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Effectiveness of Mycorrhiza, Plant Growth Promoting Rhizobacteria and Inorganic Fertilizer on Chlorophyll Content in *Glycine max* (L.) cv. Detam-4 Prida

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Abstract. This study aimed to find an effective combination of mycorrhiza, PGPR (Plant Growth Promoting Rhizobacteria), and inorganic fertilizers. Whereas the specific purpose was observed in effective mycorrhiza to increased chlorophyll content. This study used a completely randomized design (CRD) with three factors: the 1st factor is mycorrhiza application, the 2nd factor is PGPR and the 3rd factor is the application of nitrogen and phosphorus fertilizers. The data were analyzed with Analysis of Variance to determine the effect of the treatment being tried. Continued with the Least Significance Different test at a 95 % confidence level. The results indicated that the most effective application in increasing total chlorophyll content was (i) the "Commercial Mycorrhiza" without being combined with PGPR and fertilizer (TP.TR.MP:80 g mL^{-1}). (ii) "Brawijaya Mycorrhiza" is combined with PGPR without fertilizer (TP.R.MB: 83 g mL^{-1}). (iii) "Unida Mycorrhiza" without combined with PGPR and without fertilizer (TP.TR.MU: 80 g mL^{-1}).

Keywords: Environmental friendly, increase soybean productivity, microbial fertilizer,

1 Introduction

Photosynthesis is the process by which plants harvest sunlight to produce sugars from carbon dioxide and water [1]. Photosynthesis is a metabolic process in plants to form carbohydrates that use CO_2 from free air and water from the soil with the help of sunlight and chlorophyll. The process of photosynthesis will occur if there is light and an intermediate pigment, chlorophyll. Chlorophyll is a leafy or pigment green substance that has a major role in photosynthesis. The main function of photosynthesis is the formation of fat and protein energy sources from glucose. The results of the process of photosynthesis in

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the form of glucose. The chlorophyll content is a benchmark of plant growth related to crop production [2]. The higher the chlorophyll content of a plant, the higher the yield potential [3].

The main problem in increasing the production of black soybean [*Glycine max* (L.) Merr.] lies in the absorption of the element nitrogen, phosphorus and potassium. Not yet the maximum absorption of nutrients by black soybean plants will cause low yield production. Therefore, one alternative that can be done to increase production is to look for environmentally friendly technologies, namely using mycorrhiza and PGPR to optimize the absorption of nutrients, especially phosphorus and nitrogen [4, 5]. Mycorrhizal fungi can help plants absorb phosphorus and water absorption, while PGPR can provide nitrogen for black soybean plants. PGPR application can support the formation of mycorrhiza and can improve various functions adequately, such as Arturson's research inoculation with strains of the bacterium *Paenibacillus brasilensis* has been shown to increase the level of root colonization by mycorrhizal fungi and can absorb phosphorus elements so as to increase plant growth [6].

The general objective of this study is to find an effective combination of mycorrhizae, PGPR and inorganic fertilizers. While the specific purpose of this research is to find mycorrhizae that are effective in nutrient absorption to increase chlorophyll content.

2 Methods

Research has been carried out at the greenhouse of the Department of Agrotechnology, University of Darussalam, Gontor, Ponorogo, Indonesia. The design used was a completely randomized design (CRD) with three factors: the first factor was mycorrhizal application, the second factor was PGPR and the third factor was the application of nitrogen, phosphorus and potassium fertilizers (P). Mycorrhiza used in this study were collections from the Darussalam University – UNIDA (MU) Agrotechnology Laboratory, mycorrhiza produced by Brawijaya University (MB) and mycorrhiza produced by the manufacturer (commercial mycorrhiza) (MP). Whereas PGPR uses the types of *Pseodomonas* sp. (Migula, 1894) and *Bacillus Polymixa* (Prazmowski, 1880, Macé 1889) from the Agrotechnology Laboratory, Darussalam University. While the black soybean [*Glycine max* (L.) Merr.] cv. Detam-4 Prida was obtained from BALITKABI (*Balai Penelitian Tanaman Aneka Kacang dan Umbi* – Indonesian Legumes and Tuber Crops Research Institute).

