# THE APPLICATION OF RHIZOBIUM-INOCULANT ON SOYBEAN IN INDONESIA*)

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## Abstract

Soybean-inoculation with appropriate strains of *Rhizobium japonicum* is important in Indonesia and should not be over looked. Inoculation of Soybean is recommended when introducing soybean to newly cultivated area, where soybeans have never been grown. Inoculation with soil from soybeanfield have been recomended since time immemorial in Indonesia but the attention to increase the efficiency of soybean-rhizobia symbiosis started around 1936.

With the extention of cultivated area for transmigrant outside Java which are mostly poor in soybean-rhizobia population, attempt have been made to provide the transmigrant farmers with *Rhizobium* - inoculant.

That soybean inoculation is important one can follow the experience of some estate, e.g. a "Soybean Estate" in Palembang which use *Rhizobium*-inoculant to avoid failure in their production.

## Abstrak

Dalam usaha meningkatkan produksi kedelai di Indonesia, upaya inokulasi dengan strain-strain *Rhizobium japonicum* yang paling cocok merupakan salah satu upaya penting yang tidak dapat diabaikan.

Pada penanaman kedelai di daerah baru yang belum pernah ditumbuhi kedelai inokulasi dianjurkan agar tanaman kedelai yang ditanam di daerah baru itu terjamin perbintilan akarnya.

Kapankah arti dan makna inokulasi serta perbintilan akar pada tanaman kedelai telah diperhatikan oleh petani di Indonesia, tidaklah dapat ditetapkan dengan pasti. Dari beberapa informasi dapat diduga bahwa petani tradisional telah mempraktekkan pemindahan tanah (dalam jumlah kecil) yang pernah ditumbuhi kedelai ke tanah baru yang untuk pertama kalinya akan ditanami kedelai.

Perhatian ke arah meningkatkan efisiensi simbiosa *kedelai-Rhizobium* dengan penelitian-penelitian di Indonesia, baru dimulai pada tahun 1936.

Dengan perluasan areal pertanian di luar Jawa yang berhubungan dengan program transmigrasi, suatu program inokulasi pada kedelai telah dimulai pada tahun 1979.

Program ini merupakan kerjasama antara Proyek Pembinaan Pertanian Pangan Daerah Transmigrasi (P3DT) dengan Lab. Mikrobiologi Fakultas Pertanian Universitas Gadjah Mada.

Bukti tentang pentingnya masalah inokulasi pada kedelai, pengalaman yang ada pada perusahaan yang mengusahakan kedelai secara besar-besaran di Palembang (P.T. PATRATANI) dapat digunakan sebagai acuan. Untuk menghindari kegagalan dalam produksi serta mengurangi ataupun meniadakan penggunaan pupuk nitrogen, perbintilan akar yang baik harus terjamin.

Di dalam makalah ini disajikan pula penjelasan tentang seleksi beberapa strain *Rhizobium japonicum* dan cara membuat inokulum *Rhizobium*.


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# THE APPLICATION OF *RHIZOBIUM*-INOCULANT ON SOYBEAN IN INDONESIA

**Introduction**

The importance of soybean-inoculation with appropriate *Rhizobium japonicum* strains in Java was informed by Toxopeus in 1936 (Toxopeus 1936, 1938). De Jongh (1941) reported that inoculation with rhizobia is necessary when introducing soybean to new area where soybean have never been grown, particularly on "alang-alang" *(Imperata cylindrica)* fields outside Java.
Keleney (1959) and Newton(1961) have also indicated that in many areas in Indonesia the soybean plants were not so well nodulated. A respons to inoculation with soybean inoculant have been indicated in their observations.
The quality of nodulation on soybean in some parts of Central Java can be improved by inoculation with appropriate *Rhizobium japonicum* isolates that have been isolated from local varieties of soybeans (Jutono 1969a). The average nodulation ratings (see Burton and Curley 1965) were 2.1 for the uninoculated plants and 2.3 for the inoculated ones.

