JPPIPA 9(5) (2023)

Jurnal Penelitian Pendidikan IPA
Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Dual Inoculation of Rhizobium and Arbuscular Mycorrhizal Fungi Increases Soil-Total Nitrogen, Available Phosphorus, and Yield of Soybean in Vertisols

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Received: February 11, 2023
Revised: May 18, 2023
Accepted: May 25, 2023
Published: May 31, 2023

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DOI: 10.29303/jppipa.v9i5.3162

Abstract: Soybean is a protein source food crop with high economic value. So far, national production has not been able to meet domestic demand resulting in continuing import of soybean. The use of rhizobium and arbuscular mycorrhizal fungi (AMF) as biological fertilizers could be an alternative to increase soybean production. The research aimed to investigate the effect of dual inoculation of rhizobium and arbuscular mycorrhizal fungi (AMF) on root nodules formation, AMF development, total-N and available P of Vertisols, and yield of soybean. This study was designed in a completely randomized design) with 4 treatments namely no inoculation (R0), rhizobium inoculation (R1), AMF inoculation (R2), and dual inoculation of rhizobium and AMF (R3), with four replications. The variables observed were the number of effective root nodules, AMF spore density, AMF colonization, soil total-N, available P, plant height, number of pods, and seed weight. The results showed that dual inoculation of rhizobium and AMF had a better effect on the number of effective root nodules, AMF spore density, AMF colonization, soil total- N, available P, plant height, number of pods, and seed weight compared to the single inoculation and un-inoculated treatments.

Keywords: Arbuscular mycorrhizal fungi; Soybean; Rhizobium; Vertisols

Introduction

Soybean (*Glycine max* L.) is an important nutritious food source with high protein content and has important economic value. It contains around 37-42% and is high in unsaturated fatty acids (O'Keefe et al. 2015). Indonesia in particular, is one of the highest demand countries for soybean with national demand tends to increase yearly. On the other hand, the national production of soybean in Indonesia is much lower than the domestic demand. Accordingly, national production of soybean could only meet 35% of domestic demand with more than 65% of the demand for soybeans being fulfilled through imports. It also is predicted that soybean imports will continue to increase every year with an average increase of 7.73% (Ningrum et al., 2018). Due to this condition, efforts to increase soybean production have continued to be carried out, and self-sufficiency for soybean has been a part of the strategic plan for agricultural development in Indonesia.

The use of high-yielding varieties is an alternative to improve crop production. Furthermore, another important factor that also needs to be taken into account in efforts to increase soybean production is the condition of the soil that can support plant growth. In general, soil fertility in Nusa Tenggara Timur (NTT), Indonesia is low, including Vertisol which are widespread in NTT. Therefore, to get high crop yields, the fulfillment of nutrient needs is absolutely necessary. One of the environmentally friendly technologies for fulfilling plant nutrients and promoting plant growth and yield is the use of biological fertilizers.

Biofertilizer is a product containing living microorganisms that have the ability to increase soil fertility and crop production (Macik et al., 2020). Examples of microbial groups that have the potential as

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How to Cite:
Ishaq, L.F., Manehat, I. J.A., Tae, A.S.J.A., & Benggu, Y.I. (2023). Dual Inoculation of Rhizobium and Arbuscular Mycorrhizal Fungi Increases Soil-Total Nitrogen, Available Phosphorus, and Yield of Soybean in Vertisols. *Jurnal Penelitian Pendidikan IPA*, *9*(5), 2444–2451. https://doi.org/10.29303/jppipa.v9i5.3162

biological fertilizers for food legumes are rhizobium and arbuscular mycorrhizal fungi (AMF). Rhizobia is a group of N-fixing bacteria that can form a mutualistic symbiosis with leguminous plants. In this association, rhizobium will provide N to the host as an exchange for the organic carbon (Yang et al., 2022). As rhizobia is able to meet the parts of N needs of leguminous plants, it could be an alternative to reduce the use of N inorganic fertilizers (Allito et al., 2020).

