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Assessment of different inoculants of *Bradyrhizobium japonicum* on nodulation, potential N_2 fixation and yield performance of soybean (*Glycine max* L.)

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1 SUMMARY

To identify the most efficient bradyrhizobium strain for promoting soybean performance, a study was conducted in a growth chamber to assess five different inoculants and N fertilization. Soybean (*Glycine max* cv. Jutro) was subjected to inoculation with five bradyrhizobium strains *viz* DSM 1755, DSM 30131, DSM 1982, the multistrain DSM-MIX, a commercial peat inoculant, and to fertilization at 50 mg N kg^{-1} soil with $Ca(N0_3)_2$, in a completely randomized design with four replicates. At 81 days after sowing, soybean was harvested and assessed for its yield, and N_2 fixation determined by N-difference method using wheat as reference crop. Dry matter, N and grain yield, nodulation and N_2 fixation in N-fertilized soybean were lowest compared to any bradyrhizobium inoculations although these latter differed. The commercial peat inoculant performed significantly better than N fertilization with 20% and 260% increase in the rate and amount of N_2 fixed respectively. Bradyrhizobium strains DSM 1755, DSM-MIX, and DSM 30131 are preferable to the commercial inoculant. Fertilization with 50 mg N kg^{-1} soil was deleterious to soybean, while combining 10 mg N kg^{-1} soil as N starter and bradyrhizobium strain inoculation significantly improved its yields, nodulation et N_2 fixation. This practice deserves recommendation for soybean growing.

2 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill as a tropical grain legume has gained great prominence in the farming systems of many nations. In addition to being economically important as a highly nutritious food for humans and feed for animals, as forage crop and green manure, soybean plays also a key role in sustaining soil fertility in agriculture through its important potential of N_2 fixation (Amarger, 2001). Its expansion over decades into non-traditional soybean growing regions, including areas of short growing seasons such as Western Europe has been obvious. With the worldwide emphasis on sustainable agricultural systems, and the need to promote plant feedstuff for livestock given the worry raised a decade ago by the spread of the mad cow disease in Europe, the growing of soybean would likely be on upward surge.

However the success of growing soybean depends greatly on the existence of microsymbionts in the soil. Hence, inoculation with effective and efficient *Bradyrhizobium japonicum* strains to enhance effective nodulation, biological N_2 fixation, and grain yield of soybean has become a compulsory standard agricultural practice all over the world (Brockwell and Bottomley, 1995), except in Japan where most soils naturally harbor native but efficient bradyrhizobium strains

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(Minamisawa *et al.*, 1999; Amarger, 2001). In symbiotic association with *Bradyrhizobium japonicum*, this pulse can fix up to 100 - 200 kg ha^{-1} $year^{-1}$ of atmospheric N_2 (Lynch and Smith, 1993).

The problem of inoculated soybean crops in most areas, however, is the occurrence in their soils of highly competitive indigenous populations of bradyrhizobium strains which, in many cases, are less efficient N_2 fixers than the inoculated ones (Weaver and Frederick, 1974; Minamisawa *et al.*, 1992; Okereke *et al.*, 2001). Thus, on new sites without soybean history, this legume does not thrive well. In Western Europe, in particular, rhizobium strains specific to soybean are often absent or present in low numbers (Madrzak *et al.*, 1995). Beyond the specificity of its bradyrhizobium strains, failure of inoculation is due to many factors of which the poor quality of inoculants prevails (van Kessel and Hartley, 2000). The quality of inoculants depends on both the number of rhizobia they contain and their effectiveness in fixing nitrogen. Although high quality commercial inoculants are produced in powder, granular, or liquid forms in many countries, their efficiency remains highly variable (Catroux *et al.*, 2001; Amarger, 2001). Thus, following an earlier study that was conducted in Giessen, Germany (data unpublished), soybean performed quite poorly both in growth, nodulation and biological N_2 fixation. Two lines of reasoning towards this inoculation failure were set forth as (1) the commercial peat inoculant used earlier might have been of low quality, and (2) the prevailing edapho-climatic conditions during the study period were not conducive for soybean. Accordingly, Haque and Jutzi (1984) stated that whenever a legume seems to be performing below its potential, the effectiveness of the rhizobia, whether indigenous or in commercial inoculum, should be questioned.

