

## Reciprocal Grafting and Bacterial Strain Effects on Nodulation of Soybean Genotypes

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### Abstract

It is known that the soybean (*Glycine max* [L.] Merr.) genotype PI 417566 has restricted nodule development when inoculated with *Bradyrhizobium japonicum* strain USDA 110 and grown at low temperature. The current experiments were conducted to determine if the restricted nodulation of PI 417566 roots could be overcome by grafting them to a hypernodulated shoot, since it is known that grafted shoots of hypernodulated mutants induce hypernodulation on roots of normally nodulated soybean. Reciprocally- and self-grafted NOD1-3 (a hypernodulation mutant) and PI 417566 (a plant restricted nodulation genotype) plants were evaluated for nodulation in a growth chamber at constant 20°C. Seedling roots of self-grafted PI 417566 were normally nodulated when inoculated with USDA 123, while USDA 110 resulted in restricted nodulation. Grafting of NOD1-3 shoots to PI 417566 roots did not enhance nodulation when inoculated with USDA 110, but did enhance nodulation when inoculated with USDA 123. It appears that the shoot of NOD1-3 has the ability to alter autoregulatory control of nodulation of the PI 417566 genotype only when challenged with a compatible rhizobial strain.

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The shoot of PI 417566 significantly inhibited nodule formation on NOD1-3 roots when inoculated with either USDA 110 or 123. The restriction of nodulation on the PI 417566 roots inoculated with USDA 110 appears to be primarily controlled by root factors. It was concluded that restricted nodulation and autoregulatory control of nodulation appear to be separate phenomena.

Keywords: *Bradyrhizobium*, grafts, soybean, nodulation restriction, hypernodulation

## 1. Introduction

The formation of nodules on soybean roots occurs in response to infection by *Bradyrhizobium japonicum*. After rhizobial infection, nodule development is restricted by a plant process called autoregulation, in which the nodule formation on one part of the root systemically suppresses subsequent nodule formation on other root regions (Pierce and Bauer, 1983; Kosslak and Bohlool, 1984). Supernodulating and hypernodulating mutants of soybean that appear to have altered this autoregulation have been derived for Bragg and Williams parents (Carroll et al., 1985; Gremaud and Harper, 1989). These mutants have greater nodule numbers compared with their respective parents, but specific nitrogenase activity ( $C_2H_2$  reduction) tends to be lower in the mutants.

There have been numerous reports of genotype- and cultivar-specific interaction among leguminous plants and their respective symbionts (see Lohrke et al., 1995 for references). Information is limited, however, concerning factors affecting incompatible interactions between bradyrhizobia and soybean plants which result in restricted nodulation by *B. japonicum* strains. Various soybean lines have been reported to express restricted nodulation when inoculated with *B. japonicum* USDA 123 (Cregan and Keyser, 1986; Cregan et al., 1989; Keyser and Cregan, 1987; Sadowsky et al., 1995; Lohrke et al., 1995). PI 417566 is known to restrict nodulation by strain USDA 110 (Lohrke and Sadowsky, 1994).

Grafting techniques have been used to estimate the effects of shoot and root factors on several physiological aspects of plant growth and development. For instance, grafting has been shown to be of value in breeding programs to synchronize flowering in soybean (Kiihl et al., 1977). Grafting has also been used to determine that the root genotype exercises some control over drought tolerance in common bean (White and Castillo, 1989). A study on grafting between supernodulating nitrate tolerant symbiotic mutants and the parent soybean cultivar Bragg indicated that the supernodulation characteristics were controlled through a graft-transmissible shoot factor (Gresshoff and Delves, 1986; Day et al., 1989). The presence of a graft-transmissible shoot factor was also confirmed with hypernodulating soybean mutants from the

cultivar Williams (Cho and Harper, 1991). Moreover, Lee et al. (1991) reported that nodulation, nodule function, and plant growth were dependent on the shoot genotype of isolated cultivars of soybean. In contrast to shoot control of supernodulation and hypernodulation expression in soybean, the non-nodulating character was dependent on the root genotype (Cho and Harper, 1991; Mathews et al., 1992). Thus, both shoots and roots have specific involvement in the overall nodulation process.

Knowing that shoots can potentially alter nodule expression in soybean, the current investigation was carried out to evaluate the impact of the shoot and root on the nodulation process of self- and reciprocal-grafts between a hypernodulating mutant (NOD1-3) and PI 417566, inoculated with either *B. japonicum* strain USDA 110 or 123.

