A STUDY ON SOYBEAN INOCULATION +)

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S U M M A R Y

A study on soybean nodulation and artificial inoculation is carried out.

Several strains of rhizobia with different Nitrogen-fixing capacity (for soybean var.29) have been isolated from root nodules of soybean growing in Jogjakarta area.

Tests on the effects of artificial inoculation on soybean var.29 concluded : that several factors affects the nodulation and the success of artificial inoculation (e.g. organic and inorganic fertilizers).

Artificial inoculation usually ensure earlier nodulation and better nodulation if the soils were deficient in rhizobia.

Even in soils where soybean were usually grown artificial inoculation showed a good effect : the nodulation of the inoculated soybean plants were much better than the uninoculated ones.

In this study the possibilities of selecting highly effective strains of rhizobea from local strains is indicated.

It seems that the socio-economical factors rather than the technical ones which limited the application of artificial inoculation in this country.

+) "Penjelidikan tentang inokulasi pada kedelai.

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R I N G K A S A N.

Penjelidikan jang dilakukan ialah tentang nodulasi dan inokulasi sengadja pada kedelai var.29.

Didalam penjelidikan ini dikumpulkan data tambahan dari penjelidikan-penjelidikan jang pernah dilakukan oleh De JONGH dan TOXOPEUS.

Didalam membahas hasil penjelidikan ini hasil penjelidikan baru tentang arti perbintilan akar pada kedelai oleh WEBER (U.S.A.) telah dipakai pula.

Didalam penjelidikan ini penulis telah berhasil mengisolasi beberapa strain rhizobia jang berbeda dalam kemampuan penambatan N_2 dalam assosiasi symbiotis dengan kedelai var.29. Strain-strain tadi diisolasi dari bintil-bintil akar kedelai jang terdapat didaerah Jogjakarta. Kenaikan kadar N tanaman (pada umur 6 minggu) berkisar diantara 1.41 - 8.58 milligram per tanaman.

Pengudjian terhadap kedelai var.29 menjimpulkan bahwa banjak faktor mempengaruhi nodulasi dan hasil inokulasi-sengadja. Misalnja terlihat bahwa pupuk organik besar pengaruhnja pada djenis tanah jang dipakai pada pertjobaan. Nilai perbintilan akar pada penggunaan pupuk organik dapat naik dua kali lipat.

Djuga dapat ditundjukkan bahwa inokulasi-sengadja menjebabkan perbintilan akar jang lebih awal dan pada umumnja memperbaiki nilai perbintilan (nodulasi).

Pengudjian lapangan pada tanah jang telah mengandung rhizobia menundjukkan adanja pengaruh inokulasi-sengadja dengan strain-strain effektip jang dapat diduga dari mendjadi baiknja nilai perbintilan akarnja.

Dalam penjelidikan ini djuga dapat disimpulkan tentang kemungkinan memilih strain-strain effektip dari strain-strain rhizobia jang ada ("local strain").

Faktor-faktor jang perlu diatasi untuk memungkinkan praktek inokulasi-sengadja setjara luas mungkin lebih bersifat persoalan sosio-ekonomis dari pada persoalan tehnis.

Pentingnja nodulasi jang baik dapat disimpulkan dari hasil penjelidikan WEBER jang menemukan bahwa penambatan N_2 jang baik pada kedelai dapat memenuhi kebutuhan N tanaman jang berkisar diantara 1 - 74 %.

I N T R O D U C T I O N.

Several reports indicated the importance of soybean inoculation with rhizobia in some parts of Java (Indonesia) (TOXOPEUS, 1936, 1938; De JONGH, 1941, 1943).

De JONGH found that in some areas the soils were deficient in Rhizobium japonicum strains, the specific root nodule bacteria for soybeans.

KELENY (1959) reported also some response of artificial inoculation on soybean and other legumes crops in some soils in this country.

However no practice of artificial inoculation were done by the farmers in this country. The soybean plants were nodulated by native strains of rhizobia in the soils, some were effective strains and some were ineffective ones.

