BIOLOGIA PLANTARUM 35(1): 31-36, 1992

Effect of nitrogen salts on nitrate reductase activity and protein contents in wheat (*Triticum aestivum* L.)

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Abstract

The effects of different nitrogen salts on nitrate reductase activity and protein contents were investigated in three Yugoslav cultivars of wheat. The nitrate salts appeared to be a better form of nitrogen than ammonium in respect of the increase of the nitrate reductase activity and root total protein contents, whereas the treatment with ammonium salt resulted in a comparably higher shoot total protein contents. KNO_3 was the best in respect of the level of nitrate reductase activity. Different concentrations of nitrate and ammonium ions in nutrient solution, showed very similar effects on investigated parameters. NS Rana 2 cultivar had the highest values of nitrate reductase activity and protein contents.

Introduction

The assimilation of inorganic nitrogen in plants is a complex process involving a series of enzymes. Nitrate reductases NR, EC 1.6.6.1., are the enzymes which catalyze the reduction of nitrate to nitrite. The activity of NR is a function of a complex set of endogenous and ecological factors. The NR induction by NO_3 has been demonstrated in almost all plant tissues and species examined, including wheat leaf, barley aleurone layers and leaf, *Agrostemmna githago* embryos, maize tissues, rice seedlings and pea leaf (Srivastava 1980). In a typical inducting system the enzyme activity increases linearly after 30 min upon nitrate supply, reaching a maximum after 3 - 4 h. The induction in wheat is a two-phase general pattern: an initial rapid phase and a slower but longer induction phase (Jones *et al.* 1981). The concentration at which a maximum NR activity is induced varies according to plant species and tissues, for example, 3.0 mmol NO_3 for leaf of wheat, barley and maize. It is generally considered that the presence of NO_3 is essential for the induction and maintenance of NR activity. But the NH_4-grown plants have generally lower NR activity than the NO_3-grown ones. The inhibition of NR activity by ammonium has

Received 25 June 1991, *accepted* 28 September 1991.

been attributed to the inhibition of NO_3 uptake (Jackson *et al.* 1986). The objective of the present work was to investigate the NR activity and protein contents in wheat seedlings grown under different nitrogen salts conditions.

Material and methods

Growth of plants: Seedlings of three wheat (*Triticum aestivum* L.) cultivars (NS Rana 2, Jugoslavija and Žitnica) were grown in sand culture in experimental pots in a greenhouse at 28 °C under natural light. During the first two weeks, the plants were supplied only with distilled water. After that the plants were supplied two times with the nutrient solutions (the first time with 100 cm^3 each pot, and the second time, 24 h after that with 70 cm^3 of the nutrient solution). The nutrient solutions used in the experiments were the Knop solutions in which the nitrogen compound was replaced by $Ca(NO_3)_2$, KNO_3, $NaNO_3$ or $(NH_4)_2SO_4$ in concentration of 1.25 mmol l^{-1} (N-1/8), 10 mmol l^{-1} (N-1), and 30 mmol l^{-1} (N-3) of NO_3 or NH_4. The Knop solution without nitrogen compounds was marked as N-0. 48 h after the first nutrient addition, plant samples were taken for the activity assay of NR and for the estimation of leaf soluble protein content. At the same time the remained plants were harvested by washing their roots with distilled water. The above-ground parts and the roots of the plants were dried separately for further determination of their dry mass, total nitrogen and total protein content. The same experiments were carried out 3 times in different seasons during two years (Exp. I and II were performed in July and October of the first year, respectively, and Exp. III in May of the second year). Each experiment contained 6 replications.

