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A single dominant gene in McCall soybean prevents effective nodulation with *Rhizobium fredii* USDA257

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Summary

Rhizobium fredii USDA257 will effectively nodulate Asiatic and unimproved soybean cultivars, such as Peking, but most of the highly selected North American cultivars, such as McCall, produce at most rudimentary, ineffective nodules. In *R. fredii* USDA 257, a locus containing 6 open reading frames is responsible for this cultivar specific incompatibility. To examine the genetic control of incompatability on the part of the host, the soybean cultivars Peking and McCall were crossed to produce five F_1 progeny. These plants and their selfed progeny were tested for nodulation with USDA257. Resistance to nodulation was found to be conditioned by a single dominant gene. These results indicate that, in soybean, strain specific resistance to nodulation can result from 'gene(s)-for-gene' interactions.

Introduction

Bradyrhizobium japonicum (Buchanan) Jordan, and *Rhizobium fredii* (Scolla & Elkan) are compatible, symbiotic partners of soybean species, capable of inducing nitrogen fixing nodules. However, there are many examples of soybean cultivars (*Glycine max* (L.) Merr. that are ineffectively nodulated by specific strains of *B. japonicum* and *R. fredii* (Caldwell, 1966; Devine, 1984, 1987, 1988; Devine & Kuykendall, 1994; Devine et al., 1991; Ishizuka et al., 1991; Sadowsky & Cregan, 1992; Vest 1970; Vest & Caldwell, 1972). These cultivar-strain specificities resemble the cultivar-race interactions described for many plants and their microbial pathogens. Flor (1955) demonstrated that in the interaction between flax and the rust pathogen, *Melampsora lini*, restricted disease development, or incompatibility, required the presence of both a dominant avirulence allele in the pathogen and a dominant resistance allele in the host. One model of the molecular workings of these 'gene-for-gene' interactions invokes specific 'recognition' of the avirulence gene product by the product of the dominant resistance allele in the host, allowing a rapid defense response to be initiated (Gabriel & Rolfe, 1990). In those cases where a gene pathway is responsible for the production of the avirulence product, a 'gene(s)-for gene' interaction may occur. While several gene(s)-for-gene interactions have been identified in plant-pathogen combinations, in the *Rhizobium*-legume literature there are no examples of bacterial, cultivar specific avirulence genes that interact with dominant, strain specific resistance genes in the host.

A number of single dominant genes have been identified that cause soybean cultivars to 'resist' nodulation with specific strains (races) of *Bradyrhizobium japonicum*. Because the soybean cultivars sometimes develop rudimentary nodules, or cortical cell proliferations which lack the capacity to fix nitrogen, Vest et al. (1973) defined the phenotype of these interactions as ineffective or effective, rather than nod- versus nod+. The host genes have been designated *Rj2* (Caldwell, 1966), *Rj3* (Vest, 1970), and *Rj4* (Vest & Caldwell, 1972). The presence of a number of loci in soybeans that restrict nodulation with specific strains suggests that these genes may allow cultivars to 'recognize' incompatible strains.

Many soybean cultivars are ineffectively nodulated by some strains of the fast growing soybean symbiont, *Rhizobium fredii*. The ineffectively nodulated cultivar

Kent has been shown to possess a single dominant gene, *Rfg1*, that prevents nodulation with USDA205 (Devine, 1984; Devine & Kuykendall, 1994). However, the genetic determinants of incompatibility in the bacterial partner have not been investigated.

The cultivar McCall is not ineffectively nodulated by *Rhizobium fredii* USDA257, but is effectively nodulated by *R. fredii* USDA191, and both strains can effectively nodulate the cultivar Peking, exemplifying the specificity between particular cultivars and strains (Heron & Pueppke, 1984). A genetic locus has been identified in *R. fredii* USDA257 that is involved in the cultivar specific incompatibility, or avirulence response, with McCall soybean (Heron et al., 1989). The locus, located on the *sym* plasmid, consists of 7 open reading frames, designated *nolB, nolT, nolU, nolV, nolW, nolX*, and ORF4 (Meinhardt et al., 1993). With the exception of ORF4, inactivation of any of these open reading frames extends the host range of USDA257 to include fully effective nodulation of McCall, with no pleiotropic effects detected. Quite possibly all of these genes are part of a pathway required for production and excretion of a cultivar specific 'avirulence' product. Here, the genetic loci in the bacterial symbiont have been partially characterized, but the host genetics remains undefined.

