Chapter 11

Symbiotic of Nitrogen Fixation Between Acid Aluminium Tolerant *Bradyrhizobium japonicum* and Soybean

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Additional information is available at the end of the chapter

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1. Introduction

Indonesia is a tropical country in Southeast Asia region, located between the Asia and Australia continents. In most parts of Indonesia, climate variation and high of rainfall causes intensive leaching, soil becomes low content of alkaline and the pH tend to acidic. Indonesia has acid dry land area approximately 102.8 million hectares, but only 55.8 million hectares are suitable for agricultural [1]. The arid lands in Indonesia which are generally formed from mineral soil are acidic (pH 4.6 to 5.5) and poor of nutrients. One effort to increase the soil fertility and plant productivity on acid dry land with planting legumes, such as soybean. Inoculation of root nodule bacteria on soybean plant could enhance soybean quality and its productivity [2 & 3]. Some varieties of acid tolerant soybean, such as Tanggamus, Sibayak, Seulawah, Ratai, and Nanti are issued by the Research Institute for Legumes plants and Tuber Crops Indonesia could grow at acidic soil with pH 4.5-5.0 and produced soybean up to 2000 Kg/hectares on the right growing conditions [4]. Soybeans generally grow in soil at pH 5.5-6.0 while the optimum pH is 6.8. Below pH 4.7 soybean production will decline. It is related to the chemical properties of acid soil, that is high levels of aluminium, high P fixation, iron and manganese concentration increases to the toxic level, sensitive to erosion, and poor biotic status under a low pH conditions [5]. Soybean production could be increase by symbiosis with root nodule bacteria. The effectiveness of symbiotic bacteria in legume root nodules is strongly influenced by the soil conditions. Keyser and Munns [5] suggested that aluminum (Al) with a high concentration (50 μM) is one of the stress factors that can inhibit the growth and prolong the lag phase of root nodulating bacteria. Richardson *et al.* [6] also stated that the Al concentration of 7.5 μM at pH 4.8 can inhibit the expression of nod genes that play a role in nodulation. Furthermore, Johnson and Wood [7] stated that the Al^{3+} cation can bind to PO_4^{3-} of DNA thereby inhibiting

DNA replication and transcription. Therefore, strains of acid and high Al-tolerant root nodulating bacteria which have symbiotic effectiveness with soybean are needed to explore.

2. Acid aluminium tolerant *Bradyrhizobium japonicum*

Some strains of root nodulating bacteria tolerant to acid soil conditions have been reported [8]. The bacteria has ability to fix atmospheric nitrogen (N_2) and and convert into ammonium (NH_3) [9]. *Bradyrhizobium japonicum* is one of root nodule bacteria that can contribute on soybean growth by providing fixed nitrogen in nodules of soybean plants [2]. *Bradyrhizobium* is included to the family Rhizobiaceae. This family consists of four genera, namely *Agrobacterium*, *Bradyrhizobium*, *Phyllobacterium*, and *Rhizobium*. The characteristics of *Bradyrhizobium* are rod-shaped, nonspore-forming cells, motile with one polar or subpolar flagelum, aerobic, Gram-negative, cell-sized of 0.5-0.9 μm and 1.2-3.0 μm, the optimum growth temperature is 25-30°C at pH 6-7 [10]. *Bradyrhizobium* is known as slow growing bacteria with a generation time ranged 7-20 hours. The bacterial growth on yeast mannitol agar (YMA) needs 5-7 days incubation on room temperature.

Bradyrhizobium japonicum has sticky consistency and slimy (mucoid) when grown on media containing carbohydrates. The mucus is an extracellular polysaccharide that serves to maintain bacterial survival in environmental conditions with the concentration of acid and aluminum (Al) is high. Strains of *B. japonicum* have more slimy colony and generally more tolerant on acid-Al stress conditions compared to the dry type colony [8 & 11]. There are not all bacteria categorized as tolerant of acid (pH 4.0-4.5) are also a high Al tolerant. Some strains of *B. japonicum* were tolerant on an acid condition, even at the pH level 4.0-4.5. Twenty five strains of *B. japonicum* has been selected for acid tolerance (pH 4.5) consist of Al 50 μM, Mn 200 μM, Ca 50 μM, and low P 5 μM [12]. One of the *B. japonicum* (BJ) isolate namely BJ 11 wt (wild-type) has the highest tolerance on acid and had a good ability to grow on pH 4.5 media (Figure 1).

