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Salt stress induced alteration in growth characteristics of a grass *Pennisetum alopecuroides*

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Abstract

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. The present investigation was carried out to study the alterations in the growth characteristics of a grass species, *Pennisetum alopecuroides* under the influence of sodium chloride (NaCl) salinity. From the results it is clear that shoot length of *Pennisetum alopecuroides* was increased by 13.17% at 100 mM NaCl concentration while the root length was observed to be increased at 50 mM NaCl concentration by 26.93% . Maximum height of the plant was observed by 18.23% at 50 mM while shoot to root ratio was higher at 300 mM concentrations by 29.17% increase over the control. Moreover, the maximum percent increase in leaf area was recorded as 11.17% (100 mM). Fresh weight was increased by 50.92 % at 100 mM while dry weight of the experimental grass was increased by 33.64 % at the same concentration of salt to the rooting medium while moisture percentage was increased to a maximum by 24.61% at 50 mM. It appears that the grass species studied exhibit a moderate salinity tolerance as far as linear growth of plant is concerned.

Key words

Growth, *Pennisetum alopecuroides*, Sodium chloride, Salinity stress

Introduction

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality (Ouda, 2008) with increasing impact on the socio-economic fabric and health, especially of the farming communities. Sustainable management of the soil resource is one of the central issues worldwide for the welfare of the future generations (Munns, 2003). Statistics about the extent of salt affected areas vary according to authors, but estimates are in general close to one billion hectares, representing about 6% of the earth's continental extent. In addition to these naturally salt affected areas, about 77 million ha have been salinised by human activities (Ghassemi *et al*., 1995). Introduction of irrigation without proper drainage system, industrial effluents, overuse of fertilisers, removal of natural plant cover, flooding with salt rich waters, high water table and use of poor quality groundwater are some of the main reasons behind the degradation of soil due to salinity (Parida and Das, 2005). According to Fitter and Hay (1987), plants resist salinity by four different ways 1) phenological escape (seasonal adaptation) 2) exclusion (selective absorption) often seen in halophytes 3) amelioration (selective localisation) and 4) tolerance (stress acceptance).

Growth analysis is a fundamental characteristic to study plant's response to an environmental stress (Mane *et al*., 2010). Physiologically and genetically salt tolerance is complex among the variety of plants with a wide range of adaptations in halophytes and less tolerant plants (Flowers, 2004, Mane *et al*., 2010). Photosynthesis is one of the most important biochemical pathways by which plants prepare their own food material and grows (Kojo, 2004). Oxidative stress due to NaCl might contribute to the deleterious effects of salt and significant growth reductions in plants (Hernandez *et al*., 1999). Water concentrations of leaves and shoots increases significantly in plants grown at optimal levels of salinity than in plants grown at lower or higher salinities (Hester and O'leary, 2003). As a matter of fact, there has been knowledge on increase of chlorophyll content in saline environment depending on salt levels (Romero-Aranda *et al*., 2001). The total chlorophyll content decreases under NaCl salinity stress in salt stressed sorghum and maize plants. Levels of polyphenols also increases under increasing levels of salinity, which shows that the induction of secondary metabolism is one of the defence mechanisms adapted by the plants to face saline environment.

Salinity has both osmotic and also specific ion effects on plant growth (Dionisio-Sese and Tobita, 2000). The accumulation of toxic ions, Na^+ and Cl^-, in the plants is often claimed to be toxic and the main cause of growth inhibition induced by salinity (Muscolo *et al*., 2003). In general, grasses have ability to grow at various contaminated sites and have extensive root system that can firmly hold the soil and withstand under adverse environmental conditions and with this view *Pennisetum alopecuroides* was selected as an experimental species. It is native to Eastern Asia and Australia. It is commonly known as swamp foxtail or fountain grass and belongs to the grass family graminae (poaceae). Fountain grass is a warm season ornamental grass which typically grows in graceful, spreading clumps (3 to 4 feet high and the same diameter) with arching leaves draping to the ground. Foliage and attractive flower spikes of this ornamental grass provide excellent texture, colour and contrast to borders, foundations and open areas. It also grows well in moist locations such as peripheries of water gardens, ponds or streams. The present work aims to study the alterations in the growth characteristics of *Pennisetum alopecuroides* under the influence of sodium chloride salinity which will be useful to develop a salt tolerant grass species and can have better insights into phytoremediation of saline soil.

