



Nitrogen Accumulation in Lucerne (*Medicago sativa* L.) Under Water Deficit Stress

Viliana Vasileva • Emil Vasilev

received: 21 February 2013, revised: 29 April 2013, accepted: 21 May 2013

published online: 28 May 2013

© 2013 IFVC

doi:10.5937/ratpov50-3407

Summary: In order to study nitrogen accumulation in aboveground and root dry mass in lucerne (*Medicago sativa* L.) under water deficit stress, a pot experiment was carried out at the Institute of Forage Crops, Plevna, Bulgaria. The plants were grown under optimum water supply (75-80% FC) and 10-days water deficit stress was simulated at the stage of budding by interrupting the irrigation until soil moisture was reduced to 37–40% FC. Mineral nitrogen fertilization (ammonium nitrate) at the doses of 40, 80, 120 and 160 mg N kg⁻¹ soil was applied. It was found that nitrogen accumulation in dry aboveground mass was reduced to 18.0%, and in dry root mass to 26.5% under water deficit stress. Mineral nitrogen fertilization contributed to easily overcome the stress conditions of water deficit stress in lucerne.

Key words: accumulation, fertilization, lucerne, nitrogen, soil water deficit

Introduction

Lucerne (*Medicago sativa* L.) is a nitrogen-fixing crop with a capability to accumulate significantly more nitrogen in their biomass (Jarvis 2005). The application of additional nitrogen is debatable in the literature due to its nitrogen-fixing ability (Oliveira et al. 2004, Werner & Newton 2005). Unfavourable factors limit multiplication frequently. Although potential to tolerate acute conditions of water deficit stress (Busse & Bottomley 1989, Solanki & Patel 2000), due to the deeper root system, seed yield in lucerne is considerably influenced by lack of moisture (Shock et al. 1989, 2001, Frame et al. 1998) and decreased from 18 to 33% (Vasileva et al. 2006). Because of greater necessity of C and N assimilates during the reproductive stage nitrogen accumulation is an important indicator and one of the limiting factors for plant growth and seed yield formation (Serraj et al. 1999). The aim of this work was to study nitrogen accumulation in lucerne under water deficit stress.

Materials and Methods

A pot experiment was carried out on lucerne (*Medicago sativa* L.) cv. Victoria at the Institute of Forage Crops, Plevna, Bulgaria (2003-2004). Soil subtype of leached chernozem was filled in 10 L pots. The following treatments were tested with four replications: 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 deficit 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). Mineral nitrogen as ammonium nitrate equivalent to the tested doses was applied. All treatments were treated against a background of P and K, the phosphorus being applied as triple super phosphate, and potassium as potassium chloride. For treatments 1-5 the plants were maintained at 75-80% FC, and for 6-10 water deficit stress was simulated at the budding stage by interrupting the irrigation until reducing the soil moisture down to 37-40% FC.

V. Vasileva* • E. Vasilev
Institute of Forage Crops, 89 Gen. Vl. Vazov Str., Plevna 5800, Bulgaria
e-mail: viliana.vasileva@gmail.com

Samples were taken when lucerne was harvested for seeds. Dry aboveground mass and dry root mass were recorded (dried at 60°C). Nitrogen in aboveground and root dry mass was calculated as a product of aboveground and root dry mass yield and total nitrogen content (determined by standard Kjeldahl method in dry mass) (Nelson & Sommers 1980). Total nitrogen accumulation was calculated as the sum of that in aboveground and root mass. Reduction in percentage of nitrogen accumulation after water deficit stress was determined using the following formula:

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

The data were statistically processed by the LSD using SPSS 10.0 computer program.

Results and Discussion

Lucerne used soil or fertilizer nitrogen, since nitrogen assimilation required lower rates of CO₂ and energy than nitrogen fixation in starting development (Hartwig & Soussana 2001). This reflects on the nitrogen accumulation in plants.

