CONTROL OF PLANTS OF LOTUS CORNICULATUS L. ON AEROBIC AND ANAEROBIC FREE NITROGEN-FIXING BACTERIA

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ABSTRACT

The free nitrogen fixing bacteria can mobilize important soil nutrients, transforming through biological processes the unusable molecular nitrogen into an active form

and to improve soil fertility, influence many aspects of plant health and ensure their growth, showing interest for the scientific world and farmers.

But, on the other hand, this bacterial segment may be influenced by the edaphic factors and the interconnection with the plants, the growth phase, the physiological state and the root system of the plant, by the root exudates, which demonstrates the importance of the bacterial community monitoring from the area of plants influence throughout the growing periods

The aim of this study was to evaluate the influence of the age of the plants used as biofertilizer and soil moisture on the free nitrogen fixing bacterial communities (the genera *Azotobacter* and *Clostridium*) associated with the roots of the perennial plants of *Lotus corniculatus* L. There were two zones of interest, namely the area of influence of the roots of the plants (rhizosphere) but also the more distant area (edaphosphere). For the study of aerobic and anaerobic free nitrogen fixing bacteria soil samples were taken together with adjacent plants of *Lotus corniculatus* L.

The experimental variants were located in the western part of Romania, the plants being cultivated on the same soil type, but on different plots, that were in the I-IV years of culture. The influence of *Lotus corniculatus* L. plants on the free nitrogen fixing bacteria has been reported in control experimental variants. Isolation and study of this bacterial group from the 8 experimental variants was performed on a specific mineral medium, favorable for the growth of the two bacterial genera.

The results were evaluated after 5 and 10 days of incubation. Between the two assessments, there were no noticeable differences in the nitrogen fixing bacterial community, except for the stimulatory effect observed in the control vatiant and rhizosphere of the first year culture.

The plants` influence on aerobic and anaerobic free nitrogen fixing bacteria was obvious in the II and IV years of the *Lotus corniculatus* L. culture, compared

to the control variants and varies substantially depending on the age of the plant. In most analyzed soil samples, both bacterial genera, *Azotobacter* and *Clostridium* were present, confirming the known ecological relation of unilateral advantage or passive stimulation of the aerobic bacteria compared to the anaerobic clostridia. Exceptions were the samples from the cultures of the first year (rhizosphere and control), but also the rhizosphere from the culture of the year II, where only anaerobic nitrogen fixing bacteria were detected. Our results suggested that plant-soil interactions exert control over the bacteria being studied.

Keywords: *Soil, Lotus corniculatus L., Free nitrogen-fixing bacteria, Azotobacter genus, Clostridium genus*

INTRODUCTION

In nature, plants control the microorganisms` populations through root exudates and create their own rhizosphere microbiome. Besides, the studies carried out by Lei and collaborators (2019), [5] emphasized that aproximatelly 44,85% from rizosphere bacterial communities could be attributed to plant species.

Other authors considered that the crop management mode, the stage of plant development [8], the soil type and properties [2], are factors that influence the diversity and functions of the microbial community.

On the other side some authors have shown that the rhizosphere microbiome contributes to productivity, protection and improvement of host plant health, increases soil fertility and promotes plant growth [9], through the contribution of phytohormones and nutrients, in particular the biologically fixed nitrogen. Microorganisms associated with plants play an important role in global biogeochemical cycles. Of the chemical elements, nitrogen is among the key elements that ensure plant growth and productivity in aquatic and terrestrial ecosystems.

It is known that by symbiosis, established in particular between bacteria and leguminous plants, the highest amount of nitrogen is fixed. However, some research has shown, that free nitrogen-fixing bacteria can also fix significant amounts of nitrogen in different ecosystems (between 0-60 kg N/ha/year), [3].

The composition of free nitrogen-fixing micro-organisms and the activity of nitrogenase are influenced by the species, the genotype and plant rhizosphere, soil content in nutrients, soil pollution, soil moisture and temperature [4].

