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Response of soybean (*Glycine max* L.) cultivars to inoculation of *Rhizobium japonicum* in coastal sandy land

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Authors and affiliations

OKTI PURWANINGSIH, PUGUH BINTANG PAMUNGKAS AND
C. TRI KUSUMASTUTI
oktipurwaningsih71@gmail.com

Address : Department of Agrotechnology, Faculty of Agriculture,
Universitas PGRI Yogyakarta, Yogyakarta 55182, Indonesia

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Abstract

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Response of soybean (*Glycine max* L.) cultivars to inoculation of *Rhizobium japonicum* in coastal sandy land

OKTI PURWANINGSIH^{1,*}, PUGUH BINTANG PAMUNGKAS¹ AND C. TRI KUSUMASTUTI¹

¹Department of Agrotechnology, Faculty of Agriculture
Universitas PGRI Yogyakarta, Yogyakarta 55182, Indonesia
*(e-mail: oktipurwaningsih71@gmail.com)

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ABSTRACT

Soybean cultivars have various responses to the inoculation of *Rhizobium japonicum*. Inoculation of *Rhizobium japonicum* does not always have a positive response to soybean growth and yield. This study was conducted during March-June 2022 to analyze soybean cultivar's response to inoculation of *Rhizobium japonicum* in the coastal area. This research has two factors that are arranged in Complete Randomized Design (CRD). The first factor of *Rhizobium* inoculation is with and without *Rhizobium japonicum* inoculation. The second factor is soybean cultivars, namely Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning. The results indicated that soybean cultivars gave various responses to *Rhizobium japonicum* inoculation. *Rhizobium japonicum* inoculation can increase the nitrogen fixation of soybean cultivars in coastal sandy land. Anjasmara cultivar gave the best response in fixing nitrogen. Indicators of nitrogen fixation can be seen from the number of root nodules, root nodule dry weight, and shoot N uptake. These results can have implications in optimizing *Rhizobium* inoculation in coastal sandy lands to sufficient nitrogen requirements.

Key words: Cultivars, inoculation, nitrogen fixation, *Rhizobium japonicum*, soybean

INTRODUCTION

Soybean plants have special features because they are able to symbiosis with nitrogen fixator bacteria for N₂ fixation. Sufficient nitrogen needs, especially for soybean plants grown in the coastal area, can be achieved by optimizing N₂ Fixation through a symbiosis between soybean plants and *Rhizobium japonicum* bacteria (Sarwani, 2013). One of the determining factors for the success of N₂ Fixation is the compatibility between *Rhizobium* strains and soybean cultivars. The determinant factor of the successful inoculation response is the varieties used and the availability of effective and compatible bacteria (Heerwaarden *et al.*, 2017). Nitrogen in the air cannot be directly utilized by plants because it is in the form of gas. The nitrogen must be fixed in advance so that it can be used as a source of N nutrients for plants. This diazotroph organism produces a nitrogenase enzyme which plays a role as a catalyst in the decomposition of nitrogen gas and reduces it to NH₃⁺ (Wang *et al.*, 2018; Yi *et*

al., 2019) Several bacteria can fix N₂, but in agriculture, *Rhizobium* is the most important bacteria in nitrogen fixation (Sulieman and Tran, 2017).

Rhizobia causes root nodule formation in the roots of legume plants. Without legumes, plants' rhizobia cannot fix nitrogen; on the contrary, without rhizobia legumes, plants also cannot fix nitrogen. Nitrogen is fixed in root nodules and only occurs if there is a symbiotic relationship between bacteria and legume plants (Pawar *et al.*, 2018; Etesami and Adl, 2020). The symbiosis between rhizobia and the roots of legume plants will produce nitrogen-fixing organs, namely root nodules. Nodules remain active for 50-60 days, and they will experience senescence. When the bacteroid and leghemoglobin are degraded, the nodules become green or brown. The shape, size, color, texture, and location of nodules in plants are determined by the host plant (Downie, 2014).