## 2. Materials and Methods

### *Plant growth and conditions*

Two genotypes (NOD1-3, a hypernodulating mutant, and PI 417566, a restricted nodulation genotype) of soybean (*Glycine max* [L.] Merr.) and two *Bradyrhizobium japonicum* strains (USDA 110 and 123) were evaluated. Seeds were surface sterilized by immersing in 95% (v/v) ethanol for 10 sec, followed by a 3.5 min treatment with 0.5% (v/v) sodium hypochlorite, and then rinsed four times with sterilized, reverse osmosis purified, water. Germination and establishment of grafted plants were in a growth chamber programmed for 14-h photoperiods ( $500 \mu\text{mol photons m}^{-2} \text{sec}^{-1}$ ) at 28°C, and 10-h dark periods at 20°C. At 6 days after germination, grafts were made in the sand trays used for germination. Reciprocal- and self-grafts were made between seedlings of NOD1-3 and PI 417566 as described by Bezdicsek et al. (1972). Self-grafted plants were included as controls. The graft joint was held in place by a 1 cm section of plastic drinking straw (2.5 mm), slit down one side to allow expansion as the seedling grew.

Grafted seedlings were maintained in the germination trays for 5 days, covered by a transparent plastic lid to maintain a high humidity level and to facilitate graft formation. The seedlings were watered with reverse osmosis purified water as needed. Seedlings were then transplanted to 2-liter plastic pots containing a modified, minus-nitrogen Hoagland nutrient solution (Gremaud and Harper 1989) and grown in a growth chamber at constant 20°C with 14-h photoperiods and 10-h dark periods. Each pot initially contained six plants which were inoculated at transplant with 10 ml of rhizobia ( $10^8 \text{ cells ml}^{-1}$ ) grown on yeast-mannitol broth. At 10 days after transplant, plants were

evaluated for uniform growth and for some pots the number of plants was decreased to four due to poor growth of some grafted seedlings.

The nutrient solution were maintained at pH 6.5 by using ion exchange resin column systems (Harper and Nicholas, 1976). The same resin column system also served to aerate the nutrient solution. Nutrient solutions were completely changed at 7-days intervals after transplanting. Thirty days after transplanting, plants were harvested and the plant roots were detached from shoots and placed in 500 ml jars (four to six roots sample<sup>-1</sup>). The jars were sealed and injected with 50 ml acetylene through a rubber septum fitted into the lid, and incubated at 30°C for 30 min. Following incubation, 0.5 ml samples were analyzed for ethylene production by flame ionization gas chromatography (Hewlett Packard 5890A). Acetylene and ethylene peaks were quantified with a Nelson analytical 3000 series gas chromatography system (Nelson Analytical Inc., Cupertino, CA) interfaced to an International Business Machine Personal Computer for data reduction. After the assay, the nodules were counted on the root. Shoots and roots plus nodules were dried in a forced air oven for 48 h at 80°C. Dry matter of shoots and roots plus nodules were determined. Experimental data were subjected to analysis of variance using SAS.

### 3. Results

#### *Effect of self- and reciprocal-grafts on nodulation and growth of soybean genotypes inoculated with strain USDA 123*

Data of Table 1 indicate that grafting of shoots of NOD1-3 to roots of PI 417566 significantly increased nodule number, compared with self-grafted plants, when inoculated with strain USDA 123. The number of nodules formed on roots of PI 417566 plants grafted to shoots of NOD1-3 increased more than 8-fold, compared with self-grafted PI 417566 plants. In contrast, the shoot of PI 417566 plants resulted in an 86% inhibition of nodule number formed on the roots of NOD1-3, compared with self-grafts of NOD1-3.

Acetylene reduction activity was measured as an estimate of nitrogenase activity of root nodules formed by strain USDA 123 (Table 1). Reciprocal-grafts between NOD1-3 and PI 417566 did not significantly alter nitrogenase activity compared with self-grafts of the PI 417566 genotype, but all were significantly less than for self-grafts of NOD1-3. Shoot dry matter was greater for NOD1-3 than for PI 417566 with both self- and reciprocal-graft combinations (Table 1). Root plus nodule dry matter was similar among all graft combinations (Table 1).

Table 1. Nodulation, acetylene reduction activity, and dry mass accumulation of self- and reciprocally-grafted soybean NOD1-3 and PI 417566 plants inoculated with *Bradyrhizobium japonicum* USDA 123\*

Plant genotype	Nodule number (no. plant <sup>-1</sup> )	Acetylene reduction ( $\mu\text{mol C}_2\text{H}_4$ plant <sup>-1</sup> h <sup>-1</sup> )	Dry mass (g plant <sup>-1</sup> )	
			Shoot	Root + nodule
NOD1-3/NOD1-3	904 a	3.68 a	0.37 a	0.27 a
PI 417566/PI 417566	71 b	1.45 b	0.32 b	0.19 a
NOD1-3/PI 417566	665 a	2.24 b	0.48 a	0.23 a
PI 417566/NOD1-3	126 b	1.87 b	0.33 b	0.20 a

\*Each value represents the mean of three replicates. Means in the same column followed by the same letter are not significantly different at the 5% level using  $F_{\text{LSD}}$ .