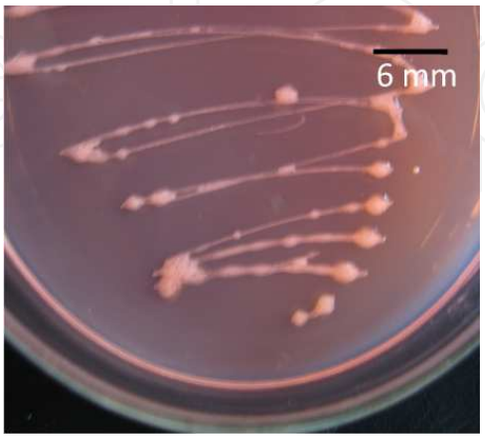

Figure 1. The growth of *Bradyrhizobium japonicum* BJ11 (wt) on pH 4.5 yeast-extract mannitol agar containing 0.0025% congo red at 10 days incubation in room temperature

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Root nodulating bacteria can be distinguished from other bacteria by growing it on media yeast extract mannitol agar (YMA) consist of 10 g/L mannitol, 0.5 g/L K_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$, 0.2 g/L NaCl, and 0.5 g/L yeast extract and containing 0.0025% congo red. Root nodulating bacteria can not absorb congo red or less, and the colony is colorless or pale white [2]. Bradyrhizobia growing on agar media are classified into three types based on the appearance of colonies, such as: small dry (SD), large mucoid (LM), large watery (LW), and dimorphism [13]. Colony of SD type is round, convex, translucent, and diameter of <1 mm. The LM type is circular, convex, slimy, relatively translucent, and diameter> 1 mm. The LW type is irregular shapes, flat, watery, translucent, and diameter> 1 mm. Dimorphism type strain is called to strain with a mixture of SD and LM type. Colony type can be used to predict tolerant or sensitive strain to acid-Al condition. A small dry colony type strain is more sensitive to acid-Al compare to large one and wet type colony [8]. BJ 11 is the slow-growing colony, circular shape, convex elevation, slimy, translucent, and diameter of colony > 1 mm, it is categorized large mucoid. Other root nodulating bacteria has fast growing and acid producing is classified as genus *Rhizobium*, whereas the slow-growing and alkaline producing reaction belong to the genus *Bradyrhizobium*. The growth reaction on YMA medium which is acidic or alkaline is determined by adding 0.0025% bromothymol blue. Colony of root nodulating bacteria that produce acid reaction is yellow [15].

3. Symbiotic effectiveness

Effective strains of *Bradyrhizobium japonicum* produce an effective root nodules on their host. Usually one strain of root nodulating bacteria is used as an inoculum for one variety of soybean plant. Selection should be done from large number of tested strains by using a suitable host-plant on soil and climatic conditions of the host habitat [2].

Symbiotic effectiveness is the relative ability of an association between legume and root nodulating bacteria. Effective nodule consist of leghemoglobin, that is an iron-containing red protein binding with O_2 that controls the partial pressure of O_2 (pO_2) in the nodule [15]. When pO_2 was below or above normal condition (0.21 atm), it could decrease the activity of N_2 fixation. Leghemoglobin is induced by the interaction between *Bradyrhizobium* with soybean.

Effective nodule tends to be large size, reddish, and able to fix nitrogen gas from air. In addition, the effective root nodules have a limited number and distribution, usually found on the main root and secondary first root [14]. Ineffective nodules tend to be small, numerous, greenish white (pale), unable to fix nitrogen from air and spread the root system [14].

Symbiotic effectiveness of acid-tolerant soybean with acid-Al tolerant *B. japonicum* could be done by using Leonard bottle modified that consists of two volumes of 700 ml bottles of ketchup. One bottle is cut at the base and used for growth media that contains sand and charcoal. Other bottle cut at the neck and is used as a reservoir for the nutrient solution [16]. The lower bottle is filled with 300 ml of N-free nutrient solution of pH 4.5 [17] and 100 ml of N-free nutrient solution poured into growing medium in the mixture form of sand and coconut shell charcoal about 480 gram. Before used, sand is sieved and washed

with clean water several times until clean and dry. Each bottle is covered with cement paper and sterilized by autoclaving at 121°C and 1 atmosphere for 2 hours. Two days sprouts of soybean in Leonard jar. Each sprout was inoculated with 10^8 cells ml^{-1} of *B. japonicum*. N-free nutrient solution and nutrient solution contained 5 mM KNO_3 used as control treatments, respectively. Symbiotic effectiveness value (SE) is measured based on percentage of dry weight of plants inoculated with tested strain toward dry weight of plants treated with KNO_3 or reference strain. *Bradyrhizobium japonicum* USDA 110 is used as a reference strain and completely genomic sequenced [18].

The effectiveness of symbiosis can be observed in several ways viz. the determination of plant dry weight, total N content, and nitrogenase activity [2]. Dry weight of the plant is still considered relevant for evaluating the effectiveness of symbiotic root nodulating bacteria with soybean plants, because plant dry weight significantly correlated with total N content [14]. Plant dry weight is usually correlated with the dry weight of root nodules. Upper plant dry weight is used as a parameter to evaluate the binding of N, because as much as 70% of the fixing N is transported to the upper plant [14].