Arbuscular mycorrhizal fungi are symbiotic mutualisms between fungi and terrestrial plants. In this symbiotic association, AMF obtains organic carbon from its host, and conversely, AMF can help the absorption of nutrients, especially phosphorus and water for its host plants (Smith and Read, 2008). It has been reported that the application of rhizobium on soybean could increase P and N content as well as the yield of plants in inoculated plants compared to un-inoculated plants (Cely et al. 2016). Furthermore, mycorrhizal application to soybeans could increase the availability of soil P content in Litosol (Wardhani et al., 2019), could increase the growth and yield of soybean under controlled and natural field conditions (Adeyemi et al., 2020), and could improve growth of soybean under abiotic stresses such as salinity (Hashem et al., 2016), drought (Ashwin et al., 2022; Lotfabadi et al., 2022), and high temperature (Jumrani et al., 2022).

The use of beneficial microbes as biological fertilizers could be in the form of a single inoculant, or as a combined inoculant of some microbes, for example, the use of rhizobium and mycorrhizae together as inoculants. Rhizobium and arbuscular mycorrhizae are two groups of microbes that commonly colonize soybean plant roots, and research results show that combined inoculation of rhizobium and AMF could increase the acquisition of N and P soybean under low P and N condition (Wang et al., 2011), and could improve growth and yield of soybean (Iglehon & Babalola, 2021; Dobo, 2022), reflecting a synergistic relationship between these two micro-symbionts.

Despite the benefits provided by dual inoculation rhizobium and AMF on crops legumes, no much study has been undertaken in Kupang district, Indonesia where the soil are less fertile. Therefore, this research aimed to investigate the effect of dual inoculation of rhizobium and arbuscular AMF on root nodules formation, AMF development, total-N, available P, and yield of soybean in Vertisols.

Method

Research had been undertaken in the glass house and Soil Laboratory of Agriculture Faculty of Nusa Cendana University from December 2020 to June 2021. The main utilities used included a compound microscope (Leica), beaker, hot plate, mortar, sieve (63, 125, 250, and 500 m), centrifuge, and laboratory equipment for soil analysis. Materials used in this study included soybean seed (Wilis variety), *R. japonicum* (extracted from fresh root nodule of soybean), commercial mycorrhizae (Mycogrow), Vertisols, 5% formaldehyde, sterile water, 70% alcohol, 0.8% NaCl, 12 % Clorox, 50% ethanol, 60% sucrose, 10% KOH, 0.3% trypan blue, glycerol, cow manure, chemical for soil analyses, and polybag.

The research was laid out in a completely randomized design with four treatments and four replicates. The treatment tested included without inoculation (R0), rhizobium inoculation (R1), mycorrhizal inoculation (R2), and dual inoculation of rhizobium and AMF (R3).

Inoculum preparation

In order to get *R. japonicum* as an inoculant, soybean was planted prior to the experiment. Effective nodules were harvested when plants were 40 days after planting. Fifteen fresh healthy effective nodules were taken, washed with sterile water, and then surface sterilized with 70% alcohol. The surface sterilized nodules were put in a ceramic mortar, 0.8% NaCl was added, and then gently crushed. The soybean seed (Wilis variety) was surface sterilized with 12% Clorox for 15 seconds followed by rinsing in sterile water three times before being inoculated with *R. japonicum*. Inoculation of seed with rhizobium was undertaken by mixing the sterile seeds with extract nodules and left for around two hours before being used. Commercial AMF (Mycogrow) was used as an AMF inoculant with the dose used being 2 g polybag^{-1} (around 66 spores).

Media preparation

The media used for planting was Vertisols from Noelbaki village, Kupang district, Nusa Tenggara Timur, Indonesia. The soil was taken diagonally from the land at 0-20 cm deep. The soil was bulked before being processed. Parts of bulked soil were used for soil chemical properties. The soil was clean from dirt, crushed into small size, and then sieved using a 2 mm sieve. The soil was sterilized with formaldehyde 5%. Ten kilogram of soil was used per polybag. Cow manure was applied as basal fertilizer with the dose of 150 g polybag $^{-1}$ equals 15ton ha $^{-1}$), and no additional inorganic fertilizer was added.

Planting

Mycorrhizal inoculation was undertaken at planting. A hole was made in the middle of sterile soil in the polybag around 7-8 cm deep, and 5-6 cm wide. Two gram of AMF inoculant was spread in hole, covered with soil around 2-3 cm thick, and three sterile seeds were placed. After that, the seeds were covered with the rest of the soil and watered until quite moist. For rhizobium treatment, AMF were not added. For AMF treatment, seeds inoculated with *R. japonicum* were used. For the control treatment, only sterile seeds were used.

