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Identification of Promiscuous Nodulating Soybean Efficient in N₂ Fixation¹

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ABSTRACT

Many developing countries lack the facilities to produce and distribute high quality rhizobia inoculants for farmers who are interested in planting soybeans [*Glycine max* (L.) Merr.]. If soybean varieties were available that could nodulate effectively with the ubiquitous, cowpea-type rhizobia, farmers could successfully grow soybeans without inoculation or fertilizer N. When 400 diverse soybean lines were tested at five sites in Nigeria for the ability to nodulate with indigenous rhizobia, only 10 were highly promiscuous, that is, capable of forming an effective symbiosis at all sites. Some entries were rated as compatible with indigenous rhizobia at one or two sites but failed to nodulate profusely at the other locations. Twenty-two isolates from nodules collected from profusely nodulated soybean plants and three other isolates prepared from cowpea nodules, were used to inoculate the 10 most compatible selections from the previous trial and two U.S. varieties, 'Bossier' and TGM 294. 'Malayan', a local Nigerian cultivar, formed an effective symbiosis with 21 of 22 soybean isolates; nodule and shoot weights in each case being greater than or equal to inoculation with Nitragin multistrain inoculant. Other accessions that displayed high degrees of promiscuity were M-381, TGM 120, TGM 119, Indo 180, and Indo 243. Whereas, Bossier formed an effective symbiosis with only one of the isolates, and TGM 294 was compatible with only 2 of the 22 rhizobia isolates. The promiscuously nodulating soybeans identified in the screening trial were also compatible with at least two of the three cowpea isolates, but Bossier and TGM 294 were compatible with none of them. When the scion of Bossier on 'Jupiter' (both of which have high yield potential) was grafted onto the root stocks of 'Orba' or Malayan (Promiscuous nodulators) enough N was fixed to meet the requirements of high yielding genotypes. These results indicate that by genetically incorporating promiscuity into varieties with high yield potential one would not necessarily reduce yield potential.

Additional index words: Rhizobia, *Glycine max* (L.) Merr., Microbiology

DEMAND is increasing in many tropical countries for soybean [*Glycine max* (L.) Merr.] products for animal and human consumption. Improved high-

yielding soybean varieties must be inoculated with *Rhizobium japonicum* to realize their yield potential on soils where soybeans have not been previously cultivated (1, 4, 6, 8, 9), but many tropical countries do not have the facilities or trained personnel to produce high-quality inoculants. Importing packaged inoculants may solve the production problem, but equally serious problems of inoculant storage and distribution will remain.

A practical alternative to the use of inoculants in less developed countries may be the development of soybean varieties capable of forming an effective symbiotic relationship with indigenous rhizobia. Earlier work at the International Institute of Tropical Agriculture (IITA) has shown that some varieties from Nigeria, Tanzania, and Indonesia nodulate without inoculation in soils where the crop has never been cultivated (8). Similar observations have been recorded in Thailand (7) and in the USA (9). Thus, it appears that the ability to form effective symbiotic relationships with cowpea-type rhizobia does exist within the soybean germplasm. Soybean varieties that are compatible with a range of rhizobia are said to be promiscuous.

The objectives of this research were to identify soybean genotypes compatible with indigenous rhizobia in a range of tropical environments and to evaluate the efficiency of the symbiosis under greenhouse and field conditions.

MATERIALS AND METHODS

Screening of Germplasm

Field trials were conducted at five diverse environments in Nigeria, ranging from the high rainfall, acid-soil zone (4°N Lat) to the semiarid Northern Guinea Savannah (11°N Lat) (Table 1). All the soils used were low in N and had no history of soybean cultivation. The land was plowed, fertilized with 300 kg/ha single superphosphate and 100 kg/ha muriate of potash and harrowed.

Four hundred soybean accessions of diverse geographical origin were each planted in a single 4-m row with two replications at all sites. Ten plants were dug from each row at

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Table 1. Climatic conditions and soil properties of testing sites in Nigeria where soybean germplasm was examined for promiscuous nodulation.

