IDENTIFICATION OF PROMISCUOUS NODULATING SOYBEANS EFFICIENT IN N_2 FIXATION [1]

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ABSTRACT

Index words: rhizobia, inoculation with R. *japonicum*, host rhizobia compatibility.

A diverse soybean germplasm which included many local cultivars was tested for compatibility with rhizobia indigenous to diverse environments in Nigeria. Only 10 of 400 accessions screened were capable of forming an effective symbiosis at all testing sites. Some entries were rated as compatible with indigenous rhizobia at one or two sites but failed to nodulate profusely at the other testing locations. This indicates that the indigenous rhizobia population is probably different across environmental zones. This diversity must be recognized in order to prevent the development of soybean material that is site-specific for nodulation.

Rhizobia isolates were prepared from nodules collected from different soybean accessions grown in the previous screening trial. The 10 selections identified as promiscuous in the screening trial were more compatible with the rhizobia isolates than Bossier and TGM 294 (two U.S. bred lines). Malayan, a local Nigerian cultivar, formed an effective symbiosis with 21 out of the 22 isolates and the efficiency of the symbiosis was equal to inoculation with the commercial inoculant - Nitragin. Ohter accessions that displayed high degrees of promiscuity were TGM 579, 120, 119, 725, 710 and 737. In this greenhouse trial Bossier was capable of forming an effective symbiosis with only one of the isolates and TGM 294 was compatible with only 2 of the 22 rhizobia isolate. The promiscuous nodulating soybeans identified in the screening trial were also compatible with at least 2 out of 3 isolates prepared from cowpea nodules; whereas, both Bossier and TGM 294 failed to form an effective symbiosis with the isolates prepared from cowpea nodules.

Grafting the scion of Bossier or Jupiter (high yield potential varieties) onto the root stocks of Orba or Malayan (promiscuous nodulating varieties) revealed that the symbiosis formed between indigenous rhizobia and Orba and Malayan roots was capable of supplying sufficient N to support the requirements of high yielding genotypes. These results indicate that if promiscuity was genetically incorporated into high yield potential varieties, one would not necessarily sacrifice yield potential.

INTRODUCTION

There is an increasing demand for soybean products for both animal and human consumption in many tropical countries. At present, most of the demand for soybean concentrates is being fulfilled by importation resulting in the drain of already severly restricted foreign reserves. The successful production of soybean in the tropics is dependent upon being able to produce and process the crop cheaper than importing the processed products. Consequently, it is essential that the N required for high yielding soybeans be provided from biological N_2 fixation and not expensive nitrogenous fertilizers.

Numerous studies, both in temperate and tropical environments, have shown that improved high-yielding soybean varieties require inoculation with *Rhizobium japonicum* to realize their yield potential when grown in soils where the crop has not been previously cultivated (1, 4, 6, 9, 10). This imposes a serious constraint as most tropical countries do not have the facilities or trained personnel required for inoculant production and quality maintenance. Importation of packaged inoculums may provide a solution to the production problem but storage, distribution and education of the farmer present severe limitations.

A more practical alternative to the use of inoculums comprised of *R. japonicum* strains may be the development of soybean varieties that are capable of forming an effective symbiosis with indigenous rhizobia. Earlier work at this institute has shown that some local varieties from Nigeria, Tanzania and Indonesia can nodulate without the aid of an inoculant of *R. japonicum* in soils where the crop has never been cultivated (8). Similar observations have been recorded in Thailand (7) and in the U.S. (9). Thus, it appears that genetic diversity for the ability to form an effective symbiosis with diverse rhizobia species exists within the soybean germplasm.

The objectives of this research were to identify soybean genotypes that are capable of establishing an effective symbiosis with rhizobia indigenous to diverse tropical environments and evaluate the efficiency of the symbiosis under greenhouse and field conditions.

