J. Ecophysiol. Occup. Hlth. 10 (2010) 177-182

Salt Stress Induced Alteration in Photosynthetic Pigments and Polyphenols of *Pennisetum alopecuroides* (L.)

A.V. Mane, B.A. Karadge[1] and J.S. Samant[2]

Department of Environmental Sciences, Fergusson College, Pune 411 004, M.S.
[1]Department of Botany, Shivaji University, Kolhapur, M.S.
[2]Department of Environmental Sciences, Shivaji University, Kolhapur, M.S.

Abstract: *Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. In the present investigation effect of NaCl salinity on the photosynthetic pigments and polyphenols of the leaves of the Pennisetum alopecuroides (L.) Spreng var. Mourdy was studied. The total chlorophyll (chl.) content of the mature leaves was increased only by 4.7%, 9.2% and 1.5% at 25, 50 and 100 mM, respectively but later on decreased with increasing concentrations of NaCl. Chlorophyll. 'a' appears to be more sensitive to salinity in the experimental grass species than chlorophyll. 'b'. Maximum increase in carotenoid content observed was 56.3%. The maximum increase in polyphenols was 111.6% at 300 mM concentration. Electrical conductivity of the pot soil increased to a maximum by 525 % in the pot soils of Pennisetum alopecuroides following external addition of NaCl at 300 mM, while, total dissolved solids (TDS) content increased to 868 %. An increase in the photosynthetic pigments at lower salt levels might be due to the osmotic adjustment mechanism developed by the grass species to incumbent with salt stress. The increased levels of polyphenols at elevated salinity might be due to the accumulation of secondary metabolites.*

Key Words: Pennisetum alopecuroides, Photosynthetic pigments, Polyphenols, Salinity

Introduction

Abiotic stress is the negative impact of non-living factors on the living organisms in a specific environment. It is well established that abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality (Ouda *et al.*, 2008) with increasing impact on the socio-economic fabric and health, especially of the farming communities. Statistics about the extent of salt affected areas vary, but estimates are 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 hectares have been salinised by human activities (Ghassemi *et al.*, 1995).

Oxidative stress due to Sodium chloride (NaCl) might contribute to the deleterious effects of salt and significant growth reductions in plants (Hernandez *et al.*, 1999). Photosynthesis is one of the most important biochemical pathways by which plants prepare their own food material and grows. 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 sorghum and maize plants (Sadale, 2007; Kate, 2008). Salinity stress causes changes in chloroplast ultrastructure (Keiper *et al.*, 1998). Levels of polyphenols also increases under increasing levels of salinity, which shows that the induction of secondary metabolism is one of the defence mechanism adapted by plants to face saline environment (Sadale, 2007; Kate, 2008). The present investigation was carried out to see the deleterious effects of NaCl salinity on the photosynthetic pigments and the polyphenols of the leaves of *Pennisetum alopecuroides* (L.). Spreng var. Mourdy.

Materials and Methods

The seedlings of *Pennisetum alopecuroides* (L.) Spreng var. Mourdy were collected from Government nursery, Kagal (Dist. Kolhapur, Maharashtra). The seedlings were uniformly cut to a minimum height 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 four weeks of their normal growth, salinity stress was measured. The plants were treated with increasing concentrations of Sodium chloride i.e. 25, 50, 100, 200 and 300 mM concentrations. Every alternate day, they were watered with a double amount of water to maintain the uniform salt concentration in the pots and to cope up with the loss of water by evaporation from the soil surface and by transpiration from the plant surface. Chlorophylls of mature leaves were estimated following the method of Arnon (1949). Carotenoids were estimated from the same extract and calculated by using the formula suggested by Kirk and Allen (1965). Statistical analysis of the data was carried out by using GraphPad software.

Results and Discussion

Chlorophyll. The effect of NaCl salinity on chlorophyll content of mature leaves of the *Pennisetum alopecuroides* is shown in Table 1. It was observed that the total chlorophyll content of the mature leaves increased slightly by 4.7%, 9.2% and 1.5% at 25, 50 and 100 mM concentrations respectively, and decreased further with increasing concentrations of NaCl. Chlorophyll 'a': chlorophyll 'b' ratio was found to be slightly increased upto 50 mM salt concentration. Highest tested salt concentration (300 mM) was negatively influential on chlorophyll 'a': 'b' ratio. Chlorophyll 'a' appears to be more sensitive to salinity than chlorophyll 'b'.

