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Original Research Paper

Salinity Induced Changes in Catalase, Peroxidase and Acid Phosphatase in Four Grass Species

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ABSTRACT

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality, and puts various problems to the plants either at the population, organism or even at the molecular level. In the present investigation seedlings of *Cymbopogon nardus* (L.) Rendle, *Pennisetum alopecuroides* (L.) Spreng var. Mourdy and *Vetiveria zizanioides* (L.) Nash were treated with increasing concentrations of sodium chloride i.e., 25, 50, 100, 200 and 300 mM and changes in the activities of catalase, peroxidase and acid phosphatase were determined. The activity of catalase was stimulated by 200 and 300 mM NaCl but it was initially decreased at lower levels of salinity. In *Cymbopogon* (36.30%), *Cynodon* (3.07%) and *Pennisetum* (0.94%), it was observed to be increased, while it was decreased in the leaves of *Vetiveria* (60.38%) at 300 mM NaCl concentration. The elevated activities of CAT under NaCl stress (300 mM and 200 mM) level probably came from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes which indicates the relationship between salt tolerance and antioxidant defence system. The details of the activities of other two enzymes are discussed in the present paper.

INTRODUCTION

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality (Shani & Dudley 2001, Ouda 2008) with increasing impact on the socio-economic fabric and health, especially of the farming communities. Salinity is a general term used to describe the presence of elevated levels of different salts such as sodium chloride, magnesium and calcium sulphates and bicarbonates in soil and water. Sehgal & Abrol (1994) report that 187.2 Mha area in India is degraded, of which 162.4 Mha is degraded by water and wind erosion and 21.7 Mha by salinity and water logging. The remaining 4 Mha area is affected by the depletion of nutrients. The United States Salinity Laboratory Staff (1954) defined a saline soil as one having electrical conductivity of saturation extract of soil greater than 4 mS/cm or equivalent to approximately 40 meq/L and an exchangeable sodium percentage less than 15%. Usually, pH of saline soils remains below 8.5.

Salinity puts various problems to the plants either at the population, organism or even at the molecular level. Physiologically and genetically salt tolerance is a complex among the variants of plants with a wide range of adaptations in halophytes and less tolerant plants (Flower 2004). Plants maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. Salt stress induces the increased activities of superoxide dismutase, ascorbate

peroxidase, catalase and glutathione reductase in rice, representing a higher antioxidative capacity to NaCl, which is one of the salt tolerant mechanisms adapted by rice to cope with it (Hoai et al. 2005). Similarly, increase in the activities of superoxide dismutase, catalase, peroxidase (Gao et al. 2008, Arora et al. 2008) and polyphenol oxidase (Agarwal & Pandey 2004) is reported. With increasing salinity up to 150 mM NaCl, the hydrogen peroxide content and the activity of guaiacol-specific peroxidase increases markedly, but in contrast, catalase activity with increasing salinity is not correlated with hydrogen peroxide content in mulberry (Poontariga et al. 2003). In contrast to this, salinity stress decreased the activities of antioxidant enzymes in lem peroxisome of the wild salt tolerant tomato species *Lycopersicon pennellii* (Mittova et al. 2004). Decreased ROS production contributes to the salinity associated reduction in maize's leaf elongation, acting through a mechanism which is associated with pH change (Andre et al. 2004).

MATERIALS AND METHODS

The seedlings of *Cymbopogon nardus* (L.) Rendle; *Pennisetum alopecuroides* (L.) Spreng var. Mourdy and *Vetiveria zizanioides* (L.) Nash were collected from Govt. nursery, Kagal (Dist. Kolhapur, Maharashtra) while those of *Cynodon dactylon* (L.) Pers. were collected from Shivaji University campus. The seedlings were uniformly cut to a minimum height required for their growth and were transplanted into 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 treatment were commenced. The plants were treated with increasing concentrations of sodium chloride i.e., 25, 50, 100, 200 and 300 mM. 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.

