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NITROGEN FIXATION ON SOYBEAN CULTIVAR PLANTED IN COASTAL AREA

ABSTRACT

The natural resources of the coastal area in Indonesia have the potential to be developed as an agricultural land with the support of both cultivation technology and land processing which one of them is by using *Rhizobium japonicum* bacteria to meet the need of nitrogen in soybean plants. This study aims to determine the characteristics of nitrogen fixation in various soybean cultivar planted in the coastal area.

The research was conducted in Mancingan, Parangtritis, Kretek, Bantul, DIY. The study was done using Completely Randomized Design which consisted of two factors and repeated three times. Factor I was *Rhizobium japonicum* inoculation (with inoculation and without inoculation); factor II was 10 various cultivar of soybean (Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning).

The results showed that the inoculation of *Rhizobium japonicum* in soybean cultivars in coastal area can increase the number of root nodule, dry weight of root nodule, dry seed weight per plant, and harvest index. Burangrang cultivar planted in coastal area is the most responsive to *Rhizobium japonicum* inoculation among other soybean cultivars tested.

Key words: Nitrogen fixation, soybean cultivar, coastal area

INTRODUCTION

Soybean is a strategic food commodity besides being both a protein source from food and energy source for alternative fuel (Adie and Krisnawati, 2014). However Indonesia still has to import the soybean to meet the domestic demand. During the period of 2010 - 2014 the demand was ± 2.3 million tons of dry beans/ year, while soybean production in 2015 was only 963.183 ton / ha (BPS, 2016). Every year Indonesia still has to import soybean ± 1.3 million tons. The soybean production tends to decrease as the decreasing land area for cultivation which transferred into residential and industrial estate. Also the farmers prefer to cultivate other food crops such as rice and corn. To reduce the dependence on the import, it is necessary to increase the soybean production by expanding the soybean cultivation area (the extensification). The rice field area in Indonesia in 2015 covering ± 8 million ha, while the harvest area of soybean is only 614 thousand ha (BPS, 2016). Therefore it is necessary to make an effort to increase the area for soybean cultivation by utilizing the marginal land that has not been widely used in Indonesia such as coastal area.

Indonesia as an archipelagic country has a long coastline of about 81.000 km. Coastal areas have the potential of various natural resources that can be developed such as fisheries, livestock, agriculture and tourism sector. The utilization of coastal areas for agricultural sector experiencing some constraints which are salinity, low soil moisture, and low content of nutrient and organic materials. During the day, the soil temperature can reach 55 -60°C. Also the aeration which runs faster

causing slower accumulation of organic matter as well as the ability to hold the water (Kertonegoro, 2006). Meanwhile, the sunlight and strong wind which carry water vapor with the high salinity lead to limited variety of the cultivated plants in coastal area. So that soil and salinity conditions are the important issue in planting the crops in the coastal areas.

Soybean, as a strategic commodity in Indonesia, has high sensitivity to salinity and it become the main problem as it can affect the plant growth due to ionic and osmotic stress. The salinity stability condition can decrease the germination, fresh seed weight, seed growth, and dry weight (Farhoudi and Tafti, 2011). The coastal land has the nature of margin to the soil texture; the ability to hold water; chemical content; and soil organic matters. The effort to utilize the coastal area is by improving the physical, chemical, and soil organisms so that soil-water-plant interaction can be well occurred. Another effort is by managing the interaction between the plants and atmosphere (Gunadi, 2002). According to Sudihardjo (2001) cit. Hartati (2008), based on criteria of CSR / FAO (1983), the actual suitability of the Southern coastal area of DIY is classified into not suitable or marginally suitable for food crops and vegetables.

The problem of low nutrient content in coastal area can be managed by the addition of organic fertilizer. Besides, it can also be optimized by the application of *Rhizobium japonicum* bacteria. The cultivation of soybean in coastal area requires large amount of nutrient especially N. The nitrogen requirement can be fulfilled if the fixation of N_2 can be optimally occurred by the plants. The N_2 fixation is determined by the suitability of the *Rhizobium* strain and the soybean cultivar. Therefore, it is necessary to conduct a study to find out the response of various soybean cultivars to *Rhizobium* inoculation which suitable to be developed in coastal area. The results of this study are expected to be used as the way to optimize the utilization of marginal land in Parangkusumo coastal area of Bantul Regency for the development of soybean crops using low energy input by maximizing the symbiosis between soybean plants and *Rhizobium* bacteria to fix N_2 .

The fixation of N_2 occurs because of the symbiotic between the plants and prokaryotic bacteria diazotroph, the bacterium that can inhibit the molecules of nitrogen gas in the air (MacDicken, 1994). The diazotroph organism produces a nitrogenase enzyme that acts as a catalyst in the decomposition of nitrogen gas and reduces into NH^{3+} . The symbiosis between *Rhizobia* and leguminous plant roots will produce nitrogen-blocking organs which called as root nodules. In the root nodules, there are cells that are slightly enlarged contains bacteroid and among them there are more smaller cells contained starch. The shape, size, color, texture and location of the root nodule on the plants will be determined by the host plant (Dierolf, et al., 2001). The effectiveness of *rhizobia* is determined by the color of the root nodules, the pink root nodules are more effective in fixing the nitrogen than the white ones. This will affect the growth and yield of the host plants (Karaca and Uyanoz, 2012). Until 2016 the government has released 83 superior varieties of soybean that have superiority in morphological and agronomic characteristic. The new improved varieties released in 2014-2016 were Pearl 2 and Pearl 3 (black soybean); Demas 1 (Admix Soybean soybean); Dena 1 and Dena 2 (Shade tolerant soybean); Devon 1

(high isoflavone soybean) ; Dega 1; and Deja 1 and Deja 2 which are water-tolerant (Susanto and Nugrahaeni, 2017).

The results of Douka's research, et al. (1986) and Aaron and Ammar (2001) showed that soybean cultivars will respond differently to *Rhizobium* inoculation. The study of *Rhizobium* inoculation on several varieties of *Pisum sativum* L. showed that the three varieties of *Pisum sativum* L. which tested have a good result on root nodules; dry weight of root nodules; dry weight of root, and seed yield (Ali, et al., 2008).

Therefore, we aims to examine the characteristics of nitrogen fixation on various soybean cultivars planted in the coastal area.

MATERIALS AND METHODS

The research was conducted in Mancingan, Parangtritis, Kreek, Bantul DIY. The study was done in Complete Randomized Design (CRD) consists of two factors in three replications. Factor I was *Rhizobium japonicum* inoculation (without inoculation and with inoculation of soybean legin). Factor II was the various cultivar of soybeans(Grobogan cultivars, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning). The research obtained 20 combinations of the treatments.

The planting media used was beach sand and cow manure (ratio 1: 1) in polybag (20 cm x 30 cm). The polybags used were installed with plastic plates to avoid the contamination. Before planting, the soybean seeds were inoculated with soybean legins containing *Rhizobium japonicum* bacteria. Before the inoculation, soybean seeds were soaked in sugar solution so that soybean legin will be more easily attached to the seed. The inoculation treatment was done by mixing the seeds with soybean legin. The inoculation of soybean seeds is carried out in a shady place (not directly exposed by the sunlight).

The variables observed were the number of root nodules, dry weight of root nodules, and N canopy uptake that were performed at the maximum vegetative growth. The observations on the growth variables were performed at the time of vegetative growth including the leaf area and dry weight of the plant. The observations on the yield variables was done at harvest time including the weight seeds per plant, weight of 100 seeds, and harvest index.

The data were analyzed by using analysis of variance at 5% significant level and followed with DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSIONS

The soil analysis was done to find out the nutrient content in the soil. The analysis was conducted on the sand soil samples in the research location and planting media in the form of soil mix and manure. The soil analysis showed that the application of manure can increase the nutrient content of C-organic, N total, P₂O₅ and K₂O (See Table 1).

Insert Table 1 here.

The Nitrogen Fixation Ability

The ability of soybean cultivars in fixing N₂ is determined by the activity of the root nodules. The more root nodules are active, the higher ability of soybeans in fixing N₂. In this study the ability of soybean in fixing N₂ was observed from the number of root nodules; dry weight of root nodules; and N canopy uptake. Table 2 shows the interaction between *Rhizobium japonicum* inoculation and various type of soybean cultivar in the formation of root nodules. This indicates that soybean cultivars provide different response to the *Rhizobium japonicum* inoculation. Anjasmara and Grobogan cultivar showed the best result on the number of root nodules and nodule dry weight. This result was supported by the study done by Purwaningsih (2012) on agricultural land in Banguntapan which showed that Anjasmara cultivar inoculated with *Rhizobium japonicum* has the highest number of root nodules and significantly different from the Wilis, Sinabung, Gepak Yellow, Argomulyo, and Grobogan.

Insert Table 2 here.

The study conducted by Purwaningsih (2012) clasified Anjasmara, Argomulyo, Gepak Kuning cultivar into the groups of soybeans cultivar that resulted higher the fixation of nitrogen and seed yield followed by inoculation with *Rhizobium japonicum*. While the Grobogan, Sinabung, Wilis cultivar are clasified into the less-responsive group. These study indicates that in different environment, soybean cultivar will respond differently to the inoculation of *Rhizobium japonicum*.

