

A comparative study on

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in collaboration with
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DEPARTMENT OF AGRICULTURE (MINISTRY OF AGRICULTURE)
CENTRAL REGION AGRICULTURAL CENTRE (MINISTRY OF AGRICULTURE)
NORTHEAST AGRICULTURAL CENTRE (MINISTRY OF AGRICULTURE)
OFFICE OF THE ATOMIC EMERGY FOR PEACE

RESEARCH PROGRAMME NO. 44
PRODUCTION AND UTILIZATION OF GRAIN LEGUMES

RESEARCH PROJECT NO. 44/3
SYMBIOTIC NITROGEN FIXATION OF GRAIN LEGUMES

# REPORT NO. 3
# A COMPARATIVE STUDY ON THE EFFECTIVENESS OF LOCAL AND INTRODUCED *RHIZOBIUM* STRAINS ON "S. J. 2" SOYBEAN

BY
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TROPICAL AGRICULTURAL PRODUCTS INSTITUTE

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# F O R E W O R D

Cooperative Research Programme No. 44 involves cooperation between ASRCT and many other agencies as set out on the cover of this report.

The present study involved cooperative work between ASRCT, (Tropical Agricultural Products Institute) and the Central Region Agricultural Centre, Ministry of Agriculture. The primary objective of this investigation was to compare the effectiveness of the local and introduced strains of Rhizobium japonicum on pot-grown "S.J. 2" soybean.

# A COMPARATIVE STUDY ON THE EFFECTIVENESS OF LOCAL AND INTRODUCED RHIZOBIUM STRAINS ON "S.J.2" SOYBEAN

By Srivan Chomchalow* and Barry Norman+

SUMMARY

By modified "Leonard's jar" assembly, seven strains of introduced Rhizobium japonicum received from the United States (US 38, US 62, US 94, US 110, and US 112), and from Australia (CB 1795 and CB 1809) were inoculated onto "S.J. 2" soybean seeds in an attempt to compare the effectiveness of these introduced strains which have been known to be effective in inducing nodulation on foreign soybean varieties. A locally isolated strain from Chai Nat (CN 1) was also tested. It was found that strain CB 1795 was the best and strains US 38, US 110, and CB 1809 were equally effective on the bases of nodulation, dry weight per plant, and nitrogen uptake per plant. Strain CN 1 was effective in nodulation but its ability to fix atmospheric nitrogen was inferior to that of some of the introduced strains.

## INTRODUCTION

Although most legumes are able to form nodules along their roots in the presence of Rhizobium bacteria already existing in the soil, soybean (Glycine max) is somewhat different since it needs a specific strain of Rhizobium japonicum. It is generally accepted that in areas where no soybean has ever been grown before, the roots of soybean plants are devoid of nodules. This is also applicable to areas where other legumes were grown before soybean. This phenomenon has discouraged the cultivation of soybean in new areas because the yield would be poor even with some fertilizer application. In the areas where soybean has been grown before, the amount of nodules would increase greatly if effective strains of R. japonicum are inoculated onto the seeds at the time of seed sowing. When effective nodules are formed, symbiotic nitrogen fixation then begins, and, in this way,

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enriches the soil with available nitrogen at a relatively low cost. Consequently, the yield of soybean would naturally be increased.

The present investigation was conducted in an attempt to compare the effectiveness on "S.J.2" soybean of a number of strains introduced from the United States and Australia and the locally isolated strain of R. japonicum. Preliminary study by the senior author at ASRCT, using the modified "Leonard's jar" assembly described by Norris (1968) has indicated that all of these introduced strains were effective in inducing nodulation on "S.J.2" soybean.

## MATERIALS AND METHODS

### (i) Experimental design

The design of the experiment was a randomised complete block with six replications. There were 11 treatments as follows:

1. Uninoculated - Control
2. " - Low N (4 kg/rai or 25 kg/ha)
3. " - High N (16 kg/rai or 100 kg/ha)
4. Inoculated - CN 1
5. " - US 38
6. " - US 62
7. " - US 94
8. " - US 110
9. " - US 112
10. " - CB 1795
11. " - CB 1809

### (ii) Experimental procedures

Leonard's jar assembly. A modified Leonard bottle-jar assembly (Leonard 1944; Norris 1968) was used (Figure 1). This consisted on an inverted plastic litre bottle (with the bottom removed) which rested in a wire support within the top of a plastic lined aluminium container. The neck of the bottle was plugged with soft tissue and the bottle filled with sterile growth media in which the plants were grown.