Stages the chlorophyll content test has been carried out as follows; leaf sampling on the 40 d after the application of treatment by taking samples of the leaves of the bottom, middle and top of the plant, then weighed 1 g. Each sample was crushed with cold mortal and 1 mL of boric acid was added, the results of the grinding were transferred to the ependof and 4 mL of distilled water was added. Furthermore centrifuged at 6 000 rad s^{-1} for 25 mL. The resulting chlorophyll (pellet) extract was transferred to the reaction tube and 96 mL of 5% ethanol was added. Chlorophyll extracts were incubated in a dark room for 30 min. The chlorophyll content of absorbance was measured using a spectrophotometer at wavelengths of 649 nm and 665 nm.

The amount of chlorophyll-a was calculated using the Formula (1), and the amount of chlorophyll-b was calculated using the Formula (2)

$$(13.7 \times A665) - (5.76 \times A649) \tag{1}$$
$$(25.8 \times A649) - (7.60 \times A665) \tag{2}$$

while the amount of chlorophyll the total is calculated using the formula chlorophyll-a plus chlorophyll-b.

The observational data were analyzed with Analysis of Variance (ANOVA) to determine the effect of the treatment being tried. If the results of the analysis have a significant effect then proceed with the LSD test (Least Significance Different) at a 95 % confidence level [7].

3 Results and discussions

3.1 Level of group dynamics

Chlorophyll is a leafy or pigment green substance that has a major role in photosynthesis. The main function of photosynthesis is the formation of fat and protein energy sources from glucose. The results of the process of photosynthesis in the form of glucose. The process of chlorophyll synthesis through photoreduction of protochlorophyllide to chlorophyllide a and subsequently by esterification of phytol to form chlorophyll a is catalyzed by the chlorophyllase enzyme [8]. Chlorophyll content is a benchmark of plant growth related to crop production. The higher the chlorophyll content of a plant, the higher the yield potential [3].

Chlorophyll-a is the main pigment in photosynthesis which functions to electron donors in the photosynthetic electron transport chain. Chlorophyll-a most effectively absorbs the spectrum with wavelengths of 429 nm and 659 nm. This chlorophyll reflects the blue-green color. Factors that influence the chlorophyll content are nutrients N, Mg, Fe and Light. All green plants contain chlorophyll-a and chlorophyll-b, chlorophyll-a making up 75 % of the total chlorophyll [8]. The results showed that there were very significant differences between mycorrhizal, PGPR and fertilizer treatments according to Analysis of Variance (ANOVA) analysis.

Table 1. Clorophyll a content

No.	Treatment	Average	Notation
1	TP TR TM	27	bc
2	TP TR MU	29	d
3	TP TR MB	28	c
4	TP TR MP	27	bc
5	TP R TM	29	d
6	TP R MU	28	c
7	TP R MB	26	a
8	TP R MP	28	c
9	P TR TM	29	d
10	P TR MU	29	d
11	P TR MB	32	e
12	P TR MP	26	a
13	P R TM	29	d
14	P R MU	26	a
15	P R MB	28	c
16	P R MP	28	c

Notes: Figures in the same column and followed by the same letter are not significantly different based on the LSD 5 % further test.

The results of the analysis of chlorophyll-a absorbance value of mycorrhizal treatment without fertilizer and without PGPR were the highest "Unida Mycorrhiza" (TP.TR.MU:g mL^{-1}), while the lowest treatment of mycorrhizal treatment (TP.TR.MP: 27 g mL^{-1}). The highest mycorrhizal treatment plus PGPR without chlorophyll-a fertilizer is

"Unida Mycorrhiza" and "Commercial Mycorrhiza" (TP.R.MU: 28 g mL^{-1}), while the lowest is "Brawijaya Mycorrhiza" (TP.R.MB: 26 g mL^{-1}). The treatment of mycorrhizae plus fertilizer without PGRP the highest value of chlorophyll a content is "Brawijaya Mycorrhiza" (P.TR.MB: 32 g mL^{-1}), while the lowest is the "Commercial Mycorrhiza" (P.TR.MP: 26 g mL^{-1}). The highest treatment of mycorrhizae plus fertilizer and PGPR chlorophyll-a content is control (P.R.TM: 29 g mL^{-1}), while the lowest is "Unida Mycorrhiza" (P.R.MU: 26 g mL^{-1}).