The plants were watered based on the water needs of the plant (Blaney and Criddle method) using sterile water. Plants were thinned into two plants per polybag when they were 14 days after sowing (DAS). Plants were harvested when they were 110 DAS, and harvesting was undertaken four times. Soil, root and nodules were taken for analysis at the last harvest time. The variables observed included soil total-N and available P, number of effective root nodules, AMF spore density (expressed as number of spore in 100 g soil), AMF colonization, plant height, number of pods, and seed weight. Isolation of AMF spore was based on methods described by Brundrett et al. (1996). For AMF colonization observation, roots were cleared with 10% KOH and stained with 3% trypan blue dye. AMF colonization was counted using a gridline intersection method (Brundrett et al., 1996). Data were analyzed using ANOVA followed by 5% Duncan Multiple Range Tests (DMRT) when the ANOVA test was significant.

Result and Discussion

Soil Analysis

The result of soil analysis before the experiment was described in Table 1. Soil organic-C content is 3.64% and categorized as high. Soil pH is slightly alkaline (7.97), total-N is high (0.58%), and available P is categorized as medium (30.25 mg.kg^{-1}).

Table 1. Initial soil analysis (before treatment)

Observation	Value	Categorization*
Organic-C (%)	3.64	High
pH (H_2O)	7.97	Slightly Alcaline
Total-N (%)	0.58	High
Available P (mg.kg^{-1})	35.25	Medium

*Based on Hardjowigeno (1995).

Effective root nodules

Statistical analysis indicated that inoculation had a significant effect on the number of effective root nodules of soybean. The highest root nodules were at rhizobium inoculation, accounted for 105.2, which was not significantly different from dual inoculation of rhizobia and AMF with the number of nodules was 97.8. The lowest root nodules were at the control treatment (16.75), which was not significantly different from the AMF inoculation treatment (21.5) (Figure 1).

The highest root nodules formation at rhizobia inoculation treatment was not surprising as the bacteria was introduced to the seed. The high amount of nodules on rhizobia inoculation treatment could indicate that the introduced rhizobia was infective and developed well. *Rhizobium japonicum* is an obligate N_2-fixing bacteria colonizing the root of soybean. In this association, rhizobium will benefit the host plant through the fixed N, while the plants will provide organic-C to the bacteria. As N is critically important for photosynthesis, an adequate supply of N will result in a high accumulation of photosynthate which is important for supporting not only plant growth (Tisdale et al., 1999) but also the micro-symbiont. In this study, it is interesting that the dual colonization of rhizobia and AMF treatment also gave a high number of root nodules, indicating a positive synergism effect between rhizobia and AMF. The result of this study is in accordance with the study previously reported (Iglehon & Babalola, 2021; Dobo, 2022). Accordingly, plant-rhizobium will benefit from AMF through the acquisition of P and other nutrients that can increase the photosynthesis rate resulting in more allocation of organic carbon for rhizobia development including root nodules formation.

The lowest number of root nodules was at the un-inoculated (control) treatment. This is not surprising as in the control treatment no rhizobia inoculant was introduced to stimulate early root nodule formation.

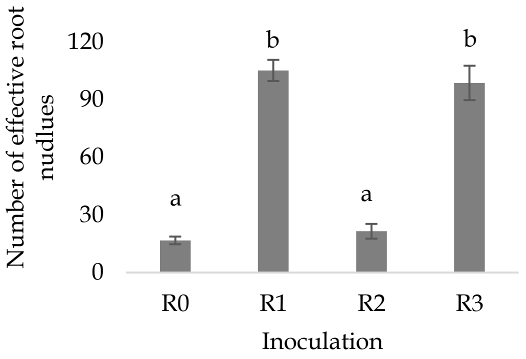

Figure 1. Number of effective nodules of soybean with treatments; un-inoculated (R0), inoculated with rhizobium (R1), inoculated with AMF, and inoculated with rhizobium and AMF (R3). Bar followed with same letter is not significantly different at 5% DMRT.