MATERIALS AND METHODS

Screening of germplasm: Field trials were conducted at five locations in Nigeria. The testing sites were chosen to represent diverse ecologies ranging from the high rain-fall acid-soil zone (4°N) to the semi-arid northern Sudan (11°N). Care was taken to select sites with soils low in N and without a history of soybean cultivation. At all sites, the land was plowed, fertilized with 300 kg/ha single superphosphate and 100 kg/ha muriate of potash and harrowed.

Approximately four hundred soybean accessions were selected, based upon origin, to represent a diverse germplasm. Each accession was planted in a single row 4 m long with two replications. At 60 days after planting (DAP), 10 plants were carefully dug from each row and visually evaluated for nodule number, nodule size and plant vigor. Nodule number was rated on a scale of 1-3 with 1= less than 10 nodules / plant; 2= 10-20 nodules and 3= more than 20 nodules/plant. Nodule size was also rated on a 1-3 scale with 1= most of the nodules were less than 2 mm in diameter; 2= nodules were 2-5 mm diameter and 3= nodules greater than 5 mm in diameter. Accessions with a nodule number rating of 3 and a nodule size rating of 2 or 3 were evaluated for nodule effectiveness by examining the bacteroid-central tissue for leghaemoglobin.. Ten nodules from the promising accessions were rated for effectiveness using a 1-3 scale with 1= at least 7 of the 10 nodules were devoid of leghaemoglobin (green center); 2= an even distribution of green and red central tissue and 3= at least 7 of the 10 nodules contained bright red central tissue. Plant vigor was also rated at 60 DAP on a 1-3 scale with 1= stunted accessions with yellow leaves; 2= plants intermediate in vigor with light green leaves and 3= vigorus growing plants with dark green leaves.

<u>Testing of rhizobia isolates</u>: Nodules were collected at each testing site from profusely nodulated accessions with dark green leaves in the germplasm screening. Nodules were placed in a screw top vial, dried over silica gel, and stored at 5°C in a dessicator until isolation. Rhizobia isolates were prepared according to standard procedures (11). Re-hydrated nodules were surface sterilizaed will 0.1% acidified mercuric chloride and rinsed repeatedly with sterile water. One nodule was crushed on a sterile slide and an extract (in some cases, the entire crushed nodule) streaked onto a yeast extract mannitol (YEM) agar plate. After the appearance of rhizobia colonies (5-10 days), an aliquot was streaked on YEM agar slants and retained as stock cultures. Isolates were tested for rhizobia using cowpeas as host plants in a Leonard jar assembly. Eighteen isolates prepared from soybean nodules and three isolates from cowpea nodules were multiplied in YEM broth for 12 days at which time there were approximately 10^9 cells/ml.

Soil low in N was collected from the field and fertilized with 50 ppm P and K. The soil was air-dried and then heat sterilized for seven days at 110° C. Three kgs of soil were added to sterile greenhouse pots (washed in 1% sodium hypochlorite). Soybean seeds were surface sterilized with 0.1% sodium hypochlorite and 6 seeds planted per pot. The pots were watered with distilled water and inoculated with various rhizobia isolates by adding 20 ml of broth to each pot at 7 days after planting. The inoculum was added to several holes in each pot to ensure an even distribution. The experimental design was a split-plot with rhizobia isolates as main plots and varieties as sub-plots. A 1 M area separated the main plots in order to reduce contamination. The pots were thinned to 2 plants at 10 DAP. The experiment was terminated at 40 DAP and the shoots dried at 80°C for 72 hrs. The roots were carefully removed and nodules collected, washed and dried at 80°C. Shoot and nodule dry weights were recorded and the data subjected to analysis of variance.