Hence, this study was initiated under controlled conditions with the objectives (1) to select the most efficient bradyrhizobium strain from different inoculants and (2) to compare it with N fertilization towards sustaining soybean growth, nodulation and N_2 fixation.

3 MATERIALS AND METHODS

The experiment was carried out at the experimental station of the Institute of Plant Nutrition of the Justus Liebig University, Giessen, Germany, in a growth chamber set at 24-26 °C, an average of 3000 lux through sodium lamps and a 12-h photoperiod regime. Plants were grown on a N-poor subsoil from Kleinlinden, soil of which the main physico-chemical characteristics were 32.3% sand, 45.0% silt and 22.7% clay; pH ($CaCl_2$): 6.3; 6.5 and 47.4 mg kg^{-1} P and K (in calcium acetate-calcium lactate-acetic-acid solution) respectively; 0.279% total C; 0.029% N; and 11.8 cmol kg^{-1} of CEC. Prior to sowing, the soil was sieved to discard coarse components and amended with a basal dressing consisting of P, K, Ca, Mg and a combination of micronutrients at the rates shown in Table 1, and then loaded in pots. The soil used had no previous record of soybean growing.

Table 1: Amounts and sources of nutrients applied in air-dried soil as basal dressing in the experiment

Element	Rate (mg kg^{-1} soil)	Source	Amount applied(mg kg^{-1} soil)
K & P	750 & 298	K_2HPO_4 (1)	1674.85
Mg	60	$MgSO_4.7H_2O$	616.65
Ca	125	$CaCl_2.2H_2O$ (2)	195.62
B	0.5	H_3BO_3	2.82
Cu	1.25	$CuSO_4.5H_2O$	4.94
Mn	2.5	MnSO4.HH2O	7.68
Zn	2.5	$ZnSO_4.7H_2O$	11.17
Mo	1.25	$(NH_4)_6Mo_7O_{24}.4H_2O$	2.19
Co	0.125	$CoCl_2.6H_2O$	0.50

(1) Single source of K and P (2) Complementary Ca source to Nitrate of Ca

3.1 Experimental treatments: Soybean [*Glycine max* (L.) Merr.] cv. Jutro was subjected to six treatments replicated four times, in 4-kg plastic pots arranged in a completely randomized design. Treatments consisted of a series of inoculations with *Bradyrhizobium japonicum* DSM 1755, *B. japonicum* DSM 1982 (syn. TAL 379), *B. japonicum* DSM 30131 (syn. ATCC 10324), a multistrain made up of the above three DSM strains referred to as DSM-MIX, a commercial peat inoculant (CO-PEAT), and N-fertilization (N-FERT) at 50 mg N kg^{-1} soil using $Ca(NO_3)_2$. Summer wheat (*T. aestivum* cv. Thasos WeW) served as non-N_2-fixing reference crop and was likewise fertilized with N at the same rate. The three DSM bradyrhizobium strains were supplied in lyophilized form by the German Collection of Microorganisms and Cell Cultures (DSMZ, *Deutsche Sammlung von Mikroorganismen und Zellkulturen* GmbH), Braunschweig, Germany, and certified effective on soybeans. The commercial granular inoculant with peat as carrier and soybean seeds were obtained from *IG Saatzucht GmbH & Co.* KG, München, Germany. Each vacuum-dried culture of *B. japonicum* strain was aseptically extracted from vials and cultured on yeast extract mannitol agar slants in 5 ml pre-sterilized test tubes caped with metallic lids for preservation. Then each strain was grown on yeast extract mannitol broth and kept in a CERTOMAT® HK incubator shaker at 26 °C for 5 days. The DSM multistrain was made by thoroughly mixing in equal volume the cultured broths of the three original *B. japonicum* DSM strains i.e. 1755, 1982 and 30131). The commercial inoculant was prepared by thoroughly mixing 10 g of the peat medium with 50 ml distilled water.