The activity of catalase (E.C. 1.11.1.6) in the fresh mature leaves was determined by the method described by Sadasivam & Manickam (1991) with slight modifications. Peroxidase (E.C. 1.11.1.7) was determined following the method described by Maehly (1954). The activity of enzyme acid phosphatase (E.C.3.1.3.2) from fresh mature leaves was determined following the method of McLachlam (1980). Activity of each enzyme is expressed as Δ O.D./h/mg protein. The enzyme protein was estimated according to Lowry et al. (1951). Statistical analysis of the data was carried out by using GraphPad software.

RESULTS AND DISCUSSION

The influence of NaCl salinity on the activity of catalase in the leaves of *Cymbopogon nardus*, *Cynodon dactylon*, *Pennisetum alopecuroides* and *Vetiveria zizanioides* is shown in Table 1. The activity of this enzyme was stimulated by 200 and 300 mM NaCl but it was initially decreased at lower levels of salinity. In *Cymbopogon* (36.30%), *Cynodon* (3.07%) and *Pennisetum* (0.94%) it was observed to be increased, while it was decreased in the leaves of *Vetiveria* (60.38%) at 300 mM NaCl concentration. Catalase is a common enzyme found in nearly all living organisms, where it functions to catalyse the decomposition of hydrogen peroxide to water and oxygen. Catalase can also oxidise different toxins, such as formaldehyde, formic acid, phenols and alcohols. In doing so, it uses hydrogen peroxide according to the following reaction.

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

Hydrogen peroxide is a harmful by-product of many normal metabolic processes and to prevent damage, it must be quickly converted into other less toxic substances. To end this, catalase is

Table 1: Effect of sodium chloride salinity on catalase activity in the leaves of four grass species.

Sr. No.	Name of the species	Sodium chloride (mM)					
		Control	25	50	100	200	300
1.	*Cymbopogon nardus*	0.898 (±0.072)	0.548*** (±0.044)	0.325*** (±0.047)	0.271*** (±0.034)	1.103* (±0.093)	1.223*** (±0.072)
2.	*Cynodon dactylon*	1.025 (±0.035)	0.461*** (±0.023)	0.322*** (±0.037)	1.020 (±0.037)	1.057 (±0.043)	0.455*** (±0.244)
3.	*Pennisetum alopecuroides*	1.452 (±0.100)	0.516*** (±0.029)	0.448*** (±0.050)	0.337*** (±0.032)	0.836*** (±0.043)	0.455*** (±0.048)
4.	*Vetiveria zizanioides*	1.080 (±0.052)	0.667 (±0.035)	0.271*** (±0.037)	0.154*** (±0.021)	0.720*** (±0.068)	0.428*** (±0.064)

Each value is expressed as Δ O.D. min/mg/protein
Values in parentheses indicate standard deviation
Each value is a mean of three determinations
*Significant (p = 0.01 to 0.05)
**Very Significant (p = 0.001 to 0.01)
***Extremely Significant (p < 0.001)

Table 2 Effect of sodium chloride salinity on peroxidase activity in the leaves of four grass species.

Sr. No.	Name of the species	Sodium chloride (mM)					
		Control	25	50	100	200	300
1.	*Cymbopogon nardus*	0.158 (±0.025)	0.120 (±0.024)	0.112 (±0.009)	0.212 (±0.033)	0.293** (±0.041)	0.273* (±0.052)
2.	*Cynodon dactylon*	0.147 (±0.030)	0.115 (±0.022)	0.103 (±0.017)	0.201 (±0.033)	0.244** (±0.027)	0.118 (±0.020)
3.	*Pennisetum alopecuroides*	0.104 (±0.014)	0.097 (±0.013)	0.086 (±0.014)	0.172** (±0.024)	0.184** (±0.010)	0.176** (±0.026)
4.	*Vetiveria zizanioides*	0.103 (±0.009)	0.091 (±0.010)	0.081 (±0.011)	0.148 (±0.037)	0.132 (±0.024)	0.084 (±0.020)

Each value is expressed as Δ O.D. min/mg/protein
Values in parentheses indicate standard deviation
Each value is a mean of three determinations
*Significant (p = 0.01 to 0.05)
**Very Significant (p = 0.001 to 0.01)
***Extremely Significant (p < 0.001)

Table 3: Effect of sodium chloride salinity on acid phosphatase activity in the leaves of four grass species.