Grafting Experiment: Seeds of Bossier, Jupiter, Orba and Malayan were surface sterilized and planted in sterile vermiculite. Grafting was conducted 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 transferred to room temperature (20-25°C) but away from direct sunlight for another 3 days. Survival rate was approximately 90%. Six healthy, grafted plants were transplanted into metal drums containing 40 kg of soil. The soil was collected from a farmer's field where soybeans had never been grown. The soil was fertilized with 50 ppm P, 50 ppm K, 25 ppm S and 1 ppm B (wt/wt). The experiment consisted of 12 graft combinations grown in soil uninoculated and inoculated with *R. japonicum*. The inoculated treatments were prepared by mixing 5 g of the commercial inoculant (Nitragin) per pot in the surface 10 cm prior to transplanting. The plants were thinned to 4 plants/pot one week after transplanting. Five replications of each treatment were harvested at 50 days after transplanting for shoot dry weight, shoot N content and nodule mass. Total N was conducted on ground samples as described by Ferrari et al. (5). Another five replications of each treatment were grown until maturity for seed yield.

RESULTS AND DISCUSSION

Screening of Germplasm: The testing sites were selected to represent diverse ecological zones with distinct native vegetation. The rational for testing across environments was to insure that only accessions compatible with diverse rhizobia would be selected. The leguminous flora native or adapted to the various ecological zones is diverse; consequently, it is assumed that rhizobia compatible with these hosts and environments are also diverse. Cowpeas which are known to be compatible with a wide range of rhizobia species have been observed to nodulate freely at all testing sites.

The distribution patterns of the visual ratings for nodulation and plant vigor are presented in Table 1. In the humid, acid soil environment at Onne, approximately 100 accessions contained 20 or more nodules/plant; however, 70% of these selections had small nodules most of which were devoid of leghaemoglobin. Only, 35 accessions or 8% of the total population were selected for compatibility with rhizobia indigenous to the Onne environment. At Yandev, 60 accessions formed more than 20 nodules/plant but only 20 of these accessions contained large, effective nodules. There were 28 entries that did not display N deficiency symptoms but 8 of these selections were poorly nodulated indicative that they were extracting N from the soil and probably growing in small N pockets. Only 5% of the total population was considered as compatible with rhizobia indigenous to the Yandev area. The nodulation pattern at Ibadan differed from Onne and Yandev as all accessions that contained 20 or more nodules/plant also had large effective nodules. The absence of small, ineffective nodules on some of the accessions as observed at Onne and Yandw is unexplainable. Based upon plant vigor scores, 48 accessions were selected; however, 16 of them appeared to be escapes as they contained only a few, small nodules. At Ibadan, 8% or 32 accessions were rated as compatible with the indegenous rhizobia. The Mokwa testing site was the most severe as the screening was conducted on a sandy soil low in organic matter with a cation exchange capacity of less than two. Thirty-two accessions were profusely nodulated but only 20 of these entries contained large, effective nodules. Due to the low N status of the soil at Mokwa, all entries that were growing vigorously with dark green leaves also contained more than 20 nodules/plants with a diameter greater than 5 mm. At the Mokwa environment, only 5% of the accessions were capable of forming an effective symbiosis with the indigenous rhizobia. In the semi-arid region near Funtua, 12% of the population nodulated profusely but 25% of these accessions had small, ineffective nodules. Approximately, 15% or 58 entries displayed good shoot growth but only 36 of these selections were heavily nodulated with effective rhizobia. The large number of escapes i.e. 22 accessions may be due to the lack of uniformity of the soil at this site and a previous history of N fertilizer applications.

