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Research Notes

Supernodulating Soybean Mutant Alleles, *nts382* and *nts1007*, Show No Recombination with the same Restriction Fragment Length Polymorphism Marker

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Our linkage analysis with restriction fragment length polymorphism markers showed that the two supernodulating mutants, *nts382* and *nts1007*, have mutations at the same locus; this supports genetic complementation data (A. C. Delves, B. J. Carroll, and P. M. Gresshoff, J. Genet. 67:1-8, 1988).

Additional keywords: allelism, genome, nitrate-tolerant symbiosis.

Several supernodulating soybean mutants were isolated from the cultivar Bragg (*Glycine max* L. (Merr.)) background through ethyl methane sulfonate mutagenesis (Carroll *et al.* 1985a,b; Gresshoff and Delves 1986). After separate M2 family analysis and M2 segregation, each of these symbiotic mutants was judged to be the result of a separate mutational event. These supernodulating *nts* (nitrate-tolerant symbiosis) mutants exhibit increased nodulation (up to 40 times) compared to their wild-type progenitor, Bragg, even in the presence of nitrate. Similar mutants were isolated by Buzzell *et al.* (1990), Gremaud and Harper (1989), and Akao and Kouchi (1992).

When the supernodulating mutants were crossed with the wild-type cultivar Bragg, all F1 plants exhibited wild-type nodulation, indicating that the *nts* gene is recessive (Delves *et al.* 1988). The *nts* locus in the F2 population segregated as a single Mendelian gene (three wild-type/one supernodulating). Complementation studies were conducted on nine of these supernodulating mutants; crosses were made in a diallel pattern (Delves *et al.* 1988). No complementation was noted when the supernodulating *nts* lines were intercrossed; all F1 plants were supernodulating. This suggested that the same gene was affected in each line. One of the problems with interpreting these results was the inability to distinguish between the self- and cross-fertilized seeds that produced F1 plants. There were no morphological markers available to confirm that one-half of the genotype from the male parent had been incorporated through the cross. Accordingly, the conclusion of allelism was based on a probabilistic argument; the failure to complement was noted in all nts1007 × nts382 supernodulating soybean crosses, whereas the frequency of cross-fertilization was relatively high (as judged in parallel crosses of identical pollen with wild-type tester plants). Restriction fragment length polymorphisms (RFLPs) provide molecular markers that may be used as genetic markers and may establish linkage between a phenotype and a particular banding pattern (Keim *et al.* 1990, 1989).

Recently, we showed through analysis of an F2 population from the supernodulating mutant, nts382, that the genetic locus controlling supernodulation in soybean cosegregates tightly with the cloned molecular marker pUTG-132a (Landau-Ellis *et al.* 1991). Here, we extend these studies to another independently isolated *nts* mutant, nts1007, and lend molecular mapping support to the complementation studies of Delves *et al.* (1988) by showing that the *nts382* and *nts1007* alleles cosegregate at the same frequency with the same molecular marker.

RFLP analysis was done on the F2 populations of the supernodulating mutants (nts382 and nts1007) crossed with a distantly related soybean line *G. soja* Siebold & Succ. (PI468.397). Crosses were made under field conditions in the summer of 1988. In each case, *G. soja* with purple flowers, small black seeds, and vining growth habit was used as the male parent. The female parents, mutants of the cultivar Bragg, had white flowers, buff-colored seeds, and an upright growth habit. This wide cross provided indicators for confirming a successful cross. The F1 seeds were harvested and grown in the greenhouse in the fall. Plants were confirmed as true F1 hybrids by their intermediate growth habit, purple flower color, and heterozygous RFLP patterns. These plants were grown to produce F2 seed.

A population of 82 F2 plants was grown from the nts382 × *G. soja* cross (C16). Twenty of these plants had the supernodulating phenotype. A population of 57 F2 plants was grown from the nts1007 × *G. soja* cross (A3). Fifteen of these plants had the supernodulating phenotype, which confirms the Mendelian inheritance pattern seen for nts382. DNA from each of the supernodulating segregants was purified (Dellaporta *et al.* 1983), restricted with appropriate endonucleases (known to generate polymorphisms), and separated by agarose gel electrophoresis (5 μg of DNA

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per lane, 0.9% agarose, 1× TAE electrophoresis buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0), 35 V, 15 hr). The DNA from the gel was then transferred onto Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA) via vacuum blotting (LKB VacuGene; LKB, Bromma, Sweden). Membranes were probed with radioactively ([α-^{32}P]dCT, random primer method; Boehringer Mannheim, Indianapolis, IN) labeled inserts from clones known to be polymorphic between the two parents. Hybridizations were performed at 60° C, according to Zeta-Probe instructions (Bio-Rad). Membranes were exposed to Kodak X-Omat AR film from 24 to 72 hr at −70° C.

Probes were made from plasmid clones (Iowa State University), which detected polymorphisms between the experimental line A81-356022 (*G. max*) and PI468.916 (*G. soja*). We generated inserts by digesting genomic soybean DNA (*G. max*) with *Pst*I and cloning the 0.5- to 3-kb fragments into the vector pBS+ (Stratagene Inc., La Jolla, CA). These probes make up a large portion of a linkage map that currently covers 30 linkage groups, 293 loci, and 2,400 centimorgans (cM).

We found that both nts382 and nts1007 supernodulating phenotypes were linked to several markers in a region of tentative linkage group E; this suggests that they represent mutations at the same (*nts*) locus. Recombination fractions were estimated by the method of maximum likelihood (Weir 1990).

There was approximately 10% recombination between the *nts* locus and probe pA-36 (Table 1). The insert from probe pA-36 is approximately 1,300 bp and detects a polymorphism between *G. max* (Bragg and mutants) and *G. soja* PI468.397, when DNA is digested with *Hin*dIII. The autoradiographs (Landau-Ellis *et al.* 1991) of hybridizations reveal that *G. soja* and *G. max* share a common band of 6.8 kb. The polymorphic bands are 2.2 and 3.5 kb for *G. soja* and *G. max*, respectively. The heterozygotes exhibit all three bands.

No recombination was detected between the probe pUTG-132a and the *nts* locus for our sample sizes (Table 1). This probe contains an insert of approximately 1,750 bp and is polymorphic for our DNA samples when restricted with the enzyme *Dra*I. In the autoradiographs (Landau-Ellis *et al.* 1991), *G. soja* exhibits one intense band at approximately 2.6 kb. *G. max* shows three bands: one intense band at 2.9 kb and two faint bands at 2.6 kb and 500 bp. All probes tested thus far show the same RFLP banding pattern for Bragg, nts382, and nts1007, emphasizing their common background.

We did not find recombination between probe pUTG-132a and the *nts* locus when 35 *nts* segregants were evaluated from a total F2 population of 139 plants (combined A3 and C16). This suggests close proximity of the marker to the *nts* locus.

Table 1. Ratios of restriction fragment length polymorphism banding patterns represented in the supernodulating segregants of the F2 populations of two crosses C16 (nts382 × *Glycine soja*) and A3 (nts1007 × *G. soja*)

	Banding patterns[a]		
Probe	C16 supernodulators	A3 supernodulators	Percentage of recombination[b]
pA-36	15:4:0	13:1:1	10 ± 4
pUTG-132a	20:0:0	15:0:0	0 ± 4

[a] Banding patterns are represented in ratios of *G. max* type/heterozygous/*G. soja* type. Only 19 of the C16 plants are represented for pA-36 because one slower maturing plant was not scored.

[b] Percentage of recombination is expressed as the maximum likelihood estimate ± the standard error.

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