The ability of soybean plants in fixing nitrogen can be determined by the uptake of N canopy. Table 3 shows that there is no interaction between *Rhizobium japonicum* inoculation and soybean cultivar to the N canopy uptake. This is due to the N content present in the canopy is not only derived from the N₂ fixation but also comes from the N present in the planting medium. *Rhizobium japonicum* inoculation has no significant effect on the uptake of N canopy as the low environmental stresses of soil moisture and high salinity causing ineffective to the nitrogen fixation. This is supported by the result of correlation analysis which shows that N canopy absorption is not significantly correlated with the number of root nodules ($r = 0.25ns$) and dry weight of root nodule ($r = 0.23ns$), so that N contained in the leaf is suspected derived from the N uptake from the soil.

Insert Table 3 here.

Nitrogen requirements for the plants can be obtained through the N_2 fixation, both NH_4^+ and NO_3^- absorption (Gerendas and Sattelmachner, 1990). Only nitrogen in the form of ammonium ions and nitrates is available for plants taken through rooting systems (Salisbury and Ross, 1991).

The characteristic of soil moisture in planting medium used in this study is low as the ability of sand particles in binding the water is low as well (even after the application of manure). The environmental conditions also tend to high evaporation as the water stress affects the nitrogen fixation (Yelton et al., 1983). In general, water stress affects the formation and function of root nodules as it will inhibit the movement of bacteria to reach the root surface or encourage attachment of soil particles on the root surface thus will inhibit the attachment of *Rhizobium* and the host plants. Drought can cause nodule death as it can damage the plasmodesmata that connect to the root tissue and the host plant cells, and the damage can continue in the form of cell around the cells containing the bacterium in the nodule. The water strain results in decreasing activity of nitrogenase (Bordeleau and Prevost, 1994). The availability of water plays an important role in transporting the nitrogen fixation out of the root nodules. The results of nitrogen tethering that is not immediately transported out of the root nodules will accumulate in the root nodules thus inhibiting the efficiency of nitrogen fixation.

Plant Growth Analysis

The variables of leaf area and dry weight were observed when the plants reach maximum vegetative stage (See Table 4).

Insert Table 4. here.

The result showed that the inoculation of *Rhizobium japonicum* only give a significant effect on leaf area and dry weight of Burangrang cultivar. The *Rhizobium japonicum* inoculation did not show any significant result on the leaf area and dry weight of Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning cultivar.

Burangrang cultivar has a narrow leaf as it accumulates lower dry matter (resulting from photosynthesis) than other cultivars. So the leaf area and dry weight will increase significantly after inoculated with the *Rhizobium japonicum*.

The Seed Yield

The inoculation of *Rhizobium japonicum* in soybean can increase the harvest index and dry weight of seeds per plant. This is related to the ability of plants to supply nutrients for the plant growth, especially for the seed formation. Soybean requires N nutrients for growth, especially in the forming and filling period of seed. Lack of water and nutrients in this period will affect the yield. The harvesting index describes the ability of plants to channel photosynthate to economical yield for the seed

formation. The inoculated plants have more ability to transfer the photosynthesis result for seed formation than the uninoculated one. The harvest index and dry seed weight can be seen in Table 5.

Insert Table 5 here.

The inoculation improves seed yield significantly as the plants have more ability to deliver assimilates to economical yield (high harvest index) not because of its ability in forming assimilates (dry weight of the plant). The correlation analysis showed negative and significant correlation with the plant dry weight ($r = -0.33$). While the harvest index correlated positively and significantly with the seed weight per plant ($r = 0.75^{**}$). The smaller dry weight of the plants, the more harvest index and the weight of the seeds per plant will be obtained.

CONCLUSIONS

The inoculation of *Rhizobium japonicum* in some soybean cultivars planted in coastal area can effectively increase the number of root nodules and dry weight of the root nodule (the N_2 blocking organ in nitrogen fixation). The ability to fulfill the nitrogen need in plants is followed by the increasing of crop yield. The inoculated cultivar has higher harvest index and seed weight per plant than uninoculated ones. Burangrang cultivar is more responsive to *Rhizobium japonicum* inoculation in coastal area than Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning cultivar.

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Table 1. Nutrient content (N, P, K) and pH

No	Variables Observed	Soil of coastal area	Soil of coastal area + 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/100g
5.	K ₂ O potential	9	58	mg/100g

Table 2. The Average number and dry weight of root nodule on various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Number of root nodule		Dry weight of root nodule (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
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
	(+)		(+)	

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

Table 3. N uptake of canopy (%) of various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Uninoculated	Inoculated	Average
Grobogan	1,64	1,90	1,77 abcd
Burangrang	1,97	2,46	2,22 ab
Argomulyo	1,93	1,93	1,93 abcd

Anjasmara	2,27	2,28	2,28 a
Dena 1	1,58	1,88	1,73 bcd
Gema	1,73	2,29	2,01 abc
Kaba	1,76	1,51	1,63 cd
Wilis	2,10	2,18	2,14 abc
Sinabung	2,25	1,75	2,00 abc
Gepak Kuning	1,32	1,66	1,49 d
Average	1,85 p	1,99 p	(-)

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level.

Table 4. The Effect of *Rhizobium japonicum* inoculation on leaf area, fresh weight of plant, dry weight of soybean cultivars.

Cultivars	Leaf Area (cm ²)		Dry weight of root nodule (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Grobogan	11.66 d	12.60 cd	7.70 pq	12.28 p
Burangrang	3.70 e	15.22 abc	0.91 r	10.15 pq
Argomulyo	16.34 ab	17.78 a	8.60 pq	7.55 pq
Ajasmara	14.99 abc	15.53 abc	8.75 pq	8.95 pq
Dena 1	14.18 bcd	15.82 abc	8.76 pq	8.14 pq
Gema	17.15 ab	15.89 ab	10.07 pq	7.42 pq
Kaba	17.56 a	15.95 ab	8.23 pq	6.53 pq
Wilis	14.91 abc	15.17 abc	7.08 pq	6.76 pq
Sinabung	15.16 abc	15.74 abc	8.39 pq	6.80 pq
Gepak kuning	15.50 abc	15.95 ab	8.86 pq	5,39 qr
	(+)		(+)	

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

Table 5. The Average of dry seed weight per plant and harvest index

Cultivar	Dry seed weight per plant (g)				Harvest index	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Average
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
Rerata	0.68 q	0.98 p		0.11 y	0.16 x	
	(-)			(-)		

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ABSTRACT

The natural resources of the coastal area in Indonesia have the potential to be developed as an agricultural land with the support of both cultivation technology and land processing which one of them is by using *Rhizobium japonicum* bacteria to meet the need of nitrogen in soybean plants. This study aims to determine the characteristics of nitrogen fixation in various soybean cultivar planted in the coastal area.

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INTRODUCTION

Soybean is a strategic food commodity besides being both a protein source from food and energy source for alternative fuel (Adie and Krisnawati, 2014). However Indonesia still has to import the soybean to meet the domestic demand. During the period of 2010 - 2014 the demand was ± 2.3 million tons of dry beans/ year, while soybean production in 2015 was only 963.183 ton / ha (BPS, 2016). Every year Indonesia still has to import soybean ± 1.3 million tons. The soybean production tends to decrease as the decreasing land area for cultivation which transferred into residential and industrial estate. Also the farmers prefer to cultivate other food crops such as rice and corn. To reduce the dependence on the import, it is necessary to increase the soybean production by expanding the soybean cultivation area (the extensification). The rice field area in Indonesia in 2015 covering ± 8 million ha, while the harvest area of soybean is only 614 thousand ha (BPS, 2016). Therefore it is

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necessary to make an effort to increase the area for soybean cultivation by utilizing the marginal land that has not been widely used in Indonesia such as coastal area.

Indonesia as an archipelagic country has a long coastline of about 81.000 km. Coastal areas have the potential of various natural resources that can be developed such as fisheries, livestock, agriculture and tourism sector. The utilization of coastal areas for agricultural sector experiencing some constraints which are salinity, low soil moisture, and low content of nutrient and organic materials. During the day, the soil temperature can reach 55 -60°C. Also the aeration which runs faster causing slower accumulation of organic matter as well as the ability to hold the water (Kertonegoro, 2006). Meanwhile, the sunlight and strong wind which carry water vapor with the high salinity lead to limited variety of the cultivated plants in coastal area . So that soil and salinity conditions are the important issue in planting the crops in the coastal areas.

Soybean, as a strategic commodity in Indonesia, has high sensitivity to salinity and it become the main problem as it can affect the plant growth due to ionic and osmotic stress. The salinity stability condition can decrease the germination, fresh seed weight, seed growth, and dry weight (Farhoudi and Tafti, 2011). The coastal land has the nature of margin to the soil texture; the ability to hold water; chemical content; and soil organic matters. The effort to utilize the coastal area is by improving the physical, chemical, and soil organisms so that soil-water-plant interaction can be well occurred. Another effort is by managing the interaction between the plants and atmosphere (Gunadi, 2002). According to Sudihardjo (2001) cit. Hartati (2008), based on criteria of CSR / FAO (1983), the actual suitability of the Southern coastal area of DIY is clasified into not suitable or marginally suitable for food crops and vegetables.

