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Short communication

Multi-Strain Versus Single-Strain Inoculation: Effect on Strain Effectiveness and Competition for Soybean Nodulation

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Abstract

The objective of the study was to investigate the effectiveness of symbiotic associations of soybean variety Kolubara with three different *Bradyrhizobium japonicum* strains (2/1, D-122, 2b), as well as the competitive ability of the strains in the nodulation process. The experiment was conducted in the field. Strain 2/1 was the best competitor under field conditions – 64% nodule occupancy. This amount was the highest in the case of the multi-strain inoculations in the field trials.

Keywords: *Bradyrhizobium japonicum*, soybean, competition, effectiveness

1. Introduction

Leguminous plants have the ability to provide a great part of their requirement for nitrogen (N) via symbiotic nitrogen fixation, so long as their root nodules are formed by effective strains of nodule bacteria. Efficient symbiosis can be achieved by inoculating legume seeds with appropriate

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effective strains. Two inoculum types are currently produced in the world – those with a single-strain and those with a group of strains. In Australia inoculants contain one strain only (Roughley and Pulsford, 1982), whereas in the USA (Burton and Martinez, 1980) and Yugoslavia (Milic and Mrkovacki, 1994) inoculants used contain a mixture of highly efficient strains of *B. japonicum*.

Somasegaran and Bohlool (1990) reported that systematic studies and comparisons dealing with the effect of single-strain and multi-strain inoculations as well as those concerned with the identification of strains in nodules are few. This restricts information and limits generalizations on symbiotic associations between soybean and *B. japonicum*. In our country the competitive ability of *Rhizobium meliloti* strains in double and triple inocula was defined by Vojinovic et al. (1989a, b).

Experiments under field conditions were conducted in order to determine the efficiency of individual strains, their different combinations and mixtures, as well as to evaluate the competitive ability of *B. japonicum* strains in symbiotic association with soybean.

2. Material and Methods

Bradyrhizobium japonicum cultures

Three wild-type *B. japonicum* strains (2b, 2/1, and D-122) from the collection of the Institute of Field and Vegetable Crops in Novi Sad (NSCNFM) were used in the trial. Strains 2b and 2/1 had been isolated from the soil in the Region of Backa, while strain D-122 had been obtained from the USDA *Rhizobium* collection in Beltsville MD (USA). The selection of these strains was conditioned by their resistance to different antibiotics (Mrkovacki, 1990), as well as by their belonging to different serological groups (Mrkovacki, 1992).

Inoculum preparation and inoculation

Individual strains were cultured for 96 hours separately at 28°C in a Demolon broth containing: K_2HPO_4 1.0 g; K_2SO_4 0.25 g; KH_2PO_4 0.5 g; $CaCl_2$ 0.1 g; NaCl 0.1 g; $FeSO_4$ 0.01 g; $MnSO_4$ 0.01 g; Na-boratum 0,0001 g; Na Molybdat 0,0001 g; yeast extract 2g; distilled water 1 l; 2 ml 3% alkaline solution bromthymolblue; pH 7.2. For field experimentation each culture was injected aseptically into gamma-irradiated peat to prepare pure peat cultures. Final concentration of strains in peat inoculants were 10^8 cells per seed. Experiments consisted of three single-strain inoculations, three two-strain combinations (2/1

+ 2b; 2/1 + D-122; 2b + D-122), and a multi-strain inoculation – a mixture of all three strains used as a single-inoculant. Two-strain combinations and three-strain combinations contained the identical number of cells per 1 ml (the proportion of 1:1:1 (V:V:V)). Soybean seeds of the variety Kolubara (bred at the Institute of Field and Vegetable Crops in Novi Sad) were inoculated at sowing. Uninoculated plants were used as the control treatment. At harvesting, at the stage of flowering (62 days), the plants were cut at the soil surface, dried (at 65°C for 48 h), weighed, ground, and total nitrogen was determined by Kjeldahl. The roots were washed free of soil, after which nodules from selected plants were picked for the purposes of fresh weight determination and strain identification.

Field experimental treatments, block design and harvest

This experiment consisted of two main treatments, an inoculated treatment and the uninoculated control. It was conducted in the chernozem soil at Rimski Sancevi (N 0.16%, P 12.8 mg, K 19.59 mg/100 and organic matter 2.41%), where soybean had not been cultivated for 20 years. A randomized block design was used. Each treatment was set up in triplicate.

Nodule identification

The nodule occupancy by the inoculant strains was determined by resistance to antibiotics. Twenty nodules were typed from each replication of multi-strain inoculation treatment to determine inter-strain competition. Ten nodules per replication were typed for single-strain inoculations. The nodule identifications were converted to percentages.

Statistical analysis

Shoot and nodule dry weights and total N were analysed for differences by Duncan's Multiple Range analysis. Uninoculated controls were not included in the analysis.

