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The effect of differentiated nitrogen fertilization on nitrate reduction in broccoli heads of 'Lord F_1' in spring cultivation

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ABSTRACT

Broccoli (*Brassica oleracea* L. var. *italica*) 'Lord F_1' was grown at the Agricultural University experimental farm in the Cracow area in the spring growing cycle of three successive years. In harvested broccoli heads activities of nitrate reductase (NR) and nitrite reductase (NiR) as well as NO_3^- and NH_4^+ ions contents were determined. The nitrogen fertilizer rate introduced into the soil did not significantly affect the average activity of both enzymes, responsible for nitrate reduction. The application of mineral N increased nitrate level in broccoli heads, however, decreased NH_4^+ content. The foliar nutrition with 2% urea, applied five times during the growing period significantly increased the activity of NR in all investigated treatments, but did not influence the activity of NiR as compared with

non-fed plants. Foliar application lowered the average nitrate content in broccoli heads but the significant effect was found only in the case of the full rate of mineral N. This factor did not significantly affect ammonium ions content in the analyzed plant organs. Strong interdependence was observed between the activity of the determined enzymes, as well as of NO_3^- and NH_4^+ and the weather conditions accompanying the plant cultivation in the particular years of experiment.

INTRODUCTION

The rate of nitrate reduction is an important factor which modifies the content of this compound in plant tissue. Two key enzymes are responsible for nitrate assimilation: nitrate (NR, EC 1.6.6.1) and nitrite reductase (NiR, EC 1.7.7.1). Both the expression of NR genes and the rate of nitrate reduction in the plant tissues can be considerably modified by some factors such as light, presence of NO_3^- ions, day length, sugar level or certain nitrogen metabolites (Lillo et al. 2004). Moreover, the form of nutritive nitrogen (N-NO_3, N-NH_4, N-NH_2) also affects the activity of NR and NiR. This interdependence was shown, among others, in cabbage (Rożek et al. 1999) or lettuce (Sady et al. 1995) leaves.

The aim of the present investigations was to study the effect of the foliar nutrition with urea on nitrate and nitrite reductase activities in relation to the changes of NO_3^- and NH_4^+ ions in the heads of broccoli 'Lord F_1', grown at differentiated fertilization with the mineral nitrogen. The broccoli yield and some indices of its quality were described earlier (Wojciechowska et al. 2005).

MATERIAL AND METHODS

Broccoli (*B. oleracea* L. var. *italica*) of 'Lord F_1' was grown at the Agricultural University experimental farm in the Cracow area in the spring growing cycle of three successive years (1999 – 2001). Plants were cultivated on brown soil of pH 6.9 containing of 2.2% of organic matter. Mineral nutrition was based on the results of chemical analyses of the soil samples. The content of soil available P, K, Ca, Mg, and N (N-NO_3 + N-NH_4) was supplemented to the level of 60 mg P dm^{-3}, 200 mg K dm^{-3}, 1150 mg Ca dm^{-3}, 125 mg Mg dm^{-3}, and 150 mg N dm^{-3} in the case of full rate, respectively. Nitrogen fertilizer selected was ammonium nitrate. Natural N content in the soil equaled to 12-28 mg per dm^3 depending on the year of study.

The following treatments in two combinations (untreated and treated with 2% urea) were introduced in the experiment: (1) the control (natural soil content of

N), (2) half of the calculated rate of N (75 mg N dm^{-3}) introduced to the soil before planting of seedlings (broadcast), (3) half of the calculated rate of N introduced to the soil before planting of seedlings (broadcast) + another dose during the growth (top-dressing) and (4) full rate of N (150 mg dm^{-3}) introduced to the soil before planting of seedlings.

Foliar nutrition with 2% urea was carried out five times during the growing cycle. Broccoli seedlings were planted on 30.03.1999, 3.04.2000, 3.04.2001, and harvested on 10.06.1999, 13.06.2000, and 11.06.2001. Each treatment consisted of 100 plants. The experiment was carried out in four replications. Six plants were randomly taken from each replication for chemical analyses. In broccoli heads the nitrate and nitrite reductase activity, nitrate and ammonium ions as well as free amino acid content were estimated.

Nutritive macroelements of the soil were detected in the 0.03 M acetic acid extract (Nowosielski 1988). The nitrogen level in the soil was measured by the microdestillation method of Bremner; K, Mg and Ca content was determined spectrometrically using the Carl Zeiss AAS-1 apparatus, while P level was detected colorimetrically.