The problem of low nutrient content in coastal area can be managed by the addition of organic fertilizer. Besides, it can also be optimized by the application of *Rhizobium japonicum* bacteria. The cultivation of soybean in coastal area requires large amount of nutrient especially N. The nitrogen requirement can be fulfilled if the fixation of N_2 can be optimally occurred by the plants. The N_2 fixation is determined by the suitability of the Rhizobium strain and the soybean cultivar. Therefore, it is necessary to conduct a study to find out the response of various soybean cultivars to *Rhizobium* inoculation which suitable to be developed in coastal area. The results of this study are expected to be used as the way to optimize the utilization of marginal land in Parangkusumo coastal area of Bantul Regency for the development of soybean crops using low energy input by maximizing the symbiosis between soybean plants and *Rhizobium* bacteria to fix N_2 .

The fixation of N_2 occurs because of the symbiotic between the plants and prokaryotic bacteria diazotroph, the bacterium that can inhibit the molecules of nitrogen gas in the air (MacDicken, 1994). The diazotroph organism produces a nitrogenase enzyme that acts as a catalyst in the decomposition of nitrogen gas and reduces into NH_3^+ . The symbiosis between Rhizobia and leguminous plant roots will produce nitrogen-blocking organs which called as root nodules. In the root nodules, there are cells that are slightly enlarged contains bakteroid and among them there are more smaller cells contained starch.

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Therefore, we aims to examine the characteristics of nitrogen fixation on various soybean cultivars planted in the coastal area.

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The planting media used was beach sand and cow manure (ratio 1: 1) in polybag (20 cm x 30 cm. The polybags used were installed with plastic plates to avoid the contamination. Before planting, the soybean seeds were inoculated with soybean legins containing *Rhizobium japonicum* bacteria. Before the inoculation, soybean seeds were soaked in sugar solution so that soybean legin will be more easily attached to the seed. The inoculation treatment was done by mixing the seeds with soybean legin. The inoculation of soybean seeds is carried out in a shady place (not directly exposed by the sunlight).

The variables observed were the number of root nodules, dry weight of root nodules, and N canopy uptake that were performed at the maximum vegetative growth. The observations on the growth variables were performed at the time of vegetative growth including the leaf area and dry

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weight of the plant. The observations on the yield variables was done at harvest time including the weight seeds per plant, weight of 100 seeds, and harvest index.

The data were analyzed by using analysis of variance at 5% significant level and followed with DMRT (Duncan's Multiple Range Test).

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RESULTS AND DISCUSSIONS

The soil analysis was done to find out the nutrient content in the soil. The analysis was conducted on the sand soil samples in the research location and planting media in the form of soil mix and manure. The soil analysis showed that the application of manure can increase the nutrient content of C-organic, N total, P₂O₅ and K₂O (See Table 1).

Insert Table 1 here.

The Nitrogen Fixation Ability

The ability of soybean cultivars in fixing N₂ is determined by the activity of the root nodules. The more root nodules are active, the higher ability of soybeans in fixing N₂. In this study the ability of soybean in fixing N₂ was observed from the number of root nodules; dry weight of root nodules; and N canopy uptake. Table 2 shows the interaction between *Rhizobium japonicum* inoculation and various type of soybean cultivar in the formation of root nodules. This indicates that soybean cultivars provide different response to the *Rhizobium japonicum* inoculation. Anjasmara and Grobogan cultivar showed the best result on the number of root nodules and nodule dry weight. This result was supported by the study done by Purwaningsih (2012) on agricultural land in Banguntapan which showed that Anjasmara cultivar inoculated with *Rhizobium japonicum* has the highest number of root nodules and significantly different from the Wilis, Sinabung, Gepak Yellow, Argomulyo, and Grobogan.

Insert Table 2 here.

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The study conducted by Purwaningsih (2012) clasified Anjasmara, Argomulyo, Gepak Kuning cultivar into the groups of soybeans cultivar that resulted higher the fixation of nitrogen and seed yield followed by inoculation with *Rhizobium japonicum*. While the Grobogan, Sinabung, Wilis cultivar are clasified into the less-responsive group. These study indicates that in different environment, soybean cultivar will respond differently to the inoculation of *Rhizobium japonicum*.

The ability of soybean plants in fixing nitrogen can be determined by the uptake of N canopy. Table 3 shows that there is no interaction between *Rhizobium japonicum* inoculation and soybean cultivar to the N canopy uptake. This is due to the N content present in the canopy is not only derived from the N₂ fixation but also comes from the N present in the planting medium. *Rhizobium japonicum* inoculation has no significant effect on the uptake of N canopy as the low environmental stresses of

soil moisture and high salinity causing ineffective to the nitrogen fixation. This is supported by the result of correlation analysis which shows that N canopy absorption is not significantly correlated with the number of root nodules ($r = 0.25ns$) and dry weight of root nodule ($r = 0.23ns$), so that N contained in the leaf is suspected derived from the N uptake from the soil.

Insert Table 3 here.

Nitrogen requirements for the plants can be obtained through the N_2 fixation, both NH_4^+ and NO_3^- absorption (Gerendas and Sattelmachner, 1990). Only nitrogen in the form of ammonium ions and nitrates is available for plants taken through rooting systems (Salisbury and Ross, 1991).

The characteristic of soil moisture in planting medium used in this study is low as the ability of sand particles in binding the water is low as well (even after the application of manure). The environmental conditions also tend to high evaporation as the water stress affects the nitrogen fixation (Yelton et al., 1983). In general, water stress affects the formation and function of root nodules as it will inhibit the movement of bacteria to reach the root surface or encourage attachment of soil particles on the root surface thus will inhibiting the attachment of *Rhizobium* and the host plants. Drought can cause nodule death as it can damage the plasmodesmata that connect to the root tissue and the host plant cells, and the damage can continue in the form of cell around the cells containing the bacterium in the nodule. The water strain results in decreasing activity of nitrogenase (Bordeleau and Prevost, 1994). The availability of water plays an important role in transporting the nitrogen fixation out of the root nodules. The results of nitrogen tethering that is not immediately transported out of the root nodules will accumulate in the root nodules thus inhibiting the efficiency of nitrogen fixation.

Plant Growth Analysis

The variables of leaf area and dry weight were observed when the plants reach maximum vegetative stage (See Table 4).

Insert Table 4. here.

The result showed that the inoculation of *Rhizobium japonicum* only give a significant effect on leaf area and dry weight of Burangrang cultivar. The *Rhizobium japonicum* inoculation did not show any significant result on the leaf area and dry weight of Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning cultivar.

Burangrang cultivar has a narrow leaf as it accumulates lower dry matter (resulting from photosynthesis) than other cultivars. So the leaf area and dry weight will increase significantly after inoculated with the *Rhizobium japonicum*.

The Seed Yield

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The inoculation of *Rhizobium japonicum* in soybean can increase the harvest index and dry weight of seeds per plant. This is related to the ability of plants to supply nutrients for the plant growth, especially for the seed formation. Soybean requires N nutrients for growth, especially in the forming and filling period of seed. Lack of water and nutrients in this period will affect the yield. The harvesting index describes the ability of plants to channel photosynthate to economical yield for the seed formation. The inoculated plants have more ability to transfer the photosynthesis result for seed formation than the uninoculated one. The harvest index and dry seed weight can be seen in Table 5.

Insert Table 5 here.

The inoculation improves seed yield significantly as the plants have more ability to deliver assimilates to economical yield (high harvest index) not because of its ability in forming assimilates (dry weight of the plant). The correlation analysis showed negative and significant correlation with the plant dry weight ($r = -0.33$). While the harvest index correlated positively and significantly with the seed weight per plant ($r = 0.75^{**}$). The smaller dry weight of the plants, the more harvest index and the weight of the seeds per plant will be obtained.

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CONCLUSIONS

The inoculation of *Rhizobium japonicum* in some soybean cultivars planted in coastal area can effectively increase the number of root nodules and dry weight of the root nodule (the N_2 blocking organ in nitrogen fixation). The ability to fulfill the nitrogen need in plants is followed by the increasing of crop yield. The inoculated cultivar has higher harvest index and seed weight per plant than uninoculated ones. Burangrang cultivar is more responsive to *Rhizobium japonicum* inoculation in coastal area than Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning cultivar.

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Table 1. Nutrient content (N, P, K) and pH

No	Variables Observed	Soil of coastal area	Soil of coastal area + 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/100g
5.	K ₂ O potential	9	58	mg/100g

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Table 2. The Average number and dry weight of root nodule on various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Number of root nodule		Dry weight of root nodule (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
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
	(+)	(+)		

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

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Table 3. N uptake of canopy (%) of various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Uninoculated	Inoculated	Average
Grobogan	1,64	1,90	1,77 abcd
Burangrang	1,97	2,46	2,22 ab
Argomulyo	1,93	1,93	1,93 abcd
Anjasmara	2,27	2,28	2,28 a
Dena 1	1,58	1,88	1,73 bcd
Gema	1,73	2,29	2,01 abc
Kaba	1,76	1,51	1,63 cd
Wilis	2,10	2,18	2,14 abc
Sinabung	2,25	1,75	2,00 abc
Gepak Kuning	1,32	1,66	1,49 d
Average	1,85 p	1,99 p	(-)

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Table 4. The Effect of *Rhizobium japonicum* inoculation on leaf area, fresh weight of plant, dry weight of soybean cultivars.

