Response of Local Bean from Tanimbar on Nitrogen Fixing Azotobacter

Reginawanti Hindersah*, Priyanka Asmiran

Abstract— Food security in Tanimbar Island, Maluku Tenggara Barat Regency, is depend on local bean as carbohydrate, fat and protein sources in their diet. Appropriate fertilization is needed to support sustainable bean productivity due to low nitrogen content of Tanimbar's soil. The use of biofertilizer, a nitrogen fixing bacteria *Azotobacter*, is a way to increase soil nitrogen availability by lowering inorganic fertilizer dose. The objective of this preliminary study was to verify the influence of *Azotobacter chroococcum* inoculation on germination of seven local bean varieties of Tanimbar Island. The results of the study showed that the germination percentage of all bean variety were 100% at day three but Azotobacter inoculation lowered that of two bean varieties. Each cultivar of local bean demonstrated different germination responses to Azotobacter inoculation. This bioassay determined that negative response of some local bean varieties on Azotobacter biofertilizer inoculation was evidence. Only red cow pea which demonstrated positive response on bacterial inoculation.

Index Terms— *Azotobacter*, Biofertilizer, Germination, Phytohormone

1 INTRODUCTION

Tanimbar which cover area of 3,333 km² is a bigger island in Tanimbar Archipelago, Maluku, Indonesia. Daily Tanimbar community consumption is included a variety of bean in their diet. Pulses are important food crops which has significant economic value, while contributing to the sustainable management of soils. In some village in remote area, pulses (dried beans) are an irreplaceable food in dry season. Farmers in general grow native common bean and mung bean varieties due to their resistance to low nutrient and drought condition. Despite of intensive beans cultivation, farmer awareness to pulse productivity is limited. Farmers cultivate beans in low to medium fertility dry land soil with rainfall as the only source of water. Most of cultivated land is located in steep area with slightly acid to slightly alkaline soil, low in organic matter, average in total nitrogen, and low in available phosphorous and potassium. Several area of beans cultivation contained organic carbon only between 1.3%-1.8%, and total nitrogen of around 0.38%. Based on local soil fertility, nutrient external input is necessary to maintain bean productivity and pulse availability in local market.

Soil beneficial microbes are an important input to support good soil nutrient management strategy [2] and to decrease the dependency of farmers on chemical fertilizer. The used of soil beneficial microbe in crop production enables to maintain soil quality in sustainable agriculture. Soil microbe, especially Plant Growth Promoting Rhizobacteria (PGPR), is important biofertilizer to enrich soil nutrient and phytohormone availability for plant uptake and growth [7].

Free living nitrogen-fixing *Azotobacter* is easily isolated from most of either legume or nonlegume crops rhizosphere, and since decades is used as biofertilizer in dryland as well as wetland. Novel insight of use of *Azotobacter* in agriculture is exopolysachharide production [6] which improve soil aggregation and nutrient uptake; and biological control against soil plant pathogen *Rhizoctonia solani* [4].

Important beneficial bacteria in legume is indeed symbiotic nitrogen-fixing *Rhizobia* spp. Dual inoculation of *Azotobacter-Rhizobium* is supposed to improve nodulation, growth and yield of commercialized bean is clearly demonstrated (3, 12, 9). However, there is still limited study concerning the effect of *Azotobacter* on the seedling growth of local bean in Indonesia. Seed inoculation with *Azotobacter* is a way to place the bacteria into soil so that they colonize seedling root as soon as seed germination. The objective of this preliminary study was to verify the effect of *Azotobacter chroococcum* inoculation on germination of seven local bean cultivar grown in Tanimbar Island.

2 MATERIAL AND METHOD

Seed of seven varieties of local bean (Fig 1) were collected from farmer in Tanimbar Selatan Subdistrict, Maluku Tenggara Barat District, Maluku Province at May 2017. A total of four varieties belong to cow pea (*Vigna Unguiculata* L.), three varieties are belong to mung bean (*Vigna radiata* (L) Wilczek,). Primary metabolites content of some varieties was analysis before the experiment (Table 1) to show nutritional benefit of common bean and mung bean. All seed were kept in 4°C at dark co

TABLE 1
Nutrient content of two varieties of Cow pea and Mung bean

Beans variety	Nutrient (%)			Water content (%)	Ash content (%)
	Protein	Fat	Carbohydrate		
Yellow Cow pea (*Vigna unguiculata* L)	36.15	2,87	46,13	12,37	3,24
Red Cow pea (*Vigna unguiculata* L)	34,85	3,74	45,50	12,73	3,18
Red mung bean (Vigna radiata)	38,17	2,08	45,39	11,41	2,94
Black mung bean (V. radiata)	36,22	2,58	46,05	11.31	2.90

- *Reginawanti Hindersah is a Lecturer of Faculty of Agriculture Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363, Indonesia. E-mail: reginawanti@unpad.ac.id*
- *Priyanka is Master Graduate of Soil Science Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363 , Indonesia.*

All the seed codes were listed below and their morphology are shown in Fig 1.

