salt tolerance, the greenness of the grids for biomarkers like MDA, EL and H_2O_2 and redness for RWC, PRO and SUG was considered; which means a species for which the grids are more reddish for RWC, PRO and SUG and less greenish for MDA, EL and H_2O_2 could be considered the most tolerant of all. However, this was easily recognized in the cluster analysis due to grouping of the studied species on the basis of their responses to biochemical markers.

The ranges of fold change values in the clusters are represented by the colored bars. Results suggested the probable interrelations among biochemical parameters subjected to NaCl stress and variable salt tolerance between all grass genera.

Based on their salt sensitivities, the grasses formed 2 distinct groups (Figure 4). One group was comprised of *Axonopus*, *Chrysopogon*, *Oplismenus* and *Thysanolaena*. The remaining grasses with varying response patterns to NaCl solutions formed the second group and were classified into 3 subgroups: *Arundo* and *Capillipedium; Arundinella* and *Setaria*; and *Digitaria*, *Cynodon*, *Eragrostis* and *Imperata*.

Discussion

This rapid screening for salinity tolerance in the forage grasses has been attempted as a simple method of identifying the most salt-tolerant grasses for introduction into areas with increasing soil salinity and decreasing productivity. Previously, Zulkaliph et al. (2013) in their studies with turfgrasses ranked the different species of grasses for salinity tolerance on the basis of shoot and root growth, leaf firing, i.e. yellowing of leaves resulting from cell death due to osmotic imbalances, turf color and turf quality. We estimated salinity tolerance of the grasses primarily by a salt sensitivity index (SSI), determined by evaluating the effects of NaCl solutions on leaf discs over 96 hours. This type of bioassay has been used previously in several transgenesis experiments to evaluate the tolerances of transgenic plants relative to the wild type plants from which they were bioengineered (Bhaskaran and Savithramma 2011; Yadav et al. 2012).

The amount of chlorophyll leached out from the leaf discs into the NaCl solution was used as an indicator of the effect of NaCl on leaf tissues. The decrease in chlorophyll concentration in plants subjected to NaCl treatment has been inversely correlated with salinity tolerance. For instance, the decrease in Chlorophyll a: Chlorophyll b ratio in salt-tolerant *Najas graminea* was lower than in *Hydrilla verticillata* and *Najas indica* (Rout et al. 1997). In the present study, we quantified the amount of chlorophyll in the leaf discs in both control and treatment sets and the values were used to reciprocate the sensitivity of grasses towards NaCl treatment. Greater salt sentivity index values denoted greater susceptibility of the grasses towards NaCl. Overall, the results of the bioassay indicated that among the grasses tested, *Imperata*, *Cynodon* and *Digitaria* could be considered as less sensitive or resistant on the basis of SSI values at 100 and 200 mM NaCl. SSI therefore presents an easy and rapid technique to screen out the potential salt-tolerant forage grasses.

The 6 biomarkers we selected to analyze the salt-tolerance potential of the forage grasses, namely relative water content (RWC), proline and soluble sugar concentrations, membrane lipid peroxidation, electrolyte leakage and H_2O_2 concentration, proved useful in indicating differences between species in ability to tolerate saline conditions both simply and rapidly.

While RWC of any plant always decreases with the increase in NaCl concentration, a lower decrease in RWC is a valuable marker in the selection of salt-tolerant species (Ziaf et al. 2009). In our study, lowest decreases in RWC were observed in *Cynodon*, *Eragrostis* and *Imperata* across all concentrations and durations of NaCl treatments, identifying them as salt-tolerant species. In contrast, accumulation of proline and soluble sugars is considered to be positively correlated with salinity tolerance (Karsensky and Jonak 2012; Hayat et al. 2012). Accumulation of higher levels of proline has been reported in the halophytes, *Mesembryanthemum crystallinum* and *Sporobolus virginicus* when compared with the glycophytes carrot and rice (Thomas et al. 1992; Tada et al. 2014). In the present study, apart from *Axonopus*, *Chrysopogon* and *Oplismenus*, proline accumulation increased in all grasses subjected to NaCl treatment. We also observed that soluble sugar accumulation decreased in *Arundo*, *Axonopus*, *Capillipedium*, *Oplismenus*, *Setaria* and *Thysanolaena* across all concentrations of NaCl and durations of exposure. In contrast, accumulation of soluble sugars increased in *Digitaria*, *Imperata* and *Arundinella* subjected to NaCl treatments for 3, 6 and 9 days. Nedjimi (2011) also correlated the accumulation of greater amounts of soluble sugars in the forage grass *Lygeum spartum* with osmotic adjustment and protection of membrane stability that conferred salinity tolerance.