Cultivars	Leaf Area (cm ²)		Dry weight of root nodule (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Grobogan	11.66 d	12.60 cd	7.70 pq	12.28 p
Burangrang	3.70 e	15.22 abc	0.91 r	10.15 pq
Argomulyo	16.34 ab	17.78 a	8.60 pq	7.55 pq
Ajasmara	14.99 abc	15.53 abc	8.75 pq	8.95 pq
Dena 1	14.18 bcd	15.82 abc	8.76 pq	8.14 pq
Gema	17.15 ab	15.89 ab	10.07 pq	7.42 pq
Kaba	17.56 a	15.95 ab	8.23 pq	6.53 pq
Wilis	14.91 abc	15.17 abc	7.08 pq	6.76 pq
Sinabung	15.16 abc	15.74 abc	8.39 pq	6.80 pq
Gepak kuning	15.50 abc	15.95 ab	8.86 pq	5.39 qr
	(+)		(+)	

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Table 5. The Average of dry seed weight per plant and harvest index

Cultivar	Dry seed weight per plant (g)		Harvest index			
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Average
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
Rerata	0.68 q	0.98 p		0.11 y	0.16 x	
	(-)				(-)	

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NITROGEN FIXATION ON SOYBEAN CULTIVAR PLANTED IN COASTAL AREA

ABSTRACT

The natural resources of the coastal area in Indonesia have the potential to be developed as an agricultural land with the support of both cultivation technology and land processing which one of them is by using *Rhizobium japonicum* bacteria to meet the need of nitrogen in soybean plants. This study aims to determine the characteristics of nitrogen fixation in various soybean cultivar planted in the coastal area.

The research was conducted in Mancingan, Parangtritis, Kretek, Bantul, DIY. The study was done using Completely Randomized Design which consisted of two factors and repeated three times. Factor I was *Rhizobium japonicum* inoculation (with inoculation and without inoculation); factor II was 10 various cultivar of soybean (Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning).

The results showed that the inoculation of *Rhizobium japonicum* in soybean cultivars in coastal area can increase the number of root nodule, dry weight of root nodule, dry seed weight per plant, and harvest index. Burangrang cultivar planted in coastal area is the most responsive to *Rhizobium japonicum* inoculation among other soybean cultivars tested.

Key words: Nitrogen fixation, soybean cultivar, coastal area

INTRODUCTION

Soybean is a strategic food commodity besides being both a protein source from food and energy source for alternative fuel (Adie and Krisnawati, 2014). However Indonesia still has to import the soybean to meet the domestic demand. During the period of 2010 - 2014 the demand was ± 2.3 million tons of dry beans/ year, while soybean production in 2015 was only 963.183 ton / ha (BPS, 2016). Every year Indonesia still has to import soybean ± 1.3 million tons. The soybean production tends to decrease as the decreasing land area for cultivation which transferred into residential and industrial estate. Also the farmers prefer to cultivate other food crops such as rice and corn. To reduce the dependence on the import, it is necessary to increase the soybean production by expanding the soybean cultivation area (the extensification). The rice field area in Indonesia in 2015 covering ± 8 million ha, while the harvest area of soybean is only 614 thousand ha (BPS, 2016). Therefore it is necessary to make an effort to increase the area for soybean cultivation by utilizing the marginal land that has not been widely used in Indonesia such as coastal area.

Indonesia as an archipelagic country has a long coastline of about 81.000 km. Coastal areas have the potential of various natural resources that can be developed such as fisheries, livestock, agriculture and tourism sector. The utilization of coastal areas for agricultural sector experiencing some constraints which are salinity, low soil moisture, and low content of nutrient and organic materials. During the day, the soil temperature can reach 55 -60°C. Also the aeration which runs faster

causing slower accumulation of organic matter as well as the ability to hold the water (Kertonegoro, 2006). Meanwhile, the sunlight and strong wind which carry water vapor with the high salinity lead to limited variety of the cultivated plants in coastal area. So that soil and salinity conditions are the important issue in planting the crops in the coastal areas.

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The variables observed were the number of root nodules, dry weight of root nodules, and N canopy uptake that were performed at the maximum vegetative growth. The observations on the growth variables were performed at the time of vegetative growth including the leaf area and dry weight of the plant. The observations on the yield variables was done at harvest time including the weight seeds per plant, weight of 100 seeds, and harvest index.

The data were analyzed by using analysis of variance at 5% significant level and followed with DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSIONS

The soil analysis was done to find out the nutrient content in the soil. The analysis was conducted on the sand soil samples in the research location and planting media in the form of soil mix and manure. The soil analysis showed that the application of manure can increase the nutrient content of C-organic, N total, P₂O₅ and K₂O (See Table 1).

Insert Table 1 here.

The Nitrogen Fixation Ability

The ability of soybean cultivars in fixing N₂ is determined by the activity of the root nodules. The more root nodules are active, the higher ability of soybeans in fixing N₂. In this study the ability of soybean in fixing N₂ was observed from the number of root nodules; dry weight of root nodules; and N canopy uptake. Table 2 shows the interaction between *Rhizobium japonicum* inoculation and various type of soybean cultivar in the formation of root nodules. This indicates that soybean cultivars provide different response to the *Rhizobium japonicum* inoculation. Anjasmara and Grobogan cultivar showed the best result on the number of root nodules and nodule dry weight. This result was supported by the study done by Purwaningsih (2012) on agricultural land in Banguntapan which showed that Anjasmara cultivar inoculated with *Rhizobium japonicum* has the highest number of root nodules and significantly different from the Wilis, Sinabung, Gepak Yellow, Argomulyo, and Grobogan.

Insert Table 2 here.

The study conducted by Purwaningsih (2012) clasified Anjasmara, Argomulyo, Gepak Kuning cultivar into the groups of soybeans cultivar that resulted higher the fixation of nitrogen and seed yield followed by inoculation with *Rhizobium japonicum*. While the Grobogan, Sinabung, Wilis cultivar are clasified into the less-responsive group. These study indicates that in different environment, soybean cultivar will respond differently to the inoculation of *Rhizobium japonicum*.

The ability of soybean plants in fixing nitrogen can be determined by the uptake of N canopy. Table 3 shows that there is no interaction between *Rhizobium japonicum* inoculation and soybean cultivar to the N canopy uptake. This is due to the N content present in the canopy is not only derived from the N₂ fixation but also comes from the N present in the planting medium. *Rhizobium japonicum* inoculation has no significant effect on the uptake of N canopy as the low environmental stresses of soil moisture and high salinity causing ineffective to the nitrogen fixation. This is supported by the result of correlation analysis which shows that N canopy absorption is not significantly correlated with the number of root nodules ($r = 0.25ns$) and dry weight of root nodule ($r = 0.23ns$), so that N contained in the leaf is suspected derived from the N uptake from the soil.

Insert Table 3 here.

Nitrogen requirements for the plants can be obtained through the N_2 fixation, both NH_4^+ and NO_3^- absorption (Gerendas and Sattelmachner, 1990). Only nitrogen in the form of ammonium ions and nitrates is available for plants taken through rooting systems (Salisbury and Ross, 1991).

The characteristic of soil moisture in planting medium used in this study is low as the ability of sand particles in binding the water is low as well (even after the application of manure). The environmental conditions also tend to high evaporation as the water stress affects the nitrogen fixation (Yelton et al., 1983). In general, water stress affects the formation and function of root nodules as it will inhibit the movement of bacteria to reach the root surface or encourage attachment of soil particles on the root surface thus will inhibit the attachment of *Rhizobium* and the host plants. Drought can cause nodule death as it can damage the plasmodesmata that connect to the root tissue and the host plant cells, and the damage can continue in the form of cell around the cells containing the bacterium in the nodule. The water strain results in decreasing activity of nitrogenase (Bordeleau and Prevost, 1994). The availability of water plays an important role in transporting the nitrogen fixation out of the root nodules. The results of nitrogen tethering that is not immediately transported out of the root nodules will accumulate in the root nodules thus inhibiting the efficiency of nitrogen fixation.

Plant Growth Analysis

The variables of leaf area and dry weight were observed when the plants reach maximum vegetative stage (See Table 4).

Insert Table 4. here.

The result showed that the inoculation of *Rhizobium japonicum* only give a significant effect on leaf area and dry weight of Burangrang cultivar. The *Rhizobium japonicum* inoculation did not show any significant result on the leaf area and dry weight of Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning cultivar.

Burangrang cultivar has a narrow leaf as it accumulates lower dry matter (resulting from photosynthesis) than other cultivars. So the leaf area and dry weight will increase significantly after inoculated with the *Rhizobium japonicum*.

The Seed Yield

The inoculation of *Rhizobium japonicum* in soybean can increase the harvest index and dry weight of seeds per plant. This is related to the ability of plants to supply nutrients for the plant growth, especially for the seed formation. Soybean requires N nutrients for growth, especially in the forming and filling period of seed. Lack of water and nutrients in this period will affect the yield. The harvesting index describes the ability of plants to channel photosynthate to economical yield for the seed

formation. The inoculated plants have more ability to transfer the photosynthesis result for seed formation than the uninoculated one. The harvest index and dry seed weight can be seen in Table 5.