Nitrate and ammonium ions level was determined using an ionoselective electrodes (ORION) in cooperation with UNICAM-9460 ionometer. Nitrate and nitrite reductase activity in broccoli heads were measured in vitro by Buczek method (1984). All analyses were statistically evaluated using Duncan`s test, for significance at $p = 0.05$.

RESULTS

The application of foliar nutrition with urea caused significant increase in nitrate reductase (NR) activity in broccoli heads in comparison with untreated plants, except for the treatment with the full rate of N, used as a pre-plant dose (Fig. 1A). In general, no distinct effect of the increasing fertilizer dose on NR activity was observed. Neither the differentiated fertilization with N mineral nor the foliar nutrition significantly affected nitrite reductase (NiR) activity in broccoli heads (Fig. 1B).

The lowest activity of nitrate reductase was observed in broccoli heads harvested in 1999, irrespective of the rate of nitrogen fertilizer (Fig. 2A). In case of NiR the highest and the lowest activities of this enzyme were found in 1999 and in 2001, respectively (Fig. 2B).

In Fig. 3 the activities of NR and NiR were compared in broccoli plants treated and untreated with urea, excluding the soil nitrogen fertilization. In 2000 and 2001, the foliar nutrition of plants almost doubled the activity of nitrate reductase. In

case of nitrite reductase, urea application significantly increased its activity in broccoli heads harvested in 1999 while decreased this value in 2001.

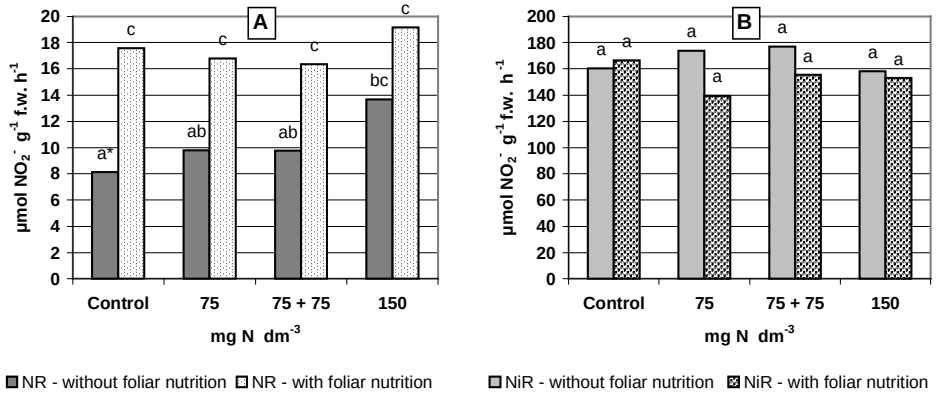

Figure 1. The effect of soil nitrogen fertilization and foliar urea application on nitrate reductase activity (NR) (A) and nitrite reductase activity (NiR) (B) in broccoli heads, irrespective of the year of study. *Values marked with the same letters do not differ significantly

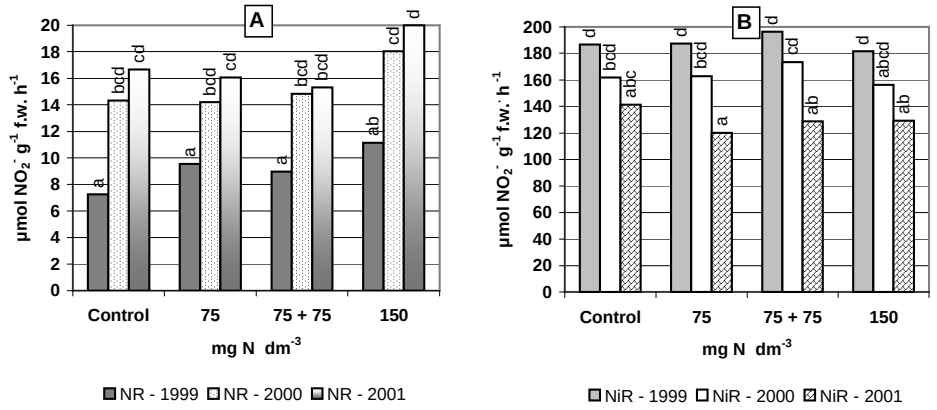

Figure 2. The effect of soil nitrogen fertilization on nitrate reductase activity (NR) (A) and nitrite reductase activity (NiR) (B) in broccoli heads in successive years of study, irrespective of foliar urea application