3.2 Seedling inoculation and management: To avoid the risk of rhizobial cross-contamination among treatments, the lids of the plastic pots bore three holes for raising three plants. Soybean seeds were pre-germinated on a sandy soil bed for 10 days. Then three seedlings were transplanted into each pot in corresponding holes and accordingly inoculated by spraying 3 ml of the appropriate strain suspension on its roots. The soil was immediately hand-compacted around the stem base and thoroughly watered. For wheat, 12 seedlings were raised per pot out of 30 sown earlier. Over the growth period, plants were watered to 75% soil water-holding capacity. Three weeks after planting, inoculated soybean showed N deficiency by yellowing leaves in contrast with N-fertilized ones, and subsequently received 10 mg N kg^{-1} soil per pot as a corrective measure. The recovery was fast noticing. Wheat plants also received this additional N rate. At 81 days after sowing, all plants were harvested. Plant parts were separated into leaves plus stems, pods with seeds, roots, and nodules. After nodule counting, all plant parts were oven-dried at 65 °C for up to 36 h, and then weighed. Seeds were collected from pods. Each plant part was separately ground and analyzed for N content by the Kjeldahl method, using the Büchi 322/342 system (Germany) with subsequent, separate titration of N out of boric acid as a receiving solution. Dry-matter (DM) and N harvest indexes were calculated in relation to soybean grain yields. Nodulation and yield-related parameters as well as N_2 fixation, estimated by the N-difference method (Peoples *et al.*, 1989), were assessed. Data monitored were subjected to the analysis of variance, using IRRISTAT 3.1 statistical package, while a comparison of treatment means at 5% probability level was performed using the Duncan multiple range test (DMRT) or the least significant difference test (LSD) as appropriate.

4 RESULTS

4.1 Biomass yield: At 81 days after sowing, the five inoculation treatments affected similarly soybean biomass yields (Table 2). However, leaf DM yield under inoculation with the bradyrhizobium strain DSM 1755 differed significantly ($p<0.05$) from that of peat inoculant. N fertilization resulted in over 4-fold highly significant ($p<0.01$) root DM of plants than any bradyrhizobium inoculation. In contrast, it led to significantly ($p<0.01$) lower shoot and total DM yields than all the other treatments (Table 2). The performance of wheat as non N_2-fixing reference crop over the same growth period is shown in Table 3.

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Table 2: Effect of different inoculants of *Bradyrhizobium japonicum* and N fertilization on soybean biomass yield

Treatment	Root DM	Leaves DM	Shoot DM	Total DM
	-------- g.pot^{-1} --------			
DSM 1755	1.58 a	8.32 b*	24.85 b	27.35 b
DSM 1982	1.58 a	6.12 a	22.71 b	25.05 b
DSM 30131	1.60 a	5.26 a	22.94 b	25.14 b
DSM-MIX	1.60 a	6.95 ab	24.58 b	27.02 b
CO-PEAT	1.44 a	6.35 ab	23.01 b	25.16 b
N-FERT	6.65 b**	6.55 ab	16.80 a **	20.78 a **
Mean	1.91	6.59	22.48	25.08
CV (%)	25.1	18.7	8.3	8.4

In a column, means sharing a same letter do not significantly differ at 5% level by Duncan Multiple Range Test. Asterix denote significance of difference at 5% (*) and 1% (**) with CO-PEAT as the control

Table 3: Dry matter and N yield of wheat (*Triticum aestivum* var. Thasos) used as non-N_2-fixing crop under N fertilization

Variables	Units	Means[a]
Root DM	g.pot^{-1}	17.2 ± 3.2
Shoot DM	»	14.1 ± 1.0
Root N	%	0.43 ± 0.02
Shoot N	%	0.48 ± 0.02
Root N Yield	mg.pot^{-1}	73.4 ± 12.7
Shoot N Yield	»	68.2 ± 7.0
Total DM	g.pot^{-1}	31.3 ± 4.1
Total N Yield	mg.pot^{-1}	141.6 ± 19.7

[a] Data are means of 4 replicates ± S.E.

