Influence of mixture of *Arbuscular Mycorrhiza* fungi and rhizobia on growth and yield of soybean at tidal swamp land

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Abstract. Tidal swamp land is a marginal land which has the potential for enhancement of many agricultural crop growths and yields. The objective of this study was to investigate the effect of a bio-fertilizer containing *Arbuscular mycorrhizae* fungi and a *Bradyrhizobium yuanmingense* on productivity of soybean at the tidal swamp land. The field experiment was established in a completely randomized design with three treatments and three replications per treatments. The treatments include fertilization (P) consisting of a 100% of standard dose of NPK (P1) and the use of biological fertilizers combined with NPK; 100% of standard dose (P2) and 50% of the standard dose (P3) per hectare. The results showed that the yield of soybean treated with bio-fertilizer and combined with 50% standard dosages of NPK could increase production up to 44.7% compared to P1.

1. Introduction

Soybean (*Glycine max* (L.) Merril) is one of important legume crops in Indonesia and becomes strategic commodity due to the volume of demand and a large and diverse usage. Soybean demand increases rapidly as the rate of population increase with the average demand is about 3.1 million tons in 2017. Most of domestic demand was fulfilled by importing soybean from out of the country. The contribution of domestic soybean production is only 30% [1]. Limited area for soybean plant was due to competition with other crops, therefore it is important to find a suboptimal land to increase soybean production.

There are two main strategies to gain domestic soybean production, i.e intensification and extensification. Tidal swamp land is one type of suboptimal land that has high potential for agricultural development, in particular crops. About 9.9 million hectares out of about 20.1 million hectares of tidal swamp land in Indonesia is potential for land extensification [2]. The use of tidal swamp land for the development of crops is constrained by physical, chemical, and biological properties of the soil as a limiting factor for plant growth, such as low pH, high content of iron (Fe$^{2+}$), low phosphorus (P), shallow pyrite (FeS$_2$) layer, high salinity, excess water volumes, and low population of microorganism [3].

Large amounts of chemical fertilizers are generally used to improve growth and yield of crops (soybean). However, increasing costs of these fertilizers and environmental concerns related to their use have led to the development of alternative strategies. The use of beneficial soil microorganisms could reduce the amount of fertilizer input by increasing the efficiency of nutrient availability and plant growth promoting activities. One strategy to improve the availability of nutrients in tidal swamp land is bio-fertilizer application.

*Bradyrhizobium yuanmingense* is a group of nitrogen-fixing bacteria symbiotic with roots of legume and is effective in the formation of nodules in soybean roots. It has been reported that *B. yuanmingense*
can result in the maximum N accumulation (139 kg N/Ha) [4]. The use of *Bradyrhizobium* in tidal swamp lands with limestone treatment at South Sumatera could increase soybean production up 161% [5].

*Arbuscular mycorrhizae* are fungi colonize the interior of the root and form specialized structures for nutrient exchange with the host. Fungal hyphae extend from the root and explore the soil more efficiently. *Arbuscular mycorrhizae* can provide the plant with supplemental phosphorus (P), nitrogen (N) and micronutrients since the plant roots alone are not able to maximize the interception of nutrients. Specifically, in soybean, AM have been shown to improve the overall water status and nutrient of the plant [6].

The objective of this study was to investigate the effect of a bio-fertilizer containing *Arbuscular mycorrhizae* fungi and *Bradyrhizobium yuanmingense* combined with chemical fertilizer on productivity of soybean at tidal swamp land.

2. Materials and Methods

2.1. Source of microbes

Microbes used as active ingredients for research activity in the field are *Bradyrhizobium yuanmingense* B64 and *Arbuscular mychorrizae* fungi. Both of these microbes are culture collection of Laboratory of Plants Symbiotic Microbes, Research Center for Biotechnology-LIPI. These two cultures have been shown to increase the biomass weight and symbiotic effectiveness of soybean [7].

