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The Use of Acid-Aluminium Tolerant *Bradyrhizobium japonicum* Formula for

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Abstract**—**Land with low pH soil spread widely in Indonesia can be used for soybean (*Glycine max*) cultivation, however the production is low. The use of acid tolerant soybean and acid-aluminium tolerant nitrogen-fixing bacteria formula was an alternative way to increase soybean productivity on acid soils. *Bradyrhizobium japonicum* is one of the nitrogen fixing bacteria which can symbiose with soybean plants through root nodule formation. Most of the nitrogen source required by soybean plants can be provided by this symbiosis. This research was conducted to study the influence of acid-aluminium tolerant *B. japonicum* strain BJ 11 formula using peat as carrier on growth of Tanggamus and Anjasmoro cultivar soybean planted on acid soil fields (pH 5.0-5.5). The results showed that the inoculant was able to increase the growth and production of soybean which were grown on fields acid soil at Sukadana (Lampung) and Tanah Laut (South Kalimantan), Indonesia.

Keywords**—***Bradyrhizobium japonicum*, acid-aluminium tolerant mutant, Tanggamus cultivar soybean, acid soils

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I. Introduction

LEGUME such as soybean could increase soil fertility and plant productivity. Inoculation of root nodule bacteria, such as *Bradyrhizobium japonicum*, on soybean plantation could enhance soybean quality and its productivity [1], [2]. A wide variation of *B. japonicum* tolerance to acid soil conditions have been reported on many agriculturally important legumes from various countries [3]. Bacteria can increase plant production because its ability to fix nitrogen and provide for plant. Atmospheric nitrogen (N_2) was fixed by the bacteria into ammonium (NH_3) in a nodule, so that the nitrogen can be used by the plant for its growth [4].*Bradyrhizobium japonicum* is one of root nodule bacteria that can contribute on plant growth by providing fixed nitrogen in nodules of soybean plants [1]. Some strains of *B. japonicum* were tolerant on an acid condition, even at the pH level 4.0-4.5 [5]. Twenty five strains of *B. japonicum* had been selected for acid tolerance using either solid and broth medium [6]. In the Leonard bottle experiment, indigenous *B. japonicum* strain 11 (BJ 11) significantly could increase dry weight of the upper crop and nitrogen uptake of soybean cultivar Slamet higher than standard strain from USA, USDA 110 [7]. Furthermore, pot experiments on acid soil pH 4.7 showed that inoculation of BJ 11 could increase plant height, shoot and root weight, number of flowers, pods, seeds, seeds dry weight, and shoot and seed nitrogen content [8].This research was conducted to study to study the influence of acid-Al tolerant *B. japonicum* formula using peat as carrier on growth of Tanggamus and Anjasmoro cultivar soybean planted on field acid soil (pH 5.0-5.5).

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II. Materials and Methods

Materials

Isolate acid tolerant *B. japonicum*, BJ 11, was maintained at IPB Culture Collection, Biology Department, Faculty of Science and Mathematics, Bogor Agricultural University. Tanggamus and anjasmoro cultivar soybean seeds were obtained from Research Institute for Food Crops and Genetic Resources. Peat as inoculant carrier was obtained from Indonesian Research Institute for Legumes and Tubers. Physical and chemical analysis of soils and peats was done in Indonesian soil Research Institute, Bogor.

Medium and Formula Preparation

Isolates *B. japonicum* 11 (BJ 11) was grown on *Yeast Mannitol Agar* (YMA) that consist of mannitol (10 gL^{-1}), K_2HPO_4 (0.5 gL^{-1}), $MgSO_4 \cdot 7H_2O$ (0.2 gL^{-1}), NaCl (0.2 gL^{-1}), yeast extract (0.5 gL^{-1}), added with congo red (0.0025%) and rifampicin (50 $\mu g\ ml^{-1}$). The isolates were resistant to rifampicin [9]. The isolates were incubated for about 7-8 days at the room temperature. Then they were inoculated into *Yeast Mannitol Broth* (YMB) on an Erlenmeyer, and incubated for five days with an 125 rpm shaker. Formula contained 10^8-10^{10} cell/gram BJ 11 mixed with peat, gum arabic, limestone, and packed in plastic (0.5 Kg/plastic pack).

