Proc. Indian natn. Sci. Acad. **B52** No. 4 pp 491–494 (1986)

Effect of Zinc Deficiency on Enzyme Activities of Nitrate Reduction and Ammonia Assimilation of *Oryza sativa* L. and *Pennisetum americanum* L. Leeke†

Y SEETHAMBARAM[1] and V S R DAS[2]
Centre for Photosynthetic Studies, School of Life Sciences, University of Hyderabad, Hyderabad 500 134

(Received 17 February 1986; after revision 24 April 1986)

The influence of zinc deficiency on the enzyme activities of nitrate reductase (NR) and nitrite reductase (nitrate reduction); glutamine synthetase (GS) and glutamate synthase (GOGAT) (ammonia assimilation) were investigated in rice and pearl millet. The enzyme activities of both nitrate reduction and ammonia assimilation were reduced in pearl millet (a C_4 plant) under zinc deficiency, whereas only nitrate-reducing capacity was inhibited in rice (a C_3 plant) without any effect on ammonia assimilatory power. The inhibition of the affected enzymes was marginal in 15-day old plants and increased with the increasing age. The observed activities of GS and GOGAT in rice are discussed in relation to the enhanced photorespiration under zinc deficiency.

Key Words: **Rice, Pearl millet, Zinc deficiency, Nitrate reductase, Nitrite reductase, Glutamine synthetase, Glutamate synthetase**

Introduction

Reduction of dry matter production under zinc deficiency has been reported in Soybean (Ohki 1978) and in tropical legumes (Johnson & Simons 1979). Nitrogen assimilation is an important aspect of plant metabolism that governs the dry matter production and grain yield in various crops. Ghildiyal et al. (1981) have reported the reduction of nitrate reductase activity in mustard under zinc deficiency. However, the reasons for the lowered activity of this enzyme under zinc deficiency are not known. Also, the stage of the growth at which the enzymes of nitrogen assimilation are affected under zinc deficiency is not clear. The present study was undertaken in rice (*Oryza sativa* L.) (a C_3 plant) and pearl millet (*Pennisetum americanum* L. Leeke) (a C_4 NADP-ME type) with the objectives of finding the influence of zinc deficiency on (i) the enzymes—nitrate reductase, nitrite reductase, glutamine synthetase and glutamate synthase, (ii) the stage of the growth at which these enzymes are affected (if any), and (iii) the reasons for the lowered activity of nitrate reductase.

Materials and Methods

Rice (Var. 4–14) and pearl millet (Var. RBS-2) plants were grown in pot culture in a greenhouse under natural photoperiod (day temperature 28–35°C, night temperature 20–25°C) employing the sand culture technique as described by Seethambaram and Das (1984), at 0, 0.025, 0.05 and 0.1 ppm levels of zinc. The enzyme activities were determined in 15-, 23-, and 30-day old plants. All the

† This paper forms a part of the approved Ph.D. Thesis submitted by Y S to the University of Hyderabad

[1] Department of Biochemistry, College of Agriculture, Rajendranagar, Hyderabad 500 030
[2] Department of Botany, Sri Venkateswara University, Tirupati 517 502

leaves of the plant formed the sample at 15th day whereas only 3rd and 4th leaves (from top) were used as a sample at the later two stages.

In vitro NR (EC 1.6.1.1) was assayed by the method of Hageman and Hucklesby (1971). The colour developed by diazo-coupling reagents was measured at 540 nm.

The *in vivo* NR assay was done as described by Brunetti and Hageman (1976). 0.3 g leaf tissue (10 mm diameter discs) was vacuum-infiltrated in 5 ml of 0.1 M KNO_3, 0.1 M potassium phosphate (*p*H 7.5) and 0.3% Triton X-100 and incubated in the dark at 30°C. Aliquots were removed at time intervals for nitrite determination. Activity was expresed as μmol NO^-_2 formed/g fresh weight/hr.

Nitrite reductase (EC 1.6.6.4) extracted in 10 mM Tris-HCl buffer (*p*H 8.0), containing 1mM EDTA and 10 mM dithiothreitol, was assayed with dithionite—reduced methyl viologen as the reductant (Losada & Paneque 1971).