Figure 1. The modified Leonard jar assembly for testing nitrogen-fixing effectiveness of Rhizobium strains. From left, the component parts, nutrient aluminum can with plastic lining and holder, cover paper, rubber band; plastic bottle with bottom removed, tissue plug, paper sheath; assembly, filled with sand and nutrient solution; complete set after sterilization.

Growth medium. The medium was a 2:1 (v:v) mixture of sand and vermiculite. The medium was acid washed (1% sulphuric acid for 5 minutes) and thoroughly rinsed in distilled water. The washed medium was sterilized by dry heating at 90-100°C for 12 hours. A total of 510 g of medium was added to each bottle.

Nutrient solution. The nutrient solution was that used in the earlier study (Chomchalow 1971). Approximately 200 ml was poured onto the top of the medium and the rest of the 700 ml placed in the plastic lined container.

Seed treatment. Seeds were surface sterilized in water at 62-64°C for 5 minutes. A preliminary study by Chomchalow (1971) at the ASRCT Laboratory indicated that this treatment has a disinfectant action and, in addition, more rapid and even germination is obtained. Four seeds were sown in each bottle and thinned to two plants per bottle after establishment.

Rhizobium cultures. Cultures were prepared using liquid yeast-extract-mannitol medium. Densely turbid cultures were obtained in six days. One ml of the appropriate culture was transferred to the inoculation treatments.

Nitrogen treatments. Ten ml of ammonium nitrate solution was applied to the appropriate nitrogen treatments. The solution contained 5.7 mg N/10 ml for Low N and 22.0 mg N/10 ml for the High N treatment.

(iii) Methods of collecting data

Flowering commenced 34 days after sowing and plants were harvested 55 days after sowing. The entire plant was removed from the apparatus and growth medium washed from the roots by shaking vigorously in 1% HCl solution. The following data were then recorded:

Nodulation. All visible nodules, regardless of size and position, were counted. Observations were also made on the external and internal colour of the nodules as well as the size of the nodules on the tap and lateral roots.

Dry weight of whole plant. The plants were dried in a hot air oven at 60-70°C until no decrease in weight was obtained.

Amount of nitrogen uptake. After the dry weight had been obtained, the two plants in each pot were ground. The amount of nitrogen per gramme of the dried sample was determined, employing Kjeldahl method of analysis, and calculated into the amount of nitrogen uptake per plant.

(iv) Time and place

This experiment was carried out from 6 November to 30 December 1970 at the Central Region Agricultural Centre.

## RESULTS

Table 1 presents the data relating to the effect of Rhizobium strain on nodulation, dry weight, and nitrogen uptake per plant.

(i) Nodulation

Highly significant differences in the average number of nodules per plant were found among different strains of Rhizobium. Strains CB 1795 and CN 1 nodulated profusely and were significantly better than all strains except US 38 and CB 1809. The strains US 62, US 94, and US 112 nodulated poorly.

(ii) Dry weight of whole plant

CB 1795 was the outstanding strain, significantly outyielding all other strains. US 38 was the next best which was superior to the remaining strains except US 110 and CB 1809. Strains US 94, US 112, and US 62 were among the poorest in dry matter production.

(iii) Nitrogen uptake per plant

As in dry weight of whole plant, CB 1795 outyielded all other strains. Strains US 110 and US 38 produced more nitrogen than the remaining strains, with the exception of CB 1809. The amount of nitrogen produced by strains CN 1, US 62, US 94, US 112 did not differ from that of the uninoculated controls.

TABLE 1
EFFECT OF RHIZOBIUM STRAIN ON NODULATION, DRY WEIGHT, AND NITROGEN UPTAKE PER PLANT OF "S.J.2" SOYBEAN[1/]

| Treatment | Nodulation (nodules/plant) | Dry weight (mg/plant) | Nitrogen uptake (mg/plant) |
|---|---|---|---|
| 1. No N | 0.0 c | 699 d | 10.4 d |
| 2. Low N | 0.0 c | 756 d | 12.8 d |
| 3. High N | 0.7 c | 916 b c d | 11.9 d |
| 4. CN 1 | 25.7 a | 825 c d | 20.1 c d |
| 5. US 38 | 17.3 a b | 1390 b | 39.4 b |
| 6. US 62 | 3.8 c | 735 d | 10.2 d |
| 7. US 94 | 0.7 c | 707 d | 10.2 d |
| 8. US 110 | 10.2 b c | 1378 b c | 41.7 b |
| 9. US 112 | 1.8 c | 712 d | 12.1 d |
| 10. CB 1795 | 26.6 a | 2075 a | 70.2 a |
| 11. CB 1809 | 15.8 a b | 1368 b c | 33.3 b c |
| L.S.D. 5% | 12.1 | 568 | 19.0 |

[1/] Means with a letter in common are not significantly different at the 5% level.