Research on the response of soybean cultivars to the inoculation of *Rhizobium japonicum* in the Bantul DIY Indonesia area

showed that the cultivars studied gave different responses (Purwaningsih *et al.*, 2013; Purwaningsih *et al.*, 2019). Research conducted at Savelugu-Nanton and Karanga in Nigeria showed that inoculated soybeans by *Rhizobium* increased yields ranging from 200 kg/ha (Lal *et al.*, 2016; Ronner *et al.*, 2016). Another research showed that *Rhizobium* inoculation increased soybean yields of more than 192% of plants without inoculation (Tairo and Ndakidemi, 2013). Fifty-six percent of crop yields from farmers increased by around 200 kg/ha when given additional *Rhizobium* inoculation (Ulzen *et al.*, 2018).

This research is vital to analyze the effect of *Rhizobium japonicum* inoculation on nitrogen fixation and soybean yields in the coastal sandy area. The impact of this research can optimize *Rhizobium japonicum* inoculation on sandy beach land, and the selection of varieties positively responds to this inoculation.

MATERIALS AND METHODS

Study Site

The research was carried out in the coastal area of Parangtritis Village, Kretek District, Bantul Regency, Yogyakarta, Indonesia. The height of the research site was 25 m above sea level and located between 14°04'50" - 27°50'50" South Latitude and 110°10'41" - 110°34'40" East Longitude. The research team came from the Department of Agrotechnology, Faculty of Agriculture, Universitas PGRI Yogyakarta, Indonesia. The research was conducted from March – June 2022.

Material and Experimental Design

The materials used in this research were soybean seeds from 10 cultivars, soybean legins, manure, plastic plates, polybags, urea fertilizer, SP36, and KCl. The tools used in this research were digital scales, rulers, ovens, pH meters, and leaf area meters. The research consisted of two factors arranged in a Completely Randomized Design (CRD), repeated three times. The first factor was the inoculation of *Rhizobium japonicum*, consisting of two levels: without inoculation and inoculation with *Rhizobium japonicum*. The

second factor was soybean cultivars, consisting of 10 cultivars namely Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning.

Research Procedures

The media used was the soil of the coastal area mixed with manure with a ratio of 1:1. The mixture of soil and manure was put into a polybag with a plastic plate as the base. Fertilization was performed when planting with a dose of 25 kg/ha Urea, SP36 75 kg/ha, and KCl 100 kg/ha. Before planting, the soybean seeds were first inoculated with soybean legin. Inoculation was done by mixing soybean legin with soybean seeds. To find the nutrient content in the growing media, soil analysis was carried out analysis of the shoot's N uptake using the Kjeldahl method.

Statistical Analysis

The observation was made on the number of root nodules, root nodules dry weight, absorption N canopy, dry canopy weight, seed weight per planting. The data obtained were analyzed by analysis of variance (ANOVA) at the level of 5%. Duncan's Multiple Range tests at the 5% accurate level were performed to determine the real difference between treatments.

RESULTS AND DISCUSSION

Analysis of Nutrient Content

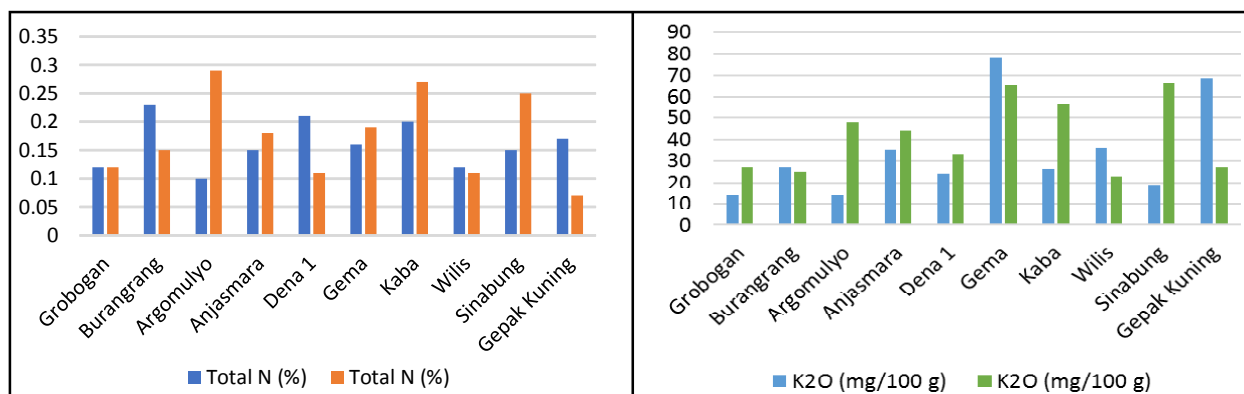
Soil analysis was carried out to determine the macronutrient content contained in the soil media used in the research. The results of the initial soil analysis can be seen in Table 1.