Insert Table 5 here.

The inoculation improves seed yield significantly as the plants have more ability to deliver assimilates to economical yield (high harvest index) not because of its ability in forming assimilates (dry weight of the plant). The correlation analysis showed negative and significant correlation with the plant dry weight ($r = -0.33$). While the harvest index correlated positively and significantly with the seed weight per plant ($r = 0.75^{**}$). The smaller dry weight of the plants, the more harvest index and the weight of the seeds per plant will be obtained.

CONCLUSIONS

The inoculation of *Rhizobium japonicum* in some soybean cultivars planted in coastal area can effectively increase the number of root nodules and dry weight of the root nodule (the N_2 blocking organ in nitrogen fixation). The ability to fulfill the nitrogen need in plants is followed by the increasing of crop yield. The inoculated cultivar has higher harvest index and seed weight per plant than uninoculated ones. Burangrang cultivar is more responsive to *Rhizobium japonicum* inoculation in coastal area than Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning cultivar.

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Table 1. Nutrient content (N, P, K) and pH

No	Variables Observed	Soil of coastal area	Soil of coastal area + 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/100g
5.	K ₂ O potential	9	58	mg/100g

Table 2. The Average number and dry weight of root nodule on various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Number of root nodule		Dry weight of root nodule (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
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
	(+)		(+)	

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

Table 3. N uptake of canopy (%) of various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Uninoculated	Inoculated	Average
Grobogan	1,64	1,90	1,77 abcd
Burangrang	1,97	2,46	2,22 ab
Argomulyo	1,93	1,93	1,93 abcd

Anjasmara	2,27	2,28	2,28 a
Dena 1	1,58	1,88	1,73 bcd
Gema	1,73	2,29	2,01 abc
Kaba	1,76	1,51	1,63 cd
Wilis	2,10	2,18	2,14 abc
Sinabung	2,25	1,75	2,00 abc
Gepak Kuning	1,32	1,66	1,49 d
Average	1,85 p	1,99 p	(-)

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level.

Table 4. The Effect of *Rhizobium japonicum* inoculation on leaf area, fresh weight of plant, dry weight of soybean cultivars.

Cultivars	Leaf Area (cm ²)		Dry weight of root nodule (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Grobogan	11.66 d	12.60 cd	7.70 pq	12.28 p
Burangrang	3.70 e	15.22 abc	0.91 r	10.15 pq
Argomulyo	16.34 ab	17.78 a	8.60 pq	7.55 pq
Ajasmara	14.99 abc	15.53 abc	8.75 pq	8.95 pq
Dena 1	14.18 bcd	15.82 abc	8.76 pq	8.14 pq
Gema	17.15 ab	15.89 ab	10.07 pq	7.42 pq
Kaba	17.56 a	15.95 ab	8.23 pq	6.53 pq
Wilis	14.91 abc	15.17 abc	7.08 pq	6.76 pq
Sinabung	15.16 abc	15.74 abc	8.39 pq	6.80 pq
Gepak kuning	15.50 abc	15.95 ab	8.86 pq	5,39 qr
	(+)		(+)	

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

Table 5. The Average of dry seed weight per plant and harvest index

Cultivar	Dry seed weight per plant (g)				Harvest index	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Average
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
Rerata	0.68 q	0.98 p		0.11 y	0.16 x	
	(-)			(-)		

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (-): there is no interaction

• [ipas] The Effect of Rhizobium japonicum on The Growth of Soybean Cultivars in Coastal Area

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THE EFFECT OF *Rhizobium japonicum* INOCULATION ON THE GROWTH OF SOYBEAN CULTIVAR PLANTED IN COASTAL AREA

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ABSTRACT

The natural resources of the coastal area in Indonesia have the potential to be developed as an agricultural land with the support of both cultivation technology and land processing, which one of them was done by using *Rhizobium japonicum* bacteria to meet the need of nitrogen in the soybean plants. This study aimed to determine the characteristics of nitrogen fixation in various soybean cultivars planted in the coastal area.

The research was conducted in Mancingan, Parangtritis, Kretek, Bantul, DIY. The study was designed in Completely Randomized Design which consisted of two factors and was repeated three times. Factor I was *Rhizobium japonicum* inoculation (with inoculation and without inoculation); factor II was 10 various cultivars of soybean (Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning).

The results showed that the inoculation of *Rhizobium japonicum* in soybean cultivars in a coastal area can increase the number of root nodule, dry weight of root nodule, dry seed weight per plant, and harvest index. Burangrang cultivar planted in the coastal area was the most responsive to *Rhizobium japonicum* inoculation among other soybean cultivars tested.

Key words: Nitrogen fixation, soybean cultivar, coastal area

INTRODUCTION

Soybean is a strategic food commodity as it both a protein source from food and energy source for alternative fuel (Adie and Krisnawati, 2014). However, Indonesia still has to import the soybeans to meet domestic demand. During the period of 2010 - 2014 the demand was ± 2.3 million tons of dry beans/ year, while soybean production in 2015 was only 963.183 ton/ha (BPS, 2016). Every year Indonesia still has to import soybean ± 1.3 million tons. The soybean production tends to decrease as the land area for cultivation is being transferred into the residential and industrial estate. Also, the farmers prefer to cultivate other food crops such as rice and corn. To reduce the dependence on the import, it is necessary to increase the soybean production by expanding the soybean cultivation area (the extensification). In 2015 the rice field area in Indonesia cover around ± 8 million ha, while the harvest area of soybean is only 614 thousand ha (BPS, 2016). Therefore it is necessary to make an

effort to increase the area for soybean cultivation by utilizing the marginal land that has not been widely used in Indonesia such as coastal area.

Indonesia as an archipelagic country has a long coastline of about 81.000 km. Coastal areas have the potential of various natural resources that can be developed such as fisheries, livestock, agriculture, and tourism sector. The utilization of coastal areas for agricultural sector is experiencing some constraints which are salinity, low soil moisture, and low content of nutrient and organic materials. During the day, the soil temperature can reach 55 -60°C. Also, the aeration which runs faster, the structure and particles of the soil are loose so the soil porosity is very large. These condition cause slower accumulation of organic matter as well as the ability to hold the water (Kertonegoro, 2006). Meanwhile, the sunlight and strong wind which carries water vapour with the high salinity lead to the limited variety of the cultivated plants in the coastal area. So the soil and salinity condition are the most important issues in planting the crops in the coastal areas.

Soybean, as a strategic commodity in Indonesia, has high sensitivity to salinity and it becomes the main problem as it can affect the plant growth due to ionic and osmotic stress. The effort to utilize the coastal area is by improving the physical, chemical, and soil organisms so that soil-water-plant interaction can be well occurred. Another effort is by managing the interaction between the plants and the atmosphere (Gunadi, 2002). According to Sudihardjo (2001), based on criteria of CSR / FAO (1983), the actual suitability of the Southern coastal area of DIY is classified into not suitable or marginally suitable for food crops and vegetables.

Either to improve the physical and chemical properties in a coastal area can be managed by the addition of organic matter (Gunadi, S., 2002). Besides, it can also be optimized by the application of *Rhizobium japonicum* bacteria. The cultivation of soybean in a coastal area requires a large amount of nutrients especially N. The nitrogen requirement can be fulfilled if the fixation of N₂ can be optimally occurred by the plants. The N₂ fixation is determined by the suitability of the Rhizobium strain and the soybean cultivar. Therefore, it is necessary to conduct a study to find out the response of various soybean cultivars to *Rhizobium* inoculation which suitable to be developed in a coastal area. The results of this study are expected to be used as the solution to optimize the utilization of marginal land in Parangkusumo coastal area of Bantul Regency for the development of soybean crops using low energy input by maximizing the symbiosis between soybean plants and *Rhizobium* bacteria to fix N₂.

The fixation of N₂ occurs because of the symbiotic between the plants and prokaryotic bacteria diazotroph, the bacterium that can inhibit the molecules of nitrogen gas in the air (MacDicken, 1994). The diazotroph organism produces a nitrogenase enzyme that acts as a catalyst in the decomposition of nitrogen gas and reduces into NH³⁺. The symbiosis between the Rhizobia and leguminous plant roots will produce nitrogen-blocking organs which called as root nodules. In the root nodules, there are cells that are slightly enlarged contains bakteroid and among them, there are smaller cells contained starch. The shape, size, color, texture, and location of the root nodule on the plant s will be determined by the host plant (Dierolf, et al., 2001). The effectiveness of rhizobia is determined by the

colour of the root nodules, the pink root nodules are more effective in fixing the nitrogen than the white ones. This will affect the growth and yield of the host plants (Karaca and Uyanoz, 2012). Until 2016 the government has released 83 superior varieties of soybean that have superiority in morphological and agronomic characteristic. The new improved varieties released in 2014-2016 were Pearl 2 and Pearl 3 (black soybean); Demas 1 (Admix Soybean soybean); Dena 1 and Dena 2 (Shade tolerant soybean); Devon 1 (high isoflavone soybean) ; Dega 1; and Deja 1 and Deja 2 which are water-tolerant (Susanto and Nugrahaeni, 2017).

