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EFFECT OF MINERAL NITROGEN FERTILIZATION ON GROWTH CHARACTERISTICS OF LUCERNE UNDER INDUCED WATER DEFICIENCY STRESS

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Abstract

Utility of lucerne crop fertilization with nitrogen fertilizer has been discussed in literature with controversy. In this study experiment was conducted to determine the effect of mineral nitrogen at the doses of 40, 80, 120 and 160 mg N/kg on some characteristics of lucerne under induced water deficiency stress at the stage of budding in a pot trial. It was found that mineral nitrogen at the doses of 120 and 160 mg N/kg soil increased the productivity of dry top mass by 17 and 23% in conditions of optimum moisture, and by 9% in conditions of water deficiency stress at the dose of 80 mg N/kg soil. Application of mineral nitrogen at the dose of 120 and 160 mg N/kg soil increased the quantity of dry root mass by 43 and 38% for the conditions of optimum moisture, and by 54-56% for conditions of water deficiency stress. Mineral nitrogen fertilizing at the dose of 40 mg N/kg soil had the lowest suppressive effect on the nodulation (11%). The dose of 160 mg N/kg soil was found to be toxic to nodulation. The dose of 80 mg N/kg soil, at which the crop had the lowest sensitivity to water deficiency stress, was optimal for lucerne development.

Introduction

The question whether lucerne as a nitrogen-fixing crop needs additional application of nitrogen and what kind and quantity of nitrogen or will rely only on that obtained from nitrogen fixation, is debatable in literature (Oliveira *et al*., 2004). Lucerne has the ability to utilize significantly more nitrogen than other legumes through its deep rooting characteristics (Jarvis, 2005). Tufenkci *et al*. (2006) reported positive effect of nitrogen fertilization on lucerne growth. This crop has a longer duration of active vegetative growth and needs frequent irrigations than other forage crops (Solanki & Patel, 2000). The question of application of nitrogen fertilizers stands more sharply in conditions of water deficiency stress, which is a typical phenomenon of the Bulgarian agriculture. Although lucerne is more tolerant than most of forage legumes, its dry mass productivity, as well as other characteristics are influenced negatively by the water deficiency stress, particularly in the year of establishment (Frame *et al*., 1998; Humphries & Auricht, 2001; Zahran, 1999, 2001). Decreased capacity of lucerne plants to obtain nutrients and nitrogen from soil makes them more dependent on fertilizer nitrogen than on that fixed by nodules (Sidiras *et al*., 1999). Moreover mineral nitrogen lessens the sensitivity of lucerne to water deficiency stress (Lodeiro *et al*., 2000; Radeva 1991; Zahran, 1999, 2001). In our previous studies we have reported the effect of mineral nitrogen on seed production of lucerne (Vasileva, 2008; 2010) and effect of mineral nitrogen and manure of the development of lucerne (Vasileva *et al*., 2006). The objective of this study was to investigate the effect of mineral nitrogen fertilization on some characteristics in lucerne grown for forage under induced water deficiency stress at the stage of budding.

Material and Methods

The experiment was carried out in the Institute of Forage Crops, Pleven Bulgaria (2003-2004) on lucerne (*Medicago sativa* L.) variety Victoria in pots. Pots of 10 L capacity and soil subtype of leached chernozem were used. Sowing was conducted by hand at a depth of 2-3 cm with a number of about 20 viable seeds. After emergence, 4 well developed plants per pot were left and excess were removed. The following variants were tested with 4 replications: Under optimum water supply (75-80% WHC): 1. Control 1- unfertilized- (C1); 2. Soil + 40 mg N kg/soil (N40); 3. Soil + 80 mg N kg/soil (N80); 4. Soil + 120 mg N kg/soil (N120); 5. Soil + 160 mg N kg/soil (N160). In another similar set plants were kept under 10-day water deficiency stress at the stage of budding (37-40% WHC) by interrupting the irrigation until reduction of soil moisture down to 37-40% WHC. Mineral nitrogen was applied as ammonium nitrate (g) equivalent to the above mentioned doses. Triple super phosphate and potassium chloride at 1.1 g each were applied as a background. Two cuts (after 80 days intervals) for forage were harvested to analyze the effect of nitrogen fertilization on lucerne.