Glutamine synthetase (EC 6.3.1.3) was assayed based on glutamyl hydroxamate synthesis according to O'Neal and Joy (1973b). Glutamate synthase (EC 2.6.1.53) activity was determined by measuring the formation of glutamate from 2 oxoglutarate according to Wallsgrove et al. (1977). Reaction mixture contained 10 mM 2-oxoglutarate, 10 mM glutamine, 10 mM amino oxyacetate, 100 μg methyl viologen and 100 μl enzyme in a total volume of 0.5 ml. The reaction was started by the addition of 0.1 ml of a solution containing 16 mg sodium dithionite and 16 mg sodium bicarbonate/ml. The separation of glutamate formed, was achieved by chromatography in 75% (W/W) phenol in the presence of ammonia vapour. The chromatograms were dried after development and then sprayed with ninhydrin reagent of Atfield and Morris (1961). The glutamate spots were cut out, eluted and its extinction at 500 nm determined in a Gilford spectrophotometer.

Results

Enzymes of Nitrate Reduction (table 1)

The enzyme activity was reduced at low levels of zinc. Whereas the *in vitro* NR and nitrite reductase activities were not disturbed in the early stages of growth, the *in vivo* NR activity was reduced. But the activities of all these enzymes were inhibited during the subsequent growth. Rice and pearl millet grown at 0 ppm zinc exhibited an *in vitro* NR activity of 58 and 61% respectively as against their controls (0.1 ppm Zn plants), whereas the corresponding values for *in vivo* NR were 46 and 51% at the 30th day of the growth. The enzymes of nitrate reduction were more affected in rice than in pearl millet at low levels of zinc.

Enzymes of Ammonia Assimilation (table 2)

Zinc nutrition had no effect on the activities of GS and GOGAT of rice, at any stage of the growth measured. However, the activities of these

Table 1 *Activities of* in vitro *NR,* in vivo *NR and nitrite reductase in 15, 23 and 30-day old plants of rice and pearl millet grown at different concentrations of zinc. Values are averages of four observations and critical differences shown below each column (P<0.05). NR expressed as μ moles nitrite formed/g (fresh weight)/hr, nitrite reductase expressed as μmoles nitrite reduced/g (fresh weight)/hr*

Zinc level (ppm)	*in vitro* NR			*in vivo* NR			Nitrite reductase		
days	15	23	30	15	23	30	15	23	30
Rice									
0.0	7.8	5.0	4.5	1.6	1.2	0.9	24.5	17.4	11.3
0.025	7.9	6.1	5.6	1.8	1.4	1.2	25.0	21.4	16.9
0.05	8.2	6.6	6.8	1.8	1.6	1.6	24.6	23.6	21.8
0.1	8.2	7.5	7.8	2.0	1.9	1.9	25.2	26.8	25.6
LSD (*P*<0.05)	—	0.8	0.6	0.3	0.2	0.2	—	1.9	2.3
Pearl millet									
0.0	13.9	9.9	8.7	2.8	2.0	1.7	44.3	32.7	24.3
0.025	14.4	11.2	10.6	3.0	2.3	2.1	44.8	37.9	30.2
0.05	14.6	12.5	11.9	3.2	3.8	2.6	45.4	40.2	39.8
0.1	14.5	13.9	14.2	3.4	3.1	3.3	45.6	46.8	45.8
LSD (*P*<0.05)	—	1.4	1.2	0.3	0.3	0.2	—	2.4	2.8

LSD = Least significant difference

Table 2 *Effect of zinc nutrition on the activities of glutamine synthetase and glutamate synthase of rice and Pearl millet at 3 different growth stages. Values are the means of four determinations*

Enzyme	Zinc level (ppm)		Rice			Pearl millet		
		(days)	15	23	30	15	23	30
Glutamine synthetase	0.0		26.5	27.1	28.1	26.2	20.0	14.3
(μmoles r-glutamyl	0.025		28.2	26.6	27.6	27.8	22.9	19.4
hydroxamate/g fr wt/hr)	0.05		29.1	27.3	28.3	30.2	29.1	23.5
	0.1		28.5	26.8	27.8	31.6	32.3	29.8
LSD ($P < 0.05$)			NS	NS	NS	2.7	4.6	5.2
Glutamate synthase	0.0		5.2	4.9	5.6	3.8	3.1	2.3
(μmoles glutamate/g	0.025		4.9	5.3	5.2	4.1	3.9	3.2
fr. wt/hr)	0.05		5.4	5.1	5.4	4.5	4.5	4.1
	0.1		5.3	4.8	4.9	4.7	5.2	4.8
LSD ($P < 0.05$)			NS	NS	NS	0.5	0.8	0.7

enzymes were significantly reduced in pearl millet under low levels of zinc at all the growth stages. The differences for varying levels of zinc treatment were not significant in rice. In one-month-old plants of pearl millet that were grown without zinc, the activities of GS and GOGAT were found to be about 48% of their controls (0.1 ppm zinc plants).

