Indian J. Plant Physiol., Vol. 11, No. 3, (N.S.) pp. 295-299 (Jul.-Sept., 2006)

SHORT COMMUNICATION

A CORRELATIVE ANALYSIS OF OXALATE DEGRADATION AND EARLY NITRATE ASSIMILATION IN GRAIN SORGHUM GROWN UNDER SODIUM CHLORIDE STRESS

S. SINGH[1], S. N. MISHRA[2] AND C. S. PUNDIR[1]*

[1]Biochemistry and [2]Plant Biochemistry and Molecular Biology Research Laboratory, Department of Bio-Sciences, MD University, Rohtak-124 001

Received on 11 May, 2006, Revised on 7 Aug., 2006

Seedlings of grain sorghum (*Sorghum vulgare* Var. CSH-14) were grown upto 10 days in Hoagland's solution with different levels of NaCl (0, 20, 50 and 100 mM). The changes in oxalate degradation through oxalate oxidase (OXO) and the activities of enzymes of nitrate assimilation pathway, viz. nitrate reductase (NR) and nitrite reductase (NiR) were examined in leaves. The activity of oxalate oxidase correlated statistically with the activities of the enzymes of nitrate assimilation suggesting thereby a possible link between oxalate oxidation and nitrate assimilation. The degradation of oxalate by oxalate oxidase increased with increasing level of NaCl upto 50 mM concentration with increasing production of H_2O_2 in leaves. Activities of nitrate reductase and nitrite reductase were suppressed by NaCl salinity, showing a significant negative correlation with oxalate degradation. This shows that oxalate degradation and nitrate assimilation in grain sorghum are simultaneously affected by salt stress.

Key words: **Nitrate reductase, nitrite reductase, oxalate oxidase, sodium chloride stress, sorghum.**

The study of metabolic pathways related to cellular mechanisms of adaptations to salinity stress in crop plants is important. Oxalic acid in plants is generated as a byproduct in respiratory breakdown of carbohydrate and protein catabolism. It is also an intermediate product of carbon dioxide assimilation in C_4 pathway. Oxalic acid is degraded by two enzymes in plants; oxalate decarboxylase which decarboxylyses one mol of oxalic acid to one mol each of CO_2 and formate with the help of ATP and CoA and oxalate oxidase which aerobically oxidizes one mole of oxalate to one mole of H_2O_2 and two moles of CO_2. Oxalate degradation by oxalate oxidase in the germinating cereals has been correlated with salt tolerance and had a broad range of studies related to adaptation in higher plants (Lane *et al.* 1993). A possible role of oxalate oxidase in decreased N_2-fixation by bacteroids in faba beans under water stress has been reported by Trinchant and Rigaud (1996). They suggested that oxalate was an important source of carbon for nitrogen fixation but under water restricted conditions, oxalate degradation was induced by activation of oxalate oxidase while activity of nitrogenase decreased. Nitrate is the main form of nitrogen available to crop plants in normal conditions and the productivity of plants is largely influenced by assimilation of nitrate in plant system (Mishra and Srivastava 1983). Since the productivity of crops is adversely affected by salt stress, uptake and reduction of nitrate becomes critical for plants to adapt and grow in saline conditions (Rao and Gnanam 1990). NaCl is known to affect nitrogen assimilation in various plants by modifying nitrate reductase and nitrite reductase. Suppression of nitrate reductase by NaCl stress has been reported in maize (Khan and Srivastava 2000) and barley (Yamashita and Matsumoto 1996).

*Corresponding author, E-mail: pundircs@rediffmail.com

Nitrite reductase activity was decreased in *Sorghum vulgare* (Rao and Gnanam 1990) and tolerant rice cultivars (Katiyar and Dubey 1992) under NaCl stress. The present investigations were carried out to find the relationship between oxalate oxidase and the nitrate reductase and nitrite reductase for early nitrate assimilation in grain sorghum under NaCl stress.

