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Isolation, trapping, and application of arbuscular mycorrhizal fungi to increase of phosphorus efficiency, growth, and productivity of soybean under saturated soil culture on acid sulphate soil

Munif Ghulamahdi[1*], Maya Melati[1], Purwono[1], Irdika Mansur[1], Ridwan Muis[1], A. Haitami[2], Tatsuhiko Shiraiwa[3], Paul Taylor[4]

[1] Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia
[2] Departement of Agrotechology, Universitas Islam Kuantan Singingi, Riau, Indonesia
[3] Graduate School of Agriculture, Laboratory of Crop Science, Kyoto University, Japan
[4] Faculty of Science, University of Melbourne, Australia
* Corresponding author's e-mail: mghulamahdi@yahoo.com

ABSTRACT

This study investigated the effects of arbuscular mycorrhizal fungi (AMF) inoculation and varying phosphorus (P) doses on two soybean cultivars (Tanggamus and Anjasmoro) under saturated soil culture and dry culture conditions in acid sulfate tidal soils. The experiment, conducted in Jambi Province, Indonesia, from May to August 2019, employed a split-split plot design with three factors: AMF inoculation, soybean variety, and phosphorus fertilizer application rates (0, 36, 72, and 108 kg P_2O_5 ha^{-1}). Results showed that AMF inoculation of the Tanggamus variety at 36 kg P_2O_5 ha^{-1} improved soil available phosphorus, plant P uptake and content, P uptake efficiency, inoculant relative efficiency, stem diameter, and branch count. This combination also enhanced stover dry weight, accelerated flowering, and harvest age, reduced empty pod count, and improved P use efficiency. The Tanggamus variety at 36 kg P_2O_5 ha^{-1} demonstrated improvements in growth parameters and P-related efficiencies. AMF inoculation at 36 kg P_2O_5 ha^{-1}positively impacted multiple soil and plant characteristics, including available P, P uptake and content, potassium uptake, and various efficiency measures. It also improved yield components such as pod count, 100-seed weight, and seed weight per plot and hectare. Notably, soybean yield in saturated soil culture surpassed that in dry culture, suggesting the potential of this method for addressing challenges in acid-sulfate tidal soils, particularly those with limited P availability and pyrite layers.

Keywords: tidal land, cultivation, suboptimal land, pyrite.

INTRODUCTION

The harvested area of soybeans has been shrinking, from 660,823 hectares in 2010 to 550,793 hectares in 2013. In 2015, the harvested area of soybeans increased, reaching 624,848 hectares. The productivity level has been rising since 2010, and in 2015 it reached 15.73 quintals per hectare, with national soybean production reaching 982,967 tons. The national soybean demand in 2015 was estimated to reach 2.5 million tons. Most of this demand would be met by imports. The import volume up to 2013 reached 1.8 million tons (Badan Pusat Statistik, 2015). Soybean productivity increased by 29.5%, but during the period from 1992 to 2013, there was a 66.7% decrease in the harvested area. As a result, domestic production was only able to meet 30% of the national demand. Therefore, efforts to expand the planted area are necessary. A highly feasible alternative is to expand the planted area into tidal swamp lands. (Ghulamahdi, 2016). Tidal swamp ecosystems face a significant challenge in the limited availability of macronutrients, particularly N, P, and K. This phenomenon occurs despite the high total nutrient content, especially phosphorus. A case study conducted in type B tidal lands of Banyuasin Regency, South Sumatra, revealed that while the total phosphorus content reached 106.2 ppm, only 13.1 ppm was plant-available (Sefrila et al., 2022). This situation illustrates the complexity of soil fertility in tidal swamp ecosystems, where high nutrient content does not necessarily translate to adequate nutrient availability for plant growth.

Tidal swamp lands have limitations such as nutrient deficiency, low pH, the presence of pyrite layers, and so on, but they possess potential resources necessary for soybean growth and production, such as abundant sunlight and water. The limitations of marginal lands like these tidal swamps must be anticipated with environmentally friendly farming systems. Their implementation in the field will improve the health of the agroecosystem, including biodiversity, biological cycles, and soil biological activity (Sutanto, 2015). The use of AMF is one of the cultivation techniques that can serve as an alternative to increase soybean production.

The mechanism of the relationship between AMF and plant roots is as follows: AMF spores germinate and subsequently infect the plant roots, then grow and develop to form long, branching hyphae. This hyphal network has a much wider reach than the plant's root system itself. These AMF hyphae, with their more extensive range, then function as an extension of the plant roots in absorbing water and nutrients from the soil.

