

## Inheritance of Promiscuous Nodulation in Soybean

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### ABSTRACT

The development of functional root nodules resulting in  $N_2$  fixation in soybean [*Glycine max* (L.) Merr.] can be induced by two strains of rhizobia, *Bradyrhizobium japonicum* (*B. japonicum*) and *Bradyrhizobium* spp. (cowpea-type). Genetic control of response to each type has been recognized in soybeans with two categories identified after inoculation with cowpea-type rhizobial strains: (i) promiscuous, which produces functional nodules,  $N_2$  fixation, and green leaves; and (ii) nonpromiscuous, which forms no or nonfunctional nodules and yellow leaves. Using leaf color, segregation patterns indicated that nonpromiscuity was dominant with two alleles segregating at each of two independent loci. With this genetic model, the expression of promiscuity requires the presence of both recessive alleles at each locus. Since the cowpea strain is indigenous to the soils in many tropical areas, especially Africa, the development of promiscuous soybean cultivars would greatly increase soybean production without commercial seed inoculation.

SOYBEAN SYMBIOTICALLY associates with rhizobia to form root nodules that fix  $N_2$  thereby dispensing with the need for application of nitrogenous fertilizers. Two types of rhizobial inoculants, namely *B. japonicum* and *Bradyrhizobium* spp. (cowpea-type), are capable of establishing this symbiotic relationship. Genetic variation for the response to cowpea-type rhizobia has been reported with two responses observed: (i) promiscuous, which forms functional nodules capable of  $N_2$  fixation after cowpea-type rhizobial inoculation and has green leaves without nitrogen fertilization; and (ii) nonpromiscuous, which forms nonfunctional or no nodules after cowpea-type rhizobial inoculation and has yellow leaves without nitrogen fertilization (Gwata et al., 2003; Pulver et al., 1985). In many tropical soybean production areas, nonpromiscuous genotypes are usually inoculated with compatible commercial inoculants of rhizobia (*B. japonicum*) to develop functional nodules. However, in many of these soybean production regions, particularly Africa, commercial *B. japonicum*-type rhizobial inoculants and nitrogenous fertilizers are not readily available. Cowpea-type rhizobia, but not *B. japonicum*, are indigenous or ubiquitous in tropical soils in Africa (Kueneman et al., 1984), making the use of promiscuous cultivars very desirable since inoculant or nitrogen fertilizers are not necessary for maximum production. Therefore, improved soybean cultivars selected for promiscuous nodulation with indigenous rhizobia would be useful to improve production with minimum cost. Knowledge of the promiscuity trait is essential to efficiently introgress promiscuity

into nonpromiscuous lines with superior agronomic performance (Kueneman et al., 1984).

Information on the inheritance of the promiscuous trait is very limited. The trait was initially observed in the cultivar Herton 147 (Corby, 1967) that had been released in 1942 for commercial fodder production in Zimbabwe (Gwata and Nziramasanga, 2001). This observation suggested that Herton 147 was segregating for the promiscuous trait. In a study conducted in uninoculated sandy soils in the field, segregation was observed in a cross between promiscuous and nonpromiscuous genotypes with no genetic analysis reported (Kueneman et al., 1984). Inheritance studies using ureide accumulation (Kueneman et al., 1984) and acetylene reduction (Chowdhury and Doto, 1981) screening techniques for promiscuous nodulation also obtained inconclusive results. Based partly on these observations, it was concluded that promiscuous nodulation in soybean was probably regulated by a few major genes (Kueneman et al., 1984). The objective of this study was to determine the inheritance of promiscuous nodulation using leaf color as a measure of  $N_2$  fixation effectiveness.

### MATERIALS AND METHODS

Seeds of a nonpromiscuous cultivar Soma used in this study were obtained from the Crop Breeding Institute, Harare, Zimbabwe. Soma has white flowers with the hilum black in color. Seeds of a promiscuous cultivar SamSoy were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria. SamSoy has purple flowers and a brown hilum color. Purple flower color is dominant over white flower color (Hartwig and Hinson, 1962). Black hilum color is dominant over brown (Specht and Williams, 1978). Both traits were used to validate cross-pollinations.

