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BRADYRHYZOBIUM JAPONICUM STRAINS ACTIVITY UNDER DIFFERENT NH^+_4 AND NO_3^- LEVEL IN NUTRITION MEDIUM

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Summary

The nodulating soybean isolines are capable obtain partial or complete N-fertiliser requirements through fixation of the atmospheric nitrogen. The symbiotic assimilation is strongly inhibited by high concentration N- compounds in soil and nodules are either absent or they are inactive. The plants were planted in greenhouse on 1/2, 1 fold and 3 fold N doses in Reid-York´s and Knop´s nutrient solution. The small plants were inoculation by 7 *B.japonicum* strains. 3 fold level of N in both forms (NH^-_4 and NO_3^-) affected inhibition of nodulation. The tested strains reacted differently in nitrogenase activity, uptake amount of N, rate of growth and respiration rate in relation to level and form of N ions in solution.

***Key words*:** soybean; *Bradyrhizobium* strains activity; NH^-_4 and NO_3- level in nutrition medium, N_2 symbiotic fixation.

Introduction

Bradyrhizobium japonicum as a specific microsymbiont to soybean initially absent in Slovak soils, but usually survives well after introduction. Our recent results (Kubová et al., 1999), also Obaton (1996) show good survive and next high activity of B. japonicum in a field even 6 years after recent soybean planted.

The form of nutrition that plants prefer depends on environmental conditions and on the age of plants. Energy expenses to NH^+_4 assimilation are lower than for other nitrogen forms, and represents more favourable source of nitrogen. However, it was found out that only nitrogenase activity has direct positive effect on the content soluble proteins and grain yield.

Nitrate reductase (NR, E.C.: 1.6.6.4) and nitrogenase (NG, E.C.: 1.18.2.1) co-exist in legume root nodules. Their activity decreases under conditions of high NH^+_4 and NO^-_3 concentration in nutrient medium (Caba et all. 1994).

B. japonicum strains differ in many parameters- compatibility to soybean cultivars, nodulation, symbiosis efficiency, sensitivity, or resistance to environmental factors.

The goal of this research was to find out the nodulation and metabolic activity of 6 *Bradyrhizobium japonicum* strains and their mix - Rizobin, their relationship to physiological processes of soybean plants under different level of N- nutrition and its form, to find out strains sensitivity and resistant to high level of N-nutrition.

Material and methods

Bradyrhizobium japonicum strains multiplied on peat substrate were obtained from collection of RIPP Praha - Ruzyně. Next strains were applied:D 216, D 536, D 538, D 574, D 575, D 597 and Rizobin.

The soybean seeds cv. *Maple Arrow* after disinfection by concentrated H_2O_2 were put on specific germinator. Small plants were transferred to plastic pockets with shutter that enabled to fix plant shoots. Before putting the plants into pockets their root system was dipped into responsible rhizobial preparation.

The plants were planted in greenhouse on half, I fold and 3 fold N doses in Reid-York´s (NH^+_4 - ions) and Knop´s nutrient solution (NO^-_3 - ions), plus control with distilled water. Nutrient solution and water were added according to the need of plants. The measurement of physiological and metabolic processes was done in V 3 stage according Fehr and Cavins scal.

The activity of nitrogenase (NG) was determined by the acetylene reduction assay on gas chromatograph Chrom . The growth characteristic of plants as a length of shoot and root, number of leaves, dry weight, protein content is result of interactive work of all factors and symbiosis efficiency. The respiration rates of leaves and roots were estimated by Warburg´s manometric method and calculated in mm^3 $O_2.g^{-1}$ of dry matter.min^{-1}.

Results

The first nodules on root system appeared 35 days after inoculation. Amount of N taken up by plant during experiment is following: 15.6 mg N in the 1/2 level of NH_4^+ ; 15.1mg N in the 1/2 level of NO_3^- ; 30.0 mg N in the 1x level of NH_4^+ ; 21.8 mg N in the 1x level of NO_3^- ;61.06mg N in the 3x level of NH_4^+ ; 70.7 mg N in the 3x level of NO_3^-.

The differences are caused by need and ability of plants to uptake, transport and utilise N supplied in nutrient solution. High level of NH_4^+ caused consuming away of shoots. Influence of 3x level of NO_3^- was not as much drastic. We should assume that soybean is better adapted to higher level of NO_3^- in environment.

Nodulation is strongly influenced by N concentration in nutrition medium. 3 fold level of N in both forms ($NH_4^+$and NO_3^-) affected absolutely inhibition of nodulation (Fig.1). Half level of N in cation form stimulated nodulation. Very significantly

reacts to it *B. japonicum* strains D_{216}, D_{597} and Rizobin. Even at anion N form were bacteria of Rizobin very virulent up to 1 x level of N. NO_3^- form in higher concentration is well accepted by D_{538} strain. Generally should be stated that for good nodulation and nitrogenase activity is more suitable 1/2 level of N in cation form (Fig 2). The tested strains reacted differently. At NH_4^+ form was the highest nitrogenase activity with strain D_{574} and Rizobin and in NO_3^- form was high activity even at 1 x level with strains D_{538}, D_{216} and Rizobin.
The respiration rate is not in realation to high activity of nitrogenase, growth and nodulation (Fig. 3, 4). The respiration rate was increased for roots and leaves at high N concentration. (3x level N H_4^+). It refer to metabolism disorders and destruction of organism structures.