*Bradyrhizobium yuanmingense* B64 was isolated from nodules of soybean. The culture was maintained on Yeast Mannitol Agar (YMA). For production, *B. yuanmingense* B64 was grown in Yeast Mannitol Broth (YMB) medium with composition (per liter medium): 10 g Mannitol, 0.5 g K$_2$HPO$_4$, 0.5 g yeast extract 0.2 g MgSO$_4$. 7H$_2$O, 0.1g NaCl, 1 L distil water and pH was adjusted to 6.8 ± 0.2 at temperature 27°C. Furthermore, this bacterial culture was injected into 100 ml sterile plastic. For application purposes, inoculants population is at least $10^7$ cfu per ml.

The *Arbuscular mycorrhizal* inoculums were a mix of chopped root segments, spores and hyphae/mycelium biomass of *Scutellospores and Acaulospores* grown in pot culture for 3-months. Bio-fertilizer containing *Arbuscular mycorrhizal* inoculums were wrapped in 1 kg plastic packaging.

2.2. Source of soybean variety

Seeds of soybean variety Anjasmoro for this research were obtained from Jambi Assessment Institute for Agricultural Technology (BPTP Jambi). Soybean variety Anjasmoro is a potentially high-yielding (2.03-2.25 ton/ha), harvesting duration is 82.5-92.5 days, resistance to leaf rust is moderate and weight of 100 seeds is 14.8-15.3 g.

2.3. Experimental Design

The research was conducted at tidal swamp land in Simpang Village, Berbak Sub district, Tanjung Jabung Timur Regency, Jambi Province. The experiment was conducted by comparing soybean cultivation method by farmers using chemical fertilizers at the field with bio-fertilizer application. There was no treatment without fertilization in this experiment. The field experiment was established in a completely randomized design with three treatments and three replications per treatments. The size of each plot is 10 × 40m and planting distance is 40 × 15cm. The treatments include fertilization (P) consisting of a 100% of standard dose of NPK (P1) and the use of biological fertilizers combined with NPK; 100% of standard dose (P2) and 50% of the standard dose (P3) per hectare. The standard doses of NPK fertilizer for soybean planting are 50 kg Urea/ha, 75 kg SP-36/ha and 50 kg KCl/ha. NPK fertilizer was applied 1 time simultaneously at 3 days after planting. Two weeks before treatment, the soil was given dolomite of 300 kg/ha.

Soybean planting was done by mixing soybean seed with *B. yuanmingense* B64 and *Arbuscular mycorrhizae* inoculums until all the seeds were coated and then embedded 2–3 soybean seeds per hole. The dosage of fertilizer is 500 ml/ha *B. yuanmingense* B64 and 5 kg/ha of *Arbuscular mycorrhizae* inoculums.
2.4. Growth measurement

Before harvesting 16 plants per plot were measured for the height, number of branch, and pods. Number of root nodules was observed 45 days after planting. Dry grain yield (ton/ha) was obtained from calculation of 2 × 5 m tiled plot. The data were analysed with analysis of variance (ANOVA).

3. Result and Discussion

3.1. The chemical characteristic of soil

The average pH of soil at planting location was acid (4.8), and is below the range of optimum for soybean growth with medium [5.3] C-organic (Table 1) [8]. Exchangeable Al was high, above the level that can be tolerated by most soybean variety of 0.44-0.88 me/100g [9]. Aluminium saturation was high, above the critical threshold of 30%. Low availability of P might be related to low soil pH and high Al saturation [10]. Organic matter as indicated by organic-C content was medium.

| Parameters           | Value       | Notes     |
|----------------------|-------------|-----------|
| pH (H₂O)             | 4.8         | Acid      |
| C-organic            | 5.3         | Medium    |
| N (%)                | 0.17        | Low       |
| P available (ppm P2O5) | 19.8  | Low       |
| S available (ppm SO₄)  | 98.51       | High      |
| K-dd (me/100 g)      | 0.15        | Low       |
| Ca-dd (me/100 g)     | 2.47        | Low       |
| Mg-dd (me/100 g)     | 0.85        | Medium    |
| Al-dd (me/100 g)     | 2.18        | High      |
| H-dd (me/100 g)      | 0.5         | Low       |
| Fe (ppm)             | 311.4       | Very high |
| Mn (ppm)             | 13.64       | High      |
| KTKE (me/100 g)      | 6.34        | Low       |
| Kej-Al (%)           | 34.34       | High      |
| Kej-Na (%)           | 2.18        | -         |

