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Influence of Different Soil Cultivation and Fertilizing on the Content of Plastid Pigments and Nitrate Reductase Activity in Lucerne for Forage

A. ILIEVA. I. PACHEV and T. KERTIKOV
Institute of Forage Crops, BG - 5800 Pleven, Bulgaria

Abstract

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In a field trial carried out in the Second experimental field of the Institute of Forage Crops in Pleven the influence of mineral fertilizing ($N_6P_{10}K_8$, $N_{2.3}P_{10}K_{3.5}$, $N_{3.5}P_8K_{5.0}$ and Amophos) and different soil cultivations (12-15 cm loosening and ploughing at 12-15 cm, 22-24 cm, 18-22 cm, 30-35 cm) on the nitrate reductase activity (NR) and content of plastid pigments was studied. It was found that the NR activity in leaves exceeded 4 to 8 times the activity in stems and roots of lucerne. The different rates of mineral fertilizing and soil cultivation exerted a different influence on the NR activity and total content of plastid pigments. The application of $N_6P_{10}K_8$ by approved technology increased the NR activity and total content of plastid pigments to the greatest degree under ploughing at 22-24 cm.

The maximum value of NR activity (15.08 µmol NO_2-/g fr.w./h and high content of plastid pigments (313.6 mg/100 g fresh mass) were observed for Amophos application under 18-22 cm ploughing. A medium positive correlation was found between NR activity and content of plastid pigments (r=0.40).

The two-factor variance analysis of the results showed the strong influence of mineral fertilizing - 54.6 % on the NR activity and the slighter influence of the soil cultivation on the content of plastid pigments.

Key words: legume plants, lucerne, soil cultivation, plastid pigments

Introduction

Legume plants have two sources of nitrogen nutrition - nitrogen fixation of atmospheric nitrogen in symbiosis with bacteria of genera Rhizobium and Bradyrhizobium and assimilation of soil nitrogen. mainly in the form of nitrates (Arrese-Igor et al.. 1990). It is known that the application of nitrogen fertilizers greatly inhibits nitrogen-fixing capacity of plants (Kots et al.. 1990; Streeter. 1988). so the question

of the quantity of "start" doses of mineral nitrogen applied into lucerne remains debatable. The metabolism of nitrogen applied into soil passes through several stages and depends on the nitrate reductase activity. The nitrate reductase is a key enzyme catalyzing the stage limiting the intensity in the total process of nitrate assimilation that often limits plant growth and productivity. The nitrate reductase activation requires nitrate. light and photosynthesis (Kaiser et al.. 1999).

The objective of the study was to follow the change in the nitrate reductase activity and plastid pigment content in lucerne for forage at different fertilizer rates and soil cultivation.

Material and Methods

The plant samples were taken from a field trial with lucerne variety "Obnova 10" grown in treatments with different fertilizer rates and soil cultivation. The trial was laid out by the long plot method in the Second experimental field of the Institute of Forage Crops in Pleven in 2003 with the following fertilizer treatments:

1. Control - unfertilized
2. $N_6P_{10}K_8$ - by approved technology
3. $N_{2.3}P_{10}K_{3.5}$ - nitrogen 1/2 in year 1 and 1/2 in year 3, phosphorus and potassium as reserve application
4. $N_{2.3}P_{10}K_{3.5}$ - nitrogen presowing. potassium as reserve application. phosphorus 1/3 in each of years 1. 2 and 3
5. $N_{3.5}P_8K_{5.0}$
6. Amophos - 25.0 kg/ha- estimated at fertilizing rate $N_{2.7}P_{12}K_0$, and treatments of soil cultivation:
   - loosening at 12 - 15 cm
   - ploughing at 12 - 15 cm
   - ploughing at 22 - 24 cm - control
   - ploughing at 18 - 22 cm
   - ploughing at 30 - 35 cm.

The *in vivo* nitrate reductase activity (NR) was determined in the leaves. stems and roots after Jaworski (1971) and the content of plastid pigments after Zelenskiy and Mogileva (1988). A two-factor variance analysis (ANOVA) was used to find the influence of mineral fertilizing and soil cultivation on the NR activity and total content of plastid pigments. The percentage of total variation was estimated after Manolov et al. (1999).

Results and Discussion

Lucerne belongs to species accomplishing nitrate reduction in the plant leaves stems and roots (Arrese-Igor et al.. 1991). In our trial. the NR activity in the leaves exceeded 6-8 times the activity in the stems and 4-7 times that in the roots of lucerne (Figure 1). In the opinion of Kaim et al. (1983) almost 75 % of assimilated nitrates are reduced in the plant leaves.

