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Chemical composition, nitrate reductase activity and plastid pigments content in lucerne under the influence of ammonium and nitrate form mineral nitrogen

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Abstract. A pot trial was carried out at the Institute of Forage Crops, town of Pleven, Bulgaria (2003–04). Whereas ammonium and nitrate forms of mineral nitrogen are assimilated for the plants, the influence of these two forms of mineral nitrogen on the chemical composition, nitrate reductase activity and plastid pigments content of lucerne in conditions of optimum moisture and water deficiency stress was tested. Urea as a source of ammonium nitrogen and potassium nitrate as a source of nitrate nitrogen were used. Rates of 70, 140 and 210 mg N kg^{-1} soil were applied. It was found that mineral nitrogen in ammonium form, applied at the doses of 140 and 210 mg N kg^{-1} soil at optimum moisture increased crude protein content by 5–13%, and in the nitrate form at the same doses, by 3–7%. Crude protein content under water deficiency stress increased by 4–21% for ammonium, and by 3–12% for the nitrate form of mineral nitrogen. When the plants were supplied insufficiently with nitrogen, water deficiency stress more strongly deteriorated the chemical composition of lucerne, crude protein decreased by 6% and crude fiber increased by 10%. The application of mineral nitrogen under optimum moisture decreased calcium and phosphorus content, and the decrease was bigger for the nitrate form (up to 10% for calcium, and 23% for phosphorus). Under water deficiency stress the content of calcium and phosphorus decreased, but there were no differences for two forms of mineral nitrogen. At the optimum moisture and water deficiency stress, there was a similar tendency to reduce nitrate reductase activity in leaves, when applied mineral nitrogen at a dose of 70 mg N kg^{-1} soil in both forms, and to increase at the doses of 140 and 210 mg N kg^{-1} soil. The total content of plastid pigments increased as compared to unfertilized control, when mineral nitrogen was applied in both forms.

Key words: ammonium nitrogen, nitrate nitrogen, lucerne, crude protein, nitrate reductase activity, plastid pigments

INTRODUCTION

Lucerne is the most widely used forage crop and main source of protein in Bulgaria. It produces more protein per unit area as compared to other forage crops, characterized by high content of minerals and vitamins, resistance to drought, and when the moisture was restored, its growth activated (Hall et al., 1988; Barnes et al., 1995; Keskin et al., 2009).

Legumes have two sources of nitrogen nutrition − symbiotic nitrogen fixation of atmospheric molecular nitrogen in symbiosis with *Rhizobium* or *Bradyrhizobium* sp.,

and assimilation of soil nitrogen (mainly in the form of nitrates), using the enzyme nitrate reductase (Arrese-Igor et al., 1990). These enzyme systems are able to convert, depending on the source of nitrogen (Kretovich, 1997).

In the early stages of plant development, the main manner of recovery of mineral nitrogen is the nitrate assimilation, which is needed from available nitrogen in the soil, delivered through nitrogen fertilizer (Streeter, 1998). Ammonium and nitrate forms of mineral nitrogen are assimilated by the plants (Brown, 2005). Their influence on lucerne, with both optimum and a limited water supply, is insufficiently studied. The nitrate form of mineral nitrogen had a depressive effect on nodulation to a lesser extent as compared to ammonium (Vasileva & Kostov, 2006).

There are limited data in literature for the influence of both forms of mineral nitrogen on the chemical composition, nitrate reductase activity and plastid pigments content of lucerne, grown in conditions of water deficiency stress (Kot's et al., 1990; Kot's, 2001), which is the aim of this study.

MATERIALS AND METHODS

A pot trial was carried out at the Institute of Forage Crops, Pleven (2003–04) with lucerne variety Victoria. Pots of 10 L, filled with leached chernozem soil subtype from the region of Pleven were used. Four plants were grown in each pot. The applied quantities of mineral N in its two forms (ammonium and nitrate) were equivalent to 70, 140 and 210 mg kg^{-1} soil. Carbamide (urea) − $CO(NH_2)_2$ was used as a source of nitrogen, mainly in the ammonium form, and potassium nitrate KNO_3 as a source of nitrate. We assumed that after 10–day mineralization the amide nitrogen from the ureate had been transformed into ammonium.