Location	Latitude	Mean rainfall mm/year	Soil pH (H ₂ O)	Vegetation zone
Onne	4°51'	2355	4.3	Tropical rain forest
Yandev	7°23'	1288	5.1	Southern Guinea Savanna (Eastern Nigeria)
Ibadan	7°26'	1215	6.0	Transitional zone (forest- derived savanna)
Mokwa	9°18'	1110	5.6	Southern Guinea Savanna (Western Nigeria)
Funtua	11°38'	1005	5.8	Northern Guinea Savanna

† Soils at Onne are acidic and derived from coastal sandy sediments; soils from Yandev and Mokwa are loamy sand and derived from sandstone; soils from Ibadan are sandy loam and derived from basement complex rocks, mainly banded gneiss; soils from Funtua are of loamy texture and are derived from eolian drift.

60 days after planting (DAP) and evaluated for nodule number, nodule size, and plant vigor. Nodule number was rated on a scale of 1 to 3, with 1 = less than 10 nodules/plants, 2 = 10 to 20 nodules, and 3 = more than 20 nodules/plant. Nodule size was also rated on a 1 to 3 scale, with 1 = most nodules having less than 2 mm in diameter, 2 = most nodules 2 to 5 mm diam, and 3 = most nodules greater than 5 mm in diameter. Accessions with high nodule number and mass were further evaluated for "apparent nodule effectiveness" by examining the central bacteroid tissue for leghaemoglobin. Ten nodules from each of these accessions were rated on a 1 to 3 scale, with 1 = at least seven nodules devoid of leghaemoglobin (green center), 2 = even distribution of green and red central tissues, and 3 = bright red central tissue in at least seven nodules. For plant vigor the rating scale was 1 = stunted accessions with yellow leaves, 2 = plants intermediate in vigor with light-green leaves, and 3 = vigorously growing plants with dark green leaves.

Testing of Rhizobial Isolates

Twenty-five strains of indigenous rhizobia were tested for compatibility with 10 soybean hosts selected for broad-spectrum compatibility in the germplasm screening. A multistrain, commercial inoculant, Nitragin®, and a no inoculation treatment were used as controls. Inoculant treatments were also applied to two improved high-yielding varieties developed in the USA ('Bossier' and TGM 294) and responses to inoculation treatments were compared; Bossier is a selection from 'Lee' and TGM 294 is derived from a cross of Lee 68 × Hill and was selected in Mississippi, USA, and reselected in Nigeria. For inoculant production nodules were collected at each testing site from profusely nodulated accessions with dark green leaves. Nodules were placed in screw top vials containing silica gel. Rhizobia isolates were cultured according to standard procedures (10). Cultures were maintained on yeast extract monitol (YEM) plates or slants. Isolates were authenticated as rhizobia using cowpeas as the host plant in Leonard jar assemblies. The 22 isolates were prepared from soybean nodules (four from Onne, three from Ibadan, six from Mokwa, and nine from Funtua), together with three isolates from cowpea collected at Funtua). Prior to inoculation isolates were multiplied to approximately 10⁹ cells/mL in YEM broth.

Soil (Alfasol [Oxic Paleustalf, Egbeda Series] derived from banded gneiss) low in N was collected from the field and fertilized with 50 mg of P and K per kg of soil. The soil was air-dried, heat-sterilized for 7 days at 110°C and added to 20 cm greenhouse pots previously washed in 1% sodium

hypochlorite. Soybean seeds were surface sterilized by emersion in 0.1% sodium hypochlorite. After rinsing repeatedly in distilled water six seeds were planted in each pot. Pots were watered with distilled water and inoculated with various isolates of *Rhizobium* by adding 20 mL of broth to each pot 7 days after planting. Multiple indentations were made in the soil, and the inoculum was added to each indentation to ensure even distribution. Two replications of each treatment were arranged in a split-plot design with isolates of *Rhizobium* as main plots and varieties as subplots. A 1-m border separated the main plots to reduce contamination. The plants were thinned to two per pot at 10 DAP. The experiment was terminated 40 DAP and the shoots were dried at 80°C for 72 h. The roots were removed, the nodules collected, washed and dried at 80°C. Then shoot and nodule dry weights were recorded, and the data subjected to analysis of variance (ANOVA).

Grafting Experiment

Seed of Bossier and 'Jupiter' (two typical nonpromiscuous varieties) and of 'Orba' and 'Malayan' (both promiscuous) were surface sterilized and planted in sterile vermiculite. Grafting was done on 4-day-old seedlings using a technique similar to the "straw-band" procedure (3).