The obtained data on the NR activity showed that it changed under the influence of fertilizing and soil cultivation (Table 1). In the control (the treatment without fertilizing) the highest NR activity was observed in the treatments with surface soil cultivation - 12-15 cm loosening and 12-15 cm ploughing (8.0 µmol NO_2^-/g fr.w./h - 8.48 µmol NO_2^-/g fr.w./h. respectively). That could be explained by the fact that under these cultivation the nutrient concentrations in the upper soil layers almost did not change and there were some quantities of available nitrogen. But when turning the layer (under deep ploughing) the most enriched surface layer of soil fell at a greater depth and moved away from the active root layer. The application of mineral fertilizers containing nitrogen increased the NR activity in all treatments

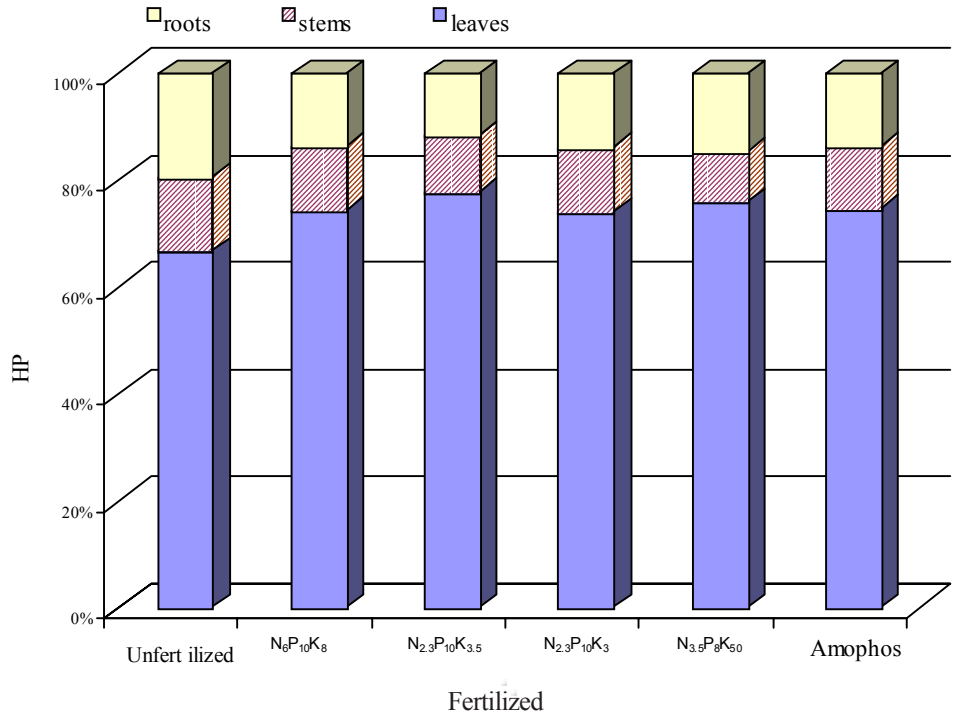

Fig. 1. Nitrate reductase activity in the organs of lucerne plants at different fertilizing rates

Table 1

Nitrate reductase activity in lucerne leaves under fertilizing and different soil cultivations (on average for 2004-2005)

Treatment	12-15 loosening		12-15 ploughing		22-24 ploughing		18-22 ploughing		30-35 ploughing	
		% of C		% of C		% of C		% of C		% of C
Unfertili-zed– C	8	-	8.48	-	6.16	-	6.44	-	5.76	-
$N_6P_{10}K_8$	8.72	109	10.58	124	11.56	187.7	7.16	111.2	8.84	153.5
$N_{2.3}P_{10}K_{3.5}$	10.36	129.5	10.92	128.8	11	178.6	11.76	182.6	10.04	174.3
$N_{2.3}P_{10}K_{3.5}$	9.96	124.5	10.76	126.9	10.76	174.7	9.68	150.3	7.56	131.3
$N_{3.5}P_8K_{50}$	9.2	115	10.32	121.7	8.16	132.5	9.24	143.5	12.48	216.7
Amophos	11.96	149.5	11.92	140.6	9.96	161	15.08	234.2	9.96	172.9

