J. Mycopathol. Res. **42(1)** : 59-63 (2004)

In vitro study on genetic influence of host-root in modification of incompatible strain to nodulate restrictive soybean genotype in presence of compatible strain

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The detrimental trait of non-nodulating line of soybean [*Glycine Max* (L.) Merr.] is known. The restrictive nodulation of rj_1 rj_1 soybean cultivar divided the strains of *Bradyrhizobium japonicum* into rj_1-incompatible one. It was found, by using a mixture of genetically marked strains as inoculum, that the presence of a strain possessing the ability to nodulate rj_1 rj_1 plants facilitates nodulation of these plants by a strain incapable of nodulation when used alone. The present study was undertaken to determine rj_1-compatible strains of *B. japonicum* produce any diffusable compound that after the ability of the rj_1-incompatible strain to nodulate rj_1 rj_1 soybean plant when the situation becomes (1) an rj_1-incompatible strain grown in the culture filtrate of rj_1-compatible strain grown in the culture filtrate of rji-compatible strains. (2) rj_1-incompatible strain culture in U-tube across a membrane filter of polycarbonate membrane of 0.08 pore size from rji-compatible strain or (3) rji-incompatible strain cultured indialysis tubing suspended in broth cultures of a rji-compatible strain and then inoculating 7 days old seedling. It was concluded that rj_1-compatible strain do not produce any diffusable compounds that alter the ability of rji-incompatible strains to nodulate rji rji-host genotypes. The entirely different happenings is only be possible when rji- compatible strain exerts its effect in close proximity to nodule site.

Key words : Genetic influence, host root, soybeans, compatible strain

INTRODUCTION

Williams and Lynch in 1954 successfully developed one non-nodulating line of soybean by mutation. They also reported its recessive allele, rj_1 in soybean which produce normal nodulation with some strains of *Bradyrhizobium japonicum*. Clark (1957) reported that some strains of *B. japonicum* form significant number of nodules in rj_1 rj_1 plants of soybean. But there were still strains that were unable to produce any nodule in this rj_1rj_1 plant. It was letter designated as a rj_1-compatible and rji-incompatible strain of *Bradyrhizobium japonicum*. Clarks' rj_1 rj_1 plant when inoculated with a mixed strain of *Bradyrhizobium japonicum* having Bu/S_4-streptomycin penicillin and chloramphenicol succeptible (Bu/S_4-SPCS) and Bu/S_5-streptomycin penicillin and chloramphenicol resistant strains (Bu/S_5-SPCS) as these containing the rj_1 compatible strain (BU/S_4 SPCS). Banerjee (1974) while studying the homeostatic control in nodulation observed the appearance of certain unknown stains in the nodule of the soybean host when inoculated with mixture of *B. japonicum* strains. The appearance of new strains in the nodule might be due to genetic transformation of the bacteria (Doctor and Modi, 1976). but when a heritable rj_1 strain used alone such happenings of genetic transformatics dose not arise.

It is hypothesized that nodulation by the normally rj_1-incompatible strain might be due to either the modification of the plant root by the rj_1-compatible strain rendering it indiscrimately receptive to nodulation or to the production of some diffusable compound by the rj_1-compatible strain endowing normally the rj_1-incompatible strain with the ability to nodulate the rj_1 rj_1 genotype.

The present experiments have been undertaken to determine whether the compatible strain that nodulate rj_1 rj_1 plant produce any diffusable com-

pound *in vitro* that can modify the nodulating ability of a strain that normally was not capable of rodlating rj_1 rj_1 plants.

MATERIALS AND METHODS

The experiments were conducted in three different phases.

In the first set of experiments, three strains of *B. japonicum* viz. Bu/S_6, Bu/S_8 and Bu/S_{22} were taken. The first two strains were rj_1-compatible and strain Bu/S_{22} was rj_1-incompatible. Seven-day-old broth cultures of these strains (100 ml, AIE HM broth) were centrifused at 10,000 rpm for 10 minute (SORVAIL-RC-5 superspeed refrigerated centrifuge was used). The supernated fluid was filtered sterilized by passage through a membrane filter with an average pore size of 0.2 mμ.