In our study nitrogen accumulation in dry aboveground mass under optimum water supply increased with the increase of doses of mineral nitrogen fertilization (Table 1). For the experimental doses of 40 and 80 mg N kg⁻¹ soil, the increase as compared to unfertilized control was by 6.7 and 9.5% and for the doses of 120 and 160 mg N kg⁻¹ soil by 22.3 and 24.2%, respectively.

Doses of 40 and 80 mg N kg⁻¹ soil did not significantly affect nitrogen accumulation in dry aboveground mass under water deficit stress. For doses of 120 and 160 mg N kg⁻¹ soil there was an increase in nitrogen accumulation as compared to unfertilized control by 6.1 and 13.8%, respectively.

Nitrogen accumulation in dry root mass decreased to 35.1% with increase of doses of mineral nitrogen fertilization under optimum water supply (Table 2). It is related to the directing of assimilates to leaf mass and needs of nitrogen

for seeds with high protein content (Tufenkci et al. 2006).

Under the conditions of water deficit stress for the doses of 120 and 160 mg N kg⁻¹ soil, nitrogen in dry root mass decreased by 24.9 and 30.7%, respectively.

Our results showed that plants insured with nitrogen easily overcome water deficit stress. In confirmation of that are the findings of Radeva (1991) and Antolin et al. (1995) according to which lucerne plants fertilized with mineral nitrogen and subjected to water deficit stress showed better growth and productivity as compared to these depend only on the nitrogen of symbiotic N₂ fixation. Nitrogen is important for growth and formation of seeds with high protein content and water deficit affects supply of plants with nitrogen.

Table 1. Nitrogen accumulation in dry aboveground mass in lucerne

Treatments	Optimal water supply (75-80% FC)	Water deficit stress (37-40% FC)
N0PK(C1)	591.3	558.7
N40PK	631.2	543.6
N80PK	647.3	574.2
N120PK	722.9	592.8
N160PK	734.3	635.6
Average	665.4	581.0
LSD at P< 0.05	25.8	16.2

Table 2. Nitrogen accumulation in dry root mass in lucerne

Treatments	Optimal water supply (75-80% FC)	Water deficit stress (37-40% FC)
N0PK(C1)	357.9	274.6
N40PK	360.7	265.0
N80PK	307.8	267.7
N120PK	245.6	206.3
N160PK	232.4	190.2
Average	300.9	240.8
LSD at P< 0.05	25.5	17.1

Total nitrogen accumulation (N in dry aboveground + N in dry root mass) varied in narrow range and was less affected by mineral nitrogen fertilization under both conditions of water supply (Figure 1).

Although lucerne is more tolerant than most of forage legumes, some characteristics like nitrogen in yield are influenced negatively by the water deficit stress (Serraj et al. 1999, Zahran 1999, 2001, Humphries & Auricht 2001). Root mass of the plants was more sensitive to water deficit stress as compared to aboveground mass. Nitrogen accumulation in dry aboveground mass

decreased to 18.0%, but in dry root mass to 26.5% (Table 3).

Some authors (Cihacek 1994, Petkova 1994) consider lucerne needs nitrogen despite its nitrogen-fixing ability. This is particularly true for the stress conditions of water deficit. Water deficit stress strongly reduced nitrogen in dry root mass in the plants of unfertilized control (by 23.3%). The decrease for doses of 80 and 120 mg N kg⁻¹ soil was 13.0 and 16.0%, respectively. Under experimental doses of 80 and 120 mg N kg⁻¹ soil lucerne showed a slightly overcome the negative effect of water