Artificial inoculation with highly effective strains is desirable to ensure effective nodulation, since a high gain of nitrogen can be expected if the plant were nodulated by effective strains of rhizobia.

WEBER's experiments (1966) concluded that there is no known way of substituting commercial nitrogen for nodule nitrogen that will result in a significant increase in soybean yield and that more than 3 pounds of fertilizer nitrogen are required to substitute for 1 pound of nodule nitrogen.

He also found that the amount of nitrogen fixed symbiotically by soybeans ranged from 1 to 142 pounds per acre (1.121 to 159.182 kilograms per hectare), that is, 1 to 74% of the N uptake respectively.

Effective nodulation is therefore a major economic factor in soybean production.

In Indonesia farmers almost never used fertilizers nitrogen for their soybean crops, so that the nitrogen of the plant are derived from the soil and from the air if the plant were

nodulated.

The purpose of this study is to get additional facts for the evaluation of the status of soybean inoculation in Indonesia.

Since most of the data on soybean inoculation were dated back to 1941/1943, additional data were collected for reconfirmation. The most crucical problem which have to be considered before practicing artificial inoculation in large scale is the mass production of inoculant.

Experiments conducted by the author of this paper indicated that rhizobia culture is easy to prepare, although with the most simplest apparatus (JUTONO, 1968).

The preparation of legume-inoculant have been studied by other research workers in this country (NEWTON,1961; WIRJOATMODJO, 1964). With the present facilities of the laboratory of Agric. Microbiology in Jogjakarta it is possible to produce rhizobia inoculant in large scale.

MATERIALS AND METHODS.

1. Isolation of rhizobia from soybean root nodules, and Nitrogen-fixing capacity test.

Rhizobia strains were isolated from root nodules of soybeans by the usual method (ALLEN, 1953; FRED & WAKSMAN, 1928).

Two kind of media were used: mannitol-phosbate agar and yeast-water mannitol agar.

Well isolated colonies with rhizobia characteristics were selected from the isolation plates and reisolated in a second one. The isolates were then transferred to agar-slants (yeast-water mannitol agar). Each pure culture thus obtained was further tested for its nodule forming ability and for its nitrogen fixing capacity. Seeds of

soybean var.29 were used for these experiments. Tall gloss cylinders filled with river sand plus modified Crone solution (Nitrogen free) were used for testing the nodule forming ability and the nitrogen fixing capacity of the isolates.+) The sand and the culture solution were sterilized separately by the usual method.

Examination of the nodules on the roots were done after three weeks growing period of the soybean plants, while the Nitrogen analyses (with the conventional Kjeldahl method) after six weeks.

2. <u>The effect of artificial inoculation on soybean nodulation (Pot experiment I)</u>.

The purpose of this experiment is to know the effect of artificial inoculation on the number of nodules and also on the quality of the nodulation.

Forty pots filled with rhizobia deficient soil were used; twenty of them were planted with inoculated seeds while the others with uninoculated ones. The seeds were sterilized firstly with mercurichloride solution for 5 minutes and then washed several times in running water (steril water). The inoculated seeds contained about 100,000 rhizobia cells.

After three weeks of growing period the number of nodules were counted and nodulation was rated on an arbitary scale from 0 to 4. A "4" rating indicated clusters of large nodules around the tap root; a "0" rating signified no nodules. Rating "1", "2" and "3" were intermediate (see BURTON and CURLEY, 1965).

+) The procedure of inoculation of the seeds was as follows : Rhizobia cells taken from the agar slant culture were suspended in steril a water containing 6% sucrose, and then diluted with the same solution to get a final concentration of 100,000,000 cells per millilitre. 0.1 ml of this suspension was then mixed thoroughly with 100 seeds of soybean in steril petridish. Each seed will received 100,000 cells of rhizobia.