Chlorophyll content in plants correlates directly to the healthiness of plant (Zhang *et al.*, 2005). Tantawy *et al.* (2009) also observed the decrease in total chlorophyll content in tomato with the increasing level of salinity. Djanaguiraman *et al.* (2006) observed a decrease in chlorophyll content in rice under saline conditions. They observed that chlorophyll 'b' was degraded at a higher rate than chlorophyll 'a' in the leaves exposed to NaCl, which resulted in an increase in chlorophyll 'a':'b' ratio. This can be explained by the fact that the first step in chlorophyll 'b' degradation involves its conversion to chlorophyll 'a' (Fang *et al.*, 1998). The decrease in chlorophyll content of sorghum leaves as a result of salinity; especially in the salt-sensitive genotype could be a result of oxidative stress (Lacerda *et al.*, 2003). Some studies have shown that salt stress inhibits PS II activity (Everard *et al.*, 1994). Whereas, other studies have indicated that salt stress has no effect on PS II (Brugnoli and Lauteri, 1991; Abadoa *et al.*, 1999). Parvaiz and Riffat (2005) have reported a decrease in chlorophyll content in *Pisum sativum* as the concentration of salt increased from 50 to 200 mM, which could be associated with the accumulation of Na^+ in the leaves. Reduction in chlorophyll content coupled with increased salt concentration was also reported in *Picea mariana*, *Picea glauca* and *Pinus banksiana* (Croser *et al.*, 2001). Present investigation shows that the total chlorophyll content in the leaves of *Pennisetum alopecuroides* increases at lower levels of salinity. It could be due to the osmotic adjustment mechanism developed by the grass. A decrease at higher salt concentrations might be associated with disruption in cellular functions, membrane deterioration and damage to photosynthetic electron transport chain due to accumulated ions.

Table 1. Effect of sodium chloride on chlorophyll content of the leaves of Pennisetum alopecuroides

Parameters	Sodium Chloride (mM)					
	Control	25	50	100	200	300
Chlorophyll 'a'	157.06 (±2.55)	170.35** (±3.24) +8.46	181.12*** (±2.86) +15.32	152.51 (±4.13) -2.90	126.91*** (±3.92) -19.19	103.16*** (±3.72) -34.32
Chlorophyll 'b'	97.24 (±1.80)	95.85 (±2.03) -1.43	96.55 (±3.72) -0.71	105.63* (±0.97) +8.63	104.26* (±2.53) +7.22	105.12* (±3.20) +8.11
Total Chlorophyll	254.23 (±2.62	266.12*** (±2.81) +4.68	277.59*** (±0.94) +9.19	258.07 (±3.75) +1.51	231.10*** (±1.90) -9.10	208.22 (±2.09) -18.10
Chlorophyll 'a' : Chlorophyll 'b' ratio	1.62 (±0.05)	1.78 (±0.06)	1.88 (±0.10)	1.44 (±0.05)	1.22 (±0.07)	0.98 (±0.06)

Each value is expressed as mg 100^{-1} g fresh tissue Values in parenthesis indicate standard deviation. Each value is a mean of three determination.

* Significant (p = 0.01 to 0.05)

** Significant (p = 0.001 to 0.01)

*** Significant (p < 0.001)

Table 2. Effect of sodium chloride on carotenoid and polyphenol content of the leaves of *Pennisetum alopecuroides*

Name of the species	Sodium Chloride (mM)					
	Control	25	50	100	200	300
Carotenoids	13.17 (±0.56)	20.53*** (±0.96) +55.87	20.59*** (±1.36) +56.28	8.48*** (±0.89) -35.63	4.69*** (±0.61) -64.37	2.93*** (±0.49) -77.73
Polyphenols	371.31 (±22.08)	254.67** (±14.68) -31.41	326.60 (±25.42) -12.04	405.33 (±33.63) +9.16	672.63*** (±23.57) +81.15	785.58*** (±26.0) +111.57

Each value is expressed as mg 100^{-1} g fresh tissueValues in parenthesis indicate standard deviation. Each value is a mean of three determinations

* Significant (p = 0.01 to 0.05)

** Significant (p = 0.001 to 0.01)

*** Significant (p < 0.001)

Carotenoids: Results indicated that carotenoid content of the leaves of *Pennisetum alopecuroides* was increased at lower levels of salinity. Maximum increase in carotenoid content was 56.3% (Table 2). Carotenoids have two major functions in photosynthesis as they protect chloroplast from photo-oxidative damage and also act as accessory light harvesting pigments The response shown by the plants with respect to accumulation of carotenoids under the salinity stress varies from plant to plant. A reduction in carotenoids content due to salinity stress has been shown in mulberry (Agastian *et al.* 2000) and in *Aegiceros corniculatum* However, increase in carotenoid content under saline conditions has been shown in broad bean leaves and lupine plants (El-Zeiny, 1990; Hamada and El-Enany, 1994). Present investigation further revealed that total carotenoid content in the leaves of *Pennisetum alopecuroides* was reduced at higher NaCl salinity. This could be due to the photo-oxidative damage.