The nitrogen quantities were added as follows: for 70 mg N kg^{-1} soil as NH_4^+-N – 1.502 g $CO(NH_2)_2$ pot^{-1}; for 140 mg N kg^{-1} soil as NH_4^+-N – 3.004 g $CO(NH_2)_2$ pot^{-1}; for 210 mg N kg^{-1} soil NH_4^+-N – 4.506 g $CO(NH_2)_2$ pot^{-1}. For 70 mg N kg^{-1} soil as NO_3^--N – 5.185 g KNO_3 pot^{-1}; for 140 mg N kg^{-1} soil as NO_3^--N – 10.37 g KNO_3 pot^{-1} and for 210 mg N kg^{-1} soil as NO_3^--N – 15.55 g KNO_3 pot^{-1}. The following treatments in four replications were tested: Under optimum water supply – 75–80% Field Capacity (FC): 1. $Control_1$- unfertilized with nitrogen + PK (C_1); 2. NH_4^+– N_{70} + PK; 3. NH_4^+- N_{140} + PK; 4. NH_4^+- N_{210} + PK; 5. NO_3^- – N_{70} + PK; 6. NO_3^- – N_{140} + PK; 7. NO_3^- – N_{210} + PK; Under 10–day water deficiency stress- 37–40% Field Capacity: 8. $Control_2$ – unfertilized with nitrogen + PK (C_2); 9. NH_4^+– N_{70} + PK; 10. NH_4^+– N_{140} + PK; 11. NH_4^+– N_{210} + PK; 12. NO_3^- – N_{70} + PK; 13. NO_3^- – N_{140} + PK; 14. NO_3^- – N_{210} + PK.

All treatments were laid out on a background of phosphorus and potassium fertilizing (P– 110 mg P kg^{-1} soil; K– 110 mg P kg^{-1} soil). Phosphorus was applied as triple super phosphate and potassium as potassium nitrate.

At the budding stage, 10–day water deficiency stress was imposed by stopping the irrigation till moisture dropped to 37–40% FC. Two cuts for forage were harvested. The content of crude protein was determined according to Kjeldahl method (CP =N x 6.25), crude fiber − Weende method (AOAC, 1990), phosphorus − by the hydroquinone method, and calcium − complexometrically. The activity of nitrate reductase enzyme was determined *in vivo* by the method of Javorski (1971), and the plastid pigments

content by the method of Zelenskii & Mogileva (1980). The data from the two experimental years were statistically processed (standard error) using SPSS 10.0 computer program.

RESULTS AND DISCUSSION

Nitrogen fertilization is one of the main factors influencing the chemical composition of plants. An important quality indicator for the productivity of legumes (especially lucerne) is the crude protein content (Berando, 1992; Popovic et al., 2001; Vasilev, 2004). Data from our study show that under optimal moisture, mineral nitrogen in both forms at a dose of 70 mg N kg^{-1} soil did not affect crude protein content in the aboveground mass (Table 1). Applied as ammonium form at doses of 140 and 210 mg N kg^{-1} soil increased crude protein content as compared to unfertilized control by 5 and 13%, and as a nitrate form at the same doses, by 3 and 7%.

With increasing doses of mineral nitrogen fertilization under conditions of water deficiency stress, crude protein content increased by 4 to 21% for the ammonium form, and by 3 to 12% for nitrate. Increases are greater than those at the optimim moisture content. Increasing the crude protein content, which is the sum of all nitrogen compounds (proteins and non-protein) under water deficiency stress, is associated with the synthesis of new stress proteins, key enzymes of biosynthesis of osmolitics, detoxication enzymes, other proteases and amino acids (mainly proline) that increase the adaptation capacity of plants (Yamaguchi–Shinozaki et al., 2002). Similar results were obtained in another study (Ilieva & Vasileva, 2010). Water deficiency stress decreased the crude protein content mostly in treatments without nitrogen fertilization (6%).

Table 1. Crude protein and crude fiber content in aboveground mass of lucerne in two forms of mineral nitrogen and water deficiency stress.

Treatments	Crude protein		Crude fiber	
	g kg^{-1} DM	% C1 and C2	g kg^{-1} DM	% C1 and C2
	Optimum moisture content (75–80% FC)			
$Control_1$ (C_1)	174.3	–	250.9	-
NH_4^+ - N_{70}	175.0	+1	285.4	+14
NH_4^+ - N_{140}	182.4	+5	263.8	+5
NH_4^+ - N_{210}	197.4	+13	259.6	+3
NO_3^- - N_{70}	176.0	+1	265.8	+6
NO_3^- - N_{140}	179.3	+3	268.7	+7
NO_3^- - N_{210}	185.8	+7	260.7	+4
SE (P= 0.05)	3.09		4.01	
	Water deficiency stress (37–40% FC)			
$Control_2$ (C_2)	164.5	-	276.5	-
NH_4^+ - N_{70}	169.9	+4	280.6	+2
NH_4^+ - N_{140}	192.7	+17	265.6	-4
NH_4^+ - N_{210}	199.0	+21	263.8	-4
NO_3^- - N_{70}	168.8	+3	252.4	-9
NO_3^- - N_{140}	178.0	+9	258.5	-6
NO_3^- - N_{210}	184.1	+12	258.3	-6
SE (P= 0.05)	4.90		3.85	