In 1999, both the reciprocal crosses of the nonpromiscuous  $\times$  promiscuous parents were produced in the greenhouse. In 2000, the  $P_1$ ,  $P_2$ , the backcrosses of both reciprocal  $F_1$  populations [ $BC_1(P_1) = F_1(\text{female}) \times P_1$  and  $BC_1(P_2) = F_1(\text{female}) \times P_2$ ], and  $F_2$  populations were produced. The  $F_2$  population of each reciprocal cross was obtained by mixing the seed from at least 19  $F_1$  plants. The seeds of each generation (except for the backcrosses) were then divided into two portions. In July 2001, the seeds (from the first portion) of all populations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ , and first backcrosses) of the crosses were planted in Conetainers (Stuewe and Son, Inc., Corvallis, OR) (4.0-cm diameter by 20.5-cm depth) filled with sterile washed sand in the greenhouse at the University of Florida, Gainesville, FL, with day and night temperatures of approximately 28 and 20°C, respectively, without supplemental light. This set of genetic materials constituted Experiment 1. At planting, seeds of all populations were inoculated with a cowpea-type rhizobial strain AB-16D.

The rhizobial strain, AB-16D, was obtained from NifTAL, Paia, HI. This strain was known to form effective nodules with the promiscuous cultivar SamSoy but not with the nonpromis-

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**Abbreviations:** BC, backcross; F, filial generation; P, parent; YMA, yeast-mannitol agar.

cuous cultivar Soma (Gwata et al., 2004). The strain was maintained in the laboratory by subculturing in petri dishes periodically (every 60 d) on yeast-mannitol agar (YMA) growth medium reported by Vincent (1970). The petri dishes (15 by 100 mm) containing the YMA growth medium were sealed, inverted, and incubated at room temperature after streaking and inoculating them with rhizobia.

The inoculum was prepared by excising (under sterile conditions) two pieces of agar (20 by 15 mm) supporting rhizobial colonies from the subculturing dishes that were then placed in bottles containing 300 mL sterile distilled water. Two drops of the surfactant Tween 80 (polyoxyethylene sorbitan monooleate) (Aldrich Chemical Company, Milwaukee, WI) were added to each bottle before shaking vigorously to disperse the rhizobial cells.

One week after emergence, 0.1 g of microelement fertilizer (3.72% Fe, 9.28% Mg, 0.002% Mo, 2.32% Mn) was applied to each plant. Two weeks after emergence and at weekly intervals thereafter, 0.1 g of N-free (0–10–20) fertilizer was applied to each plant.

Six weeks after emergence, the leaves of each plant in each population were classified as either green (effective nodulation exhibiting  $N_2$  fixation) or yellow (no or ineffective nodules exhibiting absence of  $N_2$  fixation). In a previous study designed to examine the relationship between nodulation and leaf color in soybean, promiscuous genotypes exhibiting dark green leaves, formed effective (functional) nodules but the nonpromiscuous genotypes formed ineffective (nonfunctional) or no nodules resulting in distinctly chlorotic yellow leaves (Gwata et al., 2004). Similarly, a positive correlation between nodule dry weight and  $N_2$  fixation in soybean was reported (Nicolas et al., 2002).

In September 2001, the remainder of the seeds (second portion) of four populations ( $P_1$ ,  $P_2$ ,  $F_1$ , and  $P_2$ ) were planted (as Experiment 2) under the same growth conditions as described above. Similarly, the plants were classified 6 wk after emergence.

In each experiment, each genotype represented an experimental unit. Chi-square ( $\chi^2$ ) tests for goodness-of-fit (Steel and Torrie 1980) were applied using several phenotypic ratios which are associated with three genetic models (3:1 = 2 alleles, 1 locus, dominant gene action; 13:3 = 2 alleles, 2 independent loci, dominant gene action and epistasis between loci and 15:1 = 2 alleles, 2 independent loci, dominant gene action and function).

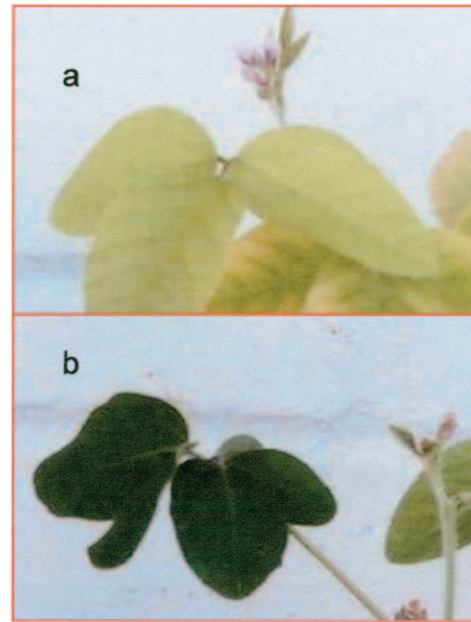
## RESULTS

The leaf color differences between the promiscuous and nonpromiscuous were very distinct and easily classified with the promiscuous, green and the nonpromiscuous, yellow (Fig. 1).