Sr. No.	Name of the species	Sodium chloride (mM)					
		Control	25	50	100	200	300
1.	*Cymbopogon nardus*	0.407 (±0.011)	0.397 (±0.040)	0.331 (±0.033)	0.854*** (±0.064)	0.366 (±0.056)	0.181*** (±0.030)
2.	*Cynodon dactylon*	0.226 (±0.027)	0.087** (±0.014)	0.128* (±0.017)	0.217 (±0.029)	0.516*** (±0.043)	0.283*** (±0.050)
3.	*Pennisetum alopecuroides*	1.067 (±0.125)	0.392** (±0.058)	0.510** (±0.067)	1.678** (±0.181)	1.924*** (±0.238)	0.747 (±0.161)
4.	*Vetiveria zizanioides*	0.212 (±0.023)	0.146 (±0.029)	0.31* (±0.035)	0.437*** (±0.048)	0.442*** (±0.035)	0.164 (±0.040)

Each value is expressed as Δ O.D. min/mg/protein
Values in parentheses indicate standard deviation
Each value is a mean of three determinations
*Significant (p = 0.01 to 0.05)
**Very Significant (p = 0.001 to 0.01)
***Extremely Significant (p < 0.001)

frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al. 1996). The changes in CAT activity may depend on the species, the development and metabolic state of the plant, as well as on the duration and intensity of the stress (Chaparzadeh et al. 2004). According to Gao et al. (2008) increased CAT activity in *Jatropha curcas* seedlings especially in radicals is thought to play a critical role in plant salt tolerance. Tolerance of kikuyu grass to long term salt stress is associated with induction of antioxidant defences (Adele et al. 2003). Cherian & Reddy (2003) noticed the activities of SOD and CAT remained below in NaCl treated callus tissues as compared to control in *Suaeda nudiflora* Moq. Niknam et al. (2006) noticed that salt stress caused to increase CAT activity in the seedlings of *Trigonella foenum-graecum* up to 150 mM, but 200 mM NaCl caused to decrease it slightly. However, there was a pronounced salt induced increase in CAT activity in calli. Kim et al. (2004) observed that the higher Na^+ content of the leaves of salt tolerant *Setaria viridis* could induce increase in active oxygen species and an increase in the activities of antioxidant enzymes such as SOD and CAT could lead to the increase in antioxidant protection and a decrease in oxidative damage. Increase in catalase was also noticed in sunflower var. Hisun-33 and SF-187 by Noreen et al. (2009).

An increase in the activity of catalase at the higher levels of salinity in *Cymbopogon*, *Cynodon* and *Pennisetum* could be associated with an increase in the level of H_2O_2, which might be formed due to increased superoxide dismutase activity. The elevated activities of CAT under NaCl stress (300 mM and 200 mM) level probably came from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes which indicates the relationship between salt tolerance and antioxidant defence system. While initial decrease at lower levels of salinity and then an increase at higher levels (still the values were below those in the control) of salinity in *Vetiveria* indicates the strong adjustment developed by the leaves to tolerate higher saline environment.

The influence of NaCl salinity on the activity of peroxidase in the leaves of *Cymbopogon nardus*, *Cynodon dactylon*, *Pennisetum alopecuroides* and *Vetiveria zizanioides* is given in Table 2. It is clear from the results that the activity of this enzyme was considerably increased especially at 100 and 200 mM NaCl salinity in the grasses. It was the highest in *Cymbopogon*, *Cynodon and Pennisetum* respectively as 85.74, 66.39 and 76.68% at 200 mM NaCl concentration while it was higher by 43.39% at 100 mM NaCl concentration over the control. Passardi et al. (2005) were of the opinion that increased POD activity might enable plants to protect themselves against salt stress.