Most of the soybean in Indonesia were grown on rice fields and only a few on dry land. There are two main pattern of soybean cultivation that are practiced by the Indonesian farmers. The very extensive culturing pattern and the intensive one. The extensive pattern which is still practiced in some parts of Java is the most simple one, e.g. the soybean seeds were just sown after rice harvesting without any soil tillage (see also De Vries 1932). Growing soybean in rice fields has some advantage, in that the plant could benefitted the residual effects of previous applied fertilizers for the rice plants. To increase the soybean yield in Indonesia the intensibe pattern is recomended, and more attention should be given to optimize the soybean-rhizobia symbiosis, besides the applications of nonnitrogenous fertilizers (phosphorus and potasium), pest control and population density trials.

## STATE OF THE LEGUME-RHIZOBIA SYMBIOSIS STUDIES IN INDONESIA

The spiralling cost of nitrogenous fertilizers require that we increase our attention on the biological nitrogen fixation and its application.

The Minister of State in Research and Technology of Indonesia has proposed to the Indonesian Institute of Science to establish a Nation Wide Program in Research and Development on Biological Nitrogen Fixation. A workshop was held at the National Biological Institute/Indonesian Institute of Science in Bogor on the 29$^{th}$ of April, 1981 to initiate the aforementioned program.
Thirty participants, representing 4 universities and 7 research institutions have attended the workshop. From the reports presented in the work shop, 31 on going research projects conducted by universities and research institutions can be identified. Nine of which are research on aspects of the soybean rhizobia symbiosis, seven projects are on aspects of free-living nitrogen fixing organisms and 5 on other legume-rhizobia symbiosis.

The results of the workshop was a document of a Nation Wide Program on the research and development on Biological Nitrogen Fixation to be proposed to Minister of State in Research and Development of Indonesia.

On the 24th of September 1981 a meeting was held at the Pajajaran University in Bandung as the follow up of the workshop previously held. Nineteen reports on legume-rhizobia symbiosis and 5 reports on nonsymbiotic nitrogen fixing organisms were discussed.

It can be concluded sofar that progress have been made, and by integrated effort valuable results will be obtained, particularly the practical aspects of the biological nitrogen fixation in support of our agriculture.

## THE PRACTICE OF INOCULATION WITH RHIZOBIA ON SOYBEAN (PROBLEM AND PROSPECTS)

### General

The literature survey has indicated that attention on the efficiency of nitrogen fixation of the soybean-rhizobia symbiosis in Java (Indonesia) started in 1936 (see Toxopeus 1936). From various sources University is assigned to provide soybean-*Rhizobium* inoculant for the transmigrant farmers outside Java.

A peat-clay base *Rhizobium*-inoculant have been produced in our laboratory. This type of inoculant were distributed to the transmigrants is small policelonium bag and were provided with procedures of application which was printed on the bag.

The quality of the inoculant is the some as the one supplied for the soybean plantation of the P.T. Patratani in Palembang by our laboratory. Since 1978 this plantation have been using this type of inoculant.

### The production of inoculant

Inoculant can take the form of liquid or solid cultures. Liquid culture is easy to produced but the bacteria do not survive well (Vincent 1970).

Solid materials such as agar, compost, coir dust, peat or peat-soil are used as the carrier of the solid bacterial cultures.

a. The isolation and selection of the *Rhizobium*

The methods of isolation and selection of *Rhizobium* strains in our laboratory were as follows :

The source of the *Rhozobium* strains were healthy root nodules from local varieties of soybean and from imported soybean varieties of the Intsoy Program Trials in Central Java. The isolations were conducted by conventional method (Allen 1953).

After the performance trials in pot cultures, the nitrogen activity of the nodulated plants were assayed by Gas Liquid Chromatograph (see Wacek 1976). Out of 164 isolates of *Rhizobium* for soybeans, 25 were classified as the promising ones. Further elucidation on several soybeans (including var. 27, var. 29, Taichung, Orba, Improved Pelican Americana, and 4 for other local varieties of soybeans) concluded that 10 isolates were able to produce effective nodules on all varieties tested. The average nodulation rating were not less than 2.5 (see Burton and Curley 1965). It is indicated also that certain varieties have some preference to some isolates. These selected isolates are the strains of Rhizobium to be used for the preparation of inoculant, singly or in the form of composite cultures.

**b. The production of inoculant**

We have used the biphasic system for growing rhizobia for the preparation of the peat-clay base inoculant (see Jutono 1969b). The biphasic growth system consisted simply of a layer of solid medium overlaid medium in Erlenmeyer flasks. Concentrated liquid cultures can be obtained with this method (see table 1). Bacterial cells in the liquid phase is still easy to be suspended in water then bacterial cells *(Rhizobium)* from agar slant cultures.