Productivity of dry top mass was recorded by drying to constant temperature at 60^{o}C. After washing of the root system of plants in laboratory conditions, the quantity of dry root mass was recorded (drying at 60^{o}C), as well as the nodulation- by counting and determination of nodule number per plant (Somesagaron & Hoben, 1991). Efficacy of nitrogen fertilizing was calculated according to formulae of Bowen & Zapata (1991). The crop sensitivity index of lucerne to water deficiency stress (CSI) was determined according to the formula of Dimitrova & Mehandzhieva (1990) and Hiller & Clark (1971). Averaged data from the two cuts during the two experimental years were statistically analyzed using SPSS10.0 computer program.

Results and Discussion

Under optimum moisture, the mineral nitrogen increased the productivity of dry top mass in first cut, the increase being by 12 and 13% in variants with fertilizing at the doses of 40 and 80 mg N/kg soil, and by 32 and 34% in those with 120 and 160 mg N/kg soil (Table 1). In second cut, significant increases in top dry mass was recorded as compared to unfertilized, the increases at the

higher tested doses (120 and 160 mg N/kg soil) were found 9 and 17%, respectively. On average for the two cuts, the productivity of dry top mass increased by 5-6% at the doses of 40 and 80 mg N/kg soil, and by 17 and 23% at the doses of 120 and 160 mg N/kg soil (Table 1).

Table 1. Dry mass productivity of lucerne, grown for forage under increasing doses of mineral nitrogen.

Variants	First cut	Second cut	Average	-, Decrease
	g/pot			%
	Optimum moisture (75-80% WHC)			
Control 1 PK	11.9	19.2	15.6	-
N40PK	13.3	19.4	16.4	-
N80PK	13.5	19.5	16.5	-
N120PK	15.7	20.9	18.3	-
N160PK	16.0	22.4	19.2	-
SE (p=0.05)	0.77	0.60	0.66	
	Water deficiency stress (37-40% WHC)			
Control 2 PK	8.5	17.6	13.0	16
N40PK	8.5	18.2	13.3	18
N80PK	9.8	18.6	14.2	14
N120PK	6.9	19.9	13.4	27
N160PK	5.4	19.3	12.3	36
SE (p=0.05)	0.76	0.4	0.30	

$N_{40,80,120,160}$ mg N kg/soil; N applied as an ammonium nitrate, P as a triple super phosphate; K as a potassium chloride

The application of mineral nitrogen in conditions of water deficiency stress decreased the productivity of dry top mass by 19 and 36% for doses of 120 and 160 mg N/kg soil. At the low tested dose no difference was observed, while at the dose of 80 mg N/kg soil, the dry top mass was 15% greater (Table 1). In second cut the quantity of dry top mass increased by 4 to 13%. The data obtained on average for the two cuts showed that the application of mineral nitrogen did not exert significant influence on the productivity of dry top mass, while the dose of 80 mg N/kg soil increase upto 9%. The induced water deficiency stress decreased considerably the values of that parameter in both cuts. In first cut- 27 to 66% at the doses of 80 and 160 mg N/kg soil, and to a smaller extent, by 5 to 14%, in the second cut.

The application of mineral nitrogen exerted a stronger influence on the quantity of dry root mass. The doses of 80 and 160 mg N/kg soil increased the root dry mass by 44 and 61%, and in second cut, by 30 and 47% at the dose of 160 and 120 mg N/kg soil (Table 2). On average for the two cuts, the increase as against the unfertilized control was by 23% at the doses of 40 and 80 mg N/kg soil, and by 38 and 43% at the doses of 160 and 120 mg N/kg soil. In conditions of water deficiency stress, the application of mineral nitrogen increased the productivity of dry root mass in both cuts. In first cut, it was up to 57% for dose of 80 mg N/kg soil, in second cut up to 59% for dose of 120 mg N/kg soil.

Table 2. Dry root mass productivity of lucerne, grown for forage under increasing doses of mineral nitrogen.

Variants	First cut	Second cut	Average	-, Decrease
	g/pot			%
	Optimum moisture (75-80% WHC)			
Control1 PK	4.89	13.5	9.2	-
N40PK	5.79	16.8	11.3	-
N80PK	7.06	15.6	11.3	-
N120PK	6.50	19.8	13.2	-
N160PK	7.85	17.6	12.7	-
SE (p=0.05)	0.5	1.04	0.69	
	Water deficiency stress (37-40% WHC)			
Control2 PK	4.28	9.1	6.7	27
N40PK	5.35	12.6	9.0	21
N80PK	6.74	13.9	10.3	9
N120PK	6.38	14.5	10.5	21
N160PK	6.64	12.3	9.5	26
SE (p=0.05)	0.46	0.91	0.68	