Based on the soil analysis results in Table 1, it could be seen that giving manure to the soil could increase the content of organic C-nutrients, total N, P_2O_5 , and K_2O . Macronutrient elements of the growing media used in this research as listed in Table 1, column 4. The soil analysis results at the end of the research are presented in Fig. 1.

Rhizobium inoculation has positive effects directly or indirectly on the physical and chemical properties of the soil so that it can increase soil fertility, soil organic essential

Table 1. Nutrients content (N, P, K) and soil pH in soils of coastal area and soils of coastal area + manure

S. No	Test Parameters	The soil of the coastal area	The soil of the coastal area and manure	Unit
(1)	(2)	(3)	(4)	(5)
1.	pH (H ₂ O)	6.20	7.71	
2.	C-organic	0.04	0.60	%
3.	N-total	0.06	0.18	%
4.	P ₂ O ₅ potential	152	209	mg/100 g
5.	K ₂ O potential	9	58	mg/100 g

Fig. 1. Soil analysis after research (Total N and K₂O).

sources, and increase nutrient sources N (Khaitov *et al.*, 2016; Abdiev *et al.*, 2019).

Nitrogen Fixation Ability Analysis

Cultivar ability in N₂ Fixation is determined by the nodules' activity. The more active nodules the Soybean had, and more the ability to fixate N₂. In this research, soybean's ability to fixate N₂ is examined from nodules number, nodules weight, and root's N uptake. The result of the examination of nodule number, weight, and N uptake can be seen in Table 3.

The result of variance analysis in Table 2 shows that inoculation *Rhizobium japonicum* interacted with soybean cultivar in the formation of nodules. Inoculation *Rhizobium japonicum* has the most effect on nodule formation for Anjasmara and Grobogan cultivars. Anjasmara and Grobogan cultivar inoculated with *Rhizobium japonicum* has the most number of nodules and the most weight compared to other cultivars whether it is inoculated or not by *Rhizobium japonicum*.

Grobogan, Burangrang, Kaba, Wilis, Sinabung, and Gepak Kuning are not inoculated with *Rhizobium* and cannot form

Table 2. Average number of nodules and nodules weight per plant

Cultivars	Nodules number		Nodules Weight (g)	
	Without Inoculation	Inoculation	Without Inoculation	Inoculation
Grobogan	0.00 b	26.00 a	0.00 r	0.32 q
Burangrang	0.00 b	12.80 b	0.00 r	0.15 qr
Argomulyo	1.40 b	1.60 b	0.02 r	0.03 r
Anjasmara	0.40 b	35.87 a	0.00 r	0.61 p
Dena 1	0.40 b	7.47 b	0.00 r	0.07 r
Gema	0.47 b	7.80 b	0.01 r	0.18 qr
Kaba	0.00 b	7.33 b	0.00 r	0.13 qr
Wilis	0.00 b	4.47 b	0.00 r	0.09 r
Sinabung	0.00 b	4.07 b	0.00 r	0.04 r
Gepak Kuning	0.00 b	5.93 b	0.00 r	0.09 r
	(+)	(+)		

Remarks: the number that is followed with the same letter on the same column and the same line shows that there are no differences according to the DMRT test on the real stage of 5% (+): Interaction.