3. Results

The response of plants inoculated with single-strains under field conditions did not vary significantly, except in the case of the number of nodules. All three strains had similar effectiveness (nodule mass, total N in the above-ground plant parts, and the amount of N fixed) (Table 1). In the study of the double

inoculants, the combinations 2/1 + 2b and 2/1 + D-122 proved to be more effective than the combination 2b + D-122. The greatest amount of N fixed was in the case of multi-strain inoculation (2b + 2/1 + D-122). The order of the strains' competitiveness (2/1, D-122, 2b) was also confirmed by their percentage nodule occupancy in the double inoculants (strain 2/1 66.6% and 79.16%, strain D-122 33.3% and 71.4%, strain 2b 20.83% and 28.6%) (Table 2). Average of % nodule occupancy showed that the strain 2b is significantly different from the two other strains. The most competitive was strain 2/1 in the equal-strain inoculation treatment which formed 64.28% of the nodules per plant, followed by D-122 (21.43%) and 2b (14.28%) (Table 3). The percentage of nodules occupied by a particular strain indicates the extent of its participation in N fixation in the host plant. Thus, the largest contribution to the effectiveness of the multi-strain inoculant was probably that of strain 2/1, since it had the highest percentage nodule occupancy.

4. Discussion

Little is known about the mechanisms of competition for nodule occupancy between various strains of nodule bacteria. Two basic interdependent mechanisms can be formulated. One is direct interaction between two bacteria that includes both passive parameters, such as adaptability to soil conditions and resistance to stress, and such active factors as inter- and intra-specific competition of bacteria for soil nutrients. The other mechanism is individual interaction between the plant and the microorganism, which has been described in the literature more often. Strain differences in competitiveness with a single plant cultivar have been well documented (Josephson and Pepper, 1984; Shoushtari and Pepper, 1985; Pepper et al., 1989).

Clayet-Marel et al. (1995) determined the competitiveness of two *B. japonicum* strains under controlled conditions. A strain introduced into the soil as an overpopulation remained clearly dominant for the nodule occupancy.

A strategy being considered to overcome limited benefits from nitrogen fixation for soybean is to use varieties that are restricted to nodulation with soil strains, but nodulate normally with inoculant bradyrhizobia (Ferrey et al., 1994). The case of nodulation restriction with some strains in particular lines of hosts within an inoculation group has been termed genotype-specific nodulation (GSN). Sadowsky et al. (1995) have shown that certain genotypes restrict nodulation by *B. japonicum* both before and after the formation of nodule primordia. Onishchuk and Simarov (1995) suggest the possibility of selecting effective strains of nodule bacteria with improved competitive traits for nodulation.

Table 1. Effect of inoculation and inoculant composition on the effectiveness of three strains of *B. japonicum* on soybean

Inoculation	Shoot dry[a] WT (g)	Number[a] of nodules	Nodule fresh[a] WTN (mg)	Shoot N[a] (mg)	N fixed[a] (mg)
2b	3.26	27.60 B	164.3	131.0	54.38 AB
2/1	3.34	32.20 A	209.3	133.5	57.20 AB
D-122	3.45	31.33 AB	193.0	131.5	56.42 AB
2b+D-122	3.07	29.80 AB	154.7	118.2	41.10 B
2/1+D-122	3.43	32.47 A	207.3	131.7	56.99 AB
2b+2/1	3.46	28.20 AB	172.3	136.1	60.58 AB
2b+2/1+D-122	3.67	30.57 AB	177.3	151.31	75.18 A
LSD (0.05)[b]	0.9001 NS	3.938	70.87 NS	32.21 NS	26.91
Uninoculated	2.78	0.4	0	85.41	0

[a]Values are given per plant. Plants were 62 days old at the time of harvesting. [b]LSD least significant difference. NS = Not significant.

Table 2. Competition for nodule occupancy between *B. japonicum* strains

Inoculant strain	% Nodule occupancy by		
	2/1	2b	D-122
2/1+2b	79.16	20.83	–
2b+D-122	–	28.60	71.40
2/1+D-122	66.60	–	33.30
Average	72.88 A	24.71 B	52.35 A

Table 3. Competition for nodule occupancy by strains of *B. japonicum* against each other in three strain inoculation

Inoculant strain	% Nodule occupancy by multi-strain[a]
2b	14.28 C
2/1	64.28 A
D-122	21.43 B

[a]Equal number of all strains.

Diatloff and Brokwell (1976) showed that the interaction of the soybean variety Hardee with *Bradyrhizobium* restricts nodulation by certain serological groups of *B. japonicum* (Cregan and Keyser, 1986; Devine, 1985). Such restricted nodulation, according to Sadowsky et al. (1991), is caused by the absence of a single bacterial gene, nol A.

In our study the highest percentage nodule occupancy was that of *B. japonicum* strain 2/1, which belongs to a different serological group than strains 2b and D-122. In order for us to be able to determine if it is this strain that the variety Kolubara selects, further studies must include different soybean genotypes as well.

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