Legend as shown in Table 1

Previous studies reported that nitrate form of nitrogen inhibited the nodule development, as compared to the ammonium, but at doses over 140 mg N/kg soil completely inhibited the nodule formation (Athar & Johnson, 1996a, b; Vasileva, 2008, 2010). In this study increase level of mineral nitrogen suppressed the nodulation (Table 3). In first cut, at optimum moisture and at doses of 80 and 120 mg N/kg soil, the decrease was by 66 and 82%, and at the highest experimental dose there was no nodulation. In second cut, the same tendency was observed and the decrease was 52 to 54%. In conditions of water deficiency stress the application of mineral nitrogen also suppressed the nodulation with increase of the tested doses (Table 3). The water deficiency stress decreased the nodule number in first cut by 39% at the dose of 80 mg N/kg soil, and in second cut, by 38% at the dose of 120 mg N/kg soil.

The data on nodule number on average for the two cuts (Fig. 1) and under the two types of moisture showed that with increase of the doses of nitrogen fertilizing, the percentage of nodule formation decreased. Under water deficiency stress nitrogen decreased the nodulation by 11, 25 and 37% at the doses of 40, 80 and 120 mg N kg soil, respectively. Lucerne is high yielding fodder crop, but drought determines to a great extent the quality of lucerne in arid and semi arid regions. The application of organic nitrogen showed to overcome depressive effect of water deficiency with regards to forage production, root dry mass (Vasileva & Kostov, 2002) although it suppressed the nodulation.

Table 3. Nodulation of lucerne, grown for forage under increasing doses of mineral nitrogen.

Variants	First cut	Second cut
	Nodule number/pot	
	Optimum moisture (75-80% WHC)	
Control 1 PK	202	336
N40PK	142	180
N80PK	69	160
N120PK	37	154
N160PK	0	0
SE (p=0.05)	36.4	53.3
	Water deficiency stress (37-40% WHC)	
Control 2 PK	133	317
N40PK	121	166
N80PK	42-68	131
N120PK	25-82	96
N160PK	0	0
SE (p=0.05)	26.5	51.7

Legend as shown in Table 1

Table 4. Efficacy of soil-plant system under increasing doses of mineral nitrogen (lucerne, grown for forage).

Variants	N use efficacy (an agronomic parameter)[a]	Crops sensitivity index (a stress parameter)[b]
	Optimum moisture (75-80% WHC)	
Control 1 PK	-	-
N40PK	818	-
N80PK	412	-
N120PK	305	-
N160PK	240	-
SE (p=0.05)	130	
	Water deficiency stress (37-40% WHC)	
Control 2 PK	-	0.162
N40PK	667	0.184
N80PK	355	0.140
N120PK	223	0.268
N160PK	154	0.358
SE (p=0.05)	114	0.004

[a], mg harvested top mass/mg N applied; [b], CSI= (g top mass productivity under optimum moisture content- g top mass productivity under water deficiency stress)/g top mass productivity under optimum moisture content

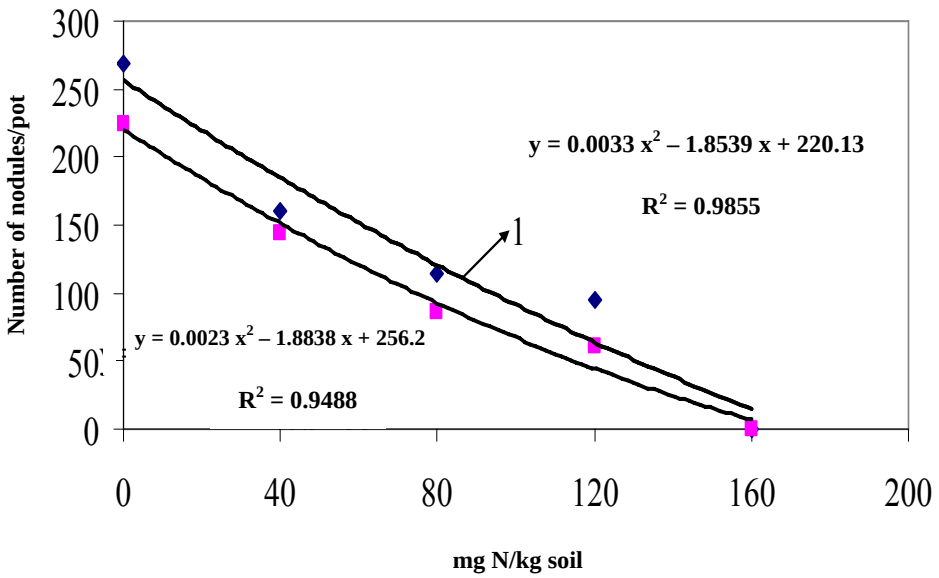

Fig. 1. Nodulation of lucerne, grown for forage (average for two cuts). 1. Optimum moisture. 2. Water deficiency stress.

