Examination of mycorrhizal inoculum for improving maize tolerance to water stress in pot culture using zeolite and Andisol

V R Cahyanı¹, Suryanti, D F Setiawan, Suntoro, S Minardi, Purwanto and Rahayu

¹Department of Soil Science, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
E-mail: vitaratri@staff.uns.ac.id

Abstract. Arbuscular mycorrhiza (AM) is known as beneficial microsymbiont that capable to support plant growth by increasing nutrient uptake and improving plant tolerance toward diverse adverse conditions, such as water stress. The present study examined single and mixture mycorrhizal inoculum sources for improving maize tolerance to water stress in pot culture using zeolite and Andisol. Pot culture experiment was conducted using a Factorial Completely Randomized Design with three factors: A: Media type (A₀: zeolite, A₁: Andisol), B: AM inoculum sources (B₁: No inoculum, B₂: Inoculum from Andisol, B₃: Inoculum from eight soil types), C: Media moisture level (C₁: 50%, C₂: 70% of field capacity), with three replications. Each pot contained 300 g of media that sterilized using formaldehyde 2%, Maize were grown until maximal vegetative phase (77 days after planting). The results showed that maize growth on Andisol was higher than on zeolite, inoculum from Andisol were resulted in higher effect than a mixture of inoculum from eight soil types. On zeolite media (A₀), inoculum B₁ showed to improve plant tolerance to water stress at moisture level of C₁ as indicated by plant dry weight (PDW) that 38.6% higher than B₂ and 31.9% higher than B₃, whereas on Andisol (A₁), inoculum B₁ resulted PDW 13.4% higher than B₂ and 38.4% higher than B₃. By AM inoculation in pot culture, maize growth on zeolite media at C₁ was lower than at C₂, whereas maize growth on Andisol at C₁ was higher than at C₂ conditions.

Keyword: Andisol, Maize, Mycorrhizal inoculum, Water stress, Zeolite

1. Introduction
Symbiosis of arbuscular mycorrhiza (AM) with plant roots has been shown to improve the tolerance toward water stress in various plant species, such as for sunflower [1], tomato [2], and wheat [3]. The research on the effect of AM to support maize tolerance toward water stress have also been reported by Renxin et al [4] which comprised of two growth substrates (weathered mine spoil and spontaneously combusted mine spoil), three intensities of drought stress (80%, 60%, 40%), and two mycorrhizal inoculations (non-inoculated and inoculated).

The mechanisms to improve drought tolerance by AM can be mediated by the increasing of nutritional status from developing root surface area, especially P [5]; [6]; [7] by increasing resistance to withering [8], by increasing proline accumulation levels in roots [9]; [10]. The other possible mechanism of drought resistance is by increasing photosynthetic activity as detected by the enhancing of photosynthetic pigment content (chlorophyll) [11]; [12].

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Andisol as one type of soil that occupied around 5.4 million Ha in Indonesia [13] and around 124 million Ha in the world [14] is very potential land area to be subjected for intensification and extensification programs to increase agricultural productivity. The main limitation of the utilization of Andisol is P fixation by allophane. The other major constraint for Andisol in Indonesia is the limitation of water availability. Research on the effect of AM in supporting plants in water stress conditions on Andisol soils is still very limited. The present study aimed at examining the effects of single and mixture mycorrhizal inoculums on maize growth at water stress conditions (50% field capacity) and normal moisture conditions (70% field capacity) using two types of media (zeolite and Andisol) in greenhouse pot culture experiment. Zeolite was used for the comparison media, this is very important to be investigated, since commercial mycorrhizal inoculums are commonly cultured on zeolite media.