Fifty ml volumes of filtrates of each strain was then taken seperately and were enriched with 0.5 ml. of 10% arabinose solution. It was then inoculated with one ml. of a suspension of strain Bu/S_{22} prepared by suspending a two-week-old slant culture of 5 ml. of AIE HM broth. Fifty ml. of culture filtrate were also enriched with arabinose but remained uninoculated and was considered as control. After nine days incubation the control set did not show any turbidity which indicated the effective sterilization. The inoculated cultured filtrates were incubated in a rotary shaker for nine days and showed sufficient turbid culture. This culture eas used to inoculate the seeds of "Clark" rj_1 rj_1 grown "Leonard" jar sand culture in the green house. At the same time seeds of normally isoline of 'Clark' were inoculated with each of these culture to determine their effectivity. As the degree of nodulation varies with temperature, special attention was taken for seasonal variation. For reference check, inoculation of Clark rj_1 rj_1 plant was done with these three strains of *B. japonicum* seperately. At 30 days after inoculation plants were uprooted carefully for counting nodules.

In the second set of experiments an U-tube was taken, having 2.0 cm in diameter, two end of which was seperated from direct contact by an 0.08 mμ pore size, polycarbonate membrane. Instead of three strains in the first experiment, strain No. Bu/S_5, an rj_1 incompatible strain, and strain no Bu/S_8, rj_1 compatible strain, were taken and were grown in fresh arabinose broth. (a) In one set, one arm containing 10 ml. of fresh broth of strain Bu/S_5 and other arm strain Bu/S_8. (b) In another set, both arm containing the broth Bu/S_5 strain. (c) In the third set, one arm containing Bu/S_8 strain and the other arm uninoculated broth. The cultures were incubated for 5 days at 30°C on a rotary shaker and then used to inoculate soybean n Leonard jar sand culture. Thirty six Leonard jars with four clark rj_1 rj_1 seeds per jar and four fars with normal 'clark' variety of soybean were each inoculated with 2 ml. of the strain Bu/S_5 culture of set (a). Similary another group of thirty six jars were inoculated, with Bu/S_5 strain from set (b). Four jars planted with Clark rj_1 rj_1 seeds were inoculated, with control culture of strain Bu/S_8 of set (c). Plants were grown vermiculite for 5 weeks in the green-house without supplementary nutrients. Each plant was uprooted carefully to examine the presence of nodules.

In the third set of experiments some dialysis tubes having length of 2.5 (molecular exclusion size 12,000), closed at one end were used. 50 ml. of AIE HM broth was added in the dialysis tube and it was suspended in Fernbach flask containing 1000 ml of AIE HM broth. The other end of the dialysis tube remained open and extended through the plug of the flask. The whole system is sterilized properly. Broth inside each membrane sac was inoculated with rj_1 incompatible strain Bu/S_5 SPCR of *B. japonicum* derived from the strain Bu/S_5 and carrying antibiotic resistance marker for streptomycin, penicillin and cloramphenicol (Chandra, 1974). Broth outside the membrane sac was inoculated either with strain Bu/S_5-SPCR or with strain Bu/S_4 SPCS (An rj_1 compatible strain), marked with succeptible for streptomycin penicillin and chloramphenicol (Chandra, 1974). The flask/ membrane sac culture was incubated for 7 days at 23°C with continuous aeration with sterile air. The culture within membrane sac then used to inoculate7 days old seedlings of 'Clark' rj_1 rj_1 and 'Clark' soybean in vermiculate culture in Leonard jars. Thirty Leonard jars each containing four healthy seedlings was inoculated 10 ml of

inoculum. Some 'Clark' rj_1 rj_1 soybean seedlings were inoculated with Bu/S_4 - SPCS strain as reference check to understand the environmental factors for nodulation. Samples from the broth inside the membrane sac was plated in the agar medium containing seperately streptomycin, penicillin and chloramphenicol confirmed that strain BU/S_4 - SPCS had not penetrate the membrane barrier. Plants were grown in the growth chamber at 24°C with 16 hrs DL. No additional nutrients were supplied. Plants were uprooted carefully to examine the nodules 28 days after inoculation. It is to mention that the pregerminated seedlings were used to nullify any putative factor conditioning the rj_1 compatibility during the interval between seed inoculation and development of receptive roots.