Under the same N fertilization rate, wheat produced relatively high total DM yield compared to soybean plants under either treatment. Wheat root DM amounted to some 2.6 fold that of the best corresponding N-fertilized soybean, and to as much as 9 fold the average soybean root yield. Nevertheless, wheat shoot DM as well as N yields were lower than those of soybean under any treatment (Tables 2, 3).

4.2 Nitrogen accumulation in soybean under inoculation and N application: Soybean plant parts at maturity revealed large differences in N concentration, regardless of treatments applied (Table 4). While root and leaves percent N were similar (1.36%), pods had the poorest N concentration that averaged less than 1%. However, highest N amounting to 3.8 and 4.5-fold that of either leaves or roots were found in soybean nodules and grains, respectively. N accumulation in those parts, in relation to their respective DM yields, ranked in the decreasing order as grains > pods > leaves > nodules > roots (Table 4).

Table 4: Effect of different inoculants of *Bradyrhizobium japonicum* and N fertilization on soybean N concentration and yield

TREATMENT	Root	Nodule	Leaves	Pods	Grain	Root	Nodule	Leaves	Pods	Grain
	-------- % N --------					-------- mg $N.pot^{-1}$ --------				
DSM 1755	1.72**c	5.06*	1.61 b	0.88 ab	6.35 b	27.0* b	46.9 b	141.9 b	143.9 bc	673.1 b
DSM 1982	1.35 b	5.05*	1.41 ab	0.74 a	6.50 b	21.3 ab	38.1 b	87.3 ab	119.7 b	628.9 b
DSM 30131	1.37 b	5.43	1.21 ab	0.73 a	6.41 b	21.6 ab	33.3 b	64.6 a	128.7 b	727.7 b
DSM-MIX	1.42 b	5.20	1.50 b	1.11 b	6.53 b	22.6 ab	44.3 b	106.6 ab	195.4 c	692.6 b
CO-PEAT	1.30 b	5.70	1.41 ab	0.84 ab	6.35 b	18.5 a	36.4 b	89.6 ab	141.0 bc	668.6 b
N-FERT	1.04* a	5.34	1.00 a	0.61 a	4.82**a	35.3**c	18.2*a	66.4 a	63.8 a	269.4** a
Mean	1.37	5.3	1.36	0.82	6.16	24.41	36.29	92.7	132.08	610.0
CV(%)	10.7	8.0	21.9	27.9	6.2	20.6	25.9	41.3	28.3	11.0

Asterix denote significance of difference at 5% (*) and 1% (**) with CO-PEAT as the control

In a column, means sharing a same letter do not significantly differ at 5% level by Duncan Multiple Range

The different bradyrhizobium inoculants differed in affecting both the percent and N yield across soybean parts. The strain DSM 1755 was significantly ($p<0.05$) the best overall treatments in root % N while other DSM inoculants compared similarly well with the commercial peat inoculant. The latter treatment (Table 4) scored however the highest % N in nodules (5.7%). Except in root N accumulation, the application of N fertilizer did not increase N content of soybean across plant parts as compared to all inoculations with bradyrhizobium strains (Table 4).

4.3 Soybean nodulation and N_2 fixation : Any inoculation produced significantly ($p<0.01$) more nodules in soybean than N fertilization (Table 5).