No segregation for nodulation was observed in the parental populations (Table 1). No differences in dominance expression were present between the reciprocal crosses of SamSoy and Soma with nonpromiscuity dominant in both.

In each experiment involving each reciprocal cross, the  $F_2$  segregation pattern statistically fit a 15:1 ratio (Table 2). The absence of heterogeneity between experiments and the pooled data results further confirmed this conclusion that the data fitted a 15:1 ratio indicating that this trait was controlled by the segregation of two alleles at two independent loci with dominant gene action and function.

A relatively small backcross population was available



**Fig. 1.** Differences in leaf color (at 6 wk after germination) between (a) nonpromiscuous (with ineffective or no nodules) and (b) promiscuous (with effective nodules) soybean parental genotypes grown in N-depleted medium inoculated with the cowpea-type rhizobial strain AB-16D.

for testing the genetic models. In the backcross population involving the nonpromiscuous parent, all the 52 plants observed were consistently yellow and no segregation was observed (Table 3). However, the genetic segregation pattern for promiscuous nodulation in the backcross population involving the promiscuous parent indicated the expected 3:1 ratio (Table 3).

## DISCUSSION

The results of the genetic analysis of the  $F_2$  segregation pattern indicated that promiscuous nodulation is controlled by two alleles at each of two independent loci with a dominant gene action and function. Therefore, green ( $N_2$ -fixing) genotypes would be double recessive. Any individual possessing at least one dominant allele at either locus would be nonpromiscuous. The results of the genetic analysis of the backcross population supported this genetic model for the control of promiscuous nodulation. The genes involved in controlling promiscuous nodulation are temporarily designated *gene 1* and *gene 2*.

Duplicate dominant gene inheritance is generally associated with species of tetraploid origin such as wheat (*Triticum aestivum* L.) and peanut (*Arachis hypogaea* L.).

**Table 1.** Phenotype (based on leaf color) of parental and  $F_1$  reciprocal crosses of soybean populations grown in N-depleted medium inoculated with the cowpea-type rhizobial strain AB-16D.

Population	Number of plants		
	Yellow	Green	Total
$P_1$ (Soma)	20	0	20
$P_2$ (SamSoy)	0	24	24
$F_1$ (Soma $\times$ SamSoy)	19	0	19
$F_1$ (SamSoy $\times$ Soma)	26	0	26

**Table 2. Segregation pattern for promiscuous nodulation in soybean F<sub>2</sub> populations based on differences in leaf color of plants grown in N-depleted medium inoculated with the cowpea-type rhizobial strain AB-16D.**

Reciprocal cross <sup>†</sup>	Experiment	Number of F <sub>2</sub> plants			Ratio	X <sup>2</sup>	P value
		Yellow	Green	Total			
S × SS	1	23	3	26	3:1	2.51	0.200–0.1000
					13:3	0.89	0.500–0.0300
					15:1	1.16	0.300–0.2000
	2	76	6	82	3:1	13.67	0.001–0.0001
					13:3	7.03	0.010–0.0010
					15:1	0.16	0.700–0.5000
	combined	99	9	108	3:1	16.00	0.001–0.0001
					13:3	7.69	0.001–0.0001
					15:1	1.26	0.300–0.2000
	heterogeneity X <sup>2</sup>				3:1	16.18	0.001–0.0001
13:3					7.92	0.001–0.0001	
15:1					1.32	0.300–0.2000	
SS × S	1	65	4	69	3:1	13.57	0.001–0.0001
					13:3	7.60	0.010–0.0010
					15:1	0.31	0.700–0.5000
	2	105	6	111	3:1	22.73	0.001–0.0001
					13:3	12.97	0.001–0.0001
					15:1	0.13	0.900–0.7000
	combined	170	10	180	3:1	36.30	0.001–0.0001
					13:3	20.57	0.001–0.0001
					15:1	0.15	0.700–0.5000
	heterogeneity X <sup>2</sup>				3:1	36.30	0.001–0.0001
					13:3	20.57	0.001–0.0001
					15:1	0.44	0.700–0.5000
	pooled	272	16	288	3:1	58.07	0.001–0.0001
13:3					26.74	0.001–0.0001	
15:1					0.24	0.700–0.5000	

<sup>†</sup> S, nonpromiscuous cultivar Soma; SS, promiscuous cultivar SamSoy.