Nitrate reductase assay: Fresh leaves of wheat seedlings were cut into pieces. The leaf pieces (0.2 g) were placed into a 15 cm^3 bottle containing 2.5 cm^3 of incubation mixture. The incubation mixture contained 100 mmol l^{-1} of potassium phosphate buffer (pH 7.7), 100 mmol l^{-1} potassium nitrate and 1 % (v/v) isopropanol. The bottle was sealed up with a rubber stopper. Through one hypodermic needle, N_2 gas was exhausted out the bottle. The mixture was flushed with N_2 gas for 5 min and then incubated in the dark at 30 °C for 1 h. At the end of the incubation, 0.5 cm^3 of the mixture was taken and put into a colour reagent mixture containing 0.025 % (m/v) N-(1-naphtyl)ethylenediamine dichloride, and 0.5 % (m/v) sulphanilamide in 1.5 N HCl. After 10 min of reaction, absorbance of the mixture was measured at 540 nm. In the case the colour of the mixture was too dark for the measurement, distilled water was added to dilute the mixture. Standard absorbance for the nitrite colour complex is 55 mmol l^{-1} at 540 nm (Farnden and Robertson 1980, Coombs and Hall 1982). NR activity was calculated from the produced nitrite and expressed in nmol NO_2 g^{-1} (fr.m.) min^{-1}.

Determination of leaf soluble protein content: Leaf soluble protein content was determined according to Lowry *et al.* (1951).

Determination of total nitrogen and total protein content: The total nitrogen content of dry shoot and roots was determined by the Kjeldhal method. The protein content [mg(protein) g^{-1}(dry m.)] was calculated from the total nitrogen content [mg(nitrogen) g^{-1}(dry m.)] multiplied by the factor of 5.7.

Statistical analysis: The obtained results on the NR activity, protein content and nitrogen content in the plants were analyzed by the analysis of variance analysis for randomized complete-design. The results were treated as 3 factors (cultivar, nitrogen salts and nitrogen concentration) with 3 or 4 levels for each (3×4×4). The LSD values (p = 0.05 and p = 0.01) were calculated for testing the significance of the difference between the treatments and the control.

Results and discussion

The plants treated with nitrate salts had significantly higher NR activities than those treated with ammonium salts (Table 1). Of the three nitrate salts used, KNO_3 appeared to be the best one for the induction of NR activity. The treatment with $Ca(NO_3)_2$ resulted in the NR activities which were higher than with $NaNO_3$. Our results show that higher NR activities were induced by treatment with KNO_3 more than with $NaNO_3$ and $Ca(NO_3)_2$. This is consistent with Blevins *et al.* (1978a, 1978b), who reported NR activity in wheat seedlings 2 to 3 times higher in the presence of KNO_3 than $NaNO_3$. K^+ ion has been found to stimulate NR activity (Oji and Izana 1969) and, in addition, it is also important for NO_3 translocation as an accompanying cation (Gaudinová 1983). It is considered that Ca^{2+} may have a direct effect on NR (Dekock *et al.* 1979) and indirect effect on the accumulated nitrate (Paulser and Harper 1968). On the other hand, Na^+ is thought to have no effect on NR activity (Pfluger and Wiedemann 1977). In general, leaf soluble protein content was a more stable parameter than the enzyme activity, in response to the change of the forms of nitrogen salts in the nutrient solutions. The shoot total protein contents were higher in the plants treated with ammonium salt than in those treated with nitrate salts. The effect of different types of nitrate salts on root total protein contents was not significant.

Table 1. Effect of different nitrogen salts on the mean values of the nitrate reductase (NR) activity and proteins contents for all nitrogen concentrations and wheat cultivars.

N salt	NR activity [nmol(NO_2^-) g^{-1}(f.m.) s^{-1}]	Leaf soluble protein [mg g^{-1}(fr.m.)]	Shoot total protein [% d. m.]	Root total protein [% d.m.]
$Ca(NO_3)_2$	6.77	19.1	21.1	7.7
KNO_3	8.30	19.3	20.7	7.4
$NaNO_3$	5.90	19.3	20.6	7.5
$(NH_4)_2SO_4$	2.20	19.6	21.5	7.3
5 % LSD	0.36	0.6	0.3	0.3
1 % LSD	0.47	0.7	0.4	0.4

Table 2. Effect of different nitrogen concentrations on mean activities of nitrate reductase (NR) and protein content for all nitrogen salts and wheat cultivars.