Muscolo et al. (2003) observed the higher POD activity in both roots and leaves of kikuyu grass grown under saline conditions showing that POD is involved in the mechanism of reducing oxidative stress and kikuyu is capable of effectively scavenging the ROS, resulting in the production of certain secondary metabolites which enable the plant to withstand salt stress. Rodriguez-Rosales et al. (1999) observed the increase in the activity of ascorbate peroxidase in tomato plants under high salt concentration. The increased total peroxidase activity in the medium of salt adapted cells of tomato reflected the changing mechanical properties of the cell wall, which in turn, could be related to the salt adaptation process, since cell wall properties are known to be modified by salt stress (Sancho et al. 1996). The increase in the activity of peroxidase in the leaves of the grasses under salinity stress may be regarded as an inhibition of stimulated secondary metabolism. It may also be involved in scavenging the reactive oxygen species, particularly in plants grown under stress condition.

The influence of NaCl salinity on the activity of acid phosphates in the leaves of *Cymbopogon nardus*, *Cynodon dactylon*, *Pennisetum alopecuroides* and *Vetiveria zizanioides* is given in Table 3. It is clear from the results that the activity of this enzyme was considerably increased over the control

especially at 100 and 200 mM NaCl salinity. It was the highest in *Cymbopogon* (109.94%) at 100 mM while in *Cynodon* (128.61%), *Pennisetum* (80.37%) and *Vetiveria* (108.27%) at 200 mM NaCl concentration.

An increased level of phosphatase activity accompanied by a decrease in phosphorus under salt-stress has been reported in some varieties of wheat calli (Szabo-Nagy et al. 1992). The increased acid phosphatase activity at the elevated levels of salinity in the leaves of the grasses might be due a decrease in available in the rooting medium phosphorus under salt-stress and the scavenging mechanism for phosphate from organic sources under phosphate limiting conditions as might be developed in the leaves.

CONCLUSION

The elevated activities of CAT under NaCl stress (300 mM and 200 mM) level probably came from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes which indicates the relationship between salt tolerance and antioxidant defence system in the grass species investigated. The increase in the activity of peroxidase under salinity stress may be regarded as an inhibition of stimulated secondary metabolism and may also be involved in scavenging the reactive oxygen species. The increased acid phosphatase activity at the elevated levels of salinity in the leaves of the grasses might be due a decrease in phosphorus under salt-stress and the scavenging mechanism for phosphate from organic sources under phosphate limiting conditions.

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REFERENCES

Adele, M., Sidari, M. and Panuccio, R.M. 2003. Tolerance of Kikuyu grass to long term salt stress is associated with induction of antioxidant defences. Plant Growth Regulation, 41: 57-62.

Agarwal, S. and Pandey, V. 2004. Antioxidant enzyme responses to NaCl stress in *Cassia Angustifolia*. Biologia Plantarum, 48: 555-560.

Andre, A. A., Aguez, R., Alicia, R., Coardoba, O. L. and Taleisnik, E. 2004. Decreased reactive oxygen species concentration in the elongation zone contributes to the reduction in maize leaf growth under salinity. Journal of Experimental Botany, 55: 1383-1390.

Arora, N., Bhardwaj, R., Sharma, P. and Arora, H. K. 2008. 28-homobrassinolide alleviates oxidative stress in salt treated maize (*Zea mays* L.) Plants. Braz. J. Plant Physiol., 20: 153-157.

Chaparzadeh, N., Amico, M. L., Nejad, R. K., Izzo, R. and Izzo, F. N. 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol. Biochem., 42: 695-701.

Cherian, S. and Reddy, M.P. 2003. Evaluation on NaCl tolerance in callus cultures of *Suaeda nudiflora* Moq. Biol. Plant., 46: 193-198.

Flowers, T. J. 2004. Salt tolerance is complex genetically and physiologically. Journal of Experimental Botany, 55: 307-319.