Table 3. Average of N uptake (g) by shoots per plant

Cultivar	Without inoculation	Inoculation	Average
Grobogan	4.06 d	29.69 a	16.87
Burangrang	4.06 d	19.88 b	11.97
Argomulyo	6.35 cd	6.27 cd	6.31
Anjasmara	4.73 cd	35.06 a	19.89
Dena 1	4.76 cd	13.11 bcd	8.93
Gema	4.92 cd	14.74 bc	9.83
Kaba	4.06 d	13.79 bcd	8.92
Wilis	4.06 d	11.55 bcd	7.80
Sinabung	4.06 d	9.83 bcd	6.94
Gepak Kuning	4.06 d	12.83 bcd	8.44
Average	4.51	16.67	(+)

Remarks: Figures followed with the same letter on the same column and the same line shows that there are no differences according to the DMRT test on the real stage of 5%; (+): Interaction found.

nodules. In contrast, Anjasmara, Dena 1, and Gema can form nodules but are quite inactive, as shown by their low weight. The ability of soybeans to fixate nitrogen can be seen from the shoot's N uptake. In Table 4, it is seen that there is an interaction between the inoculated soybean cultivar to the shoot's N uptake. This may be caused by the content of N in the roots, which came from the fixation result and N found in the soil. N that is found in the soil may come from the N fertilizer. The plant's need for nitrogen can be obtained from N_2 Fixation, NH_4^+ , or NO_3^- uptake (Jilling *et al.*, 2018).

Anjasmara Cultivar has a more significant ability to take N than another cultivar. Even though the Anjasmara cultivar has a bigger nodules number compared to others, it cannot be detected whether the N

proportion in the shoots came from the N_2 Fixation or from the N that is contained in the soil. If we look at Anjasmara and Argomulyo cultivars not inoculated with *Rhizobium japonicum*, they have the same shoot's N uptake as the inoculated one. As seen from the soil result analysis at the end of the research, Anjasmara and Argomulyo planting media inoculated by *Rhizobium japonicum* have a higher N total than the planting media that is not inoculated. Kaba and Sinabung cultivars that are not inoculated have higher N uptake than those inoculated ones. The result of the soil analysis conducted at the end of the research inoculated planting medium of Kaba and Sinabung cultivars have a higher N total compared to the un-inoculated soil.

Water stress affects nitrogen fixation. Generally, water stress affects the nodule formation and nodule function. Water stress conditions will be the inhibitor of bacteria movement to the surface of the roots or accelerate soil particles on the surface of the roots to barricade the closeness of *Rhizobium* with the host plant. Drought can cause the death of nodules. Drought can also damage plasmodesmata which relate nodules system with the host plant's cells; further damage can be surrounding cells tissue damage which contains a bacteroid inside. Water stress results in the degrading of nitrogenase activity (Gandhi, 2018; Liu *et al.*, 2018; Aziez, 2023). Water availability plays a role in the transportation of nitrogen fixed outside the nodules. Nitrogen fixation result that is not transported immediately out of the nodules will

Table 4. Average seed weight per plant and harvest index

Cultivars	Seed weight per plant (g)			Harvest index		
	Without Inoculation	Inoculation	Mean	Without Inoculation	Inoculation	Mean
Grobogan	0.99	1.05	1.02 ab	0.15	0.11	0.13 pgrs
Burangrang	0.00	0.66	0.33 c	0.00	0.08	0.04 s
Argomulyo	0.52	1.00	0.76 ab	0.07	0.18	0.12 pqrs
Anjasmara	0.63	0.74	0.68 b	0.08	0.09	0.09 rs
Dena 1	0.55	1.02	0.79 ab	0.07	0.14	0.11 qrs
Gema	1.00	1.12	1.06 ab	0.15	0.21	0.18 pq
Kaba	0.84	1.20	1.02 ab	0.15	0.22	0.18 pq
Wilis	0.77	0.68	0.72 ab	0.13	0.13	0.13 pqrs
Sinabung	0.59	1.08	0.83 ab	0.12	0.15	0.14 pqr
Gepak Kuning	0.89	1.28	1.09 a	0.12	0.28	0.20 p
Average	0.68 q	0.98 p		0.11 y	0.16 x	
		(-)			(-)	

Remarks: Figures followed with the same letter on the same column and the same line shows that there are no differences according to the DMRT test on the real stage of 5%; (-): No interaction found.

be accumulated in the nodules that, inhibit the nitrogen fixation efficiency.