The results of Douka's research, et al. (1986) and Aaron and Ammar (2001) showed that soybean cultivars will respond differently to *Rhizobium* inoculation. The study of *Rhizobium* inoculation on several varieties of *Pisum sativum* L. showed that the three varieties of *Pisum sativum* L. which tested have a good result on root nodules; the dry weight of root nodules; the dry weight of root, and seed yield (Ali, et al., 2008).

Therefore, we aim to examine the characteristics of nitrogen fixation on various soybean cultivars planted in the coastal area.

MATERIALS AND METHODS

The research was conducted in Mancingan, Parangtritis, Kreek, Bantul DIY. The study was designed in Complete Randomized Design (CRD) consists of two factors in three replications. Factor I was *Rhizobium japonicum* inoculation (without inoculation and with inoculation of soybean legin). Factor II was the various cultivar of soybeans (Grobogan cultivars, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning). The research obtained 20 combinations of treatments.

The planting media used was beach sand and cow manure (ratio 1: 1) in polybag (25 cm x 30 cm). The polybags used were installed with plastic plates to avoid the contamination. Before planting, the soybean seeds were inoculated with soybean legins containing *Rhizobium japonicum* bacteria. Before the inoculation, soybean seeds were soaked in sugar solution so that soybean legin will be more easily attached to the seed. The inoculation treatment was done by mixing the seeds with soybean legin. The inoculation of soybean seeds was carried out in a shady place (not directly exposed by the sunlight).

The variables observed were the number of root nodules, dry weight of root nodules, and N canopy uptake that were performed at the maximum vegetative growth. The observations on the growth variables were performed at the time of vegetative growth (8 week after planting) including the leaf area and dry weight of the plant. The N canopy uptake was analysed with Kjeldahl micro method. The observations on the yield variables were done at harvest time including the weight seeds per plant and harvest index.

The data were analysed by using analysis of variance at 5% significant level and followed with DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSIONS

The soil analysis was done to find out the nutrient content in the soil. The analysis was conducted on the sand soil samples in the research location and planting media in the form of soil mix and manure. The soil analysis showed that the application of manure (ratio 1:1) can increase the nutrient content of C-organic, N total, P₂O₅, and K₂O (See Table 1).

Insert Table 1 here.

The Nitrogen Fixation Ability

The ability of soybean cultivars in fixing N₂ is determined by the root nodules of active. The more root nodules are active, the higher of the ability of soybeans in fixing N₂ will be obtained. In this study, the ability of soybean in fixing N₂ is observed from the number of root nodules; the dry weight of root nodules; and N canopy uptake. Table 2 shows the interaction between *Rhizobium japonicum* inoculation and various type of soybean cultivars in the formation of root nodules. This indicates that soybean cultivars provide a different response to the *Rhizobium japonicum* inoculation. Anjasmara and Grobogan cultivar showed the best result on the number of root nodules and nodule dry weight. This result is supported by the study done by Purwaningsih (2012) on agricultural land in Banguntapan which showed that Anjasmara cultivar inoculated with *Rhizobium japonicum* has the highest number of root nodules and significantly different from the Wilis, Sinabung, Gepak Yellow, Argomulyo, and Grobogan.

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The ability of soybean plants on fixing nitrogen can be determined by the uptake of N canopy. Table 3 shows that there is no interaction between *Rhizobium japonicum* inoculation and soybean cultivar to the N canopy uptake. This is due to the N content present in the canopy is not only derived from the N₂ fixation but also comes from the N present in the planting medium. *Rhizobium japonicum* inoculation has no significant effect on the uptake of N canopy as the low environmental stresses of soil moisture and high salinity causing ineffective to the nitrogen fixation. This is supported by the result of correlation analysis which shows that N canopy absorption is not significantly correlated

with the number of root nodules ($r = 0.25\text{ns}$) and dry weight of root nodule ($r = 0.23\text{ns}$). This indicates that the N-content in the canopy does not only originate from N_2 fixation by root nodules.

Insert Table 3 here.

Nitrogen requirements for the plants can be obtained through the N_2 fixation, both NH_4^+ and NO_3^- absorption (Gerendas and Sattelmachner, 1990). Only nitrogen in the form of ammonium ions and nitrates is available for plants taken through rooting systems (Salisbury and Ross, 1991).

The characteristic of soil moisture in planting medium used in this study is low as the ability of sand particles in binding the water is low as well (even after the application of manure). The environmental conditions also tend to reach high evaporation as the water stress affects the nitrogen fixation (Yelton et al., 1983). In general, water stress affects the formation and function of root nodules it will inhibit the movement of bacteria to reach the root surface or encourage the attachment of soil particles on the root surface thus will inhibiting the attachment of *Rhizobium* and the host plants. Drought can cause nodule death as it can damage the plasmodesmata that connect to the root tissue and the host plant cells. The damage can continue in the form of the cell around the cells containing the bacterium in the nodule. The water strain results in decreasing activity of nitrogenase (Bordeleau and Prevost, 1994). The availability of water plays an important role in transporting the nitrogen fixation out of the root nodules. The results of nitrogen tethering that is not immediately transported out of the root nodules will accumulate in the root nodules thus inhibiting the efficiency of nitrogen fixation.

Plant Growth Analysis

The variables of leaf area and dry weight were observed when the plants reach the maximum vegetative stage at 8 week after plant. The results of variance analysis show an interaction between rhizobium inoculation and various of cultivars on the leaf area variables and plant dry weight. (See Table 4).

Insert Table 4. here.

The result showed that the inoculation of *Rhizobium japonicum* only give a significant effect on leaf area and dry weight of Burangrang cultivar. The inoculation of *Rhizobium japonicum* can increase the leaf area and dry weight of Burangrang cultivar. The *Rhizobium japonicum* inoculation do not show any significant result on the leaf area and dry weight of Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning cultivar. The inoculation of *Rhizobium japonicum* in these cultivars cannot increase the leaf area and dry weight of plants.

Burangrang cultivar has less a leaf area as it accumulates lower dry matter (resulting from photosynthesis) than other cultivars. So the leaf area and dry weight will increase significantly after inoculated with the *Rhizobium japonicum*.

The Seed Yield

The inoculation of *Rhizobium japonicum* in soybean can increase the harvest index and dry weight of seeds per plant. This is related to the ability of plants to supply nutrients for the plant growth, especially for the seed formation. Soybean requires N nutrients for growth, especially in the forming and filling period of seed. Lack of water and nutrients in this period will affect the yield. The harvesting index describes the ability of plants to distribute the results of photosynthesis to economical yield for the seed formation. The photosynthesis result for seed formation on the inoculated plants have much than the non inoculated one. The harvest index and dry seed weight can be seen in Table 5.

Insert Table 5 here.

The inoculation improves seed yield significantly. This indicates that the results of photosynthesis is mostly used for seed formation compared to dry weight of the plant. The correlation analysis shows negative and significant correlation with the plant dry weight ($r = -0.33$). While the harvest index correlate positively and significantly with the seed weight per plant ($r = 0.75^{**}$). The lower dry weight of the plants will increase the harvest index and the weight of the seeds per plant.

CONCLUSIONS

The inoculation of *Rhizobium japonicum* in some soybean cultivars planted in the coastal area effectively increased the number of root nodules and dry weight of the root nodule (the N_2 fixing organ in nitrogen fixation). The ability to fixed the nitrogen was followed by the increase of crop yield. The inoculated cultivar showed higher harvest index and seed weight per plant than non-inoculated ones. Burangrang cultivar was more responsive to *Rhizobium japonicum* inoculation in coastal area than Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning cultivar.

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Table 1. Nutrient content (N, P, K) and pH

No	Variables Observed	The soil of a coastal area	The soil of coastal area + 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/100g
5.	K ₂ O potential	9	58	mg/100g

Table 2. The Average number and dry weight of root nodule on various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Number of root nodule	The dry weight of root nodule (g)
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	Uninoculated	Inoculated	Uninoculated	Inoculated
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
	(+)		(+)	

NB: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

Table 3. N uptake of the shoot (%) of various soybean cultivar (uninoculated and inoculated with *Rhizobium japonicum*)

Cultivar	Uninoculated	Inoculated	Average
Grobogan	1,64	1,90	1,77 abcd
Burangrang	1,97	2,46	2,22 ab
Argomulyo	1,93	1,93	1,93 abcd
Anjasmara	2,27	2,28	2,28 a
Dena 1	1,58	1,88	1,73 bcd
Gema	1,73	2,29	2,01 abc
Kaba	1,76	1,51	1,63 cd
Wilis	2,10	2,18	2,14 abc
Sinabung	2,25	1,75	2,00 abc
Gepak Kuning	1,32	1,66	1,49 d
Average	1,85 p	1,99 p	(-)

Remarks: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level.

Table 4. The Effect of *Rhizobium japonicum* inoculation on leaf area, dry weight of the plant.