2. Materials and Methods

2.1. Collecting soil for media of pot culture and rhizosphere soil for inoculum sources
Andisol soil from Tengaran was used as planting media for pot culture experiment at greenhouse of Faculty of Agriculture, Sebelas Maret University. Rhizosphere soils as a inoculum sources were taken from 8 soil types (from 9 locations) as listed in Table 1. The other media type used for pot culture was zeolite that obtained from the hydroponics shop.

| Location       | Soil Type | Geographic coordinate point |
|----------------|-----------|-----------------------------|
|                |           | Latitude | Longitude          |
| Tengaran       | Andisol   | 07°15’51”S | 110°26’57”E       |
| Tawangmangu    | Andisol   | 07°09’37”S | 112°38’14”E       |
| Delanggu       | Inceptisol| 07°39’20”S | 110°44’21”E       |
| Pracimantoro   | Entisol   | 07°51’46”S | 110°54’26”E       |
| Jumantono      | Alfisol   | 07°37’47”S | 110°56’51”E       |
| Jatikuwung     | Vertisol  | 07°31’06”S | 119°50’44”E       |
| Ambarawa       | Histosol  | 07°15’48”S | 110°27’00”E       |
| Tuntang        | Oxisol    | 07°15’58”S | 110°27’06”E       |
| Bogor          | Ultisol   | 06°32’05”S | 106°20’36”E       |

2.2. Isolation and spore enumeration
Mycorrhizal spores were isolated from rhizosphere soils using a modification method of Cahyani [15] with wet sieving method and decantation followed by separation spores from the filtrate with 60% sucrose solution with centrifugation at 3000 rpm for 5 minutes.

2.3. Preparation media for pot culture
Andisol soil samples were air dried and sieved (<2mm), and filled into plastic pots (8 cm diameter and 14 cm in depth) 300 g of soil/pot culture. The second media for pot culture was zeolite that also prepared 300 g/pot culture.

2.4. Pot experiment in greenhouse
Greenhouse experiment in the present research was designed using a Factorial Completely Randomized Design with three factors: the first factor was media type (A) with 2 levels: A₀ : zeolite, A₁ : Andisol. The second factor was mycorrhizal inoculum source (B) with 3 levels: B₀ : no inoculum, B₁ : Inoculum from Andisol, B₂ : Inoculum from eight soil types and the third factor was media moisture level (C) with 2 levels: C₁ : 50%, C₂ : 70% of field capacity. Media in the pots were sterilized using formaldehyde 2% (20 ml/pot) and incubated for a week, then pots were opened for a week. Urea, rock phosphate and KCl were added at the dosages 150 kg urea Ha⁻¹, 100 kg KCl Ha⁻¹ and 150 kg rock phosphate Ha⁻¹ (0.024 g
urea/pot, 0.024 g rock phosphate/pot and 0.018 g KCl/pot). Two corn seeds were planted per pot culture for all treatment combinations with a depth of 3-5 cm. Mycorrhizal inoculum were inoculated on 7 days after planting (DAP) with a dosage of 30 spores (single sources or mixture sources)/pot culture. Plants were treated with watering according to the experimental treatment of moisture levels with 50% and 70% field capacities. Plants were harvested at maximal vegetative phase on 77 DAP.

Effect of the treatments were observed and measured including mycorrhizal effectiveness (plant height, plant fresh and plant dry weight, root fresh and root dry weight, chlorophyll content), mycorrhizal infectivity, and soil pH (H₂O, KCL, and NaF). Analysis of mycorrhizal infectivity was conducted using methods described by Phillips and Hayman [16] and Bierman and Linderman [17]. Data were analyzed by using the SPSS application for analysis of variance (ANOVA) and Duncan’s Multiple Range Test (DMRT) at level of 5%.

3. Results and Discussion

3.1. The results of ANOVA of the effects of media type, mycorrhizal inoculum source and media moisture level to pH of media, mycorrhizal infectivity and plant growth was presented in Table 2.

