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Banat's Journal of Biotechnology
2015, VI(11)

NODULATION DYNAMICS IN ALFALFA VARIETIES (*Medicago sativa* L.)

DOI: 10.7904/2068–4738–VI(11)–83

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Abstract. Eight Bulgarian alfalfa varieties (Prista 2, Prista 3, Prista 4, Obnova 10, Pleven 6, Dara, Multifoliate and Dama) and one French variety (Europe) were cultivated on slightly leached chernozem in Institute of Forage Crops (Pleven) during the period 2006–2009. The dynamics in alfalfa nodulation by years showed the greatest number of nodules on average per plant was formed during the second year of development (8.1) followed by third (6.5) and first year (6.3). Least nodules were formed in the fourth year (1.2) as the decrease to the previous three years was sensitive (by 82.9%). In all years (except first year) the intensity of nodulation decreased from spring regrowth to summer and autumn regrowth. The main quantity of nodules was situated on the lateral roots as in the first year their percentage was 52.4 and during the second, third and fourth year–86.4, 95.4 and 100% respectively. With most pronounced nodule–forming ability for the Bulgarian varieties were Obnova 10 and Multifoliate and the French variety Europe.The alfalfa nodulation correlated with important parameters: age of stand (r= –0.729), stand density (r= –0.648), number of stems per plant (r= 0.763) and weight of root mass (r= 0.384).
Keyword: alfalfa, correlations, nodulation, varieties

Introduction

Alfalfa is called "queen of forage crops" because of its remarkable ability to produce high yields of rich, palatable, nutritious forage under a wide range of soil and climatic conditions.

There are undoubtedly many factors that contribute to this plant's excellence but recognition must be given to the microsymbiont, the nodule bacteria that fix free nitrogen from atmosphere [BURTON, 1972].

Cultivated alfalfas (Medicago sativa) can nodulate in every soil with appropriate pH and in absence of abnormal concentrations of metal ions and salts [SCOTTI *et al.*, 1997].

According to Burton [BURTON, 1972] and Mueller and Teuber [MUELLER and TEUBER, 2007] calculated values of N_2 (molecular nitrogen) fixation in alfalfa vary from about 40 to 400 pounds of N_2 fixed acre per year with an average value of about 175 pounds of N_2 fixed acre per year.

In the conditions of Bulgaria symbiotic system *Rizobium meliloti–Medicago sativa* acts in all main soil types because of wide dissemination of specific alfalfa nodule bacteria in them.

The amount of symbiotically fixed nitrogen from system *R. meliloti–M. sativa* on average per year for the different soil types in Bulgaria varies from 4 to 18 kg da^{-1} [RAICHEVA–KOSTADINOVA, 1986; BUTNARIU *et al.*, 2012]. The nodules are formed on thin alfalfa roots and they are situated in the surface soil layer [MASLINKOV *et al.*, 1972; BUTU *et al.*, 2014; IANCULOV *et al.*, 2005; PUTNOKY *et al.*, 2013].

Legumes nodules are usually shed following cutting and new nodules are formed when the plants renew their growth [BURTON, 1972; ANDREEA *et al.*, 2012].

Dynamics by years shows that least nodules are formed during the first year as during the second year their numbers increased, during the third year it decreased and during the fourth year–sharply declined [VASILEVA, 2004; GAITIN *et al.*, 2013].

Maslinkov and collab. [MASLINKOV *et al.*, 1972] find that the amount and activity of nodules are closely related to water, nutritious and air regime of soil.

Under prolonged drought in the summer they die. Vasileva [VASILEVA 2004] and Vasileva and Kostov [VASILEVA and KOSTOV 2006] also report for significantly decrease in the nodule number under drought.

According to other authors [VOLENEC *et al.*, 1987] the nodule amount is determined by stand density as at high density the nodule number is reduced.

Some researchers observe significant differences among alfalfa varieties in regard to total nodule biomass [SCOTTI *et al.*, 1997], nodule number [SAIDI *et al.*, 2009] and nodulation capacity [HERNÁNDEZ *et al.*, 1995].

Some authors establish correlative dependences between nodulation and: alfalfa growth [TAN and TAN, 1986; SAIDI *et al.*, 2009], weight of dry foliar mass [HERNÁNDEZ *et al.*, 1995] and root mass [SELGE and HIGUCHI, 2000].

The purpose of present experiment was to study the dynamics in nodulation of different alfalfa varieties and its correlative dependence with other parameters.