The tetraploid origin of the soybean genome ( $n = 20$ ), likely explains in part the involvement of duplicate factors in the control of the promiscuous nodulation trait. Soybean is considered to be a diploidized tetraploid with a putative diploid ancestor ( $n = 11$ ) that underwent an aneuploid loss (to  $n = 10$ ) followed by genome duplication (Shoemaker et al., 1996; Lackey, 1980). Moreover, two recessive alleles were previously reported to control other genetic traits including nonnecrotic resistance to *Cowpea chlorotic mottle virus* (Goodrick et al., 1991) and stem canker susceptibility [caused by *Diaporthe phaseolorum* (Cooke & Ellis) Sacc.] (Kilen et al., 1985) in soybean as well as bruchid (*Callosobruchus maculatus* Fabr.) resistance (Adjadi et al., 1985) in cowpea [*Vigna unguiculata* (L.) Walp.] ( $2n = 2x = 22$ ). There-

fore, duplicate inheritance in soybean for the promiscuous character is not unexpected.

Very limited information on the inheritance of the promiscuity trait is available. A study conducted in the field with an unknown and uncharacterized mixture of indigenous rhizobial strains reported 12% “well-nodulated” soybean plants were present in a F<sub>2</sub> population (Kueneman et al., 1984) suggesting digenic inheritance. The study reported in this paper was conducted using only one rhizobial strain under greenhouse conditions. Different rhizobial strains have been shown to produce different responses on promiscuous genotypes (Sanginga et al., 2000; Kueneman et al., 1984; Pulver et al., 1982; Gwata and Wofford, unpublished data, 2001). Therefore a mixture of rhizobial strains in the rhizosphere may be

**Table 3. Segregation pattern for promiscuous nodulation in soybean backcross populations based on differences in leaf color of plants grown in N-depleted medium inoculated with the cowpea-type rhizobial strain AB-16D.**

Reciprocal cross <sup>†</sup>	Experiment	Number of plants			Ratio	X <sup>2</sup>	P value	
		Yellow	Green	Total				
S × SS	BC <sub>1</sub> (S) BC <sub>1</sub> (SS)	1	18	18	–	–	–	
					3:1	0.16	0.700–0.5000	
					13:3	0.07	0.900–0.7000	
SS × S	BC <sub>1</sub> (SS)	1	22	29	3:1	7.10	0.010–0.0010	
					13:3	0.01	0.950–0.9500	
					15:1	0.55	0.500–0.3000	
	BC <sub>1</sub> (S)	1	34	34	–	–	–	
					3:1	0.17	0.700–0.5000	
					13:3	0.62	0.500–0.3000	
	combined (nonsegregating) combined (segregating)					15:1	22.83	0.001–0.0001
						–	–	–
						3:1	0.11	0.900–0.7000
						13:3	0.55	0.050–0.3000
					15:1	22.75	0.001–0.0001	

<sup>†</sup> BC, backcross; S, nonpromiscuous cultivar Soma; SS, promiscuous cultivar SamSoy.

more effective in the nodulation process and result in somewhat altered  $F_2$  ratios.

From a plant-breeding standpoint, the leaf color method used in this study for identifying promiscuous genotypes would be most rapid and least expensive compared with other frequently used methods such as acetylene reduction (Sinclair and Serraj, 1995; Giller, 1987; Chowdhury and Doto, 1981) or xylem ureide assay (Herridge and Rose 1994; Kueneman et al., 1984; McClure et al., 1980). The leaf color differences between promiscuous and nonpromiscuous genotypes are distinct and easily distinguished under the growth conditions described in this study. Therefore, the use of this method to score promiscuous genotypes growing under nitrogen-depleted conditions should be very rapid and effective. The method may be adequate in plant-breeding programs concerned with rapid-screening methods for promiscuous nodulation.

Since cowpea-type rhizobia are indigenous to the soils in many tropical areas, especially Africa, the development of promiscuous soybean cultivars would greatly increase soybean production without commercial seed inoculation. However, one of the major challenges in developing improved promiscuous soybean cultivars in the future may be in identifying genotypes that nodulate consistently across a wide range of soybean production regions, particularly in Africa.

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