The symbiotic interaction between soybean and root nodulating bacteria played an important role in increasing the plant growth of soybean plant. Effectiveness of a root nodulating bacteria in fixing nitrogen is affected by the compatibility between bacteria and the soybean plant. Mubarik *et al*. [19] described that inoculation of BJ 11 (wt) root nodulating bacteria could increase the height of soybean plant and shoot dry weight until 37 days after planting (DAP) (Figure 2). The nodule dry weight was positively correlated with the ability of plants to fix N and shoot dry weight. The value of symbiotic effectiveness, shoot dry weight, and N uptake of BJ 11 was higher than USDA 110 as reference strain (Table 1).

Figure 2. Soybean plant (37 days after planting) grow on a Leonard bottle using N-free nutrient solution pH 4.5 + Al 50 μM: (1) without inoculation BJ 11 and (2) inoculated with BJ 11 [16]

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Treatment	Number of nodule (nodule plant $^{-1}$)	Dry-weight of nodule (g plant $^{-1}$)	Height of plant (cm)	Nitro-genase activity (µmol C_2H_4 plant $^{-1}$ hour $^{-1}$)	Dry-weight of shoot (g plant $^{-1}$)	N uptake (mg N plant $^{-1}$)	SEN(%)	SER (%)
BJ 11 (wt)	17	0.0397	71.4	12.54	0.8447	16.88	155.37	144.55
USDA 110	12	0.0241	63.0	12.21	0.6164	13.63	114.92	100.00
Control N	0	0	46.0	0	0.5509	13.58	100.00	96.26
Control NO	0	0	37.3	0	0.4561	6.91	83.88	77.55

0 =no detection, N:without BJ inoculation consist of 5 mM KNO_3, N0: without BJ inoculation and without 5mM KNO_3, Symbiotic Effectiveness (SE) against N/R.

Table 1. Effect of inoculation of *B. japonicum* on soybean cultivar Slamet at 37 DAP using N-free solution at pH 4,5 + Al 50 µM (Mubarik *et al.* 2012)

Bradyrhizobium japonicum is able to form nodules and fix nitrogen. Nodule formation on the roots of leguminous plants generally through the following stages: (i) the introduction of a suitable partner on the part of the plant and bacterial attachment to root hairs, (ii) the hair root invasion by bacteria through thread-forming infection threads, (iii) the bacteria moves to the main root through infection threads, (iv) the formation of bacteria in plant cells called bacteroids, and (v) plant and bacterial cell division that is constantly and will produce the mature root nodules [15]. Stages of nodulation (nodule formation) is controlled by the *nod* genes.

The source of energy for nitrogen fixation in bacteroids depends on host photosynthate which is transported through the membrane simbiosome in the form intermediate product of the tricarboxylic acid cycle (Krebs cycle) such as succinic acid, fumaric and malic acid which is a electron donor to produce ATP and reduce N_2. Pyruvic acid is the reductant that involved directly as an electron donor in the nitrogenase system [15]. The N_2 binding reaction that occurs in bacteroids as follows:

$$N_2 + 8e + 8H^+ + 16\ MgATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16\ MgADP + 16\ Pi$$

Complex of nitrogenase reduces the triple bond of N_2 into ammonia molecules. Nitrogenase enzyme activity can be measured by the acetylene reduction technique. Acetylene (C_2H_2) can be used as an alternative substrat to N_2. Reduction of N_2 and acetylene by nitrogenase as follows:

$$N_2 + 12\ ATP + 6e^- + 6H^+ \rightarrow 2\ NH_3 + 12\ ADP + 12\ Pi$$

$$C_2H_2 + 4\ ATP + 2e^- + 2H^+ \rightarrow C_2H_4 + 4ADP + 4Pi$$

The comparison between the substrate N_2 reduction by C_2H_2 is 3:1, and according to calculation [20] the total amount of N fixed by plants (µg) = µmol C_2H_4 x 28.

While the C_2H_2 reduction can provide a useful tool for detecting N_2-fixing activity in both legumes and non-legumes plants, the method is unsuitable for measuring N_2 fixation at field scales. There are some suitability of methods for quantifying N_2 fixation for crop legumes, such as measurement of N difference, relative ureide method, ^{15}N natural abundance, and ^{15}N isotope dilution [21]. But none of the methods for assessing N_2 fixation is perfect. Some additional informations are needed to support the N_2 fixation data, such as assessment of nodulation, growth analysis, rooting patterns of N_2 fixing and companion non N_2-fixing plants, determination of mineral N soil, and soil analysis [21].