Table 5: Effect of different inoculants of *Bradyrhizobium japonicum* and N fertilization on soybean nodulation and potential N_2 fixation

	Nodules/pot		N_2 fixed	
Treatment	no.	**g DW**	**mg.pot^{-1}**	**%**
DSM 1755	424 b	0.93* c	891.2 bc	86,0 b
DSM 1982	374 b	0.76 bc	753.6 b	84,2 b
DSM 30131	377 b	0.61 b	834.4 bc	85,6 b
DSM-MIX	410 b	0.85 bc	919.9 c	86,6 b
CO-PEAT	365 b	0.64 b	812.5 bc	85,2 b
N-FERT	66**a	0.34* a	312.0** a	65,7** a
Mean	336	0.68	753.9	82,2
CV (%)	14.1	22.6	13.3	9,6

In a column, means sharing a same letter do not significantly differ at 5% level by Duncan Multiple Range Test. Asterix denote significance of difference at 5% (*) and 1% (**) with CO-PEAT as the control

Other bradyrhizobium inoculants gave similar nodule numbers per pot as the control, peat inoculant. N fertilization of soybean significantly depressed both the number and dry weight of nodules compared to the control commercial peat inoculant. The strains DSM 1755 and DSM-MIX, however, ranked the best in increasing both nodulation parameters while N treatment was the least (Table 5).

All DSM strains performed similarly to CO-PEAT, the control, in the amount and the proportion of N_2 fixed, the reliance of soybean upon N_2 fixation being in a similar magnitude ranging from 84 to 87% of its N requirement (Table 5). The multistrain inoculant (DSM-MIX) and DSM 1755 were however the most efficient promoters of soybean biological N_2 fixation. Consistently, nodulation as well as the amount and rate of N_2 fixation were significantly ($p<0.01$) lower in N-fertilized soybean plants than in inoculated ones. Peat inoculant performed significantly better than N fertilization with some 20 % and 260 % increase in the rate and amount of N_2 fixed, respectively.

4.4 Assessment of soybean productivity : All inoculants led to similar yields of soybean in terms of numbers and DM of pods and grains per pot. Although DSM 1755 bore significantly ($p<0.05$) more pods and DSM 1982 less grains per pot, none was better than the control peat inoculant in either pods or grain yields (Table 6). They also performed similarly in promoting the DM harvest index of soybean. Nonetheless, significant differences arose among the inoculation treatments in affecting soybean N yield as well as its N harvest index for which the multistrain inoculant, DSM-MIX, and the unistrain, DSM 30131 respectively recorded the highest values. For most productive variables, soybean fertilization with N led to significantly lower yields than any bradyrhizobium inoculation. All inoculants led to similar yields of soybean in terms of numbers and DM of pods and grains per pot. Although DSM 1755 had significantly ($p<0.05$) more pods and DSM 1982 less grains per pot, none was better than the control peat inoculant in either pods or grain yields (Table 6).

Table 6: Effect of different inoculants of *Bradyrhizobium japonicum* and N fertilization on soybean productivity performance

Treatment	Pods[a] no.pot^{-1}	Pods[a] g.pot^{-1}	Grain yield no.pot^{-1}	Grain yield g.pot^{-1}	N yield mg.pot^{-1}	Harvest index (DM)	Harvest index (N)
DSM 1755	46*c	16.52 b	66 b	10.67 b	1032.8 bc	0.433b	0.704 ab
DSM 1982	39 bc	16.59 b	59*b	9.68 b	895.2 b	0.426 b	0.752 ab
DSM 30131	39 bc	17.68 b	71 b	11.36 b	976.0 bc	0.496 b	0.791 b
DSM-MIX	40 bc	17.63 b	66 b	10.61 b	1061.5 c	0.433 b	0.699 a
CO-PEAT	38 b	16.74 b	69 b	10.53 b	954.1 bc	0.456 b	0.744 ab
N-FERT	30* a	10.25** a	42**a	5.62**a	453.6 **a	0.331**a	0.682 a
Mean	38	15.90	62	9.74	895.5	0.429	0.728
CV (%)	12.6	10.1	10.8	11.9	10.4	10.5	7.6

