The Effect of *Rhizobium* and N Fertilizer on Growth and Yield of Black Soybean (*Glycine max (L) Merril*)

O Herliana1*, T Harjoso2, A H S Anwar1, A Fauzi1

1 Laboratory of Agroecology, Department of Agrotechnology. Universitas Jenderal Soedirman
2 Laboratory of Agronomy and Horticulture, Department of Agrotechnology. Universitas Jenderal Soedirman

JL.. Dr Suparno No 61 Karangwangkal Purwokerto, Jawa Tengah Indonesia 53122

*Email: o.herliana@gmail.com*

**Abstract.** The study aimed to find out the growth response and yield of black soybeans (*Glycine max (L) Merril*) on *Rhizobium* inoculation, N fertilization and the interaction of both. This research was carried out in the Experimental Farm of the Faculty of Agriculture, Jenderal Sudirman University on May to October 2018. This study arranged in factorial completely randomized design (FCRD) with two factors. The first factor is the species rhizobium (R) consisting of *Rhizobium radiobacter*, *Rhizobium pusenses*, *Rhizobium nepotum*. The second factor is the N Fertilizer (N) consisting of 0, 25%, 50%, 75%, and 100% fertilizer dosage of N recommendations (100% dosage recommendation is similar to 120 kg). Variables observed were plant height, number of leaves, leaf area, stem diameter, dry weight of root, seed weight, number of pods, pod weight, and pod weight per plant. The results showed that: Application of the type of Rhizobium neoptum isolate gave the best results on leaf area, pod weight and number of pods. The treatment of 25% N fertilizer dosage recommendations gave the best results on variable stem diameter and pod weights, N 50% fertilizer dosage recommendations gave the best results on plant height, leaf area, number of leaves and number of pods. There was no interaction between the type of isolate and dose of N fertilizer.

**Keywords:** Black soybean, Ultisol, Rhizobium, N fertilizer, Growth and Yield

1. Introduction

Black soybean (*Glycine max (L)Merril*) is one of protein sources that have long been cultivated by the Indonesian farmer [1]. Soybean production in Indonesia is below the consumption number and still unstable. The instability of black soybean production in Indonesia is caused by a decrease in soybean cultivation area. For 5 years 2011 to 2015 soybean production has fluctuated on 2011 soybean production reached 851,286 tons and decreased to 779,992 tons in 2013 and then increased again in 2015 to reach 963,183 tons [2]. Improvement efforts that can be done are by expanding the marginal land area such as coastal sand land, oxisol land and ultisol land which are useful to increase the productivity value of food crops including soybeans [3].

Ultisol is one of the marginal soil types in Indonesia which widely distributed reaching 45,794,000 ha or about 25% of the total land area of Indonesia [4]. Ultisol soil is currently the main target of agricultural expansion, but it has many limiting factor, such as low soil organic matter, soil acidity, base saturation of...
less than 35%, high Al saturation, low CEC, low N, P and K, and very sensitive to erosion [5]. Soybean can be cultivated on acid soils such as ultisol by increasing the organic matter and Rhizobium inoculation. 

*Rhizobium* is a symbiotic N-fixing bacterium that can supply almost all of soybean nitrogen needs. The results of Rhizobium symbiosis with soybean plants will form nodules or root nodules that produce N [6] Explained by Sisworo et al [7], that the amount of nitrogen fixed on soybean plants is 33% of N total plants which is equivalent to 26-33 kg/N/ha/season. Added by [8], *Rhizobium* is able to fix N by 100-300 kg / ha, which means it is able to meet the needs of 80% N and increase 10-25% of soybean production, but the effectiveness of N uptake by plants depends on the suitability of Rhizobium and soil types. 

Nitrogen is a very important fertilizer for black soybean plants in the formation of food reserves, the formation of protein content in seeds and pods of seeds. To meet the needs of soybean plants on marginal land such as ultisol, it requires Nitrogen addition 25-75 kg/ha [9]. According to [10] the lack of N causes chlorophyll formation to be inhibited so that the leaves will turn pale green. The most easily observed symptom of N deficiency is pale green leaves, smaller leaf size, in very severe conditions, all pale yellow leaves and eventually fall, and growth of dwarf plants. N-deficiency generally occurs in sand-textured soil, acid soil (low pH) where the activity of fixing microorganisms (fixation) N (Rhizobium) is disrupted so that root nodules do not develop. N-deficiency symptoms also often occur in poorly drained land because N-fixing bacteria do not develop, and N absorption is inhibited.