Bossier and Jupiter were used as scions and grafted onto their own root stocks and stocks of Orba and Malayan. Grafted plants were placed in an incubator at 15°C and 70% humidity for 2 days and then maintained at room temperature (20 to 25°C) away from direct sunlight for another 3 days. Survival rate was approximately 90%. Six healthy grafted plants of the same root-shoot treatment were transplanted into metal drums each containing 40 kg of soil. The soil (similar soil classification as described earlier) was collected from a farmer's field where soybeans had never been grown. The soil was fertilized with 50 mg of P/kg of soil, 50 mg of K, 25 mg of S, and 1 mg of B. The experiment consisted of 12 graft combinations grown in uninoculated soil and in soil inoculated with *R. japonicum* (Nitragin Co., Milwaukee, WI). Five replications of each treatment were harvested 50 days after transplanting; then shoot and nodule dry weights and shoot N content determined. Total N was estimated on ground samples as described by Farrari et al. (5). Five replications of each treatment were grown until maturity and seed yield measured.

RESULTS AND DISCUSSION

Screening Germplasm

The purpose of testing in five diverse environments with different native leguminous flora was to identify soybean germplasm that is compatible with a wide range of soil rhizobia. Cowpeas, which are compatible with a wide range of rhizobia, nodulate freely at all the testing sites.

Several accessions had poor stands in some sites and were deleted from the analysis at those sites. The distributions of the visual ratings for nodulation and plant vigor are presented in Table 2. Of the 400 accessions tested only 35, 20, 32, 20, and 36 accessions had many large, effective nodules and vigorous plant growth at Onne, Yandev, Ibadan, Mokwa, and Funtua, respectively.

There was considerable evidence of site-specific nodulation. Of the 35 accessions rated as compatible with rhizobia native to Onne, 17 did not nodulate in any of the other four environments. Four entries formed effective nodule masses at Onne and one other site; three entries nodulated at Onne and two other

Table 2. Distribution of 400 soybean accessions rated for nodule number, nodule size, and plant vigor at five diverse locations in Nigeria.

Location	Nodule number†			Nodule size‡			Plant vigor§		
	1	2	3	1	2	3	1	2	3
	%								
Onne	12	61	28	55	37	8	54	36	10
Yandev	40	45	15	55	40	5	43	50	7
Ibadan	47	45	8	52	40	8	28	60	12
Mokwa	40	52	8	60	35	5	65	30	5
Funtua	41	47	12	51	40	9	55	30	15

† Nodule number: 1 = < 10 nodules, 2 = 10–20 nodules, 3 = > 20 nodules/plant.

‡ Nodule size: 1 = < 2 mm, 2 = 2–5 mm, 3 = > 5 mm in diameter.

§ Plant vigor: 1 = yellow and stunted, 2 = light green and intermediate size, 3 = dark green with vigorous growth.

locations; and one accession nodulated at Onne and three other sites. At Yandev, 20 accessions were rated as compatible, but four of them nodulated only at Yandev and one other site. The results at Ibadan were similar: although 32 accessions were classified as compatible, four were specific to the Ibadan site, and 12 nodulated only at Ibadan and one other location. Twenty accessions were selected at Mokwa, but four of them nodulated only at Mokwa and one other site. At Funtua, 36 accessions appeared to be compatible with indigenous rhizobia, but seven nodulated only at Funtua and one other location. In summary, out of 400 accessions screened, only 10 entries were capable of forming effective symbiotic relationships with the soil rhizobia at all five locations. Three of these 10 selections are unimproved cultivars grown in Africa: 'Malayan' from Nigeria, 'Obo' from Central African Republic, and 'Hernon 237' from Tanzania. Four of the entries from Indonesia, Indo 180, Indo 216, Indo 226, and 'Orba', were also rated as compatible at all sites, but more than 70 other accessions collected in Indonesia failed to nodulate consistently. Two other promiscuously nodulating accessions (TGM 119 and TGM 120) were collected in East Africa, but their exact origins are unknown. The remaining accession (M-351) is a progeny of Malayan × 'Clemson Non-Shattering'. None of the 25 improved cultivars bred and selected in the USA were capable of nodulating well with the indigenous rhizobia at the various testing sites.

