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Consistency of Mycorrhizal Effectiveness on Maize Growth and P Uptake in Two Generations of Pot Culture Using Andisol-Based Media

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ABSTRACT

The functional roles of Arbuscular Mycorrhiza (AM) in soils with P limitations are well documented. However, the protocol to produce effective AM inocula was still limited. This research aims at obtaining the effective AM culture in handling P constraints for maize growth on Andisols. The first experiment of pot culture I was to propagate and examine the effectiveness of AM isolates by using a factorial completely randomized design with two factors, A=media (A0: zeolite; A1: representative media=Andisol Tengaran; A2: typical media=a mixture of Andisol Tengaran+Tawangmangu; A3: typical media+Bio-RP nutrition; A4: Inceptisol) and I = AM inoculum source (I0: no inoculum; I1: AM from Andisol Tengaran; I2: AM from Andisol Tengaran + Tawangmangu; I3: AM from 8 soil types), with six replications. The second experiment investigated the consistency of mycorrhizal effectiveness by reculturing AM cultures generation I to pot cultures generation II with the same composition of the respective media. The combination treatments of A1I3, A2I2, A0I3, and A4I3 (AM cultures generation I), continued by $A1I3^2$, $A2I2^2$, $A0I3^2$, and $A4I3^2$ (AM cultures generation II) showed consistently the highest AM infectivity and effectiveness on maize growth and P uptake on Andisol-based media, and on the comparison media of zeolite and Inceptisol media, respectively.

INTRODUCTION

Andisols are soil from volcanic material originating from volcanic areas and activities (Kilic et al., 2018). Andisol land in Indonesia is estimated to be around 5.4 million ha. Based on the aspects of biophysical conditions, the majority of the total area (61.99% or 3.34 million ha) consisted of mountainous regions with slopes >30%, and the rest, around 2.05 million ha or 38%, which potential for agricultural land consisting of land with flat to wavy, undulating, and hilly areas (Sukarman & Dariah, 2014).

It is known that allophanes dominate the specific characteristics of Andisols. Andisols are also characterized by low bulk density and high organic matter content (Anda & Dahlgren, 2020) high porosity, high water storage capacity, irreversible physical changes after drying (Rahman et al., 2008), a wide range of pH H_2O between 3.4 - 6.7, and high pH NaF with values ranging from 10.5 - 12.1 (Utami et al., 2012) which indicates the high content of amorphous minerals, especially allophane. The high mineral content of allophane in Andisols results in low P soil availability and is the main limiting factor for plant growth (Parfitt, 2009).

Several previous studies have reported using Arbuscular Mycorrhiza (AM) as a biofertilizer to resolve the P constraint on Andisols. AM has been proven to be capable of increasing P uptake in a variety of agricultural plants on Andisols either as a single inoculant (Martínez et al., 2019) or as a mixed inoculant with other beneficial microorganisms such as phosphate solubilizing fungi (Tamayo-

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Velez & Osorio, 2017), combined with slow-release fertilizers such as rock phosphate (Rubio et al., 2003) or compost (Borie et al., 2002). However, the common mycorrhizal biofertilizers available in the agricultural market are not targeted for the specific P constraint on Andisols. This study focused on screening AM isolates with superior functions in increasing P uptake and supporting plant growth on Andisols.

Producing mycorrhizal biofertilizer needs to consider two factors: the superiority of AM isolates (Cahyani et al., 2019) and the media suitability (Oseni et al., 2010). Superior AM isolates can be obtained as single or mixed cultures. Ortas (2010) examined the effectiveness of various types of mycorrhizae in increasing cucumber yields consisting of the treatment of single and mixture AMF during four growing seasons in different years, and the results showed that for each year, the highest production was obtained by a single inoculum. A further result was reported by Sery et al. (2016) that a mixture of AM inoculum had a higher effect than the treatment of single inoculum on the growth and yield of cassava plants under field conditions.

Some previous researchers used various types of media to produce mycorrhizal cultures, including soilless media (sand, charcoal, perlite, clay, coal marl, and vermiculite) (Gaur and Adholeya, 2000), soil media such as Mollisols (Silvani et al., 2019), and organic media such as compost (Chaiyasen et al., 2016). The composition of each media has a different effect on mycorrhizal infectivity and plant growth. In line with the research of Vestberg and Kukkonen (2009) that the treatment of various peat media (light and dark Sphagnum peat), commercial peat media (dark Carex Bryales), and their combination treatment with minerals (sand, pumice, vermiculite, perlite, clay, or zeolite) gave the different result toward mycorrhizal infectivity and daisy growth.

The AM inoculation is estimated to potentially affect the abundance, diversity, and functional activity of endophytic bacteria. On the contrary, it was also estimated that endophytic bacteria and/or fungi affect AM infection and their functional activity. Sundram et al. (2011) reported that endophytic bacteria *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 isolated from oil palm root showed the effect on spore germination and hyphal length of *G. intraradices* UT126 and *G. clarum* BR152B.

The objective of the present study is to propagate, examine, and select AM isolates whose superior functional capability in supporting plant growth especially P uptake and in handling the limitation of Andisols. The research was conducted by culturing AM isolates in two steps. In the first step, mycorrhizal spores were isolated from natural sources of the rhizospheric soils from Andisols Tengaran, from Andisols Tengaran + Tawangmangu, and eight different soil types, and then propagated in pot culture using Andisol-based media with different compositions. In the second step, the products of AM inocula in the pot cultures generation I (GI) were subjected to continued propagation in pot cultures using the same respective media compositions to produce AM cultures generation II (GII). The effectiveness of two generations of AM cultures was observed and measured on plant growth and P uptake on Andisol-based media. Maize, as a mycorrhizal-dependent plant with a fibrous root system efficiently establishes mycorrhizal infectivity, was selected as a plant host for mycorrhizal effectiveness indicator (Tawaraya, 2003; Fasusi et al., 2021; Ma et al., 2021). Besides measuring the effects of the treatments on mycorrhizal and plant growth parameters, the present study observed foliar symbiotic nitrogen-fixing bacteria (NFB) and total heterotrophic bacteria. Inceptisol and zeolite were used as comparison media in the present study. Inceptisol represented soil media with minimal P constraints (Soil Survey Staff, 2022), and zeolite described non-soil media commonly used to produce mycorrhizal biofertilizer commercially.

MATERIALS AND METHODS

The present study consisted of two steps experiment conducted from June 2020 to December 2021 at the greenhouse of the Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. The following is the sequence of research steps:

Isolation of AM Spores from Natural Habitat

AM spores were isolated from the rhizosphere of maize and grasses from eight soil types taken from nine locations (Table 1). Isolation of the AM spores was carried out using wet sieving and decantation method (Pacioni, 1992), continued with centrifugation by adding 60% glucose solution (Ianson & Allen, 1986), then the supernatant

containing AM spores was poured into a petri dish, the spores were characterized using a binocular stereomicroscope with a magnification of 400x at the genus level (Souza, 2015) and counted the density (Cahyani, 2008) (Table 2). Genus Glomus dominated AM isolates from all soil types.

Soil Sampling for Media of Pot Culture

Non-rhizospheric soil of Andisol Tengaran and Andisol Tawangmangu with a depth of 0-20 cm were sampled for the primary media of the pot culture experiment. In addition, zeolite and Inceptisol were used for the comparison treatments. Zeolite (diameter 3-5 mm) represented non-soil media, and Inceptisol represented soil with minimum P constraint. All soil samples were air-dried, crushed, and sieved (<2 mm). The chemical characteristics of all these media are presented in Table 2.