Among the free diazotranophe bacteria which have unique characteristics we can mention the genus *Azotobacter* and *Clostridium*, whose evolution is also of interest in this work, known as the mutual relationship between *Lotus corniculatus* L. and symbiotic nitrogen fixing bacteria.

The objectives of this research were (1) the study of the community of aerobic and anaerobic nitrogen-fixing bacteria in the rizosphere of Lotus corniculatus L plants in different crop years (I-IV), placed on the same soil type and (2) the evolution of the two nitrogen fixing bacteria genus, namely *Azotobacter as an*

aerobic and *Clostridium* as an anaerobic one, under the influence of the plant and soil moisture.

MATERIALS AND METHODS

The study was conducted within the boundaries of Arad county (46°22′N 21°48′E), located in the west Romania (fig. 1) characterized by a temperate continental climate with oceanic influences, with an annual average temperature of 10ºC, average annual precipitation s between 565-600 mm in the plain area [14] and the variability of telluric-edaphic factors [15].

Fig. 1 *Arad county - the place from which the soil samples were taken (Source 12,13: processed image)*

The soil samples were taken from the control plots (uncultivated) and the plots covered with *Lotus corniculatus* L.), in the flowering phase, years I-IV of the culture, from the depth of 0-20 cm, in the summer season (June - July). The community of aerobic and anaerobic free nitrogen fixing bacteria was studied during the same calendar year, from the rhizosphere of plants and from the soil (control variants) not influenced by the plant roots.

The processing and evaluation of this group of free soil bacteria was carried out in the laboratory of Microbiology, University of Agricultural Sciences and Veterinary Medicine of the Banat "King Michael I of Romania" from Timisoara.

Isolation of free nitrogen fixing bacteria from the control soil samples and from the rhizosphere was performed on Ashby liquid mineral medium [10]. The optimum growth temperature of the aerobic and anaerobic free nitrogen fixing bacteria was 28ºC, and the incubation time was 5-10 days. Bacterial growth was followed by reading and interpreting the results based on McCrady tables [10].

Soil samples moisture was determined by the thermo-gravimetric method, aided by a Sartorius scale MA-50 at 105ºC, as described by Bordean et al. 2011 [1].

RESULTS AND DISCUSSION

The community of free aerobic and anaerobic bacteria in the rhisosphere of *Lotus corniculatus* L. plants (I-IV crops) was evaluated in comparison with the control plots (not influenced by legumes). The tests were performed after the isolated bacteria were incubated 5, respectively 10 days on nutrient medium. The results showed: (1) an increase in the probable number of free nitrogen fixing bacteria in the root of the cultures in the years I to IV compared to control variants; (2) keeping a constant number of free nitrogen fixing bacteria in most cases included 10 incubation days, with the exception of the control and variants of the rhizosphere from the first culture year, where there was a slight increase of the bacteria, with 0.35-0.51%. In figures 2-4 the results obtained after 5 days of incubation are presented.

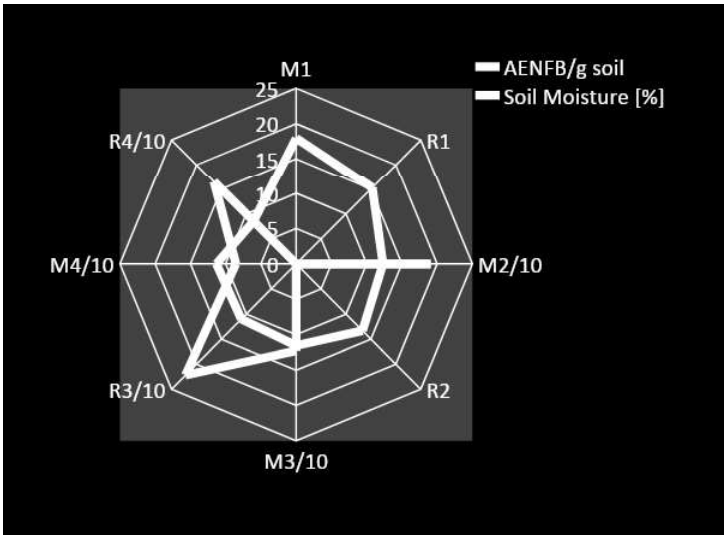


Fig. 2 *Development of Aerobic Nitrogen Fixing Bacteria from Lotus corniculatus L. culture (AENFB)*