4. Greenhouse experiments of symbiotic between acid aluminium tolerant *B. japonicum* and soybean on acid soils

Situmorang *et al*. [22] prepared media for soybean cultivication by using mixed composition of 1200 g acid soil (pH 4.5) and 800 g peat in a polybag. Peat is used as an additional organic matter to the soil. Acid soils and peat are prepared by drying and filtering using 2 mm pore of diameter. The media is sterilized by autoclave at 121˚C and 2 atm for one hour. The media is inoculated with 20% (v/w) of 10^8 cells/ml bacterial culture. Positive control media is added with 5 mM KNO_3. Plant harvest are divided into two groups, at the 50 DAP to crop nodules and 75-108 DAP to crop pods of legume. Three isolates are used viz. BJ 11 (wt), and its mutant BJ 11(5) and BJ 11(19). Wahyudi *et al.* [23] has been constructed several strains of acid-aluminium tolerant *B. japonicum* with increased symbiotic effectiveness through transposon TN5 mutagenesis, such as BJ 11(5), BJ 11(19), BJ 11 (20), and KDR 15 (37). The mutants could grow better on acid pH (4.0-4.5) and when each mutant inoculated to soybean plants will influenced better of symbiotic effectiveness, plant height, shoot and root weight, number of flowers, pods, dry weight of 100 seeds, and plant N-content [22].

Inoculation of BJ 11(19) isolate increased number of seeds and pods higher than the other treatments [22]. Acid tolerant soybean such as Slamet generally has weight 12.5 g of 100 seeds [24]. BJ 11 (19) showed the highest 13.5 g of 100 seeds. Pods that were already formed then were filled with photosynthate to form seeds. Numbers of seeds are effected by the number and size of pods. Higher number of pods also produce higher numbers of seeds [25].

Further experiments are done in acid soil plots (pH 4.5). Totally 12 plot experiments, each plot measured 1 m x 2 m x 0.2 m filled with 45 kg of acid soils (pH 4.5) and 10% (w / w) peat or rice husk as innoculant carrier. Each plot planting with soybean sprouts each with a spacing of 20 x 40 cm^2. Amount of inoculant (about 1.0 x 10^8 cells/ml) in peat-carrier is applied to each plot. Every hole on plot planted with 5 seedling soybeans and to be reduced to 3 plants at 30 days after planting. Each plot is separated by a distance of 1 m from other plot. Results of plot experiment showed that the effectiveness of symbiotic BJ 11 (19) with soybean is significantly had higher value on the plant height, dry weight of upper crop, root nodules, nodule number, nitrogenase activity, and weight of 100 seeds. Treatment of compost before planted soybean in acid soils could produce better crops and increase producing of soybean seeds compare to

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without compost (Figure 3). The compost consists of plant residues and soil microbes that can improve acid soil structure becomes more fertile and porous.

5. Viability test of acid-aluminium *B. japonicum* inoculant using peat as carrier

Viability of *B. japonicum* shoud be tested before used as an inoculant on fields experiments. Handayani *et al.* [26] conducted to test the viability of strains of acid-aluminium tolerant after a period of storages (1, 2, and 3 months) both at room temperature (± 25 °C) and 10 °C. The inoculant of *B. japonicum* BJ 11 (wt), and its mutants viz. BJ 11 (5) and BJ 11 (19), were tested by using sterilized peat as carrier (Figure 4). Peat is an decaying-organic material containing humic acid and organic-C and N which suitable for microbial growth. The result of viability test showed that there were an interaction between strain types, temperature, and a period of storage. The Inoculant of BJ 11 (19) which was stored at temperature 10 °C for 2 months showed the highest viability at 2,5 x 10^8 cell/g inoculants (Table 2).