The aim of this study was to find out 1) the growth response and yield of black soybeans inoculated with *Rhizobium*; 2) the growth response and yield of black soybeans given various dosage of nitrogen fertilizer; 3) the interaction effect of N fertilizer and type of rhizobium on the growth response and yield of black soybeans.

2. Material and Method

This research was carried out in the Experimental Farm of the Faculty of Agriculture, Jenderal Sudirman University with an altitude of 112 above sea level. The study was conducted from May to October 2018.

The materials used in this study were: black soybean seeds (malika cultivar), *Rhizobium radiobacter isolates, Rhizobium pusense, Rhizobium nepotum*, ultisol soil, alcohol, aquades, compost fertilizer, urea fertilizer, SP 36, KCl, broth media, ashy media, biocides. The tools used in this study were: test tubes, erlenmeyer, L stem, needle ose, bunsen, autoclave, pan, micropipet, sprayer, plastic sill, paper grill mat, poly bag, hoe, raffia rope, bamboo stick, thermohigrometer, Leaf Area Meters (LAM), Spectrophotometry, Lux Meters, analytical scales, meters, labels, stationery and camera.

This study was an experiment using Randomized Complete Block Design (RCBD). This study consisted of 2 factors. The first factor is the Rhizobium isolate which consists of 3 levels and the second factor is N Fertilizer Dosage which consists of 5 levels. There were 15 combinations of treatments and repeated 3 times to obtain 45 experimental units. Each experimental unit consists of 4 plants so that 180 polybags with a size of 40 x 40 cm are obtained.

The first factor is the species rhizobium (R) which consists of 3 levels, there are: $R_1 = Rhizobium radiobacter$, $R_2 = Rhizobium pusense$, $R_3 = Rhizobium nepotum$. The second factor is the dose of fertilizer N (N) which consists of 5 levels, there are: $NO = no fertilizer N$, $N1 = 25\%$ fertilizer dosage N recommendation ($0.2 \text{ g/polybag}$), $N2 = 50\%$ fertilizer dosage N recommendation ($0.4 \text{ g/polybag}$), $N3 = 75\%$ fertilizer dosage N recommendation ($0.6 \text{ g/polybag}$), $N4 = 100\%$ fertilizer dosage N recommendation ($0.8 \text{ g/polybag}$ or 75 kg/ha).

Variables observed were plant height, number of leaves, leaves area, stem diameter, dry weight of root, weight of 100 seeds, number of pods, pod weight, pod weight per plant. Data is analyzed using the Analysis of Varian (Anova) F test. If it is significantly different, proceed with Duncan's Multiple Range Test (DMRT) at the error level of 5%.

3. Result and Discussion

The matrix of the analysis of various data on growth and yield of black soybean plants on the treatment of *Rhizobium* isolates and N fertilizer doses showed diverse response.
Table 1. Matrices of Analysis of Variance to the Variables Observed

| No | Variables                  | R   | N   | RXN |
|----|----------------------------|-----|-----|-----|
| 1  | Plant height (cm)          | ns  | *   | ns  |
| 2  | Leaf number                | ns  | *   | ns  |
| 3  | Leaf area (cm²)            | *   | *   | ns  |
| 4  | Stem diameter (mm)         | ns  | *   | ns  |
| 5  | Weight of dry root (g)     | ns  | *   | ns  |
| 6  | Seed weight (g)            | ns  | ns  | ns  |
| 7  | Weight 100 seed (g)        | ns  | ns  | ns  |
| 8  | Weight of pods (g)         | *   | *   | ns  |
| 9  | Weight pods per plant (g)  | ns  | ns  | ns  |
| 10 | Number of pods (g)         | *   | *   | ns  |

Remarks: R = type of Rhizobium, N = Various N fertilizer Dose, RXN = Interaction of Rhizobium and N fertilizer, ns = not significant, *=significant

The effect of Rhizobium significantly affected to leaf area, weight of pod and number of pods. Application of N fertilizer gave significantly effect to plant height, leaf number, leaf area, stem diameter, weight of dry root, weight of pods an number of pods. Interaction both of treatment did not give significantly effect to the all variables observed.