Legend*: M1- control, the first year M2/10- control, the second year M3/10- control, the third year; M4/10- control, the fourth year; R1- rhisophere, the first year; R2- rhisophere, the second year; R3/10- rhisophere, the third year; R4/10- rhisophere, the fourth year*

R3>M2>R4>M4

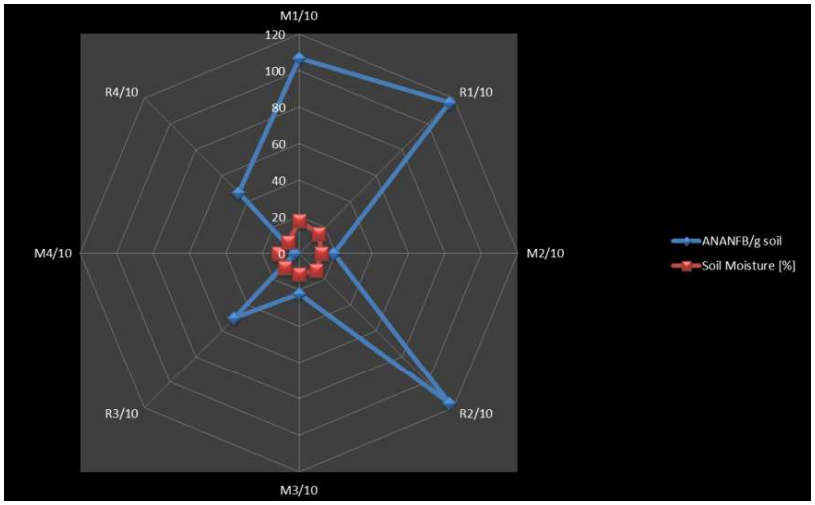
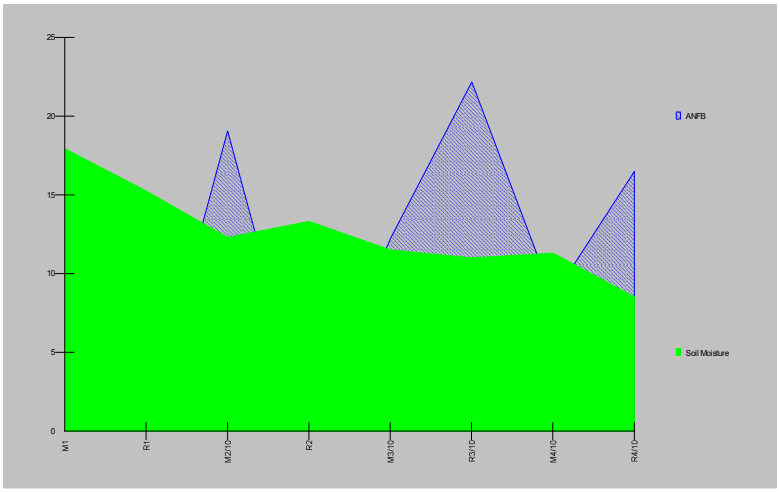


Fig. 3 *Development of Anaerobic Nitrogen Fixing Bacteria (ANANFB) from Lotus corniculatus L. culture (AENFB)*

Legend*: M1- control, the first year M2/10- control, the second year M3/10- control, the third year; M4/10- control, the fourth year; R1- rhisophere, the first year; R2- rhisophere, the second year; R3/10- rhisophere, the third year; R4/10- rhisophere, the fourth year*