Cultivars	Leaf Area (cm ²)		dry weight of the plant (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Grobogan	11.66 d	12.60 cd	7.70 pq	12.28 p

Burangrang	3.70 e	15.22 abc	0.91 r	10.15 pq
Argomulyo	16.34 ab	17.78 a	8.60 pq	7.55 pq
Ajasmara	14.99 abc	15.53 abc	8.75 pq	8.95 pq
Dena 1	14.18 bcd	15.82 abc	8.76 pq	8.14 pq
Gema	17.15 ab	15.89 ab	10.07 pq	7.42 pq
Kaba	17.56 a	15.95 ab	8.23 pq	6.53 pq
Wilis	14.91 abc	15.17 abc	7.08 pq	6.76 pq
Sinabung	15.16 abc	15.74 abc	8.39 pq	6.80 pq
Gepak kuning	15.50 abc	15.95 ab	8.86 pq	5,39 qr
		(+)	(+)	

Remark: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (+): there is interaction

Table 5. The Average of dry seed weight per plant and harvest index

Cultivar	Dry seed weight per plant (g)			Harvest index		
	Uninoculated	Inoculated	Average	Inoculated	Uninoculated	Average
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	
		(-)	(-)			

Remark: The number followed by the same letter in the same column and row shows no significant difference based on the DMRT test at 5% significant level. (-): there is no interaction

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The Effect of *Rhizobium japonicum* Inoculation on the Growth of Soybean Cultivars in Coastal Area

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ABSTRACT

The natural resources in the form of coastal area in Indonesia have the potential to be developed as agricultural land with the support of both cultivation technology and land processing which one of them can be done by using *R. japonicum* bacteria to meet the need of nitrogen in the soybean plants. This study aimed to determine the characteristics of nitrogen fixation in various soybean cultivars planted in the coastal area. The research was conducted in Mancingan, Parangtritis, Kretek, Bantul, Yogyakarta. The study was designed in Completely Randomized Design which consisted of two factors replicated three times. The first factor was *R. japonicum* inoculation (with inoculation and without inoculation) and the second factor was 10 cultivars of soybean (Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning). The results showed that the inoculation of *R. japonicum* in soybean cultivars planted in coastal area could increase the number of root nodule, dry weight of root nodule, dry seed weight per plant and harvest index. Cultivar Burangrang planted in the coastal area was the most responsive to *R. japonicum* inoculation among other soybean cultivars tested.

Keywords: Nitrogen fixation, soybean cultivar, coastal area

INTRODUCTION

Soybean is a strategic food commodity as it serves both protein source for food and energy source for alternative fuel (Adie and Krisnawati, 2014). However, Indonesia still has to import soybeans to meet the domestic demand. During the period of 2010 – 2014, the demand was \pm 2.3 million tons of dry beans year⁻¹, while soybean production in 2015 was only 963.183 ton ha⁻¹ (BPS, 2016). Every year, Indonesia still has to import soybean \pm 1.3 million tons. The soybean production tends to decrease as the land area for cultivation is being transferred into the residential and industrial estate. Also, the farmers prefer to cultivate other food crops such as rice and corn. To reduce the dependence on the import, it is necessary to increase the soybean production by expanding the soybean cultivation area (extensification of agriculture). In 2015, the rice field area in Indonesia covered around 8 million ha, while the harvest area of soybean was only 614 thousand ha (BPS, 2016). Therefore, it is necessary to make efforts to expand the

area for soybean cultivation by utilizing the marginal land that has not been widely used in Indonesia such as coastal area.

Indonesia as an archipelagic country has a long coastline of about 81,000 km. Coastal areas have the potential of various natural resources that can be developed such as fisheries, livestock, agriculture, and tourism sector. The utilization of coastal areas for agricultural sector is experiencing some constraints which are salinity, low soil moisture and low content of nutrient and organic materials. Besides, during the day, the soil temperature can reach 55–60°C and the aeration runs faster. In addition, the structure and particles of the soil are loose so the soil porosity is very large. These conditions cause slower accumulation of organic matter and lower ability to hold water (Kertonegoro, 2006). Meanwhile, the sunlight and strong wind carrying water vapor with high salinity lead to the limited cultivars able to be planted in the coastal area. Hence, the soil and salinity conditions are the most important issues in planting the crops in the coastal areas.

Soybean, as a strategic commodity in Indonesia, has high sensitivity to salinity and it becomes the main problem as it can affect the plant growth due to ionic and osmotic stress. One of the efforts to utilize the coastal area is done by improving the physical and chemical properties as well as soil organisms so that soil-water-plant interaction can be well occurred. Furthermore, the interaction between the plants and the atmosphere is necessary to be managed (Gunadi, 2002). According to Sudihardjo (2001), based on criteria of CSR / FAO (1983), the actual suitability of the Southern coastal area of Special Region of Yogyakarta (DIY) is classified into not suitable or marginally suitable for food crops and vegetables.

Improvement of the physical and chemical properties in a coastal area can be managed by the addition of organic materials (Gunadi, 2002). It can also be optimized by the application of *Rhizobium japonicum* bacteria. The cultivation of soybean in a coastal area requires a large amount of nutrients especially N. The nitrogen requirement can be fulfilled if the fixation of N₂ can be optimally conducted by the plants. The N₂ fixation is determined by the suitability of the *Rhizobium* strain and the soybean cultivar. Therefore, it is necessary to conduct a study to find out the response of various soybean cultivars to *Rhizobium* inoculation suitable to be developed in a coastal area. The results of this study are expected to be used as the solution to optimize the utilization of marginal land in Parangkusumo coastal area of Bantul Regency for the development of soybean crops using low energy input by maximizing the symbiosis between soybean plants and *Rhizobium* bacteria to fix N₂.

The fixation of N₂ occurs because of the symbiosis between the plants and prokaryotic bacteria diazotroph, the bacterium that can inhibit the molecules of nitrogen gas in the air (MacDicken, 1994). The diazotroph organism produces a nitrogenase enzyme that acts as a catalyst in the decomposition of nitrogen gas and reduces into NH₃⁺. The symbiosis between the *Rhizobia* and leguminous plant roots will produce nitrogen-blocking organs called as root nodules. In the root nodules, there are cells that are slightly enlarged containing bacteroid and among them, there are smaller cells containing starch. The shape, size, color, texture, and location of the root nodule on the plant will be determined by the host plant (Dierolf *et al.*, 2001). The effectiveness of *rhizobia* is determined by the color of the root nodules, in which the pink root nodules are more effective in fixing the nitrogen than the white ones. This will

affect the growth and yield of the host plants (Karaca and Uyanoz, 2012). Until 2016, the government has released 83 superior cultivars of soybean that have superiority in morphological and agronomic characteristic. The new improved cultivars released in 2014–2016 were Pearl 2 and Pearl 3 (black soybean); Demas 1 (Admix Soybean soybean); Dena 1 and Dena 2 (Shade tolerant soybean); Devon 1 (high isoflavone soybean); Dega 1; and Deja 1 and Deja 2 which are water-tolerant (Susanto and Nugrahaeni, 2017).

The results of Douka's research *et al.* (1986) and Aaron and Ammar (2001) showed that soybean cultivars would respond differently to *Rhizobium* inoculation. The study of *Rhizobium* inoculation on several varieties of *Pisum sativum* L. showed that the three varieties of *Pisum sativum* L. tested had a good result on root nodules; dry weight of root nodules; dry weight of root, and seed yield (Ali *et al.*, 2008). Therefore, the aim of the experiment was to examine the characteristics of nitrogen fixation on various soybean cultivars planted in the coastal area.

MATERIALS AND METHODS

The research was conducted in Mancingan, Parangtritis, Kreek, Bantul DIY. The study was arranged in Complete Randomized Design (CRD) consisting of two factors with three replications. The first factor was *R. japonicum* inoculation (without inoculation and with inoculation of soybean legin) and the second factor was the various cultivars of soybean (Grobogan, Burangrang, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning). There were 20 treatment combinations tested in the research.

The planting media used was beach sand and cow manure (ratio of 1: 1) in polybag (25 cm × 30 cm). The polybags used were installed with plastic plates to avoid the contamination. Before planting, the soybean seeds were inoculated with soybean legins were containing *R. japonicum* bacteria. Before the inoculation, soybean seeds were soaked in sugar solution so that soybean legin will be more easily attached to the seed. The inoculation treatment was done by mixing the seeds with soybean legin. The inoculation of soybean seeds was carried out in a shady place (not directly exposed to the sunlight).

The variables observed were the number of root nodules, dry weight of root nodules, and canopy N uptake that were performed at the maximum vegetative growth. The observations on the growth variables

were performed at the time of vegetative growth (8 week after planting) including the leaf area and dry weight of the plant. The canopy N uptake was analyzed with Kjeldahl micro method. The observations on the yield variables were done at harvest time including the seeds weight per plant and harvest index.

The data were analyzed using analysis of variance at 5% significance level and followed with DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSION

The soil analysis was done to find out the nutrient content in the soil. The analysis was conducted on the sand soil samples in the research location and planting media in the form of beach sand and manure mixture. The soil analysis showed that the application of manure (ratio 1:1) was able to increase the nutrient content of organic C, total N, P₂O₅, and K₂O (Table 1).