3.1 Effect of Rhizobium application to the growth and yield of black soybean

Growth and yield response of black soybean presented in Table 2. The results of variance analysis showed that the treatment of Rhizobium isolates had an effect on leaf area, pod weight and number of pods.

Table 2. Effect of Rhizobium application to the growth and yield of black soybean

| Treatment       | Growth Response Variable | PH (cm) | NL (sheet) | LA (cm²) | SD (mm) | WDR (g) |
|-----------------|--------------------------|---------|------------|----------|---------|---------|
| R radiobacter   |                          | 22.49   | 24.939     | 131.52 ab| 7.09    | 2.328   |
| R pusense       |                          | 23.03   | 24.789     | 127.544 b| 7.5     | 2.534   |
| R nepotum       |                          | 24.84   | 25.806     | 138.756 a| 7.061   | 2.546   |
| CV (%)          |                          | 12.38   | 16.15      | 23.81 *  | 7.38    | 17.32   |

| Treatment       | Yield Variable Component| SW (g) | W100S (g) | WP (g) | WPP (g) | NP (g) |
|-----------------|-------------------------|--------|-----------|--------|---------|--------|
| R radiobacter   |                         | 8.181  | 8.817     | 9.205 b| 4.598   | 44.25 b|
| R pusense       |                         | 7.509  | 9.3       | 9.652 b| 4.372   | 49.45 b|
| R nepotum       |                         | 9.062  | 9.361     | 10.459 a| 4.712   | 56.22 a|
| CV (%)          |                         | 21.32  | 15.21     | 21.6 *  | 21.26   | 23.21 *|

Remarks: PH = Plant height, NL = Number of leaves, LA = Leaf area, SW = Seed weight, SD= Stem Diameter, WDR = Weight dry of Root, W100S = Weight of 100 seeds, WP = Weight of Pods, WPP = Weight of Pods per Plant, NP = Number of Pods. The numbers followed by different letters in the same column are significantly different at p 0.05 of DMRT.
The results of the variance analysis showed that the treatment of Rhizobium isolates had an effect on leaf area variables, *R. nepotum* isolates gave the best leaf area, ie 138,756 cm² followed by *R. radiobacter* isolates and *R. pusense* isolates 131.52 and 127.544 cm² respectively. Soybean plants that have a high leaf area will affect the results of photosynthesis and then affect the growth of pods so that the pods are formed more and weighted. This shows the response of soybean plants in utilizing additional N from *Rhizobium* to form chlorophyll [6]. [11] explained that plants that were applied to *Rhizobium* isolates had a greener color than plants without *Rhizobium* isolates due to the presence of more N candles produced from N fixation by *Rhizobium* isolates. The N compound produced from the nitrogen fixation process is exported from root nodules in the form of ureides (allantoin and allantoic acid) and translocated to leaves where they are catabolized and used for chlorophyll biosynthesis.

*R. nepotum* isolates are thought to be able to adapt to ultisol (acid) so that acidic conditions do not inhibit *Rhizobium* infection and root nodule formation. According [12] a fairly acidic soil pH does not cause major problems with bacterial strains, soybean plants, and the development of rhizobium. Although the presence of high amounts of total nitrogen in the rhizosphere is known to have a detrimental effect on nitrogen nodule fixation from legumes because it can inhibit rhizobium growth. This is according to what [13], revealed that inoculation by giving *Rhizobium japonicum* culture is intended so that these bacteria are associated with soybean plants to bind N2 free from the air so that plants can be utilized. Sutejo [14] added that with the availability of nitrogen elements, the leaves formed will also increase, resulting in increased leaf area. Nitrogen which is bound by Rhizobium is then used by plants for its growth. Thus the treatment of the soil concentration can meet nitrogen requirements to support leaf area indices that are closely related to the role of Rhizobium bacteria and other microorganisms in tethering nitrogen [15].