There is considerable evidence for site-specific nodulation. Of the 35 accessions that were rated as compatible with the rhizobia native to Onne, 17 of these did not nodulate at any of the other 4 environments. Four entries formed an effective nodule mass at Onne and one other site, 3 entries nodulated at Onne and 2 other locations and one accession nodulated at Onne and 3 other sites. At Yandev, 20 accessions were rated as compatible but 4 of them nodulated at only Yandev and 5 nodulated only at Yandev plus one other site. The results at Ibadan were similar; 32 accessions were selected but 4 were specific to the Ibadan site and 12 only nodulated at Ibadan and one other location. Twenty accessions were selected at Mokwa but 4 of them nodulated at Mokwa only and 5 more only formed a symbiosis at one other site plus Mokwa. At Funtua, 36 accessions appeared to be compatible but 7 nodulated at Funtua only and 13 others were compatible with rhizobia at Funtua and one other location. In summary, out of approximately 400 accessions screened only 10 entries were capable of forming an effective symbiosis at all 5 locations. Four of these 10 selections are local cultivars: Malayan from Nigeria, TGM 618 from Central African Republic, TGM 344 from Tanzania and Orba from Indonesia. Three more entries form an Indonesia collection (TGM 710, 725 and 730) were also rated as compatible at all sites but more than 70 other accessions collected in Indonesia failed to nodulate. Two other promiscuous nodulating accessions (TGM 119 and 120) were collected in East Africa but their origins are unknown. The remaining accession (TGM 579) is a progeny of Malayan. None of the 25 improved cultivars tested which were bred and selected in the U.S. were capable of nodulating with the rhizobia indigenous to the various testing sites.

Testing of rhizobia isolates

Twenty rhizobia isolates were tested for compatibility with 10 soybean hosts selected from the germplasm screening and compared to two improved high-yielding varieties developed in the U.S. (Bossier and TGM 294). Of the isolates tested, 22 were isolated from soybean nodules collected at various locations (4 from Onne, 3 from Ibadan, 6 from Mokwa and 9 from Funtua). Three isolates prepared from cowpea nodules were collected from three different varieties grown at Funtua. The test included an uninoculated check and inoculation with the multi-strain, commercial inoculant (Nitragin). The interactions of inoculant X host plant were significant ($p \gtrsim 0.1$) for both nodule mass and shoot dry weight which is a reflexion of host-strain specificity. It is not practical to discuss the response of each host-plant and inoculant treatment due to the large number of combinations (305). The most meaningful interpretation of the data can be derived by comparing the effect of the isolates versus the uninoculated control and inoculation will Nitragin for each host plant. These comparisons are presented in Table 3. Nodule mass (dry weight) is indicative of the degree of compatibility between the host and the rhizobia isolate and shoot dry weight a measure of the effectiveness of the host-isolate combination for fixing N_2. Malayan appears to be the most promiscuous host in the test as it formed significantly more nodule mass than the uninoculated control with all 22 soybean isolates tested and twenty-one of these isolates were as effective as Nitragin as shown by shoot growth. TGM 579 (a progeny of Malayan) gave similar results; compatible with 21/22 soybean isolates and 16 of these produced shoot growth equal to Nitragin. TGM 119 and 120 formed effective symbiosis with 20 and 19 of the isolates prepared from soybean nodules, respectively. Four Indonesia accessions (TGM 710, 725, 737 and Orba) were less promiscuous but still were effectively nodulated by 11-16 of the 22 soybean isolates. TGM 618 and730 were compatible with 16 and 18 of the isolates from soybean nodules but the effectiveness of all but four of the isolates was less than inoculation with Nitragin. Bossier and TGM 294 were clearly less compatible and inefficient hosts for these indigenous rhizobia isolates when compared to other accessions. Bossier nodulated with 12 isolates but only four of these resulted in shoot growth greater than the uninoculated control and only one isolate increased the shoot growth of Bossier as equally well as Nitragin. Similar results were obtained when TGM 294 was the host. Bossier and TGM 294 were capable nodulating with only one of the three isolates from cowpea nodules and this symbiosis was ineffective. Whereas, the promiscuous accessions formed a significant nodule mass with at least 2 out of the 3 cowpea isolates (Table 3). Malayan, TGM 120 and Orba grew equally well when inoculated with the all 3 cowpea isolates as compared the inoculation with Nitragin.

It is presumptuous to conclude that the 22 soybean and 3 cowpea rhizobia isolates are representative of the entire indigenous rhizobia population at the various testing sites. However, the number of isolates tested provides convincing evidence that soybeans genotypes differ in ability to from an effective symbiosis with rhizobia indigenous to tropical soils. The accessions selected from the germplasm screening are clearly more compatible with these rhizobia isolates than Bossier and TGM 294.