3. The effect of inoculated seeds to the uninoculated ones planted in the same pot (Pot experiment II).

Large pots were used for this experiment, filled with rhizobia deficient soils. A glass tube was planted at the centre of every pot. Four plant holes were digged at equidistant from the centre, so that the holes are 15 centimetres apart from each others forming a quadrangle. Two of the holes in every pot were then planted with inoculated seeds (soybean seeds var. 29) and the two others with uninoculated ones. The glass tube at the centre of each pot were used as a device for watering the soil, so that surface stream of water can be avoided during the experiment. The number of nodules were counted and rated as above after 4 weeks growing period of the plants.

4. The effect of various treatment on soybean inoculation (Pot experiment III).

Pots were filled with soils which contain some rhizobia according to preliminary observations.

Four kinds of treatment were applied to the soils before planting the seeds. Each treatment consisted of 60 pots in which 30 pots were planted with inoculated seeds and the others with uninoculated ones. The treatments were as follows : (a) plus compost (1 ton per hectare), (b) plus sulphate ammonia (1 quintal per hectare), (c) plus sulphate ammonia and dubbelsuperphosphate (1 quintal per hectare), (d) nothing added (Check).

Nodules counting and rating and Nitrogen analysis were done after 6 weeks growing period of the plants. The number of pods were counted after 14 weeks growing period.

5. The effect of organic material on the nodulation of soybean (Pot experiment IV).

Organic materials were given at the rate of one ton per hectare. Three kind of organic material were used in these experiments :

compost, cow manure and horse manure.

There are 8 kinds of treatment, each treatment consisted of 5 pots : (a) plus compost, seeds inoculated, (b) plus compost, seeds uninoculated, (c) plus cow manure, seeds inoculated, (d) plus cow manure, seeds uninoculated, (e) plus horse manure, seeds inoculated, (f) plus horse manure, seeds uninoculated, (g) nothing added, seeds inoculated, (h) nothing added, seeds uninoculated. After 4 weeks growing period the number of nodules were counted and the nodulation were rated as above.

6. Inoculation of field grown soybean.

The seeds were planted in rows in this field trial. Twelve rows were established. The plants in the rows were 20 centimeters apart from each others and the distant between rows were 40 centimeters. The rows were numbered from 1 to 12. The rows with odd numbers were planted with inoculated seeds (soybean var. 29) and row with even numbers with uninoculated seeds.

After 5 weeks growing period, the plants were digged carefully and then washed several times in running water. The number of nodules were then counted and the nodulation rated as above.

RESULTS AND DISCUSSION.

1. Isolation of rhizobia from soybean root nodules and the Nitrogen fixing capacity test.

Several strains of rhizobia can be isolated from soybean root nodules. The isolates were capable of forming nodules on soybean var.29. From the nitrogen analysis of the plants is found that the nitrogen content of the plants increased between the range of 1.41 to 7.84 milligram per plant using these strains as inoculant. Strain rH 16[+)] taken from our culture collection in the laboratory caused the highest increase (8.58 miligram per plant). These analysis were average of three analyses of the plants taken from the sand culture at the age of 6 weeks.

Thus the author found also that there are many strains of rhizobia for soybeans (In this case for soybean var.29). TOXOPEUS (1936) and DE JONGH (1943) reported also that several strains of rhizobia can be isolated from several soybean plants in Java.

One result of TOXOPEUS experiments indicated the presence of a very ineffective strains of Rhizobium japonicum, which he considered as a paresitic strain for some soybean variety. Thus it seems that the possibilities existed to select highly effective strains from local rhizobia strains in this country.

The introduction of new varieties in new areas should be accompanied also with the introduction of its highly effective microsymbiont (rhizobia strain).

+) Number of the rhizobia strain from the Culture Collection Lab. Agric. Microbiology Faculty of Agric. Gadjah Mada University.

2. The effect of artificial inoculation on soybean nodulation (Pot experiment I).

The results of this experiment are presented in table I.