Seedlings of grain sorghum (var. CSH-14) were surface sterilized with 0.1% $HgCl_2$ and allowed to germinate on moist filter paper lined in petridish at various levels of salinity (20 mM, 50 mM and 100 mM) NaCl contained in Hoagland's solution). Untreated seedlings (0 mM NaCl) served as control. After germination, the seedlings were kept in growth chamber (Light: 75W m^{-2}, 30±3°C) and maintained at a constant photo-period of 8-10 h. Equal number (10) of seedlings were collected from each group after 4, 6, 8 and 10 days of germination and the assays performed. The leaf extract (buffer pH 7.0, 0.1M; 1:5 w/v) was centrifuged at 15000 g for 20 min at 4ºC and the supernatant was collected as source of various enzymes and metabolites. All parameters were expressed in terms of per g fw. The oxalate oxidase estimated following Satyapal and Pundir (1993) and oxalate by Kuchhal *et al.* (1993). The procedure for estimation of soluble oxalate was same as that of oxalate oxidase except that 0.1ml of purified enzyme was taken in place of standard oxalate. The estimation of nitrate reductase activity and nitrite were done by Stevens and Oaks (1973), nitrate by Wooley *et al.* (1960) and nitrite reductase activity by Rao *et al.* (1988). The change in activity of each enzyme in leaves of seedlings grown in 0 to 100 mM NaCl salinity, was determined at 4, 6, 8 and 10 days after germination. Two-way ANOVA was carried out to quantify statistical significance of effect of different NaCl levels on activities of oxalate oxidase, nitrate reductase and nitrite reductase at different days of growth. The statistical significance of correlation coefficients was used to find the possible physiological link between oxalate degradation and nitrate assimilation.

The activity of oxalate oxidase in leaves increased significantly with increase in salinity upto 50 mM NaCl treatment but decreased at 100 mM concentration, with maximum activity being at day 8 (Fig. 1). Correspondingly, levels of soluble oxalate decreased upto 50 mM NaCl while that of H_2O_2 increased with the increase in NaCl level (Fig. 1). Increase in oxalate oxidase activity had earlier been found in NaCl stressed forage sorghum seedlings (Singh *et al.* 1998). One reason for increased oxalate oxidase activity could be due to increased tissue remodelling for uptake of water from increasingly saline nature of growth medium (Lane *et al.* 1993). The activity of nitrate reductase and nitrate level decreased with the increase in NaCl concentration at all growth stages (Table 1). The NaCl caused decrease in nitrate reductase activity has been reported in other cereals like *Sorghum vulgare* (Rao and Gnanam 1990) and maize (Khan and Srivastava 2000). The most