Grafting experiment

The accessions selected for compatibility with indigenous rhizobia in the germplasm screening are all low yield-potential genotypes due to inferior agronomic traits. Malayan, TGM 579, 344, 119 and 120 are very photo-sensitive, late-maturing with excessive vegetative growth and susceptible to lodging. Under optimum conditions, these genotypes have a maximum yield potential of less than 1.0 ton/ha. The free-nodulating Indonesian cultivars are poorly adapted to the low land tropics and are characterized by early maturity, short statue with limited leaf area and a yield potential of 0.5 - 1.0 ton/ha. Although the above genotypes are capable of forming an effective symbiosis with a wide range of rhizobia, the efficiency of the symbiosis need not be high in order to supply sufficient N to satisfy their low yield potential. In contrast, non-promiscuous improved varieties such as Bossier and Jupiter have superior agronomic character but must be supplied with enough N to support a 2-3 ton/ha yield potential. If the promiscuous nodulating character(s) possessed by the low yielding accessions was genetically transfered to the improved cultivars would the resulting symbiosis be capable of providing sufficient N to support a high yield potential? This question was addressed by grafting the shoots of Bossier and Jupiter onto root stocks of Malayan and Orba.

Results from 12 graft combinations grown in soil containing only indigenous rhizobia and soil inoculated with strains of *R. japonicum* (Nitragin) are presented in Table 4. Plants with a Bossier scion grafted back onto its own root stock gave large responses to inoculation with Nitragin as evidenced by an increase of 103% in shoot growth, 632% in nodule mass and 50% in seed yield. In contrast, Bossier grafted onto the root stock of Orba and grown in uninoculated soil gave shoot growth, N content, nodule mass and seed yield equal to Bossier grafted onto Bossier and inoculated with Nitragin. Also, grafts with Orba root stocks did not respond to inoculation. Grafts of Bossier scions and Malayan root stocks were difficult to perform due to the thick hypocotyl of Bossier and the thin stem of Malayan. Vegetative growth of plants with this combination was less than the control treatment of Bossier grafted onto Bossier even when grown in inoculated soils indicating that early growth may have been affected by the mechanics of grafting. However, seed yield of Bossier grafted onto Malayan roots were equal to Bossier grafted onto Bossier and inoculated with Nitragin. Grafts using Jupiter as the scion and Malayan or Orba as root stocks and grown under uninoculated conditions produced plants that grew as well and yielded the same as Jupiter grafted onto Jupiter and grown in inoculated soil. It is evident from these studies that the symbiosis between Malayan or Orba and indigenous rhizobia is capable of supporting the yield potential of Bossier or Jupiter. Consequently, if the promiscuous nodulating character(s) can be genetically incorporated into high yielding varieties, one would not sacrifice yield potential.

CONCLUSIONS

It is apparent from these studies and earlier work (8) that variation exists in the soybean germplasm for the ability to nodulate and form and effective symbiosis with rhizobia indigenous to tropical soils. The germplasm screened for promiscuity included only 400 accessions but a deliberate attempt was made to select accessions that have been grown by farmers in areas where inoculants have not been used. Of the 10 accessions identified as promiscous, four are local cultivars from Africa and Indonesia indicative that farmers may have selected material for compatibity with native rhizobia. Local farmer s varieties maybe more probable of possessing promiscuity but the fact that it is a local cultivar does not ensure promiscuity. Varieties that freely nodulate in Northern Thailand did not form an effective symbiosis at several locations in Nigeria. Also, of more than 70 entries collected in Indonesia only five were rated as promiscuous in Nigeria. The inability of material developed in the U.S. to nodulate with rhizobia indigenous to various tropical environment may be due to the narrow genetic base of U.S. bred material and/or the continuous selection of this material in soil contaminated with introduced strains of *R. japonicum*.

