The growth response of pokem (*Setaria italica* L.) inoculated with arbuscular mycorrhizal fungi (AMF) from tailings area

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**Abstract**

The purpose of this research was to study the growth response of pokem (*Setaria italica*), which was inoculated by the arbuscular mycorrhizal fungi (AMF) from the tailings area. The method used in this research was a completely randomized design of factorial pattern. The factors consisted of AMF types (M) (M0: non-mycorrhizal, M1: *Claroideoglomus etunicatum* BGR, M2: *C. lamellosum* B1107S, M3: *C. etunicatum* L3101D), and inoculum density (I) (I: 5 g, II: 10 g per pot of planting media) with 8 replicates. The source of inoculum *C. lamellosum* B1107S and *C. etunicatum* L3101D originated from tailings in the gold mining area of Timika. The results showed that inoculation of *C. etunicatum* L3101D could increase plant growth better than *C. etunicatum* BGR and *C. lamellosum* B1107S. Significant growth occurred on the parameters of the height of the plants, leaf area, dry weight and fresh weight of the plants, relative growth rate, and phosphorus absorption by the plants. The amount of nitrogen tended to decrease with AMF treatment, but it was not significant, whereas K has insignificantly increased. The propagule density treatment increased plant growth on all parameters. The best growth occurred if the type *C. etunicatum* L3101D was inoculated with 10 g of propagule per planting media.

**Keywords:** AMF, *Claroideoglomus*, growth of plant *S. italica*, tailings

**Introduction**

Pokem (*Setaria italica* L.) is one of the plants, which is found in the East of Indonesia. This plant can be found in some regions, such as Papua (Biak Numfor) and Maluku (Buru Island) (Suharno et al., 2015a). Pokem have been cultivated by local communities in Papua and used in a variety of processed foodstuffs (Suharno et al., 2015a; 2015b). The distribution of pokem in the world is extremely wide (Li and Brutnell 2011; Suharno et al., 2015a), such as China, India, Japan, Korea, Uni Soviet, Africa, Europe, and South of Asia (Ji et al., 2010), including in some parts of America, Eurasia, and Australia (Lin et al., 2012). In addition to high nutritional value, *S. italica* can be used as a model in the process of domestication, the physiological processes associated with photosynthesis of C₄, the study of abiotic stress tolerance and the development of genomic sources, as well as models in plant growth (Suharno et al., 2015a).

The pokem in Biak Numfor are known to be symbiotic with arbuscular mycorrhizal fungi (AMF) (Suharno et al., 2015b). These fungi are part of a mycorrhizal group, which have capability of...
symbiosis with over 80% of vascular plants (Souza, 2015; Pagano and Gupta, 2016; Suharno et al., 2020). The mutualistic symbiosis between AMF and plants is known to increase the growth of plants (Smith and Read, 2008; Suharno et al., 2017; Husna et al., 2021). On the other hands, the fungi obtain energy, which is derived from the photosynthesis of host plants, so they can grow and develop well (Smith and Read, 2008). The involvement of these symbionts is widely used as a method for a variety of purposes, such as farming systems, horticulture and forestry (Smith and Read, 2008; Brundrett and Ashwath, 2013).

More specifically, AMF enhances the absorption of phosphorus (Bücking et al., 2012; Suharno et al., 2017; Suharno et al., 2020), other nutrients (Pagano and Gupta, 2016; Gao et al., 2020; Husna et al., 2021), and water (Birhane et al., 2012). It also has a role in phytoremediation of pollutants (Göhre and Paszkowski, 2006; Khan, 2006; Upadhyaya et al., 2010), tolerance to disease (Qiao et al., 2011), and improve the aggregation of soil (Li et al., 2015). In fact, mycorrhizal colonization can increase plant survival and growth of seedlings and cuttings in greenhouse conditions, improve resistance to transplanting shock, and promote earlier flowering and fruiting (Corcidi et al., 2008; Berutti et al., 2016).

AMF response in enhancing the growth of plants is very diverse. Most AMFs can significantly increase plant growth, but some do not have a significant effect (Tarbell and Koske, 2007; Bücking et al., 2012; Rohyadi et al., 2017). Positive responses often occur when the treatment of plant growth is done on the land or media, which is lacking phosphorus (Tarbell and Koske, 2007) and marginal land (Suharno et al., 2017). Therefore, the aim of this study was to determine the response of the growth of pokem plants (S. italica), which is inoculated with AMF from the tailings land.