N conc. [mmol l^{-1}]	NR activity [nmol(NO_2^-) g^{-1}(f.m.) min^{-1}]	Leaf soluble protein [mg g^{-1}(fr.m.)]	Shoot total protein [% d. m.]	Root total protein [% d.m.]
0	2.12	19.3	17.3	6.5
1.25	3.95	18.5	18.2	7.0
10.0	8.23	19.8	22.8	7.9
30.0	8.85	19.6	25.4	8.5
5 % LSD	0.36	0.6	0.3	0.3
1 % LSD	0.47	0.7	0.4	0.4

Effect of different concentrations of nitrate and ammonia on the biochemical parameters. The NR activity is very dependent on NO_3 concentration. The NR activity increased significantly with the increase of NO_3 concentration from 0 to 10 mmol l^{-1} and further from 10 to 30 mmol l^{-1}. Leaf soluble protein contents did not change significantly with the change of the concentrations of NO_3 and NH_4. Shoot total protein contents increased very significantly with the increase of the concentrations of NO_3 or NH_4 from 0 to 30 mmol l^{-1} in the nutrient solutions. The concentrations of both NO_3 and NH_4 ions exhibited significant effects on root total protein contents.

Effect of different wheat cultivars on the biochemical parameters. Among the three cultivars investigated, NS Rana 2 had the highest NR activity and Žitnica the lowest. Other researchers have also shown that there are significant genetic variations in NR activity among different cultivars of wheat (Brunetti and Hageman 1976). NS Rana 2 had the highest leaf soluble protein contents, shoot total protein content, and root total protein content, whereas Žitnica had the lowest.

Table 3. Effect of different wheat cultivars on the mean activities of nitrate reductase (NR) and protein contents for all nitrogen salts and concentrations.

Cultivar	NR activity [nmol(NO_2^-) g^{-1} (f.m.) min^{-1}]	Leaf soluble protein [mg g^{-1}(fr.m.)]	Shoot total protein [% d. m.]	Root total protein [% d.m.]
NS Rana 2	7.08	21.4	21.5	8.1
Jugoslavija	4.80	19.5	20.9	7.3
Žitnica	5.33	17.2	20.2	7.0
5 % LSD	0.30	0.5	0.3	0.3
1 % LSD	0.41	0.6	0.4	0.3

There are positive correlations between the activities of NR and shoot total protein content ($r = 0.77^*, 0.79^*, 0.71^*$) for all three cultivars, and between the activities of NR and root total protein content ($r = 0.69^*$) in NS Rana 2.

In conclusion, the nitrate salts were more effective form of nitrogen salt than the ammonium salt in regard of the level of NR activity and root total protein content,

whereas the treatment with the ammonium salt resulted in an increased shoot total protein content. Of the three nitrate salts, KNO_3 was the best one in respect of the level of NR activity, whereas $Ca(NO_3)_2$ was a better choice for root total protein content.

The values of NR activity, the shoot and root total protein content increased very significantly with the increase of the concentration of NO_3 in the nutrient solutions.

NS Rana 2 had the highest values of all the investigated parameters, and Žitnica cultivar showed the lowest values of the investigated parameter.

Table 4. Coefficients of correlation between the nitrate reductase activity and protein content

	Wheat cultivar	NR activity	Leaf soluble protein	Shoot total protein
Leaf soluble protein	NS Rana 2	0.41		
	Jugoslavija	0.20		
	Žitnica	0.32		
Shoot total protein	NS Rana 2	0.77**	0.51	
	Jugoslavija	0.79**	0.31	
	Žitnica	0.71*	0.15	
Root total protein	NS Rana 2	0.69*	0.40	0.80**
	Jugoslavija	0.59	0.25	0.69*
	Žitnica	0.56	-0.01	0.60

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