BACTERIAL INOCULATION EFFECT UPON YIELD CAPACITY IN ALFALFA AND ORCHARD GRASS

EFECTUL INOCULĂRII BACTERIENE ASUPRA CAPACITĂȚII DE PRODUCȚIE LA LUCERNĂ ŞI GOLOMĂȚ

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***Abstract**: This work presents the bacterial inoculation effect upon the mixed alfalfa and orchard grass crop. The bacterial inoculation with strains of Rhizobium meliloti, for alfalfa, and strains of Beigerinckia and Azospirillum, for orchard grass, increases this mixture's yield capacity with 24.5%, in the first year of vegetation, and with 23% in the second year. The most important effect was noticed in the case of the species Beigerinckia, which proves higher resistance to acid soils. A synergism relationship appears between the three bacterial species, enhancing the capacity of fixing atmospheric nitrogen.*

***Rezumat**: Lucrarea prezintă efectul inoculării bacteriene asupra culturii în amestec a lucernei şi golomățului Inocularea bacteriană cu tulpini de Rhizobium meliloti, pentru lucernă, şi tulpini de Beigerinckia şi Azospirillum, pentru golomăț, creşte capacitatea de producție a amestecului cu 24,5% în primul an de vegetație, şi 23%, în anul al doilea. Efectul cel mai mare s-a constatat în cazul speciei Beigerinckia, care are o rezistență mai ridicată în condițiile solurilor cu reacție mai acidă. Între cele trei specii de bacterii studiate se creează o relație de sinergism, care potențează capacitatea de fixare a azotului atmospheric.*

Key words: *alfalfa and orchard grass mixture, bacterial inoculation, Rhizobium meliloti, Beigerinckia, Azospirillum*
***Cuvinte cheie**: amestec lucernă cu golomăț, inoculare bacteriană, Rhizobium meliloti, Beigerinckia, Azospirillum*

INTRODUCTION

Within the natural and cultivated ecosystems, nitrogen fixation with the help of some various fixing systems (free, associative, symbiotic) represents a complex biological mechanism, relied on the molecular nitrogen reduction to forms of ammoniac nitrogen, which can be used by plants in protein synthesis. The practical importance of this phenomenon may be hardly assessed, because of many factors: a great variety of nitrogen-fixing microorganisms, different responses to the influence exerted by natural and technological factors, different estimations concerning the methods of fixed nitrogen quantitative determination.

Cultivated in associations, perennial leguminous species fix important amounts of biological nitrogen, which will be directly used by the gramineae species leading to dramatic reductions of nitrogen-based fertilizer doses (Byron, 2000; Ledgard et. al. 1992; Loges, 1998; Mihăescu G. et. al, 1989).

This work intends to highlight the capacity of enhancement of the atmospheric nitrogen fixation, through the bacterial inoculation with selected strains of various fixing bacteria, in alfalfa and orchard grass, cropped in pure crop and mixture.

MATERIALS AND METHODS

Our researches have been performed during 2005-2006 at S.C.D.P. Timisoara, on a brown eumesobasic soil, gleyied, slightly acid (pH=5.6), moderately supplied with phosphorus and potassium.

The experimental device was consisted of the following experimental research variants:

1. Non-inoculated alfalfa (Mt_1)
2. Alfalfa inoculated with LC-Liv2
3. Non-inoculated orchard grass (Mt2)
4. Orchard grass with *Azospirillum* (A)
5. Orchard grass inoculated with *Beigerinckia* (B)
6. Orchard grass inoculated with mixture (A + B)
7. Non-inoculated alfalfa + non-inoculated orchard grass (Mt3)
8. Alfalfa inoc. LC-Liv2 + non-inoculated orchard grass
9. Alfalfa inoc. LC-Liv2 + inoculated orchard grass (A)
10. Alfalfa inoc. LC-Liv2 + inoculated orchard grass (B)
11. Alfalfa inoc. LC-Liv2 + inoculated orchard grass (A + B)

Settlement of variants within the experimental field was carried out according to blocks method, in 4 repetitions, and each variant was fertilized before seeding, with a dose of $N_{50}P_{50}K_{50}$. Mixture structure was consisted of alfalfa 60 % and orchard grass 40%.