Increase in malondialdehyde (MDA) concentration, an indication of lipid peroxidation, is considered unfavorable for plant health, and plants, which show little increase in MDA concentration when exposed to NaCl, are considered to be salt-tolerant (Miller et al. 2010). Marked increases in MDA concentration were observed in *Axonopus*, *Capillipedium*, *Chrysopogon* and *Thysanolaena*, following exposure to salt. However, minimal increase was observed in *Cynodon* and *Eragrostis* across all concentrations and durations of treatment.

Similarly, low electrolyte leakage (EL) and limited increase in H_2O_2 concentration in response to NaCl treatment are also considered as markers of the salt

tolerance of plants (Mostafa and Tammam 2012). Accumulation of H_2O_2 in plants interferes with the normal biochemical processes inside plants. In the present study, EL in all grasses increased with the increase in NaCl concentration and duration of treatment. Least EL was observed in *Cynodon*, *Imperata* and *Arundinella*, which could be considered salt-tolerant species in comparison with the other grasses. The high increases in H_2O_2 concentration observed in *Arundo*, *Axonopus*, *Capillipedium* and *Chrysopogon* indicate that these species can be considered susceptible to salination on the basis of this trait. Comparatively, low increases in H_2O_2 concentration observed in *Imperata*, *Setaria* and *Cynodon* indicate that they can be considered salt-tolerant.

Finally, hierarchical cluster analysis using the software CLUSTER 3.0 was used to represent the inter-relations among the physiological parameters and to align the grasses on the basis of their salinity tolerance as a similar type of hierarchical cluster analysis has been performed to evaluate the natural variation in drought tolerance in bermuda grass (Shi et al. 2012) and the variation in salt tolerance in rice cultivars (Chunthaburee et al. 2016). In the present study we utilized the relative fold change values of all the parameters in forming clusters. Based on the variations of the physiological parameters, all grasses were grouped according to their NaCl tolerance that could be interpreted with the aid of the fold change values denoted by colored bars. The relationships between the physiological parameters themselves was also illustrated in the cluster analysis. The grasses were clearly divided into 2 groups - a susceptible group (*Axonopus*, *Chrysopogon*, *Oplismenus* and *Thysanolaena*) and a relatively salt-tolerant group containing the remaining grasses. Critical analysis of the second group revealed 3 subgroups of less tolerant (*Arundo* and *Capillipedium*), moderately tolerant (*Arundinella* and *Setaria*) and tolerant grasses (*Digitaria*, *Cynodon*, *Eragrostis* and *Imperata*). These results are in accordance with the findings of other workers who reported the use of some of these and other related, tolerant grasses for the reclamation and utilization of saline soils and increased forage production (Kaffka 2001; Weber and Hanks 2006).

Based on the results of hierarchical clustering, we conclude that *Imperata cylindrica*, *Eragrostis amabilis*, *Cynodon dactylon* and *Digitaria ciliaris* were relatively salt-tolerant. SSI values individually pointed towards the superior salt-tolerance of *Imperata*, *Digitaria* and *Cynodon*, whereas proline concentration indicated marked tolerance in *Cynodon*, *Arundinella*, *Imperata*, *Eragrostis* and *Setaria*. If we consider the MDA concentrations, *Cynodon*, *Arundinella*, *Imperata* and *Eragrostis* could be considered salt-tolerant. Thus, while individual biochemical markers provide good indications of the degree of salt tolerance of a species, cluster analysis, which incorporates the results with several biomarkers, provides a much more reliable indication. However, SSI values can provide an easy and rapid tool for the screening of salt tolerance. Based on our screening results, we consider that the selective propagation of the most salt-tolerant species could be utilized for the rejuvenation of native grasslands and also for the reclamation of salinity infested wastelands.

Acknowledgments

The authors are grateful to CSIR, New Delhi (Award No. 09/285(0046)/2008-EMR-I) and UGC, ERO, Kolkata (Minor Research Project No. PSW-80/12-13) for the financial support which enabled the carrying out of this work.

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(Received for publication 25 August 2016; accepted 3 June 2017; published 30 September 2017)