Discussion

The lowered *in vivo* NR activity under zinc-deficiency (table 1) was consistent with the reduced net photosynthesis (Seethambaram & Das 1985) in both the plants at all the stages of the growth. *In vitro* NR was not affected in the 15-day-old plants of rice and pearl millet by zinc nutrition suggesting that zinc-deficient cells possessed the full potential for nitrate reduction at this stage. The reduction in *in vivo* NR under zinc deficiency at this stage may be due to shortage of NADH as a result of decreased net photosynthesis. Sawhney and Naik (1972) opined that redox changes taking place in greening tissue, as a result of the hill reaction, create conditions for the enhanced synthesis of NR and nitrite reductase. Shortage of reduced ferredoxin due to lowered electron transport under zinc deficiency (Seethambaram 1983) would lead to the suppression of nitrite reductase as observed here (table 1).

The GS/GOGAT pathway suggested by Lea and Miflin (1974) is now considered to be the major pathway of photosynthetic ammonia assimilation rather than the glutamate dehydrogenase pathway. In addition to the incorporation of NH_3 produced by nitrate reduction, GS/GOGAT pathway also reassimilates the NH_3 released during photorespiration (Keys et al. 1978).

Though there was a considerable lowering in the activities of NR and nitrite reductase under zinc deficiency in rice and pearl millet, GS and GOGAT were not affected in rice while these enzymes were significantly reduced in Pearl millet (table 2). GS is activated with increase of *p*H, Mg^{2+} concentration and energy charge in light (Lea & Miflin 1979). Ferredoxin dependent GOGAT is localised in the chloroplasts (Lea & Miflin 1974). ATP and reduced ferredoxin are the limiting factors under zinc deficiency due to lowered electron-transport (Seethambaram 1983). Decreased levels of ATP and reduced ferredoxin might be responsible for the reduction in the activities of GS and GOGAT respectively in pearl millet under zinc deficiency. However, GS and GOGAT were not affected in rice.

Zinc deficiency caused enchancement of photorespiration in rice but not in pearl millet (Seethambaram et al. 1985). The increased photorespiration under zinc deficiency in rice, would eventually lead to the enhanced release of ammonia by glycine decarboxylation.

The possible explanation for the normal levels of GS/GOGAT in rice, even under zinc deficiency is that the elevated levels of these enzymes are required for a rapid reassimilation of the increased release of photorespiratory ammonia. Though energy level and reducing power are the limiting factors under zinc deficiency, the metabolism might have been so regulated to avoid the accumulation of ammonia, which at higher concentrations inhibit photosynthetic CO_2 fixation by uncoupling photophosphorylation (Good 1960). However, it is evident in our present study (data not given here) that the reduction of photophosphorylation under zinc deficiency was due to lowered rates of electron flow and not due to the uncoupling effect. Canvin (1981) was of the opinion that the capacity of the

ammonia assimilation system in C_3 leaves would quantitatively have to be geared to rates of photorespiratory ammonia release rather than to those of NH_3 formation from primary N assimilation. Gaudinova (1983) observed the reduction of NR activity in *Pisum sativum* L. (a C_3 plant) under Mg and Ca deficiencies, while GS was not affected. In senescent wheat flag leaves, GS/GOGAT pathway of ammonia assimilation and photorespiratory nitrogen cycle remained functional in spite of reduction in photosynthetic CO_2 fixation and other enzymes (Berger et al. 1985).

Our results conclude that the nitrate reduction and ammonia assimilation were reduced under zinc deficiency in pearl millet (a C_4 plant) while in rice (a C_3 plant) only nitrate reduction was affected without any influence on ammonia assimilation. It is suggested that the present activities of GS and GOGAT in rice might have geared to assimilate the increased photorespiratory ammonia under zinc deficiency.