The bacterial inoculation of alfalfa and orchard grass seeds was carried out with ameliorated strains of *Rhizobium meliloti* (LC-Liv2), for alfalfa, respectively strains of *Azospirillum* and *Beigerinckia*, for orchard grass, achieved at I.C.D.A. Fundulea. The bacterial strain for alfalfa was multiplied on agar medium, and the two strains for orchard grass on peat medium. For bacterial adherence to alfalfa and orchard grass seeds, we have applied a special adhesive at the same time with seed inoculation.

The results achieved were statistically processed and interpreted with the help of the variance analysis.

RESULTS AND DISCUSSIONS

Within the vegetal associations, consisted of gramineae and perennial leguminous mixtures for temporary pastures, the gramineae species benefit of a certain amount of biological nitrogen, produced as a result of the symbiotic relationship between the nitrogen-fixing bacteria belonging to the genus *Rhizobium* and the leguminous species. This quantity of biological nitrogen may be increased through seed bacterial inoculation, for leguminous and for gramineae species, as well. This process of atmospheric nitrogen fixation may occur endogenous in leguminous species, which have their own fixing system by which they enter a direct symbiotic relationship with bacteria, and exogenous, in the annual and perennial gramineae, where the nitrogen fixed occurs at the rhizospheric level.

During our researches, we have noticed that the nitrogen-fixing process with the help of the species *Rhizobium meliloti* and through the action of the free bacteria belonging to *Azospirillum* and *Beigerinckia*, occurs beginning with the first year of vegetation, in pure crop and in associations, as well. So, the results presented in Table 1 lead to the conclusion that the highest dry matter growth in pure crop was achieved in the variant with orchard grass inoculated with *Beigerinckia* (7%, respectively 0.44 t/ha D.M. compared to the control variant, non-inoculated).

Among the variant cropped as mixtures, we have remarked the variant with alfalfa inoculated with LC-Liv2 and with orchard grass inoculated with mixture of *Azospirillum* and

Beigerinckia, with a production growth higher with 1.57 t/ha D.M. (respectively 24.5%) than in the variant with non-inoculated mixture.

Table 1

Bacterial inoculation effect in pure and associated alfalfa and orchard grass crops upon dry matter yield (year I of vegetation)

Variants	t/ha	Diff. t/ha	%	Significance
Non-inoculated alfalfa (Mt_1)	5.92	-	100	
Alfalfa inoculated with LC-Liv2	6.12	0.20	103.4	
Non-inoculated orchard grass (Mt2)	6.27	-	100	
Orchard grass with *Azospirillum* (A)	6.29	0.02	100.3	
Orchard grass inoculated with *Beigerinckia* (B)	6.71	0.44	107.0	
Orchard grass inoculated with mixture (A + B)	5.95	-0.32	94.9	
Non-inoculated alfalfa + non-inoculated orchard grass (Mt3)	6.39	-	100	
Alfalfa inoc. LC-Liv2 + non-inoculated orchard grass	6.63	0.24	103.7	
Alfalfa inoc. LC-Liv2 + inoculated orchard grass (A)	7.18	0.79	112.4	
Alfalfa inoc. LC-Liv2 + inoculated orchard grass (B)	7.23	0.84	113.1	
Alfalfa inoc. LC-Liv2 + inoculated orchard grass (A + B)	7.96	1.57	124.5	

LSD 5% 0.92; LSD 1% 1.22; LSD 0.1% 1.59

The studied performed within the second year of vegetation have highlighted even more the positive influence exerted by bacterial inoculation with ameliorated strains upon yield capacity in gramineae and perennial leguminous species, seeded in pure crop and associations (Table 2). In the situation of a unilateral bacterial inoculation, the best results were achieved in the case of inoculation with *Beigerinckia*, compared to the *Rhizobium*-type bacteria, which has led to a yield growth similar with the first year of vegetation, or to the *Azospirillum*-type bacteria, which have exerted a negative action.