A: Yellow cow pea (*Vigna unguiculata* L.)
B: Black cow pea (*Vigna unguiculata* L.)
C: Red cow pea (*Vigna unguiculata* L.)
D: Red mung bean (*Vigna radiata* (L.) Wilczek)
E: Black mung bean (*Vigna radiata* (L.) Wilczek)
F: Green mung bean (*Vigna radiata* (L.) Wilczek)
G: White-eyed Black cow pea (*Vigna unguiculata* L.)

Azotobacter chroococcum isolated from corn rhizosphere is a collection of Soil Biology Laboratory of Universitas Padjadjaran. The bacteria maintained in Nitrogen-free Ashby media (KH_2PO_4 0,2 g, $MgSO_4.7H_2O$ 0,2 g, Mannitol 10 g, NaCl 0,2 g, $CaSO_4.2H_2O$ 0,1 g, $CaCO_3$ 5 g, Agar 10 g, Aquadest 1 L) at neutral acidity. A total of 100 mL of liquid Ashby medium in Erlenmeyer 250 mL was sterilized at 121°C for 20 minutes one day before inoculated with 5% of *Azotobacter* liquid inoculum. The culture was incubated at room temperature for three days on reciprocal shaker with agitation rate of 115 rpm prior to be used in germination test.

Petri plates-8 cm in diameter-were wrapped with used newspaper, sterilized at 180°C for three hours and kept one night in the oven at room temperature. A total of 10 mL of liquid inoculum (average density 10^7 cfu/mL) were poured evenly on the surface of filter paper on petri plate. Beans seeds were wrapped in wet cloth one night in the dark before 20 seeds were placed on filter paper. Control treatment received the same amount of sterilized water. All treatments were replicated two times. Covered Petri plate was placed in dark chamber for one day before transferred to the room chamber with natural light for two consecutive days.

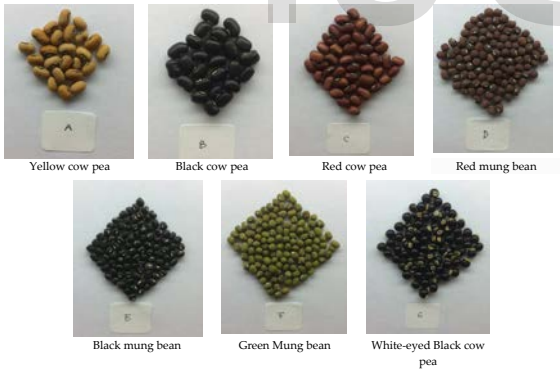

Fig 1. Seven varieties of bean grown naturally in Tanimbar Selatan for germination test

Number of germinated seed was counted at day one and three; seed germination percentage was calculated at day three. Shoot height, roots length, and leaves length were measured at day six for A to F seeds and day seven for G seeds. Average of all parameters was calculated from five seedlings in one petri plate.

3 RESULTS AND DISCUSSION

3.1 Germination rate

Azotobacter inoculation did not exhibit consistent pattern with bean varieties. At day one, number of germinated seed of certain bean varieties was reduced or increased at day one except red mung bean that showed no change of germinated seed (Table 2). At day three, germination rate of most bean varieties is 100%. In general, *Azotobacter* inoculation did not affect germination rate except that of red bean (85%) and red mung bean (95%).

Seed quality plays mostly dictate growth and yield of agricultural legume crops [16]. Variety trueness and seed purity of local bean in Tanimbar is unknown yet since farmer and local government do not develop true bean seed. Germination percentage is important to ensure yield parameters such as pod length, pod width, number of seed/pod and 100-seed weight [1]. The information of no harm effect of *Azotobacter* in germination is a first step to continue nitrogen-fixing biofertilizer development for local bean. However, the reason of germination percentage decrease of two varieties after inoculation should be studied.

TABLE 2.

Bean seedling germination after Azotobacter inoculation

Bean variety, inoculation	Number of germinated seed		Germination percentage at day three
	Day one	Day three	
Yellow cow pea, uninoculated	5,3	20	100
Yellow cow pea, inoculated	6,3	20	100
Black cow pea, uninoculated	4,3	20	100
Black cow pea, inoculated	6,3	20	100
Red cow pea, uninoculated	5,7	20	100
Red cow pea, inoculated	4,7	17	85
Red mung bean, uninoculated	5,3	20	100
Red mung bean, inoculated	5,3	19	95
Black mung bean, uninoculated	5,3	20	100
White-eyed Black cow pea, inoculated	6,0	20	100
Green mung bean, uninoculated	5,7	20	100
Green mung bean, inoculated	6,3	20	100
White-eyed black cow pea, uninoculated	5,3	20	100
White-eyed black cow pea, inoculated	6,0	20	100