## DISCUSSION

The present study attempts to compare the effectiveness of each of the seven introduced, and a locally isolated, strains of Rhizobium japonicum on "S.J.2" soybean. From the results of the experiment, strain differences were apparent for each of the three criteria used to assess the effectiveness of the Rhizobium-legume symbiosis. It is especially striking to observe that strain CB 1795 was outstanding in all respects, being significantly superior to all other strains in dry matter and nitrogen production. The local strain, CN 1, was equal to CB 1795 in the ability to form nodules. The remaining introduced strains differed markedly in their ability to nodulate effectively. The strains US 62, US 94, and US 112 failed to nodulate satisfactorily with the soybean variety "S.J.2". However, soybean varieties are rather strain specific in the ability to form an effective symbiosis. These strains of Rhizobium could be fully effective on other varieties of soybean and should be further tested on a range of promising

varieties from the soybean collection and breeding programme. The symbiotic incompetence exhibited by soybean is also present in other legumes such as mung bean. The most promising strains, other than CB 1795, were US 38, US 110, CB 1809, and the local strain, CN 1. For each of the criteria for symbiotic effectiveness studied, these strains were intermediate between CB 1795 and the three strains which failed to nodulate satisfactorily.

The locally isolated strain, CN 1, proved to be extremely aggressive; however, the ability to fix atmospheric nitrogen was inferior to that of some of the introduced strains. The variety "S.J.2" was selected in Thailand without the use of inoculum. The selection process would favour lines which had the ability to nodulate effectively with local strains of Rhizobium japonicum. The variety "S.J.2" had been grown at Chai Nat for a number of seasons. Selection for effective nodulation of this variety may already have occurred within the original Rhizobium japonicum population.

The presence of an aggressive local strain would make the successful introduction of a more efficient nitrogen-producing strain difficult. The "elite" strains are usually selected on the ability to fix nitrogen rather than the ability to establish within an existing soil Rhizobium population. However, CB 1795 appears equally as aggressive, and may be able to establish effectively in the field.

An interesting feature of the experiment was the relationship between the nodulation, nodules per plant, and dry matter production. Most studies show a positive relationship between nodules per plant and dry matter production under the conditions of this experiment. This relationship holds true for the introduced "elite" strains. The local strain is a noteworthy exception. The natural selection for survival in the soil and ability to nodulate aggressively appears to have been made also for lower nitrogen-fixing ability.

As far as the size of nodules is concerned, it was observed that the nodules on the main roots were much larger than the ones on the lateral roots. There was no difference in the colour of the nodules infected by either effective or non-effective Rhizobium. If the nodules were opened up, however, they showed marked difference. Those

effective nodules were pinkish whereas the less effective ones were paler in colour. This is probably due to the presence of haemoglobin within the effective nodules in larger amount than in the less effective ones. In the present investigation, there was no attempt to determine the amount of haemoglobin since it requires elaborate laboratory set up. However, it is anticipated that the colour of the nodule directly reflects its effectiveness. Various factors aside from the Rhizobium strains have been known to affect the amount of haemoglobin, e.g. cobalt concentration.

It is anticipated from the results of this experiment that inoculation onto "S.J.2" soybean with effective Rhizobium, particularly CB 1795, would produce greater yield of soybean on the basis of its ability to fix nitrogen. As noted in the earlier report of similar nature (Chomchalow 1971), the present investigation was conducted under laboratory conditions, and the results might not be the same if these strains were to be tested in the field, since there can be no absolute guarantee that inoculation with a good strain of Rhizobium will result in that strain successfully competing against the poor strains already in the soil. Means et al. (1961) have shown that some strains of Rhizobium have powerful competitive advantage in nodulation, which is not related to their effectiveness in nitrogen fixation. This may be the case of the locally isolated strain, CN 1, which is rather effective in nodulation but not in nitrogen fixation in the laboratory conditions. It is hoped that this particular strain would be more effective and more powerful than the added Rhizobium in the field conditions. However, it must be emphasized that the added effective Rhizobium has the powerful advantage of prior presence in large numbers in the environment of the germinating seed, and would, therefore, be able to nodulate in greater amount than the natural Rhizobium already existing in the soil. This would naturally contribute to the increase in the yield, if it had the ability to adapt itself to the local conditions.

It may also be advisable to mix several effective strains together so that in different environmental conditions, one strain may be more effective than the other, and, therefore, would be of considerable advantages to the soybean plant.

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