Table I. The effect of art. inoculation on the number and quality of nodulation of soybean var. 29 (Pot experiment I).

Tabel I. (Pengaruh inokulasi-sengadja terhadap djumlah dan kwalitas perbintilan akar pada kedelai var. 29 (Pertjobaan pot I).

Treatment of the seeds (Perlakuan terhadap bidji-bidji)	Number of plants examined (Djumlah tanaman jang diperiksa)	Number of nudoles (Djumlah bintil akar)	Number of unnodulated plant (Djumlah tanaman jg tak berbintil akar)	Average rating ($Rata^2$ nilai perbintilan)
Uninoculated (tak diinokulasi)	50	93	31	0,52
Inoculated (diinokulasi)	50	356	2	3.01

A conclusion can be drawn that artificial inoculation increased the number of nodules produced on each plant and resulting also a better nodulation. A direct contact between the seeds and the rhizobia is necessary to ensure good nodulation. These results are in agreement with previous experiments.

3. Pot experiment II.

The results of this experiment are presented in table II.

Table II. The effect of inoculated seeds to the uninoculated ones planted in the same pot (Pot expement II).

Tabel II. (Pengaruh bidji jang diinokulasi-sengadja terhadap jang tidak, jang ditanam dalam pot jang sama) (Pertjobaan pot II).

Treatment of the seeds (Perlakuan terhadap bidji[2])	Number of plants examined (Djumlah tanaman jg diperiksa)	Number of nodules (Djumlah bintilakar)	Ratio nodulated plants : unnodulated plants (Ratio tanaman berbintil akar: tanaman tak berbintil akar).	Average rating (Rata[2] nilai perbintilan akar).
Uninoculated (tak diinokulasi)	79	158	29 : 50	0.49
Inoculated (diinokulasi)	65	587	55 : 10	3.12

No effect of inoculated seeds to the uninoculated ones can be observed in this experiment. Under these conditions the rhizobia did not disseminate through the soil. Although the plants were only 15 centimeters apart from each others no direct or indirect effect can be observed to the nodulation of the plants grown from the uninoculated seeds. Since the plants were examined only four weeks after planting we cannot know wether there will be any effect after longer growing period of the plants. But it is clear from this result that artificial inoculation will ensure early nodulation. Thus inoculation with highly effective strain will start an earlier nitrogen fixation, as stated by previous research worker also.

4. Pot experiment III.

The results are presented in tabel III.

Table III. The effects of several factors on art. inoculation of soybean var. 29 (Pot experiment III).

Tabel III. (Pengaruh beberapa faktor terhadap inokulasi sengadja pada kedelai var. 29)(Pertjobaan pot IV).

Treatment of the soils (Perlakuan thd. tanah).	treatment of the seeds (Perlakuan terhadap bidji)	I +) Number of nodules for 25 plants (Djumlah bintilakar tiap 25 tanaman)	I +) Average rating (Rata² nilai per-bintilan akar	I +) Average N content in % dry matter (Kadar N dalam % berat kering)	II ++) Number of pods for 50 plants (Djumlah polongan tiap 50 tanaman).
-	uninoc. (tak diinokulasi)	187	1.59	1.44	521
-	inoc. (diinokulasi)	386	2.21	1.73	981
plus compost +++)	uninoc. (tak diinokulasi)	250	1.84	1.97	1590
	inoc. (diinokulasi)	564	3.07	2.01	2508
plus sulphate ammonia ++++)	uninoc. (tak diinokulasi)	202	1.68	1.66	926
	inoc. (diinokulasi)	426	2.56	1.89	1119

plus sulphate ammonia and dubbel-superphosphate +++++)	uninoc. (tak diinokulasi)	201	1.65	1.76	963
	inoc. (diinokulasi)	558	3.04	1.99	1779

+) Analysis done after 6 weeks growing period of the plants (Analisa setelah tanaman berumur 6 minggu)
++) Analysis done after 14 weeks growing period of the plants (Analisa setelah tanaman berumur 14 minggu)
+++) One ton per hectare.
++++) One quintal per hectare.
+++++) One quintal per hectare.

