

Communication

Cultivar-Specific Interactions of Soybean with *Rhizobium fredii* Are Regulated by the Genotype of the Root¹

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ABSTRACT

Rhizobium fredii USDA257 forms nitrogen-fixing nodules on soybean cultivar Peking, but not on cultivar McCall. This pattern of nodulation persists when McCall and Peking seedlings are cultivated together in plastic growth pouches. Reciprocal grafting experiments confirm that the root genotype, and not that of the shoot, regulates such cultivar specificity. When Peking roots are grafted onto McCall seedlings, the nodulation responses of roots similarly remain unaffected. Transposon-mutant 257DH4, which is derived from USDA257, can form nitrogen-fixing nodules on McCall. Such nodulation is blocked by the presence of USDA257 in the inoculum. Grafting experiments indicate that blocking is not due to a translocatable inhibitor produced by McCall roots or triggered by their interaction with USDA257. Thus, neither freely diffusible nor graft-transmissible substances are involved in cultivar-specific interactions of soybean with *R. fredii* and its derivatives.

Compatibility of *Rhizobium fredii* with soybean is strain and cultivar dependent (5, 11, 15). Primitive varieties, such as Peking, are nodulated widely, but many advanced cultivars remain nodule-free or form Fix⁻ nodules after inoculation with certain strains. One of these interactions involves *R. fredii* USDA257 and the improved cultivar, McCall. USDA257 curls McCall root hairs and induces foci of cortical cell divisions, but nodules are not formed (1, 3, 8–10). The incompatibility of USDA257 with McCall can be overcome by inactivation of a gene termed *nolB* and partially reversed by inactivation of another gene, termed *nolC* (3, 8; and HB Krishnan, L Meinhardt, SG Pueppke, unpublished data). Thus, mutant 257DH4 (*nolB*::Tn5) both nodulates and fixes nitrogen in combination with McCall; mutant 257DH5 (*nolC*::Tn5) produces nodules, but these fail to fix nitrogen.

Although the functions of the *nolB* and *nolC* gene products are unknown, nodulation of McCall by 257DH4 is acutely sensitive to the presence of USDA257 in the inoculum (1, 3). This has led us to hypothesize that the *nolB* gene product might interfere with nodulation of McCall and that this product, when supplied by cells of USDA257, is sufficient to

block nodulation by mutant 257DH4. Here, we report results of grafting studies and experiments in which McCall and Peking seedlings were cocultivated in plastic growth pouches. We wanted to know if the shoot or the root regulates the compatibility of soybean with USDA257 and to search for soluble and graft-transmissible substances that might function in this process.

MATERIALS AND METHODS

Seeds of soybean (*Glycine max* [L.] Merr.) cv McCall were from D. A. Whited, North Dakota State University, Fargo. Seeds of cultivar Peking were harvested from plants grown at the University of Missouri Bradford Research Farm. *Rhizobium fredii* USDA257 was originally from H. H. Keyser, U.S. Department of Agriculture, Beltsville, MD; Tn5-mutant 257DH4 has been described (1, 3). The bacteria were maintained as glycerol stocks at -70°C and routinely cultured in liquid yeast extract-mannitol medium (21) at 28 to 30°C and 125 rpm. Cultures of the mutant always contained kanamycin at 100 µg/mL. Bacterial inocula were prepared as in earlier experiments (3).

The effect on nodulation of cocultivating soybean genotypes was tested hydroponically in autoclaved plastic growth pouches, under conditions where roots and root exudates mingled freely (18). Seeds were surface-sterilized and pregerminated on water agar for 2 d (18). Seedling radicles then were dip-inoculated with USDA257 (10⁸ cells/mL) and groups of three seedlings transferred to each pouch in various combinations as given below. The pouches were incubated as described (18), and nodules were enumerated on alternate days between d 8 and d 20 after inoculation.

Seeds for plants to be grafted were surface-sterilized (18) and planted individually in sterile, 4-cm diameter × 21-cm long ConeTainers (ConeTainer Nursery, Canby, OR) containing autoclaved vermiculite. After incubation for 6 d in a growth chamber at 400 µmol/m²·s and a 12-h photoperiod, 10-cm high seedlings were paired, and a longitudinal cut of about 1.5 cm was made into the lower hypocotyl of each, so that the epidermis and upper part of the cortex was removed. Incisions were immediately pressed together and the stems firmly attached to one another with Parafilm. Pairs of grafted plants then were returned to the growth chamber.

Reciprocal grafts were produced 8 d later by excising the unwanted shoot and root from each plant pair. The grafted plant that remained was immediately bagged to reduce desic-

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Table I. Nodulation of Soybean by USDA257 Depends on the Root Genotype

Nodules were enumerated 3 weeks after inoculation. Controls, which had been treated with phosphate buffered saline (3), remained nonnodulated. The data are from two experiments, each with 15 replications per treatment.