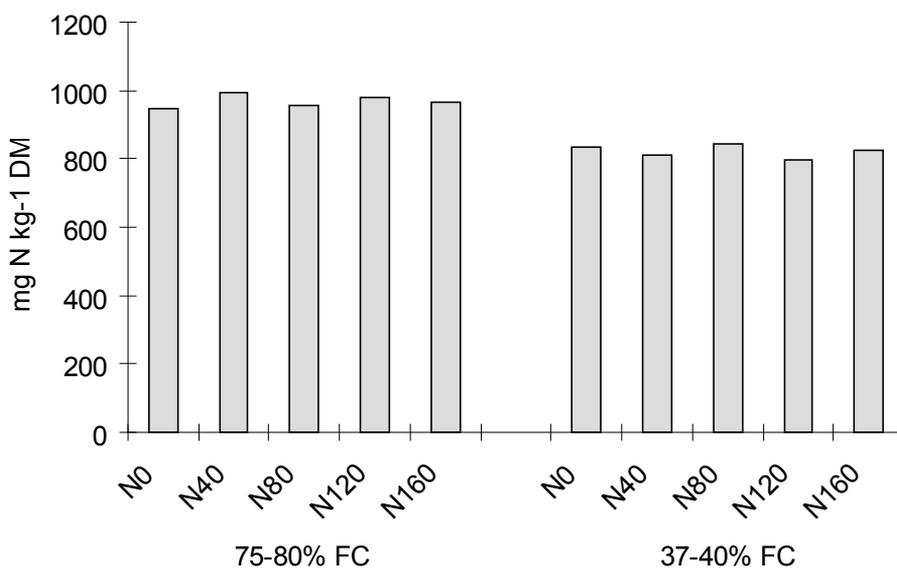


Figure 1. Total nitrogen accumulation in lucerne under optimum water supply (75-80% FC) and water deficit stress (37-40% FC)

Table 3. Percent of reduction in nitrogen in dry mass yield in lucerne after water deficit stress

Treatments	Nitrogen in yield (%)		
	Aboveground dry mass	Root dry mass	Total (aboveground + root dry mass)
N0PK(C1)	5.5	23.3	12.2
N40PK	13.9	26.5	18.5
N80PK	11.3	13.0	11.9
N120PK	18.0	16.0	18.5
N160PK	13.4	18.2	14.6

deficit stress in terms of nodulation and dry root mass (Vasileva 2010). Water deficit stress reduced total nitrogen accumulation (N in dry aboveground + N in dry root mass). This decrease was lowest (11.9%) in treatment subjected to 80 mg N kg⁻¹ soil.

Conclusions

Water deficit stress decreased nitrogen in dry aboveground mass to 18.0% and nitrogen in dry root mass to 26.5%. Mineral nitrogen fertilization at a dose of 80 mg N kg⁻¹ soil contributed to easily overcome the stress conditions of water deficit in lucerne.

References

- Antolin, M., Yoller, J., & Sanchez-Diaz, M. (1995). Effects of temporary drought on nitrate-fed and nitrogen-fixing alfalfa plants. *Plant Science*, 107, 159-165.
- Busse, M. D., & Bottomley, P. J. (1989). Growth and nodulation responses of *Rhizobium meliloti* to water stress induced by permeating and non-permeating solutes. *Applied and Environmental Microbiology*, 55, 2431-2436.
- Cihacek, L.J. (1994). Alfalfa nutrient needs and fertilization. In: *Workshop Sobre O Potencial Forrageiro Da Alfafa (Medicago sativa L.) Nos Trópicos*, Juiz de Fora, Anais. Juiz de Fora: EMBRAPA, CNPGL, 93-97.
- Frame, J. J., Charlton, J. F., & Laidlow, A. S. (1998). *Temperate Forage Legumes*. CAB International, pp. 107-159.
- Hartwig, U. A., & Soussana, J. F. (2001). Ecophysiology of symbiotic N₂ fixation in grassland legumes. In J. Isselstein, G. Spatz, M. Hofmann (eds.). *Organic Grassland Farming. Grassland Science in Europe* 6: 23-26.
- Humphries, A., & Auricht G. (2001). Breeding lucerne for Australia's southern dryland cropping environments. *Australian Journal 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-flows and Forage Utilization in Legume-Based Systems*, Proc. of the 2nd COST 852 workshop, Grado, Italy 10-12 November 2005, Wachendorf M., Helgadottir A., Parente G. (eds.), 187-198.
- Nelson, D.W., & Sommers, L. E. (1980). Determination of total nitrogen in plant material. *Agronomy Journal*, 65, 109-112.
- Oliveira, W., Oliveira, P., Corsi, M., Duarte, F., & Tsai, S. M. (2004). Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Scientia Agricola* (Piracicaba, Braz.), 61, 433-438.
- Petkova, D. (1994). Effect of the fertilization with nitrogen and some microelements on the productivity during the first year of alfalfa grown for seeds. *Plant Science*, 31(5-6), 33-37. (In Bg)
- Radeva, V. (1991). Biological reaction of lucerne varieties under the conditions of different water and nutritive regime. *Proceedings of the Eucarpia*, September 11-13, Kompolt, Hungary, 256-269.
- Serraj, R., Sinclair, T., & Purcell, L. (1999). Symbiotic N₂ fixation response to drought. *Journal of Experimental Botany*, 50, 143-155.
- Shock, C.C., Feibert, E. Pettet, F., Sadowski, A., & Saunders, M. (2001). *Relationship between water stress and seed yield of two drip-irrigated alfalfa varieties*. Oregon State University Agricultural Experiment Station, Special Report 1038, 27-38.
- Shock, C.C., Stephen, W.P., Cairo, V., Stieber, T.D., & Saunders, M. (1989). *Irrigation, insect population, and disease management for alfalfa seed production*. Oregon State University Agricultural Experiment Station, Special Report 844, 16-21.