Materials and Methods

The seedlings of grass *Pennisetum alopecuroides* (L.) Spreng var. Mourdy were collected from government nursery, Kagal (Kolhapur, Maharashtra). The seedlings were uniformly cut to a minimum height of 3 cm required for their growth and were transplanted into the earthen pots (30 cm height with a narrow base) to grow and establish under normal conditions with proper irrigation. After 4 weeks of their normal growth salinity stress was commenced. The plants were treated with increasing concentrations of sodium chloride *i.e.* 25, 50, 100, 200 and 300 mM. Different sodium chloride concentrations were prepared in tap water and every alternate day, in the morning 2 l of solutions was applied to the rooting medium. The day before treatment plants were also watered with 2 l of tap water to maintain the homogeneous distribution of salt levels in the pot soil. This was also done to cope up the loss of water by evaporation from the pot soil and by transpiration from the plant surface.

Ten plants from each treatment pot were carefully uprooted and washed thoroughly with water to remove any dirt and dust particles on the surface of the plant parts and blotted to surface dry. The growth analysis was carried out after 10 weeks. This plant material was analysed for their growth and development using various parameters such as root length (cm), shoot length (cm), shoot: root ratio, height of a plant (cm), average leaf area plant $^{-1}$ (cm^2). Meter scale and thread were used to measure the root length, shoot length and height of the plants. For biomass production the fresh weight (g), dry weight (g) and moisture content (%) of the randomly sampled ten leaves were recorded. Biomass of the roots and shoots were measured by using digital weight balance on the basis of wet weight and dry weight while leaf area was recorded by using a leaf area meter.

Statistical analysis of the data was carried out by using GraphPad software. Mean, standard deviation and percent variation was calculated. One - way analysis of variance (ANOVA) was tested for the parameters in order to see the statistical difference among the means of control values and means treatment values. Tukey-Kramer multiple comparison test of significance was carried out which suggested the variation among the column means is significant or not at different levels of significance. The data was analysed for three different levels of significance based on the 'p' values as * p = 0.01 - 0.05, ** p = 0.001 - 0.01, *** p < 0.001

Results and Discussion

According to He and Cramer (1993), growth analysis is fundamental to the characterisation of plant's response to an environmental stress. Shonjani (2002) observed the inhibition in root and in particular shoot growth with NaCl treatments for sugar beet, rice and cotton seedlings and a decrease in length of shoots was more pronounced at higher salt treatment (200 mM). From the results it is very clear that shoot length of *P. alopecuroides* was increased by 13.17% at 100 mM NaCl concentration while it was adversely affected by higher doses of salinity (Table 1). All the results obtained were statistically significant except at 25 mM when compared to the control. Significant decrease in shoot length of two maize cultivars C.6127 and DK.623 was observed by Cicek and Cakirlar (2002) under the influence of NaCl salinity. Bauci *et al*. (2003) and Khosravinejad *et al*. (2009) also noticed a significant decrease in shoot elongation in barley genotypes with increasing NaCl treatment. From the present results, it can be seen that the shoot length of the grass species was stimulated at lower levels of salinity and it appears that the grass species studied exhibit a moderate salinity tolerance as far as linear growth is concerned.

Munns *et al*. (2000) noticed that the salt tolerance of barley is correlated with the better root growth rates coupled with fast development and early flowering. From the results it can be seen that the root length of *P. alopecuroides* was observed to be increased at 50 mM NaCl concentration with an increase by 26.93 but later on showed a declining trend at higher levels of salinity and a perfect negative correlation with increasing levels of NaCl (Table 1). The values were significant at 25, 50, 100 and 300 mM salt concentration to the rooting medium when compared to the control. Stimulation of root length with increasing salt levels has been documented in many grass species, including St. Augustine grass (Meyer *et al*., 1989), barley (Bauci *et al*., 2003), *Sporobolus virginicus* (Marcum and Murdoch, 1992), *Atriplex griffithii* (Khan *et al*., 2000) and *Triticum aestivum* (Maghsoudi and Maghsoudi, 2008). From the present investigation, it is clear that the root growth of the selected grass species was more sensitive and severely affected at higher levels of salinity.