Greenhouse Experiment for the Pot Culture Generation I (GI)

The first experiment was the propagation and examination of natural AM isolates from Andisols Tengaran, from Andisols Tengaran + Tawangmangu, and from eight different soil types for producing culture Generation I (GI) on Andisol-based media. Zeolite and Inceptisols were also used as comparison media. The first greenhouse experiment was designed in a Factorial Completely Randomized Design with two factors: media composition (A) with five levels and AM inoculum source (I) with four levels. There were 20 combination treatments with six replications (Table 3).

The media used was 300 g of media/pot, which contained 240 g of soil media according to the composition treatment (80%) and 60 g of zeolite (20%), except for the zeolite media, which contained 100% zeolite (Table 3). The media was then filled into a plastic pot (8 cm diameter and 12 cm depth). Bio-RP nutrition was added only for the media A3 a week before planting. Bio-RP nutrition contained a mixture of liquid cultures of N-fixing and phosphate-solubilizing bacteria at a dosage of 10 ml/pot and rock phosphate at a dosage of 0.04 g/pot.

Two seeds of maize cultivar BISI II were planted per pot culture for all combination treatments at a depth of 3-5 cm. AM inoculums were inoculated with the composition according to the treatments (Table 3) seven days after planting (DAP) with a dosage of 50 g spores/pot culture at a depth of ± 1 cm under the maize roots in the rhizospheric area. During the growing period, soil moisture was maintained at 70% of field capacity. Maize plants in the three replications of pot treatments were harvested at the maximal vegetative phase on 70 DAP. The other three replications were extended 50 days for drying the media with no addition of water for measuring mycorrhizal spore production on 120 DAP (spore density/100 g media).

Observation, measurements, and analysis were conducted for soil chemical characteristics consisting of pH H_2O, pH KCl, pH NaF (electrophotometri method), soil available P (Bray and Kurtz, 1945), C-organic (Walkley and Black, 1934), and total Nitrogen (Kjeldahl method) (Bremner & Mulvaney, 1982). AM infectivity was measured by analyzing AM infectivity with root staining using trypan blue 0.05% in lactophenol (Phillips and Hayman, 1970) and AM spore density 70 and 120 DAP using wet sieving and decantation method (Pacioni, 1992). AM effectiveness was observed from maize growth variables consisting of plant height, fresh and dry weights, and chlorophyll content (Hendry and Grime, 1993). P concentration was measured from the shoot samples by using the acid digestion method with mixtures of nitric and perchloric acids (Zarcinas et al., 1987; Wheal et al., 2011), and P uptake was determined by multiplying the dry weight of the plant shoot by the P concentration of the plant tissue (Duffková et al., 2019).

The population of endophytic symbiotic nitrogen-fixing bacteria and total heterotrophic bacteria were isolated from the leaves of maize. The outer surface of the leaves sample was washed with tap water and immediately disinfected using ethanol 70% followed by sodium hypochlorite (NaOCl) 2.5% and rinsed with sterilized distilled water (Barra et al., 2016). The leave samples were then crushed using mortar and pestle and subjected to the isolation of culturable endophytic bacteria by plating a dilution series of the samples using the spread plate method on Yeast Mannitol Agar (YMA) and Nutrient Agar (NA) media.

Table 1. Sampling locations and soil types for Arbuscular mycorrhizal isolates sources

Location	Soil Type	Coordinate	
		Latitude	Longitude
Sukosari, Jumantono, Central Java Province	Alfisol	7^{0}37'47"S	110^{0}56'51"E
Jatikuwung, Gondangrejo, Central Java Province	Vertisol	7^{0}31'06"S	110^{0}50'42"E
Gondosuli, Tawangmangu, Central Java Province	Andisol	7^{0}39'52"S	110^{0}10'45"E
Rowoboni, Banyubiru, Central Java Province	Histosol	7^{0}15'48"S	110^{0}27'00"E
Karangduren,Tengaran, Central Java Province	Andisol	7^{0}15'51"S	110^{0}26'57"E
Asinan, Bawen, Central Java Province	Oxisol	7^{0}15'58"S	110^{0}27'06"E
Megerbaru, Ceper, Central Java Province	Inceptisol	7^{0}39'20"S	110^{0}44'21"E
Gebangharjo, Pracimantoro, Central Java Province	Entisol	7^{0}51;46"S	110^{0}54'26"E
Kentrong, Lebak, West Java Province	Ultisol	6^{0}29'01"S	110^{0}28'01"E

Table 2. Chemical characteristics of all media

Media type	pH H_2O	pH NaF	pH KCl	Available P (ppm)	Organic C (%)	N total (%)	C/N ratio	AM Spore density/100 g media
Zeolite	8.3	9.2	7.02	7.5	0	0.02	4.92	0
Andisol Tengaran	6.4	10.9	5.05	6.41	5.0	0.31	16.22	2120
Andisol Tawangmangu	6.3	10.9	5.03	6.60	3.8	0.29	13.10	3020
Inceptisol Klaten	7.7	9.3	6.41	8.36	2.3	0.26	8.75	102

Table 3. Greenhouse experimental design for pot culture generation I using Factorial Completely Randomized Design with two factors (5x4) with six replications

First factor	Media composition
A0	Zeolite
A1	Representative media (Andisol Tengaran + 20% zeolite)
A2	Typical media (Andisol Tengaran + Andisol Tawangmangu + 20% zeolite)
A3	Typical media (Andisol Tengaran + Andisol Tawangmangu + 20% zeolite) + Bio-RP nutrition
A4	Inceptisol + 20% zeolite
Second factor	**Inoculum source**
I0	No inoculum
I1	Arbuscular mycorrhizal spores from Andisol Tengaran
I2	Arbuscular mycorrhizal spores from Andisol Tengaran + Andisol Tawangmangu
I3	A mixture of arbuscular mycorrhizal spores from eight soil types (Alfisol, Andisol, Entisol, Histosol, Inceptisol, Oxisol, Ultisol, Vertisol)

Table 4. Greenhouse experimental design for pot culture generation II using Completely Randomized Design single factor consisted of 20 levels with six replications

Reculturing Inoculum GI consisted of 20 levels	
Level	**Description**
A0I0²	Media = A0, Inoculum = GI-A0I0
A0I1²	Media = A0, Inoculum = GI-A0I1
A0I2²	Media = A0, Inoculum = GI-A0I2
A0I3²	Media = A0, Inoculum = GI-A0I3
A1I0²	Media = A1, Inoculum = GI-A1I0
A1I1²	Media = A1, Inoculum = GI-A1I1
A1I2²	Media = A1, Inoculum = GI-A1I2
A1I3²	Media = A1, Inoculum = GI-A1I3
A2I0²	Media = A2, Inoculum = GI-A2I0
A2I1²	Media = A2, Inoculum = G-A2I1
A2I2²	Media = A2, Inoculum = GI-A2I2
A2I3²	Media = A2, Inoculum = GI-A2I3
A3I0²	Media = A3, Inoculum = GI-A3I0
A3I1²	Media = A3, Inoculum = GI-A3I1
A3I2²	Media = A3, Inoculum = GI-A3I2
A3I3²	Media = A3, Inoculum = GI-A3I3
A4I0²	Media = A4, Inoculum = GI-A4I0
A4I1²	Media = A4, Inoculum = GI-A4I1
A4I2²	Media = A4, Inoculum = GI-A4I2
A4I3²	Media = A4, Inoculum = GI-A4I3

Greenhouse Experiment for Pot Culture Generation II (GII)

In the second experiment, the AM culture generation I (GI) product was used as an inoculum source to produce AM culture generation II (GII). The pot culture generation II experiment was designed in Completely Randomized Design (CRD) single factor for re-culturing inoculum GI with 20 levels and 6 replications (Table 4). The media composition for Culture II was the same as the experiment for Culture I. However, the total weight of pot media for culture II was enhanced to 360 g media/pot using a plastic pot with an 11.5 cm diameter and 9.5 cm depth. The AM culture GI as the product of pot culture I used for AM inoculum source for pot culture II with a dosage of 40 g of inoculum per pot culture (10% w/w). The other procedure was the same as in the first experiment for pot culture I. In the experiment of pot culture GII, some main variables were observed, including available P in media, AM infectivity, spore density on 120 DAP, plant height, fresh and dry weights, P concentration in the shoot, and P uptake.