In this report, crosses between McCall and Peking have been analyzed for their ability to effectively nodulate with USDA257.

Materials and methods

Bacterial strains *Rhizobium fredii* USDA205, USDA257 and a Tn-5 derived mutant designated USDA257-DH5 (*nol-B*), were obtained from Dr. Steven Pueppke, University of Missouri. Bacteria were cultured in YEM broth (Vincent, 1970) at 30° and 125 rpm. Inoculum was prepared by centrifuging the bacterial cells, at 5,000 G for 10 min, and resuspending to a density of approximately 10^8 per ml in Jensen's N-free media (Vincent, 1970). Seeds of soybean cultivars McCall and Peking were obtained from Dr. Steven Pueppke, University of Missouri. Seeds of cultivar Kent were obtained from the USDA-ARS Soybean Research Lab, Urbana, IL. Crosses between the parental lines were performed in a greenhouse, with McCall serving as the male parent in all cases. Five F_1 seeds were produced. The five hybrids were screened for nodulation phenotype with both *R. fredii* USDA191 and USDA257. Concurrent screening of the F_1 hybrids was accomplished through inoculation of rooted stem cuttings; stem sections, with a single leaf attached, were rooted in sterile water and then planted in sterile pots filled with medium grade vermiculite. Replicate pots were drench inoculated with prepared bacterial suspensions. Seedlings of the F_2 and F_3 generations were also scored for nodulation with USDA257, and selected F_3 populations were scored for nodulation with *R. fredii* USDA205. All soybean seeds were surface sterilized as previously described (Pueppke, 1983) and planted, in groups of 8 to 10, into sterilized 8 inch plastic pots filled with medium grade vermiculite. Seedlings were inoculated 2 days after planting by thorough drenching with a bacterial suspension.

Plants were uprooted and scored after 28 days growth in the greenhouse. Phenotypes were scored as effectively nodulated when at least one leghemoglobin containing nodule was present. Ineffectively nodulating plants were distinctly chlorotic. Parental lines and uninoculated controls were included in all experiments.

Results

All hybrid progeny exhibited the nodulation phenotype of McCall following inoculation with *R. fredii* USDA191 (nod+) and *R. fredii* USDA257 (nod-), when tested separately by leaf cutting inoculation. The five F_1 plants were selfed to generate F_2 populations, and these were screened for nodulation phenotype with USDA257. The chi-square probability of the pooled populations was 0.5–0.3 (Table 1), supporting the hypothesis that a single dominant gene is segregating in this generation. Analysis of 55 F_3 families, generated from randomly selected F_2 seed, produced a chi-square probability of 0.70, further supporting the hypothesis of a single dominant gene (Table 2). The 31 segregating F_3 families fit a 3 : 1 ratio of ineffective : effective when scored individually, generating a chi-square probability of 0.4–0.3 (Table 3). These results support the conclusion that a single dominant gene is present in McCall which prevents effective nodulation with *R. fredii* USDA257.

In addition to *R. fredii* USDA257, other *R. fredii* strains, including USDA205, are unable to nodulate McCall. An additional nodulation test was performed to determine whether USDA205 might interact with the same dominant resistance gene in McCall. Seedlings from nine F_3 families that had been determined to be homogenous for effective or ineffective nodula-

Table 1. Phenotypic segregation of F_1 and F_2 progeny of McCall × Peking crosses, after inoculation with *Rhizobium fredii* USDA257

	Phenotype		Expected		X^2	P
	Effective	Ineff.	Effective	Ineff.	(3 : 1)	
McCall	1	148				
Peking	121	0				
F_1	0	5				
F_2 A	37	96	33.25	99.75	0.42	0.70–0.50
B	38	117	38.75	116.75	0.002	1.00
C	36	116	38	114	0.078	0.90–0.70
D	42	100	35.5	106.5	1.35	0.30–0.20
E	55	147	50.0	151.5	0.422	0.70–0.50
Pooled F_2	208	577	196.25	588.75	0.861	0.50–0.30

Table 2. Nodulation phenotype of F_3 lines of McCall × Peking after inoculation with *Rhizobium fredii* USDA257

F3 Phenotypes	No. Lines	
	Obs.	Exp.
Nonsegregating effective	11	13.75
Segregating; effective and ineffective	31	27.5
Nonsegregating ineffective	13	13.75

X^2 (1 : 2 : 1) = 0.70.
P = 0.70.