- Solanki, R.M., & Patel, R.G. (2000). Response of lucerne to different moisture regimes. Phosphate levels and sowing methods. *Indian Journal Agricultural Research*, 34, 160-163.
- Tufenkci, S., Erman, M., & Sonmez, F. (2006). Effect of nitrogen application and *Rhizobium* inoculation on the yield and nutrient uptake in lucerne (*Medicago sativa*). *Bulletin of Pure and Applied Sciences-Botany*, 25, 1.
- Vasileva, V. (2010). Effect of mineral nitrogen fertilizing on nodulation of lucerne for seeds in conditions of water deficiency stress. *Journal of Mountain Agriculture on the Balkans*, 4, 950-960.
- Vasileva, V., Kostov, O., & Vasilev, E. (2006). Development of lucerne (*Medicago sativa* L.) treated with mineral fertilizer and manure at optimal and water deficit conditions. *Comm. Appl. Biol. Sci. Ghent University*, 4, 5-17.
- Werner, D., & Newton, W.E. (2005). Soil stress factors influencing symbiotic nitrogen fixation. In: *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*. Series: Nitrogen Fixation: Origins, Applications, and Research Progress. Springer, pp. 89-102.
- Zahran, H. H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology Molecular Biology Review*, 63, 968-989.
- Zahran, H. H. (2001). Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *Journal of Biotechnology*, 91, 143-153.

Akumulacija azota u lucerki (*Medicago sativa* L.) pri vodnom deficitu

Viliana Vasileva • Emil Vasilev

Izvod: U cilju ispitivanja akumulacije azota u suvoj masi nadzemnih i podzemnih delova lucerke (*Medicago sativa* L.) pri vodnom deficitu, eksperiment u saksijama je izveden u Institutu za krmno bilje u Plevenu, Bugarska. Biljke su uzgajane pod optimalnom obezbeđenošću vodom (75-80% poljskog vodnog kapaciteta), a desetodnevni stress pri vodnom deficitu je simuliran u fazi pupoljaka prekidom dovoda navodnjavanja do smanjenja vlažnosti zemljišta na 37-40% vodnog kapaciteta. Primenjeno je mineralno azotno đubrenje (amonijum nitrat) u dozama 40, 80, 120 i 160 mg N kg⁻¹ zemljišta. Akumulacija azota pri vodnom deficitu u suvoj masi nadzemnih delova je smanjena na 18,0% a u suvoj masi podzemnih delova na 26,5%. Mineralno azotno đubrenje doprinelo je lakšem prevazilaženju stresnih uslova pri vodnom deficitu u usevu lucerke.

Ključne reči: akumulacija, azot, deficit vode, đubrenje, lucerka