Polyphenols: The influence of NaCl salinity on polyphenol content of the leaves of *Pennisetum alopecuroides* is shown in Table 2. The maximum increase in polyphenols was observed at 300 mM salt concentration. Polyphenols are a group of chemical substances found in plants, characterised by the presence of more than one phenol units or building blocks per molecule. Karadge (1981) observed a linear decrease in polyphenol content of the leaves of *Portulaca oleracea* with increasing concentrations of NaCl, whereas, Parida *et al.* (2002) observed an accumulation of polyphenols in *Bruguiera parviflora* with increasing levels of salinity. A considerable increase in polyphenol content of the leaves under NaCl salinities has been recorded in groundnut var. TMV-10, in *Aegiceros corniculatum* and in *Brassica campestris* (Karadge and Chavan, 1981; Parida *et al.*, 2004; Singh and Kumari, 2006). In order to tolerate higher levels of salinity, stress and adverse

Table 3. Electrical conductivity and total dissolved solids of the soil treated with sodium chloride (1:10 Soil Suspension in Water)

Name of the species	Sodium Chloride (mM)											
	Control		25		50		100		200		300	
	EC	TDS	EC	TDS	EC	TDS	300	TDS	EC	TDS	EC	TDS
Pennisetum	62.92	32.42	81.20*	48.18***	95.41***	52.84***	192.07***	104.94***	364.09***	221.70***	524.76***	313.77***
alopecuroides	(±1.79)	(±0.96)	(±5.01)	(±2.39)	(±3.53)	(±2.43)	(±5.41)	(±2.97)	(±8.96)	(±2.42)	(±9.25)	(±4.31)
			+29.05	+48.61	+51.63	+62.98	+205.25	+223.68	+478.62	+583.84	+733.97	+867.84

EC expressed in µS cm^{-1} and TDS expressed in mg l^{-1} Values in parenthesis indicate standard deviation
Each value is a mean of three determinations
* Significant ($p = 0.01$ to 0.05)
** Significant ($p = 0.001$ to 0.01)
*** Significant ($p < 0.001$)

conditions the increased levels of polyphenols at higher levels of salinity may induce accumulation of secondary metabolites in the present experimental species.

Electrical conductivity (EC) and total dissolved solids (TDS): The influence of NaCl salinity on EC and TDS of the treated soil was determined and recorded in Table 3. As expected, EC of the pot soil increased with the increasing conc..ntrations of NaCl. The maximum increase of 525 % in the pot soils with 300 mM NaCl concentration was observed. While at the same concentration of salt, TDS content increased by 868 %. The varying levels of EC and TDS in the pot soil might be due to the different binding capacities of the roots of the plant and competition by the roots to get nutrients from the soil. External addition of NaCl and its interactions with root and organic-inorganic ions already present in the original soil may further influence the EC and TDS levels.

It is well known that many species try to adapt under salinity stress and the selected experimental species is not an exception to this fact.

Acknowledgements

Authors are thankful to Dr. P. D. Raut, Head, Department of Environmental Science, Shivaji University, Kolhapur for providing necessary facilities. We are thankful to Dr. A.N. Sadale and Dr. N. M. Ghadge for their valuable suggestions during the investigation. Our sincere thanks also go to management of Fergusson College, Pune and the Principal, Dr. R. G. Pardeshi for constant encouragement.

Reference

Abadoa, A., Belkohodja, R., Morales, F. and Abadoa, J. (1999) Effects of salinity on the photosynthetic pigment composition of barley (*Hordeum Vulgare* L.) growth under a triple-line-source sprinkler system in the yield. *Plant Physiol.*, **154,** 392-400.

Agastian, P., Kingsley, S. J. and Vivekanandan, M. (2000) Effect of salinity on photo-synthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica.*, **38**, 287-290.

Arnon, D. I. (1949) Copper enzyme in isolated chloroplasts: polyphenol oxisdase in *Beta vulgaris. Plant Physiol.*, **24**, 1-15.

Brugnoli, B. and M. Lauteri, (1991) Effects of salinity on stomatal conductance, photosynthetic capacity and carbon isotope discrimination of salt-tolerant (*Gossypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C_3 non-halophytes. *Plant Physiol.* **95**, 628-635.

Croser, C., Renault, S., Franklin, J. and Zwiazek, J. (2001) The effect of salinity on the emergence and seedling growth of *Picea mariana*, *Picea glauca* and *Pinus banksiana. Env. Pollution.*, **115**, 9-16.