Statistical Analysis

The data were analyzed for ANOVA test with a confidence level of 95%, Duncan's Multiple Range Test (DMRT) to determine differences between treatments, and *Pearson* correlation analysis to measure the relationship between parameters.

RESULTS AND DISCUSSION

The Effect of AM Inoculum Sources on Soil Chemical Characteristics at Various Media Compositions in the Pot Cultures

The interaction of media composition and AM inoculum sources (A x I) did not significantly affect pH. However, the treatment factor of media composition (A) separately showed a highly effective effect on all soil pH variables (pH H_2O, pH KCl, and pH NaF). In contrast, the factor of treating AM inoculum sources (I) affected pH H_2O and pH NaF significantly. This phenomenon is mainly related to the differences in the basic characteristics of each media type (Zeolite, Andisol-based media, and Inceptisol). It is clearly shown in Table 5 that there was no difference in DMRT notation on the pH variables among Andisol-based media (A1, A2, A3). Still, there were differences in DMRT notation in comparing Andisol-based media to Zeolite media (A0) and Inceptisol media (A4).

The interaction of the treatments (A x I) significantly affected organic C, total N, and available P but did not significantly affect the C/N ratio (Table 5). The treatments of AM inoculation of I1, I2, and I3 resulted in the increasing organic-C, total N, and available P on all compositions of Andisol-based media (A1, A2, A3). By comparing with the respective corresponding control among the three Andisol-based media (A1, A2, and A3), the treatment of A1I3 showed the lowest increase of organic-C and total N. Still, it gave the highest increase (14.1%) of available P from 6.44 to 7.35 ppm. The treatment of A2I2 showed the second-highest increase (7.6%) of available P from 6.43 to 6.92 ppm.

On the comparison, media of zeolite, the status of available P in the pots without AM inoculation (A0I0) was at the level 7.74 ppm or higher compared with the same treatment of no AM inoculation on the three Andisol-based media (A1I0, A2I0, A3I0) which was at the range 6.29 - 6.44 ppm. The AM inoculation of I3 on zeolite media (A0I3) significantly increased available P by 15.6% (at the level 8.95

ppm) compared with no inoculation treatment (A0I0), whereas I1 and I2 gave no significant effect. These results indicated that the increase of available P by AM source I3 on zeolite media (A0I3) was higher than those on Andisol-based media (A1I3).

On the Inceptisol media without AM inoculation (A4I0), the level of available P was 8.4% higher than zeolite media (A0I0) and 30.3%–33.4% higher than Andisol-based media (A1I0, A2I0, and A3I0). Compared with A4I0, the AM inoculation treatments on Inceptisol (I1, I2, I3) showed increased available P significantly by 4.3%–4.8%. On Andisol as the main media, the increase in available P was around 4.3%–14.1%. These findings showed that the mycorrhizal effect was more effective in soils with P constraints (Andisols) than soils with minimal P constraints (Inceptisols) in the present study.

In the present study, the Pearson correlation analysis showed a negative and significant correlation between available P with organic C (r = -0.740; p < 0.01) and total N (r = -0.529; p < 0.01). These facts might be caused by the basic differences in chemical characteristics among the media: Zeolite (A0), Andisol-based media (A1, A2, A3), and Inceptisol (A3). Zeolite and Inceptisol were characterized by the content of organic C around 0.2% and 2.4%, which were lower than those in Andisol-based media (4.5%–5%), but the range of available P at the level of 8.11 ppm and 8.65 ppm which were higher than those in Andisol-based media (6.45 ppm–6.82 ppm).

The other phenomenon is shown in Table 5 in the presented data of the interaction between media composition (A) and mycorrhizal inoculum source (I). Although the statistical analysis of DMRT showed the same notation among the treatments of AM inoculum source on zeolite media. However, the treatments of AM inoculation (I1, I2, I3) tended to give higher organic-C and total N compared with no AM inoculation (I0), and the treatment of I3 tended to provide the highest levels. On Andisol-based media, the significant enhancement of available P by AM inoculation, the substantial enhancement in organic C and total N, and the treatment of I3 gave the highest increase. The significant increases of soil organic-C, total N, and available P by AM inoculation were also reported by Qiu et al. (2019) on aeolian sandy soil in reclaimed coal mining subsidence. The increasing available P related to the increasing organic-C and total N in the present study might explain the potential involved mechanism. AM inoculation affected in supporting plant growth, including the intensive development of mycorrhizal roots. The fine root turnover and mycorrhizal external mycelium turnover may act as a fundamental mechanism for transferring root-derived C to enhance soil C input (Godbold et al., 2006).

Furthermore, AM fungi exhibit the functional capability to influence increasing the content and the variety of organic acid exudation from plant roots under varying edaphic environments (Klugh & Cumming, 2007; Ma et al., 2022) and also in elevating the activities of soil enzymes (Qiu et al., 2019; Li et al., 2023). These organic acids are important in increasing available P by mobilizing or releasing P-ion from adsorbed conditions (Borie et al., 2019). In addition the enzymes stimulated the organic matter decomposition in soil.

The Effect of AM Inoculum Sources on Mycorrhizal Parameters and the Population Density of Foliar Endophytic Potential Symbiotic Nitrogen-Fixing Bacteria and Heterotrophic Bacteria at Various Media Compositions in the Pot Cultures

Table 6 shows that the interaction of the treatments (A x I) has a significant effect on AM infectivity (p < 0.05) and spore density at 70 DAP (p < 0.01) and 120 DAP (p < 0.05). Among all Andisol-based media (A1, A2, A3), on the representative (A1) and typical (A2) media, inoculum I3 gave the highest increase in AM infectivity, with the rise in A1I3 and A2I3 were 58.3% and 29.2% compared with the corresponding controls (A1I0 and A2I0). On the typical media + Bio-RP nutrition (A3), the highest AM infectivity was obtained by I2 and I3, both 54.6% higher compared to A3I0. On zeolite (A0) and Inceptisol (A4) as the comparison media, the highest AM infectivity was also obtained by the AM inoculum of I3. The treatments of A0I3 and A4I3 showed 100% and 60% higher AM infectivity compared with A0I0 and A4I0.