AMF (Arbuscular Mycorrhizal Fungi) has a wide range of habitats, and therefore, it is naturally available in various soil types and diverse growth environments. The use of local AMF is intended to accelerate environmental adaptation, thus maximizing its benefits to soybeans. Utilizing AMF from the soybean rhizosphere is meant to facilitate easier adaptation with its host when the AMF isolates are returned to the cultivation field after propagation. The use of local AMF can also be done easily and at a low cost, making it feasible for implementation at the farmer level to increase soybean productivity. Another expected impact is reduced dependence on inorganic fertilizers.

The availability of phosphorus (P) as a plant nutrient influences the percentage of AMF colonization. A slight addition of P will increase colonization. Conversely, adding P in high concentrations will reduce AMF colonization (Anggraini et al., 2022). The use of phosphate in acidic soils can increase the degree of AMF infection, improve soil fertility, and enhance crop yields (Lone et al., 2017). The application of phosphorus (P) fertilizer is crucial in increasing production. Phosphorus is an essential nutrient that is closely related to the quality of soybean seeds. The provision of P nutrients can stimulate generative growth, thereby increasing seed yield per unit area and enhancing the quality of soybean seeds. Rasyid (2012) states that P nutrients are stored predominantly in seeds and determine the vigor of soybean seeds. With good seed vigor, the potential for survival increases significantly.

Each variety is assumed to have a different response to P fertilization and ability to form symbiosis with AMF. Therefore, two soybean varieties were used: Anjasmoro and Tanggamus. The selection of the Anjasmoro variety is due to its widespread adoption among farmers. Farmers prefer the Anjasmoro variety because it is classified as a large-seeded soybean. The Tanggamus variety was chosen because it has a high adaptability to cultivation in tidal swamp areas. This has been demonstrated by several studies conducted by previous researchers, showing a relatively high productivity level compared to other varieties.

Most mineral soils in tidal swamp areas are characterized as acid sulfate soils. The pyrite content in acid sulfate soils varies and tends to increase in deeper layers. Pyrite becomes hazardous to plants when oxidized, causing a rapid decrease in pH. The oxidation of sulfidic materials containing pyrite layers in the soil begins with water deficiency in the soil, causing the groundwater level to drop below the position of the pyrite layer. This condition leads to a reaction between pyrite compounds (FeS_2) and oxygen (O_2), producing elements and compounds that are toxic to plants (Hadi, 2023).

Soybean cultivation in tidal swamp areas can be carried out using water-saturated cultivation. Shaturated soil culture (SSC) is a planting method on raised beds with continuous water supply in the trenches, resulting in water-saturated soil beneath the root zone without flooding (Du et al., 2021). SSC can also be applied in areas with adequate irrigation or in planting areas with poor drainage (Ghulamahdi, 2009).

Soybean cultivation in wetlands can increase yields by 20–80% (Indradewa et al., 2004). Broadly, Cho (2013) concludes that soybeans cultivated with flooded trenches show faster growth and higher yields compared to those cultivated with flood irrigation as practiced by farmers. This is due to: (1) receiving adequate moisture throughout their life cycle, (2) continued nodule growth until the pod-filling stage, and (3) experiencing delayed senescence and an extended reproductive phase.

Maintaining a constant water level will eliminate the negative effects of excess water on plant growth, as soybeans will acclimatize and subsequently improve their growth (Ghulamahdi et al., 2006). Plant growth increases after passing through the acclimatization period. This growth enhancement is closely related to increased nodulation and significant N_2 fixation (Ghulamahdi et al., 2006). This research aims to increase soybean productivity by improving P fertilization efficiency through the use of local AMF isolates in SSC.

MATERIAL AND METHODS

The experiment was conducted in Simpang Village, Sub District Berbak, Tanjung Jabung Timur District, Jambi Province. The experiment used a Split-Split Plot Design in a Randomized Block Design with three factors. The first factor as the main plot was inoculant, consisting of without inoculation and with inoculation. The second factor as a subplot was soybean varieties, consisting of the Tanggamus variety and the Anjasmoro variety. The third factor as sub-subplots was the phosphorus fertilizer dose, consisting of 0, 36, 72, and 108 kg P_2O_5 ha^{-1}. This experiment had 16 treatments, each repeated three times.

The soil for the cultivation of AMF was taken from the rhizosphere of soybean plants at a depth of 0–20 cm and a diameter of 20 cm, including the soybean plants growing on it. The soil for AMF propagation was taken from the rhizosphere of 20 soybean plants, with each rhizosphere sample placed in a plastic bag. The soybean variety used for cultivation was Anjasmoro. The soil samples for nutrient analysis were composite samples from 10 collection points, each weighing 500 g, taken from two depths, 0–30 cm and 30–60 cm, and placed in plastic bags.