Table - 1: Effect of sodium chloride (NaCl) on growth characteristics of *Pennisetum alopecuroides*

Growth parameters	NaCl (mM)					
	Control	25	50	100	200	300
Shoot length (cm)	44.80(±2.66)	46.20(±3.79)	49.70**(±2.49)	50.10**(±3.48)	50.70**(±3.12)	49.80**(±2.70)
	0.0[a]	+3.13	10.94	11.83	13.17	11.16
Root length (cm)	37.50(±3.24)	41.60*(±2.99)	47.60***(±2.95)	42.90**(±2.42)	35.60(±1.96)	32.40**(±3.23)
	0.0[a]	10.93	26.93	14.40	-5.07	-13.60
Shoot : Root ratio	1.20(±0.03)	1.12(±0.16)	1.05(±0.10)	1.17(±0.05)	1.43***(±0.11)	1.55***(±0.16)
	0.0[a]	-6.67	-12.50	-2.50	19.17	29.17
Height of the plant (cm)	82.30(±5.89)	87.80(±1.99)	97.30***(±3.47)	93.0***(±5.50)	86.30(±4.14)	82.96(±4.96)
	0.0[a]	6.68	18.23	13.0	4.86	0.80
Average leaf area (cm^2)	15.22(±0.77)	15.63(±1.09)	16.33(±0.34)	16.75**(±1.0)	16.92***(±0.90)	16.66**(±0.78)
	0.0[a]	2.69	7.29	10.05	11.17	9.46
Fresh weight of leaves $plant^{-1}$ (g)	1.63(±0.24)	2.17*(±0.20)	2.42**(±0.24)	2.46(±0.26)	1.62(±0.24)	1.20(±0.23)
	0.0[a]	33.13	48.47	50.92	-0.61	-26.38
Dry weight of leaves $plant^{-1}$ (g)	1.07(±0.21)	1.33(±0.20)	1.39(±0.14)	1.43(±0.19)	1.06(±0.20)	0.85(±0.19)
	0.0[a]	24.30	29.91	33.64	-0.93	-20.56
Moisture content (%)	34.33(±3.03)	38.75(±3.95)	42.78*(±1.49)	42.12*(±1.68)	34.64(±2.79)	29.07(±2.96)
	0.0[a]	12.88	24.61	22.69	0.90	-15.32

[a] The values indicates percentage variation relative to control values. Each value is a mean of ten observations and ± values in parenthesis indicate standard deviation with significant at * p = 0.01 - 0.05, ** p = 0.001 - 0.01, *** p < 0.001

Table - 2: Electrical conductivity (EC) and total dissolved solids (TDS) of the soil treated with sodium chloride (1:10 soil suspension in water)

NaCl (mM)	EC (µS)	TDS (mg l^{-1})
Control	62.92(±1.79)0.0[a]	32.42(±0.96)0.0[a]
25	81.20*(±5.01)+29.05	48.18***(±2.39)+48.61
50	95.41***(±3.53)+51.63	52.84***(±2.43)+62.98
100	192.07***(±5.41)+205.25	104.94***(±2.97)+223.68
200	364.09***(±8.96)+478.62	221.70***(±2.42)+583.84
300	524.76***(±9.25)+733.97	313.77***(±4.31)+867.84

[a] = The values indicates percentage variation relative to control values. Each value is a mean of three observations and ± values in parenthesis indicate standard deviation with significant at * p = 0.01 - 0.05, ** p = 0.001 - 0.01, *** p < 0.001, growth characteristics, *Pennisetum alopecuroides*, salinity, sodium chloride

Bauci *et al*. (2003) found an average shoot to root ratio as 5.7 in S-5 barley genotype which was gradually declined to 2.9 with increasing NaCl concentrations. In the present investigation shoot to root ratio was reduced to a minimum by 12.50% at 50 mM NaCl concentration but later on increased with higher doses of salinity and were only significant at 200 and 300 mM concentration (Table 1). Tattini *et al*. (1995) noticed a decrease in shoot to root ratio in olive plants but in contrast to this Mathangi *et al*. (2006) observed an increase in shoot to root ratio in the salt-exposed kikuyu grass. They further concluded that such an increase was due to the rapid reduction of shoot dry matter production as a result of NaCl salinity stress. Similarly, the decrease in shoot:root ratio due to NaCl salinity stress has been reported by Meloni *et al*. (2004) in *Prosopis alba* and Ping *et al*. (2005) in *Lycopersicon esculentum*. It is clear that the shoot to root ratio is considerably increased in *P. alopecuroides* especially at higher salinity levels and might be due to the more allocation of assimilates from the root to shoot.

