Impact of nitrogen fertilization on nodulation and symbiotic efficiency of indigenous *Bradyrhizobium japonicum* strains

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Abstract

Nitrogen fixation is a beneficial microbial process that greatly contribute to sustainable agricultural production and environment protection. Soil bacteria, collectively named rhizobia, are characterized by their unique ability to induce the formation of root nodules in which they convert molecular nitrogen into a usable form for plants. Soybean plants require a large amount of nitrogen for their development and achievement of high seed yields. The use of high quality rhizobial inoculants strives to optimize nitrogen nutrition of soybean with minimal environmental impacts. Selection of the most suitable *Bradyrhizobium japonicum* strains is of great importance for successful soybean inoculation as well as optimal nitrogen fertilization. The aim of the present study was to determine the impact of fertilization with different rates of mineral nitrogen on nodulation and symbiotic efficiency of indigenous *B. japonicum* strains. In the vegetation pot experiment two indigenous and one reference *B. japonicum* strain as well as different rates of mineral nitrogen were tested. Application of increased rates of mineral nitrogen reduced number of nodules and nodule dry weight. The highest nitrogen content was determined in plants grown without any mineral nitrogen fertilization but inoculated with indigenous *B. japonicum* strains.

Keywords: symbiotic nitrogen fixation, *Bradyrhizobium japonicum*, indigenous rhizobia, nitrogen fertilization

1. Introduction

An essential element of agricultural sustainability is the effective management of nitrogen in the environment. This usually involves at least some use of biologically fixed nitrogen because nitrogen from this source is used directly by the plant and therefore is less susceptible to volatilization, denitrification and leaching. Nitrogen fixation is a process which enables the conversion of molecular nitrogen to ammonia thus providing the usable form of nitrogen for plant nutrition. The largest amounts of biologically fixed nitrogen derive from symbiotic relationships between legumes and diazotrophs called rhizobia. However, some soils are free of indigenous rhizobia or existing populations are unable to induce efficient nodulation and nitrogen fixation. Although indigenous rhizobia may have some advantages over commercial strains (e.g. competiveness and better adaptation to unfavorable soil conditions) their symbiotic capacity is mostly unknown. Thus, in most cases there is a strong need for application of rhizobial inoculants, particularly in the production of economically important legumes such as soybean. The efficiency of nitrogen fixation is dependent on many factors, including environmental conditions and agricultural practices as well as compatibility between soybean cultivar and rhizobial strain and symbiotic properties of rhizobial strain. The application of mineral nitrogen fertilizers can reduce the beneficial effect of inoculation because plants prefer assimilation of easily available nitrogen from the fertilizers over nitrogen fixation. Since rhizobial strains considerably differ in important symbiotic characteristics, selection of the most suitable *Bradyrhizobium japonicum* strains is of great importance for successful soybean inoculation. The objective of this study was to evaluate the impact of nitrogen fertilization and inoculation with different rhizobial strains on nodulation and efficiency of nitrogen fixation.

2. Materials and Methods

Two-factorial vegetation experiment (4 x 4) was set up in growth chamber under controlled conditions (16 h light at 26° C and 8 h darkness at 22° C; relative humidity 65%). Surface sterilized soybean seeds (cv. Gabriela) were sown in plastic pots containing sterile mixture (1:1) of sand and vermiculite. The experiment was conducted in a randomized complete block design in three replicates with a two factorial arrangement of treatments. Factors of the experiment were as follows: (i) different rates of mineral nitrogen (0,05% KNO3 - N1; 0,025% KNO3 - N2; 0,10% KNO3 -N3; and control, without nitrogen - N0); (ii) inoculation with different *B. japonicum* strains (reference strain - S344; indigenous strains isolated from different regions of Croatia - S8, S11; and uninoculated control -S0). Plants were watered with sterile nutrient solution containing different nitrogen concentrations. Soybeans were inoculated by adding 2 ml of cell suspension (10^8 CFU/ml) per seed obtained after 7 days of incubation at 28° C.

In full blossom phase, plants were collected and the nodules were separated from the roots. Nodule number and nodule dry weight per plant were determined as well

as aerial biomass dry weight (5 plants) and total N content in aerial parts of soybean. Nodules and aerial biomass were dried in oven at 80°C to a constant weight to determine dry weight. Total N content in aerial parts was determined by Kjeldahl method. For all data obtained, appropriate analysis of variance was performed with the statistical package SAS 9.4 for Windows.

3. Results

The results of this study indicate that soybean nodulation is significantly influenced by application of mineral nitrogen. The highest values for nodule number as well as for nodule dry weight were recorded at treatments without any addition of nitrogen. The differences between N1 and N2 treatments were not statistically significant while the weakest nodulation was determined with application of the highest rates of nitrogen. Although the highest aerial biomass was determined in N1 treatment, significant differences between different nitrogen rates were not determined. Significantly reduced aerial biomass was recorded only in treatments without any nitrogen addition. Application of the highest nitrogen rate significantly increased total nitrogen content in plants compared to other nitrogen treatments.

Inoculation significantly increased soybean nodulation and nitrogen content in plants. Beneficial response to inoculation was recorded for all parameters with the exception of aerial biomass dry weight. Significant differences between rhizobial strains were not determined regarding the nodule number and nodule dry weight. However, the application of indigenous *B. japonicum* strains resulted in significantly higher nitrogen content in plants in comparison not only to uninoculated (control) treatment but also in comparison to reference *B. japonicu*m strain. Besides, interaction between mineral nitrogen application and inoculation with different strains was determined for nodulation and nitrogen content in plants. The best results were recorded in treatments where indigenous strains (S8 and S11) were used for inoculation and where the nitrogen was not added at all (data not shown).

Table 1. Mean values for nodule number, nodule dry weight, aerial biomass dry weight, nitrogen content in aerial parts and significance of their differences

	NN	NDW (g)	ABDW (g)	NC (%)
N_0	14,394 a	0,029 a	0,635 b	0,563 b
N1	8,050 b	0,010 bc	0,878 a	0,581 b
N2	9,627 b	0,015 b	0,689 a	0,561 b
N3	4,353 c	0,003 c	0,719 a	0,608 a
S_0	0,766 b	0,003 b	0, 682	0,522 c
S344	11,350 a	0,015 a	0,766	0,570 b
S8	12,458 a	0,020 a	0,720	0,622 a
S11	11,850 a	0,019 a	0,754	0,600 a

[§]- factor level means accompanied by various letters are significantly different with an error of p<0.05

Legend: S- strain; NN- nodule number; NDW-nodule dry weight; ABDW-aerial biomass dry weight; NC- nitrogen content in aerial parts

These results clearly confirmed the inhibitory effect of mineral nitrogen application on nodulation and efficiency of symbiotic nitrogen fixation. Besides, the results emphasized the importance of rhizobial strain selection for successful soybean inoculation

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