NITROGEN ACCUMULATION AND FORAGE PRODUCTION IN LUCERNE (*MEDICAGO SATIVA* L.) UNDER MINERAL NITROGEN FERTILIZATION AND WATER DEFICIENCY STRESS

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Abstract

A pot trial was conducted at the Institute of Forage Crops, Pleven, Bulgaria during 2003-2004 to study the effect of mineral nitrogen fertilization and water deficiency stress on nitrogen accumulation and aboveground and root mass of lucerne as source of forage. Mineral nitrogen fertilizer (ammonium nitrate) was applied at the doses of 40, 80, 120 and 160 mg N kg^{-1} soil. Water deficiency stress was imposed for 10 days at the budding stage of lucerne by stopping the irrigation till soil moisture dropped to 37-40% field capacity. It was found that mineral nitrogen fertilization increased nitrogen in aboveground dry mass for doses of 120 and 160 mg N kg^{-1} soil in the conditions of optimal moisture by 21 and 37%; and for doses of 80 and 120 mg N kg^{-1} soil and water deficiency stress, by 14 and 12%, respectively. Mineral nitrogen fertilization had a stronger effect on nitrogen in dry root mass yield compared to the aboveground dry mass. The negative effect of water deficiency stress on nitrogen in aboveground and root dry mass was lowest when applied 80 mg N kg^{-1} soil. The findings of this study can be utilized in lucerne cultivation under drought stress for obtaining good forage production with little nitrogen fertilization. This will save the environmental pollution as well as the cost of forage production.

Introduction

Lucerne (*Medicago sativa* L.) is one of the most important forage legume of Bulgaria (Vasileva, 2010), and the third most valuable crop in the US worth of $8 billion per annum (Monteros and Bouton, 2009). It is a perennial species that requires little or no nitrogen fertilizer because of its ability to perform symbiotic nitrogen fixation. Lucerne has the ability to utilize significantly more nitrogen than other legumes through its deep rooting characteristics and derives 40 to 70% of total plant nitrogen through symbiosis (Heichel *et al*., 1981; Jarvis, 2005). Due of its nitrogen-fixing ability, the application of additional nitrogen to this crop is debatable in the literature (Oliveira *et al*., 2004; Werner and Newton, 2005). Moreover, lucerne has potential to tolerate acute conditions of water deficiency (Busse and Bottomley, 1989; Solanki and Patel, 2000; Vasileva *et al*., 2006a). Many farmers are unable to afford chemical fertilizers due to high fertilizer cost. The outlook for fertilizer prices is that high prices will likely continue into the future (Mikkelsen, 2008). Current high prices are related to a global demand for more food, a more diverse diet for a growing world population, an era of higher energy prices, rising transportation costs, a fluctuating value of the U.S. dollar, and an increased demand for biofuel crops (Mikkelsen, 2008). Many factors are involved in producing a high-quality lucerne crop. Although some factors (like rainfall and temperature) cannot be controlled, many other critical components of the production system can be carefully managed (Boyer, 1982; Ray, 2010). High yields require maintenance of an adequate nutrient supply to meet the needs of the rapidly growing crop. As the demand for high-quality and high-yielding hay increases, closer examination of the role of proper plant nutrition is needed to remain profitable (Bouton, 2007).

The aim of this work was to study the effects of mineral nitrogen fertilization and water deficiency on nitrogen accumulation in above and belowground biomass in lucerne grown for forage.

Materials and Methods

A pot experiment was carried out on lucerne (*Medicago sativa* variety Victoria) at the Institute of Forage Crops, Pleven, Bulgaria (2003-2004). Soil subtype of leached chernozem was filled in 10 L pots. The soil used had the following agrochemical characteristics: total C, 2.8; total N, 0.23; P (P_2O_5), 5.3 mg 100 g^{-1} soil; K (K_2O), 48.4 mg 100 g^{-1} soil; pH (H_2O) 7.05.