The spore isolation was performed using 25 g of soil sample. The isolation of AMF spores from the soil samples was carried out using the wet sieving method (Finlay, 2004), followed by centrifugation based on the method by Brundrett et al. (1996). The centrifugation process produces a layer in the middle of the tube, between the water and glucose layers, which contains particles of AMF spores. This layer is then collected and poured into a set of sieves (425, 300, 150, and 53 µm) and washed with running water to remove the glucose. The remaining sediment in the sieves was poured into a Petri dish and observed under a microscope for spore counting. The identification of AMF types was based on the criteria proposed by Schenck and Perez (1990).

The trapping technique followed the method of Brundrett et al. (1996) using small culture pots. The planting medium used was a mixture of approximately 50 g of soil samples and 150 g of 1–2 mm zeolite particles. The method of filling the culture pots was as follows: the pots were first filled with 100 g of zeolite, then 50 g of soil from tidal land was added, and finally, 50 g of zeolite was used to cover the soil, forming a layer of zeolite-soil-zeolite. *Pueraria japonica* seeds, sorghum seeds, maize seeds, and soybean seeds, which were to be used as host plants, were first soaked in a Bayclin solution for 5–10 minutes to sterilize the surface. The plant inoculant used in the field is the AMF inoculant from corn plants because it makes AMF grow faster and has more roots.

The seeds were soaked in warm water (40 °C) for approximately 24 hours to break any possible dormancy. Afterward, the seeds were sown in a nursery tray for about 10 days. The seedlings were then directly transplanted into the culture pots. The maintenance of the cultures included watering, nutrient application, and manual pest control. The nutrient solution used was a compound fertilizer (25-5-20) with 1 g/2 L of water (0.05%) concentration. The nutrient solution was applied twice weekly, with approximately 20 mL for each culture pot. After 90 days, the cultures were harvested to obtain spores that would be used in the next stage of the experiment.

Soil amelioration was done by applying manure two weeks before planting. The plots that had been prepared were fertilized with cow manure at a rate of 1 ton per hectare. The seeds were planted using a dibble method at 2–3 cm depth. Each hole was filled with 3 seeds, spaced 40×12.5 cm apart, with 2 plants maintained per planting hole. Thinning was done 2 weeks after planting. The planting holes were covered with 5 grams of Arbuscular Mycorrhizal Fungi inoculant per hole. The treatments were placed in experimental plots measuring 2×3 m.

Phosphorus fertilizer was applied according to the treatment doses, along with 100 kg of KCl per hectare, which was given as a basal fertilizer at planting in furrows beside the plant rows. Manure, SP-36, and KCl were mixed and incubated for 1 week. Urea fertilization was carried out 4 times, at 3, 4, 5, and 6 weeks after planting (WAP), by spraying the leaves with a concentration of 7.5 g of urea per liter of water, using a spray volume of 400 liters of water per hectare.

RESULTS AND DISCUSSION

Isolation

The results of the soil analysis before the research were conducted on the land to be planted with soybeans in Simpang Village, Berbak District, Tanjung Jabung Timur Regency, Jambi Province (Table 1). Several indicators are necessary to relate the results of the soil analysis to the presence of AMF, including the fractions that make up soil texture, cation exchange capacity (CEC), carbon (C), nitrogen (N), phosphorus (P), and pH. This is as explained by Fraser (2009), who stated that the distribution of mycorrhizae is influenced by many factors, including soil type and structure, phosphorus and nitrogen nutrients in the soil, moisture, pH, and soil temperature. After characterization and identification using the Schenck and Perez (1990) method, two species of AMF from the genus Glomus were obtained: *Glomus fasciculatum* and *Glomus fecundisporum*, based on the main characteristics found in the AMF spores (Table 2). The images of the spores are presented in Figure 1.

The presence of AMF is important for the resilience of an ecosystem. AMF enhances plant stability and diversity, as well as improves crop productivity. Therefore, the availability of inoculants in good quantity and quality is a key factor in the broader use of AMF. The first step taken is the characterization and identification of AMF from soil samples, especially to obtain local AMF.

In dry culture (DC) condition, varieties have relatively the same response as SSC to soil P availability with treatment with P doses and treatment without and giving AMF inoculant, but the P soil availability on SSC was higher than DC. The soil P availability on SSC between 16.23–49.32 ppm and on DC only 6.02–24.25 ppm. The highest

Table 1. Example of full column size table

Variables	Depth			
	0–30 cm	Criteria	30–60 cm	Criteria
pH 1:1 H_2O	4.70	Acid	4.80	Acid
pH 1:1 KCl	4.00	Very acid	4.00	Very acid
C-org (%)	3.59	High	1.83	Low
N-Total (%)	0.33	Moderate	0.17	Low
P Bray I (ppm)	16.80	Moderate	7.80	Verylow
P HCl 25% (ppm)	164.50	Very high	73.50	Very high
Ca (me/100g)	6.61	Moderate	2.39	Low
Mg (me/100g)	4.22	High	2.73	High
K (me/100g)	0.46	-	0.14	-
Na (me/100g)	0.47	-	0.22	-
CEC (me/100g)	33.62	High	19.21	Moderate
Saturated Base (%)	34.98	Moderate	28.53	Low
Al-exc (me/100g)	0.90	Low	2.34	Moderate
H (me/100g)	0.12	-	0.30	-
Fe (ppm)	25.74	High	34.65	High
Cu (ppm)	3.66	Low	3.92	Low
Zn (ppm)	4.70	Moderate	3.43	Moderate
Mn (ppm)	19.74	Moderate	23.50	High
Sand (%)	0.73		0.54	
Dust (%)	58.94		46.47	
Clay (%)	40.33		52.99	

Note: *As per the standards established by Balittanah (2005).

