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BRIEF COMMUNICATION

Relationship between soil nitrate content and activities of NADH: and NAD(P)H:nitrate reductases in Indian mustard

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Abstract

The pattern of NADH- and NAD(P)H-specific nitrate reductase (NRs) activities in Indian mustard (*Brassica juncea* L. Czern. and Coss.) was monitored throughout growth stages. NAD(P)H:NR (EC 1.6.6.2) activity was maximum at early stages of growth (30 days after sowing, DAS), then declined gradually reaching to almost zero at 90 DAS. Contrary to this, NADH:NR (EC 1.6.6.1) activity was low at 30 DAS, then gradually increased till 90 DAS and thereafter, it became constant. The decrease in NAD(P)H:NR activity and increase in the NADH:NR activity were associated with the seasonal decrease in nitrate content in the soil.

Additional key words: *Brassica juncea* L., growth stages.

Nitrate reductase (NR) is a key enzyme involved in the first step of nitrate assimilation pathway in the plant. It catalyzes the reduction of nitrate to nitrite with pyridine nucleotide as electron donor in higher plants. The NR in most higher plant species is NADH-specific (EC 1.6.6.1), but in some plants including rice, soybean, maize, birch, barley and mustard, the NAD(P)H specific NR has also been reported. In plants, nitrate is the primary signal regulating NR induction, and the enzyme is synthesized *de novo* in response to nitrate (Abdin *et al.* 1994). In our earlier work, we have reported two forms of NR in the mustard seedlings: NAD(P)H:NR activity was found maximum at higher nitrate content, while NADH:NR activity at low nitrate content (Ahmad and Abdin 1999). On this basis, we hypothesised that the two NRs appear to be induced in sequence during growth and development of the mustard in response to soil nitrate content. The present investigation was, therefore, conducted to examine the above hypothesis and to evaluate the pattern of NADH: and NAD(P)H:NR activities in the field grown Indian mustard throughout the growing season in response to the availability of nitrate in the soil.

The seeds of mustard (*Brassica juncea* L. Czern. Coss. cv. Pusa Jai Kisan) were obtained from National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, India. The crop was raised in the experimental field during winter season of 1998-1999 using randomized block design. The plot size was 9 m^2 and the texture of the soil was sandy loam. Nitrogen, sulphur, phosphorous and potassium were applied at the rate of 100, 40, 40 and 40 kg ha^{-1}. The sowing and culturing were carried out according to the method described earlier (Ahmad *et al.* 1999). For soil nitrate determination, soil samples from five different places of each experimental plot were taken at 15, 30, 45, 60, 75, 90, 105 and 120 days after sowing (DAS). The soil samples were then oven dried for 72 h at 65 °C and extraction of nitrate from the soil samples was done following the method of Grover *et al.* (1978). Nitrate in the aliquot was reduced to nitrite by hydrazine sulphate following the method of Fishman *et al.* (1964), and nitrite was estimated by the method of Evans and Nason (1953). For assaying nitrate reductase (NR) activity in the

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Abbreviations: DAS - days after sowing; NR - nitrate reductase.
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leaves at different growth stages, leaf samples from five plants were collected at 30, 45, 60, 75, 90 and 105 DAS between 09:30 and 11:30. The assay of nitrate reductase was done as described earlier (Ahmad and Abdin 1999). Soluble protein content was determined by the method of Bradford (1976).

Soil nitrate content was maximum at 15 DAS due to the application of nitrogenous fertilizers and then declined gradually at subsequent growth stages (Fig. 1). Total nitrate reductase (NADH:NR + NAD(P)H:NR) activity in the leaves of mustard was maximum [0.835 nmol(NO_2^-) mg^{-1}(protein) s^{-1})] at 45 DAS and then declined gradually until maturity. This pattern may be attributed to nitrate availability in the soil, as NR is the substrate inducible enzyme. The NADH:NR activity was minimum at 30 DAS [0.137 nmol(NO_2^-) mg^{-1}(protein) s^{-1}], then gradually increased till 90 DAS [0.403 nmol(NO_2^-) mg^{-1}(protein) s^{-1})], and thereafter became constant. Contrary to NADH:NR activity, NAD(P)H:NR activity was maximum [0.571 nmol(NO_2^-) mg^{-1}(protein) s^{-1}] at early growth stage (30 DAS), and then gradually declined at subsequent growth stages, becoming negligible at 90 DAS [0.015 nmol(NO_2^-) mg^{-1}(protein) s^{-1}]. This interesting seasonal pattern of NADH: and NAD(P)H:NR activities can be correlated with soil nitrate content at different growth stages (Fig. 1). It is likely that while both NAD(P)H: and NADH:NR are functional during various growth stages of the plant, the former expresses in early growth stages when nitrate availability in the soil remains high, while NADH:NR expresses at later stages of plant growth when soil nitrate level becomes comparatively low. These observations confirm our earlier work (Ahmad and Abdin 1999).

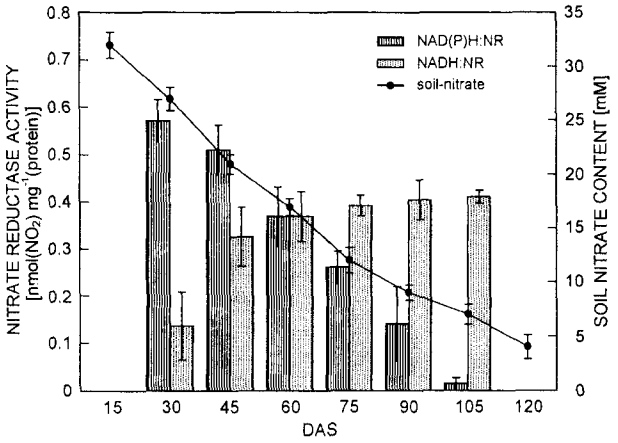

Fig. 1. Relationship between the activities of NADH:NR and NAD(P)H:NR and soil nitrate content at various growth stages. Mean ± SE; $n = 5$

References

Abdin, M.Z., Kaim, M.S., Bansal, K.C., Abrol, Y.P.: Nitrate assimilation in leaf blades and N-harvest in uniculm 'Gigas' wheat. - J. Agron. Crop Sci. **173**: 225-229, 1994.

Ahmad, A., Abdin, M.Z.: NADH:nitrate reductase and NAD(P)H:nitrate reductase activities in mustard seedlings. - Plant Sci. **143**: 1-7, 1999.

Ahmad, A. Abrol, Y.P., Abdin, M.Z.: Effect of split application of sulphur and nitrogen on growth and yield attributes of *Brassica* genotypes differing in their time of flowering. - Can. J. Plant Sci. **79**: 175-180, 1999.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Evans, H.J., Nason, A.: Pyridine nucleotide nitrate reductase from extract of higher plants. - Plant Physiol. **28**: 233-253, 1953.

Fishman, M.J., Skougstad, M.W., Scarbro, G.F., Jr.: Determination of nitrate content of water. - J. amer. Water Works Assoc. **56**: 633, 1964.

Grover, H.L., Nair, T.V.R., Abrol, Y.P.: Nitrogen metabolism of the upper three leaf blades of wheat at different soil nitrogen levels. I. Nitrate reductase activity and content of various nitrogenous constituents. - Physiol. Plant. **42**: 287-292, 1978.