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BRIEF COMMUNICATION

Iron mediated nitrate reductase activity in different parts of young maize seedlings

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Abstract

Incubation of 5-d-old maize seedlings in the half-strength Hoagland's nutrient solution containing 10 mM KNO_3 with $FeCl_3$ or $FeSO_4$ (0.5 or 2.0 mM) caused a significant increase in nitrate reductase (NR) activity and slightly increased total protein content in root, shoot and scutellum. In case of root, NADPH:NR activity was inhibited contrary to the NADH:NR activity. In spite of NR activity, nitrate uptake was inhibited from 13 to 37 % by the iron. The results presented demonstrate an isoform specific, organ specific, and to some extent salt specific responses of NR to iron.

Additional key words: NADH:NR, NADPH:NR, *Zea mays*.

Nitrate reductase (NR) activity is a rate limiting step in nitrate assimilation. It is affecting by iron supply as was observed in tomato (Brown and William 1976), rice (Sasakawa and Yamamoto 1977) and in marine phytoplankton (Timmermans *et al.* 1994). Most of these experiments were conducted with the NADH:NR (E.C. 1.6.6.1) isoform of the NR, which is of almost ubiquitous occurrence and is believed to be the main nitrate assimilating isoform. The bispecific NADPH:NR (E.C. 1.6.6.2) was also found in many species (Srivastava 1992). In maize seedlings it is present in roots and scutellum but not in leaves (Redinbaugh and Campbell 1981). This isoform is a product of a different gene than NADH:NR (Warner *et al.* 1987) and differs from NADH:NR in some constituent amino acids (Schondorf and Hachtel 1995). The physiological significance of the presence of this isoform in seedlings is not known (Srivastava 1992).

The current study was undertaken to investigate whether the activity of NR varied according to the isoform, organ of the seedling, and the iron salts used. It was hoped that the investigation will provide some important clue for the mechanism of iron action on nitrate uptake and assimilation in young seedlings of maize.

Seeds of *Zea mays* L. cv. Ganga safed-2 were surface sterilized with 1 % bleaching powder ($CaOCl_2$) and soaked in distilled water for about 1 h. Seedlings were raised in 15 cm Petri plates containing two layers of filter paper moistened with Hoagland's nutrient solution containing 10 mM KNO_3 (pH 6.5) as sole nitrogen source in dim light provided by a combination of cool fluorescent tubes and incandescent bulbs (photoperiod 12 h, irradiance approximately 27 W m^{-2}) at temperature 25 ± 3 °C for 5 d. Then the seedlings were incubated for 24 h in a nutrient medium containing 10 mM KNO_3 and 0.5 or 2.0 mM $FeCl_3$ or $FeSO_4$ as iron source under the same environmental conditions.

Crude enzyme extract was prepared by extracting freshly harvested plant parts in medium containing 1 mM EDTA in 0. 1 M phosphate buffer (pH 7.5), 5 mM cystein and 0.5 % casein at temperature 0 - 1 °C. NADH:NR was assayed according to Srivastava and Ormrod (1984), and NADPH:NR to Dailey *et al.* (1982). Total protein content of fresh material was determined by the method of Lowry *et al.* (1951). Bovine serum albumin (BSA) was used as a standard. For nitrate uptake measurement, 5 seedlings of similar size and mass were placed in a 250 cm^3 beaker containing 100 cm^3 of half strength Hoagland's nutrient solution with 10 mM KNO_3 and 0.5 or 2.0 mM of iron salts for 24 h. Nitrate content of the nutrient solution was measured with an *Orion* model *290A* ion selective electrode before and after incubation.

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Seedlings incubated in the nutrient solution with iron salts did not show any visible effects upto 3 d, however, afterward seedlings raised with $FeSO_4$ was dark green (blue green), thicker and shorter in height as compared to $FeCl_3$ incubated seedlings which were longer, slightly thin and bright green (yellow green). In a medium containing 10 mM KNO_3 and 0.5 or 2.0 mM iron salts, the NADH:NR activity increased significantly as compared to control (-Fe) (Table 1). In root and shoot the increase was higher with $FeCl_3$ than that with $FeSO_4$. While in scutellum maximum NR activity was observed with $FeSO_4$. However, in all the tissues examined, maximum increase was observed in the root.

Table 1. Effect of different iron salts added to nutrient solution with 10 mM KNO_3 and their concentrations on NADH:NR and NADPH:NR activities [nmol(NO_2^-) g^{-1}(f.m.) s^{-1}] in different parts of maize seedlings. Means ± SD (n = 6).