Table 2. Nodulation, acetylene reduction activity, and dry mass accumulation of self- and reciprocally-grafted soybean NOD1-3 and PI 417566 plants inoculated with *Bradyrhizobium japonicum* USDA 110\*

Plant genotype	Nodule number (no. plant <sup>-1</sup> )	Acetylene reduction ( $\mu\text{mol C}_2\text{H}_4$ plant <sup>-1</sup> h <sup>-1</sup> )	Dry mass (g plant <sup>-1</sup> )	
			Shoot	Root + nodule
NOD1-3/NOD1-3	434 a	3.73 a	0.48 ab	0.22 ab
PI 417566/PI 417566	6 c	0.36 c	0.42 c	0.19 b
NOD1-3/PI 417566	10 c	0.91 c	0.51 a	0.24 a
PI 417566/NOD1-3	126 b	2.50 b	0.44 bc	0.21 ab

\*Each value represents the mean of three replicates. Means in the same column followed by the same letter are not significantly different at the 5% level using  $F_{\text{LSD}}$ .

*Effect of self- and reciprocal-grafts on nodulation and growth of soybean genotypes inoculated with strain USDA 110*

Results presented in Table 2 show that grafts between the shoot of NOD1-3 and the root of the PI 417566 genotype had no effect on nodule number formed on

PI 417566 roots, compared with self-grafted PI 417566 plants. In contrast, the shoot of PI 417566 significantly decreased nodule number on the root of NOD1-3. Nitrogenase activity, as estimated by acetylene reduction assay, showed the same trend as noted for nodule number. Grafting of NOD1-3 shoots to PI 417566 roots resulted in small but significant increases in shoot and root plus nodule dry matter compared with self-grafted PI 417566 plants (Table 2). Dry matter of plant parts of reciprocal-grafted NOD1-3 and PI 417566 plants was not different from self-grafted NOD1-3 plants.

#### 4. Discussion

The current study confirmed a previous report (Sadowsky et al., 1995) that nodulation is restricted on PI 417566 when inoculated with USDA 110 and grown at low temperature. Although Lohrke et al. (1995) concluded that host-strain incompatibility for nodulation and nodule-blocking is most likely governed by an autoregulatory feedback mechanism, our observation that NOD1-3 shoots were capable of stimulating nodulation of PI 417566 when inoculated with USDA 123 (Table 1) indicates that autoregulatory control is operational in PI 417566. Previous studies have indicated that NOD1-3 shoots release autoregulatory control of nodule number when shoots are grafted to roots of a normally nodulated cultivar (Cho and Harper, 1991). Likewise, the shoot of PI 417566 exerts the typical autoregulatory control of nodulation when grafted to roots of the NOD1-3 hypernodulating line. Efforts to overcome the root restriction of USDA 110 nodulation of PI 417566 by grafting to shoots of the NOD1-3 hypernodulated mutant were not successful. These findings indicate that the root of PI 417566 plays a dominant role in restricting nodule development with USDA 110 inoculation.

This root control of restricted nodulation expression is somewhat analogous to the root control of a non-nodulating trait in *rj*<sub>1</sub> lines (Delves et al., 1986). Although no known rhizobial strain results in normal nodulation of *rj*<sub>1</sub> lines, there is evidence that sporadic nodulation occurs on *rj*<sub>1</sub> lines at high inoculum dose (Francisco and Akao, 1993; La Favre and Eaglesham, 1984; Mathews et al., 1987). As shown in the PI 417566 genotype inoculated with USDA 123 (Table 1), the root also has primary control of nodulation suppression in the NN5 non-nodulating mutant derived from Williams (Cho and Harper, 1991). The NN5 mutant is controlled by two recessive genes (*rj*<sub>5</sub> and *rj*<sub>6</sub>) (Pracht et al., 1993), and differs from *rj*<sub>1</sub> lines in nodulation response in that NN5 has never been observed to nodulate even at high inoculum doses (Francisco and Harper, 1994). The current study provides additional support for the concept that strain specific restricted nodulation of PI 417566 is root controlled, and not subject to

being overridden by a translocatable shoot-derived factor known to provide autoregulatory control in normally nodulating soybean. Another report (Balatti and Pueppke, 1990) showed that restriction of nodulation was controlled by roots when the soybean cultivar McCall was inoculated with *Rhizobium fredii*.

Delves et al. (1986) indicated that while non-nodulation in soybean is controlled by the genotype of the root, the shoot governs the nodulation of supernodulation phenotypes. The latter result was confirmed for hypernodulation phenotypes as well (Cho and Harper, 1991). Results of the present investigation support the conclusion of previous reports (Lohrke et al., 1995) that both root and shoot factors can influence the interaction of soybean genotypes and specific *B. japonicum* strains. It appears clear, however, that the root is in primary control of restricted nodulation in the genotype PI 417566 when inoculated with USDA 110 and grown at cool (20°C) temperature, and that this phenomenon is separate from the autoregulatory control of nodule number in normally nodulating soybean. This does not rule out, however, a separate feedback autoregulatory control as proposed by (Lohrke et al., 1995), and further investigation is warranted.

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