The results of the variance analysis showed that the treatment of the type of *Rhizobium* isolate had an effect on the variable weight of pods and number of pods. The best pod weight and number of pods were treated by *R. nepotum* isolates with pod weights 10.459 g and number of pods 56.22 cm followed by *R. radiobacter* and *R. pusense* with pod weights which were 9.205 g and 9.652 g and pods 44.25 and 49.45 pieces. The number of pods per plant formed is influenced by the treatment given by *Rhizobium*. This proves that bacteria that work with root nodules affect plants in forming pods. Jumrawati [16] added that the number of pods produced by soybean plants is largely determined by vegetative growth in this case such as photosynthesis rate and assimilation results. According to [17] about 80% of N availability in legumes occurs due to symbiosis with various types of *Rhizobium* bacteria. This biological N fixation has the potential to overcome N dependence from outside (N synthesis).

3.2 Effect of N fertilizer various dosage application to the growth and yield of black soybean

The treatment of N fertilizer dosage had an effect on variable plant height, leaf number, leaf area, stem diameter, weight dry of root, pod weight and number of pods.

| Treatment        | PH (cm) | NL (Sheet) | LA (cm²) | SD (mm) | WDR (g) |
|------------------|---------|------------|----------|---------|---------|
| Nitrogen 0%      | 20.379 b | 17.52 c    | 91.16 c  | 6.95 b  | 1.994   |
| Nitrogen 25%     | 23.824 ab | 22.68 bc  | 131.64 b | 7.18 b  | 2.568   |
| Nitrogen 50%     | 24.778 a | 27.25 ab   | 144.54 ab | 6.96 b  | 2.698   |
| Nitrogen 75%     | 23.175 ab | 29.41 a   | 129.24 b | 7.02 b  | 2.756   |
| Nitrogen 100%    | 25.120 a | 29.023 a  | 164.42 a | 7.96 a  | 2.332   |
| CV (%)           | 12.38 *  | 16.15 *    | 23.81 *  | 7.38 *  | 17.32*  |

Table 3. Effect of N fertilizer dosage application to the growth and yield of black soybean
| Treatment     | Yield Variable Component | SW (g) | W100S (g) | WP (g) | WPP (g) | NP (g) |
|---------------|--------------------------|--------|-----------|--------|---------|--------|
| Nitrogen 0%   | SW                       | 8.637  | 8.886     | 8.314 b| 4.659   | 33.56 c|
| Nitrogen 25%  | SW                       | 8.272  | 9.351     | 9.908 ab| 4.443   | 47.82 b|
| Nitrogen 50%  | SW                       | 7.542  | 9.624     | 9.034 ab| 4.117   | 63.25 a|
| Nitrogen 75%  | SW                       | 7.895  | 8.748     | 9.763 ab| 4.415   | 56.27 ab|
| Nitrogen 100% | SW                       | 8.908  | 9.188     | 11.509 a| 5.169   | 45.85 bc|
| CV (%)        |                          | 21.32  | 15.21     | 21.6 *  | 21.26   | 23.21 *|

Remarks: PH = Plant height, NL = Number of leaves, LA = Leaf area, SW = Seed weight, SD= Stem Diameter, WDR = Weight dry of Root, W100S = Weight of 100 seeds, WP = Weight of Pods, WPP = Weight of Pods per Plant, NP = Number of Pods. The numbers followed by different letters in the same column are significantly different at p 0.05 of DMRT.

The treatment of 25% N fertilizer dosage recommendations gave the best results on the variable stem diameter and pod weights which were 7.18 cm and 9.908 g and the N fertilizer 50% recommended the best results on variable plant height, leaf area, number of leaves and number of consecutive pods according to 24,778 cm, 144.54 cm, 27.25 pieces and 63.25 pieces. This is consistent with the statement of [18] that nitrogen is the dominant element compared to other elements in vegetative growth. N function to stimulate overall growth, especially the stems, branches and leaves. According to [19] N fertilizers at various doses play an effective role in increasing the N content in the soil so that the amount of N produced from being able to meet the N needs of soybean plants.

The treatment of 25% N fertilizer dosage recommendations was able to give the best results on variable stem diameter and pod weights which were 7.18 cm and 9.908 g. This shows that at this dose the N elements contained in urea fertilizer have affected growth, namely the formation of pods. Increased weight of pods associated with the function of nitrogen in plants. This is explained by [20] that the function of the element Nitrogen for plants is as a constituent of protein and chlorophyll. The formation of chlorophyll is useful in photosynthesis, where it acts as a synthesis of chlorophyll. Chlorophyll functions to capture sunlight which is useful for the formation of food in the process of photosynthesis. The results of photosynthesis will be used by plants for generative growth of plants such as the formation of plant pods.