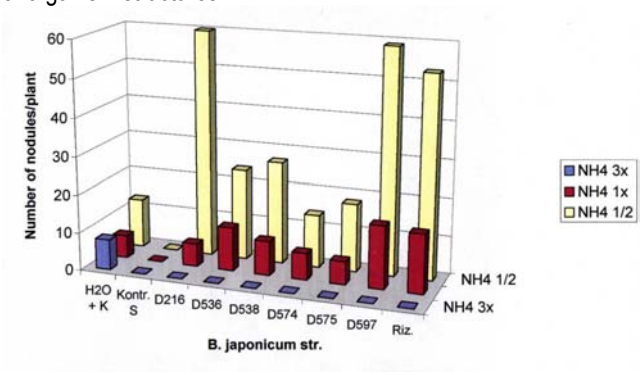

Figure 1: Number of nodules – ½, 1, 3x dose NH_4+

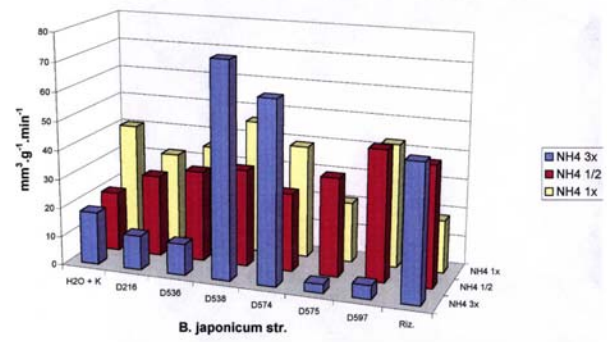

Figure 2: Nitrogenase activity – ½, 1, 3x doses NH_4+

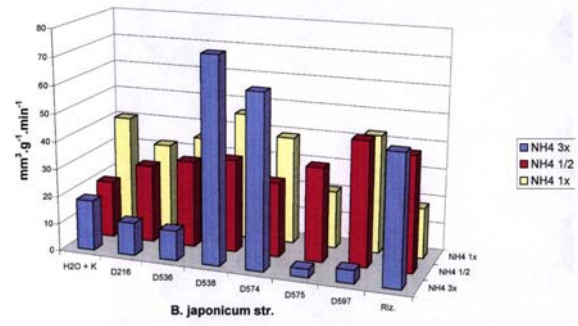

Figure 3:Respiration rate of shoots – ½, 1, 3x doses NH_4+

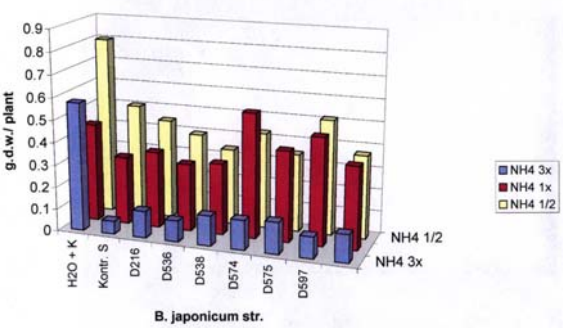

Figure 4: Dry weight of root – ½, 1, 3x NH_4+

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SOIL MICROBIAL ACTIVITY UNDER DIFFERENT MANAGEMENT

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Summary

The effects of integrated (IS) and ecological (ES) management of soil on the biomass of microorganisms (C_{mic}) and dehydrogenase activity (DHA) were investigated in the period 1999-2000. The soils used were collected from a stationary experiment established in 1990 on gley brown soil at the Experimental Station of the Slovak Agricultural University, Nitra. For each field with a different structure of crops two fertilization treatments were used : (a) no fertilization and (b) use of manure for silage maize and, within IS, also mineral fertilizers for balancing. A higher amount of microbial biomass (C_{mic}) in terms of absolute values was noted for ES but without statistical significance. Cultivated crops and the timing of soil sampling were found to have the greatest effect on the parameters observed in individual experimental years and within the two systems of soil management.

Key words: soil microbial biomass, enzymatic activity of soil, dehydrogenase, integrated management of soil, ecological management of soil

Introduction

In the recent years many works have been published in which alternative and conventional systems are compared and evaluated from different aspects (Beyer et al., 1993; Kandeler et al., 1999). The most often used parameters which are used to assess the soil biological activity is an amount of microbial biomass (Šatrůčková, 1993), which is usually supplemented with determination of the enzyme activity (Šíša, 1993; Beyer et al., 1993). A choice of sensitive indicators of the soil quality reflecting the effects of soil management should also help those who cultivate soil and take an active part in sustainability of agro-ecosystems.

Material and methods

Within the stationary experiment established on gley brown soil, the effects of two systems of soil management, namely the integrated system (IS) and the ecological system (ES), on soil biological activity were observed in the above mentioned type of soil during the growing period of 1999 - 2000. The fields chosen from the experiment to study soil biological activity are given in Table 1. For each of these fields two treatments of fertilizing were used: (a) no fertlization and (b) organic fertilization using manure for silage maize and within IS, it was also supplemented with mineral fertilizers for the purposes of balancing. Soil samples were collected 5 - 6 times in a depth of 0 - 0.2 m during the vegetation period. After being passed through a 2 mm sieve, they were analysed for:

- basic soil characteristics: oxidizable organic carbon (C_{ox}), total nitrogen (N_t), pH active and exchangeable;
- biomass carbon of soil microorganisms (C_{mic}) (Vance et al., 1987);
- dehydrogenase activity (DHA) (Casida et al., 1964);

For the statistical evaluation of results, the χ^2 test of good conformity was used, then analysis of variance was used for comparing means of the basic set and Scheffe test for testing the differences in means (Stehlíková, work being at the printers).

Table 1 Crop rotation in chosen fields of integrated and ecological systems

Year	Integrated system			Ecological system		
	Field I	Field V	Field VII	Field II	Field V	Field VII
1999	bean + alfalfa	spring barley	winter wheat	bean + alfalfa	pea	winter wheat
2000	Alfalfa	winter wheat	silage maize	Alfalfa	silage maize	pea