Testing of Rhizobia Isolates

The inoculant × host plant interaction, which reflects host-strain specificity, was highly significant ($P < 0.01$) for both nodule and shoot dry weights (Table 3). Nodule weight indicates the degree of compatibility between the host and the rhizobial isolate, and shoot dry weight is a measure of the effectiveness of fixing N_2 of the host-isolate combination. Malayan appeared to be the most promiscuous host in the test since it formed more nodule mass than the uninoculated control with all 22 soybean isolates tested and since 21 of these isolates were as effective as Nitragin as shown by shoot growth. TGM 579 (a progeny of Malayan × 'Clemson Non-Shattering') gave similar results; it was compatible with 21 out of 22 soybean isolates, and 16 of these had shoot growth equal to that of Nitragin. In contrast, Bossier and TGM 294

Table 3. Effect of inoculating sterile soil with 22 rhizobial isolates from promiscuous soybean varieties and three isolates from cowpea on nodule mass and shoot growth at 40 days of 10 soybean accessions selected for promiscuity and two non-promiscuous varieties (Bossier and TGM 294).

Soybean	No. of isolates producing nodule or shoot weights greater than the uninoculated control ($P < 0.05$)		No. of isolates producing nodule or shoot weights greater than or equal to inoculation with Nitragin ($P < 0.05$)	
	Soybean isolates† (n = 22)	Cowpea isolates (n = 3)	Soybean isolates (n = 22)	Cowpea isolates (n = 3)
Host	Nodule wt.	Shoot wt.	Nodule wt.	Shoot wt.
	wt.	wt.	wt.	wt.
Malayan	22	21	3	3
TGM 579	21	19	3	2
TGM 120	22	21	3	3
TGM 119	21	20	2	2
TGM 618	18	15	2	2
TGM 725	17	17	3	2
TGM 710	15	14	3	3
Orba	14	11	3	3
TGM 737	15	15	3	2
TGM 730	16	7	3	2
Bossier	12	4	1	0
TGM 294	10	3	1	0

† Source of inoculant: three were 22 isolates from soybeans (4 from Onne, 3 from Ibadan, 6 from Mokwa and 9 from Funtua). Three cowpeas isolates were from cowpea varieties, Vita 1, Vita 4, and Vita 5 at Funtua.

were clearly less compatible with these indigenous isolates than the other accessions. Bossier nodulated with 12 isolates; but only four of these resulted in shoot growth greater than that of the uninoculated control, and only one isolate increased the shoot growth of Bossier as much as did the Nitragin inoculant. Similar results were obtained when TGM 294 was the host. Bossier and TGM 294 nodulated with only one of the three isolates from cowpea nodules, and this symbiosis was ineffective. The promiscuous accessions, however, formed a significant nodule mass with at least two out of the three cowpea isolates (Table 3). Malayan, TGM 120, and Orba grew equally well when inoculated with all three cowpea isolates, as compared to inoculation with Nitragin.

It would be presumptuous to assume that the 22 soybean and three cowpea rhizobia tested are representative of the entire indigenous population of *Rhizobium* at the various testing sites. The number of isolates is large enough to provide convincing evidence that soybean genotypes differ in ability to form effective symbiotic relationships with rhizobia indigenous to these tropical soils. The accessions identified as promiscuous during the germplasm screening are clearly more compatible with these rhizobia than are Bossier and TGM 294.

Grafting Experiment

This study was conducted to determine if the promiscuous nodulating character(s) of the low yielding accessions were genetically transferred to the improved cultivars, would the resulting symbiosis provide sufficient N to realize high yield potential? To answer this question, shoots of high yielding varieties, Bossier and Jupiter, were grafted onto root stocks of Malayan and Orba (promiscuous varieties with low yield potential).

Table 4. Shoot dry weight, total N content and nodule dry weight at 50 days after transplanting and seed yield at maturity of inoculated (Nitragin) and uninoculated grafts using Bossier and Jupiter as scions and Orba and Malayan as root stocks.