Treatment	Nodules/Plant
shoot/root	mean No. \pm SE
McCall/McCall	0
Peking/Peking	23.5 \pm 2.3
McCall/Peking	44.7 \pm 3.6
Peking/McCall	0

cation and returned to the growth chamber. These grafts were inoculated 2 d later by pipetting 2 mL of inoculum containing 10^8 bacteria/mL onto the vermiculite at the base of each plant. Nodules were enumerated 3 weeks later.

Modified approach grafts also were produced. In this case, just the unwanted shoot was removed after 8 d, leaving a root- and shoot-containing mother plant in one ConeTainer with a second, grafted root system in an adjacent ConeTainer. The shoot-containing mother plant was inoculated immediately as described above. The grafts then were returned to the growth chamber without bagging. The shootless root system was inoculated 1 d later as described above. Nodules were enumerated 3 weeks after the second inoculation.

RESULTS

Using plastic growth pouches, we failed to detect soluble signals, from either McCall or Peking, that influenced nodulation of the other cultivar. When grown between pairs of nodulated Peking seedlings, inoculated McCall seedlings remained nodule-free. Conversely, inoculated Peking seedlings contained equivalent numbers of nodules (an average of 3.9–4.1 per plant at 20 d), regardless of whether their companions were McCall seedlings or other Peking seedlings. Inoculation of the neighboring plants with USDA257 did not influence these results.

Table I contains data from reciprocal grafting experiments designed to distinguish between root and shoot control of compatibility with USDA257. It can be seen that vermiculite supports more nodulation than does the hydroponic pouch system. More importantly, formation of nitrogen-fixing nodules is specified by the genotype of the root and not that of

the shoot. Thus, Peking shoots did not override the natural incompatibility of McCall roots with USDA257, and conversely, McCall shoots did not prevent nodulation of Peking roots. Although replacement of the normal Peking shoot with a grafted McCall shoot actually increased nodule number, this is probably a physiological effect (11–13). McCall shoots (maturity group 00) flowered and began to set pods during the course of the experiments, while those of Peking (maturity group IV) remained vegetative.

Table II summarizes the results of a series of modified approach grafting experiments with plants containing two root systems of different genotypes. Data on lines 1 and 2 confirm that the root genotype specifies compatibility with USDA257 and further show that such compatibility is insensitive to graft-transmissible factors from the other genotype. Table II also contains data on nodulation blocking. Specifically, we tested the hypothesis that translocatable signals originating from the interaction of USDA257 and McCall can block nodulation of a grafted root system by mutant 257DH4. Comparison of lines 3 and 4 (Table II) shows that the ability of the mutant to nodulate the grafted root system is reduced little if any by inoculation of the mother plant by the blocking strain. In contrast, nodulation is reduced >95% if McCall is coinoculated with USDA257 and mutant 257DH4 (1, 3).

DISCUSSION

There is increasing evidence that development of the legume-*Rhizobium* symbiosis is regulated in part by plant-derived signals (7, 16). Using both plant cocultivation experiments and grafting approaches, we have sought to identify such signals in cultivar-specific interactions of soybean with *R. fredii*. We were unsuccessful in detecting substances that are excreted by one cultivar and that modify compatibility of the second cultivar with USDA257. Similarly disappointing results have been obtained in studies of supernodulating and nonnodulating genotypes (6, 17, 19), indicating that internal signals are likely to be of more significance in these interactions.

We examined the source and distribution of internal signals by a series of grafting experiments with McCall and Peking. Compatibility with *R. fredii* was specified in all cases by the root genotype. Thus, neither a grafted shoot of a second cultivar nor a graft union with a foreign root system, inoculated or uninoculated, changed the basic nodulation response to USDA257 or 257DH4. In soybean, strain specificity conditioned by the *Rj₂* gene also is controlled by the root (2), as

Table II. Nodulation of Approach-Grafted Soybean Plants

Groups of 15 mother plants per treatment were inoculated at time 0. Modified approach-grafted roots were inoculated 24 h later, and nodules were enumerated after 3 weeks.

McCall Mother Plant		Approach Graft		
Treatment	Nodules/Plant	Genotype	Treatment	Nodules/Plant
	mean No. \pm SE			mean No. \pm SE
Control	0	Peking	USDA257	26.3 \pm 1.8
USDA257	0	Peking	USDA257	27.9 \pm 3.8
Control	0	McCall	257DH4	16.9 \pm 2.1
USDA257	0	McCall	257DH4	11.3 \pm 1.6

is nonnodulation (4, 20). The opposite regulatory abnormality, supernodulation, is governed by the shoot (4), indicating that both shoot and root factors can play the deciding role in governing nodulation.