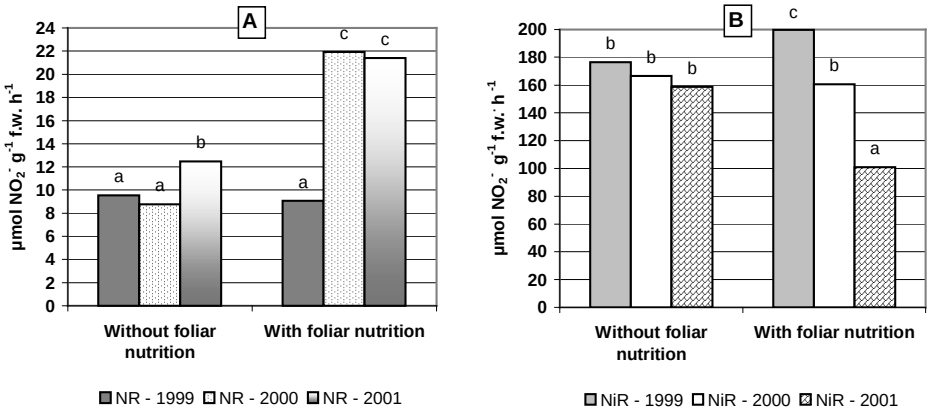

Figure 3. The effect of foliar urea application on nitrate reductase activity (NR) (A) and nitrite reductase activity (NiR) (B) in broccoli heads in successive years of the study, irrespective of mineral nitrogen in soil

In Fig. 4 the average values of three years of NO_3^- and NH_4^+ contents are presented. With the increasing dose of N_{min} introduced into the soil, a growing accumulation of nitrates in the broccoli heads non-treated by the foliar nutrition was observed. The foliar application slightly lowered nitrate contents in all treatments, however, a significant effect was found only in the case of full and divided dose of N mineral. It is worth noticing, that the highest and the lowest contents of ammonium ions were found in the control plants and in those treated with the single application of full rate of mineral N, respectively. The foliar feeding did not change this dependence.

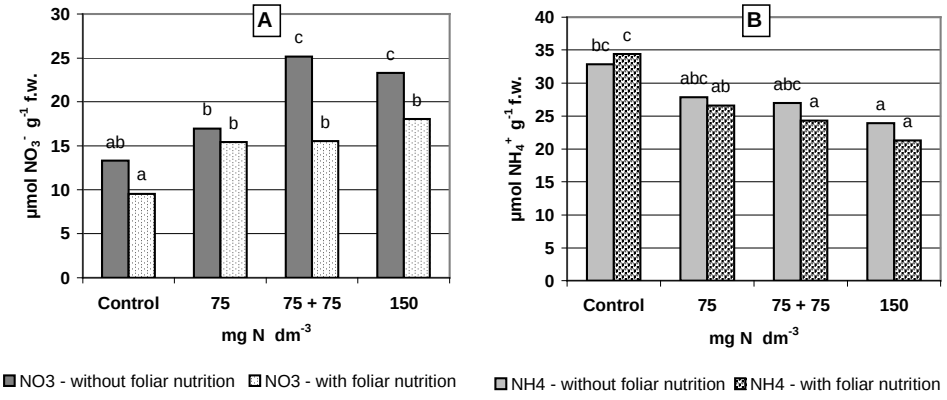

Figure 4. The effect of soil nitrogen fertilization and foliar urea application on nitrate (NO_3^-) (A) and ammonium (NH_4^+) (B) ion content in broccoli heads, irrespective of the year of the study

The broccoli heads harvested in 1999 contained the lowest level of nitrates in comparison with the other years of experiment (Fig. 5). In case of NH_4^+ the highest and the lowest contents of these ions were observed in the heads harvested in 1999 and 2000, respectively. In the two years of the study (1999 and 2001) the same regularity was noticed: the increasing dose of mineral N introduced into the soil was accompanied by the decrease of ammonium nitrogen form, measured in broccoli plant tissue.

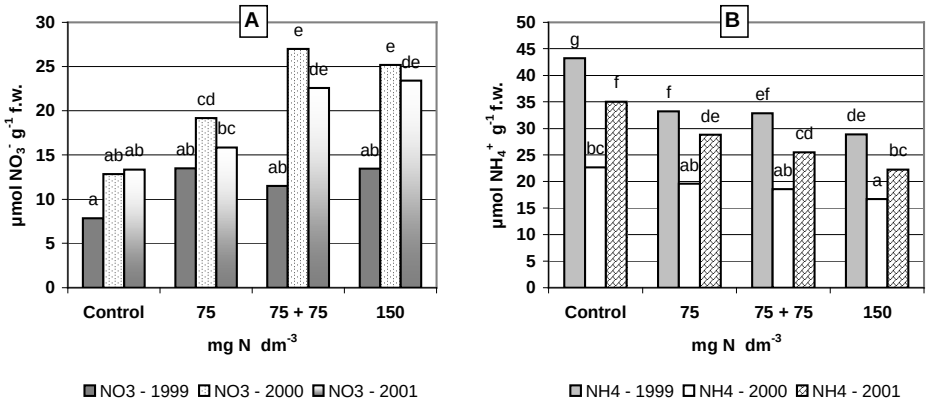

Figure 5. The effect of soil nitrogen fertilization on nitrate (NO_3^-) (A) and ammonium (NH_4^+) (B) ion content in broccoli heads in successive years of the study, irrespective of foliar urea application