AMF spore density

Statistical analysis showed that inoculation treatment had a significant effect on AMF spore density. The highest AMF spore density was at dual inoculation treatment of rhizobium and AMF inoculation accounted for 137.2 spores per 100 g soil, which was not significantly different from the single AMF inoculation treatment with 104.5 spores observed. The lowest AMF spore density was at rhizobium inoculation treatment which was not different from control treatment with the number of spores were 41.2 and 54 per 100 g soil, respectively (Figure 2).

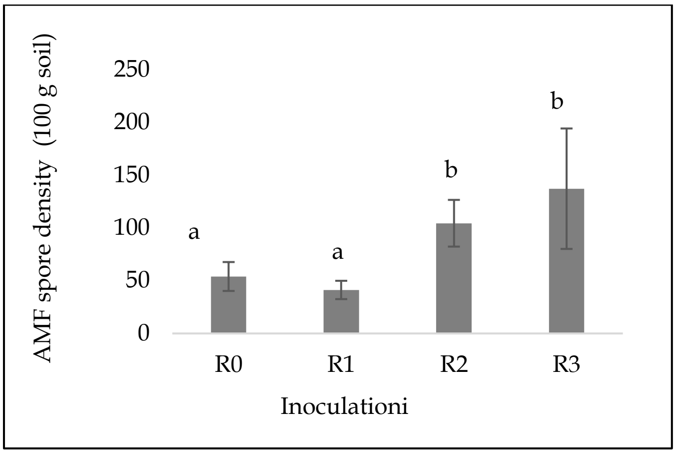

Figure 2. AMF spore density in the rhizosphere of soybean with the treatments; un-inoculated (R0), inoculated with rhizobium (R1), inoculated with AMF, and inoculated with rhizobium and AMF (R3). Bar followed with same letter is not significantly different at 5% DMRT.

AMF is known can form association with around 80% of terrestrial plants (Smith and Read, 2008), including soybean (Iglehon et al., 2021). Spore is one of the inoculum sources for mycorrhizal inoculation. Many factors could be involved in the development of AMF including soil condition, host and season (Silva-Flores et al., 2019). If the soil condition is favorable, AMF spore will germinate and form association with the compatible host. The soil condition can also influence the development of AMF and its function to the host.

In this study, the highest number of AMF spore were observed at dual inoculation of rhizobium and AMF which was not significantly different from single inoculation of AMF treatment. This might indicate that the AMF inoculant was infective so that it could infect the plants and sporulate well. With 66 spores applied for the inoculation, the number of spore density observed increased to be 137. 2 and 104.4 spores at dual inoculation of rhizobium and AMF and the single inoculation of AMF, respectively. The highest number of AMF spores at dual inoculation of rhizobium and AMF indicated a positive synergism effect between AMF and rhizobium, in other words, AMF could develop and sporulate well in the presence of rhizobium. This was in line with the number of root nodules observed where the number of root nodules was also high at the dual inoculation treatment of rhizobium and AMF. It is predicted that in this tripartite plant-rhizobium-AMF association, plants will be benefited more in the presence of rhizobium through N uptake, and the uptake of P and other plant nutrients through AMF colonization compared to the benefits from the single inoculation of either rhizobium or AMF. A sufficient supply of N, P and other soil nutrients could increase the photosynthesis rate resulting in a higher allocation of organic-C to the root for supporting rhizobium and AMF development and function, including the sporulation of AMF.

The lowest AMF spore density at rhizobium and control treatments was not surprising as there was no AMF inoculated to the plants. The presence of AMF spores in these treatments was possibly due to contamination during the experiment.

AMF colonization

Statistical analysis showed that there was a significant effect of inoculation treatment on AMF colonization in the roots of soybean. The highest AMF colonization was at dual inoculation treatment of rhizobium and AMF with 46.2% of the root being colonized by AMF, which was not different from the single colonization treatment of AMF with 43.6% of colonization. The lowest AMF colonization was at control treatment (11.6%) followed by rhizobium inoculation treatment with 22% of the root being colonized by AMF (Figure 3).