There is considerable evidence that rhizobia composition differs across environments. Several soybean accessions nodulated in one or two sites but were incompatible with rhizobia strains native to other environments. Also, rhizobia isolates prepared from nodules collected from the same or different host grown at various locations gave differential reactions when tested for compatibility with a range of hosts. The variation in rhizobia composition within and between environments must be recognized in order to prevent the development of varieties that are site-specific for compatibility. Data from the germplasm screening indicate that testing should be conducted in at least three distinct environments in order to reduce the probability of selecting material that is site-specific.

The symbiosis between indigenous rhizobia and promiscuous nodulating cultivars is effective and capable of fixing sufficient N_2 to satisfy the requirements of improved, high yielding cultivars. Consequently, improved genotypes that combine the superior agronomic traits of the U.S. material with the promiscuous nodulating ability of some local cultivars could be developed and still maintain the yield potential of the improved parent; assuming promiscuity is a heritable trait.

Although no direct evidence is available concerning the heritability of promiscuity, empirical observations indicate that the character(s) can be transferred genetically. Some national breeding programs have developed promiscuous nodulating lines, albeit unintentionally, using parents identified in the germplasm screening. Breeders in Tanzania have used TGM 344 as a parent due to its vigorous vegetative growth and produced at least three lines that maintained the promiscuity of TGM 344 (2, 8). Also, a Nigerian program employed Malayan as a parent and many of the progeny have been observed to possess promiscuity similar to Malayan i.e. TGM 579.

We have made crosses between some promiscuous accessions identified in the germplasm screening (mainly TGM 344, 120 and 119) and improved high yielding parents (Jupiter and Bossier). Selections were made in the F2 generation at Mokwa based initially upon agronomic characters and at physiological maturity these selections were examined for nodule mass. Selections were made in the F3 generation at Ibadan again for agronomic characters and nodulation. Seeds from these selection were planted again at Mokwa (F4) and further selected for favorable agronomic traits and nodule mass. Seeds from the F4 plants were increased at Ibadan and the F5 generation evaluated for yield and nodulation in multi-location yield trials in cooperation with national programs. Results from the yield trials showed that the vast majority of the entries were promiscuous and possessed improved agronomic traits. Consequently, promiscuous nodulating soybeans with improved agronomic traits have been developed and this material can be grown without the need of inoculation with *R. japonicum*. Results from the breeding for promiscuity including genetic studies will be presented in other articles.

Table 1. Climatic conditions and soil properties of testing sites where soybean varieties were examined for promiscuity.

Location	Latitude	Mean rainfall mm/yr	Great soil Group	Vegetation Zone
Onne	4°51'	2355	Typic Palendult	Tropical rain forest
Yandw	7°23'	1288	Oxic Palenstalf	Southern Guinea Savanna
Ibadan	7°26'	1215	Oxic Palenstalf	Transitional zone (forest - derived savanna)
Mokwa	9°18'	1110	Oxic Palenstalf	Southern Guinea Savanna
Funtua	11°38'	1005	Oxic Palenstalf	Northern Guinea Savanna

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

Location	Percentage of cultivars								
	Nodule No.			Nodule size			Plant vigor		
	Rating scale[1]								
	1	2	3	1	2	3	1	2	3
Onne	12	61	28	55	37	8	54	36	10
Yandw	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

[1]Rating scales: Nodule No. : 1= <10 nodules, 2= 10-20 nodules and 3= >20 nodules/plant.

Nodule Size: 1= nodules <2mm, 2= nodules 2-5mm and 3= nodules >5mm in diameter.

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

Table 3. Effect of inoculating sterile soil with rhizobium isolates from soybeans and cowpea nodules on nodules mass and shoot growth of 10 soybean accessions selected for promiscuity and 2 varieties (Bossier and TGM 294) from U.S.