Nitrogen fertilization influenced both the crude protein and crude fiber content. Crude fiber content under optimum moisture increased by 14% when nitrogen was applied at a dose of 70 mg N kg^{-1} soil in the ammonium form, and less (7%), for the other experimented doses for both conditions of water supply.

Under water deficiency stress (excluding the dose of 70 mg N kg^{-1} soil in ammonium form), crude fiber content decreased (stronger for nitrate form). Water deficiency stress had no significant effect on crude fiber for the treatments, supplied with mineral nitrogen, but crude fiber content increased significantly (by 10%) in control.

Calcium content under the influence of nitrogen fertilization was changed, probably due to the participation of this element in the processes of nitrogen assimilation (Kaiser et al., 1999). In our study, nitrogen in ammonium form at the optimum moisture does not effect the crude fiber content, but with increased doses of mineral nitrogen in nitrate form, the calcium content decreased to 10% (Fig. 1). Under water deficiency stress the calcium content (excluding that at a dose of 210 mg kg^{-1} soil in ammonium form), for the both experimented forms, decreased (4–6%). Calcium content decreased by 6–15% under the influence of water deficiency stress for the ammonium form of mineral nitrogen, and significantly less (by 3–4%) for the nitrate one.

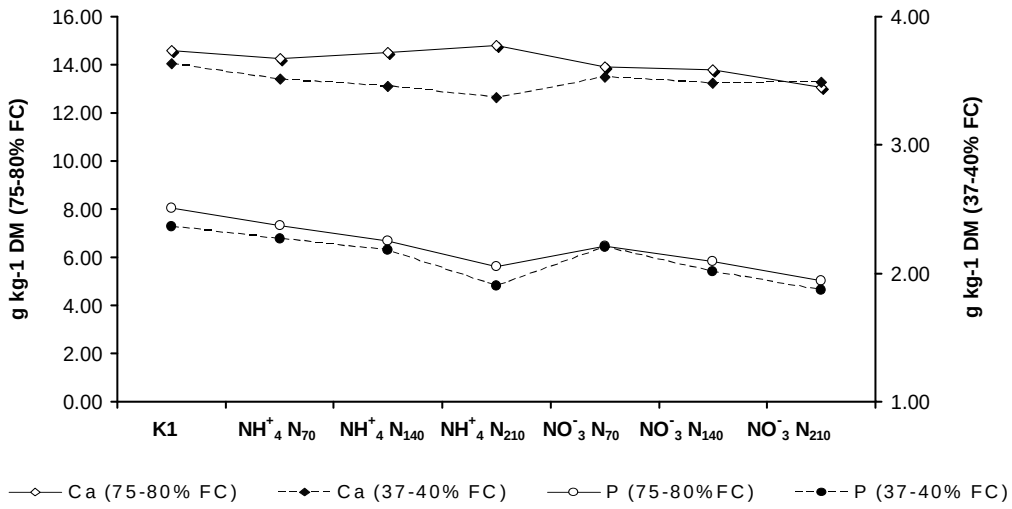

Figure 1. Calcium and phosphorus content in aboveground mass of lucerne in two forms of mineral nitrogen and water deficiency stress.

An important indicator for assessing the lack of phosphorus, as well as recommendations for appropriate fertilization, is its content in plant tissues of different parts (leaves, stems, roots) of lucerne (Jacobsen & Surber, 1995; Wang et al., 2005). Under optimum moisture with increasing doses of mineral nitrogen, phosphorus content decreased (more strongly for the nitrate form, to 23%). The tendency for the conditions of water deficiency stress was similar. Water deficiency stress decreased phosphorus content, but significant differences between tested forms of mineral nitrogen were not found.

Data showed that the level of potential activity of nitrate reductase in organs of lucerne, depend on the forms and doses of mineral nitrogen fertilizer (Table 2).