Materials and Methods

Time and location of the research

The research was conducted in the scale of a laboratory at the Taxonomy Laboratory of Biology Faculty Gadjah Mada University (GMU), Yogyakarta. Several activities were done at Silviculture Laboratory and Soil Laboratory at Seameo-Biotrop, Bogor. N, P, and K were analysed at the Laboratorium Penelitian dan Pengujian Terpadu (LPPT) GMU, Yogyakarta.

Materials and research media

The pokem seeds (S. italica) used in the isolate response test were obtained directly from Numfor Island, Biak Numfor District, Papua. According to Li and Brutnell (2011), S. italica plant is one of the most widespread species, which is used in various research because it is considered as a model in the genetic system, especially Panacoid grass.

The soil media used in this study included sandy soils with neutral pH (7.0–7.3), very low organic C and N contents, which were 0.10 to 0.14%; 0.02% respectively, very low C/N ratio (4–6), available P (P2O5) is known very high (223.8–408.8 ppm), K (0.12–0.14 cmol kg⁻¹), while CEC (cation exchange capacity) is very low (2.08–2.50 cmol kg⁻¹).

Propagules of AMF used were obtained from the tailing land of C. etunicatum and C. lamellosom. C. etunicatum BGR from SEAMEO-BIOTROP Bogor was used as another source control. These three AMF types have different numbers of spores, which are C. etunicatum BGR averaged 89 spores, C. lamellosom of 96 spores, and C. etunicatum L3101D of 87.7 spores per 100 g of soil samples. The observations of the Most Probable Number (MPN) of these three AMF types were respectively 1400, 2600, and 1700 propagules per gram of wet soil weight. According to Berutti et al. (2016), the use of propagules as inoculums is better if compared to only with spores because propagules can increase the infection at the root of plants very well.

Treatment of research

This research used a completely randomized design with two factors. The first factor was the type of treatment of AMF: M0: non-mycorrhizal, M1: C. etunicatum BGR, M2: C. lamellosom, M3: C. etunicatum L3101D. The second factor was inoculant density: I (5 g) and II (10 g) per 3 kg of planting media, each treatment was done with 8 replicates. The observation of high growth and leaf area was done once every week, while other parameters such as wet weight, dry weight, relative growth rate, spore count, percent AMF infection, N, P and K content analysis were performed at 10 weeks after planting (WAP).

Data analysis

The observed data were analyzed by analysis of variance (ANOVA) with two factors using IBM Statistical Package for Social Sciences (SPSS) software version 20.0 continued with Duncan's Multiple Range Test (DMRT) test via post hoc test with 95% confidence level ($p = 0.05$).

Results and Discussion

The results showed that AMF increased the growth of pokem plant (S. italica). AMF was able to infect the roots of pokem plant well (Figure 1). It could even develop an intraradical hypha network, form vesicles and arbuscules in cortex cells of plant roots. The colonization reached 83.5% with an average of 78.9% (Table 1), which is included very high category (Suharno et al. 2017). The highest colonization was treated with C. etunicatum L3101D, followed by C. lamellosom B1107S and C. etunicatum BGR. The height growth of plant and leaf area increased better
than control (Figures 2 and 3). AMF significantly increased plant height growth, leaf area (Table 2), grown ratio: fresh weight root and dry weight (Table 4).

The best growth of *S. italica* plant occurred in combination treatment of *C. etunicatum* L3101D and inoculation of 10 g to 20.71 cm (Table 2) derived from the tailings field, followed by *C. lamellosum* B1101S and *C. etunicatum* BGR Bogor collection. This plant height increased 37.97% compared to controls that only 15.0 cm. The increase of leaf area growth was 105.42% compared to control, i.e. 14.03 cm$^2$ in combination treatment of *C. etunicatum* L3101D and inoculation of 10 g of propagule. AMF also influences the shoot ratio: fresh weight root and dry weight (Table 4) and P adsorption of shoot plant (Table 6). However, the utilization of AMF could not increase fresh weight and dry weight (Table 3), significant absorption of N and K plant shoot (Table 5). The interaction between AMF type and inoculum density significantly affects all measured growth parameters. Inoculum density has an effect on P adsorption at the shoot of the plant and not on other parameters. The type of AMF significantly influenced the fresh shoot: roots ratio (Table 4), percent colonization, relative growth rate (Table 3), K adsorption (Table 5), and P shoot (Table 6).

The use of the type of AMF *C. etunicatum* L3101D originating from the tailings area had an effect on the increase of pokem growth, i.e. plant height, leaf area, wet weight and dry weight of plants, percent colonization, relative growth rate (RGR), and the K adsorption by plant shoot. The best interaction treatment occurred when the type *C. etunicatum* L3101D was combined with 10 g inoculum per plant media, compared to other interaction treatments (Table 7). The percentage of AMF colonization has positively impacted in the increase of RGR (Table 1). AMF type *C. etunicatum* L3101D with average colonization of 81.66% increased the relative growth rate up to 0.264, followed by *C. lamellosum* B1107S 78.62% (RGR: 0.26) and *C. etunicatum* BGR 78.40% (RGR: 0.08). However, this warning was not proportional to the absorption of nutrients.