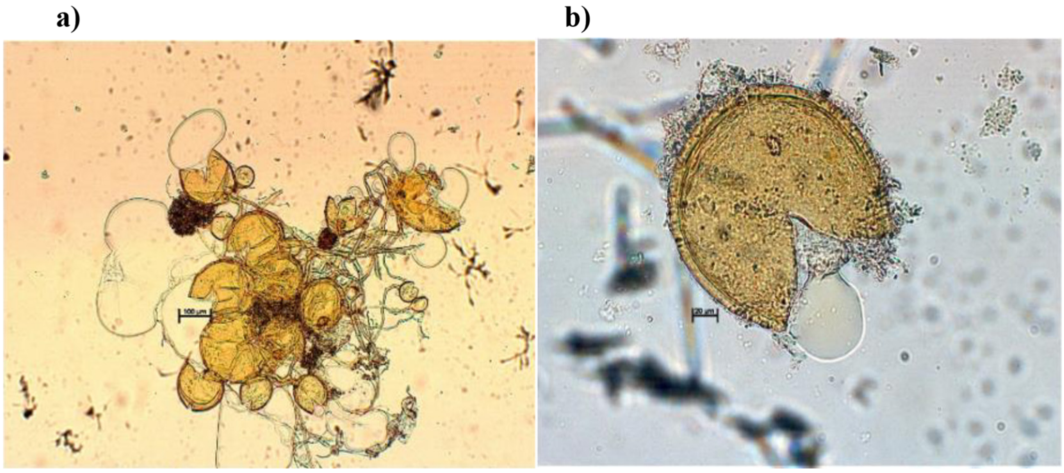

Figure 1. Species FMA: a) *Glomus fasciculatum*, b) *Glomus fecundisporum*

Table 2. The dynamics of sporulation of AMF at 70 DAP, 80 DAP, and 90 DAP in AMF trapping using different host plants

Host plant types	Number of AMF spores on the day after planting (DAP)		
	70	80	90
Zea mays	149	113.33	203.33
Pueraria javanica	7	9	2
Sorghum bicolor	21	55	168.67
Glycine max (Tanggamus)	5	4	0.33
Glycine max (Anjasmoro)	6	29.00	0.33
Glycine max (Selamet)	2	6.67	1.33
Glycine max (Pangrango Godek)	3	5.67	0.33
Glycine max (Sibayak Pangrango)	0	1.67	1
Glycine max (Wilis)	6	2.67	0.33

Table 3. Soil P availability on interaction of AMF inoculation, variety, and P under saturated soil culture

AMF Inoculation	Variety	P (kg P_2O_5 ha$^{-1)}$			
		0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
		(ppm)			
Without	Tanggamus	17.43 g	24.81 f	27.79 e	32.48 ef
	Anjasmoro	16.24 g	16.56 g	26.08 ef	26.25 ef
Inoculation	Tanggamus	24.17 f	49.33 a	30.37 d	26.55 ef
	Anjasmoro	16.81 g	36.04 b	33.03 c	25.67 ef

Note: numbers accompanied by the identical letter are not substantially different according to the Duncan Multiple Range Test with α = 5%.

soil P availability was obtained on the treatment SSC on Tanggamus variety with 36 kg kg P_2O_5 ha^{-1} dosage and with AMF inoculant than the other treatments. Soil P availability on DC only 49.17 % SSC in the highest P availability (Table 3 and 4).

P nutrient uptake

AMF inoculation on the Tanggamus variety fertilized with 36 kg P_2O_5 ha^{-1} resulted in P uptake that was 0.117 g/plant higher and significantly different compared to no AMF inoculation on the Anjasmoro variety fertilized with 0 (control)

Table 4. Soil P availability on interaction of AMF inoculation, variety, and P under dry culture

AMF Inoculation	Variety	P (kg P_2O_5 ha^{-1})			
		0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
		(ppm)			
Without	Tanggamus	6.58 ij	7.03 i	13.49 e	13.85 e
	Anjasmoro	6.03 j	6.44 ij	11.59 g	12.53 f
Inoculation	Tanggamus	9.08 h	24.26 a	17.65 c	17.07 c
	Anjasmoro	8.33 h	22.01 b	17.36 c	15.88 d

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

P_2O_5 ha^{-1}, and significantly different from other treatments. Without AMF inoculation, the Anjasmoro variety shows a more responsive reaction compared to the Tanggamus variety in terms of plant P uptake. The Anjasmoro variety will provide more significant plant uptake variable values compared to the Tanggamus variety. However, when inoculated with AMF, the Tanggamus variety responds better than the Anjasmoro variety in terms of plant P uptake. Quantitatively, the P uptake of the Tanggamus variety is superior to that of the Anjasmoro variety (Table 5).