The causes of growth reduction differ (Munns *et al*., 1995), but it is not clear which mechanism plants employ to maintain residual growth. In the present study, *P. alopecuroides* showed an increase in the plant height with a maximum by 18.23% at 50 mM NaCl concentration (Table 1). The height of the grass species increased upto 50 mM NaCl salinity but thereafter it was decreased at higher levels of NaCl. The values of the plants treated only at 50 and 100 mM were statistically significant at p<0.001 when compared with control. Salinity has both osmotic and also specific ion effects on plant growth (Dionisio-Sese and Tobita, 2000). Many plants show a decrease in plant height under the influence of NaCl salinity *e.g. Phaseolus mungo* (Dash and Panda, 2001), sugarbeet (Ghoulam and Fares, 2001), senna seedlings (Agarwal and Pandey, 2004), ornamental species *Ageratum* (Zapryanova and Atanassova, 2009) and tomato (Tantawy *et al*., 2009). According to Bohnert *et al*. (1995) the chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast which leads to decrease in turgor that lastly results into growth reduction. In the present investigation, the decrease in plant height of *P. alopecuroides* might be due to less availability of water and toxicity of sodium chloride.

Salinity usually causes a reduction in the leaf area which generally leads to a drastic reduction in net CO_2 assimilation and also an increase in thickness of the leaf (Benzioni *et al*., 1992). In our study, maximum percent increase in leaf area is recorded as 11.17% at 100 mM concentration of NaCl with significance values at 100, 200 and 300 mM concentration (Table 1). When salinity is applied to the root medium, leaf elongation is immediately inhibited for maize (Munns *et al*., 2000), rice (Yeo *et al*., 1991), tomato (Tantawy *et al*., 2009) and jowar (Stuart *et al*., 2000). Reduction in leaf area under the influence of salinity is also noticed in wheat (Passioura and Munns, 2000), maize (Cicek and Cakirlar, 2002), chickpea (Sheldon *et al*., 2004) and *Catharanthus roseus* (Jaleel *et al*., 2008). Ali *et al*. (2004) noticed the reduction in leaf area under saline conditions in rice due to decreased water uptake, toxicity of sodium and chloride in the shoot cell as well as reduced photosynthesis. From the present results, it is clear that leaf area per plant is increased at lower levels but decreased at the higher levels of NaCl salinity in the *P. alopecuroides*. The ROS production under water stress in leaf apoplast might be the reason for an increase in leaf area in the salinity stressed experimental grass.

Ceyhan and Ali (2002) observed an increase in fresh weight of lettuce plant at high salinity. Ruiz *et al*. (1999) have also reported this for orange leaf and attributed this to increased water content of leaf. In the present study, fresh weight of *P. alopecuroides* was increased by 50.92% at 100 mM NaCl while the values were significant only at 25 and 300 mM concentration (Table 1). Jaleel *et al*. (2008) found a reduction in fresh weight (about 25%) in *Catharanthus roseus* while Cicek and Cakirlar (2002) found it in maize under salt stress. Tantawy *et al*. (2009) also noticed a decrease in fresh weight of tomato plants while Khosravinejad *et al*. (2009) noticed in two barley varieties namely Afzal and EMB82-12 with increasing levels of salinity. The reduction in shoot biomass production by the plant may be due to the chlorosis and necrosis of the leaves that reduce the photosynthetically active area (De Herralde *et al*., 1998). The decrease in fresh weight of the leaves $plant^{-1}$ of the grass might be due to soil moisture stress developed under saline conditions and the suppression of growth under salinity stress during the early developmental stages.