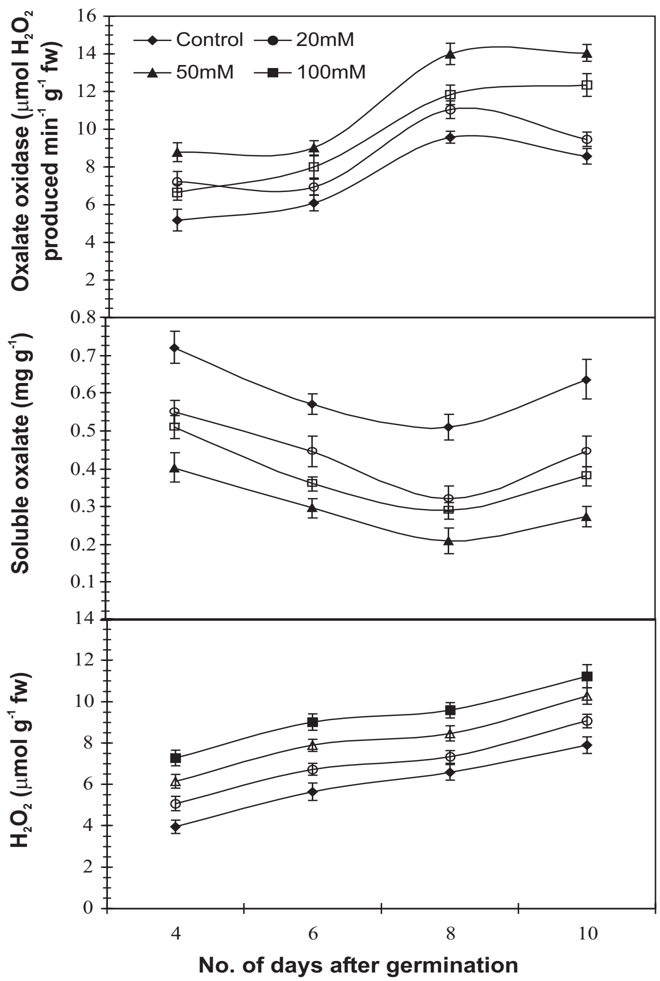

Fig. 1. Activity of oxalate oxidase and levels of soluble oxalate and H_2O_2 in leaves of grain sorghum seedling plants grown under different NaCl concentrations during early growth. All readings are average of three observations [Result of ANOVA on oxalate oxidase activity: F value of treatments= 151.34; F value odf days growth= 286.04 (both significant at 1% level of significance)]

Indian J. Plant Physiol., Vol. 11, No. 3, (N.S.) pp. 295-299 (Jul.-Sept., 2006)

Table 1. Activity of nitrate reductase and nitrate content in leaves of grain sorghum hybrid (var. CSH-14) exposed to various levels of NaCl stress, during early growth.

No. of days	Concentration of NaCl			
of growth	Control	20mM	50mM	100mM
	Nitrate reductase activity (µmol NO_2^- hr^{-1} g^{-1} fw)			
4	2.134±0.24	1.507±0.22	1.13±0.18	1.004±0.19
6	2.511±0.18	2.009±0.19	1.758±0.17	1.381±0.16
8	3.264±0.27	2.385±0.25	2.009±0.18	1.256±0.2
10	4.394±0.23	3.264±0.28	2.511±0.21	1.883±0.18
	Nitrate content (µmol g^{-1} fw)			
4	51.72±4.32	32.91±4.67	18.81±4.58	7.05±4.83
6	63.48±4.78	47.02±4.07	33.7±5.15	21.16±4.46
8	75.23±5.62	58.78±5.13	39.7±5.69	27.21±3.72
10	84.64±5.27	65.83±5.38	51.2±4.89	37.6±4.76

All values are 'mean ± SD (n=3); Result of ANOVA on nitrate reductase activity: F value of treatments= 139.55, F value of days of growth= 116.99 (Both significant at 1% level of significance)

probable reason for decrease in nitrate reductase activity could be due to decrease in nitrate (Table 1) which in turn might be due to interference in nitrate uptake by nitrate transporter. The same reason had been advocated for decrease in NR activity in wheat (Angeles Botella *et al.* 1993) and barley (Aslam *et al.* 1984). Increase in hydrogen peroxide in stressed grain sorghum might also have some role in reversible inactivation of nitrate reductase in NaCl stress (Solomonson and Barber 1990). The nitrite reductase activity and nitrite level decreased with the increase in NaCl concentration throughout the growth study (Table 2). This might be due to decrease in the nitrite supply resulting from decreased nitrate reductase activity in the leaves of sorghum seedlings. Decrease in nitrite reductase may also be due to salt *per se* (Rao and Gnanam 1990). The observed decrease in nitrite reductase activity in response to NaCl salinity is corroborated by the observation in *Sorghum vulgare* (Rao and Gnanam 1990).