The spore density at 120 DAP showed a higher level than that at 70 DAP in all treatments indicating the drying effect on pot cultures for 50 days in producing AM spores. Among Andisol-based media (A1, A2, A3), the three treatments of inoculum sources (I1, I2, I3) showed the same level of increasing spore density on the media A1 and A3. In contrast, on media A2, the treatments of I1 and I2 showed higher spore density than I3. There were no spores on the treatment of I0 (A0I0). This fact was related to no AM infection on the treatment A0I0. This finding explained that the zeolite used in the present study did not contain indigenous mycorrhizal propagules as found in soil media. Notably, the indigenous AM spores contained in the initial Andisols were at a higher level compared with initial Inceptisols, namely on Andisol Tengaran 2120 spores/100 g soil, Andisol Tawangmangu 3020 spores/100 g soil, and Inceptisol 102 spores/100 g soil.

Table 5. Effect of arbuscular mycorrhizal inoculum sources on chemical characteristics of soil/media on various media composition in the pot culture generation I

Combination Treatment	pH H_2O	pH KCl	pH NaF	Organic-C (%)	Total N (%)	C/N ratio	Available P (ppm)
F Values							
A	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
I	0.008**	0.804ns	0.020*	0.00**	0.00**	0.324ns	0.00**
A x I	0.752ns	0.424ns	0.886ns	0.003*	0.023*	0.720ns	0.00**
Media composition (A)							
A0	8.4±0.3c	7.1±0.18c	9.2±0.02a	0.20±0.03a	0.04±0.02a	5.51±4.18a	8.11±0.51d
A1	6.5±0.5a	5.0±0.83a	10.9±0.05c	5.04±0.08e	0.30±0.07c	16.67±0.47d	6.82±0.36c
A2	6.5±0.5a	5.1±0.14a	10.9±0.04c	4.56±0.26d	0.34±0.19d	13.43±0.47c	6.73±0.22b
A3	6.4±0.5a	5.2±0.28a	10.9±0.05c	4.45±0.17c	0.33±0.04d	13.50±1.22c	6.45±0.13a
A4	7.7±0.6b	6.6±0.20b	9.3±0.04b	2.41±0.13b	0.28±0.01b	8.77±0.47b	8.65±0.19e
Mycorrhizal inoculum sources (I)							
I0	7.1±0.9a	5.8±0.91	10.3±0.8b	3.19±1.82a	0.25±0.12a	11.16±4.35	7.06±0.88a
I1	7.1±0.9a	5.8±0.88	10.2±0.8a	3.28±1.84b	0.25±0.11a	11.97±4.20	7.30±0.90b
I2	7.1±0.9a	5.7±0.88	10.2±0.8a	3.37±1.90c	0.26±0.12a	12.15±4.46	7.39±0.87b
I3	7.2±0.9b	5.8±0.89	10.2±0.8a	3.47±1.97d	0.27±0.12b	11.01±4.97	7.66±1.02c
Interaction (A x I)							
A0I0	8.4±0.0	7.2±0.17	9.2±0.06	0.14±0.02a	0.03±0.01a	4.33±1.15	7.74±0.05g
A0I1	8.4±0.0	7.1±0.10	9.2±0.1	0.19±0.02a	0.04±0.02a	6.50±3.59	7.85±0.05g
A0I2	8.4±0.0	6.9±0.06	9.2±0.1	0.17±0.05a	0.03±0.02a	8.13±7.71	7.88±0.06g
A0I3	8.4±0.06	7.3±0.21	9.2±0.1	0.16±0.01a	0.05±0.01a	3.08±0.29	8.95±0.05j
A1I0	6.4±0.06	5.0±0.12	10.8±0.06	4.97±0.03gh	0.31±0.01cde	16.20±0.33	6.44±0.05ab
A1I1	6.4±0.06	5.1±0.03	10.9±0.06	4.97±0.03gh	0.30±0.01cde	16.75±0.43	6.61±0.06cd
A1I2	6.5±0.0	5.1±0.04	10.8±0.06	5.11±0.02h	0.30±0.01cde	17.04±0.52	6.87±0.08e
A1I3	6.5±0.0	5.1±0.10	10.8±0.06	5.12±0.01h	0.31±0.01cde	16.71±0.34	7.35±0.08f
A2I0	6.4±0.0	5.0±0.06	10.9±0.06	4.27±0.02d	0.32±0.01ef	13.21±0.18	6.43±0.05ab
A2I1	6.5±0.06	5.2±0.21	10.9±0.06	4.46±0.09e	0.33±0.01ef	13.65±0.17	6.80±0.05e
A2I2	6.5±0.06	5.1±0.17	10.9±0.06	4.66±0.03f	0.33±0.01ef	13.56±0.17	6.92±0.06e
A2I3	6.5±0.06	5.1±0.15	10.9±0.06	4.87±0.28g	0.34±0.02fg	13.29±0.88	6.77±0.22de
A3I0	6.4±0.0	5.3±0.45	10.9±0.06	4.27±0.04d	0.32±0.01def	13.37±0.54	6.29±0.06a
A3I1	6.4±0.0	5.3±0.29	10.8±0.1	4.37±0.05de	0.31±0.03cde	14.33±1.34	6.45±0.11abc
A3I2	6.4±0.06	5.1±0.20	10.9±0.1	4.48±0.05e	0.35±0.03gh	13.04±1.34	6.49±0.10bc
A3I3	6.5±0.0	5.1±0.21	10.8±0.06	4.68±0.12f	0.36±0.06h	13.25±2.13	6.56±0.08bc
A4I0	7.7±0.1	6.4±0.15	9.3±0.1	2.29±0.05b	0.26±0.01b	8.81±0.30	8.39±0.11h
A4I1	7.7±0.06	6.6±0.12	9.3±0.0	2.42±0.04bc	0.28±0.01bc	8.65±0.23	8.75±0.14i
A4I2	7.7±0.06	6.7±0.36	9.3±0.06	2.45±0.01c	0.27±0.01bc	8.98±0.17	8.78±0.11i
A4I3	7.7±0.06	6.6±0.06	9.3±0.06	2.45±0.22c	0.29±0.01bcd	8.45±0.96	8.79±0.14i

Remarks: ** = $p < 0.01$, * = $p < 0.05$, ns = non-significant; Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test

Table 6. Effect of arbuscular mycorrhizal inoculum sources on AM infectivity, spore density 70 DAP, spore density 120 DAP, population density of potential symbiotic nitrogen-fixing bacteria and total heterotrophic bacteria on various media compositions in the pot culture generation I