Our data have several implications for study of the cultivar specificity of USDA257 and for analysis of the blocking response. In both cases, the genetically conditioned responsiveness of root tissues to rhizobia appears to be the deciding factor in determining the outcome of the interaction. This suggests that key specificity events may be highly localized and relatively intractable—perhaps involving initiation of infection threads in root hairs or the trigger for organization of the nodule meristem (1, 14). In addition, the inability of USDA257 to interfere, from a distance, with nodulation of McCall by 257DH4, implies that blocking requires the direct participation of USDA257 cells.

LITERATURE CITED

- Balatti PA, Pueppke SG (1990) Nodulation of soybean by a transposon-mutant of *Rhizobium fredii* USDA257 is subject to competitive nodulation blocking by other rhizobia. *Plant Physiol* **94**: 000–000
- Caldwell BE, Hinson K, Johnson HW (1966) A strain-specific ineffective nodulation reaction in the soybean (*Glycine max* L. Merrill). *Crop Sci* **6**: 495–496
- Chatterjee A, Balatti PA, Gibbons W, Pueppke SG (1990) Interaction of *Rhizobium fredii* USDA257 and nodulation mutants derived from it with the agronomically improved soybean cultivar McCall. *Planta* **180**: 303–311
- Delves AC, Matthews A, Day DA, Carter AS, Carroll BJ, Gresshoff PM (1986) Regulation of the soybean-*Rhizobium* nodule symbiosis by shoot and root factors. *Plant Physiol* **82**: 588–590
- Dowdle SF, Bohlool BB (1985) Predominance of fast-growing *Rhizobium japonicum* in a soybean field in the People's Republic of China. *Appl Environ Microbiol* **50**: 1171–1176
- Eskew DL, Schrader LE (1977) Effect of rj_1rj_1 (non-nodulating) soybeans on nodulation of near-isogenic Rj_1Rj_1 plants in nutrient culture. *Can J Microbiol* **23**: 988–993
- Gresshoff PM, Delves AC (1986) Plant genetic approaches to symbiotic nodulation and nitrogen fixation in legumes. In AD Blonstein, PJ King, eds, *Plant Gene Research III*. Springer-Verlag, Vienna, pp 159–206
- Heron DS, Ersek T, Krishnan HB, Pueppke SG (1989) Nodulation mutants of *Rhizobium fredii* USDA257. *Mol Plant-Microbe Interact* **2**: 4–10
- Heron DS, Pueppke SG (1984) Mode of infection, nodulation specificity, and indigenous plasmids of 11 fast-growing *Rhizobium japonicum* strains. *J Bacteriol* **160**: 1061–1066
- Heron DS, Pueppke SG (1987) Regulation of nodulation in the soybean-*Rhizobium* symbiosis. Strain and cultivar variability. *Plant Physiol* **84**: 1391–1396
- Keyser HH, Bohlool BB, Hu TS, Weber DA (1982) Fast-growing rhizobia isolated from root nodules of soybean. *Science* **215**: 1631–1632
- Lawn RJ, Bushby HVA (1982) Effect of root, shoot and *Rhizobium* strain on nitrogen fixation in four Asiatic *Vigna* species. *New Phytol* **92**: 425–434
- Lawn RJ, Brun WA (1974) Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. *Crop Sci* **14**: 11–16
- LeGal MF, Hobbs SLA (1989) Cytological studies of the infection process in nodulating and nonnodulating pea genotypes. *Can J Bot* **67**: 2435–2443
- Lin J, Walsh KB, Johnson DA, Canvin DT, Shugin W, Layzell DB (1987) Characterization of *R. fredii* QB1130, a strain effective on commercial soybean cultivars. *Plant Soil* **99**: 441–446
- Long SR (1989) *Rhizobium*-legume nodulation: Life together in the underground. *Cell* **56**: 203–214
- Matthews A, Kosslak RM, Sengupta-Gopalan C, Appelbaum ER, Carroll BJ, Gresshoff PM (1989) Biological characterization of root exudates and extracts from nonnodulating and supernodulating soybean mutants. *Mol Plant-Microbe Interact* **2**: 283–290
- Pueppke SG (1983) *Rhizobium* infection threads in root hairs of *Glycine max* (L.) Merr., *Glycine soja* Sieb. & Zucc., and *Vigna unguiculata* (L.) Walp. *Can J Microbiol* **29**: 69–76
- Sutherland TD, Bassam BJ, Schuller LJ, Gresshoff PM (1990) Early nodulation signals of the wild type and symbiotic mutants of soybean (*Glycine max*). *Mol Plant-Microbe Interact* **3**: 122–128
- Tanner JW, Anderson IC (1963) Investigations of non-nodulating and nodulating soybean strains. *Can J Plant Sci* **43**: 542–546
- Vincent JM (1970) *A Manual for the Practical Study of Root-nodule Bacteria*. Blackwell, Oxford