Material and methods

Eight Bulgarian alfalfa variety (Prista 2, Prista 3, Prista 4, Obnova 10, Pleven 6, Dara, Multifoliate and Dama) and one French variety (Europe) were cultivated on slightly leached chernozem in IFC–Pleven during the period 2006–2009.

The sowing was carried out during spring of 2006 using the block method, in 4 replications, at row spacing 11.5 cm and sowing rate 2.5 kg da^{-1}.

For determination of nodulation (number nodule per plant) at harvest (early flowering stage) of the spring, summer and autumn regrowth's were taken soil monoliths with dimensions 20 x 200 x 30 cm (width/length/depth) as the number of reported plants was from 50 to 431.

The obtained data were processed by the method of variance analysis with software product Statgraphics.

Results and discussion

The fourth experimental years were different in regard to meteorological conditions as secured in greatest degree with respect to rainfall was 2009 (613 mm) and in least degree–2008 (469 mm) (Figure 1).

The annual average daily temperature was in the limits from 12.2 (2006) to 13.6°C (2007).

As a whole the study period (2006–2009) is characterized with higher annual average daily temperature (12.7°C) and less sum of rainfall (541 mm) compared to the same for the previous 20–year period (12.2°C and 562 mm, respectively).

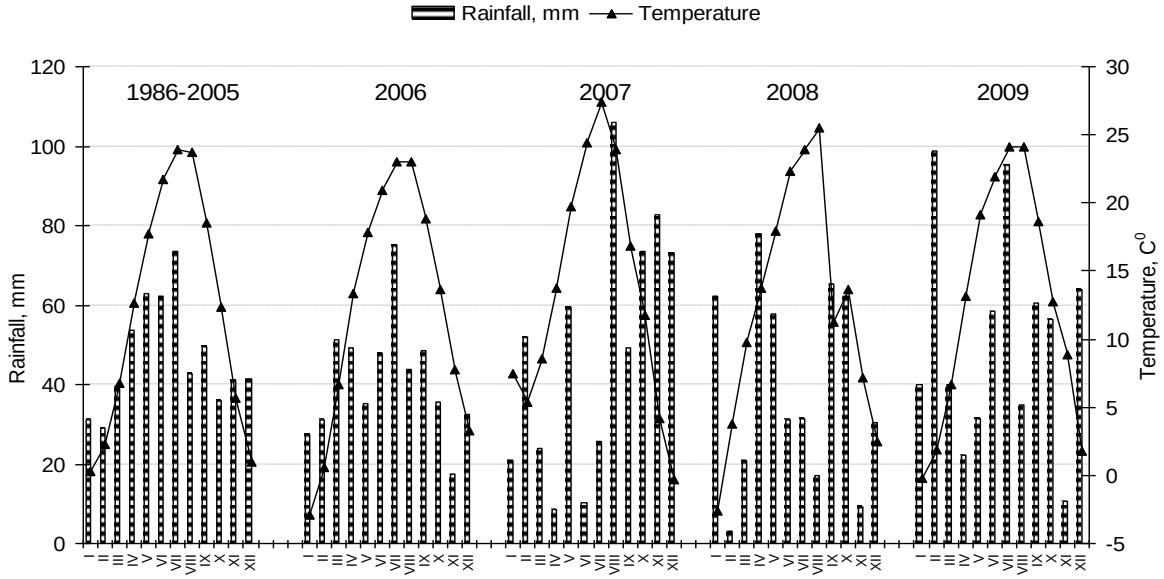

Figure 1. Meteorological characteristic

The dynamics in nodulation by years (Figure 2) showed the greatest nodule number on average per plant during the second year of alfalfa development (8.1) followed by third (6.5) and first year (6.3) (Figure 2).

Least nodules were formed in the fourth year (1.2) as the decrease

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compared to previous three years was sensitive (by 82.9 %).

Similar results are reported from other authors [JENKINS and BOTTOMLEY, 1984; BUTNARIU *et al.*, 2005].