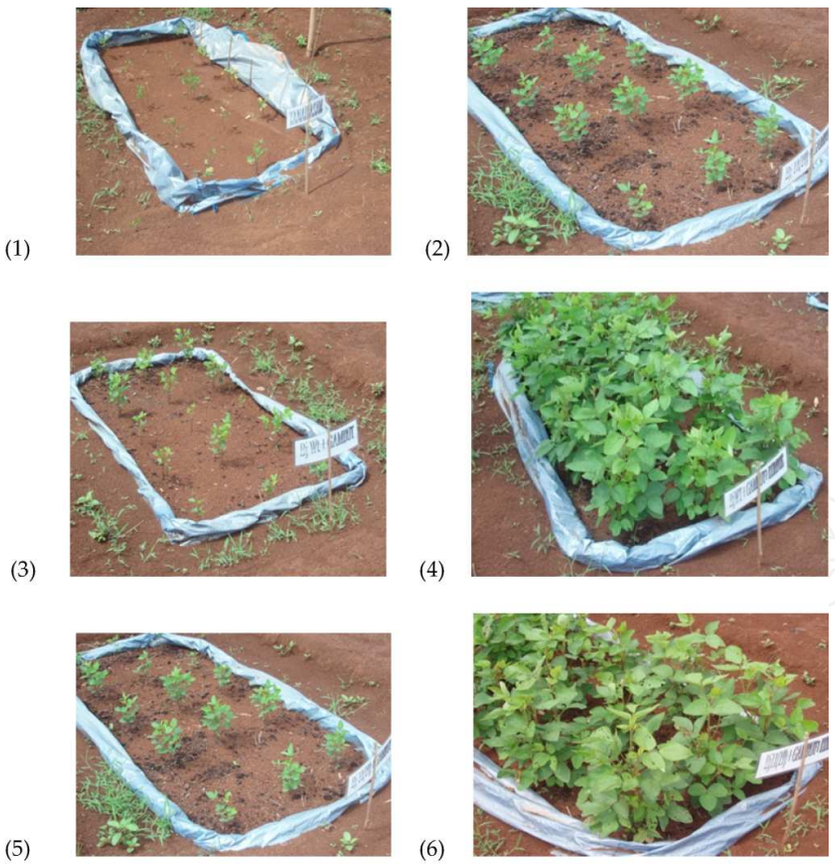

Figure 3. Growth of acid tolerant soybean variety Slamet 38 DAP on plot experiments: (1) control without inoculation, (2) control without inoculation + compost, (3) inoculation with BJ 11 (wt), (4) inoculation with BJ 11 (wt) + compost, (5) inoculation with BJ 11 (19), (6) inoculation with BJ 11 (wt) + compost.

Strain	Temperature	Storage periode (months)		
		1	2	3
BJ 11 (5)	Room	9.8×10^7 cdef	2.8×10^7 f	1.3×10^8 abcdef
	10 ºC	1.4×10^8 abcdef	1.2×10^8 abcdef	7.6×10^7 def
BJ 11 (19)	Room	2.4×10^8 ab	2.0×10^8 abc	1.1×10^8 bcdef
	10 ºC	1.8×10^8 abcd	2.5×10^8 a	1.8×10^8 abcd
BJ11 (wt)	Room	1.6×10^8 abcde	1.1×10^8 bcdef	4.2×10^7 ef
	10 ºC	1.3×10^8 abcdef	1.1×10^8 bcdef	1.9×10^8 abcd

Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05)

Table 2. Viability of three acid aluminium tolerant *B. japonicum* strains (cell. g^{-1}) stored at room temperature (± 25 °C) and 10 °C at 3 months storage [26]

Figure 4. The formula of inoculant acid-aluminium tolerant *B. japonicum* containing 10^9 cells g^{-1} using peat as carrier. Each pack contains 0.5 kg of inoculant for 10 kg of soybean seeds

6. Field trial of application of acid-aluminium tolerant *B. japonicum* on soybean

There are three locations for field trials to apply of the formula acid-aluminium tolerant *B. japonicum* on soybean viz. Jasinga (West Java), Sukadana (Province Lampung), and Tambang

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Ulang (Province South Kalimantan). Planting sites prepared a total area 1 hectare. Before planting on the field, the chemical contents of the soil and total plate count of soil bacteria are analyzed (Table 3 & 4). There are not found indigenous *B. japonicum* on all of field trial locations before symbiotic effectiveness treatments.

Parameter	Soil contents		
	Jasinga	Sukadana	Tambang Ulang
C (%)	1.18	0.73	2.54
N (%)	0.16	0.11	0.20
P (%)	0.0342	0.0142	0.0749
Mg (%)	0.02	0.01	0.02
K (%)	0	0	0.01
Ca (%)	0.23	0.14	0.67
C/N (%)	7.38	6.64	12.7
Al-dd	2.56	0.88	0.15
Capacity of cation exchange	7.55	2.45	7.14
pH : aquadest	4.61	5.56	6.18
pH :KCl	4.72	4.76	4.81

Table 3. Chemical properties of soil at the field trial locations

Field location	Numbers of cell (cfu/ml)	Numbers of *B. japonicum* (cfu/ml)
Jasinga-West Java	4.0×10^5	0
Sukadana -Lampung	5.9×10^5	0
Tambang Ulang -South Kalimantan	7.4×10^5	0

Table 4. Total plate count of bacteria and total of indigenous *B. japonicum* isolated from soil on planting sites

The field trial was conducted to examine the efficiency of BJ 11 (wt) and BJ 11 (19) on the growth, nodulation and yield of soybean variety Tanggamus and Anjasmoro. Tanggamus is one of leading variety which can adapt to dry acid soil, Anjasmoro generally showed good adaptation on paddy fields.

The seeds were coated with the inoculum formula before sowing. Seeds were sown by hand in each hole and planted 3 seeds per hole at a depth of 3 cm, distance of hole 20 cm x 40 cm. Fertilizer was placed at other hole besides of seeds hole. Watering was carried regularly if no rain. Removal of weeds or grasses are done as far as possible.

Soybean seed are sown by hand in a hole at soil. There were three seeds per polybag. Soybean seeds were selected based on the same size and healthy (able to shoot). Some treatments were conducted to soybean seed as follows: 1. inoculated by *B. japonicum* galur BJ 11, 2. inoculated by BJ 11 and application with 100 % N fertilizer; 3. inoculated by *B. japonicum* galur BJ 11 and

application with 50 % N fertilizer + 50% compost; 4. Control treatment: without inoculant, without inoculant + 100% N fertilizer, without inoculant + 50 % N fertilizer + 50% compost.