RESULTS AND DISCUSSION

Table 1 : Influence of culture filtrate of rji-compatible strains of *B. japonicum* (Bu/S_6 and Bu/S_8) on nodulation of an rji in compatible strain (Bu/S-22)

Inoculum Strain	Culture filtrate strain	Clark - rj_1 rj_1 No. of plants	Clark - rj_1 rj_1 Nodule per plant	Clark No. of plants	Clark Nodules per plant
Bu/S-22	Bu/S_6	131	0.05	8	5.75
Bu/S-22	Bu/S_8	130	0.02	8	8.25
Bu/S-22	Bu/S-22	105	0.06	4	5.50
Reference checks					
Bu/S_6	None	5	3.50	NT*	NT
Bu/S_8	None	5	4.50	NT	NT
Bu/S-22	None	5	0.00	NT	NT

*NT = Not tested.

It was evident from Table 1, that these three strains were viable and able to nodulate in normal 'Clark' - soybean plant. The results from reference check indicated that the environmental conditions were conducive for nodulation of this genotype. Strain Bu/S-22 showed detectable efficacy neither when it grown in the filtrate of Bu/S_6 and Bu/S_8 (Compatible strains) nor when grown in its own filtrate.

This indicated that no extracellular product was retained in the filtrate of *Bradyrhizobium* strains Bu/S_6 and Bu/S_8 that could modify or influence the receptivity of roots of the tested plant (rj_1 rj_1 - clark) to render the strain Bu/S-22 to become compatible for nodulation.

Table 2 : Showing the effect of Bu/S_8 (rj_1-compatible strain) on Bu/S_5 (rji-incompatible strain) seperated by polycarbonate membrane in U-tube

Strain U tube					
Arm A (inoculum source)	Arm B (across membrane)	Clark - rj_1 rj_1 No. of plant	Clark - rj_1 rj_1 Nodule /plant	Clark No. of plant	Clark Nodule /plant
Bu/S_5	Bu/S_8	140	0.0	15	7.00
Bu/S_5	Bu/S_5	140	0.0	15	6.50
Reference checks					
Bu/S_8	UB*	15	4.5	NT*	NT

UB = Uninoculated broth NT = Not tested.

From the second set of experiments (Table 2) it was observed that environmental conditions were conducive as it was found nodutation in 'Clark' rj_1 rj_1 plants by strain Bu/S_8 grown across the membrane from uninoculated broth. The viability of Bu/S_5 strain was proved as it nodulate the normal 'Clark' soybean grown across the membrane from either strain Bu/S_5 or Bu/S_8. Comparing nodulation performance of Bu/S_5 strain grown either with Bu/S_8 strain or with Bu/S_5 in the opppsote arm it was clear that Bu/S_8 has no positive effect to change the nodulation potentiality of Bu/S_5 strain on clark rj_1 rj_1 soybean plant.

Table 3 : Effect of rji-compatible strain (Bu/S_4-SPCS) on rji-incompatible strain Bu/S_5-SPCR) seperated by membrane sac-system. Inoculated 7 days seedlings

Position of strain** in membrane system		Clark - rj_1 rj_1		Clark	
Intra	Extra	No. of plant	Nodule /plant	No. of plant	Nodule /plant
Bu/S_5 SPCR	Bu/S4-SPCS	75	0.05	2	18.0
Bu/S_5 SPCR	Bu/S_5-SPCR	5	0.01	12	21.3
Reference checks					
Bu/S_5 SPCR	Bu/S_4-SPCR	20	6.5	NT*	NT