*Table 1.* **Growth of Rhizobium japonicum (strain Rh. 16) in the biphasic growth system with various agar : broth ratio*)**

| Ratio of agar : broth | Agar (ml) | Broth (ml) | Number of cells per ml ($x10^9$) at 1 week in the flasks | Total number of cells in the liquid phase ($x 10^9$) at 1 week | Number of cells per ml in the biphasic flasks ($x 10^9$) at 1 week |
|---|---|---|---|---|---|
| — | 0 | 10 | 0.29 | 2.90 | 0,29 |
| — | 0 | 15 | 0.31 | 4.65 | 0.31 |
| 1 | 15 | 15 | 6.31 | 97.50 | 3.25 |
| 2 | 20 | 10 | 7.73 | 77.30 | 2.58 |
| 3 | 30 | 10 | 20.50 | 205.00 | 51.20 |
| 4 | 30 | 7.50 | 24.92 | 186.90 | 49.80 |

*) Volume of flasks 150 ml; Mannitol Yeast Extract as culturing medium The cultures were incubated at room temperature (28—30°C) and were shaked on a shaking machine for 6 hours each day during incubation period.

Cell numberswere average of triplicates of surface planting on Manitol Yeast Extract agar.

*Table* 2. **Yields of R. japonicum isolates in the biphasic system with agar : broth ratio of 3 : 1 *)**

| Strain No. | Origin of the isolates (variety of soybean) | Number of cells per ml after 5 days ( X $10^9$)**) |
|---|---|---|
| 1. | Var. 29 | 3.1 |
| 2. | ditto | 2.8 |
| 3. | ditto | 2.9 |
| 4. | from Treub Lab Bogor | 3.2 |
| 5. | ditto | 2.9 |
| 6. | Taichung | 4.1 |
| 7. | ditto | 2.2 |
| 8. | soybean Intsoy Program | 2.9 |
| 9. | ditto | 4.2 |
| 10. | ditto | 3.7 |
| 11. | ditto | 3.5 |
| 12. | ditto | 2.8 |
| 13. | ditto | 0.9 |
| 14. | Var. Orba | 0.8 |
| 15. | ditto | 3.2 |
| 16. | Local var. black seed | 0.5 |
| 17. | ditto | 3.4 |

*) Cultures in 150 ml flasks, incubated at room temperature.
**) Initial cell concentration, $10^4$ cells per ml.

The ratio of volume of agar : liquid phase and the depth of the agar were the critical value in the biphasic growth system (see Elizabeth A. Tyrrell *et al* 1958). The optimum ratio should be determined experimentally. With 250 ml flasks, 150 ml agar medium overlaid with 45 ml liquid medium are considered satisfactory in our laboratory.

For the preparation of peat clay base medium, a mixture composing of 90 per cent of peat and 10 percent of clay (Grumusol) were mixed throuroughly and lime (powder) were added to obtain a final pH of 7.0 after one week incubation. Sterilized liquid medium composing of mannitol, $K_2HPO_4$, $MgSO_47H_2O$ and NaCl were added to the sterilized peat-clay mixture and steril water were added to obtain a final mixture content of 50% (wet base). A diluted suspension taken from the liquid phase of the biphasic growth system of the *Rhizobium* culture were inoculated into the aforementioned peat clay base medium. After two weeks of incubation at room temperature the culture is ready to use.
The shelf-live of the peat culture is presented in table 3.

*Table 3.* **Shelf-life of peat cultures at storage temperature**

| Strorage Temperature | Numbers of viable Rhizobium cells after incubation period of *) | | | | |
|---|---|---|---|---|---|
| | 0 month | 1 month | 3 months | 6 months | 9 months |
| 28 — 30°C | $1.2 \times 10^8$ | $9.2 \times 10^7$ | $7.3 \times 10^6$ | $5.1 \times 10^5$ | $3.2 \times 10^3$ |
| 4°C | $1.1 \times 10^8$ | $8.7 \times 10^7$ | $7.6 \times 10^7$ | $8.9 \times 10^6$ | $5.7 \times 10^6$ |

*) Average of triplicates, and expressing the cell number for 1 gram of peat clay base inoculant.