Gaetani, G., Ferraris, A., Rolfo, M., Mangerini, R., Arena, S. and Kirkman, H. 1996. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood, 87: 1595-1599.

Gao, S., Ouyang, C., Wang, S., Xu, Y., Tang L. and Chen, F. 2008. Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. Plant Soil Environ., 54: 374-381.

Hoai, T.T.N., Shim, I.E., Sung, K.K. and Usui, I.K. 2005. Effects of Salt stress on ion accumulation and antioxidative enzyme activities of *Oryza sativa* L. and *Echinochloa oryzicola* Vasing. Weed Biology and Management, 5: 1-7.

Kim, Y., Joji, A.,Takuji, N., Norikazu, N., Shinji, S. and Usui, K. 2004. Antioxidative responses and their relation to salt tolerance in *Echinochloa oryzicola* Vasing and *Setaria virdis* (L.) Beauv. Plant Growth Regulation, 44: 87-92.

Lowry, O.H., Rosenbrough, N.J., Furr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Maehly, A.C. 1954. In: Methods in Biochemcial Analysis. Ed. Glick, D., Interscience Publishers. Inc., New York, 385-386.

McLachlam, K. D. 1980. Acid phosphatase of intact roots and phosphorus nutrition in plants. Aust. J. Agric. Res., 31: 441-448.

Mittova, V., Micha, G., Moshe, T. and Micha, V. 2004. Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. Journal of Experimental Botany, 55: 1105-1113.

Muscolo, A., Sidari, M. and Panuccio, M.R. 2003. Tolerance of Kikuyu grass to long term salt stress is associated with induction of antioxidant defences. Plant Growth Regul., 41: 57-62.

Niknam, V., Razavi, N., Ebrahimzadeh, H. and Sharifizadeh, B. 2006. Effect of NaCl on biomass, protein and proline contents, and antioxidant enzymes in seedlings of two *Trigonella* species. Biologia Plantarum, 50: 591-596.

Noreen, S., Ashraf, M., Hussain, M. and Jamil, A. 2009. Exogenous application of salicylic acid enhances antioxidative capacity in salt stressed sunflower (*Helianthus annuus* L.) plants. Pak. J. Bot., 41: 473-479.

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. International Journal of Natural and Engineering Sciences, 2: 57-62.

Passardi, F., Cosio, C., Penel, C. and Dunand, C. 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep., 24: 255-265.

Poontariga, H., Darinee, P., Kannarat, R. and Rangsi, C. 2003. Salinity effects on antioxidant enzymes in mulberry cultivar. Science Asia, 29: 109-113.

Rodriguez-Rosales, M.P., Kerkeb, L., Bueno, P. and Donaire, J.P. 1999. Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane H^+ ATPase and antioxidant enzyme activities of tomato *Lycopersicon esculantum* (Mill.) Calli. Plant Sci., 143: 143-150.

Sadasivam, S. and Manickam, A. 1991. Biochemical Methods. Wiley Eastern Limited, New Delhi, 2nd Ed., pp. 107-110.

Sancho, M.A., Milrad, D.F.S., Pliego, F., Valpuesta, V. and Quesada, M.A. 1996. Total peroxidase activity and isoenzymes in the culture medium of NaCl adapted tomato suspension Cells. Plant Cell Tiss. Org. Cult., 44: 161-167.

Sehgal, J. and Abrol, I. P. 1994. Soil degradation in India: Status and Impact. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Bombay, Calcutta.

Shani, U. and Dudley, L.M. 2001. Field studies of crop response to water and salt stress. Soil Sci. Soc. Am. J., 65: 1522-1528.

Szabo-Nagy, A., Galiba, G. and Erdei, L. 1992. Induction of soluble phosphatases under ionic and nonionic osmotic stresses in wheat. Journal of Plant Physiology, 140: 629-633.

United States Salinity Laboratory Staff 1954. Diagnosis and Improvement of Saline and Alkali Soils. U.S.D.A. Handbook No. 60. Washington D.C., USA.