### Table 1. Percentage of colonization and relative growth rate of *S. italica* plant.

| Types of AMF         | % colonization | RGR              |
|----------------------|----------------|------------------|
|                      | Inoculum       | Average          | Inoculum       | Average          |
|                      | 5 g            | 10 g             | 5 g            | 10 g             |
| *C. etunicatum* BGR | 74.72 b        | 78.40 b          | 0.023 e        | 0.138 d          | 0.081 l          |
| *C. lamellosum* B1107S | 77.75 ab     | 79.49 ab         | 0.313 a        | 0.212 c          | 0.263 k          |
| *C. etunicatum* L3101D | 79.86 ab     | 83.46 a          | 0.238 b        | 0.289 a          | 0.264 k          |
| Average              | 77.44          | 79.45 (+)        | 0.191          | 0.167 (+)        |                  |
| Average of treatment | 78.95 p        | 0.203 p          | 0.019 q        |                  |
| Average of control   | 1.25 q         |                  | 11.39          |
| CV (%)               | 0.92           |                  |

Note: CV (%) = coefficient of variance. RGR = relative growth rate. The numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5% contrast test (+) / (-): interaction types and inoculums significant or not significant.
Figure 2. The height growth of pokem \((S. \text{italica})\) inoculated with the AMF.

Figure 3. The growth of inoculated pokem plant leaf area \((S. \text{italica})\) with the AMF.

Table 2. The height growth and leaf area of \(S. \text{italica}\) plant inoculated with AMF from tailings.

| Types of AMF                  | Height of plant (cm) | Leaf area (cm\(^2\)) |
|-------------------------------|----------------------|-----------------------|
|                               | Inoculum            | Average               | Inoculum            | Average               |
|                               | 5 g                  | 10 g                  | 5 g                  | 10 g                  |
| \(C. \text{etunicatum}\) BGR | 17.9 b               | 16.3 c                | 171.0                | 8.3 d                 | 8.0 d                 | 8.2                     |
| \(C. \text{lamellosum}\) B1107S | 16.3 c               | 16.4 c                | 16.4                 | 9.3 c                 | 10.8 b                | 10.1                    |
| \(C. \text{etunicatum}\) L3101D | 16.3 c               | 20.7 a                | 18.5                 | 6.7 e                 | 14.0 a                | 10.4                    |
| Average                       | 16.9                 | 17.8                  | (+)                  | 16.9                  | 17.8                  | (+)                     |
| Average of treatment          | 17.3 p               | 9.5 p                 |
| Average of control            | 15.0 q               | 6.8 q                 |
| CV (%)                        | 2.9                  | 6.2                   |

Notes: CV (%) = coefficient of variance. The numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5% contrast test (+) / (-): interaction types and inoculums significant or not significant.
treatment increased the adsorption of P by 34.2% compared to control; this condition increased up to 62.1% in interaction treatment of C. etunicatum BGR with 5 g of inoculum per plant media. However, the utilization of C. etunicatum BGR has a percentage of colonization and the relative growth rate of plants, which are lower than that of C. etunicatum L3101D and C. lamellosum B1107S. This is because the absorption ability of other elements such as N and K is not effective.

N adsorption by shoot was not influenced by the AMF type, whereas the C adsorption by shoot plant was significantly increased by C. etunicatum L3101D treatment compared to C. etunicatum BGR and C. lamellosum B1107S. In fact, the uptake of N by shoot with inoculation of AMF tended to decrease (Table 5). P adsorption by shoot was affected by AMF treatment, inoculum density, type of AMF, and interaction of both treatments (Table 6). AMF treatment increased the adsorption of P by 34.2% compared to control; this condition increased up to 62.1% in interaction treatment of C. etunicatum BGR with 5 g of inoculum per plant media. However, the utilization of C. etunicatum BGR has a percentage of colonization and the relative growth rate of plants, which are lower than that of C. etunicatum L3101D and C. lamellosum B1107S. This is because the absorption ability of other elements such as N and K is not effective.
The response of plant growth to AMF varies for each type of plant (Rohyadi et al., 2017; Setyaningsih et al., 2018; Begum et al., 2019). Some results of this study are different from those previous study by Suharno et al. (2017) and Wilujeng et al. (2020) on maize. Type AMF *C. lamellosum* has a better effect on the growth of maize compared to *C. etunicatum*. The inoculum response of *C. etunicatum* BGR to maize growth was lower than both but better than control (Suharno et al. 2017). This may occur due to the differences in the response between species of AMF and host plants physiologically (Sancayaningsih et al., 2000; Suharno et al., 2017; Setyaningsih et al., 2018).