AMF inoculation on the Tanggamus variety fertilized with 36 kg P_2O_5 ha^{-1} resulted in a P content that was 0.254% higher and significantly different compared to no AMF inoculation on the Anjasmoro variety fertilized with 0 kg P_2O_5 ha^{-1}, and significantly different from other treatments. The fluctuation in P content of the Anjasmoro soybean variety is more pronounced compared to the Tanggamus variety across various P doses when not inoculated with AMF.

AMF inoculation with a P dose of 36 kg P_2O_5 ha^{-1} on both Anjasmoro and Tanggamus varieties increases available soil P, consequently enhancing P uptake by the plants. The increase in P uptake by the plants elevates the P content in plant tissues, thereby improving the relative efficiency of P nutrient uptake and the relative efficiency of the inoculant (Table 6).

Table 5. P uptake by soybean plants after AMF inoculation, several soybean varieties, and various P doses

AMF Inoculation	Variety	Dosage P (kg P_2O_5 ha^{-1})			
		0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
		(g plant $^{-1}$)			
Without	Tanggamus	0.088 def	0.098 cd	0.102 c	0.098 cd
	Anjasmoro	0.051 h	0.059 gh	0.081 ef	0.065 g
Inoculation	Tanggamus	0.091 de	0.156 a	0.128 b	0.118 b
	Anjasmoro	0.078 f	0.094 ef	0.086 def	0.084 ef

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Table 6. P levels of soybean plants after AMF inoculation, several soybean varieties, and various P doses

AMF Inoculation	Variety	Dosage P (kg P_2O_5 ha^{-1})			
		0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
		(%)			
Without	Tanggamus	0.324 cde	0.324 cde	0.328 cd	0.322 cdef
	Anjasmoro	0.185 i	0.251 h	0.311 def	0.275 g
Inoculation	Tanggamus	0.328 cd	0.438 a	0.366 b	0.344 c
	Anjasmoro	0.296 f	0.316 def	0.314 def	0.301 ef

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Efficiency of P usage

AMF inoculation on the Tanggamus variety fertilized with 36 kg P_2O_5 ha^{-1} increased available soil P, consequently enhancing P uptake by the plant. AMF inoculation on the Tanggamus variety fertilized with 36 kg P_2O_5 ha^{-1} also increased the plant tissue P content, thereby improving the relative efficiency of P nutrient uptake and inoculant relative efficiency. P absorbed by plants in the form of inorganic ions quickly transforms into organic phosphorus compounds. This P is mobile or easily translocated between plant tissues. The optimal P content in plants during vegetative growth is 0.3–0.5% of the plant's dry weight. P is one of the macronutrients required by plants, playing a crucial role in various life processes such as photosynthesis, carbohydrate metabolism, and energy flow within the plant (Khan et al., 2017). AMF inoculation on the Tanggamus variety fertilized with 36 kg P_2O_5 ha^{-1} resulted in a relative efficiency of P nutrient uptake of 174.82%, which was significantly different from other treatments (Table 7).

The availability of phosphorus for plants heavily depends on the concentration of primary orthophosphate (H2PO4-) and secondary orthophosphate ($HPO4^{2-}$) ions. The absorption of these ions is influenced by soil pH (Tian et al., 2021). According to Petzoldt (2020), at low pH levels, the absorption of primary orthophosphate ions is more dominant compared to secondary orthophosphate ions. P is a macronutrient required in large quantities. The amount of P in plants is smaller compared to Nitrogen and Potassium. P plays a role in the formation of fats and albumin, as a component of nucleic acids, phospholipids, NAD and NADP coenzymes, and ATP. It also counteracts the adverse effects of nitrogen, promotes the development of fine roots and root hairs, and enhances disease resistance. Low P levels in plants result in P deficiency, which reduces protein synthesis, as P is an energy source for converting assimilates into nucleoproteins. This deficiency leads to sugar accumulation in the plant's vegetative parts, promoting anthocyanin formation and causing leaves to turn dark green. Older leaves turn dark brown when they fall (Landi, 2015).