Combination Treatment	AM infectivity (%)	Spore density 70 DAP (per 100 g media)	Spore density 120 DAP (per 100 g media)	Potential symbiotic nitrogen-fixing bacteria (log10 CFU/g)	Total heterotrophic bacteria (log10 CFU/g)
F Values					
A	0.00**	0.00**	0.00**	0.00**	0.00**
I	0.00**	0.00**	0.00**	0.00**	0.00**
A x I	0.011*	0.007**	0.039*	0.00**	0.032*
A0I0	0±0.0a	0±0.00a	0±0.00a	4.78±0.01a	4.49±0.00a
A0I1	28±6.93b	130±20.00ab	220±20.00a	4.86±0.07b	4.52±0.00ab
A0I2	35±4.62bc	157±15.28a	263±15.28a	4.89±0.01bc	4.59±0.00ab
A0I3	41±2.31c	130±10.00a	220±20.00a	4.92±0.01cd	4.54±0.01ab
A1I0	60±4.00de	4017±15.28f	5433±40.15c	4.99±0.01ef	4.91±0.01def
A1I1	81±2.31hijk	4007±20.82f	6300±26.58de	5.06±0.01gh	5.05±0.01fg
A1I2	85±2.31hijk	4500±10.00i	6603±15.28de	5.09±0.01h	5.10±0.01g
A1I3	95±9.24k	4347±15.28h	6503±50.77de	5.09±0.02h	5.03±0.01efg
A2I0	72±4.00efgh	4333±101.16h	6380±504.78de	4.95±0.06de	4.79±0.00cd
A2I1	77±4.62fghi	5053±92.38j	6710±181.93e	5.03±0.03fg	5.03±0.01efg
A2I2	76±12.00fghi	5243±51.32k	6767±15.28e	5.15±0.00i	4.91±0.00def
A2I3	93±6.11jk	5040±69.28j	6567±321.46de	5.07±0.00h	4.92±0.01def
A3I0	55±2.31d	4090±78.10fg	5997±681.35c	4.90±0.01bc	4.84±0.02c
A3I1	68±8.00efgh	4083±46.19fg	6040±727.53de	5.05±0.00gh	4.90±0.01def
A3I2	85±12.86hijk	4150±36.06g	6567±57.74de	5.08±0.00h	4.86±0.02de
A3I3	85±2.31hijk	4097±58.59fg	6403±25.17de	5.01±0.01defg	4.95±0.01defg
A4I0	55±14.05d	380±75.50c	473±300.55b	4.90±0.01bc	4.67±0.01bc
A4I1	67±8.33ef	610±79.37d	650±20.00c	5.05±0.02gh	4.93±0.00defg
A4I2	80±4.00fghij	797±15.28e	807±11.55c	5.08±0.01h	4.83±0.00c
A4I3	88±10.58ijk	733±56.86e	750±43.59c	4.97±0.00e	4.92±0.00def

Remarks: ** = $p < 0.01$, * = $p < 0.05$, ns = non-significant; Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test

AM infectivity showed a positive and significant correlation with spore density at 70 DAP (r = 0.632; p < 0.01) and 120 DAP (r = 0.623; p < 0.01), hence the increasing AM infection or infectivity was followed by the increasing reproduction of AM spores on all media. A similar result was found in the study of Fasusi et al. (2021), who reported that the propagation of three AM species in the mixture of sterile soil and blended vermiculite media using maize as a host plant showed that the increasing AM infectivity by those three AM species followed by the increasing spore density.

The results of the Pearson correlation analysis in the present study revealed that AM infectivity and spore density indicated a negative and significant correlation with available P (r = -0.299; p < 0.05 and r = -0.883; p < 0.01). This fact was closely related to the status of available P on Andisol-based media, which was lower than those on zeolite and Inceptisol media and resulted in higher AM infectivity and spore density. Urcoviche et al. (2015) also reported that *G. etunicatum* and *R. clarus* inoculations in *Mentha crispa* on sandy sedimentary rocks soil under low levels of P treatment resulted in higher AM infectivity (61.2% and 43.4%) and higher spore density (38.1% and 15%) compared with AM infectivity and spore density under high P treatment.

The interaction of the treatments (A x I) showed the significant effects on the population density of foliar endophytic potential symbiotic nitrogen-fixing bacteria (endophytic sNFB) (p < 0.01) and foliar endophytic heterotrophic bacteria (p < 0.05) (Table 6). Among all combination treatments, the AM inoculation treatments (I1, I2, and I3) showed significantly higher population density of those two groups of endophytic bacteria compared to no inoculation treatment (I0). Among Andisol-based media, by comparing with each corresponding control, the highest population density of endophytic sNFB was obtained by A1I2 and A1I3 on media A1, A2I2 on media A2, and A3I2 on media A3. The highest population density of endophytic heterotrophic bacteria was obtained by A1I2, A2I1, and A3I3.

The population density of endophytic sNFB and endophytic heterotrophic bacteria on treatment A0I0 (on zeolite media without AM inoculation) was at the lowest level compared to all other treatments on all media. The AM inoculation treatments (I1, I2, I3) on zeolite media (A0) resulted in the same population density of endophytic heterotrophic bacteria. However, the highest endophytic sNFB was yielded by AM inoculum I3. As for Inceptisol media (A4), the highest population density of endophytic sNFB was obtained by A4I2, whereas A4I1 obtained the highest endophytic heterotrophic bacteria. Thus, the treatment with the highest population density of foliar endophytic sNFB on each composition media was not always followed by the most elevated foliar endophytic heterotrophic bacteria.

The treatment with the higher population density of endophytic sNFB and endophytic heterotrophic bacteria in the present study was closely related to the higher AM infectivity and spore density. The Pearson correlation analysis supported these facts that endophytic sNFB and endophytic heterotrophic bacteria had positive and significant correlation with AM infectivity (r = 0.788; p < 0.01 and r = 0.768; p < 0.01) and spore density at 120 DAP (r = 0.723; p < 0.01 and r = 0.807; p < 0.01). Kim et al. (2010) reported similar results, that inoculation of the mixture of three AM fungi *Acaulospora longula, Glomus clarum,* and *G. intraradiaces* on red pepper increased the population density of leaf methylotrophic bacteria significantly compared to the control, but did not significantly increase the population density of leaf total heterotrophic bacteria even though the population density was tended to be higher on AM inoculated plant compared to the control. Agnolucci et al. (2019) resulted that *F. mosseae* inoculation to two wheat cultivars (Odisseo and Saragolla) strongly differentiated the root endophytic bacterial communities and the abundance of each genus from the relevant control.

The growth of endophytic sNFB and endophytic heterotrophic bacteria from different media compositions of pot culture with the same inoculum are presented in Fig. 1a and Fig. 1b. In contrast, the growth of those from the same media composition of pot culture with different inoculum are presented in Fig. 2a and Fig. 2b. It was clearly shown that the morphology and color of the bacterial colonies were different among the media composition of pot culture and inoculum sources, either for endophytic sNFB (on YMA media) or endophytic heterotrophic bacteria (on NA media). Further studies are needed to investigate these differences in the growth of colonies. Herrera et al. (2020) reported that some endophytic bacteria isolated from mycorrhizal root segments of six terrestrial orchids were able to solubilize phosphate, produce siderophore and indole acetic acid, and some others were able to inhibit the growth of potentially pathogenic fungi. In the present study, endophytic sNFB and endophytic heterotrophic

bacteria showed positive and significant correlation with plant fresh weight (r = 0.815; p < 0.01 and r = 0.806; p < 0.01), plant dry weight (r = 0.799; p < 0.01 and r = 0.796; p < 0.01), and P uptake (r = 0.715; p < 0.01 and r = 0.722; p < 0.01). Further investigation is important to elucidate the functional roles of these endophytic bacteria in the interaction with host plants and mycorrhiza.

AM Inoculum Sources on Maize Growth and P Uptake at Various Media Compositions in AM Culture

Maize growth, as observed and measured from plant height, fresh and dry weights, chlorophyll content, P concentration in plant tissue, and P uptake, were significantly affected by the interaction of the treatments of media composition and AM inoculum source (Table 7). Among Andisol-based media, the highest maize growth as presented by all those variables was yielded by the treatment of A1I3 on representative media (A1), A2I2 on typical media (A2), and A3I3 on typical media + Bio-RP nutrition (A3). By comparing those treatments with their respective corresponding controls, namely A1I3 with A1I0, A2I2 with A2I0, and A3I3 with A3I0, it was shown that the treatment of A1I3 gave the highest increases on all maize growth variables. As represented from the main variables of the maize growth, the treatment of A1I3 resulted in the increase of plant height, plant fresh, dry weights, and P uptake by 36.5%, 70.2%, 45.9%, and 143.2% compared to the corresponding control of A1I0, respectively.