Table 3. Chi-square test of nodulation response of segregating F_3 families of McCall × Peking after inoculation with *Rhizobium fredii* USDA257

31 Pooled Families	No. Lines	
	Obs.	Exp.
Effective	203	190.5
Ineffective	559	571.5

X^2 (3 : 1) = 1.01.
P = 0.40–0.30.

tion phenotype with USDA257 were inoculated with USDA205. A total of 510 seedlings were inoculated, 255 with USDA257, and 255 with USDA205. In all cases those families that were uniformly nodulated by USDA257 were also uniformly nodulated by USDA205, and those uniformly resistant to nodulation by USDA257 were completely resistant to nodulation by USDA205. If nodulation of McCall by USDA205 and USDA257 were restricted by two independently segregating genes the probability of a particular F_3 family being uniformly nod+ or nod- with both USDA257 ahd USDA205 is only 0.25. The probability of all nine families being equivalent in phenotype with the two strains is then 0.25^8, assuming independent, unlinked genes. These results suggest that, instead, McCall carries a single gene, or locus, that restricts nodulation by both USDA257 and USDA205.

To test whether the cultivar Kent restricts at least some *R. fredii* strains due to the presence of the avirulence locus identified in USDA257, seedlings of Kent were inoculated with either *R. fredii* USDA257 or the *nol*-B mutant USDA257-DH5. Six of six seedlings were ineffectively nodulated by USDA257, and six of six were effectively nodulated by USDA257-DH5.

Discussion

Analysis of progeny from crosses between Peking and McCall indicates that McCall carries a single, dominant gene conditioning resistance to nodulation with USDA257. In soybean, six genes that control nodulation phenotypes have previously been identified. In three cases, Rj1 (Pueppke & Payne, 1987) Rj5 and Rj6 (Pracht et al., 1993), the alleles that inhibit nodulation are recessive, and are not strain specific. Dominant alleles that block nodulation include Rj2 (Caldwell, 1966), Rj3 (Vest, 1970) and Rj4 (Vest & Caldwell, 1972). In each case these alleles interact with specific strains of *Bradyrhizobium japonicum*. The gene iden-

tified in McCall is unlikely to be an allele of any of these genes.

Recently, in the cultivar Kent, Devine and Kuykendall (1994) have identified *Rfg1*, a gene whose dominant allele interacts specifically with *Rhizobium fredii* strain USDA205 to condition ineffective nodulation. This raises the question; does McCall carry the dominant *Rfg1* allele, and is it responsible for restricting nodulation with USDA257? The fact that nine F_3 families reacted identically to inoculation with either USDA205 or USDA257 suggests that a single locus conditions incompatibility with both strains. While it is possible that two closely linked loci are present in McCall which independently restrict nodulation with these strains, there is no instance of linkage among the other nodulation genes identified in soybean (Devine & O'Neill, 1989; Devine & Kuykendall, 1994). We have shown that Kent is ineffectively nodulated by USDA257 and effectively nodulated by the *nol*-B mutant, USDA257-DH5. Lastly, all tested *R. fredii* strains, including USDA205, contain DNA sequences with homology to the USDA257 *sym* plasmid locus identified as being responsible for the inability of this strain to effectively nodulate McCall (Meinhardt et al., 1993). Thus, it appears that the *Rfg1* locus is common to both cultivars Kent and McCall and that the avirulence locus identified in USDA257 interacts with this locus in both cultivars in a gene(s)-for-gene like manner. The widespread incompatibility between advanced North American soybean cultivars and *R. fredii* strains (Devine, 1985; Balatti & Pueppke, 1992) may be largely due to this particular genetic interaction.

The intriguing presence of a 'gene(s)-for gene' interaction between soybean and *Rhizobium fredii* may represent a relic from the evolution of pathogenesis into symbiosis. Alternatively, it may reflect similar selection pressures, involving similar molecular mechanisms, in the evolution of symbiosis and pathogenesis (Devine, 1988).

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