Treatments	NADH:NR			NADPH:NR	
	root	shoot	scutellum	root	scutellum
Control (-Fe)	0.244 ± 0.017	0.161 ± 0.011	0.230 ± 0.004	0.692 ± 0.021	0.370 ± 0.008
0.5 mM $FeCl_3$	0.373 ± 0.009	0.195 ± 0.031	0.256 ± 0.007	0.498 ± 0.006	0.458 ± 0.033
2.0 mM $FeCl_3$	0.411 ± 0.004	0.231 ± 0.006	0.276 ± 0.005	0.478 ± 0.011	0.398 ± 0.031
0.5 mM $FeSO_4$	0.365 ± 0.018	0.185 ± 0.008	0.295 ± 0.021	0.395 ± 0.019	0.915 ± 0.009
2.0 mM $FeSO_4$	0.335 ± 0.020	0.210 ± 0.006	0.305 ± 0.017	0.321 ± 0.014	0.835 ± 0.022

NADPH:NR was detected only in root and scutellum. In root, activity was entirely different than that of NADH:NR. NADPH:NR activity decreased at almost all Fe concentrations used. Maximum decrease was observed by 2.0 mM $FeSO_4$. On the other hand in scutellum NADPH:NR activity was increased remarkably as compared to NADH:NR activity (Table 1).

The total protein content was affected slightly by the supply of iron. In root, protein content was slightly increased and there was not remarkable difference between both the salts used. In shoot and scutellum, total protein content was maximum at 0.5 mM $FeSO_4$ (Table 2). It has been reported earlier that iron application stimulates the root growth in rice *via* increasing the carbohydrate and protein content (Ueda 1956, Shetty and Miller 1966). Seedlings incubated in the nutrient solution with iron salts for 24 h showed inhibition (13 to 37 %) of nitrate uptake (Table 2).

Table 2. Effect of different iron salts added to nutrient solution with 10 mM KNO_3 and their concentrations on total protein content [mg g^{-1}(f.m.)] and nitrate uptake [μmol(NO_3^-) seedling^{-1} d^{-1}] in different parts of maize seedlings. Means ± SD (n = 6).

Treatments	Protein content			Nitrate uptake
	root	shoot	scutellum	
Control (-Fe)	8.31 ± 0.86	24.41 ± 0.66	32.37 ± 1.01	91.00 ± 1.56
0.5 mM $FeCl_3$	11.21 ± 0.52	29.46 ± 0.93	33.61 ± 0.62	57.00 ± 1.28
2.0 mM $FeCl_3$	11.64 ± 0.49	28.91 ± 0.76	34.82 ± 0.45	65.00 ± 0.98
0.5 mM $FeSO_4$	10.65 ± 0.91	31.11 ± 1.21	35.66 ± 0.68	79.00 ± 1.33
2.0 mM $FeSO_4$	11.89 ± 0.84	30.83 ± 0.88	34.32 ± 0.57	61.00 ± 0.69

The experiments described demonstrate an organ specific effect of iron on NR activity in maize seedlings, and also the variation in effects according to the NR isoforms. Whatever the mechanism of regulation of NADPH:NR by iron may be, the response contrary to NADH:NR in root indicate its complementary role in nitrate assimilation. Apparently, when one isoform of NR is inhibited, the other takes over the job of nitrate assimilation. However, significant increase in NADPH:NR activity in scutellum, which in intact seedlings is not an assimilatory organ (Srivastava 1974), hints some function of this isoform other than the nitrate assimilation. The differential response of NADH:NR and

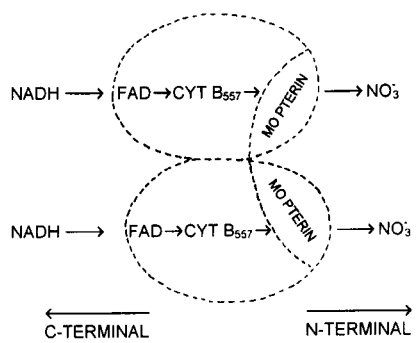

Fig. 1. Model of NADH nitrate reductase and electron transport.

NADPH:NR activity was reported earlier (Pandey *et al.* 1997). Maize leaves NR is able to catalyse ferric citrate reduction (Campbell and Smarelli 1978, Campbell and Redinbaugh 1984). In higher plants, iron is an integral component of nitrate reductase (Srivastava and Singh 1995) in the form of cytochrome b_{557} (Fig. 1) and cytochrome P_{450}. The elucidation of mechanism of iron effect is under investigation in our laboratory.

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