| Treatments | pH H₂O | pH KCl | pH NaF | MI | PHe | PFW | PDW | RFW | RDW | CC |
|------------|-------|-------|-------|----|-----|-----|-----|-----|-----|----|
| A          | 0.00**| 0.005**| 0.00**| 0.00**| 0.00**| 0.00**| 0.00**| 0.00**| 0.00**| 0.00**|
| B          | 0.00**| 0.00**| 0.00**| 0.00**| 0.765ns| 0.02*| 0.014*| 0.00**| 0.016*| 0.00**|
| C          | 0.014*| 0.100ns| 0.201ns| 0.049| 0.919ns| 0.2ns| 0.976ns| 0.534ns| 0.687ns| 0.111ns|
| Interaction (AxBxC) | 0.969ns| 0.125ns| 0.736ns| 0.03*| 0.827ns| 0.088ns| 0.008*| 0.056ns| 0.314ns| 0.00**|

**: p<0.01; *: 0.01≤p≤0.05; ns: >0.05 (non significant), A: Media type, B: Mycorrhizal inoculum source, C: Media moisture level, MI: Mycorrhizal infectivity, PHe: Plant Height, PFW: Plant Fresh Weight, PDW: Plant Dry Weight, RFW: Root Fresh Weight, RDW: Root Dry Weight, CC: Chlorophyll Content

Based on the ANOVA results, it was found that the interaction of media type, mycorrhizal inoculum source, and media moisture level showed the significant effect to mycorrhizal infectivity (MI), plant dry weight (PDW), and chlorophyll content (CC), and did not affect significantly to the pH of plant media.

The results DMRT 5% on the difference effects among treatment levels of each treatment factors separately are presented in Table 3.

Table 3 showed the significant difference effects from treatment levels of factor of media type (A) and factor of mycorrhizal inoculum source (B) to all variables measured in the present study with exception for factor B on plant height (PHe). Maize growth on Andisol (A₁) was higher than on zeolite (A₀), inoculum from Andisol (B₁) were resulted in higher effect than a mixture of inoculums from eight soil types (B₂). Treatment factor C only affected significantly on two variables of pH H₂O and mycorrhizal infectivity, in which pH H₂O and mycorrhizal infectivity were higher at C₁ than C₂.
3.2. The effect of the treatments to pH of media
Based on ANOVA and followed DMRT 5% (Table 2 and Table 3), the present results indicated that zeolite showed the significant higher pH H₂O and pH KCL than Andisol. According to Sprynskyy et al. zeolite is a mineral consisting of hydrated alumina silicate crystals containing alkali or alkaline soil cations in a three-dimensional framework [18] and zeolites have pores that filled by K ions, Na, Ca, Mg and H₂O molecules [19] For pH NaF, Andisol shows higher value of pH NaF as related that this soil has a high amorphous clay mineral of allophane as supported by Kenan et al. [20].

3.3. The effect of the treatments to mycorrhizal infectivity and plant growth
ANOVA and followed DMRT 5% results (Table 2 and Table 4) showed that the interaction of media type, inoculum source, and media moisture level gave significant effects on MI, PDW, and CC.

Correlation analysis between MI and PDW, between MI and CC, and between PDW and CC, resulted positive correlation with a correlation coefficient of \( r = +0.813, p<0.01 \) and \( r = +0.850, p<0.01 \), respectively. It meant that the increase of MI significantly contributed to the increase of PDW and CC, and the increase of PDW was significantly correlated with the increase of CC. These findings explained the mechanisms played by mycorrhiza for supporting maize tolerance to water stress in the present study.

On zeolite media, MI was higher under 50% field capacity than 70% field capacity. That was accordance with the results of the Zhu et al [21] that under severe drought stress, the AM colonization rates were significantly higher than those observed under well-watered conditions. Treatment inoculum of B₁ (mycorrhizal indigenous of Andisol) resulted in the higher mycorrhizal infectivity than B₂ (Inoculum from eight soil types) and B₀ (no inoculum) under both condition C₁ and C₂. Loit et al. [22] reported the similar findings that the indigenous/the natural AM indicated the higher infectivity comparing with the introduction inoculums after the application of organic matter.

On zeolite media, PDW and CC represented as plant growth variables under C₂ conditions were significantly higher than under C₁ conditions (Table 3). According Liu et al [23], treatment of moisture level at 65–70% field capacity on alluvial soil maintained high photosynthesis thus increasing wheat yields. While, plant dry weight with treatment inoculum of B₁ under condition C₂ (70% field capacity)
showed the highest result than B2 and B0. Niwa et al. [24] in their study showed that indigenous AM fungi as a biotic factor well adapted to the local environment.