In this study, it was found that there was a different response between mycorrhizae with other mycorrhizae towards fertilizer and PGPR. The interaction of fertilizers between plants and microorganisms in accumulating and translocating metabolites between stems and roots is an indicator of the symbiotic status between plants and microorganisms. In Juge's research on soybean plants showed that inoculation of three microorganisms when applied together had a negative impact on the shoot biomass of soybeans. The association of three microorganisms will reduce the root depth for nodulation during formation and reduce the number of nodules at the end of the experiment which can show competition when the three microorganisms are applied together [9]. "Unida Mycorrhiza" in increasing chlorophyll-a content is more effectively applied alone without a mixture of fertilizer and PGPR (TP.TR.MU: 29 g mL^{-1}) in Table 1. Application of "Brawijaya Mycorrhiza" more effectively combined with fertilizers without PGPR in increasing chlorophyll-a content compared to with other combinations, and get the highest value (P.TR.MB: 32 g mL^{-1}). For the application of "Commercial Mycorrhiza" is more effectively combined with PGPR without fertilizer compared to other combinations and can increase the chlorophyll-a content by 28 g mL^{-1} (TP.R.MP).

Table 2. Clorophyll b content

No	Treatment	Average	Notation
1	TP TR TM	52	cdefg
2	TP TR MU	46	bcde
3	TP TR MB	46	bcd
4	TP TR MP	53	cdefg
5	TP R TM	45	bc
6	TP R MU	49	bcdef
7	TP R MB	58	g
8	TP R MP	51	bcdefg
9	P TR TM	45	bc
10	P TR MU	42	b
11	P TR MB	26	a
12	P TR MP	55	defg
13	P R TM	44	bc
14	P R MU	55	defg
15	P R MB	47	bcde
16	P R MP	47	bcde

Notes : Figures in the same column and followed by the same letter are not significantly different based on the LSD 5 % further test.

Chlorophyll-b is an accessory pigment that is also responsible for the photosynthesis process. Chlorophyll has a function to collect light energy and enter chlorophyll-a during the process of photosynthesis. Chlorophyll-b is the most effective absorbing spectrum with wavelengths of 455 nm and 642 nm. Chlorophyll-b reflects the yellow-green color.

The results of research on the chlorophyll-b content in Table 2 show that mycorrhizal application without fertilizer and without PGPR is ineffective. This study found a decrease in the content of chlorophyll-b when "Unida Mycorrhiza" and "Brawijaya Mycorrhiza", except the "Commercial Mycorrhiza". Applications of mycorrhizae show no significant difference from the treatment without mycorrhiza. Mycorrhizal treatment combined with PGPR without fertilizer shows that the effective application is "Brawijaya Mycorrhiza" (TP.R.MB: 58 g mL^{-1}) while the lowest is the application without mycorrhiza (TP.R.TM: 45 g mL^{-1}). This study found that the application of mycorrhizae combined with PGPR without fertilizer is still able to increase the chlorophyll-b content of both "Unida Mycorrhiza" or "Commercial Mycorrhiza". Mycorrhizal application treatment combined with fertilizer without PGPR shows that there is a decrease in chlorophyll-b content except the treatment of "Commercial Mycorrhiza" (P.TR.MP: 55 g mL^{-1}). The most significant reduction was the treatment of "Brawijaya Mycorrhiza" (P.TR.MB: 26 g mL^{-1}). Mycorrhizal application treatment Fertilizer and PGPR showed that there was an increase in chlorophyll-b content when compared with those without mycorrhizal application, and the highest value was obtained by "Unida Mycorrhiza" treatment (P.R.MU: 55 g mL^{-1}).

Table 3. Total clorophyll content

No	Treatment	Average	Notation
1	TP TR TM	79	bcde
2	TP TR MU	80	cde
3	TP TR MB	74	bcd
4	TP TR MP	80	cde
5	TP R TM	73	bc
6	TP R MU	78	bcde
7	TP R MB	83	e
8	TP R MP	79	bcde
9	P TR TM	74	bc
10	P TR MU	71	b
11	P TR MB	58	a
12	P TR MP	81	cde
13	P R TM	73	bc
14	P R MU	81	cde
15	P R MB	75	bcde
16	P R MP	75	bcd

Notes : Figures in the same column and followed by the same letter are not significantly different based on the LSD 5 % further test.