Each treatments were done at 150-200 m^2 and replicated two times per treatment. Mineral fertilization 100% N treatment consisted of 100 Kg ha^{-1} urea + 200 Kg ha^{-1} TSP (trisodium phosphate) + 100 Kg ha^{-1} KCl. For 50% N consisted of a half dose of urea + 200 Kg ha^{-1} TSP + 100 Kg ha^{-1} KCl + compost 1000 Kg ha^{-1}. Compost was spread out at land surface one week before seeds planting. The compost only consisted of decaying plants and decomposed by microbes. There are not found rhizobia in compost, and consist of phosphate solubilizing bacteria as much as 320 $cell.ml^{-1}$. Urea used twice at one planting period viz a half dose at seeds planting and the rest at 30 days after planting (DAP) [27].

Growth parameters such as plant height at 30 days after planting (DAP), number of pods at 90 DAP, total number of seeds, total of seed weight, and weight of 100 seeds numbers of pods compare to control were determined. Growth parameters were measured from 10 plants per treatments. Data were analyzed using completely randomized design and the means at $p<0.05$ level of significance.

The results of field experiments showed that there were a significant effect of *B. japonicum* inoculation for soybean variety Tanggamus and Anjasmoro which grown at Jasinga –West Java, Tanah Laut-South Kalimantan and Sukadana-Lampung compared to control, without inoculants and fertilizer (Table 5, 6 & 7). Inoculation BJ 11 formula showed a better response on soybean growth than control, treatment without fertilizer and inoculant. Plants inoculated with BJ 11 (wt) and its mutant BJ 11 (19) showed higher plant height, number of pods, and seeds, weight of 100 seeds compare to control. To improve field-scale of soybean production in acid soils still need N- fertilizer, but the application of inoculant *B. japonicum* can reduce a half of N fertilizer.

Anjasmoro									
Treatment	**Plant height at 45 DAP (cm)**	**Number of branch**	**Number of flower**	**Plant height at 90 DAP (cm)**	**Number of branch at 90 DAP**	**Number of pods**	**Number of seed**	**Total of seed weight (g)**	**Weight of 100 seeds (g)**
BJ 11 (19) + 1 N	30.6 d	1 c	28.2 cd	34.2 c	1.5 c	10.8 c	17.5 d	2.83 cd	13.42 b
BJ 11 (19) + 1/2 N + C	35.8 b	0.7 cd	31.2 bc	41 b	2.6 a	16.2 b	29.4 bc	4.33 bc	12.97 b
BJ 11 (19)	31.9 cd	0.3 de	19.9 e	37.5 bc	1.4 c	10 c	18.3 d	2.27 de	12.60 b
BJ 11 (WT) + 1 N	40.1 a	1.7 b	35.4 b	46.5 a	2.3 ab	19.7 b	35 b	4.55 b	13.21 b
BJ 11 (WT) + 1/2 N + C	42 a	2.3 a	45.2 a	51.9 a	2.5 a	31.3 a	51.1 a	6.29 a	12.55 b
BJ 11 (WT)	33.6 bc	0.4 de	21.8 de	34.c1	0.1 d	5.5 c	7.1 e	1.02 e	15.06 a
1 N	41.3 a	1.6 b	36.4 b	38.1 bc	1.6 bc	16.6 b	25,5 bcd	3.43 bcd	12.75 b
1/2 N + C	42.2 a	2.1 ab	29.9 bc	38.1 bc	1.7 bc	17.1 b	29.8 bc	4.23 bc	13.17 b
Control	26.4 e	O e	20.5 e	34.7 c	1.8 bc	10.9 c	21.8 cd	2.95 cd	13.26 b

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Anjasmoro									
Treatment	**Plant height at 45 DAP (cm)**	**Number of branch**	**Number of flower**	**Plant height at 90 DAP (cm)**	**Number of branch at 90 DAP**	**Number of pods**	**Number of seed**	**Total of seed weight (g)**	**Weight of 100 seeds (g)**
BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl); ½ N = 50 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl; C = compost. Control = without fertilizer (NPK) and inoculants. Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05).									
Tanggamus									
Treatment	Plant height at 45 DAP (cm)	Number of branch	Number of flower	Plant height at 90 DAP (cm)	Number of branch at 90 DAP	Number of pods	Number of seed	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 (19) + 1 N	30.6 d	1 c	28.1 cd	32.6 cd	1.6 cd	14.5 bc	23.9 cd	2.37 cd	7.95 b
BJ 11 (19) + 1/2 N + C	35.8 b	0.7 cd	31.2 bc	33.2 cd	1 d	12.1 cd	17.8 d	1.38 de	7.11 b
BJ 11 (19)	31.9 cd	0.3 de	19.8 e	36.1 bc	1.6 cd	21.8 a	29.2 bc	2.68 bcd	8.32 b
BJ 11 (WT) + 1 N	40.1 a	1.7 b	35.4 b	43 a	2.1 bc	23.5 a	34.9 abc	3.72 ab	10.74 a
BJ 11 (WT) + 1/2 N + C	42.1 a	2.4 a	45.1 a	42 ab	2.8 ab	24. 8 a	38.6 ab	3.42 abc	9 ab
BJ 11 (WT)	33.6 bc	0.4 de	21.8 de	41.7 ab	3.1 a	25.9 a	42.6 a	4.02 a	8.95 ab
1 N	41.3 a	1.6 b	36.3 b	31.7 cd	1.3 cd	19.5 ab	25.1 cd	2.32 cd	8.66 ab
1/2 N + C	42.2 a	2.1 ab	29.9 bc	29.4 cd	0.9 d	10.6 cd	15.3 de	1.39 de	9.42 ab
Control	26.4 e	O e	20.5 e	27.4 d	0.1 e	5.5 d	5 e	0.42 e	8.44 ab

BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl); ½ N = 50 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl; C = compost. Control = without fertilizer (NPK) and inoculants. Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05).

Table 5. Growth of Anjasmoro and Tanggamus cultivar soybean plants on treatment with acid-aluminium tolerant *B. japonicum* formula on acid soil at Jasinga- West Java

Anjasmoro									
Treatment	**Plant height at 30 DAP (cm)**	**Plant height at 90 DAP (cm)**	**Number of leaf at 90 DAP**	**Number of branch at 30 DAP**	**Number of branch at 90 DAP**	**Number of pod at 90 DAP**	**Number of seed**	**Total of seed weight (g)**	**Weight of 100 seeds (g)**
BJ 11 (19) + 1 N	38.3 de	66.8 a	33.1 a	3 ab	3.3 ab	54.8 a	109.2 a	17.3 ab	14.7 abc
BJ 11 (19) + 1/2 N + C	38.3 de	61.5 b	28 bc	2.7 abc	3.1 ab	60.2 a	71.5 c	10.4 cd	13.5 bc
BJ 11 (19)	41.6 bc	52.8 c	26.4 cd	2.2 c	3 ab	41.2 b	123.1 a	18.7 a	13.4 bc
BJ 11 (WT) + 1 N	53 a	68.9 a	22.6 de	2.5 abc	2.3 d	34.7 bc	81 bc	14.1 bc	15 abc

Anjasmoro									
Treatment	**Plant height at 30 DAP (cm)**	**Plant height at 90 DAP (cm)**	**Number of leaf at 90 DAP**	**Number of branch at 30 DAP**	**Number of branch at 90 DAP**	**Number of pod at 90 DAP**	**Number of seed**	**Total of seed weight (g)**	**Weight of 100 seeds (g)**
BJ 11 (WT) + 1/2 N + C	44. 3 b	71.2 a	29 abc	2 c	3 ab	58.2 a	75.9 c	12.3 cd	15.3 ab
BJ 11 (WT)	40.9 cd	69.9 a	32 ab	3.3 a	3.6 a	67.7 a	101.1 ab	17.5 ab	13.3 c
1 N	53.4 a	50.8 cd	21.6 ef	3 ab	2.9 bc	27.9 cd	46.3 d	6 e	16.1 a
1/2 N + C	35.8 e	47.6 d	21.2 ef	2.3 bc	3.1 ab	27.2 cd	63.1 cd	9.2 de	13.3 c
Control	41 cd	45.8 d	17.3 f	3.2 a	2.4 cd	15.6 d	40.6 d	5 e	14.6 abc

BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl); ½ N = 50 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl; C = compost. Control = without fertilizer (NPK) and inoculants. Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05).

Tanggamus									
Treatment	Plant height at 30 DAP (cm)	Plant height at 90 DAP (cm)	Number of leaf at 90 DAP	Number of branch at 30 DAP	Number of branch at 90 DAP	Number of pod at 90 DAP	Number of seed	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 (19) + 1 N	28.6 d	62.5 a	32.9 a	1.2 ab	3 ab	37.9 abc	49.3 bcd	4.3 bc	11.0 ab
BJ 11 (19) + 1/2 N + C	30.4 cd	59.4 ab	24.8 b	1.5 a	2.7 bc	33.6 bcd	56.2 bc	4.9 bc	10.4 b
BJ 11 (19)	51.1 c	50.5 c	18.5 c	1.5 a	2.2 c	21.8 e	47.1 cd	4.8 bc	10.3 b
BJ 11 (WT) + 1 N	33.8 ab	57.3 b	31.8 a	1.7 a	3.2 ab	41.1 ab	74.9 a	6.9 a	11.4 ab
BJ 11 (WT) + 1/2 N + C	35 a	59 ab	32.6 a	1.7 a	2.7 bc	45.1 a	64.9 ab	6.8 a	10.9 ab
BJ 11 (WT)	33.9 ab	55.4 b	25 b	0.6 bc	2.6 bc	31.6 cd	64.4 ab	5.8 ab	12.1 a
1 N	34.9 a	45.8 c	23.3 bc	1.9 a	2.5 bc	21.7 e	36.7 d	3.5 c	10.3 b
1/2 N + C	32.4 bc	45.7 c	21.1 bc	1.7 a	2.7 bc	19.8 e	38.8 d	4.1 c	11.4 ab
Control	28.8 d	48.9 c	22.7 bc	0.5 c	3.5 a	26.3 de	50.3 bcd	4.4 bc	10.6 b

BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl); ½ N = 50 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl; C = compost. Control = without fertilizer (NPK) and inoculants. Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05).