Field Trial

The seeds were coated with the 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 were done as far as possible. Soybean seed were sown by hand in a hole at soil. There are three seeds per polybag. Soybean seeds were selected based on the same size and healthy (able to shoot). Some treatments were done 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: without inoculant, without inoculant + 100% N fertilizer, without inoculant + 50 % N fertilizer + 50% compost. Each

treatments were done at 150-200 m^2 land replicated two times per each. As for mineral fertilization treatment 100 % N consisted of 100 Kg ha^{-1} urea + 200 Kg ha^{-1} TSP + 100 Kg ha^{-1} KCl. As 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 spread out at land surface one week before seeds planting. Urea used twice at one planting period i.e. a half dose at seeds planting and the rest at 30 days after planting (DAP).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.

II. Results And Discussion

Isolate Growth and Formula of Inoculants

Isolate BJ 11 was able to grow on YMA which were added with 0.0025% congo red and 50 μg/ml rifampicin after 7 days incubated on room temperature. Morphology of *B. japonicum* colonies were mucoid, not quite able to reserve congo red, and curve elevated (Fig. 1a). Formula of BJ 11 on peat as carrier contained 10^9-10^{10} cell/gram BJ 11 and packed in 0.5 Kg/plastic pack (Fig. 1b).

(a) (b)

Fig. 1 (a) Colony of *B. japonicum* BJ 11 after 7 days of incubation on YMA + 0.0025% congo red + 50 μg/ml rifampicin, (b) formula of BJ 11 on peat as carrier.

Bradyrhizobium japonicum strain BJ 11 catagorized as slowly growth bacterium because its colony on solid medium was seen after 7 days of incubation. The isolate could not absorb congo red at yeast mannitol agar (YMA) added with congo red [1]. BJ 11 also secreted exopolysaccharides in a large amount of mannitol [10], the colony was appeared sticky and mucoid colony. Role of exopolysaccharide to resistance at acid condition [11].

Field Trial

A field trial was conducted to examine the efficiency of BJ 11 on the growth, nodulation and yield of soybean cultivar Tanggamus and Anjasmoro. Tanggamus is one of leading cultivar which can adapt to dry acid soil, Anjasmoro also showed good adaptation on paddies fields. The experiments took place at Sukadana (Lampung) and Tanah Laut (South Kalimantan). Chemical properties of the soil showed at Tabel 1. There were not found indigenous *B. japonicum* on field trial location and compost before treatments (Table 2 & 3).

Table I
Chemical properties of soil at the trial locations

Parameter	Soil contents	
	Lampung	South Kalimantan
C (%)	0.73	2.54
N (%)	0.11	0.20
P (%)	0.0142	0.0749
Mg (%)	0.01	0.02
K (%)	0	0.01
Ca (%)	0.14	0.67
C/N (%)	6.64	12.7
Al-dd	0.88	0.15
Capacity of cation exchange	2.45	7.14
pH : aquadest	5.56	6.18
pH :KCl	4.76	4.81

Table II
Total Plate Count Of Cell Bacteria And Indigenous *B. japonicum* In Field Location

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

Table III
Composition bacteria on compost used in this experiments

No	Microbes	Cell (cfu/ml)
1	*Lactobacillus*	0
2	Phosphate solubilization bacteria	320
3	*Rhizobium/Bradyrhizobium*	0
4	Actinomycetes	0
5	Yeast	0

Analysis data showed there were significant effect of *B. japonicum* inoculation for soybean grown at Tanah Laut-South Kalimantan and Sukadana - Lampung compared to control, without inoculants and fertilizer (Table 4 & 5). Inoculation BJ 11 formula showed better response on soybean growth than control, treatment without fertilizer and inoculant.