Graft combination Scion/root	Inoculation treatment	Shoot dry wt. g/plant	N content mg/plant	Nodule dry wt. g/plant	Seed yield g/plant
Jupiter/Jupiter	-	12.8 a	385 a	110 a	17.0 a
Jupiter/Jupiter	+	17.5 b	531 b	538 b	22.3 b
Jupiter/Malayan	-	18.6 bc	593 b	486 b	22.8 b
Jupiter/Malayan	+	17.5 b	574 b	477 b	23.7 b
Jupiter/Orba	-	20.2 bc	626 b	728 c	21.4 b
Jupiter/Orba	+	21.3 c	643 b	715 c	22.1 b
Bossier/Bossier	-	8.9 a	259 a	85 a	15.8 a
Bossier/Bossier	+	18.1 d	660 d	622 d	23.7 bc
Bossier/Malayan	-	12.0 b	390 b	310 b	19.9 b
Bossier/Malayan	+	15.0 c	517 c	457 c	23.5 bc
Bossier/Orba	-	18.3 d	617 cd	684 d	24.5 c
Bossier/Orba	+	19.7 d	730 d	647 d	21.0 bc

* Means followed by unlike letters with the same scion are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Results from 12 graft combinations grown in soil containing only indigenous rhizobia or inoculated with *R. japonicum* are presented in Table 4. Bossier scions grafted onto their own root stocks responded to inoculation, as evidenced by an increase of 103% in shoot growth, 632% in nodule weight and 50% in seed yield. When Bossier was grafted onto root stock of Orba and grown in uninoculated soil, the shoot growth, N content, nodule weight, and seed yield were similar to those for Bossier grafted onto Bossier and inoculated. The treatments with Orba root stocks did not respond to inoculation. Grafts of Bossier scions and Malayan root stocks were difficult to perform because of the thick hypocotyl of Bossier and thin stem of Malayan. Vegetative growth of this combination was less than that of the homologous grafts (Bossier grafted onto Bossier), even in inoculated soils, so early growth may have been affected by the grafting. However, seed yield of Bossier grafted onto Malayan roots equaled that of Bossier grafted onto Bossier and inoculated. Grafts of Jupiter scions onto Malayan or Orba root stocks in uninoculated soil produced plants that grew and yielded as well as Jupiter-Jupiter grafts and grown in inoculated soil. It is evident from these studies that the symbiosis between Malayan or Orba and indigenous rhizobia can supply the N needed for agronomically superior plants to realize their full yield potential. Consequently, incorporation of the promiscuous nodulating character into high yielding varieties should result in N₂ fixation adequate for high yields without inoculants or N-fertilizer. In developing countries where cowpea-type rhizobia exist, use of improved promiscuous soybean varieties is likely to result in greater stability in production. If commercial inoculants fail the promiscuous varieties will still grow well.

Heritability studies on the promiscuity character are incomplete, but empirical observations indicate that the character(s) can be transferred genetically. Some national breeding programs have already developed promiscuous nodulating lines, albeit unintentionally. Breeders in Tanzania, who have used 'Hernon 271' as a parent because of its vigorous veg-

etative growth, have produced at least three lines with the promiscuity of Hernon 271 (2,8), while in a Nigerian program that utilized Malayan as a parent, it was observed that many of the progeny (M-381, M-79, M-90, M-216, and M-98) are highly promiscuous (D. Adedzwa, 1983 personal communication). Several progenies of crosses made in Australia involving the variety 'Gilbert' were found to be promiscuous in Zambia (F. Javaheri, 1981 personal communication).

We have made crosses between some promiscuous accessions identified in the germplasm screening (mainly Hernon 271, TGM 120, and TGM 119) and improved high yielding parents (Jupiter and Bossier). Selections were made in the F₂ generation at Mokwa, based initially upon agronomic characters, then at physiological maturity on nodule mass. When Bossier, which has very limited compatibility with diverse rhizobia, was used as a parent, only 13% of the F₂ progeny were well nodulated. When Jupiter, which is intermediate in promiscuity, was used as a parent, 33% of the F₂ progeny were well nodulated. Selections were made in the F₃ generation at Ibadan, again for agronomic characters and nodulation. Seeds (F₄) from these selection were planted at Mokwa and further selected for favorable agronomic traits and nodule mass. Seeds from the F₄ plants were increased at Ibadan, and F₅ bulk populations (with F₅ heterogeneity) were evaluated for yield and nodulation in multi-locality yield trials. The vast majority of the entries were highly promiscuous and gave vigorous plant growth even on low-N soil.

It is clear, then, that promiscuously nodulating soybeans with improved agronomic traits can be developed and that this material can be grown without inoculation with *R. japonicum*. Details on the results of breeding for promiscuity, including genetic studies, will be presented in other articles.

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