The utilization of a single AMF type, combined AMF (consortium) and inoculum density have an effect in increasing biomass (Valsalakumar et al., 2007; Treseder, 2013), including maize (Sancayaningsih et al., 2000; Suharno et al., 2017). In peanuts (*Arachis hypogea*), the role of AMF is evident in the growth of dry weight of plant shoot (Doley and Jite, 2012). The high propagule density determines the increase of dry weight of the host plant at the beginning of the growth of the maize plant (Sancayaningsih et al., 2000). In addition, high density of AMF inoculum in the soil, it is important to achieve faster plant root colonization, maximal growth, better product and can encourage at least initial stimulus inputs such as fertilizer or without underground processing, avoiding longer fallow periods, and burning crop residues (Smith and Smith, 2011; Suharno et al., 2020; Gao et al., 2020).

In general, the type of AMF originating from the tailings field is able to increase the growth of *S. italica* plants well. This is because the used media was similar to the tailing land, which is dominated by sand (sandy soil). The potential of two types of AMF, i.e. *C. etunicatum* L3101D and *C. lamellosum* B1107S can be utilized in the rehabilitation of similar land. Moreover, so far, the types of AMF used in the rehabilitation of marginal areas and tailings land are still limited. Types of AMF *C. lamellosum* from tailings land (Suharno et al., 2017) and sandy soils (Li et al., 2015) have not been widely utilized for this purpose.

### Table 6. The content of P of plant shoot *S. italica* inoculated with AMF from tailing.

| Types of AMF        | Inoculum (mg kg⁻¹) | Average | 5 g   | 10 g   | 2194.42 k |
|---------------------|---------------------|---------|-------|--------|------------|
| *C. etunicatum*     |                     |         |       |        |            |
| BGR                 | 2394.08 a           | 1994.76 ab |       | 2194.42 k |
| *C. lamellosum*     |                     |         |       |        |            |
| B1107S              | 1844.01 b           | 1883.95 b | 1863.98 l |       |
| *C. etunicatum*     |                     |         |       |        |            |
| L3101D              | 2019.63 bc          | 1752.84 c | 1866.23 l |       |
| Average             | 2085.91 x           | 1877.18 y | (+)  |        |            |
| Average of treatment|                    |         |       | 1981.54 p |            |
| Average of control  |                    |         |       | 1476.61 q |            |
| CV (%)              |                     |         |       | 1.98   |            |

Notes: The same letters in the same column show no significant difference between treatment with DMRT at 5% level.

### Table 7. Response of the growth of pokem inoculated with AMF type originated from Timika tailings at 10 WAP.

| Types of AMF        | Inoculum (g) | Height plant (cm) | Leaf area (cm²) | Wet weight of shoot (mg) | Dry weight of total (mg) | Relative growth rate (RGR) |
|---------------------|--------------|-------------------|-----------------|--------------------------|--------------------------|---------------------------|
| *C. etunicatum*     | 5            | 17.09 b           | 8.31 b          | 26.50 ab                 | 30.25 b                  | 0.023 c                   |
|                     | 10           | 16.26 b           | 7.98 b          | 26.25 ab                 | 30.50 b                  | 0.138 bc                  |
| *C. lamellosum*     | 5            | 16.34 b           | 9.28 b          | 25.30 ab                 | 32.25 b                  | 0.313 a                   |
|                     | 10           | 16.44 b           | 10.82 ab        | 27.50 ab                 | 30.50 b                  | 0.212 ab                  |
| *C. etunicatum*     | 5            | 16.34 b           | 6.69 b          | 28.00 ab                 | 26.50 bc                 | 0.238 ab                  |
|                     | 10           | 20.71 a           | 14.03 a         | 38.00 a                  | 43.50 a                  | 0.290 a                   |
| Control             | 0            | 15.01 b           | 6.83 b          | 20.00 b                  | 24.75 c                  | 0.046 c                   |

Notes: The same letters in the same column show no significant difference between treatment with DMRT at 5% level.

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**Conclusion**

Type of AMF *C. lamellosum* B1107S and *C. etunicatum* L3101D originating from the tailings field is able to increase the growth of *S. italica*. So far, only a few types of AMF are often used to increase the effectiveness of plant growth. Therefore, *C. lamellosum* B1107S can be used as an alternative source of inoculum. Future research can be
concentrated in the compatibility test of this AMF with various plant species.

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