Chlorophyll content is a benchmark of plant growth related to crop production [1]. The results of the study of total chlorophyll content of mycorrhizal applications without fertilizer and without PGPR showed that all mycorrhizal applications can increase the total chlorophyll content of both "Unida Mycorrhiza", "Brawijaya Mycorrhiza" and "Commercial Mycorrhiza". This has also been explained by Hassani in his research inoculation of mycorrhizal fungi and genus *Bacillus* can increase chlorophyll content and P uptake at the initiation of tuber potato plants. Interaction between mycorrhizae and plants can increase P uptake and chlorophyll content by 1 % compared to without mycorrhizal application [10]. The process of taking phosphate nutrients in the soil by mycorrhiza involves hyphae. Phosphorus nutrient flow in hyphae follows cytoplasmic flow, while nutrient transfer from fungi to host plants through subtle branches in host cells [11]. Increased phosphorus due to mycorrhizal inoculation in plants will affect photosynthetic activity, mycorrhizal infection in plant roots can increase nutrient translocation to the top of the plant resulting in an increase in the rate of photosynthesis and the use of assimilates in the canopy and an increase in photosynthate supply from leaves to roots. Further, phosphorus is found as phosphate in some plant minerals and is a key element of protoplasm. Phosphorus is present in water as orthophosphate [12]. Phosphate exists in three forms namely H_2PO4, HPO_4^2, and PO_4^{3-}. Phosphate is generally absorbed by plants in the form of primary orthophosphate ions $H_2PO_4^-$ or secondary orthophosphate HPO_4^{2-} whereas PO_4^{3-} is more difficult to be absorbed by plants.

The highest treatment of mycorrhizal application is unida mycorrhiza (80 g mL^{-1}), while the lowest treatment value is the treatment without mycorrhiza. The treatment of mycorrhizal application combined with PGPR and without fertilizer shows that the brawijaya mycorrhiza gets the highest value (TP.R.MB: 83 g mL^{-1}), while the lowest PGPR application without Mycorrhizae and without fertilizer (TP.R.TM: 73 g mL^{-1}). Vafadar's research explains that using mycorrhizae and PGPR can significantly increase leaf dry mass production and stevioside concentration. The application of double inoculation of mycorrhizal fungi and N-binding bacteria is most effective for increasing plant growth, plant nutrient uptake, total chlorophyll content and stevioside content [13]. Mycorrhizal application treatment combined with fertilizer without PGPR shows that mycorrhizal application has decreased total chlorophyll content except the application of mycorrhizal (P.TR.MP: 81 g mL^{-1}), while the lowest mycorrhizal treatment is "Brawijaya Mycorrhiza" (P.TR.MB: 58 g mL^{-1}).

Mycorrhizal application treatment combined with PGPR and fertilizer shows that all mycorrhizal applications experience an increase in total chlorophyll content, the highest mycorrhizal application is "Unida Mycorrhiza" (PRMU: 81 g mL^{-1}), while the lowest mycorrhizal application is PGPR application and fertilizer without mycorrhizae (PRTM: 73 g mL^{-1}). This study found that the most effective application of mycorrhizae in increasing total chlorophyll content was "Commercial Mycorrhiza" without combined with PGPR and fertilizer (TP.TR.MP: 80 g mL^{-1}) because if combined with PGPR and fertilizer it would decrease the total chlorophyll content (PRMP: 75 g mL^{-1}). The most effective application of "Brawijaya Mycorrhiza" in increasing total chlorophyll content is "Brawijaya Mycorrhiza" combined with PGPR without fertilizer (TP.R.MB: 83 g mL^{-1}), if the application of "Brawijaya Mycorrhizae" combined with fertilizer will dramatically decrease the total chlorophyll content (P .TR.MB: 58 g mL^{-1}). The most effective application of "Unida Mycorrhiza" in increasing total chlorophyll content is "Unida mycorrhiza" without combined with PGPR and without fertilizer (TP.TR.MU: 80 g mL^{-1}), if unida mycorrhiza is combined with PGPR and fertilizer can still increase the total chlorophyll content (PRMU: 81 g mL^{-1}) and if "Unida Mycorrhiza" is combined with fertilizer without PGPR, it will decrease the total chlorophyll content (P.TR.MU: 71 g mL^{-1}).

4 Conclusion

The most effective application in increasing total chlorophyll content was (i) the "commercial mycorrhiza" without being combined with PGPR and fertilizer (TP.TR.MP:

80 g mL^{-1}). (ii) "Brawijaya mycorrhiza" is combined with PGPR without fertilizer (TP.R.MB: 83 g mL^{-1}). (iii) "Unida mycorrhiza" without combined with PGPR and without fertilizer (TP.TR.MU: 80 g mL^{-1}).

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