** Culture used as inoculum *NT = Not tested.

In the third set of experiments, the control set up i. e. inoculation of 'Clark' rj_1 rj_1 plant with Bu/S_4-SPCS strain, formation of nodules indicated that the environmental conditions were conducive for nodulation of this genotype (Table 3). Moreover, production of nodules in all the treatments by strain No Bu/S_5-SPCR inoculated to normal clark-soybean plant proved the viability and potentiality of the test strain. It was found that nodulation was almost nil on clark rj_1 rj_1 plant inoculated with strain Bu/S_5-SOCR irrespect of whather. Bu/S_5-SPCR was cultured across the membrane from the rj_1

compatible strain BU/S_4 SPCS or from culture of strain No. BUu/S_5 SPCR. But appreciable number of nodules appeared when this clark-rj_1 rj_1 plant was inoculated with Bu/S_4-SPCS strain. This indicated that diffusable substances from Bu/S_4-SPCS (a compatible strain) did able to modify the nodulation ability of Bu/S_5-SPCR strain. No trace of Bu/S_4-SPCS strain was found.

Vest (1970) suggested that Rj_3 gene conditioning ineffective nodulation in soybean. Caldwell (1966) observed the gene Rj_2 control this ineffective nodulation in 'Hardee' variety of soybean. Vest and Caldwell (1972) suggested that the genes Rj_1 and Rj_4 condition an incompatible nodulation response with specific serological groups of strains of *R. japonicum*. In those cases cortical proliferation or rudimentary nodules was initiated but aborted at an early state of development. But with the rj_1 rj_1 genotype of plant no microscopic evidence of nodule initiation and subsequent abortion was observed. Therefore, when rj_1 rj_1 soybean plant was inoculated with either rj_1-compatible or with incompatible strains, the roots of the plants will be characterised either by fully development nodules or by complete absence of nodules. Such observations indicate that the gene rji blocks the early stage of nodule initiation. Nevetheless, the presence of viable rhizobia in the rhizosphere of the rj_1 rj_1 plants do not impose any antagonism by the plant genotype towards *Rhizobium*. Therefore, it may be said that a specific incompatibility condition is nesessary in the early stages of nodulation rather than imposition of antagonistic effect by the rj_1 rj_1 plant genotype for rhizobial metabolism.

Carroll *et al.* (1986) isolated three non-nodulating mutants in soybean. These are nod 49, nod 772 and nod 39. Complementation analysis indicated that nod 49, nod 72 and rj_1 loci are located in the same complementary group and could induce few subepidermal cell division with no infection thread formation. These mutants could not induce root hair curling. Being at per with this observation it may be apprehended that incompatible strains failed to induce root hair curling which is prerequisite to nodule formation.

In the third set of experiments the use of dialysis membrane prevented the entry of any deoxyribonucleic acid. Therefore, occurence of any transformation of strain Bu/S_5-SPCR will be null and void. It may be suggested that the occurence of nodules, though few in number, in the roots of clark rj_1 rj_1 plant by Bu/S_5-SPCR strain may be one of the following possible mechanisms.

i) Inherent infectivity of the test strain with clark rj_1 rj_1 plant is at a very lowest level.

ii) Chance of mutation of strain Bu/S_5-SPCR to become compatible (Lorkiewicz *et al.*, 1971).

iii) Localized somatic mutation of the test strain by the influence of host root cell and lastly.

iv) Rhizospheric environment prevent the influence of the rj_1 gene.

As there was no chance of any diffusable compound by the compatible strain to change the normal ability of the incompatible strain to nodulate rj_1 rj_1 clark host genotype it may be conjectured that nodulation of rj_1-incompatible strain may had been due to host-root may play an important role in the synthesis of some putative nodulation factors. To make it possible direct contact of rj_1-compatible strain with the nodulation site is required to alter the incompatibility character of rji strain to become compatible.

ACKNOWLEDGEMENTS

The author is greatful to USDA, Beltsille, MD 20705 for providing suitable soybean cultivars, Cultivation of Science, Jadavpur University, W. Bengal for excellent technical assistance. Thanks due to Nodule Research Laboratory, BCKV, WB for providing specific bacterial strains and laboratory facilities.

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(Accepted for publication December 28 2003)