AMF inoculation enhances soybean root colonization, with 48.75% more colonization observed compared to non-inoculated plants, a statistically significant difference. This increased root colonization leads to higher dry biomass weight in soybeans. Plants inoculated with AMF show a dry biomass weight that is 3.96 g/plant higher, which is significantly different from non-inoculated plants (Table 8). This outcome is logical, as AMF assists host plants in absorbing essential nutrients for photosynthesis, while the plant, in turn, provides photosynthates necessary for AMF survival. This symbiotic relationship greatly benefits plant growth, particularly evident in the increased dry biomass weight of soybean plants.

A phosphorus dose of 72 kg P_2O_5 ha^{-1} resulted in a higher dry weight of plant biomass, which was 3.38 g/plant heavier and significantly different

Table 7. Efficiency of P fertilizer use in AMF inoculation, several soybean varieties, and various P doses

AMF Inoculation	Variety	Dosage P (kg P_2O_5 ha-1)		
		0 (Control)	36 (P_2O_5)	72 (P_2O_5)
		(%)		
Without	Tanggamus	107.21 e	119.27 d	107.79 e
	Anjasmoro	63.72 h	88.53 g	69.27 h
Inoculation	Tanggamus	174.83 a	141.12 b	130.75 c
	Anjasmoro	103.34 ef	96.68 f	88.53 g

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Table 8. Effect of AMF inoculation on root colonization and dry weight of soybean stalks

FMA Inoculation	Root colonization (%)	Dry weight of the stover (g/plant)
Inoculation	63.76 a	30.89 a
Without Inoculation	15.01 b	26.93 b

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

from the 0 kg P_2O_5 ha^{-1} dose (Table 9). The 36 kg P_2O_5 ha^{-1} dose increased soybean stem diameter. Therefore, for soybean growth, the single application of 72 kg P_2O_5 ha^{-1} is the phosphorus dose that provides the best effect on soybean plants. This aligns with several studies stating that phosphorus dose affects plant growth parameters such as plant height and productive branches (Said and Rale, 2020), dry weight of biomass (Rosi et al., 2020), plant phosphorus content, plant nitrogen content, plant dry weight, and dry weight of root nodules (Zainudin et al., 2019).

Pandey et al. (2013) state that plants deficient in phosphorus exhibit stunted growth due to cell division failure, resulting in inhibited growth and low-quality yields. Malhotra et al. (2018) assert that phosphorus deficiency in young plants leads to stunted growth. Phosphorus is mobile within plants, so in cases of deficiency, it is translocated from older leaves to younger ones. This results in impeded growth, and plants are unable to produce optimally. The phosphorus content in plants ranges from 0.1–0.5%, which is lower than nitrogen and potassium levels. Marschner (2012) indicates that the phosphorus requirement for optimal plant growth is between 0.3–0.5% of the plant's dry weight during vegetative growth. At concentrations higher than 1% in dry matter, plants are likely to experience toxicity.

Loudari et al. (2022) state that the amount of phosphorus absorbed by plants is influenced by their morphological, anatomical, and physiological characteristics. Furthermore, soil structure significantly affects growth in the process of nutrient uptake from the soil. Phosphorus can be considered the key to life. Its presence in plants serves both as an energy reserve function and as a constituent of important compounds. Thus, Turuko (2014) states that phosphorus fertilization can improve vegetative growth and increase plant dry weight. Additionally, Saputra et al. (2019) assert that phosphorus fertilization can also enhance generative growth, particularly in the formation of flowers, fruits, and seeds. In leguminous plants, phosphorus has a specific function in the symbiotic process between rhizobium bacteria and the plant, thereby increasing nitrogen fixation by rhizobium bacteria. Adeyemi et al. (2021) report that phosphorus fertilization can increase the number, size, and dry weight of soybean root nodules, as well as plant growth and yield. Consequently, the adequacy of phosphorus in plants significantly influences plant performance.

Table 9. Effect of P dose on dry weight of soybean stover

Dosage P (kg P_2O_5 ha^{-1})	Dry weight of the plant (g $plant^{-1}$)
0	27.06 d
36	29.47 b
72	30.44 a
108	28.67 c

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Soybeans inoculated with AMF respond to increased fertilizer doses by increasing the number of filled pods up to a dose of 36 kg P_2O_5 ha^{-1}, while those not inoculated with AMF continue to increase up to a dose of 72 kg P_2O_5 ha^{-1}. The increase in the number of pods in AMF-inoculated soybeans is more significant compared to non-inoculated ones as the phosphorus dose increases. Quantitatively, AMF-inoculated soybeans will produce more pods with a relatively lower fertilizer application (Table 10).

The Anjasmoro soybean variety shows a better response to AMF inoculation in terms of the number of empty pods compared to the Tanggamus variety. When inoculated with AMF, the Anjasmoro variety is able to reduce the number of empty pods it produces. Generally, the Anjasmoro soybean variety has a higher number of empty pods compared to the Tanggamus variety. The application of AMF inoculation is capable of reducing the number of empty pods in soybeans (Table 11).