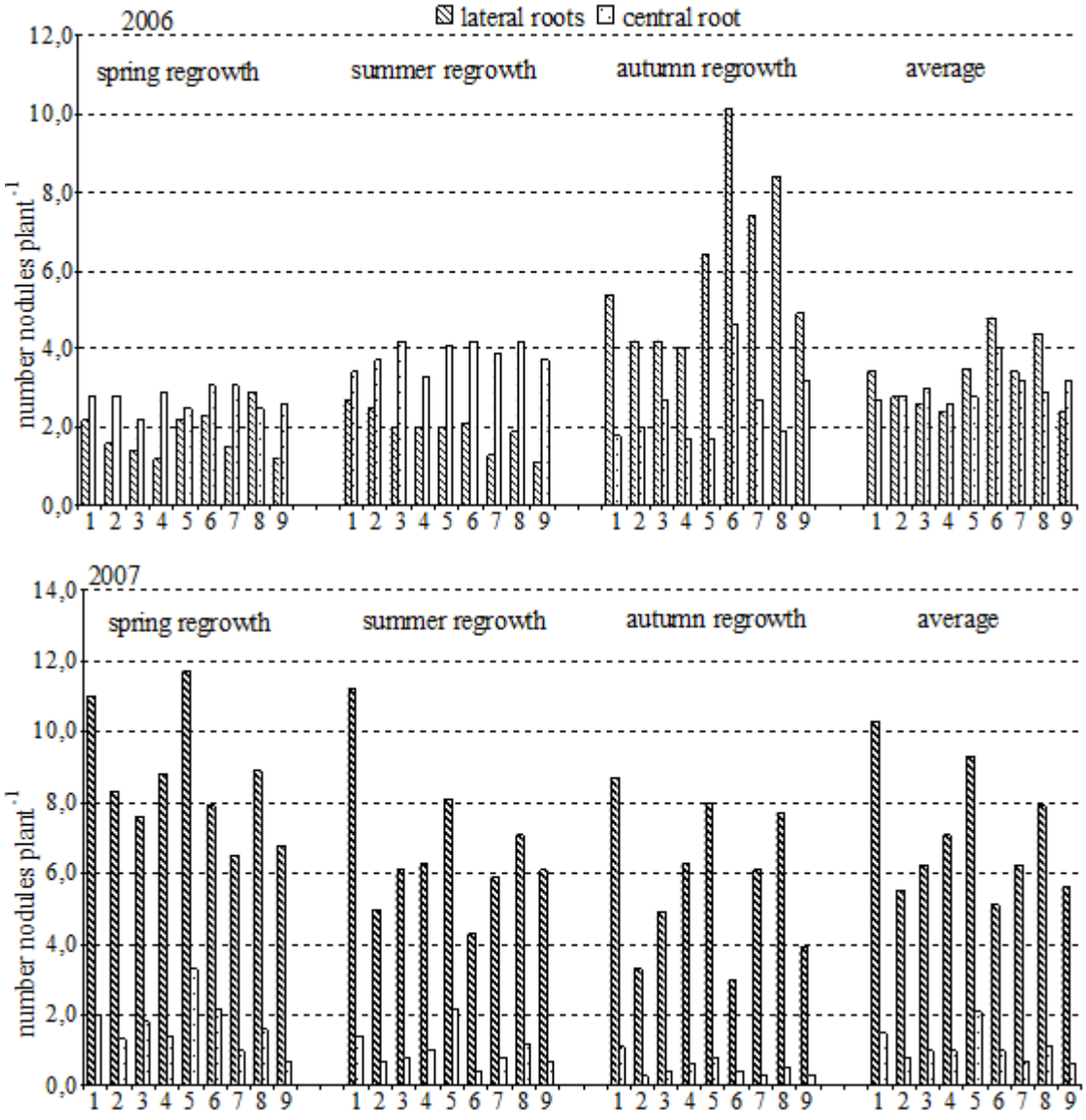
According to Vasileva [VASILEVA, 2004] less number of nodules during the first year is determined by weaker development of plants and their root system which suggested weaker potential for delivery of energetic carbon sources for the needs of nodulation and itself biological nitrogen fixation.