The treatment of 50% N fertilizer dosage recommendations was able to give the best results on variable plant height, leaf area, number of leaves and number of pods. This shows that at this dose the N elements contained in urea fertilizer have affected growth in several growth variables. According to [21], one of the roles of N elements is the formation and growth of vegetative organs, namely the stems, leaves, and roots with the availability of these N elements, so the leaves formed will also increase which will increase the leaf area of the plant. The width of the leaves formed will affect the accumulation of asimilates produced.

[20] explains that seed development is more influenced by N supply during seed formation. [21] study of pod filling and seed formation is highly dependent on N availability, both N taken by *Rhizobium* bacteria from the air and N available in the soil and also influenced by the availability of P elements. If availability of N is in a balanced condition it will result in the formation of amino acids and proteins increases in the formation of seeds so that the pod is fully filled. Nitrogen is absorbed by plants through the soil, initially stacked on the stem and leaves. After the pods are formed, N is collected into pods, with the older part N (30 - 90%) being absorbed into the seeds.

The results of the variance analysis showed that there was no interaction between the application of *Rhizobium* isolates and the dose of N. fertilizer. It was thought that *Rhizobium*'s ability to infect roots and
nodule formation was inhibited by urea fertilization. According to [22] at the age of 4-5 days *Rhizobium* begins to form root nodules, so that by over-fertilizing nitrogen causes stress in the number and size of root nodules resulting in reduced effectiveness of *Rhizobium* in infecting and nodulation which plays a role in fixation of nitrogen (N2) and reduce the amount of fixation nitrogen. [23] added that inoculation with nitrogen fixation bacteria did not always succeed in increasing large production. This statement of [24] explained his study that the application of *Rhizobium* did not have a significant effect on the number of pods per plot.

4. Conclusion

Application of isolate *R. nepotum* gave the best results on leaf area of 138, 756 cm², pod weight 10.459 g and number of pods 56.22 pieces. The treatment of 25% N fertilizer dosage recommendations gave the best results on variable stem diameter and pod weights which were 7.18 cm and 9,908 g N 50% fertilizer dosage recommendations gave the best results on variable plant height, leaf area, number of leaves and number of pods respectively at 24.77 cm, 144.54 cm, 27.25 strands and 63.25 pieces. There is no interaction between the type of isolate and the dose of N.

Acknowledgement

The authors would like to thank the Universitas Jenderal Soedirman through LPPM for the funding of *Riset Peningkatan Kompetensi* that has been given so that the results of this scientific work can be realized.

References

[1] Mapegau. 2006. Effect of Water Stress on Growth and Yield of Soybean Plants (Glycine max L. Merr). *Jurnal Ilmiah Pertanian Kultura* 40(1) (In Indonesia)

[2] Central Agency Statistics. 2016. Statistics Official News No. 43/7 / Th. XIX

[3] Mariska I, Sjamsudin E, Sopandi D, Hutami S, Husni A, Kosmiatim M, and Vivi NA. 2004. Increased resistance of soybean plants to aluminum. *Jurnal Litbang Pertanian*. 23(2): 46-52. (In Indonesia)

[4] Prasetyo, B. H. and Suriadikarta, D. A. 2006. Characteristics, Potential, and Technology of Ultisol Management for the Development of Dryland Agriculture in Indonesia. *J. Litbang Pertanian*. 2(25). 39 p. (In Indonesia)

[5] Munir, M. 1996. *Indonesia’s Main Lands. Characteristics, Classification and Utilization*. Pustaka Jaya. Jakarta.

[6] Sucayhono D. and D. Harwono. 2014. Conformity Between Black Soybean and Symbiotic N-Bacteria Bacteria. *Prosiding Seminar Hasil Penelitian Tanaman Aneka Kacang dan Umbi*. 2014. (In Indonesia)

[7] Sisworo, W.H., M.M. Mutrosuhardjo, H. Rasyid and R.J.K. Myers. 2001. Application of Biofertilizer and Chemical Fertilizers: An Integrated Approach. *AgroBio Bulletin*. Bulletin AgroBio. Balai Pemeliharaan Bioteknologi Tanaman Pangan. Bogor. (In Indonesia)

[8] Sutanto. 2002. *Penerapan Pertanian Organik: Pemasyarakatan dan Pengembangannya*. Kanisius. Yogyakarta. 219p (In Indonesia)

[9] Soil Research Center. 2010. Recommendations for fertilizing soybean crops in various types of land use. http://balittanah.litbang.deptan.go.id. (On line). (accessed on June 24, 2018).