Healthy, viable and uniform seeds of lucerne (produced in the Institute of Forage Crops) were sorted and twenty seeds were sown by hand at a depth of 2-3 cm in each pot. After germination, the seedlings were thinned keeping four equally vigorous plants per pot. The experimental design had 4 replication. There were two categories of treatment – those under optimum water supply and those with water deficiency. Under optimum water supply – (75-80% of field capacity (FC): 1. Control 1- unfertilized- N0PK (C1); 2. Soil + 40 mg N kg^{-1} soil (N40PK); 3. Soil + 80 mg N kg^{-1} soil (N80PK); 4. Soil + 120 mg N kg^{-1} soil (N120PK); 5. Soil + 160 mg N kg^{-1} soil (N160PK). Under 10- day water deficiency stress (37-40% FC): 6. Control 2- unfertilized- N0PK (C2);

7. Soil + 40 mg N kg^{-1} soil (N40PK); 8. Soil + 80 mg N kg^{-1} soil (N80PK); 9. Soil + 120 mg N kg^{-1} soil (N120PK); 10. Soil + 160 mg N kg^{-1} soil (N160PK).

For treatments 1 to 5, the plant were maintained at 75-80% field capacity, and for 6 to 10, water deficiency stress was simulated at the budding stage by interrupting the irrigation until reducing the soil moisture down to 37-40% of field capacity. Mineral nitrogen as ammonium nitrate equivalent to the tested doses was applied. The plants were also supplied with triple super phosphate at the rate of 2.62 g and potassium chloride at the rate of 1.83 g.

Two cuts for forage were harvested after a period 12 and 16 weeks of growth. Nitrogen in shoot and root dry masses was calculated as a product of shoot and root dry mass yield with total nitrogen content determined by standard Kjeldahl method) in dry mass (Nelson and Sommers, 1980). Total content of nitrogen in yield was calculated as the sum of that in shoot and root masses. Reduction in percentage of nitrogen in yield after water deficiency stress was determined using the following formula:

The data from two experimental years were statistically processed using SPSS 10.0 computer program.

Results and Discussion

Nitrogen fertilization is essential to provide optimal conditions for intensive fixation and nitrogen accumulation in plants (Kretovich, 1983). The accumulation of nitrogen is an important indicator of legumes and in lucerne in particular (Bliss, 1993).

Results show that nitrogen in aboveground dry mass at optimal moisture increased with increasing the doses of mineral nitrogen fertilization (Table 1). It was found that mineral nitrogen fertilization increased nitrogen in aboveground dry mass for doses of 120 and 160 mg N kg^{-1} soil in the conditions of optimal moisture by 21 and 37%;

Mineral nitrogen fertilization at the doses of 40 and 160 mg N kg^{-1} soil under water deficiency stress did not affect on nitrogen in aboveground dry mass yield. The increase compared to unfertilized control was 14 and 12% at the doses of 80 and 120 mg N kg^{-1} soil, respectively.

Given that the effect of mineral nitrogen rather localized on the roots than on the whole nitrogen nutrition of the plant, nitrogen in root dry mass yield increased significantly. This amount varied between 15 and 58% compared to unfertilized control under optimal moisture, and between 42 and 81% under water deficiency stress. With the increasing the doses of nitrogen fertilization under optimal moisture, nitrogen in aboveground and root dry mass increased progressively, as the differences for all treatments were significant (Figure 1). It exceeded to unfertilized control and varied from 7 to 43%.

Table 1. Nitrogen accumulation in shoot and root dry masses of lucerne for forage after mineral nitrogen fertilization.

$$\text{Reduction in N accumulation (\%)} = \frac{\text{N accumulation control – N accumulation after nitrogen fertilization}}{\text{N accumulation in control}} \times 100$$

Treatments	Nitrogen in yield			
	Shoot dry mass	Promotion/Reduction over control	Root dry mass	Promotion over control
	mg N kg^{-1} dry mass	%	mg N kg^{-1} dry mass	%
		optimal moisture (75-80% FC)		
N0PK (Control 1)	417.8	-	170.0	-
N40PK	436.1	+ 4	195.1	+ 15
N80PK	449.6	+ 8	200.3	+ 18
N120PK	505.1	+ 21	261.9	+ 54
N160PK	571.1	+ 37	268.8	+ 58
SE (P=0.05)	27.9		19.5	
		water deficiency stress (37-40% FC)		
N0PK (Control 2)	329.7	-	128.6	-
N40PK	327.7	- 1	182.2	+ 42
N80PK	376.2	+ 14	189.7	+ 47
N120PK	368.3	+ 12	233.0	+ 81
N160PK	337.3	+ 2	209.3	+ 63
SE (P=0.05)	10.1		17.3	

According to Antolin *et al.* (1995) crops treated with nitrogen and subjected to water deficiency stress had better growth and productivity as compared to those depending only on nitrogen from symbiotic N_2-fixation.