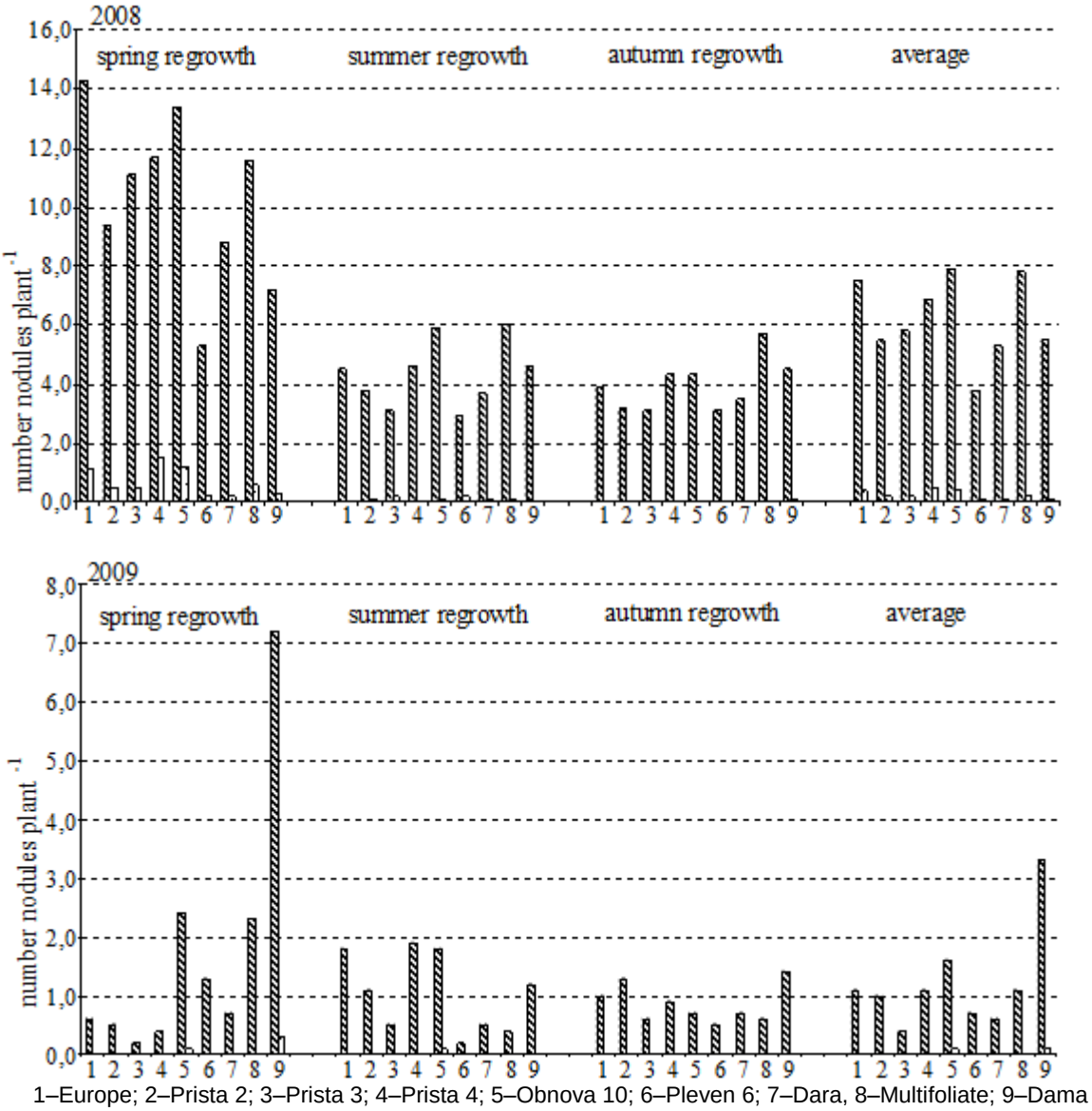
In regard to the nodule distribution on root system was found that average for the first year more than half of them were situated on lateral roots (52.4 %).

From spring regrowth (4.6 nodules per plant) to autumn regrowth (8.6 nodules per plant) was observed an increase in the nodule forming ability of alfalfa as maximum number of nodules (over average for the ninth varieties) formed Pleven 6, Multifoliate and Dara varieties and minimum–Prista 4.

During second and third year of alfalfa development were formed by 28.6 and 3.2 % more nodules compared to the first year.

The mean values in regard to the nodulation during indicated two years were highest in Europe, Obnova 10 and Multifoliate and lowest–in Pleven 6.





1–Europe; 2–Prista 2; 3–Prista 3; 4–Prista 4; 5–Obnova 10; 6–Pleven 6; 7–Dara, 8–Multifoliate; 9–Dama

Figure 2. Nodulation in alfalfa varieties

As opposed to the first year during the second, third and fourth year the intensity of nodulation was reduced from spring regrowth to summer and autumn regrowth by mean values of 42.9 and 51.9% (compared to spring regrowth) respectively.

Average for the four–year experimental period with most pronounced nodule–forming ability for the Bulgarian varieties were Obnova 10 and Multifoliate and French variety Europe.

The variation in nodulation among varieties during the first three years was mean (VC from 18 to 27 %) and during fourth year–strong (VC=73 %).