[10] Taufiq A. 2014. *Identification of the Problems of the Land of Soybean Plants*. Balai Penelitian Tanaman Aneka Kacang dan Umbi. http://balitkabi.litbang.pertanian.go.id/wp-content/uploads/2015/04/keharana_kedelai_a_taufiq_reduced-1.pdf accessed June 24, 2018

[11] Ndusha B. N., N.K. Karanja, P.L. Woomer, J. Walanglulu, G.N. Mushagalusa and J.M. Sanginga. 2017. Effectiveness of rhizobia strains isolated from South Kivu soils (Eastern D.R. Congo) on nodulation and growth of soybeans (*Glycine max*). *African Journal of Soil Science* 5(3): 367-377

[12] Solomon T. L. M. Pant and T. Angaw. 2012. Effects of Inoculation by Bradyrhizobium japonicum Strains on Nodulation, Nitrogen Fixation, and Yield of Soybean (*Glycine max* L.Merill) Varieties on
Nitisols of Bako, Western Ethiopia. Research Article of International Scholarly Research Network ISRN Agronomy 2012

[13] Sisworo, W.H., M.M. Mutrosuhardjo, H. Rasyid and R.J.K. Myers, dalam Simanungkalit. 2001. Aplikasi Pupuk Hayati dan Pupuk Kimia: Suatu Pendekatan Terpadu. Buletin AgroBio. Balai Pemeliharaan Bioeteknologi Tanaman Pangan. Bogor.

[14] Sutejo, M. 2002. Pupuk dan Cara Pemupukan. Rineka Cipta. Jakarta.

[15] Anggriani R., G.B. Non Shamdas and L. Tanggae. 2017. The Effect of Rhizobium on the Former Soil Origin of Soybean (Glycine max L.) Plant on the Next Soybean Growth for Its Use as a Learning Media. E-JIP BIOL 5(2): 119-141. (In Indonesia)

[16] Jumrawati. 2010. Effectiveness of Rhizobium sp. on the Growth and Yield of Soybean Plants on Saturated Water. Agriculture Service of Central Sulawesi Province. (In Indonesia)

[17] Hamdi H.Z. 2009. Enhancement of Rhizobia–Legumes Symbioses and Nitrogen Fixation for Crops Productivity Improvement P. In M. S. Khan et al.(eds). Microbial Strategies for Crop Improvement. 28 (11): 227-254

[18] Gardner, D.T., R W. Miller. 2004 Soils in Our Environment Prentice Hall. New Jersey. Journal of biogeography 550 p.

[19] Zainal M., A. Nugroho and N.E Suminarti. 2014. Response of Growth and Yield of Soybean (Glycine max (L.) Merill) at Various N Fertilization Levels and Chicken Cage Fertilizers. Jurnal Produksi Tanaman. 2 (6): 484-490

[20] Lakitan, B. 1993. Basics of Plant Physiology. PT Raja Grafindo Persada. Jakarta. 205 p

[21] Surtininggsih, T., Farida, dan T. Nurhariyati. 2009. Biofertilisasi Bakteri Rhizobium pada Tanaman Kedelai (Glycine max(L.) Merr.). Berk. Penel. Hayati, 15 : 31–35.

[22] Adisarwanto, T. 2008. Kedelai. Penebar Swadaya. Jakarta (In Indonesia)

[23] Zainal M., A. Nugroho and N.E Suminarti. 2014. Response of Growth and Yield of Soybean (Glycine max (L.) Merill) at Various N Fertilization Levels and Chicken Cage Fertilizers. Jurnal Produksi Tanaman 2(6):484-490. (In Indonesia)

[24] Mayani, N. and Hapsoh. 2011. Potential of Rhizobium and Urea Fertilizer to Increase Soybean Production (Glycine Max (L)) in Rice Fields. J.Ilma Pertanian Kultivar, 5(2). 67-75. (In Indonesia)