Rodriguez and Miller (2000) observed the lowered dry weights of leaves, stems and roots of Bermuda grass under salt stress. Similarly, dry weight of *P. alopecuroides* was decreased at higher levels of salinity but showed a maximum increased by 33.64% at 100 mM NaCl (Table 1). No any statistical significance was observed in the values of dry weight of the plants when compared with control value. Jaleel *et al*. (2008) found 26% reduction in dry weight of *Catharanthus roseus* due to salinity. Cicek and Cakirlar (2002) also noticed a decrease in total dry weight in 30 day-old maize plants under saline environment while Saboora and Kiarostami (2006) found it in nine different wheat genotypes. Tantawy *et al*. (2009) noticed a decrease in dry weight of tomato plants while Khosravinejad *et al*. (2009) observed such reduction in two barley varieties namely Afzal and EMB82-12 under the influence of salinity. Munns (2003) concluded that the reduction in dry weight of the plants might be attributed to the inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. From the present investigation, it is clear that the dry weight of the leaves $plant^{-1}$ of *P. alopecuroides* was increased due to salinity with clear-cut reduction only at 300 mM NaCl concentration. Such stimulation in dry matter production under the influence of salinity might be due to the accumulation of inorganic ions and organic solutes for osmotic adaptation.

Higher levels of salts can produce decreased water uptake in plants (Volkmar *et al*., 1998). It is very clear from the results that moisture percentage of *P. alopecuroides* increases due to NaCl salinity but there is a drastic reduction in it at 300 mM salt level. It was increased to a maximum by 24.61% at 50 mM while the significant values were observed at 50 and 100 mM NaCl to the rooting medium (Table 1). Rodriguez *et al*. (1997) observed a rapid decrease in leaf water potential, stomatal conductance and leaf relative water content in tomato plants. According to Sheldon *et al*. (2004) the most significant impact of increased NaCl on the growth of wheat was the osmotic effect in reducing plant water uptake. The decrease in water potential due to soil salinity causes the plants to use inorganic ions such as Na^+ and K^+ and, synthesize organic compatible solutes (Chinnusamy *et al*., 2005). Increased moisture content under saline environment shows the higher water content per unit leaf area, which may dilute the accumulated salts in the cell sap (Hagemeyer, 1997). The decrease in moisture content in the leaves of the experimental grass at higher levels of salinity stress might be due to osmotic stress induced by NaCl which could not be maintained by the plants under severe stress while increase at lower levels might be the adaptation of plants to osmotic adjustment which maintains water uptake and turgor with the accumulation of organic solutes.

Electrical conductivity (EC) is a numerical expression of the ability of water to carry an electrical current which varies with the number and types of ions in the solution expressed in μS while total dissolved solids (TDS) are simply the sum of the cations and anions concentration expressed in mg l^{-1}. It is obvious that EC and TDS of the pot soil increases with the increasing levels of NaCl (Table 2). The EC and TDS content was increased from 62.92 μS in the control soil to 524.76 μS in the pot soil added with 300 mM of NaCl while TDS content was increased from 32.42 mg l^{-1} in the control soil to 313.77 mg l^{-1} in the pot soil added with 300 mM of NaCl. All the values were observed to be significant at all treatments. The EC and TDS content was increased to a maximum by 733.97 and 867.84% in the soil treated with highest salt concentration (300 mM). The varying levels of EC in the pot soil might be due to the different binding capacities of the roots of the plants and competition by the roots to get nutrients from the soil while the varying levels of TDS in the pot soil culture were obviously due to the external additions of NaCl and it's interactions with root and organic-inorganic ions already present in the original soil. Similar increase in EC and TDS of the soil treated with increasing concentrations of NaCl to rooting medium were earlier reported by Gokhale *et al*. (2008).

From the present results, it is clear that the shoot and root length of the *P. alopecuroides was* stimulated at lower levels of salinity however at higher levels of salinity inhibition in shoot length and root length was observed which might be due to the toxicity of sodium chloride. It was also very well observed that the lower levels of salt concentration *i.e.* 25 and 50 mM is stimulatory and enhances the of growth *P. alopecuroides* which clearly indicates the favourable nature of the sodium chloride for the selected grass species. The increase in shoot to root ratio especially at higher salinity levels might be due to the more allocation of assimilates from the root to shoot. The increase in leaf area of the grass under the influence of salinity might be due to the production of toxic substances mainly involving reactive oxygen species. The decrease in fresh weight of the leaves plant^{-1} of the grass under the higher levels of salinity might be due to soil moisture stress developed under saline conditions while increase in dry matter production at higher levels of salinity might be due to the accumulation of inorganic ions and organic solutes for osmotic adjustment. Similarly, decrease in moisture content in the leaves of the experimental grass at higher levels of salinity stress might be due to osmotic stress induced by NaCl which could not be maintained by plant. Overall, it appears that the grass species *P. alopecuroides* exhibit a moderate salinity tolerance as far as linear growth is concerned.

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