Djanaguiraman, M., Sheeba, J. A., Shanker, A. K., Durgadevi, D. and Bangarusamy, U. (2006) Rice can acclimate to lethal level of salinity by pretreatment with sublethal level of salinity through osmotic adjustment. *Plant Soil.*, **284**, 363-373.

El-Zeiny, H. A. (1990) Effect of different combinations of saline water irrigation and nitrogen levels on growth, photosynthetic pigments content, amino acids and yield of lupine plants, *Lupinus termis L. Egypt. J. Agron.*, **15**, 171-188.

Everard, J. D., Gucci, R., Kann, S.C., Flore, J. A. and Loescher, W. H. (1994) Gas Exchange and Carbon Partitioning in the Leaves of Celery (*Apium graveolens* L.) at Various Levels of Root Zone Salinity. *Plant Physiol.*, **106**, 281-292.

Fang, Z., Bouwkamp, J. C. and Solomos, T. (1998) Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris L. J. Exp. Bot.*, **49**, 503-510.

Ghassemi, F., Jakeman, A. J. and Nix, H. A. (1995) Salinisation of land and water resources: human causes, extent, management and case studies. Australian National Uinversity, Canberra, Australia and CAB International, Wallingford, Oxon, UK.

Hamada, A. M. and El-Enany, A. E. (1994) Effect of NaCl salinity on growth, pigment and mineral element contents and gas exchange of broad bean and pea plants. *Bio. Plant.*, **36**, 75-81.

Hernandez, J. A., Campillo, A., Jimenez, A., Alarcon, J. J. and Sevilla, F. (1999) Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytol.*, **141**, 241-251.

Karadge, B. A. (1981) Physiological studies in succulents (physiological studies in *Portulaca* *oleracea* L., Ph. D. thesis submitted to Shivaji University, Kolhapur (India).

Karadge, B. A. and Chavan, P. D. (1981) Salt tolerance studies in groundnut (*Arachis hypogaea* L.) Variety TMV.10. *Biovigynam.*, **7**,137-144.

Kate, V. V. (2008) Physiological and biochemical studies in some medicinal plants: *Tribulus terrestris* L. and *Pedalium murex* L. Ph. D. (Botany) thesis submitted to Shivaji University, Kolhapur, Maharashtra.

Keiper, F. J., Chen, D. M. and De, F. L. F. (1998) Respiratory, photosynthetic and ultrastructural changes accompanying salt adaptation in culture of *Eucalyptus microcorys*. *J. Plant Physiol.*,**152**, 564-573.

Kirk, J. O. T. and Allen, R. L. (1965) Dependence of salinity stress on the activity of glutamine synthetase and glutamate dehydrogenase in Triticale seedlings. *Polish J. Env. Studies.*, **14**, 523-530.

Lacerda, C. F., Cambraia, J., Oliva, M. A., Ruiz, H. A. and Prisco, J. T. (2003) Solute accumulation and distribution during shoot and leaf development in two *sorghum* genotypes under salt stress recovery. *Environ. Exp. Bot.*, **54**, 69-76.

Ouda, S. A. E., Mohamed, S. G. and Khalil, F. A. (2008) Modeling the effect of different stress conditions on maize productivity using yield-stress model. *Intern. J. Natural Eng. Sci.*, **2**, 57-62.

Parida, A. K., Das, A. B. and Mohanty, P. (2004) Defence potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. *J. Plant Physiol.*, **161**, 531-542.

Parida, A., Das, A. and Das, P. (2002) NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguira perviflora*, in hydroponic cultures. *J. Plant. Biol.*, **45**, 38-36.

Parvaiz A. and Riffat, J. (2005) Effect of salt stress on growth and biochemical parameters of *Pisum sativum* L. Archives of Agronomy and Soil *Science.*, **51**, 665-672.

Romero-Aranda, R., Soria, T. and Cuartero, J. (2001) Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science.*, **60**, 265-272.

Sadale, A. N. (2007) Physiological studies in *Sesbania grandiflora* L. Poir: Stress Physiology. Ph. D. (Botany) thesis submitted to Shivaji University, Kolhapur, Maharashtra.

Singh, A. K. and Kumari, B. (2006) Physiological basis of salinity tolerance in rapeseed (*Brassica campestris*. Var. Toria) during seedling growth. *Physiol. Mol. Biol. Plants.*, **12**, 167-171.

Tantawy, A. S., Abdel-Mawgoud, A. M. R., El-Nemr, M. A., Chamoun, Y. G. (2009) Alleviation of salinity effects on tomato plants by application of amino acids and growth regulators. *Eur. of Scientific Res.*, **30**, 484-494.

Zhang, M., Qiu, Z. and Liu, X. (2005) Remote sensed spectral imagery to detect late blight in field tomatoes. *Precision Agric.*, **6**, 489-508.