## THE RESULTS OF RHIZOBIUM—INOCULANT APPLICATIONS

The role of symbiotic nitrogen fixation of soybean-rhizobia symbiosis may not be overlooked. Inoculation with appropriate strains of *Rhizobium japonicum* will ensure earlier nodulation and an earlier start of nitrogen fixation. In table 4 is shown that direct contact of the Rhizobium and the root is necessary and that dissemination of the rhizobia in the soil is limited.

*Table 4.* **The effect of inoculated plants to the uninoculated ones, planted in the same pot at 20 cm distant (In sterilized soils).**

| Var. of soybeans | Treatment of seed *) | Number of plants examined | Total number of nodules | Ratio of nodulated unnodulated plants | Average rating of nodulated plants |
|---|---|---|---|---|---|
| Vr. 29 | Uninoc. | 69 | 132 | 25 : 44 | 0.6 |
| | inoc. | 75 | 641 | 63 : 12 | 3.4 |
| Orba | Uninoc. | 71 | 126 | 30 : 41 | 0.5 |
| | inoc. | 80 | 701 | 66 : 14 | 3.6 |
| Local var. | Uninoc. | 80 | 99 | 34 : 46 | 0.5 |
| (black seed) | inoc. | 68 | 570 | 51 : 17 | 3.8 |
| Improved | Uninoc. | 62 | 80 | 20 : 42 | 0.7 |
| Pelican | inoc. | 70 | 502 | 56 : 14 | 3.1 |

*) Uninoc. means uninoculated
Inoc. means inoculated with a composite inoculant, composing of four selected strains of *Rhizobium japonicum*.

Improvement of the nodulation quality is obvious when the plants are provided with its suitable partners of *Rhizobium* although the soil harbour already *Rhizobium* bacteria.

From another trial on soil of low fertility level it was apparent that inoculation improve the performance of the plants. There is an increase in the number of pods and seed produced and some improvement on the seed quality (see table 5).

*Table 5.* **The effect of inoculation on pods and seed production of field grown soybean (Soybean var. Orba).**

| Treatment | Average nodulation rating | Number of pods for 100 plants | Number of seeds for 100 plants | Dry weight of seeds for 100 plants (grams) | Weight of 1000 seeds (grams) **) |
|---|---|---|---|---|---|
| Uninoculated | 2.2 | 2,988 | 7,495 | 616.1 | 81.2 |
| Inoculated | 3.4 | 4.563 | 10,957 | 930.3 | 95.2 |

**) The weight of 1000 seeds used was 114.7 grams.

The results of the application of inoculant on soybean is not yet complete, but positive results have been informed.

From the PATRATANI in Palembang which have been using *Rhizobium* inoculant, satisfactory results have been informed to us. The average yield of their soybean crops were not less then 2.1 ton dry seeds per hectare. We have provide *Rhizobium* inoculant for this estate the end of 1978.

A positive results was also reported from Seed Farm I, Unit II Transmigration Project Batu Raja, Martapura (South Sumatra) by Budianto and Ibnu Subroto(personal communication). The average increase in yield were ranging from 1.5 to 2.0 times by using the *Rhizobium* inoculant. Before the supply of *Rhizobium* iunoculant the farmers in this area were advised to used soil inoculant (taken from previous grown soybean fields). The recomended dose for soil inoculant is 1 kilogram soil for every 9 kg seeds.

Sofar there are only some reports indicating no effect (or respons) on soybean production by using *Rhizobium* inoculant. Before further conclusions can be drawn data is still needed. The possibility is that the soil harbour already with appropriate *Rizobium* strains for the soybean varieties used in that area.

## CONCLUSION

An increased attention on Biological Nitrogen Fixation is indicated in Indonesia. An coordinated effort to apply the available knowledge of Biological Nitrogen Fixation in agriculture is of paramount importance.

The practical aspects of Nitrogen Fixation, particularly the legume-rhizobia symbiosis should be popularized. Legume should be treated as a particular group of plants posessing the ability to fix $N_2$ form the air.

Application of selected strains of legume-bacteria (rhizobia) by improved method of inoculation may well be one of the simpelst and most economic ways of increasing the yields of soybeans in this country.

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