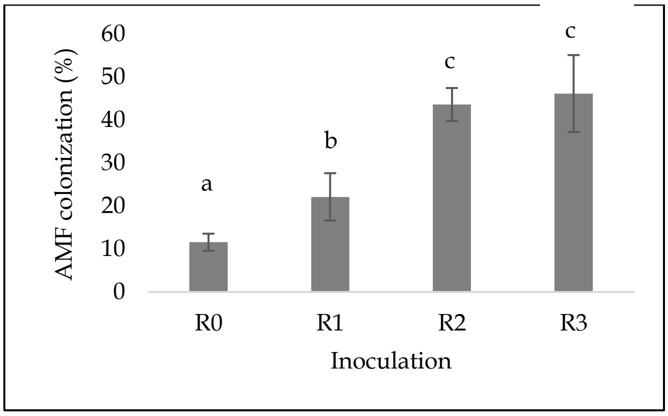

Figure 3. AMF colonization in the root of soybean with the treatments; un-inoculated (R0), inoculated with rhizobium (R1), inoculated with AMF, and inoculated with rhizobium and AMF (R3). Bar followed with same letter is not significantly different at 5% DMRT

The presence of AMF in the root of the host plant could reflect that AMF could form an association with the host plants. Moreover, the number of spores and the degree of AMF colonization in the root, in addition to plant metabolisms and growth, are usually used as indicators to evaluate the success of inoculation treatment. The higher AMF colonization could indicate that the introduced AMF are compatible with the host plant.

In this study, the highest AMF colonization was observed at dual rhizobium and AMF colonization. This may indicate that AMF could develop well in the presence of rhizobium expressing a synergism relationship between these two microorganisms. The highest colonization of AMF in the dual inoculation treatment of rhizobium and AMF could also be related to the highest number of AMF spores observed at this treatment. Spore is one of the propagules of AMF which can readily germinate and colonize the plant when the environmental condition is favorable (Smith & Read, 2008). The higher number of AMF spores produced, the more possibilities they could colonize the host plant.

The lowest AMF colonization was at control and rhizobium inoculation treatment was not surprising as there was no rhizobium and AMF introduced to these two treatments. The presence of AMF in the root of soybean might be due to contamination during the experiment. A few AMF spores were also observed in these treatments as explained above. Although contamination occurred during the experiment it is still tolerable as AMF the colonization was less than 25%.

Soil-total N

Statistical analysis showed that inoculation treatment had a significant effect on soil total-N. The highest total-N was at dual inoculation treatment of rhizobium and AMF with the total-N was 1.05% which was categorized as very high. This was followed by rhizobium inoculation treatment with a total-N was 0.86% which was also classified as very high. Soil total-N content of 0.63% (categorized as high), and 0.52% (categorized as high) were observed at AMF inoculation and control treatments, respectively (Table 2).

Table 2. Soil N-total with inoculation of rhizobium and AMF treatment

Inoculation treatment	Total-N (%)	Categorization*
R0 (control)	0.52 a	High
R1 (rhizobium)	0.86 c	Very high
R2 (AMF)	0.63 b	High
R3 (rhizobium + AMF)	1.05 d	Very high

The number followed by the same letter is not significantly different at 5% DMRT.
*Based on Hardjowigeno (1995).

Compared to the result of the initial soil analysis (before the experiment) (Table 1), there was a significant increase in total-N at inoculation treatment, whereas, a slight decrease occurred at the control treatment. This indicated that the inoculation in the form of single inoculation of rhizobium, AMF, and dual inoculation of rhizobium and AMF resulted in improving soil nutrients, particularly N. The result also showed that the dual inoculation of rhizobium and AMF gave the highest total N in the soil. This result was consistent with the variable number of root nodules observed. The higher contribution of the total–N to the soil at dual inoculation treatment of rhizobium and AMF compared to the single inoculation of rhizobium might reflect the effect of synergisms association between rhizobium and AMF. Previously, it has been reported that dual inoculation of mycorrhizae and rhizobia could increase the acquisition and transfer of N on soybean and maize in intercropping planting. Furthermore, the root nodule formed in the dual inoculation of mycorrhizae and rhizobia were also higher in the dual inoculation compared to the single inoculation of rhizobium (Meng et al., 2015).

In this study, the higher amount of soil total-N content in the dual rhizobium and AMF inoculation treatment might be related to the higher amount of effective root nodules formed. Effective nodules indicate that rhizobium is active in performing its function in fixing N. The higher amount of effective root nodules formed might indicate the higher amount of N being fixed by rhizobium resulting in a higher amount of N supply to the host and possibly N release to the soil.