The results presented in Fig. 6 indicate that irrespective of mineral nitrogen level in soil, foliar feeding distinctly reduced nitrate concentration in broccoli heads in 2000 and 2001. Moreover the plants responded to urea application with the reduced content of NH_4^+ ions only in 1999, while in the other years no significant effect of foliar urea treatment on ammonium accumulation was noticed.

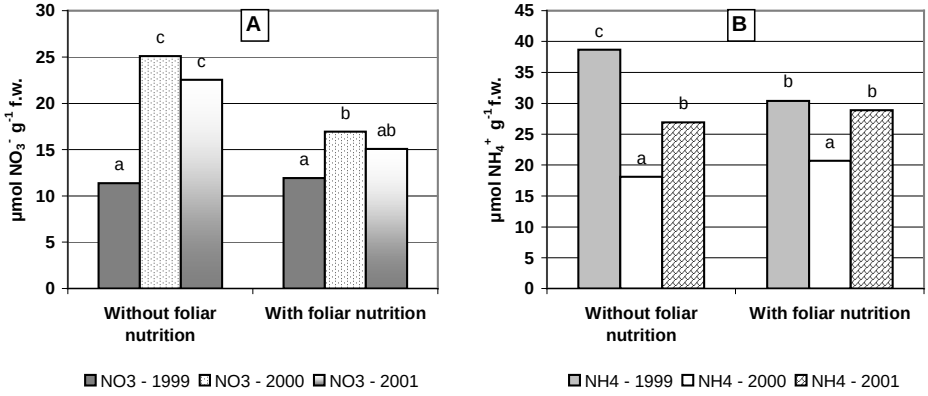

Figure 6. The effect of foliar urea application on nitrate (NO_3^-) (A) and ammonium (NH_4^+) (B) ion content in broccoli heads in successive years of the study, irrespective of mineral nitrogen in soil

DISSCUSION AND CONCLUSIONS

The purpose of the study was to explain the changes in activities of the key enzymes responsible for nitrate reduction in broccoli heads regarding the differentiated nitrogen nutrition. In the previous work it had been shown that foliar feeding with urea might be considered as the effective way of lowering the nitrate level in broccoli heads (Wojciechowska et al. 2005). The results presented in this study indicate that one of the reasons of that dependence in broccoli plants fed by urea seems to be the simultaneous increase in nitrate reductase activity. The reduction of the nitrate content in the ground lettuce as the result of urea foliar treatment was also reported by Wojciechowska (2004). However in the quoted experiment no significant dependence between nitrates and NR activity in lettuce leaves was found. It seems to be possible that the fertilization factors assumed in the present experiment changed the concentrations of both nitrogen forms within broccoli heads as well as their mutual relation which might have influenced the activity of nitrate reductase. The mutual influence between nitrate and ammonium forms was expressed by the significant correlation coefficients such as -0.3987 and -0.5930 for the non-fed and fed with urea plants, respectively (Table 1). The NR activity, negatively and positively correlated with NO_3^- and NH_4^+ concentrations, observed in broccoli heads untreated with urea seems particularly interesting. In the plants fed with urea no significant correlation between NR activity and NH_4^+ ions content was noted. These results are not in accordance with those obtained in lettuce leaves (Wojciechowska 2004). Such observations confirm that some correlations determining nitrate metabolism depend to a high degree on the plant species and its organ (Wojciechowska and Rożek 2006).

Table 1. Correlation coefficients between nitrate reductase activity (NR), nitrite reductase activity (NiR), nitrate ion content (NO_3^-) and ammonium ion content (NH_4^+) in spring growing broccoli heads due to foliar urea application

	Without foliar nutrition			With foliar nutrition		
	NO_3^-	NH_4^+	NR	NO_3^-	NH_4^+	NR
NH_4^+	- 0.3987*	-	-	- 0.5930**	-	-
NR	- 0.5631**	0.6750**	-	- 0.3494*	n.s.	-
NiR	n.s.	n.s.	0.3624*	n.s.	n.s.	0.7310**

n.s. – not significant
* – significant at p = 0.05
** – significant at p = 0.01

The mutual relation of nitrate and ammonium ions significantly affected the activity of NR in broccoli heads (though it did not influence NiR activity). This phenomenon seemed to be explained by the findings of Schuster et al. (1989).