Plant Yield Analysis

Plant yield analysis was conducted using seed weight and harvest index. Analysis results show no interaction between *Rhizobium japonicum* inoculation with soybean cultivar in seed weight and harvest index. This is because plant yield is affected by numerous factors besides environmental factors and genetics. However, the soybean cultivar that has been inoculated with *Rhizobium japonicum* has a more significant average both in seed weight and harvest index number. The seed weight and harvest index result can be seen in Table 4.

Plant yield is affected mainly by genetic factors. However, the seed weight will not be maximum without enough assimilating supply on filling pods and seed growth period, including nitrogen availability for the plant. *Rhizobium japonicum* inoculation in soybeans can increase seed weight per plant (Fig. 2) and harvest index. This relates to the plant's ability to supply nutrients for plant growth, mainly in seed formation. The soybean plant needs N nutrients to grow, especially in pod formation and pod filling periods. This period's lack of water and nutrients will affect the plant yield. The Harvest index shows the translocated assimilate for seed forming. Soybean that is inoculated with *Rhizobium japonicum* can translocate more assimilate for seed forming compared to Soybean that is not inoculated with *Rhizobium japonicum*.

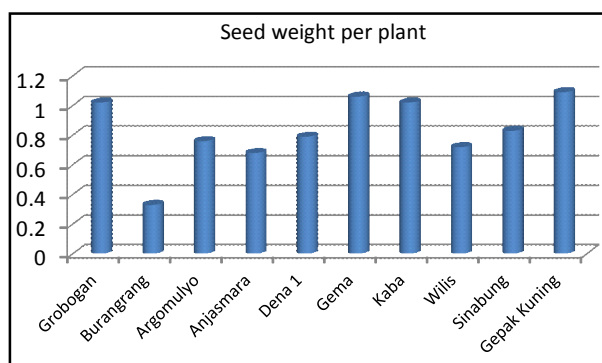


Fig. 2. Seed weight per plant (g) of soybean cultivar in the coastal area.

The analysis shows no correlation between seed weight with nodule number and

dry seed weight. Seed weight per plant correlates negatively with the shoot's N uptake ($r = -0.31^*$). The increase of the shoot's N uptake will decrease the dry seed weight per plant, and this is because the measurement of N uptake is conducted when vegetative growth is at its maximum, so the N uptake is used for vegetative growth.

Correlation result analysis shows that the harvest index correlates positively with dry seed weight per plant ($r = 0.75^*$). This shows that the harvest index is affected by seed weight per plant, seed weight increase will increase the harvest index. Seed weight per plant is affected by the number of pods per plant formed, where pod number is affected by *Rhizobium* inoculation, where inoculation can raise pod number by 13.22% compared without inoculation. The increase in pod number is affected by nitrogen function in the plant. Nitrogen forms protein and chlorophyll. Chlorophyll formation benefits the photosynthesis process, where this element plays a role in chlorophyll synthesis.

CONCLUSION

Inoculation of *Rhizobium japonicum* on soybean cultivars on coastal sandy land can increase nitrogen fixation, with indicators of root nodules formed and shoot N uptake. Plants inoculated with *Rhizobium japonicum* had a higher number of root nodules, nodule dry weight, and shoot N uptake than those not inoculated. Grobogan and Anjasmara cultivars gave a better response than other cultivars. Both cultivars were able to produce root nodules and absorb more N than the other cultivars. Inoculation of *Rhizobium japonicum* increased seed weight per plant to increase crop production.

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