The activity of oxalate oxidase was statistically correlated with early activity of nitrate reductase and nitrite reductase and a significant negative correlation was shown in both cases (Fig. 2). This indicated strong

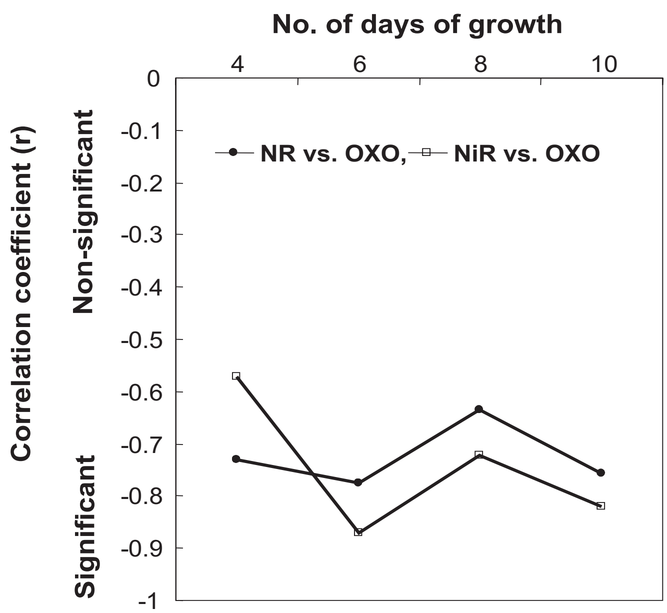

Fig. 2. Correlation between oxalate oxidase (OXO) and early nitrate assimilating enzymes (NR, i.e. nitrate reductase and NiR, i.e. nitrite reductase) in grain sorghum seedling plants grown under different NaCl concentrations during early growth. All correlation coefficients above 0.7 are statistically significant on the basis of 4 degrees of freedom for each correlated pair of data on a day of growth

Table 2. Activity of nitrite reductase and nitrite content in leaves of grain sorghum hybrid (var. CSH-14) exposed to various levels of NaCl stress, during early growth.

No. of days	Concentration of NaCl			
of growth	Control	20mM	50mM	100mM
	Nitrite reductase activity (nmol NO_2^- hr^{-1} g^{-1} fw)			
4	26.33±1.41	18.9±1.34	12.42±1.39	6.86±1.22
6	28.19±1.39	22.62±1.22	14.28±1.17	8.71±1.19
8	29.11±1.17	23.55±1.14	17.06±1.24	12.42±1.17
10	31.9±1.06	26.33±1.3	20.77±1.09	15.67±1.22
	Nitrite content (nmol g^{-1} fw)			
4	41.43±4.81	30.4±4.26	15.07±2.51	7.53±3.27
6	56.49±5.52	44±4.82	26.9±2.96	15.07±2.82
8	67.79±5.64	54.1±4.72	38.9±3.31	29.38±3.06
10	78.1±5.92	64.2±4.28	52±3.02	40.68±2.79

All values are mean ± SD (n=3); Result of ANOVA on nitrite reductase activity: F value of treatments= 481.53, F value of days of growth= 88.44 (Both significant at 1% level of significance).

possibility of simultaneous regulation of oxalate degradation and nitrate assimilation, as evident from significant coefficient correlation values. The elevated oxalate oxidase under salt stress might be due to increased carboxylation of CO_2 in first carboxylation step of C_4 cycle from phosphoenol pyruvate (PEP) by stimulating PEP carboxylase, as had earlier been observed in wheat (Thind and Malik 1988), resulting into oxalic acid production and accumulation which might, then, induce oxalate oxidase. This would regulate oxalate level by degrading it to H_2O_2 and CO_2 and thus higher level of H_2O_2 in stressed tissues. At the same time, under salt stress, reduced nitrate uptake and Cl^- mediated inhibition of nitrate movement from storage pool to metabolic pool and direct reversible inactivation of nitrate reductase by superoxide radicals (Solomonson and Barber 1990) could slow down nitrate assimilation. Thus, oxidative stress might indirectly stimulate oxalate oxidase reactions along with suppression of nitrate assimilation at the same time. The maintenance of C/N ratio is of critical importance to plant growth. Hence, the decreased nitrogen assimilation due to already mentioned reasons must have occurred simultaneously with increased oxalate oxidase as part of decreased carbon fixation.