The soil properties of planting area indicated unfavourable condition for soybean and become limiting factor for soybean growth and yield. The use of suitable varieties, appropriate combination and dosage of fertilizer for planting soybeans on tidal swamp land is expected to increase productivity and utilization of land for agriculture. Productivity on tidal swamp land is only about 0.8 tons ha⁻¹ in conventional cultivation of soybean production in the fields containing of pyrite. Management of tidal swamp land have generally remained as monoculture with the risk of crop failure due to plant pest, climate change and dynamics of prices. There are some ways to improve efficiency of land for sustainable agriculture such as intercropping planting pattern, water and nutrients, weeds, pest, and disease control [11].

In management of tidal land, the presence of adequate water could help reduction of pyrite oxidation that threatened the soybean growth. Pyrite oxidation is a chemical process in the soil layer that induced by excessive soil tillage, soil cracks or excessive drainage that can increase soil acidity and solubility of toxic compounds such as Al³⁺ and Fe²⁻ resulting in nutrient deficiency and also the decline of photosynthetic rate of cultivated plants [12].
Soybean var. Anjasmoro has high potency genetically. Production of pods per plant is determined by interaction between genetic and environment factors such as soil fertility, water availability and crop management [13]. Soybean var. Anjasmoro is able to adapt well with agro-ecological characteristic of tidal swamp land [14].

3.2. The growth and yield of soybean

Soybean plant showed positive response on bio-fertilizer inoculation. The inoculation treatment by *Arbuscular mycorrhizae* and *B. yuanmingense* B64 combined with 50% NPK (P3) showed similar response to plant height compared to 100% treatment (P1) (Table 2, Figure 1). Another treatment of bio-fertilizer inoculation combined with 100% chemical fertilizer (P2) showed the highest response on plant height. These results indicated that bio-fertilizer application affect significantly on the increase of height plant combined with 50 or 100% of chemical fertilizer.

### Table 2. The growth and yield of soybean at tidal swamp land

| Treatments | Height of plant (cm) | Number of branches/plants | Number of pods/plants | Number of nodules/plants | Yields (t/ha) |
|------------|----------------------|---------------------------|-----------------------|--------------------------|--------------|
| P1         | 54.57 ± 4.43a        | 2.3 ± 0.3a                | 50 ± 6.27a            | 42 ± 3.5a                | 1.23 ± 0.15a |
| P2         | 75 ± 3.5b            | 3.71 ± 0.47b              | 114 ± 6.32c           | 74 ± 4.16b               | 1.89 ± 0.11b |
| P3         | 57 ± 3.1a            | 3.57 ± 0.4b               | 70 ± 3.92b            | 72 ± 4.69b               | 1.78 ± 0.08b |

The values are expressed as mean ± SE. Mean values in each column by different letters are significantly different by LSD test.

![Figure 1. The height of plant, number of pods and nodules of soybean at tidal swamp land](image)

Inoculation by mixtures *Arbuscular mycorrhizae* and *B. yuanmingense* B64 combined with NPK (P2 and P3) affects significantly on number of branches, pods and roots nodules (Table 2). Both treatments gave higher response than 100% chemical treatment (P1). The highest response was shown by treatment bio-fertilizer combined 100% NPK on number of pods. In this study, inoculation treatment reached the average number of 72 nodules per plant. A total of 16 plants were measured per plot. This result was lower than using of *Bradyrhizobium japonicum* that can obtain nodules up to 104 per plant [15].
**Rhizobium** on soybean plants helps formation of nodules. The root nodules increase supply of N for plants in the process of growing roots, stem and leaves [16]. Application of bio-fertilizer combined NPK also influenced grain yields. Grain yields of P3 treatment (bio-fertilizer + 50% NPK) was almost equal with P2 treatment (bio-fertilizer + 100% NPK) (Figure 2). P3 treatment could increase production 44.72%, while P2 treatment increased 53.66% compared with P1. However, a combination of inoculant and ½ chemical fertilizer gave the best grain yield on soybean [17].