According to Cho et al. (2023), one of the functions of phosphorus in plants is its role in

Table 10. Number of filled pods in the interaction of AMF inoculation with P dose

FMA Inoculation	Dosage P (kg P_2O_5 ha^{-1})			
	0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
Without inoculation	66.01 c	71.01 bc	76.84 bc	75.52 bc
Inoculation	76.52 bc	90.03 a	81.01 ab	80.04 ab

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Table 11. Number of empty pods in the interaction of AMF inoculation with soybean varieties

FMA Inoculation	Soybean variety	
	Tanggamus	Anjasmoro
Without inoculation	2.42 b	4.03 a
Inoculation	1.69 b	2.44 b

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

pod filling, which prevents empty pods and can increase soybean seed production. Similarly, Egli (2005) states that the number of filled pods increases as the total number of pods on soybean plants increases.

Soybeans inoculated with AMF increase their 100-seed weight up to a phosphorus dose of 36 kg P_2O_5 ha^{-1}, while soybeans not inoculated with AMF continue to increase their 100-seed weight up to a dose of 72 kg P_2O_5 ha^{-1} (Table 12). Therefore, it is evident that using AMF requires a smaller increase in phosphorus dosage. According to Khofiyya (2022), AMF can create favorable root conditions to achieve good growth. Bagyaraj et al., (2015) state that mycorrhizal roots can enhance nutrient uptake, especially phosphorus. AMF can improve plant nutrition, including phosphorus uptake, through infected root systems. This condition can enhance plant growth, ultimately increasing the number of filled pods, reducing empty pods, increasing seed weight, and simultaneously improving soybean production per hectare.

Soybeans inoculated with AMF were more responsive to increasing P doses up to 36 kg P_2O_5 ha^{-1}, but increasing doses did not show an apparent effect on increasing seed weight per plot. A dose of 36 kg P_2O_5 ha^{-1} on soybeans inoculated with AMF increased yield per plot compared to soybeans given the same dose but not inoculated with AMF (Table 13).

The Anjasmoro variety has a weight of 100 seeds that is 5.03 g heavier and significantly different compared to the Tanggamus variety. The Tanggamus variety has a weight of 139.37 g heavier tile seeds and is significantly different compared to the Anjasmoro variety. The Tanggamus variety has a production per ha of 0.58 tons more and is significantly different compared to the Anjasmoro variety. In these three variables, the Tanggamus variety has better values compared to the Anjasmoro variety (Table 14).

According to Jhan et al. (2011), the difference in growth potential among varieties is determined by their genetic factors. Dumas et al. (2003) explained that the performance of a plant in a given growth environment is the result of cooperation between genetic and environmental factors. The performance of a genotype in the same

Table 12. Weight of 100 soybean seeds in the interaction of AMF inoculation with P dose

FMA Inoculation	Dose P (kg P_2O_5 ha^{-1})			
	0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
	(g)			
Without inoculation	12.91 e	13.06 cde	13.27 abc	13.19 bcd
Inoculation	13.42 ab	13.51 a	13.29 abc	13.01 de

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Table 13. Soybean seed weight per plot in the interaction of AMF inoculation with P dose

FMA Inoculation	Dose P (kg P_2O_5 ha^{-1})			
	0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
	(g/2.4 m^2)			
Without inoculation	582.01 b	635.02 b	687.01 ab	671.51 b
Inoculation	683.52 ab	823.53 a	728.51 ab	717.54 ab

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Table 14. Effect of soybean varieties on the weight of 100 seeds, seed weight per plot, and seed weight per hectare of soybeans

Soybean variety	Weight 100 seeds (g)	Seed weight/2.4 m^2 (g)	Productivity (tons ha^{-1})
Tanggamus	10.69 b	760.76 a	3.18 a
Anjasmoro	15.72 a	621.39 b	2.57 b

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

environment can also differ, so understanding the extent of the interaction between genotype and environment is crucial in breeding programs and their development. Fageria et al., (2008) stated that differences in yield potential are also determined by variations in nutrient uptake among varieties, plant age, and growth phases.

Saputra et al. (2012) reported that variety influences the growth and production of soybeans. Their research findings showed that among the variables observed, the black soybean variety Malika demonstrated superior performance compared to the Anjasmoro variety in terms of the number of productive branches, number of filled pods, and production per hectare. The Anjasmoro variety only excelled in plant height and 100-seed weight variables.