Nitrogen Fixation Ability

The ability of soybean cultivars in fixing N₂ is determined by active root nodules. The more root nodules are active, the higher ability of soybeans in

fixing N₂ will be obtained. In this study, the ability of soybean in fixing N₂ was observed from the number of root nodules; the dry weight of root nodules; and canopy N uptake. Table 2 shows the interaction effect between *R. japonicum* inoculation and various type of soybean cultivars on the formation of root nodules. This indicates that soybean cultivars provide a different response to the *R. japonicum* inoculation. Cultivar Anjasmara and Grobogan showed the best result on the number of root nodules and nodule dry weight. This result is supported by the study done by Purwaningsih (2012) on agricultural land in Banguntapan which showed that cultivar Anjasmara inoculated with *R. japonicum* had the significantly higher number of root nodules compared to cultivar Wilis, Sinabung, Gepak Yellow, Argomulyo, and Grobogan.

The study conducted by Purwaningsih (2012) classified cultivar Anjasmara, Argomulyo, Gepak Kuning into the groups of soybeans cultivar that resulted higher nitrogen fixation and seed yield as inoculated with *R. japonicum*. Meanwhile, cultivar Grobogan, Sinabung, Wilis are classified into the

Table 1. Nutrient content (N, P, K) and pH

No	Variables Observed	The soil of a coastal area	The soil of coastal area + 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 100g ⁻¹
5.	K ₂ O potential	9	58	Mg 100g ⁻¹

Table 2. The Average number and dry weight of root nodule on various soybean cultivar (Not inoculated and inoculated with *R. japonicum*)

Cultivar	Number of root nodule		The dry weight of root nodule (g)	
	Not inoculated	Inoculated	Not inoculated	Inoculated
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: Values in each variable followed by the same letters are not significantly different based on the DMRT test at 5% significance level. (+): there is interaction

less-responsive group. This study indicates that in a different environment, soybean cultivar will respond differently to the inoculation of *R. japonicum*.

The ability of soybean plants in fixing nitrogen can be determined by the canopy N uptake. Table 3 shows that there is no interaction effect between *R. japonicum* inoculation and soybean cultivar on the canopy N uptake. This is due to the N content present in the canopy is not only derived from the N₂ fixation but also comes from the N present in the planting medium. *R. japonicum* inoculation had no significant effect on the canopy N uptake as the low environmental stresses of soil moisture and high salinity caused ineffectiveness nitrogen fixation. This is supported by the result of correlation analysis which shows that canopy N uptake is not significantly correlated with the number of root nodules ($r = 0.25$ ns) and dry weight of root nodule ($r = 0.23$ ns). This indicates that the N-content in the canopy does not only originate from N₂ fixation by root nodules.

Nitrogen requirements for the plants can be obtained through N₂ fixation, both NH₄⁺ and NO₃⁻ absorption (Gerendas and Sattelmachner, 1990). Only nitrogen in the form of ammonium ions and nitrates is available for plants taken through rooting systems (Salisbury and Ross, 1991).

The soil moisture content in the planting medium used in this study was low and so was the ability of sand particles in binding the water (even after the application of manure). The environmental conditions also tend to reach high evaporation as the water stress affects the nitrogen fixation (Yelton *et al.*, 1983). In general, water stress affects the formation and function of root nodules, inhibiting the movement of

bacteria to reach the root surface or encourage the attachment of soil particles on the root surface thus will inhibiting the attachment of Rhizobium and the host plants. Drought can cause nodule death as it can damage the plasmodesmata that connect to the root tissue and the host plant cells. The damage can continue in the form of the cell around the cells containing the bacterium in the nodule. The water strain results in decreasing activity of nitrogenase (Bordeleau and Prevost, 1994). The availability of water plays an important role in transporting the nitrogen fixation out of the root nodules. The results of nitrogen tethering that is not immediately transported out of the root nodules will accumulate in the root nodules thus inhibiting the efficiency of nitrogen fixation.

Plant Growth Analysis

The variables of leaf area and dry weight were observed when the plants reached the maximum vegetative stage at 8 weeks after planting. The results of analysis of variance showed interaction effect between rhizobium inoculation and various of cultivars on the leaf area and plant dry weight. (See Table 4).

The result showed that the inoculation of *R. japonicum* only gave significant effect on leaf area and dry weight of cultivar Burangrang. The inoculation of *R. japonicum* can increase the leaf area and dry weight of Burangrang cultivar. The inoculation of *R. japonicum* did not show any significant result on the leaf area and dry weight of cultivar Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung, Gepak Kuning cultivar. The inoculation of *R. japonicum* in these cultivars

Table 3. N uptake of the shoot (%) of various soybean cultivar (uninoculated and inoculated with *R. japonicum*)

Cultivar	Not inoculated	Inoculated	Average
Grobogan	1.64	1.90	1.77 abcd
Burangrang	1.97	2.46	2.22 ab
Argomulyo	1.93	1.93	1.93 abcd
Anjasmara	2.27	2.28	2.28 a
Dena 1	1.58	1.88	1.73 bcd
Gema	1.73	2.29	2.01 abc
Kaba	1.76	1.51	1.63 cd
Wilis	2.10	2.18	2.14 abc
Sinabung	2.25	1.75	2.00 abc
Gepak Kuning	1.32	1.66	1.49 d
Average	1.85 p	1.99 p	(-)

Remarks: Values in the same column or row followed by the same letters are not significantly difference based on the DMRT test at 5% significance level.

Table 4. The Effect of *R. japonicum* inoculation on leaf area, dry weight of the plant

Cultivar	Leaf Area (cm ²)		The dry weight of the plant (g)	
	Not inoculated	Inoculated	Not inoculated	Inoculated
Grobogan	11.66 d	12.60 cd	7.70 pq	12.28 p
Burangrang	3.70 e	15.22 abc	0.91 r	10.15 pq
Argomulyo	16.34 ab	17.78 a	8.60 pq	7.55 pq
Anjasmara	14.99 abc	15.53 abc	8.75 pq	8.95 pq
Dena 1	14.18 bcd	15.82 abc	8.76 pq	8.14 pq
Gema	17.15 ab	15.89 ab	10.07 pq	7.42 pq
Kaba	17.56 a	15.95 ab	8.23 pq	6.53 pq
Wilis	14.91 abc	15.17 abc	7.08 pq	6.76 pq
Sinabung	15.16 abc	15.74 abc	8.39 pq	6.80 pq
Gepak Kuning	15.50 abc	15.95 ab	8.86 pq	5.39 qr
	(+)		(+)	

Remarks: Values in each variable followed by the same letters are not significantly different based on the DMRT test at 5% significance level. (+): there is interaction

Table 5. The The Average of dry seed weight per plant and harvest index

Cultivar	Dry seed weight per plant (g)			Harvest index		
	Not inoculated	Inoculated	Average	Inoculated	Not inoculated	Average
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 pgrs
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 pgrs
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: Values in the same column or row followed by the same letters are not significantly different based on the DMRT test at 5% significance level.

was not able to increase the leaf area and dry weight of plants.

Cultivar Burangrang has smaller leaf area as it accumulates lower dry matter (resulting from photosynthesis) than other cultivars. Therefore, the leaf area and dry weight will increase significantly after inoculated with the *R. japonicum*.

Seed Yield

The inoculation of *R. japonicum* in soybean was able to increase the harvest index and dry weight of seeds per plant. This is related to the ability of plants to supply nutrients for the plant growth, especially for the seed formation. Soybean requires N nutrients

for growth, especially in the forming and filling period of seed. Lack of water and nutrients in this period will affect the yield. The harvest index describes the ability of plants to distribute the results of photosynthesis to economical yield for the seed formation. The inoculated plants had higher amount of photosynthesis results for seed formation than non-inoculated ones. The harvest index and dry seed weight can be seen in Table 5.

The inoculation improved seed yield significantly. This indicated that the results of photosynthesis were mostly used for seed formation than for dry weight of the plant. The correlation analysis showed significant negative correlation between harvest index and plant

dry weight ($r = -0.33$). On the contrary, harvest index correlated positively with seed weight per plant ($r = 0.75^{**}$). Lower dry weight of the plants will increase the harvest index and the weight of the seeds per plant.

CONCLUSIONS

The inoculation of *R. japonicum* in some soybean cultivars planted in the coastal area effectively increased the number of root nodules and dry weight of the root nodule (the N₂ fixing organ in nitrogen fixation). The ability to fix nitrogen was followed by the increase of crop yield. The inoculated cultivar showed higher harvest index and seed weight per plant than non-inoculated ones. Cultivar Burangrang was more responsive to *R. japonicum* inoculation in coastal area than Grobogan, Argomulyo, Anjasmara, Dena 1, Gema, Kaba, Wilis, Sinabung and Gepak Kuning.

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#36371 Review

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Authors	Okti Purwaningsih, C. Tri Kusumastuti, Y. Sulistyo Nugroho, Casper Yoda Morib 
Title	The Effect of Rhizobium japonicum on the Growth of Soybean Cultivars in Coastal Area
Section	Research Articles
Editor	Rudi Murti 

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Title	The Effect of Rhizobium japonicum on the Growth of Soybean Cultivars in Coastal Area
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Alamat : Jl. PGRI I Sonosewu No 117 Yogyakarta

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Nama : Dr. Okti Purwaningsih MP
Jabatan : Lektor IIIc
Alamat : Kemetiran Lor GT II/ 678 RT 051/ RW 014 Pringgokusuman
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