References

Athar, M. and D.A. Johnson. 1996-a. Nodulation, biomass production and nitrogen fixation in alfalfa under drought. *Journal of Plant Nutrition,* 19: 185-199.

Athar, M. and D.A. Johnson. 1996-b. Competitive ability of *Rhizobium meliloti* strains from Pakistan and Nepal for nodulation in three alfalfa accessions. *Journal of Applied Botany*, 70: 128-133.

Bowen, G.D. and F. Zapata. 1991. Efficiency in uptake and use of N by plants. In: *Proceeding Series Stable Isotopes in Plant Nutrition Soil Fertility and Environmental Studies.* Vienna, Austria, pp. 349-362.

Dimitrova, Y. and A. Mehandjieva. 1990. Possibilities of SDI-method of investigating water-yield dependence. *Plant Sciences,* 27: 53-63.

Frame, J J., J.F. Charlton and A.S. Laidlow. 1998. *Temperate Forage Legumes.* CAB International, pp. 107-159. London.

Hiller, E.A. and R. N. Clark 1976. Stress day Index to characterize effects of water stress on crop yields. Transactions of the ASAE. 757-761.

Humphries, A.W. and G.C. Auricht. 2001. Breeding lucerne for Australia's southern dryland cropping environments. *Australian Journal of Agricultural Research,* 52: 153-169.

Jarvis, S.C. 2005. N flow and N efficiency in legumes based systems: A system overview. In: *Sward dynamics, N-Flow and Forage Utilization in Legume-Based System.* (Eds.): M. Wachendorf, A. Helgsdottit & G. Parente. Proc. 2nd COST 852 Workshop, Grado, Italy. pp. 187-198.

Lodeiro, A.R., P. Gonzales, A. Hernandez, L. Balague and G. Favelukes. 2000. Comparison of drought tolerance in nitrogen-fixing and inorganic nitrogen-grown common beans. *Plant Science*, 154: 1, 31-41.

Oliveira, W.S., P.P.A. Oliveira, M. Corsi, F.R.S. Duarte and S.M. Tsai. 2004. Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti. Scientia Agricola*, 61: 1-7.

Radeva, V. 1991. Biological reaction of lucerne varieties under the conditions of different water and nutritive regime. In: *Proceedings of the Eucarpia*, September 11-13, Kompolt, Hungary, 256-269.

Sidiras, N., C. Avgoulas, D. Bilalis and N. Tsougrianis. 1999. Effect of Tillage and fertilization on Biomass, Roots, N-Accumulation and Nodule Bacteria of Vetch (*Vicia sativa* cv. Alexander) Journal Agronomy and Crop Science, 182: 209-216.

Somesagaron, P. and H.J. Hoben. 1991. Methodologies for Screening Soil-improving Regimes. Marriane Serrantonio, Rodale Instititute.

Solanki, R.M. and R.G. Patel. 2000. Response of lucerne to different moisture regimes. Phosphate levels and sowing methods. *Indian J. Agric. Res.,* 34: 160-163.

Tufenkci, S., M. Erman and F. Sonmez. 2006. Effect of nitrogen application and *Rhizobium* inoculation of yield and nutrient uptake in lucerne (*Medicago sativa*). *Indian J. Agric. Res.,* 76:

Vasileva, V. 2008. Seed productivity of lucerne after fertilizing with ammonium and nitrate form of mineral nitrogen. *Journal of Mountain Agriculture on the Balkans,* 11: 728-734.

Vasileva, V. 2010. Effect of mineral nitrogen fertilization on nodulation of lucerne grown for seeds under water deficient conditions. *Journal of Mountain Agriculture on the Balkans,* 13: 950-960.

Vasileva, V. and O. Kostov. 2002. Effect of water deficiency stress and N in yield of lucerne (*Medicago sativa* L.) *Ecology and Future,* 1: 104-107.

Vasileva, V., O. Kostov O. and E. Vasilev. 2006. Development of lucerne (*Medicago sativa* L.) treated with mineral fertilizer and manure at optimal and water deficit conditions. *Communication in Applied Biological Science*, Ghent University, 71: 5-17.

Zahran, H.H. 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews*, 63: 968-989.

Zahran, H.H. 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *Journal of Biotechnology*, 91: 143-153.

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