with fertilizing. but in a different way depending on the soil cultivation (Table 1). The highest value of NR activity when applying $N_6P_{10}K_8$ by approved technology was obtained under ploughing at 22-24 cm (11.56 µmol NO_2-/g fr.w./h). It is evident from the comparison of the two treatments with application of reduced doses of mineral fertilizers (treatments 3 and 4) that under cultivation of 12-15 cm loosening. 12-15 cm ploughing and 22-24 cm ploughing the values of the NR activity were similar for both treatments. Under ploughing at 18-22 cm and 30-35 cm the application of nitrogen as 1/2 in the first year and 1/2 in the third year (treatment 3) exerted a greater influence on the enzyme activity. The maximum value - 11.76 µmol NO_2^-/g fr.w./h was obtained under ploughing at 18-22 cm. In the treatment with application of Amophos the NR activity was high as compared to the unfertilized control under surface soil cultivations. Under ploughing at 18-22 cm it reached the highest value - 15.08 µmol NO_2-/g fr.w./h. Under deeper ploughing at 22-24 cm and 30-35 cm the values of NR activity were lower - 9.96 µmol NO_2^-/g fr.w./h.

The nitrate reduction is a process dependent on photosynthesis. Our data on the total content of plastid pigments showed (Table 2) that in the control the content was the highest under 12-15 cm loosening (310.7 mg/100 g fresh mass) which corresponded with the high NR activity in the leaves in this treatment (Table 1). Similar tendency was also observed for the application of $N_6P_{10}K_8$ - the highest content of plastid pigments was under ploughing at 22-24 cm (314.5 mg/100 g fresh mass). The application of $N_{2.3}P_{10}K_{3.5}$ (treatment 3). $N_{3.5}P_8K_{5.0}$ and Amophos increased the total content of plastid pigments as compared to the control for all soil cultivation. except for 12-15 cm loosening. A medium positive correlation (r=0.40) was found between the NR activity and total content of plastid pigments.

The two-factor variance analysis of the

Table 2

Content of plastid pigments in lucerne plants under different soil cultivation and fertilizing (on average for 2004-2005)

Treatment	12-15 loosening		12-15 ploughing		22-24 ploughing		18-22 ploughing		30-35 ploughing	
	mg/ 100g	% of C	mg/ 100g	% of C	mg/ 100g	% of C	mg/ 100g	% of C	mg/ 100g	% of C
Unfertilized – C	310.7	-	297.2	-	281.8	-	298.9	-	272.7	-
$N_6P_{10}K_8$	287.6	92.6	297.4	100.1	314.5	111.6	290.1	97	271.5	99.5
$N_{2.3}P_{10}K_{3.5}$	302.6	97.4	308.5	103.8	289.7	102.8	305.8	102.3	311.2	114.1
$N_{2.3}P_{10}K_{3.5}$	284.1	91.4	291.1	97.9	284.3	100.9	293	98	313.7	115
$N_{3.5}P_8K_{50}$	278.9	89.8	318.1	107	291	103.3	310.4	103.8	293.6	107.7
Amophos	288.1	92.7	315.9	106.3	299.2	106.2	313.6	104.9	296.9	108.9

Table 3

Two-factor variance analysis

Source of variation	SS	df	MS	F	P-value	F crit	Effect of factor on variation. %
Nitrate reductase activity							
Fertilizing	65.69927	5	13.13985	5.421567	0.0026	2.710891	54.6
Soil cultivations	6.098987	4	1.524747	0.629118	0.647356	2.866081	5.07
Error	48.47253	20	2.423627				
Total	120.2708	29					

results showed that the fertilizing is a factor exerting a strong influence on the NR activity - 54.6 % (Table 3). The soil cultivation. as an independent factor. did not exert a significant influence on this parameter - 5.07 %. There was a much smaller influence of the studied factors on the content of plastid pigments. The high values of errors showed that under field conditions many factors exerted a complex influence on the NR activity and content of plastid pigments.

Conclusions

- The NR activity in the leaves exceeded 4 to 8 times the activity in the stems and roots of lucerne.
- The different rates of mineral fertilizing and soil cultivation exerted a different influence on the NR activity and total content of plastid pigments.
- A medium positive correlation was found between the NR activity and content of plastid pigments (r=0.40).
- The two-factor variance analysis of the results showed that the fertilizing is a factor exerting a strong influence on the NR activity - 54.6 %. The soil cultivation. as an independent factor. did not exert a significant influence on this parameter - 5.07 %.

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