In addition to the higher amount of effective nodules, the higher content of total-N soil observed may also be attributed to hyphae uptake. Marschner & Dell (1994) have reported that about 24% of the total N uptake in mycorrhizal plants could be related to the uptake and delivery by the external hyphae. Plants with higher content of N in the plant tissue could contribute more N to the soil through the root exudate release and decomposition of plant parts including root nodules and plant biomass.

At the control treatment, soil total-N was the lowest compared to inoculation treatments, which may also be related to the lower amount of effective nodules formed in this treatment. Moreover, the soil total-N after the experiment at control treatment was slightly lower compared to the initial soil analysis result. On the other hand, all inoculation treatments gave soil total-N much higher compared to the initial soil total-N. This may indicate that the contribution of rhizobium and AMF either as a single inoculant or dual inoculant is more beneficial to soil fertility than without inoculation.

Soil available-P

The result of ANOVA showed that inoculation rhizobium and AMF significantly affected soil available-P. The highest soil available P was at dual inoculation treatment of rhizobium and AMF accounted for 86.1 mg kg^{-1} followed by the single inoculation of AMF with 81.3 mg kg^{-1}. The lowest available P was at the control treatment (Table 3).

Table 3. Soil available P with inoculation of rhizobium and AMF treatment

Inoculation treatment	Available P (mg kg^{-1})	Categorization *
R0 (control)	21.40 a	High
R1 (rhizobium)	23.32 a	Medium
R2 (AMF)	81.27 b	Very high
R3 (rhizobium + AMF)	86.09 c	Very high

The number followed by the same letter is not significantly different at 5% DMRT.
* Based on Hardjowigeno (1995).

Compared with soil available-P before treatment, there was a high increase of soil available-P both at dual inoculation rhizobium and AMF treatment and the single inoculation of AMF. AMF are known can improve the acquisition of P and other soil nutrition to the host plants (Smith and Read, 2008). In addition, AMF can increase the availability of P through the release of phosphatase enzymes and organic acids which can increase the solubility of P (Wang et al., 2016).

In this study, the mycorrhizal inoculation treatment significantly increased soil available P, particularly, the dual inoculation treatment of rhizobium and mycorrhizae was able to increase soil available P higher than the single mycorrhizal inoculation treatment. The higher increase of soil available P in the dual inoculation treatment of rhizobium and mycorrhizae indicated that these two types of microbes were able to synergize positively. Although it is not yet known with certainty the mechanism of synergism that occurs in these two microbes so that they can increase soil available P, research results have proven a positive synergistic effect between rhizobium and mycorrhiza in increasing P uptake and also plant growth of several legume plants, including soybean (Havugimana et al., 2016).

Both at un-inoculated and the rhizobium inoculation treatments, there was a decrease in the soil available P content compared to the soil available P before the experiment. This maybe because some of the available P in the soil had been absorbed by plants, and possibly there was no or relatively small contribution of P through Rhizobium activity, root exudates, or from manure applied as basal fertilizer.

Growth and yield of soybean

The results of statistical analysis showed that the inoculation treatment had a significant effect on plant growth (expressed as plant height) and soybean yield (expressed as the number of pods and seed weight). The best growth in plant height was at the dual inoculation of rhizobium and AMF (92.7 cm), followed by mycorrhizal inoculation (73.1 cm) and rhizobium inoculation (64.1 cm). The un-inoculated treatment gave the lowest plant height (53.6 cm). The highest number of soybean pods was at the dual inoculation of rhizobium and AMF treatment (264), which was not significantly different from the rhizobium inoculation treatment (214.7) and the mycorrhizal inoculation treatment (196.7). The lowest number of pods was in the treatment without inoculation (control) but not significantly different from the AMF inoculation treatment. The highest seed weight was at the dual inoculation treatment of rhizobium and AMF (42.2 g) which was significantly different from the single inoculation treatment of rhizobium and AMF as well as from un-inoculated treatments (Table 4). There was an increase of 59, 34, and 37% of seed weigh in dual inoculation treatment of rhizobium and AMF compared to the control, rhizobium and AMF treatments, respcetively. Although the single inoculation of rhizobium inoculation and AMF inoculation was not significantly different from the un-inoculated treatment, there was an increase of 13.7 and 11.6% in seed weight of the single inoculation treatment of rhizobium and AMF, respectively (Table 4).

