

Communication

Regulation of the Soybean-*Rhizobium* Nodule Symbiosis by Shoot and Root Factors¹

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ABSTRACT

The availability of soybean mutants with altered symbiotic properties allowed an investigation of the shoot or root control of the relevant phenotype. By means of grafts between these mutants and wild-type plants (cultivar Bragg and Williams), we demonstrated that supernodulation as well as hypernodulation (nitrate tolerance in nodulation and lack of autoregulation) is shoot controlled in two mutants (nts382 and nts1116) belonging most likely to two separate complementation groups. The supernodulation phenotype was expressed on roots of the parent cultivar Bragg as well as the roots of cultivar Williams. Likewise it was shown that non-nodulation (resistance to *Bradyrhizobium*) is root controlled in mutant nod49. The shoot control of nodule initiation is epistatically suppressed by the non-nodulation, root-expressed mutation. These findings suggest that different plant organs can influence the expression of the nodulation phenotype.

The development of N-fixing nodules on legume roots upon invasion of *Rhizobium* (or *Bradyrhizobium*) bacteria is subject to regulation by factors both external and internal to the plant host. In particular, the extent of nodulation is restricted by a process termed autoregulation, in which the formation of nodules on one part of the root systemically inhibits subsequent nodule formation in other root regions (3, 15). Nodulation is also severely restricted by the presence of nitrate in the soil (7). Our laboratory has recently isolated several soybean mutants with altered symbiotic features, including some tolerant to nitrate (nts³) which also supernodulate (3, 4), and others which do not form any nodules (nod⁻) (1, 8).

Clearly nodulation is subject to control by plant factors; the sites of this control are unknown, although some experimentation has implicated shoot-root interactions (11–13). By means of grafts between these mutant and wild-type plants, we show here that supernodulation in soybean is controlled in two separate mutants by the shoot and that the genotype of the root controlled the non-nodulation phenotype of the plant. These findings will facilitate further biochemical and molecular analysis of these mutants at a tissue-specific level. Crosses made between these mutants and the wild-type parental cultivar Bragg indicated that

the altered genes in the nts mutants were different between the two nts mutants used and that one, from nts1116, is at least a partial dominant, whereas the other, from nts382, is a recessive (8).

MATERIALS AND METHODS

Plant Material. The isolation and preliminary characterization of the mutants was described by Carroll *et al.* (3, 4). This study used *Glycine max* cultivar Bragg (wild-type, nod⁺, fix⁺), and the derived mutants nts382 (nitrate tolerant, supernodulating, nod⁺⁺⁺, fix⁺), nts1116 (nitrate tolerant, but only hypernodulating, nod⁺⁺, fix⁺) and nod49 (non-nodulating with normal inoculant doses (approximately 10⁷ bacterial per plant) of *Bradyrhizobium japonicum* strain USDA110 or CB1809, allelic to rj₁, Table II). Genetic analysis of all three mutants has shown that the lines have bred true for a minimum of 5 generations. Preliminary analysis suggests that nts382 and nts1116 are in separate complementation groups. Mutant lines were selected from M2 families derived from ethyl methanesulfonate-treated M1 seeds.

Growth Conditions and Nodulation Tests. Plants used for Table I were inoculated with *B. japonicum* strain USDA110 (approximately 10⁷ bacteria per plant) and were cultured in 25 cm pots filled with vermiculite:sand mixture (1:2 ratio). Glasshouse temperatures were held between 14 and 30°C and incandescent 100 W bulbs extended the photoperiod to 16 h near summer conditions). The pots received 1.2 L of nutrient solution as described by Herridge (10) three times a week (Table I). The nutrient solution was either N-free (nitrate absent) or was supplemented with 5 mM KNO₃ (nitrate present). Plants were harvested 45 d after planting and nitrogenase activity was determined as described by Carroll *et al.* (3).

Grafted plants used for Tables III and IV were also grown under glasshouse conditions of 2 per pot in a 3:1 mixture of sterilized sand and vermiculite. Data are thus compatible with those of Table II. Grafting occurred 10 d after sowing using a wedge-shaped graft with the cotyledons left on the scion. Grafts were held in place by a polythene sleeve covering the whole graft union and plants were placed immediately after grafting under an intermittent, automatic misting system for 10 d to prevent desiccation before the grafts had functionally rejoined. Plants were then transferred to a glasshouse, inoculated with *B. japonicum* strain USDA110 as a slurry of bacteria, peat and water (at approximately 10⁷–10⁸ bacteria per plant). Watering was daily with a complete nutrient solution containing 7.5 mM KNO₃ to run off. Plants were harvested 9 weeks after sowing, nodules were picked and counted from each plant and dry weights obtained after material was oven dried. Plants for Table V were grown as

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³ Abbreviation: nts, nitrate tolerant symbiosis.