Acknowledgement

One of the authors (YS) is grateful to the Andhra Pradesh Agricultural University, for the grant during the period of this investigation.

References

Atfield G N and Morris C J O R 1961 Analytical separations by high-voltage paper electrophoresis. Amino acids in protein hydrolysates; *Biochem. J.* **81** 606–614

Berger M G, Woo K C, Wong S C and Fock H P 1985 Nitrogen metabolism in senescent flag leaves of wheat (*Triticum aestivum* L.) in the light; *Pl. Physiol.* **78** 779–783

Brunetti N and Hageman R H 1976 Comparison of *in vivo* and *in vitro* assays of nitrate reductase in wheat (*Triticum aestivum* L.) seedlings; *Pl. Physiol.* **58** 583–587

Canvin D T 1981 Photorespiration and nitrogen metabolism; in *Nitrogen and Carbon Metabolism* pp 178–194 ed. J D Bewley (The Hague: Dr W Junk Publishers)

Gaudinova A 1983 The effect of mineral nutrition on nitrogen assimilation by intact plants of *Pisum sativum* L.; *Biol. Plant.* **25** 449–455

Ghildiyal M C, Sirohi G S and Pandey M 1981 Growth and nitrate reductase activity in mustard varieties as affected by zinc nutrition; *Indian J. Pl. Physiol.* **24** 112–113

Good N E 1960 Activation of Hill reaction by amines; *Biochem. Biophys. Acta* **40** 502–517

Hageman R H and Hucklesby D P 1971 Nitrate reductase from higher plants; in *Methods in Enzymology* Vol 23 pp 491–503 ed. A Sanpietro (New York: Academic Press)

Johnson A D and Simons J G 1979 Diagnostic indices of zinc deficiency in tropical legumes; *J. Pl. Nutr.* **1** 123–149

Keys A J, Bird I F, Cornelius M J, Lea P J, Wallsgrove R M and Miflin B J 1978 Photorespiratory nitrogen Cycle; *Nature* **275** 741–743

Lea P J and Miflin B J 1974 Alternative route for nitrogen assimilation in higher plants; *Nature* **251** 614–616

—— and —— 1979 Photosynthetic ammonia assimilation; in *Encyclopedia of Plant Physiology* New Series Vol. 6 pp 445–456 ed. M Gibbs and E Latzko (New York: Springer-Verlag)

Losada M and Paneque 1971 Nitrite reductase; in *Methods in Enzymology* Vol 23 pp 487–491 ed. A Sanpietro (New York: Academic Press)

Ohki K 1978 Zinc concentration in Soybean as related to growth, photosynthesis and carbonic anhydrase activity; *Crop Sci.* **18** 79–82

O'neal D and Joy K W 1973b Glutamine synthetase of pea leaves. I. Purification, stabilization and *p*H optima; *Arch. Biochem. Biophys.* **159** 113–122

Seethambaram Y 1983 The Biochemistry of Photosynthesis and Nitrogen Assimilation in Relation to performance of Rice and Pearl millet under zinc deficiency; Ph.D. Thesis, University of Hyderabad, Hyderabad

—— and Das V S R 1984 Influence of zinc deficiency on levels of superoxide dismutase, catalase and lipid peroxidation in rice and Pearl millet; *Indian J. Biochem. Biophys.* **21** 248–250

—— and —— 1985 Photosynthesis and activities of C_3 and C_4 photosynthetic enzymes under zinc deficiency in *Oryza sativa* L. and *Pennisetum americanum* L. Leeke; *Photosynthetica* **19** 72–79

——, Rao A N and Das V S R 1985 The levels of carbonic anhydrase and photorespiratory enzymes under zinc deficiency in *Oryza sativa* L. and *Pennisetum americanum* L. Leeke; *Biochem. Physiol. Pflanzen* **180** 107–113

Sawhney S K and Naik M S 1972 Role of light in the synthesis of nitrate reductase and nitrite reductase in rice seedlings; *Biochem. J.* **130** 475–485

Wallsgrove R M, Harel E, Lea P J and Miflin B J 1977 Studies on glutamate synthase from the leaves of higher plants; *J. expl. Bot.* **28** 588–596