Nitrate reductase activity in stems and roots was significantly lower than that in leaves in all experimental treatments. There is a similar tendency for a decrease in nitrate reductase activity in leaves for the treatments with mineral nitrogen at a dose of 70 mg N kg^{-1} soil in both forms, and increasing in high doses. Nitrate reductase activity in leaves increased by 63% and 73% for ammonium, and by 15 and 75% for the nitrate form of mineral nitrogen, when applied 140 and 210 mg N kg^{-1} soil under optimum moisture. Under water deficiency stress the greatest increase in enzyme activity (by 57% for ammonium and by 51% for nitrate form) was reported in treatments with 140 mg N kg^{-1} soil. Water deficiency stress decreased nitrate reductase activity in leaves by 4 and 20% for mineral nitrogen in ammonium form at doses 140 and 210 mg N kg^{-1} soil.

Table 2. Nitrate reductase activity in leaves, stems and roots of lucerne in two forms of mineral nitrogen and water deficiency stress.

Treatments	Nitrate reductase activity					
	Leaves		stems		roots	
	µmol NO_2 g^{-1} fresh weight	% to C1, C2	µmol NO_2 g^{-1} fresh weight	% to C1, C2	µmol NO_2 g^{-1} fresh weight	% to C1, C2
	Optimum moisture content (75–80% FC)					
Control$_1$ (C_1)	44.0	-	7.9	-	3.15	-
NH_4^+ - N_{70}	43.0	- 2	5.9	- 25	4.03	+ 28
NH_4^+ - N_{140}	71.6	+ 63	23.4	+ 196	6.90	+ 119
NH_4^+ - N_{210}	76.3	+ 73	12.0	+ 52	4.25	+ 35
NO_3^- - N_{70}	37.3	- 15	7.1	- 10	4.65	+ 48
NO_3^- - N_{140}	50.7	+ 15	15.2	+ 92	5.10	+ 62
NO_3^- - N_{210}	77.2	+ 75	30.4	+ 284	4.75	+ 51
SE (P= 0.05)	6.52		3.48		0.43	
	Water deficiency stress (37–40% FC)					
Control$_2$(C_2)	44.0	-	9.1	-	3.48	-
NH_4^+ - N_{70}	44.1	-	10.2	+ 12	3.83	+ 9
NH_4^+ - N_{140}	68.9	+ 57	23.9	+ 162	2.48	- 29
NH_4^+ - N_{210}	60.7	+ 38	14.9	+ 64	3.85	+ 10
NO_3^- - N_{70}	44.0	-	10.7	+ 17	2.90	- 17
NO_3^- - N_{140}	66.2	+ 51	12.1	+ 33	3.90	+ 11
NO_3^- - N_{210}	62.7	+ 43	25.5	+ 180	5.23	+ 49
SE (P= 0.05)	4.27		2.55		0.33	

The values of nitrate reductase activity in stems and roots of plants varied depending on the tested treatments. An increase of nitrate reductase activity as compared to control was observed in most treatments. Our experimental data support the view of Andrews et al. (1984) and Kot's et al. (1996), that when nitrogen fertilizers were applied, the reduction of nitrate increased in stems of the lucerne plants. The reduction of nitrates is a process closely associated with photosynthesis. The content of chlorophylls *a* and *b*, carotenoids, and therefore the total content of plastid pigments in both levels of water supply and mineral nitrogen, increased as compared to the

unfertilized control. This increase varied depending on the dose.

Under water deficiency stress the content of carotenoids, which enter in the antioxidant defense system of plants, decreased in all treatments with mineral nitrogen. For the nitrate form of mineral nitrogen, the decrease was greater (Table 3).

Water deficiency stress did not affect the total content of plastid pigments for the control and treatment with mineral nitrogen in ammonium form at a dose of 210 mg N kg^{-1} soil. For the treatments with mineral nitrogen in ammonium form at doses of 70 and 140 mg N kg^{-1} soil, an increase of total content of plastid pigments by 9 and 4% was observed. This content decreased by 16% and 8%, with application of mineral nitrogen in nitrate form at doses of 140 and 210 mg N kg^{-1} soil (Fig. 2).

Table 3. Plastid pigments content in leaves of lucerne in two forms of mineral nitrogen and water deficiency stress.