Table 4. The effect of the treatments to mycorrhizal infectivity and plant growth

| Treatment   | MI (%)     | PDW (g/plant) | CC (mg/g)  |
|-------------|------------|----------------|------------|
| A0B0C1      | 0±0a       | 0.504±0.180ab  | 0.017±0.004a |
| A0B0C2      | 0±0a       | 0.672±0.041abc | 0.023±0.001ab |
| A0B1C1      | 18±7.6bc   | 0.658±0.082abc | 0.029±0.002ab |
| A0B1C2      | 17±7.6bc   | 0.84±0.061cde  | 0.47±0.003c  |
| A0B2C1      | 15±5.0bc   | 0.475±0.041a   | 0.033±0.002b |
| A0B2C2      | 12±2.9ab   | 0.722±0.131bcd | 0.030±0.004b |
| A1B1C1      | 17±2.9ab   | 0.953±0.197def | 0.064±0.008d |
| A1B1C2      | 18±2.9bc   | 0.850±0.047cde | 0.061±0.002d |
| A1B2C1      | 55±5.0d    | 1.319±0.048g   | 0.147±0.005h |
| A1B2C2      | 47±15.3d   | 1.006±0.142ef  | 0.111±0.016f |
| A2B1C1      | 52±12.6d   | 1.163±0.058fg  | 0.126±0.006g |
| A2B1C2      | 27±10.4c   | 0.966±0.283def | 0.090±0.010e |

Numbers followed by the same letter show no significant difference in 5% DMRT, A: Media type, B: Mycorrhizal inoculum source, C: Media moisture level, MI: Mycorrhizal infectivity, PDW: Plant dry weight, CC: Chlorophyll content.

On Andisol media, MI was higher under C1 conditions than C2 conditions. In general, AM symbiosis alleviates drought stress via direct water uptake and transport through fungal hyphae to the host plants [25] and increase in the root hydraulic conductance [26]. The treatment of inoculum B2 indicated higher mycorrhizal infectivity under both C1 and C2 conditions than treatments of inoculum B2 and B0. The use of indigenous AM (B1) was more infective, this result was supported by Selvakumar et al. [27] that AM colonization by indigenous inoculum was higher than introduced inoculum which showed high environmental adaptation.

On Andisol media, PDW under C1 conditions was higher than under C2 conditions. Sanchez-Romera et al. [28] showed that under drought conditions, the plant might adapt by modifying of the root system and physiological processes such as hydraulic properties to increase water absorption. Inoculum B1 showed the significant higher results of PDW and CC under condition C1 than inoculum of B2 and B0. The present findings explained that the roles of mycorrhiza to support plant growth and plant tolerance were contributed by enhancing photosynthetic activity. Although PFW, RFW, RDW was not significantly affected by the inoculum treatment, however inoculum B1 tended to gave the higher result comparing with B2 and B0. The enhancing root system and root functions were also estimated contributing to support maize tolerance. Estrada et al. [29] reported similar results that native AMF species were more effective to support maize tolerance on saline conditions than the introduced species.

4. Conclusion

Maize growth on Andisol with all the treatment of AM inoculation was higher than on zeolite under both conditions of moisture levels of 50% field capacity (C1) and 70% field capacity (C2). In principal, maize growth on Andisol under condition of C1 was higher than C2, conversely maize growth on zeolite under C2 was higher than C1. On the two media types, zeolite and Andisol, inoculum B1 showed the highest infectivity (as represented by the percentage of root infection) and the highest effectiveness to support plant growth (as represented by plant dry weight (PDW) and chlorophyll content (CC)) under both conditions of C1 and C2. Mycorrhizal infectivity and PDW, mycorrhizal infectivity and CC, and.
PDW and CC showed significant positive correlation, respectively, indicating the strong contribution of AM for supporting plant growth and plant tolerance to water stress.

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