The authors separated four izoforms of nitrate reductase from the mustard cotyledons. Two of them (NR_1 and NR_2) were identified only in the presence of NO_3^- as the source of nitrogen, while the others (NR_3 and NR_4) in the presence of NH_4^+. When both nitrogen forms were applied in equal concentrations, NR_3 form cooperated with NR_1 and NR_2 forms, while the NR_4 activity (appearing only in the presence of NH_4^+) was completely inhibited by the equimolar concentration of NO_3^-. The results of this work confirm that NH_4^+ ions might stimulate nitrate reductase activity in plant tissues.

The presented results are the evidence of a much stronger effect of the weather conditions in the particular years of experiment on NR and NiR activities as compared with the fertilization factor. The weather conditions in 1999 – 2001 in the area of the experiment, which strongly differentiated in relation to both the number of sunny days and total rainfalls in the particular months of the spring season were presented in the previous work (Wojciechowska et al. 2005). The most favorable weather regarding the mentioned factors occurred in 2001, and in 2000 and just in these years high activity of nitrate reductase in broccoli heads was observed. Light energy supplied to the plants is one of the main factors modifying the activity of nitrate reductase. A high activity of this enzyme, particularly in the green parts of the plants is closely connected with photosynthesis (Kaiser and Brendle-Benish 1991). Products of photosyntetic cycle, such as sugars and their phosphate derivatives act as internal signal molecules which control the activity of kinases and phosphatases modifying NR by the reversible phosphorylation (Kaiser et al. 2002).

The differences in nitrate content and NR activity noted in the particular years of the present investigations could not be, however, justified by variable light conditions only. For example, in 1999 the average nitrate content determined in broccoli heads was lower in comparison with the other years and was accompanied by the lowest activity of nitrate reductase. These data seem to be especially controvertial because just in 1999 the lowest number of the sunny days was reported (Wojciechowska et al. 2005). It is commonly known that such external factors should enhance nitrate accumulation. Poor accumulation of nitrate might have been resulting from the long-term dry weather hence the uptaking of these constituents from the soil was drastically reduced. The significant decrease of nitrate uptake and, thus, the decrease of its accumulation in plants, caused by the long-term limiting of water supply was described by Buljovcic and Engels (2001).

To sum up, the results of the study showed that nitrate reduction in broccoli heads depended to more extent on foliar nutrition with urea than on N rate introduced to the soil. Urea application significantly increased the activity of NR and decreased nitrate content in broccoli heads in the years of the study with favorable weather conditions regarding rainfalls and the number of sunny days. The foliar nutrition strongly modified correlations between NR and NiR activities as well as NR activity and ammonium ions content in broccoli heads.

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WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA AZOTOWEGO NA REDUKCJĘ AZOTANÓW W RÓŻACH BROKUŁA ‘LORD F_1’

Streszczenie: Brokuł ‘Lord F_1’ uprawiano na polu stacji doświadczalnej Akademii Rolniczej w Krakowie w cyklu wiosennym przez trzy kolejne lata. Tuż po zbiorze plonu, w różach oznaczono aktywność reduktazy azotanowej (NR) i azotynowej (NiR) oraz zawartość jonów NO_3^- i NH_4^+. Zróżnicowane dawki doglebowe azotu mineralnego nie wpływały jednoznacznie w istotny sposób na aktywność enzymów odpowiedzialnych za redukcję azotanów i azotynów. Zastosowanie N_{min} wpływało jednakże na obniżenie zawartości jonów NH_4^+ w różach brokuła. Dolistne dokarmianie 2%-owym roztworem mocznika zastosowanym pięciokrotnie w czasie wegetacji w istotny sposób zwiększało aktywność NR we wszystkich obiektach, ale nie miało wpływu na aktywność NiR w porównaniu z roślinami nie dokarmianymi. Dokarmianie dolistne obniżyło zawartość NO_3^- w różach, przy czym istotną różnicę wykazano tylko w obiekcie z zastosowaniem pełnej dawki azotu w nawożeniu doglebowym. Czynnik ten nie wpływał w istotny sposób na zawartość jonów amonowych w analizowanych organach roślin. Zaobserwowano ścisły związek aktywności obu enzymów i stężenia badanych jonów w różach z warunkami pogodowymi towarzyszącymi uprawie roślin w poszczególnych latach badań.

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