Treatments	Plastid pigments					
	chlorophyll a	chlorophyll b	chlorophyll a+b	% to C1, C2	Carote-noids	% to C1, C2
	mg 100 g^{-1} fresh weight					
	Optimum moisture content (75–80% FC)					
Control$_1$ (C_1)	130.1	85.5	215.6	-	39.9	-
NH_4^+ - N $_{70}$	155.5	100.5	256.0	+19	51.5	+29
NH_4^+ - N$_{140}$	140.4	89.4	229.8	+7	54.4	+36
NH_4^+ - N $_{210}$	159.3	102.1	261.5	+21	54.2	+36
NO_3^- - N $_{70}$	139.4	97.1	236.5	+10	51.1	+28
NO_3^- - N $_{140}$	177.1	113.9	291.0	+35	51.6	+29
NO_3^- - N $_{210}$	150.2	101.0	251.2	+17	48.6	+22
SE (P= 0.05)	5.87	3.49	9.27		1.86	
	Water deficiency stress (37–40% FC)					
Control$_2$ (C_2)	127.0	83.6	210.6	-	42.4	-
NH_4^+ - N $_{70}$	167.7	119.1	286.9	36	49.2	+16
NH_4^+ - N$_{140}$	148.2	98.1	246.4	+17	48.9	+15
NH_4^+ - N $_{210}$	153.6	108.6	262.2	+25	52.7	+24
NO_3^- - N $_{70}$	142.0	110.0	252.0	+20	43.8	+3
NO_3^- - N $_{140}$	141.4	98.9	240.3	+14	46.0	+8
NO_3^- - N $_{210}$	136.2	93.2	229.4	+9	45.4	+7
SE (P= 0.05)	4.93	4.47	9.16		1.34	

Our data recording changes in chemical composition, nitrate reductase activity and plastid pigments content, support the view of Kot's (2001), that the ammonium form of mineral nitrogen was more rapidly included into the metabolic processes of plants as compared to nitrate, becoming the nitrogen of amides and amino acids.

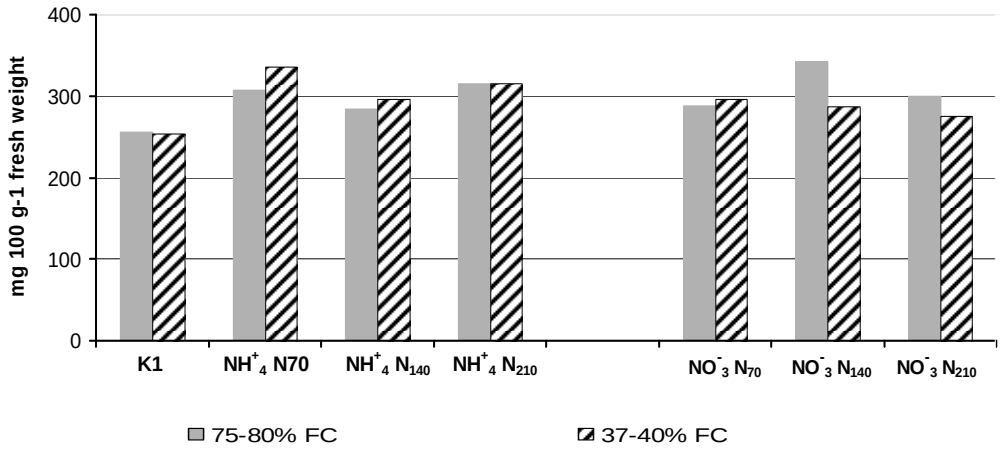

Figure 2. Total content of plastid pigments in leaves of lucerne in two forms of mineral nitrogen and water deficiency stress

CONCLUSIONS

Mineral nitrogen in ammonium form, applied at the doses of 140 and 210 mg N kg^{-1} soil at optimum moisture, increased crude protein content by 5–13%, and in the nitrate form at the same doses, by 3–7 %. Crude protein content under water deficiency stress increased by 4–21% for ammonium, and by 3–12% for nitrate form of mineral nitrogen.

Fiber content increased to 14% at a dose of 70 mg N kg^{-1} soil in the ammonium form, and less (7%) at dose of 140 mg N kg^{-1} soil.

When the plants were supplied insufficiently with nitrogen, water deficiency stress more strongly deteriorated the chemical composition of lucerne: crude protein content decreased by 6%, and crude fiber increased by 10%.

Under optimum moisture the application of mineral nitrogen decreased calcium and phosphorus content, and the decrease was bigger for the nitrate form (up to 10% for calcium, and 23% for phosphorus). Under water deficiency stress the content of calcium and phosphorus decreased, and there were no differences for two forms of mineral nitrogen.

A similar tendency for the decreasing of nitrate reductase activity in the leaves was observed for application of mineral nitrogen at a dose of 70 mg N kg^{-1} soil in its two forms, and increasing of this activity for the dose of 210 mg N kg^{-1} soil. The total content of plastid pigments increased when nitrogen in ammonium and nitrate form was applied.

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