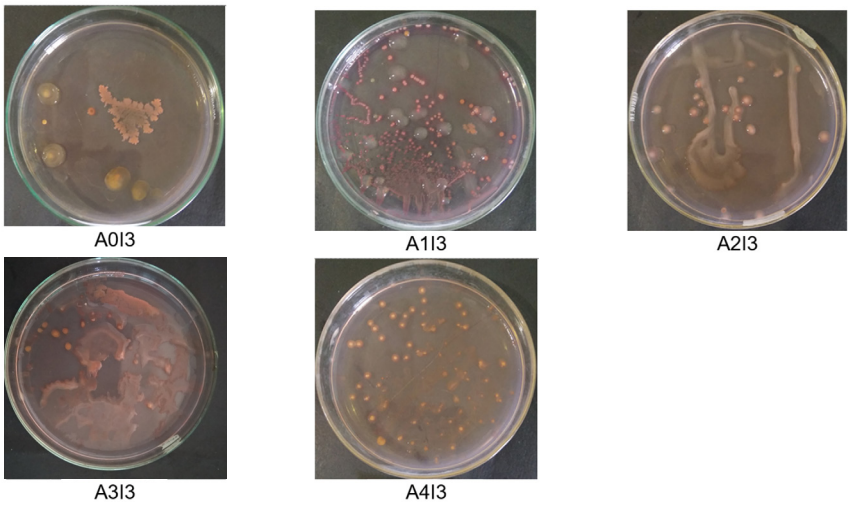

Fig. 1a. The growth of bacterial colonies on yeast mannitol agar (YMA) from the different media compositions of pot cultures of A0, A1, A2, A3, and A4 with the same AM inoculum of I3

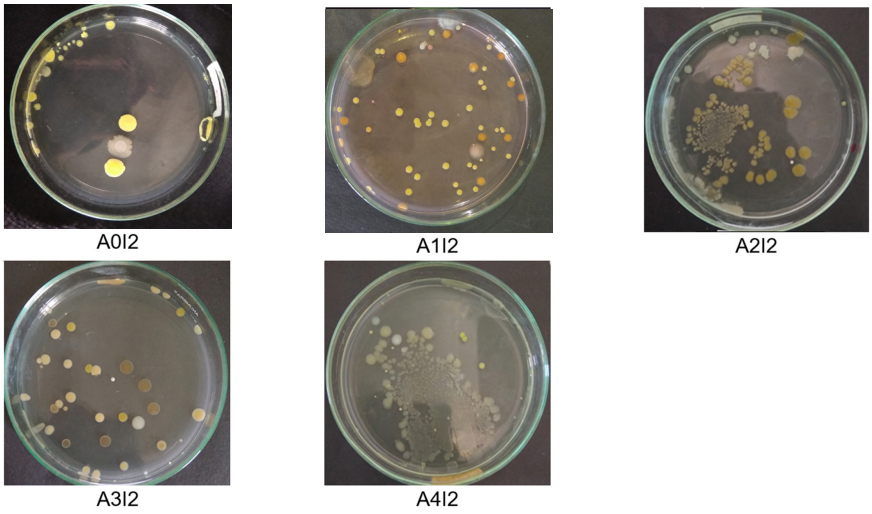

Fig. 1b. The growth of bacterial colonies on nutrient agar (NA) from the different media compositions of pot cultures of A0, A1, A2, A3, and A4 with the same AM inoculum of I2

On zeolite media (A0), compared with no inoculation treatment (I0), the AM inoculation treatments (I1, I2, I3) showed the same notation of DMRT on plant fresh and dry weight. Still, the AM inoculum I3 tended to have higher plant fresh and dry weights and significantly increased P uptake by 380% (Table 7). On Inceptisol media (A4), the AM inoculum of I3 showed especially the highest plant fresh and dry weights and P uptake. The treatment of A4I3 resulted in higher plant fresh and dry weights and P uptake by 67.2%, 34.4%, and 306.2% compared with A4I0, respectively.

The direct and/or indirect involvements of mycorrhiza for the maize grown in the present study could also be explained via the metabolic mechanisms indicated by the increasing chlorophyll content and shoot P concentration (Table 7). The photosynthetic activity was enhanced as stimulated by the increase of the chlorophyll content by AM inoculation. Hyphae of mycorrhiza have been reported in many studies to play an essential role in increasing P uptake, either in the efficient and extensive soil exploration, P uptake, and delivery to the roots (Thonar et al., 2011; Sawers et al., 2017) or in affecting the functional profiles of hyphosphere soil microbiome (Wang et al., 2023). The present study revealed the positive and significant correlation of fresh plant weight and dry weights and P uptake with chlorophyll content (r = 0.791; p < 0.01, r = 0.829; p < 0.01, and r = 0.764; p < 0.01) and shoot P concentration (r = 0.922; p < 0.01, r = 0.931; p < 0.01, and r = 0.930; p < 0.01). These results were supported by Vafadar et al. (2014), who reported that *Glomus intraradices* inoculation yielded significantly higher total chlorophyll and shoot N, P, K concentration compared with control and the increasing of those parameters promoted higher photosynthetic rates and shoot growth, and ultimately improved the biomass of *Stevia rebaudiana.*

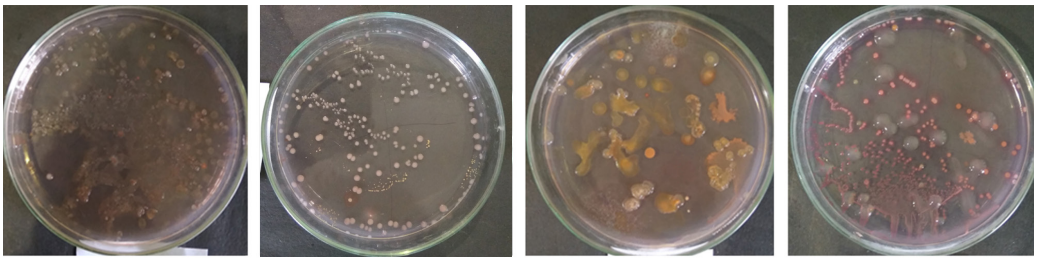

Fig. 2a. The growth of bacterial colonies on yeast mannitol agar (YMA) from the same media composition of pot cultures of A1 with the different AM inoculums of I0, I1, I2, and I3

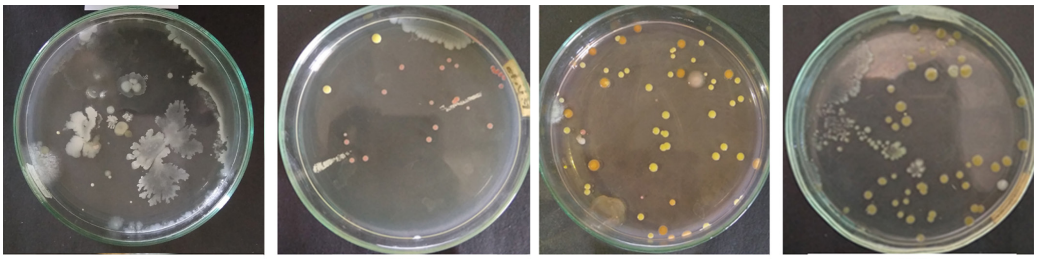

Fig. 2b. The growth of bacterial colonies on nutrient agar (NA) from the same media composition of pot cultures of A1 with the different AM inoculums of I0, I1, I2, and I3