From these results we found that artificial inoculation resulting an increase of the Nitrogen content of the plant and also the number of pods increased significantly. From the nodulation rating can be concluded that artificial inoculation ensure good nodulation. From the results can be concluded also that the soils were low in nitrogen and available phosphate. Compost give the best results in this type of soil used for these experiments. The stimulating effect of compost or organic fertilizer may be due to its mineral contents such as N, P, K and also trace elements. Thus to increase soybean yield many factors must be also considered beside the use of appropriate strain of rhizobia. That the bacteria (rhizobia) plays a very important role in the N economy of the plant (soybean plant) can be seen if we compared the results from inoculated seeds with uninoculated seeds plus N fertilizer.

5. Pot experiment IV.

The results are presented in table IV.

Table IV. The effects of organic material on the nodulation of soybean (Pot experiment IV) +)

Tabel IV. (Pengaruh bahan organik terhadap nodulasi(perbintilan akar) kedelai (Pertjobaan pot IV) +)

Treatment of the soils (perlakuan terhadap tanah)	Treatment of the seeds (perlakuan terhadap biji²)	Number of nodules for 10 plants (Djumlah bintil akar tiap 10 tanaman)	Number of effective nodules for 10 plants (Djumlah bintil efektip tiap 10 tanaman)	Average rating for 10 plants (Rata² nilai perbintilan akar).
Plus Compost (diberi kompos)	uninoculated (tak diinokulasi)	69	43	1.8
	inoculated (diinokulasi)	212	179	2.9
Plus Cow manure (diberi pupuk sapi)	uninoculated (tak diinokulasi)	78	52	1.9
	inoculated (diinokulasi)	197	165	3.0
Plus horse manure (diberi pupuk kuda)	uninoculated (tak diinokulasi)	81	47	1.8
	inoculated (diinokulasi)	165	138	2.8
Check (kontrol)	uninoculated (tak diinokulasi)	49	28	1.2
	inoculated (diinokulasi)	117	98	2.7

+) The organic materials were given at the rate of one ton per hectare
(Bahan organik diberikan dalam djumlah 1 ton per hektar).

The results indicated the importance of organic material in this type of soil used for the experiments. The stimulating effect of organic material on the nodulation is very clear, although the seeds were uninoculated the number of nodules can be increased about twice by addition of organic material only. As have been mentioned above (pot experiment III) the stimulation may be due to the content of trace elements and minerals. Organic material may increased also the waterholding capacity of the soil.

It should be mentioned here that root nodules with red pigments (leg-haemoglobine) were considered as effective nodules.

6. Inoculation of field grown soybean.

The results of this experiment are presented in table V.

Table V. The effect of artificial inoculation on the nodulation of soybean (field experiment).
Tabel V. (Pengaruh inokulasi sengadja terhadap nodulasi (perbintilan akar) kedelai (Pertjobaan lapangan).

Treatment of seeds (perlakuan thd bidji[2])	Number of nodules for 1000 plants (Djumlah bintil akar tiap 1000 tanaman)	Number of plants with nodulation rating of (Djumlah tanaman jang nilai perbintilannja sama dengan ...). 0	1	2	3	4	Average rating (Rata[2] nilai perbintilan akar)
uninoculated (tak diinokulasi)	7123	109	183	396	229	83	(1.92)
inoculated (diinokulasi)	13143	53	91	71	483	302	(2.89)

From the results can be concluded that the number of nodules increased about twice by artificial inoculation only, although the soils harbor sufficient rhizobia strains. From the nodulation rating is indicated that the quality of the nodulation can be improved by artificial inoculation.

GENERAL DISCUSSION AND CONCLUSIONS.

From these experiments can be concluded that a response of artificial inoculation on soybean is indicated although the soils used have grown soybean often and harbor sufficient soybean rhizobia. In many instances artificial inoculation ensure good nodulation of the soybean plants.