In general, the combined inoculation treatment of rhizobium and mycorrhiza gave the best plant growth and yield compared to the single inoculation treatment of rhizobium or AMF. Better plant growth and yield in the dual inoculation of rhizobium and mycorrhiza compared to the single inoculation or un-inoculated treatment can be attributed to the positive synergism of these two microbes in their interaction and their effects on plants. In this study, the positive synergism between rhizobium and AMF in interaction could be reflected in the formation of a greater number of effective root nodules, a higher number of AMF spores, and a higher AMF colonization in the combined inoculation treatment. In addition, the results of soil chemical analysis showed that higher soil total-N and soil available P were observed at the dual inoculation treatments.

Better development of rhizobium and AMF in dual inoculation of rhizobium and mycorrhizae treatment reflected a positive synergistic relationship between rhizobium-mycorrhizal which was not only beneficial to these two microbes but also to the growth and yield of the host plant. In the rhizobium-mycorrhizal-plant relationship, plants will be benefited through increased absorption of N, P, and other nutrients leading to an increase in chlorophyll content and photosynthesis rate (Ashwin et al., 2022). The increasing photosynthesis rate will result in more accumulation of organic C which in turn not only can support the development and activity of these two microbes but also can provide better plant growth and yield.

Table 4. Effect of inoculation treatment on plant height, number of pods, and seed weight of soybean

Inoculation trtreatment	Plant height (cm)	Number of pods	Seed weight (g)
R0 (control)	53.6 a	145 a	27.6 a
R1 (rhizobium)	64.1 b	214.7 b	31.4 a
R2 (AMF)	73.1 c	196.7ab	30.8 a
R3 (rhizobium+ AMF)	92.7 d	264 b	42.2 b

The number followed by the same letter is not significantly different at DMRT 5%.

The legume soybean forms symbiotic association with both *R. japonicum* and AMF which is referred as to tripartite symbiotic relationship (Abd-Alla et al., 2014). In this tripartite symbiotic relationship, around 4-16% of photosynthetic carbon is allocated for growth, activity, and C reserves for these two microbes (Kaschuk et al., 2009). The author reported that in legume plants rhizobium could increase the photosynthetic rate by 28%, AMF by 14% and the combination of rhizobium and AMF could increase the photosynthetic rate by 51%. Rhizobium was able to increase leaf P content by 13%, mycorrhizal by 6% and the combination of rhizobium and AMF was able to increase leaf P by 41%. The results of that study show that inoculation of rhizobium and AMF in legume plants can increase photosynthesis far beyond the allocation of C for these two microbes so that the plants will benefit because the amount of photosynthates for plant growth exceeds the photosynthate used by rhizobium and AMF (Kaschuk et al., 2009).

The findings of the current study correlate with some studies previously reported (Wang et al., 2011; Havugimana et al., 2016; Iglehon & Babalola, 2021; Dobo, 2022) that dual inoculation of rhizobium and AMF gave better impact on soybean growth and yield compared to the single inoculation of either rhizobium or AMF. However, since this study was undertaken in a controlled environment, future study needs to expand in the field to validate whether the dual colonization of rhizobium and AMF would also provide the similar trend as in the controlled environment.

Conclusion

Rhizobia and AMF is one of the most important ecological mutualism among tripartite legume symbiosis. Dual inoculation of rhizobium and AMF gave a better improvement in soil properties (total-N and available P), growth, and yield of soybean than the single inoculation of rhizobium and mycorrhiza. More studies need to be undertaken in the field condition where environmental factor are more favorable than in containerized media.

Acknowledgments
The authors are grateful to Universitas Nusa Cendana, Kupang, Indonesia, for the facilitation provided. The authors are also grateful to the reviewers for their corrections and suggestions regarding the improvement of the manuscript quality.

Author Contributions
Conceptualization, methodology, performed experiment, and wrote manuscript, Lily F. Ishaq and Yoke I, Benggu; Performed experiment and data collection, Ingracia J. A. Manehat; data analysis, interpreted data and wrote manuscript, Anthonius S. J. Adu Tae.

Funding
This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

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