Table 7. Effect of arbuscular mycorrhizal inoculum sources on maize growth on various media compositions in the pot culture generation I

Combination Treatment	Generation I (GI)					
	Plant height (cm)	Plant fresh weight (g/pot)	Plant dry weight (g/pot)	Chlorophyll content (mg/g)	P concentration (%)	P Uptake (mg/plant)
F Values						
A	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
I	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
A x I	0.00**	0.00**	0.011*	0.030*	0.00**	0.00**
A0I0	19.4±1.06a	2.648±0.63a	0.686±0.03a	0.003±0.00a	0.008±0.00a	0.010±0.00a
A0I1	22.2±0.62b	3.052±0.14a	0.766±.09a	0.004±0.00a	0.010±0.00b	0.014±0.01a
A0I2	23.2±0.76b	2.978±0.39a	0.770±0.06a	0.004±0.00a	0.012±0.00c	0.015±0.00a
A0I3	24.0±1.00b	3.584±0.30a	0.966±0.02a	0.005±0.00a	0.012±0.00c	0.048±0.01ab
A1I0	36.7±1.53c	7.414±0.92cdef	2.364±0.09defg	0.101±0.00cde	0.022±0.00e	0.259±0.02efg
A1I1	46.4±0.96ij	8.729±0.51efg	2.708±0.03ghi	0.096±0.01cd	0.027±0.00fgh	0.311±0.00fg
A1I2	44.8±0.68hi	11.193±0.66h	2.980±0.20ij	0.099±0.01cde	0.032±0.00i	0.449±0.01h
A1I3	50.1±0.90m	12.617±1.08h	3.450±0.48l	0.126±0.02de	0.041±0.00k	0.630±0.16i
A2I0	40.2±1.26c	7.414±0.68cdef	2.259±0.20cdef	0.087±0.00bc	0.021±0.00de	0.257±0.02efg
A2I1	46.8±0.62ij	9.134±1.34fg	2.837±0.13hij	0.113±0.03cde	0.029±0.00h	0.340±0.01g
A2I2	47.7±0.58k	11.730±2.00h	3.222±0.32kl	0.123±0.00cde	0.034±0.00ij	0.493±0.03h
A2I3	49.3±0.89kl	9.071±0.48fg	2.816±0.05ghi	0.161±0.04e	0.033±0.00i	0.452±0.02h
A3I0	38.3±0.58cd	5.895±0.59bc	1.584±0.28b	0.098±0.01cde	0.024±0.00d	0.127±0.04bc
A3I1	41.8±1.76efg	7.273±1.08cde	1.965±0.18bcd	0.093±0.02c	0.025±0.00f	0.180±0.03cde
A3I2	41.7±1.53efg	6.708±0.78bcd	1.907±0.18bc	0.099±0.00cde	0.025±0.00de	0.167±0.04cd
A3I3	42.0±1.05efg	7.452±2.24cdef	2.200±0.22cdef	0.105±0.02cde	0.026±0.00fg	0.224±0.03def
A4I0	40.9±1.59ef	5.541±3.39b	2.045±0.08cde	0.066±0.01b	0.021±0.00de	0.129±0.01bc
A4I1	43.2±1.86fgh	8.038±1.55defg	2.538±0.52fghi	0.087±0.01bc	0.028±0.00gh	0.307±0.06
A4I2	43.5±1.06fgh	7.826±0.78defg	2.480±0.48efgh	0.093±0.01c	0.026±0.00fg	0.271±0.06fg
A4I3	43.3±2.91gh	9.262±1.09g	2.749±0.10ghi	0.103±0.01cde	0.036±0.00j	0.524±0.08h

Remarks: ** = $p < 0.01$, * = $p < 0.05$, ns = non-significant; Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test

Table 8. Effect of arbuscular mycorrhizal inoculum sources on available P, AM infectivity, spore density on 120 DAP, plant fresh and dry weights, P concentration, and P uptake on various media compositions in the pot culture generation II

Generation II (GII)							
Combination Treatment	**Available P (ppm)**	**AM infectivity (%)**	**Spore density on 120 DAP (per 100 g media)**	**Plant fresh weight (g/pot)**	**Plant dry weight (g/pot)**	**P concentration (%)**	**P uptake (mg/plant)**
ANOVA	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
A0I0	7.50±0.08e	0±0.00a	0 ±0.00a	2.840±0.26a	0.830±0.01a	0.007±0.00a	0.015±0.00a
A0I1	7.84±0.06f	33±2.31b	210±1.00b	3.451±0.29a	0.963±0.09a	0.020±0.00b	0.057±0.02a
A0I2	7.75±0.09f	44±6.11c	313±1.53c	3.941±0.57a	1.055±0.08a	0.021±0.00c	0.077±0.02a
A0I3	8.92±0.05i	45±4.00c	290±3.61bc	4.218±0.76a	1.094±a	0.021±0.00c	0.082±0.04a
A1I0	6.83±0.08b	64±4.00d	7040±3.61j	17.971±4.03bc	4.871±0.20bcd	0.030±0.00e	0.857±0.16bc
A1I1	6.96±0.08bc	81±2.31efg	7003±1.53i	24.283±1.57cde	6.363±0.75ef	0.031±0.00f	1.228±0.04def
A1I2	6.95±0.05bc	92±8.00ij	7513±1.53kl	24.158±3.63cde	6.216±0.22def	0.034±0.00h	1.266±0.34ef
A1I3	7.47±0.25e	96±4.00j	7287±1.53j	33.469±3.24fg	7.872±1.59gh	0.041±0.00k	2.167±0.03gh
A2I0	6.52±0.05a	64±4.00d	6847±4.73h	12.806±1.45b	4.420±0.14b	0.021±0.00c	0.565±0.04b
A2I1	6.88±0.08bc	84±4.00efghi	7640±6.00m	17.157±0.88bc	4.626±0.16bc	0.025±0.00d	0.701±0.02bc
A2I2	7.20±0.08d	91±2.31hij	7690±5.29m	30.285±5.62efg	6.465±1.23ef	0.035±0.00hi	1.435±0.28f
A2I3	6.99±0.06bcd	88±10.58ghij	6853±13.43h	25.283±4.11cdef	5.429±0.45bcde	0.033±0.00g	1.213±0.15def
A3I0	6.61±0.35a	64±4.00d	6637±11.85g	17.032±3.92bc	4.304±1.18b	0.021±0.00c	0.524±0.12b
A3I1	7.11±0.05cd	85±6.11fghi	7333±4.93j	27.992±6.07defg	6.225±0.55def	0.035±0.00I	1.444±0.23f
A3I2	6.95±0.11bc	77±2.31ef	7393±1.15jk	23.509±3.67cde	5.965±0.69cdef	0.024±0.00d	0.878±0.07bcd
A3I3	7.02±0.06bcd	80±4.00efgh	7430±6.56k	23.513±0.22cde	6.228±0.53def	0.032±0.00f	1.232±0.02def
A4I0	8.36±0.08g	49±2.31c	690±3.51e	20.693±0.08bcd	7.010±0.73fg	0.024±0.00d	0.998±0.10cde
A4I1	8.55±0.11gh	76±4.00e	763±2.52d	34.699±9.36g	8.932±1.55h	0.038±0.00j	2.168±0.46gh
A4I2	8.66±0.14h	83±2.31efgh	813±8.14e	28.872±8.46defg	8.827±1.16h	0.038±0.00j	1.929±0.41g
A4I3	8.96±0.08i	88±4.00ghij	740±5.03f	35.353±10.01g	10.125±0.54i	0.042±0.00k	2.454±0.24h

Remarks: ** = $p < 0.01$, * = $p < 0.05$, ns = non-significant; Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test

The present study confirmed that the treatments which yielded the highest effectiveness on maize growth, namely A1I3, followed by A2I2 on Andisol-based media, A0I3 on zeolite media, A4I3 on Inceptisol media, also yielded the highest AM infectivity and the high level of spore density. Pearson correlation analysis revealed that there was a strong and positive correlation between P uptake with AM infectivity (r = 0.761; p < 0.01) and with spore density at 120 DAP (r = 0.485; p < 0.01). In addition, P uptake showed a positive and significant correlation with soil chemical properties of organic C (r = 0.650; p < 0.01) and total N (r = 0.651; p < 0.01). Thus, this finding explained the contribution of AM in supporting maize growth, especially P uptake, by direct and/or indirect mechanisms via the roles of the uptake capacity of the developing AM hyphae/mycelium and via its influences in inducing nutrients availability in the rhizosphere.