There were an association between soybean and root nodule bacteria which were played an important role in increasing the plant growth. Nitrogen fixation by the bacteria provided fixed nitrogen to the plants and then support growth and development of plants. Plants inoculated with BJ 11 isolate approximately had higher plant height, number of pods, and seeds, weight of 100 seeds. Inoculation of *Rhizobium* effectively influenced the formation and development of pods [11]. Pods that were already formed then were filled with phosynthate to form seeds. Numbers of seeds were effected by the number and size of pods. Higher number of pods were also gave higher numbers of seeds [8].

TABLE IV
GROWTH OF ANJASMORO AND TANGGAMUS CULTIVAR SOYBEAN PLANTS ON TREATMENT WITH ACID-ALUMINIUM TOLERANT *B. JAPONICUM* FORMULA ON ACID SOIL AT SUKADANA- LAMPUNG

Anjasmoro

Treatments	Plant height at 30 DAP (cm)	Number of pods at 90 DAP	Total number of seeds	Total of Seed weigh t (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	46.7 a	34.8 bc	73.9 a	9.5 ab	16.1 a
BJ 11 + 1/2 N + C	40.6 b	58.2 a	66.2 ab	7.2 bc	11.2 ab
BJ 11	25.3 d	67.7 a	35.2 d	3.1 de	8.8 b
1 N	43.2 ab	27.9 cd	53.8 bc	7 c	11.7 ab
1/2 N + C Without fertilizer and inoculants	40.9 b 33.6 c	27.2 cd 15.6 d	57.4 bc 42.9 cd	6.4 c 4.1 d	10.1 ab 9.6 b

Tanggamus

Treatments	Plant height 30 DAP (cm)	Number of pods 90 DAP	Total number of seeds	Total of seed weigh t (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	31.1 c	51.6 a	128.5 a	9.7 b	7.8 a
BJ 11 + 1/2 N + C	36.1 a	58.3 a	130.1 a	10.1 b	7.4 a
BJ 11	26.3 d	21.3 b	43.3 bc	3 c	7.1 a
1 N	33 abc	69.1 a	145.3 a	16.1	7.3 a
1/2 N + C Without fertilizer and inoculants	32.3 bc 29.7 c	60.4 a 35.2 b	129 a 73.1 b	10.6 b 5.5 bc	7.9 a 7.8 a

TABLE V 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

Anjasmoro

Treatments	Plant height 30 DAP (cm)	Number of pods 90 DAP	Total number of seeds	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	53 a	34.7 bc	81 bc	14.1 bc	15 abc
BJ 11 + 1/2 N + C	44. 3 b	58.2 a	75.9 c	12.3 cd	15.3 ab
BJ 11	40.9 cd	67.7 a	101.1 ab	17.5 ab	13.3 c
1 N	53.4 a	27.9 cd	46.3 d	6 e	16.1 a
1/2 N + C Without fertilizer and inoculants	35.8 e 41 cd	27.2 cd 15.6 d	63.1 cd 40.6 d	9.2 de 5 e	13.3 c 14.6 abc

Tanggamus

Treatments	Plant height 30 DAP (cm)	Number of pods 90 DAP	Total number of seeds	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	33.8 ab	41.1 ab	74.9 a	6.9 a	11.4 ab
BJ 11 + 1/2 N + C	35 a	45.1 a	64.9 ab	6.8 a	10.9 ab
BJ 11	33.9 ab	31.6 cd	64.4 ab	5.8 ab	12.1 a
1 N	34.9 a	21.7 e	36.7 d	3.5 c	10.3 b
1/2 N + C Without fertilizer and inoculants	32.4 bc 28.8 d	19.8 e 26.3 de	38.8 d 50.3 bcd	4.1 c 4.4 bc	11.4 ab 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.

Based on experiments, weight of 100 seeds of Anjasmoro cultivar tent to higher than Tanggamus cultivar (Table 5). Using recommended fertilizer, Tanggamus and Anjasmoro cultivar commonly had about 12 g/100 seeds and 15 g/100 seeds, respectively. In this experiment, treatment with BJ 11 + 1/2 N + C using Anjasmoro cultivar achieved 15.3 g/100 seeds, and treatment only with B J 11 achieved 12.1 g/seeds using Tanggamus cultivar. In conclusions, inoculation BJ 11 was able to increase the growth and production of soybean plants which were grown on acid soil fields.

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