Soybean	No. of isolates producing nodule mass or shoot growth[1] greater than the uninoculated control.				No. of isolates producing nodule mass or shoot growth[2] greater than or equal to inoculation with Nitragin			
	Soybean isolates[3] (n=22)		Cowpea isolates[3] (n=3)		Soybean isolates[3] (n=22)		Cowpea isolates[3] (n=3)	
Host	Nodule Mass	Shoot wt.	Nodule Mass	Shoot wt.	Nodule Mass	Shoot wt.	Nodule Mass	Shoot wt.
Malayan	22	21	3	3	21	21	3	3
TGM 579	21	19	3	2	17	16	2	2
TGM 120	22	21	3	3	2	19	0	3
TGM 119	21	20	2	2	14	20	2	2
TGM 618	18	15	2	2	4	4	0	1
TGM 725	17	17	3	2	5	16	1	2
TGM 710	15	14	3	3	0	13	2	1
Orba	14	11	3	3	5	11	0	3
TGM 737	15	15	3	2	9	15	1	2
TGM 730	16	7	3	2	1	5	0	2
Bossier	12	4	1	0	0	1	0	0
TGM 294	10	3	1	0	2	2	0	0

[1] Nodule dry wt. or shoot dry wt. is significantly greater than the uninoculated control at $p \geq .05$.

[2] Nodules dry wt. or shoot dry wt. is equal to or greater than Nitragin commercial inoculant treatment at $p \geq .05$.

[3] Host of derivation, there were 22 isolates from soybeans; 4 from Onne, 3 from Ibadan, 6 from Mokwa and 9 from Funtua. Three cowpeas isolates were from Vita 1, 4 & 5 at Funtua.

Table 4. Shoot dry weight, total nitrogen 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 concent. mg/plant	Nodule dry wt. mg/plant	Seed yield g/plant
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
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

Means followed by unlike letters with the same scion are significantly different at $p \geq .05$ according to Duncan's Multiple Range Test.

REFERENCES

1. Abel, G.H. and L.W. Erdman. 1964. Response of Lee soybeans to different strains of *Rhizobium japonicum*. Agron. J. 56:423-424.

2. Auckland, A.K. 1966. Soybeans in Tanzania I. The explotation of hibridization for the improvement of soybean. J. Agric. Sci. Camb. 69;455-464.

3. Bezdicek, D.F., B.H. Magee, and J.A. Schillinger. 1972. Improved reciprocal grafting techniques for soybeans (*Glycine max* L.). Agron. J. 64:558.

4. Chesney, H.A.D., M.A. Khan, and S. Bisessar. 1973. Performance of soybean in Guyana as affected by inoculum *Rhizobium japonicum* and nitrogen. Turrialba 23 (1): 91-96.

5. Ferrari, A. 1960. Nitrogen determination by a continuous digestion and analysis system. N.Y. Acad. Sci. 87:792-800.

6. Kang, B.T. 1975. Effects of inoculation and nitrogen fertilizer on soybean in Western Nigeria. Exp. Agri. 11(1):23-31.

7. Na Lampong, A. 1976. Inter-relationship between soybean varieties and indigenous rhizobium strains in Northeast Thailand. Proc. of a Confer. for Asia and Oceania. Chiang Mai, Thailand. INTSOY Publications Series No.10 pp. 198-199.

8. Pulver, E.L., F. Brockman, and H.C. Wien. 1982. Nodulation of soybean cultivars with *Rhizobium* spp. and their reponse to inoculation with *R. japonicum*. Crop Sci. 22:1065-1070.

9. Sears, O.H., and W.R. Carrol. 1927. Cross-inoculation with cowpea and soybean nodule bacteria. Soil Sci. 24:413-319.

10. Van Rensburg, H.J., B.W. Strijdom, and M.M. Kriel. 1976. Necessity for seed inoculation of soybean in South Africa. Phytophlactica 8:91-96.

11. Vincent, J.M. 1970. A manual for the practical study of root-nodule bacteria. Blackwell Scientific Publications, Oxford, Edinburgh. 164 p.