[a] Pods with enclosed seeds, In a column, means sharing a same letter do not significantly differ at 5% level by Duncan Multiple Range Test.. Asterix denote significance of difference at 5% (*) and 1% (**) with CO-PEAT as the control

4.5 Rank performance of treatments on soybean: The ranking of inoculation and N fertilization treatments based on ten selected key parameters that included productive and reproductive biomass, nodulation, N_2 fixation and harvest index of soybean is shown in Table 7. Out of all treatments imposed on soybean, N fertilization led to a consistent poor performance of soybean across all tested variables, while variations in ranks occurred rather with all bradyrhizobium inoculations. On overall basis, in promoting soybean performance, all treatments ranked in the decreasing order as DSM 1755 > DSM-MIX = DSM 30131 > CO-PEAT > DSM 1982 > N-FERT.

Table 7: Ranking of the different inoculants of *Bradyrhizobium japonicum* and N fertilization based on their effect on soybean production performance (with 1 and 6 being lowest and highest ranks)

Treatment	Parameters used: TDM	NN	NDW	GN	GDM	TNY	HIDM	HIN	QNF	PNF	Rank
DSM 1755	6	6	6	3	5	5	4	3	5	5	**6**
DSM 1982	2	3	4	2	2	2	2	5	2	2	**2**
DSM 30131	3	4	2	5	6	4	6	6	4	4	**4**
DSM-MIX	5	5	5	3	4	6	3	2	6	6	**5**
CO-PEAT	4	2	3	4	3	3	5	4	3	3	**3**
N-FERT	1	1	1	1	1	1	1	1	1	1	**1**

Legend: TDM: total DM, NN: nodule number; NDW: nodule dry weight, GN: grains no., GDM: grain DM, TNY: total N yield, HIDM: dry-matter harvest index, HIN: nitrogen harvest index; QNF and PNF: amount and percent of N_2 fixed

5 DISCUSSION

Measuring the benefits of rhizobial inoculation should include several traits of legume performance (Hardarson *et al.*, 1984a), but as noted by Caldwell (1969), full evaluation of strains requires growing host soybean plants to completion as strains may vary in their symbiotic effectiveness over the growing season. Accordingly, we used a wide array of variables to efficiently discriminate the different bradyrhizobium inoculant strains over the life cycle of soybean. Soybean total DM yields exhibited no significant difference among the five bradyrhizobium strains tested, thus corroborating early findings by Hardarson *et al.* (1984a) that this parameter was generally not a sensitive measure of rhizobial treatments benefit to legumes. This is due to the fact that very different nutritional treatments are often translated into small differences in DM yield, especially on a single crop cultivar as is the case in this study. N accumulation in soybean (Tables 4, 6) led rather to noticeable differences among the bradyrhizobium strains as DSM 1755 and the multistrain DSM-MIX were better than the commercial peat inoculant. Total N yield is therefore a better parameter than DM yield in assessing the ability of different bradyrhizobia to enhance N status of soybean, confirming results reported by Hardarson *et al.* (1984a).