Table 2

Bacterial inoculation effect in pure and associated alfalfa and orchard grass crops upon dry matter yield (year II of vegetation)

Variants	t/ha	Diff. t/ha	%	Significance
Non-inoculated alfalfa (Mt_1)	10.12	-	100	-
Alfalfa inoculated with LC-Liv2	10.40	0.28	102.7	-
Non-inoculated orchard grass (Mt2)	6.77	-	100	-
Orchard grass with *Azospirillum* (A)	6.09	-0.68	89.9	-
Orchard grass inoculated with *Beigerinckia* (B)	7.19	0.42	106.2	-
Orchard grass inoculated with mixture (A + B)	7.51	0.74	110.9	-
Non-inoculated alfalfa + non-inoculated orchard grass (Mt3)	9.06	-	100	-
Alfalfa inoc. LC-Liv2 + non-inoculated orchard grass	11.37	2.31	125.4	**
Alfalfa inoc. LC-Liv2 + inoculated orchard grass (A)	12.02	2.96	132.6	***
Alfalfa inoc. LC-Liv2 + inoculated orchard grass (B)	10.91	1.85	120.4	*
Alfalfa inoc. LC-Liv2 + inoculated orchard grass (A + B)	11.15	2.09	123.0	*

LSD 5% 1.68; LSD 1% 2.23; LSD 0.1% 2.90

Interesting results concerning the effect of the bacterial inoculation were achieved for alfalfa and orchard grass mixtures, indifferently of the bacterium species used. So, the highest yield growth was achieved in the variant with alfalfa inoculated with LC-Liv2 and orchard grass inoculated with *Azospirillum*, respectively 2.96 t/ha (32.6%), statistically assured. In this situation, we may notice, between the two bacteria species (*Rhizobium meliloti* and *Azopirillum*), a benefic inter-specific relationship which enhances the capacity of fixing the atmospheric nitrogen. This relationship occurs in the case of the inoculation with *Rh. meliloti* and *Beigerinckia*, leading to a growth of 1.85 t/ha DM (20.4%), statistically assured. In the

case of seed inoculation with all three bacteria species, we have achieved a growth of 2.09 t/ha, respectively 23% higher than in the control variant, not inoculated.

If we would make a hierarchy concerning the positive influence exerted by the three nitrogen-fixing bacteria upon yield capacity in alfalfa and orchard grass, cultivated in pure crop and in mixtures, too, we may consider that the bacteria belonging to the species *Beigerinckia*, through unilateral inoculation and also in association with *Rhizobium meliloti* and *Azospirillum*, have the strongest effect. This observation may be due to the fact that *Beigerinckia*, unlike *Azospirillum* and especially *Rhizobium*, finds optimal growth conditions in acid soils, too (similar to the soil on which we have performed our researches), with a high alumina and iron content (DÖBEREINER and DAY, 1974).

CONCLUSIONS

Through the bacterial seed inoculation, we may achieve a significant increase of dry matter yield in the mixture consisted of alfalfa and orchard grass;

Among the variants studied, we have remarked the variants with alfalfa inoculated with LC-Liv2 (*Rhizobium meliloti*) and orchard grass inoculated with *Beigerinckia*, which have led in the second year of vegetation a growth of 2.96 t/ha DM (32.6%), statistically assured, compared to the non-inoculated variant;

A synergic relationship appears between the three nitrogen-fixing bacteria used in inoculation, enhancing the fixation of atmospheric nitrogen and the increase of growth capacity, in alfalfa and orchard grass association.

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