Table 6. Growth of Anjasmoro and Tanggamus cultivar soybean plants on treatment with acid-aluminium tolerant *B. japonicum* formula on acid soil at Tambang Ulang-South Kalimantan

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Anjasmoro

Treatment	Plant height at 30 DAP (cm)	Plant height at 45 DAP (cm)	Number of branch at 30 DAP	Number of branch at 90 DAP	Number of pod at 90 DAP	Number of seed	Total of seed weight (g)	Weight of 100 seed (g)
BJ 11 (19) + 1 N	40.9 b	61.5 ab	1.5 ab	2.4 a	54.9 a	60.6 ab	7.6 bc	12.1 ab
BJ 11 (19) + 1/2 N + C	46.7 a	66.2 a	1.9 a	2.9 a	60.2 a	64.6 ab	10.3 a	13.6 ab
BJ 11 (19)	22.4 d	67.8 a	0 c	0 b	41.2 b	13.4 e	1 e	10.5 ab
BJ 11 (WT) + 1 N	46.7 a	50.2 bc	1.4 ab	2.4 a	34.8 bc	73.9 a	9.5 ab	16.1 a
BJ 11 (WT) + 1/2 N + C	40.6 b	44.4 c	1 b	2.4 a	58.2 a	66.2 ab	7.2 bc	11.2 ab
BJ 11 (WT)	25.3 d	64.7 a	0.2 c	0.2 b	67.7 a	35.2 d	3.1 de	8.8 b
1 N	43.2 ab	68.4 a	1.5 ab	3.0 a	27.9 cd	53.8 bc	7 c	11.7 ab
1/2 N + C	40.9 b	64.3 a	1.6 ab	2.1 a	27.2 cd	57.4 bc	6.4 c	10.1 ab
Control	33.6 c	55.3 abc	1 b	0.9 b	15.6 d	42.9 cd	4.1 d	9.6 b

BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl); ½ N = 50 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl; C = compost. Control = without fertilizer (NPK) and inoculants. Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05).

Tanggamus

Treatment	Plant height at 30 DAP (cm)	Plant height at 45 DAP (cm)	Number of branch at 30 DAP	Number of branch at 90 DAP	Number of pod at 90 DAP	Number of seed	Total of seed weight (g)	Weight of 100 seed (g)
BJ 11 (19) + 1 N	32.4 bc	61 a	3.5 ab	4.3 ab	62.3 a	131.6 a	10.7 b	7.4 a
BJ 11 (19) + 1/2 N + C	35.4 b	60.3 a	3.7 ab	4.5 a	61.4 a	127 a	10.2 b	7.3 a
BJ 11 (19)	25.1 d	34.5 f	0.5 d	1.2 c	18.4 b	33.1 c	2.2 c	6.8 a
BJ 11 (WT) + 1 N	31.1 c	51 bcd	3.1 b	4.7 a	51.6 a	128.5 a	9.7 b	7.8 a
BJ 11 (WT) + 1/2 N + C	36.1 a	57 ab	3.9 ab	4.3 ab	58.3 a	130.1 a	10.1 b	7.4 a
BJ 11 (WT)	26.3 d	42.1 e	1.2 cd	1.8 c	21.3 b	43.3 bc	3 c	7.1 a
1 N	33 abc	53.6 bc	4.4 a	4.8 a	69.1 a	145.3 a	16.1	7.3 a
1/2 N + C	32.3 bc	59.4 a	3.1 b	4.3 ab	60.4 a	129 a	10.6 b	7.9 a
Control	29.7 c	47.2 d	1.6 c	3.1 b	35.2 b	73.1 b	5.5 bc	7.8 a

BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl); ½ N = 50 Kg.Ha^{-1} urea + 200 Kg. Ha^{-1} TSP and 100 Kg.Ha^{-1} KCl; C = compost. Control = without fertilizer (NPK) and inoculants. Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05).

Table 7. Growth of Anjasmoro and Tanggamus variety soybean plants on treatment with acid-aluminium tolerant *B. japonicum* formula on acid soil at Sukadana- Lampung

7. Conclusion

Effectiveness symbiotic between soybean and acid-toleran aluminium root nodule bacteria, such as *Bradyrhizobium japonicum* BJ 11 played an important role on increasing the plant growth on acid soil (pH> 4.5). The bacteria provided fixed nitrogen to soybean plant and then support growth and development of plants. Soybean plants inoculated with *B. japonicum* strain BJ 11 (wild-type) and its mutant BJ 11 (19) showed better growth than control without inoculation in greenhouse and field trial experiments. B. japonicum inoculant on peat as carrier showed high viability and stability during storages.

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