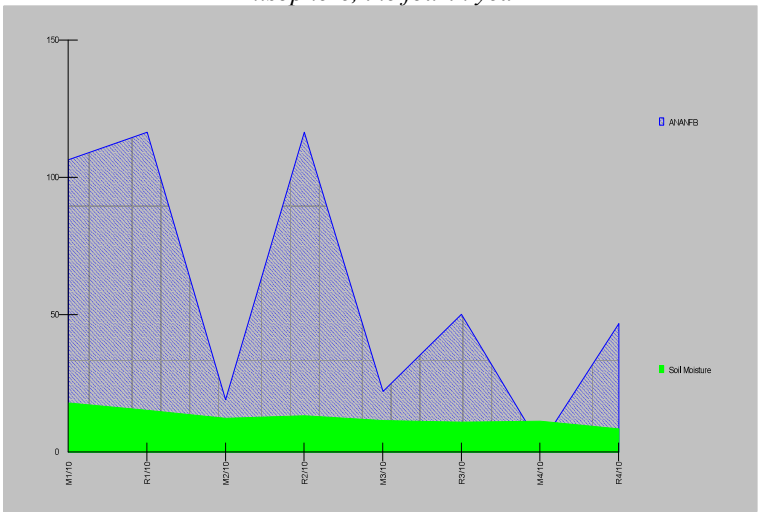
R2>R1>M1>R3>R4> M3> M2>M4

From figures 2 and 3 it was observed: (a) the increase of the number of anaerobic free nitrogen fixing bacteria compared to the aerobic ones, which were absent in variants M1, R1 and R2, (b) the decrease in the number of nitrogen fixing bacteria concomitant with the culture year, (c) and the reduction of the number of fixing bacteria from the low humidity variants, especially from the M4 variant.



a. *ANFB – Aerobic free nitrogen fixing bacteria from Lotus corniculatus L. culture*

Legend*: M1- control, the first year M2/10- control, the second year M3/10- control, the third year; M4/10- control, the fourth year; R1- rhisophere, the first year; R2- rhisophere, the second year; R3/10- rhisophere, the third year; R4/10- rhisophere, the fourth year*



b. *ANANFB – Anaerobic free nitrogen fixing bacteria from Lotus corniculatus L. culture*

Legend*: M1- control, the first year M2/10- control, the second year M3/10- control, the third year; M4/10- control, the fourth year; R1- rhisophere, the first year; R2- rhisophere, the second year; R3/10- rhisophere, the third year; R4/10- rhisophere, the fourth year*

Fig. 4 *The influence of humidity on the evolution of free nitrogen fixing bacteria*

According to the literature data the nitrogen fixing microorganisms are sensitive to the changes that occur in their living environment, which is also observed in the case of samples from the variants with lower humidity; even though they are adapted to various environmental conditions and play an important role in the biogeochemical cycle of nitrogen, they rarely dominate in terrestrial ecosystems [11].

Even if we did not report the results to other crop plants, the fact that the probably number is larger in the rhizosphere, both in the case of aerobic and anaerobic fixing bacteria, enable us to consider that there is an obvious effect of the cultivated plants on this bacterial segment. In support of these results we also add the studies of Mirza and colleagues (2014), [7] who argue that the response of diazotrophs is a direct consequence of changes in plant communities.

CONCLUSION

In agreement with our studies, the same authors pointed out that the microbial communities involved in nitrogen fixation are influenced by the rhizosphere, the plant species, their life span, but also the physico-chemical properties of the soil.

The growth coefficient of aerobic free nitrogen fixing bacteria varies and does not show a constant line depending on soil moisture, whereas anaerobic free nitrogen fixing bacteria are correlated with soil moisture value (Fig. 4). Merlo et al. (2014), [6] argue that soil moisture is one of the major abiotic factors that control the abundance of nitrogen-fixing bacteria.

From this study it can be concluded that the plant, through the root exudates, had somewhat the control over the free nitrogen fixing bacteria in the rhizosphere, in all 4 years of culture, compared to the control variant, even if the summer season, which was quite dry, also influenced these bacteria. In particular, we noticed that the anaerobic bacteria have an ascending tendency, compared to the aerobic ones, especially in the variants in which the moisture content was higher (R1, M1, R2).

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