Response of each legume seed on *Azotobacter* was not similar (Fig 2.); surprisingly, bacterial inoculation mostly showed negative impact on either shoot height and root lengths. Red cow pea and Red Mung bean were showed increase in height of seedling after grown on filter paper received Azotobacter liquid culture; as much as 10,5% and 24% respectively (Fig 2a). Decrease in seedling height following inoculation was markedly demonstrated by yyellow cow pea, black cow pea, green mung bean and white-eyed black cow pea (Fig 2a). The biggest decline in height of seedling was shown by white-eyed black cow pea; 48%. Similar to shoots heights, *Azotobacter* inoculation tend to decrease roots length, only two bean varieties were responsive to bacterial inoculation. The roots length of black cow pea and red cow pea increased up to 27% and 44% respectively in petri plate with Azotobacter while that harmful effect of *Azotobacter* on other beans was demonstrated (Fig 2b).

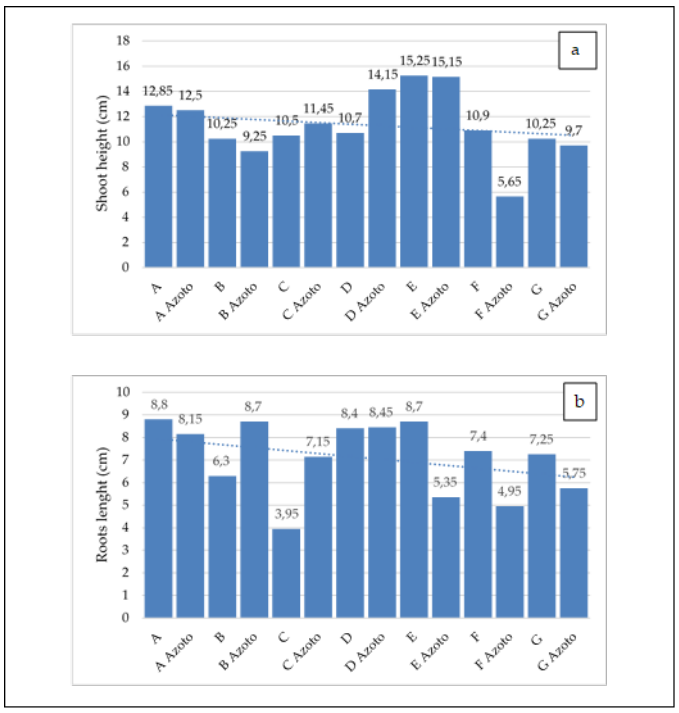

Fig 2.
Shoots height (a) and roots length (b) of eight legume seedlings following Azotobacter inoculation. A: Yellow cow pea, B: Black cow pea, C: Red cow pea, D: Red mung bean, E: Black mung bean, F: Green mung bean, G: White-eyed Black cow pea

The shoot height of black mung bean and roots length of red mung bean were remained constant after inoculation. The data in Fig 2 showed that the best effect of Azotobacter inoculation was only on the seedling growth of red cow pea which has higher shoot and longer roots after inoculation.

When Azotobacter are applying as seed inoculants, they multiply in the rhizosphere and take a part in nitrogen fixation, an important step of nutrient cycling-and benefit crop growth. Treating seedling roots of several plant species with cultures of Azotobacter chroococcm in this bioassay changed seedling growth and development. Biofertilizer Azotobacter enable to affect leaves and roots development by plant growth regulators, well known as phytohormone [10, 14]. Supernatant fluids of A. chroococcum contain cytokinins of kinetin and benzyladenine-9-glucoside group [8]. Auxin (Indole Acetic Acid), gibberellin-like substances and cytokinin-like substances were produced by Azotobacter [5].

A number of factors are controlling seed germination, including plant hormones, which are produced by both plant and soil bacteria; plant hormones will interact with plant genes to affect seed germination [11]. The phytohormone in *Azotobacter* cultures probably induced imparity hormone composition in seeding rhizosphere since geminated seeds naturally excrete indole acetic acid and gibberellin [11]. Based on this result, it was concluded that there is a negative effect of *Azotobacter* on germination, as well as it is not supposed. Despite of the richness of protein in the local bean of Tanimbar (Table 1), comprehensive study of characteristic of those local beans are very limited. Some intensive research should be done to investigate the cause of this harmful effect to ensure that nitrogen availability could be supplied by nitrogen fixing bacteria. This study should be including the rhizosphere effect and the trueness of local seed.

4 Conclusions

Based on in vitro laboratory experimental, grown local seed on paper filter inoculated with *Azotobacter* caused decreased of germination parameters. Germination percentage of all bean variety were 100% at day three but *Azotobacter* inoculation lowered that of two bean varieties. Each cultivar of local bean demonstrated different germination responses on Azotobacter inoculation. Most of varieties showed decrease in shoots height and roots lenghts, only one variety, red cow pea demonstrated positive response on inoculation. The study determined that negative effect of *Azotobacter* on growth of some local bean varieties was evidence.

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