Table III. Control of Supernodulation by the Shoot of Cultivar Bragg

Plants were raised and harvested as described in "Materials and Methods." Each entry is the mean \pm SD of 4 plants.

Graft (Shoot/Root)	Nodule No. per Plant	Nodule Mass <i>mg dry wt nodule g⁻¹ dry wt plant</i>
nts382/nts382	249 \pm 90	139 \pm 101
nts1116/nts382	71 \pm 18	110 \pm 5
Bragg/nts382	11 \pm 5	2 \pm 1
nts382/nts1116	251 \pm 46	182 \pm 16
nts1116/nts1116	64 \pm 6	14 \pm 5
Bragg/nts1116	8 \pm 3	3 \pm 1
nts382/Bragg	182 \pm 35	56 \pm 28
nts1116/Bragg	48 \pm 4	9 \pm 2
Bragg/Bragg	8 \pm 1	2 \pm 1

Table IV. Control of Supernodulation by the Shoot of Cultivar Williams

Plants were raised and harvested as described in "Materials and Methods." Each entry is the mean \pm SD of 4 plants.

Graft (Shoot/Root)	Nodule No. per Plant	Nodule Mass <i>mg dry wt nodule g⁻¹ dry wt plant</i>
Williams/Williams	19 \pm 4	2 \pm 1
Williams/nts382	14 \pm 4	2 \pm 2
nts382/Williams	409 \pm 76	125 \pm 52
Williams/nts1116	12 \pm 2	0.1 \pm 0.1
nts1116/Williams	100 \pm 11	27 \pm 5
Bragg/Williams	16 \pm 3	2 \pm 1
Williams/Bragg	14 \pm 5	2 \pm 1

Table V. Control of Non-Nodulation by the Root

Plants were raised and harvested as described in "Materials and Methods." Each data point is the mean \pm SD of 8 plants.

Graft (Shoot/Root)	Nodule No. per Plant	Nodule Mass <i>mg dry wt nodule g⁻¹ dry wt plant</i>
Bragg/Bragg	26 \pm 8	49 \pm 9
Bragg/nod49	0	0
Bragg/nts382	87 \pm 15	53 \pm 17
nod49/nod49	0	0
nod49/Bragg	23 \pm 7	41 \pm 9
nod49/nts382	69 \pm 17	53 \pm 9
nts382/nts382	284 \pm 96	88 \pm 25
nts382/nod49	0	0
nts382/Bragg	177 \pm 40	127 \pm 23

Bragg, even in the absence of *Bradyrhizobium* (5).

Nitrate sensitivity of nodule formation is a complex, yet unexplained phenomenon which involves both localized root effects and possible transmitted effects (2). Since the grafting experiments of Tables II and IV were carried out in the presence of high concentrations of nitrate, it appears that the shoot also plays a role in the sensitivity of the symbiosis to nitrate. To date we have isolated 12 supernodulating soybean mutants, each from a separate mutagenic event, and all display some tolerance to

nitrate. Taken together, the results show that nitrate inhibition and autoregulation of nodulation are functionally if not generally linked. Mediation of the nitrate response may involve the autoregulatory shoot signal implicated by the data of Tables II and IV.

In contrast to the nts mutants, the inability of our non-nodulation mutant to nodulate is strictly determined by the root (Table V). Regardless of whether the grafted shoot was that of Bragg or nts382, mutant nod49 root stocks failed to develop nodules. Mutant nod49 itself seems to be blocked at the root hair curling stage (*hac*⁻) and no infection events are normally seen (14). Grafting mutant nod49 shoots onto Bragg roots did not alter nodulation significantly compared to Bragg controls (23 versus 26 nodules per plant) and mutant nod49 shoots inhibited nodulation on mutant nts382 roots to a similar extent as did Bragg shoots (69 versus 87 nodules per plant). Thus the ability of mutant nod49 shoots to autoregulate nodulation is unaltered. Instead, the root itself expresses the mutation which prevents bacterial invasion. Similar root control of non-nodulation was previously reported in a naturally occurring soybean line *rj*₁ (6).

The results from these simple grafting and hybridizing experiments demonstrate that the development of the soybean-*Bradyrhizobium* symbiosis is under the control of both shoot and root factors and that these factors interact. They furthermore indicate that the molecular analysis of the symbiosis should not only target onto the affected organ, but that other plant parts and perhaps commonly used developmental pattern and signals do play an important role in the regulation of the symbiosis (also see Ref. 8.).

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