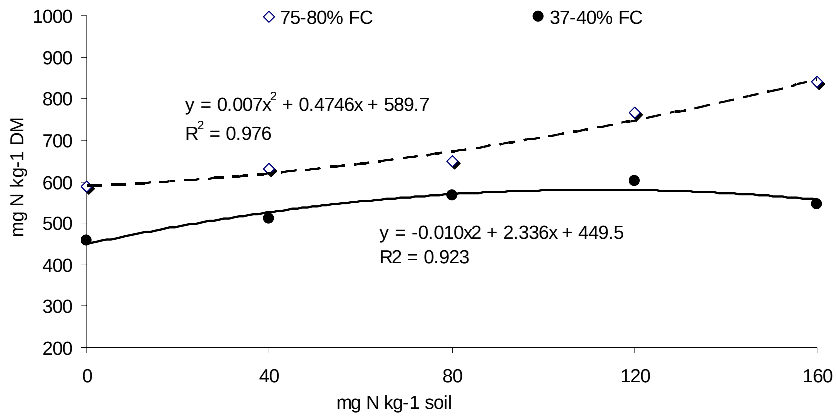

Fig. 1. Curve trend, equation and prognostic effect of the relationship between mineral nitrogen fertilization and nitrogen yield in lucerne for forage.

With the increasing the doses of nitrogen fertilization under optimal moisture, nitrogen in shoot and root dry masses increased progressively, as the differences for all treatments were significant (Figure 1).

In our study the relationship between mineral nitrogen quantities and nitrogen accumulation under optimal moisture (R^2=0.976) was greater, compared to this under water deficiency stress (R^2=0.923). Nitrogen accumulation under water deficiency stress increased with the increasing of the doses of fertilization to 120 mg N kg^{-1} soil.

Although lucerne is more tolerant than most of forage legumes, its dry mass productivity as well as other characteristics, like nitrogen in yield, are influenced negatively by the water deficiency stress, particularly in the year of establishment (Frame *et al*., 1998; Humphries and Auricht, 2001; Zahran, 1999, 2001).

Results of our study show nitrogen in aboveground dry mass under water deficiency stress decreased to 41% for the highest experimented dose (Table 2). This decrease was lowest (16%) in treatment subjected to 80 mg N kg^{-1} soil. Nitrogen in root dry mass decreased slightly as compared to that in aboveground dry mass. It was 5 and 7% for the doses of 80 and 40 mg N kg^{-1} soil, and 11 and 22%, for 120 and 160 mg N kg^{-1} soil, respectively. The highest decrease was observed for the untreated plants (24%). Nitrogen in aboveground and root dry mass decreased the least (13%) at the dose of 80 mg N kg^{-1} soil, which corresponds to whole development of lucerne (Vasileva *et al*., 2006b).

Table 2. Percent of reduction in nitrogen in dry mass yield after water deficiency stress.

Treatments	Nitrogen in yield (%)		
	Aboveground dry mass	Root dry mass	Total (aboveground dry + root dry mass)
N0PK (Control 1)	21	24	22
N40PK	25	7	19
N80PK	16	5	13
N120PK	27	11	22
N160PK	41	22	35

Conclusion

Mineral nitrogen fertilization increased nitrogen in aboveground dry mass yield in the treatment supplied with doses of 120 and 160 mg N kg^{-1} soil in the conditions of optimal moisture, by 21 and 37%; and for doses of 120 and 80 mg N kg^{-1} soil and water deficiency stress, by 12 and 14%. Mineral nitrogen fertilization showed strong effect on nitrogen in root dry mass yield, as it exceeded in the unfertilized control under optimal moisture between 15 and 58%, and under water deficiency stress, between 42 and 81%. Water deficiency stress had stronger effect on nitrogen in aboveground dry mass compared to that in root dry mass. For the doses of 80 and 40 mg N kg^{-1} soil, the decrease in nitrogen in dry root mass yield was by 5 and 7%, and for 120 and 160 mg N kg^{-1} soil, by 11 and 22%, respectively. Water deficiency stress had the strongest effect on unfertilized plants, reducing nitrogen in root dry mass yield by 24%. An optimal for the lucerne development was mineral nitrogen, applied at the dose of 80 mg N kg^{-1} soil, where water deficiency stress affects at least on nitrogen in aboveground and root dry mass. The findings of this study can be utilized in lucerne cultivation under drought stress for obtaining good forage production with little nitrogen fertilization. This will save the environmental pollution as well as the cost of forage production.

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