The dynamics in nodule distribution showed that the greater part from them was situated on lateral roots as in the first year their percentage was 52.4 and during the second, third and fourth year–86.4, 95.4 and 100% respectively.

In the conditions of present study it would be difficult to be pointed dependence among the rainfall amount by years, their distribution during vegetation (by regrowth's) and nodulation.

Well–pronounced was correlation (r= –0.729) between the age of alfalfa stand and nodulation as for different varieties the values were from –0.507 to –0.998 (Figure 3).

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Based on that correlative dependence the varieties could be divided in two groups:

- I group (nodulation was in strong dependence on stand age and "r" is over –0.666 – Pleven 6, Dara, Prista 2, Dama, Prista 3 and Multifoliate; and
- II group (nodulation was in mean dependence on stand age and "r" was between –0.333 and –0.666)– Europe, Obnova 10, Prista 4 (arrangement in the two groups is in descending order).

Analysis of results showed that the alfalfa nodulation correlated also with other important parameters:

- positively–with weight of root mass (r= 0.384) and number of stems per plant (r= 0.763); and
- negatively–with stand density expressed as number of plant per unit area (r= –0.648).

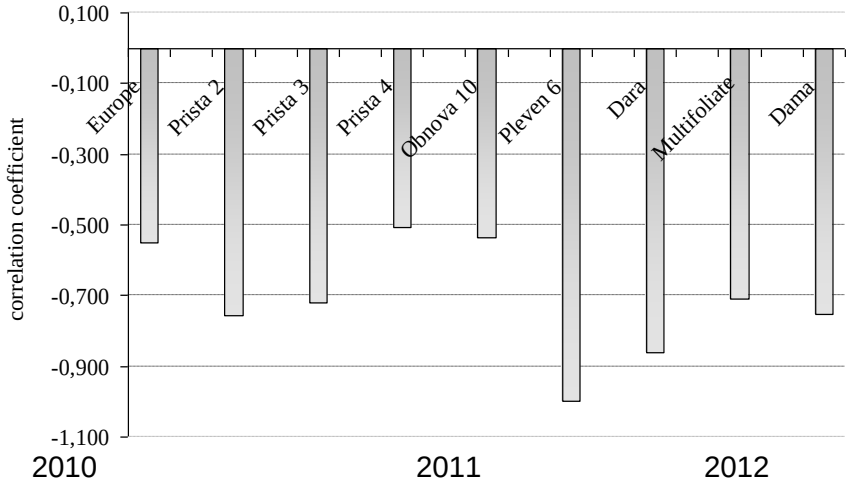

Figure 3. Correlation between nodulation and stand age in alfalfa varieties

Variance analysis determined the years of study as a factor with the strongest influence (72.5 % from the total variation) on alfalfa nodulation (Table 1).

Table 1.

Analysis of variance for alfalfa nodulation

Source of variation	Degrees of freedom	Sum of squares	Influence of factor (%)	Mean square	Significance
Total	71	654.4	–	–	–
Variants	35	620.5	94.8	17.7	*
Factor A–Years	3	474.6	72.5	158.2	*
Factor B–Varieties	2	54.0	8.3	27.0	*
AxB	24	92.0	14.1	3.8	*
Pooled error	35	30.7	–	2.1	–

*p≤5%

Considerably less but nevertheless significant was the influence of factor B (varieties)–8.3 %.

The interaction year–variety was significant for the process of nodulation and it had greater impact to factor B.

Conclusions

The dynamics in alfalfa nodulation by years showed the greatest number of nodules on average per plant was formed during the second year of development (8.1) followed by third (6.5) and first year (6.3).

Least nodules were formed in the fourth year (1.2) as the decrease to the previous three years was sensitive (by 82.9 %).

In all years (except first year) the intensity of nodulation decreased from spring regrowth to summer and autumn regrowth.

The main quantity of nodules was situated on the lateral roots as in the first year their percentage was 52.4 and during the second, third and fourth year– 86.4, 95.4 and 100 % respectively.

With most pronounced nodule–forming ability for the Bulgarian varieties were Obnova 10 and Multifoliate and the French variety Europe.

The alfalfa nodulation correlated with important parameters: age of stand (r= –0.729), stand density (r= –0.648), number of stems per plant (r= 0.763) and weight of root mass (r= 0.384).

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Received: November, 30, 2014
Article in Press: April 7, 2015
Accepted: Last modified on May 18, 2015