An important observation herein is that the systematic N analysis in all separate above- and below-ground parts of soybean shows a clear picture of N pool in the plant with the key N source (nodules) and N sink (grains) being predominantly higher in N (>5-6% N) than any straw. Another contribution lies in showing that nodules and roots amounted on average to 6.8% of soybean N yield, in addition to an early result of 5% obtained by Hardy and Halveka (1976). Purposely, Senaratne and Hardarson (1988) pointed out that the information on the proportion of plant N in the roots of legumes is scanty, most likely due to difficulty of recovering the below-ground parts. Soybean reproductive organs contained 83% of total N in the plant, as they made up almost two-thirds (63.4%) of its total DM. This conforms to earlier observation by Sinclair and Wit (1975) that N requirement of soybean is one of the highest of agronomic crops and is greatest when the seeds are developing. Such a high predominance of the reproductive organs is a result of great translocation of N from vegetative parts (Kang, 1975; Garcia and Hanway, 1976). In agreement with Hardarson *et al.* (1984a), the N harvest index was sensitive in revealing differences among bradyrhizobium strains in affecting soybean performance, whereas the corresponding index for DM yield was unable to discriminate them. Thus DSM-MIX had the highest N harvest index, although all inoculated plants showed it higher than uninoculated ones. This concurs with Morris and Weaver (1983) that the relative proportion of seed N derived from remobilization of vegetative tissue N or N_2 fixation is also affected by the *B. japonicum* strain present. Soybean harvest index for N that varied from 0.68 to 0.79 was lower than 84 - 88% obtained by Osaki *et al.* (1991) in Japan, but higher than 44 - 50.8% reported by Lynch and Smith (1993). The discrepancy of this study data with those of Osaki *et al.* (1991) is due to the inclusion of all reproductive organs, i.e. pods with enclosed seeds, in their estimation of this index, while this study considered only grain N. For harvest index of DM, however, this study result (63%) was above some 50% commonly encountered in field-grown soybean (Osaki *et al.* 1991). As all inoculated soybean received one-fifth of the N rate applied to N-fertilized ones, but performed better than the latter for most parameters, it could be thought that 50 mg N kg^{-1} soil was a high rate to depress nodulation and symbiotic N_2 fixation. This indicates the sensitivity of soybean to high inorganic N rates which is well documented (Hardarson *et al.*, 1984b; Senaratne *et al.*, 1988). It is therefore clear from the foregoing data that the addition of 10 mg N kg^{-1} soil to alleviate seedlings N deficiency observed three weeks after transplantation was appropriate as N starter for promoting soybean growth, N_2 fixation and grain yield, under bradyrhizobium inoculation.

Bradyrizobium strains varied in their ability to form nodules on soybean, probably due to the variation in the efficiency of each *B. japonicum* strain (Okereke *et al.*, 2001). Nodulation and subsequent N_2 fixation were higher in the inoculated soybean plants than in the uninoculated ones, showing that for enhanced symbiotic N_2 fixation, soybean should be inoculated by *B. japonicum* before planting. Increased nodulation and N_2 fixation after any bradyrhizobium inoculation may also be due to the fact that the experimental soil had not been planted to soybean before. This shows that a good inoculation response by soybean is more likely in soils that have not been previously inoculated with these strains or planted to soybean. On the other hand, the poor performance of N-fertilized soybean could be due to the inhibitory effect of high N rates and to the absence/ insufficiency of native bradyrhizobia in the experimental soil. Indeed, given that the extra plants used as super control (no inoculation and no N fertilizer) grew too stunted such that they could not survive over the growth period, it is doubtful that this soil originally contained any specific bradyrhizobium to nodulate soybean. Nevertheless, as the N-fed soybean nodulated and fixed N to an acceptable level although significantly below any inoculation, it is most likely that a cross-contamination occurred during the growth period, but this could not however supply enough bradyrhizobium strains populations in soil to efficiently promote soybean to a comparable level with well inoculated plants. In this respect, Singleton and Tavares (1986) observed that low populations of rhizobia in soil could not sufficiently promote legume nodulation and increase symbiotic N_2 fixation.

In conclusion, differences did exist among bradyrhizobium strains in their effectiveness. Even peat inoculant, as the least, outperformed significantly N fertilization with great increases both in the rate and amount of N_2 fixed. Its low performance in our early greenhouse experiment could most likely be ascribed to prevailing cold weather that favored poor soybean development. Nevertheless, the bradyrhizobium strains DSM 1755, the multistrain DSM-MIX, and DSM 30131 should be preferred to the commercial inoculant. Nitrogen fertilization at 50 mg N kg^{-1} soil was deleterious to soybean, while combining application of 10 mg N kg^{-1} soil as N starter with any bradyrhizobium strain inoculation improved significantly soybean growth, N yields, nodulation, N_2 fixation and grain yields. This practice deserves field validation and further recommendation for soybean growing in this area of Germany.

6 ACKNOWLEDGEMENT

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