Soybean varieties had a highly significant effect on plant height at 45 and 60 days after planting (DAP), and a significant effect on the total number of pods per plant, number of filled pods per plant, 100-seed weight, seed weight per plot, and yield potential. The Kipas Putih variety produced better soybean yields compared to Anjasmoro. Variety plays a crucial role in soybean production, as achieving high yields is largely determined by genetic potential. The yield potential in the field is influenced by the interaction between genetic factors and environmental management. If the growing environment is not managed properly, the high yield potential of superior varieties cannot be achieved. The interaction between AMF inoculation and P (phosphorus) dosage on soybean seed weight per hectare showed that the Tanggamus variety was more responsive in symbiosis with AMF compared to the Anjasmoro variety. This was evident from the dosages tested to increase seed weight per hectare for both varieties. The Tanggamus soybean variety achieved the highest seed weight at a dosage of 36 kg P_2O_5 ha^{-1}, while the Anjasmoro variety reached its highest seed weight at a dosage of 72 kg P_2O_5 ha^{-1} (Table 15). Therefore, it is evident that P fertilization is still necessary, albeit at a lower dosage. This aligns with several studies reporting that P dosage affects flowering time, 100-seed weight, and the number of filled pods (Fouda, 2017), as well as the number of filled pods and seed production per plot of soybeans (Egli, 2013).

According to Lu and Tian (2017), among essential nutrients, the role, requirement, and use of P fertilizer is generally very prominent, second only to N. P plays a crucial role in plant growth and yield increase. Malhotra (2018) suggests that the roles of P include: involvement in metabolism and as a component of complex compounds; acting as an activator, cofactor, and determining enzyme effects; and playing a role in physiological processes. Based on the soybean seed weight per hectare variable, the optimum dosage required for soybean growth and production is 49.5 kg P_2O_5 when inoculated with AMF. However, without AMF inoculation, the optimum dosage is 79.50 kg P_2O_5. Thus, the utilization of AMF saves 60% of P fertilization (Table 16).

Determining the optimum dosage is necessary to meet the P requirements of cultivated soybeans (Cahyono and Minardi 2021). According to

Table 15. Soybean seed weight per hectare in the interaction of AMF inoculation with P dose

FMA Inoculation	Dosage P (kg P_2O_5 ha^{-1})			
	0 (Control)	36 (P_2O_5)	72 (P_2O_5)	108 (P_2O_5)
	(tons ha^{-1})			
Without inoculation	2.45 b	2.66 b	2.87 ab	2.81 b
Inoculation	2.86 ab	3.44 a	3.05 ab	2.98 ab

Note: numbers sharing the same letter are not significantly different according to the Duncan Multiple Range Test with α = 5%.

Table 16. Determination of the optimum dose of P fertilizer for soybean plants with and without AMF inoculation in water-saturated cultivation based on seed weight variables

Variables	FMA Inoculation	Functions	Optimum dosage (kg P_2O_5 ha^{-1})
Productivity (ton ha^{-1})	Without Inoculation	Y = -0.0001X2 + 0.0159X + 2.1043	79.50
	Inoculation	Y = -0.0001X2 + 0.0099X + 3.223	49.50

Kamara (2010), the nutrient P is essential for pod and seed formation processes. Soybeans grown in fertile soil generally produce between 100–200 pods per plant.

Pandey (2018) states that P functions as a raw material for the formation of certain proteins; assists in assimilation and respiration; and accelerates flowering, seed maturation, and fruit ripening. Sanz-saes et al. (2017) assert that P fertilizer plays a direct role as an energy carrier, so P-deficient soil will reduce the energy that can be transferred by plants, which will decrease the rate of photosynthate production. This reduction in photosynthates results in many empty pods due to lack of energy for filling soybean pods.

CONCLUSIONS

1. AMF inoculation of the Tanggamus variety at a dosage of 36 kg P_2O_5 ha^{-1} enhanced soil accessible phosphorus, plant phosphorus uptake, plant phosphorus content, phosphorus nutrient uptake efficiency, relative inoculant efficiency, stem diameter, and the number of soybean branches.
2. AMF inoculation with the Tanggamus variety increased relative inoculant efficiency, dry biomass weight, accelerated flowering and harvest time, reduced the number of empty pods, and improved P use efficiency.
3. The Tanggamus variety with a dose of 36 kg P_2O_5 ha^{-1} increased available P, plant P content, plant P uptake, relative inoculant efficiency, relative P nutrient uptake efficiency, stem diameter, and number of branches. It also accelerated flowering and harvest time, and improved P use efficiency.
4. AMF inoculation with a dose of 36 kg P_2O_5 ha^{-1} increased available P, plant P uptake, plant P content, K uptake, relative inoculant efficiency, and relative P nutrient uptake efficiency. It also enhanced dry biomass weight, accelerated flowering and harvest time, increased the number of filled pods, 100-seed weight, seed weight per plot, seed weight per hectare, and improved P use efficiency.
5. When soybeans are inoculated with AMF, the optimum P dose is 49.50 kg P_2O_5 ha^{-1}. However, without AMF inoculation, the optimum P dose increases to 79.50 kg P_2O_5 ha^{-1}

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