The dissemination of rhizobia through the soil seems very limited, except under certain moisture content. It is clear that direct contact between the root of germinated soybean seed with its rhizobia strain will started earlier nodule formation, and that means an early start of nitrogen fixation.

If one compared the results of these experiments with the ones reported by previous research workers in soybean inoculation in Java (TOXOPEUS and De JONGH) it seems that the role of nitrogen fixation in increasing the yield of soybean in Java (Indonesia) may not be overlooked.

The soybean plants derived its N from the soil and from the air. Under high soil N, about 20% of the plant N was fixed symbioticcaly, but when the available soil N was low 66% of the N was air derived (SEARS and LYNCH, 1951).

A very intensive study was done by WEBER (1966b) using nodulating and nonnodulating soybean isolines to evaluate the magnitude of N fixation (symbiotic) by soybean. From WEBER's experiment is concluded that :

a) nodulating and nonnodulating isolines offer a unique tool for measuring symbiotic relationships.

b) Nitrogen fixation is more desirable for plant growth than nonsymbiotic N.

c) Energy required for N fixation was less than that required for absorption of NO_3 and its subsequent reduction to $-NH_2$.

To get a general picture about soybean nodulation in Indonesia we have to study firstly the soybean culture pattern in this country.

There are two main pattern : (a) an intensive culture pattern,
(b) an extensive culture pattern.

In the last mentioned pattern the soybean seed is sown just before or after rice is harvested without any soil tillage (De VRIES, 1932).

Under this very extensive culture pattern it is difficult to ensure a high yield. The yield is about 1,5 - 3,5 quintals per hectare. If the seed contain about 34% protein (equivalent to 6% N), then 1,5 - 5,5 quintals dry seeds contain about 9 - 21,5 kilograms N (equivalent to 45 - 107.5 kilograms sulphate ammonia). Thus a depletion of soil-N will be the result after soybean crops if the Nitrogen fixing apparatus (root nodules) did not work efficiently, or if no Nitrogen fertilizers were applied. Farmers in this country rerely use N, P and K fertilizers for their soybeans as are other crops. Soybeans grown on rice fields usually benefit from the residual effects of previous application of fertilizers. In this extensive culture pattern one cannot evaluates properly the beneficial effects of artificial inoculation. Artificial inoculation should be considered as an intensification factor. Therefore intensification in soybean cultivation includes : (a) the use of improved varieties, (b) soil tillage, (c) improved culture methods, (d) application of fertilizers, (e) plant protection, (f) artificial inoculation with effective strains of rhizobia.

In other countries (e.g. U.S.A. and Australia) farmers usually inoculated their soybean crops because they fully aware that effective nodulation (N fixation) is a major economic factor in soybean production. Application of selected strains of rhizobia (legume-bacteria) by improved methods of inoculation may well be one of the simplest and most economic ways of increasing the yields of soybeans, and therefore an increased research attention to this aspect of soybean production is indicated (JOHNSON et al, 1965).

As have been cited in the introductory part of this paper the N fixed symbiotically by soybean range from 1.121 kilograms to 159.182 kilograms N per hectare, that is, 1 to 74% of the Nitrogen uptake respectively.

Before artificial inoculation can be introduced successfully in this country the most important factors should be considered :

(1) the socio-economical conditions of the small farmers which grow soybean as catch crop.
(2) the selection of the highly effective strains of rhizobia for each soybean variety in this country.
(3) the selection of a proper method of artificial inoculation.

From a survey on the presence of rhizobia in Jogjakarta soils and the effects of artificial inoculation on soybeans, can be concluded that from the 50 soils samples tested, 26 soils showed a response of artificial inoculation, although most of the soils samples (42 samples) were taken from rice fields where soybean were usually grown after rice (unpublished data from the author's experiments, 1963).
Thus artificial inoculation as a factor to increase the soybean production is indicated in this country.

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