![Figure 2. The number of branches and yields of soybean at tidal swamp land](image)

Soybean plant treated by P2 (bio-fertilizer + NPK 100%) was higher than the other treatments. This indicates that growth of soybean especially plant height influenced by nutrients availability in the soil. However, grain yield by P3 treatment was able to compensate with P2 treatment, even higher than P1 treatment. This indicates that utilization of bio-fertilizer combined with NPK 50% of standard dosage is proven effective to reduce NPK fertilizer usage, since the production of soybean obtained almost the same with 100% fertilizer of standard dosage.

The use of bio-fertilizer with active ingredient *Bradyrhizobium yuanmingense* B64 and *Arbuscular mycorrhizae* can improve the efficiency of NPK fertilizer usage. Application of bio-fertilizer combined with NPK 50% of standard dosage is able to support growth and yield (1.78 t/ha) of soybean var. Anjasmoro at tidal swamp land. The other study stated that soybean treated with bio-fertilizer consist of *Bradyrhizobium* and *Arbuscular mycorrhizae* could produce up 4.22 t/ha [18].

This study showed that dual inoculation with *Rhizobium* and mycorrhizae gave synergistic effects on soybean growth which was consistent with the result of [19]. *Rhizobium* symbiosis is involved in the fixation of atmospheric N, whereas mycorrhizae improves the ability of a plant to absorb P and other nutrients. *Rhizobium* fixing nitrogen occurs when atmospheric nitrogen is converted to ammonia by enzyme nitrogenase and microbial genes are required to fixate nitrogen to be widely distributed into the environment [20]. The interaction between root nodules and symbiotic bacteria have been studied through proteomics during the signal exchange and symbiotic growth [21].

Mycorrhized plant demonstrably have higher access to soil nutrients, mainly P. In case of soil elements limitation, it is probable that more associations between plants and mycorrhizal fungi are established, attempting to increase the volume of root exploration and to have more access to nutrients. The root colonization by mycorrhizal fungi can act simultaneously with the process of biological fixation.
of nitrogen on soybean. The soybean production and mycorrhizal dependence are higher in presence of *Rhizobium* [22].

Several studies have reported a significant increase in soybean growth parameters and yield due to the inoculation of Bradyrhizobial isolates [4]. Appunu [23] stated that N₂ fixation by *B. yuanmingense* is one way to achieve N fertilizer efficiency for soybean crops. The other symbiotic microorganism has been successfully used in soybean are *Bradyrhizobium*, genera symbiotic N-fixing bacteria and mycorrhizae inoculant [17]. A successful *Rhizobium*-legume symbiosis and mycorrhizae inoculation can be determined by many factors such as species compatibility, habitat niche availability and competition with other microbes.

These results suggest that soybean growth and yield by inoculation *B. yuanmingense* and mycorrhizae was efficient and low-cost in terms of nitrogen fixation and phosphate solubilizing. These are important criteria for isolate selection as bio-fertilizer agent. The use of chemical fertilizers can be significantly reduced which would contribute to economical, environmentally friendly and sustainable agricultural practices, and also increased productivity of agriculture at tidal swamp land. The potential yield of soybean var Anjasmoro on tidal swamp land can compete with production from non-marginal soil. Production of soybean var. Anjasmoro on tidal swamp land reached 1.2–1.8 t ha⁻¹ while on irrigated and rainfed rice fields reached 1.71–1.81 and 1.2–1.3 t ha⁻¹ [24].

4. Conclusion

The use of bio-fertilizer with active ingredients *Arbuscular mycorrhizae* fungi and *Bradyrhizobium yuanmingense* B64 was effective to reduce chemical fertilizer up to 50% of the standard dose commonly applied to soybean cultivation by farmers. Soybean var. Anjasmoro treated with this bio-fertilizer and combined with 50% of standard dosages of chemical fertilizer could increase production up to 44.7% compared to farmer’s method.

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