The formula of AM inocula with high effectiveness in increasing maize growth, especially for P uptake or in handling P limitation on Andisols in the present study, was obtained by A1I3 (combination of a representative media of Andisol Tengaran with a mixture of AM inoculum isolated from eight soil types), then followed by A2I2 (combination of typical media (a mixture of Andisol Tengaran and Andisol Tawangmangu) with a mixed AM inoculum isolated from Andisol Tengaran and Andisol Tawangmangu. Compared with the standard commercial zeolite media, the present finding showed that the highest effectiveness on maize growth, especially for P uptake on zeolite media, was obtained by A0I3, followed by A0I2. These findings indicated that AM inocula comprised of more than one source could overcome P constraints on Andisol compared with AM inoculum single source from Andisol Tengaran (I1). Many previous studies have proved that a mixture or a combination of different native AM species induced a better effect in alleviating abiotic stresses and in improving plant fitness than a single species (Zhang et al., 2018; Crossay et al., 2019; Crossay et al., 2020; Parihar et al., 2020). On the contrary, in the previous study, Cahyani et al. (2022) reported that the highest functional ability in supporting maize growth, primarily to deal with P limitation on acid soil-based media, was indicated by a single consortium of AM inoculum from Alfisol (I1) then followed by a mixed consortia of AM inoculum from Alfisols, Oxisols, and Ultisols (I2) on the representative media of Alfisols.

Propagation of the Second Generation of AM Inocula (Pot Culture II) Using AM Cultures Generation I as Inoculum Sources

The principal differences between propagation AM inoculum for pot culture generation I and II were as follows: (1) Media for pot culture generation I: 300 g (consisted of 240 g soil according to the treatment composition and 60 g zeolite), for pot culture generation II: 360 g (with the same composition for the respective treatment); (2) AM inoculum for pot culture generation I: AM spores isolated from natural rhizospheric soil with the source composition according to the treatment, AM inoculum for pot culture generation II: AM inocula from product pot culture generation I (a mixture of AM propagules with the media as carrier).

The ANOVA and DMRT data results in the pot culture generation II (Table 8) showed that the treatments significantly affected available P in soil/media, AM infectivity and spore density, and all maize growth variables. On Andisol-based media, the highest available P, AM infectivity, and maize growth were reached by the treatment of $A1I3^2$, with the value of available P 7.47 ppm, AM infectivity 96%, maize plant fresh weight 33.47 g/pot, plant dry weight 7.87 g/pot, shoot P concentration 0.041%, and P uptake 2.17 mg/plant. The treatment of $A2I2^2$ showed the highest spore density at 120 DAP of 7690 spores/100 g media, whereas the other mycorrhizal and maize growth variables showed the second highest level after $A1I3^2$. As for zeolite media, the treatment of $A0I3^2$ showed the highest level of available P, AM infectivity, and maize growth, as indicated by plant fresh and dry weights, shoot P concentration, and P uptake. The treatment $A0I2^2$ showed the highest spore density, 120 DAP, the same highest level of AM infectivity and shoot P concentration, and the second highest level of other maize growth variables compared with $A0I3^2$. In addition, on Inceptisol media, the treatment $A4I3^2$ showed the highest level of all variables of mycorrhizal and maize growth parameters.

On Andisol-based media in generation II, the increase of four primary variables: soil available P, spore density at 120 DAP, plant dry weight, and P uptake of $A1I3^2$ compared with $A1I0^2$ were 9.4%, 3.5%, 61.6% 152.9%, respectively (Table 8), whereas in generation I, the increase of those main variables of A1I3 compared with A1I0 were 14.1%, 19.7%, 45.9%, and 143.2%, respectively (Table 5, Table 6, Table 7). Thus, plant dry weight

and P uptake were higher in pot culture generation II than in generation I, while the soil available P and spore density variable were higher in pot culture generation I than in generation II. On zeolite media in generation II, the increase in four main variables: available P, spore density, plant dry weight, and P uptake of $A0I3^2$ compared with $A0I0^2$ were 18.9%, 100%, 31.8%, and 446.7% (Table 8), whereas in generation I, the increase of those main variables of A0I3 compared with A0I0 were 15.6%, 100%, 40.8%, and 380% (Table 5, Table 6, Table 7). These findings indicated that on zeolite media, the increase of available P and P uptake in generation II was higher than in generation I. In this pot culture generation II, Pearson correlation analysis revealed that P uptake showed a positive and significant correlation with available P ($r = 0.260$; $p < 0.05$) and AM infectivity ($r = 0.731$; $p < 0.01$).

Based on the obtained data from pot culture generation I and II, the treatment of A1I3, A2I2, A0I3, A0I2 (pot culture generation I) and the treatment of $A1I3^2$, $A2I2^2$, $A0I3^2$, $A0I2^2$ (pot culture generation II) showed as the superior AM inocula and consistent in their effectiveness for two generations as indicated in all mycorrhizal and maize growth parameters.

CONCLUSION

In the experiment of pot culture generation I, the highest maize growth and P uptake on the Andisol-based media, reflecting the highest effectiveness of AM cultures on those media, were reached by the treatments of A1I3 and A2I2. The treatments of A1I3 yielded the highest levels of all variables of AM infectivity, maize growth, and P uptake. In contrast, the treatments of A2I2 yielded the highest AM spore density and the second highest level for the other mycorrhizal and maize growth variables after A1I3. On the comparison media, the treatments of A0I3 and A4I3 indicated the highest effectiveness on maize growth and P uptake on zeolite and Inceptisol media, respectively. The present findings revealed that I3 (AM native isolates from 8 soil types) showed as the superior AM isolates that yielded the highest maize growth and P uptake on all types of media. In addition, the present study also revealed that the highest effectiveness of A1I3, A2I2, and A0I3 on maize growth was supported by the highest population density of endophytic sNFB in the maize leaves of those treatments on the respective media composition.

In the pot culture generation II experiment, for the Andisol-based media, the treatments of $A1I3^2$ and $A2I2^2$ showed the highest AM infectivity and spore density, and the highest effectiveness on maize growth and P uptake. As for the comparison media, the treatments of $A0I3^2$ and $A4I3^2$ also showed the consistently highest efficacy on maize growth and P uptake on zeolite and Inceptisol media, respectively. The percentage increase of maize growth and P uptake of the superior AM cultures in pot culture generation II were higher than in pot culture generation I. Further study is needed to confirm the effectiveness of AM cultures in the application using the targeted plant and soil media.

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