ACKNOWLEDGEMENTS

The authors are grateful to M/s Nath Seeds Ltd., Aurangabad (Mah), India, for supplying the seeds of grain sorghum CSH-14 for this work.

REFERENCES

Angeles Botella, M., Cruz, C., Martins-Loucao, M.A. and Cerdá, A. (1993). Nitrate reductase activity in wheat seedlings as affected by NO_3^-/NH_4^+ ratio and salinity. *J. Plant Physiol.* **142:** 531-536.

Aslam, M., Huffaker, R.C. and Rains, D.W. (1984). Early effects of salinity on nitrate assimilation in barley seedlings. *Plant Physiol.* **76:** 321-325.

Katiyar, S. and Dubey, R.S. (1992). Influence of NaCl salinity on behaviours of nitrate reductase and nitrite reductase in rice seedlings differing in salt tolerance. *J. Agron. Crop Sci.* **169:** 289-297.

Khan, M.G. and Srivastava, H.S. (2000). Nitrate application improves plant growth and nitrate reductase activity in maize under saline conditions. *Indian J. Plant Physiol.* **5:** 154-158.

Kuchhal, N.K., Satyapal and Pundir, C.S. (1993). Partial purification, characterization and possible physiological role of sorghum leaves oxalate oxidase *Indian J. Plant Physiol.* **36:** 159-162.

Lane, B.G., Dunwell, J.M., Ray, J.A., Schmitt, M.R. and Cuming, A.C. (1993). Germin, a protein marker of early development is an oxalate oxidase. *J. Biol. Chem.* **268:** 12239-12242.

Mishra, S.N. and Srivastava, H.S. (1983). Increase in nitrate reductase activity, organic nitrogen and protein content of maize leaves supplied with nitrate and ammonia. *Z. Pflanzenphysiol.* **113:** 91-93.

Rao, R.K. and Gnanam, A. (1990). Inhibition of nitrate and nitrite reductase activities by salinity stress in *Sorghum vulgare*. *Phytochem*. **29:** 1047-1049.

Rao, R.K., Mannan, R.M., Gnanam, A. and Bose, S. (1988). Inhibition of nitrate and nitrite reductase in wheat by Sandoz 9785. *Phytochem.* **27:** 685-688.

Satyapal and Pundir, C.S. (1993). Purification and properties of an oxalate oxidase from leaves of grain sorghum hybrid CSH-5. *Biochem. Biophys. Acta* **1161:** 1-5.

Singh, S., Thakur, M., Malik, V., Goyal, L. and Pundir, C.S. (1998). Influence of NaCl stress on oxalate oxidase activity in germinating seeds of forage sorghum leaves. *Indian J. Plant Physiol.* **3:** 317-319.

Solomonson, L.P. and Barber, M.J. (1990). Assimilatory nitrate reductase: functional properties and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41:** 225-253.

Stevens, D.L. and Oaks, A. (1973). The influence of nitrate on the induction of nitrate reductase in maize roots. *Can. J. Bot.* 51: 1255-1258.

Thind, S.K. and Malik, C.P. (1988). Carboxylation and related reactions in wheat seedlings under osmotic stress. *Plant Physiol. Biochem. (India)* **15:** 58-63.

Trinchant, J.C. and Rigaud, J. (1996). Bacteroid oxalate oxidase and soluble oxalate in nodules of faba beans (*Vicia faba* L.) submitted to water restricted conditions: Possible involvement in nitrogen fixation, *J. Exp. Bot.* **47:** 1865-1870.

Wooley, J.I., Hicks, G.P. and Hageman, R.H. (1960). Rapid determination of NO_3^- and NO_2^- in plant material. *J. Agric. Chem.* **8:** 481-482.

Yamashita, K. and Matsumoto, H. (1996). Salt stress-induced enhancement of anion efflux and anion transport activity in plasma membrane of barley roots. *Soil Sci. Plant Nutr.* **42:** 209-213.