Combined application of native mycorrhizal and cellulolytic fungi to manage drought effects on maize

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Abstract. Drought become the foremost abiotic stress limiting plant growth and leading to crop-yield loss. This study was aimed to evaluate the effect of native arbuscular mycorrhizal (AM) and cellulolytic fungi (CF) on the growth, yield, mycorrhizal colonization, and the phosphorous (P) and proline contents of maize under drought. This study was conducted using a 3 x 3 factorial arranged in randomized complete block design with three replications. The treatments were inoculation of native AM fungi (no AM fungi, Acaulospora tuberculata, and Gigaspora cf. gigantea) and native cellulolytic fungi (no CF, Talaromyces pinophilus strain MR107 and Talaromyces pinophilus isolate OK3SP103P) evaluated under 50% field capacity (FC). Our results clearly indicated that increasing plant height, plant diameter, mycorrhizal colonization, and P content and decreasing proline content were affected either by native AM or cellulolytic fungi, but dry weight of 100-grain was only increased by native cellulolytic fungi. Combined inoculation of native AM and cellulolytic fungi improved P content and mycorrhizal colonization. Gi. cf. gigantea and T. pinophilus strain MR107 were more potential native inoculants to eliminate negative effect of water stress on maize.

1. Introduction

Increasing the global demand of food to feed 9.1 billion people by 2050 [1] may create a food crisis in the future if do not followed by increasing crop production. One of the major constraints to the global crop production is the availability of water [2]. Drought is considered as the main environmental factor limiting plant growth and leading to reduce more than 50% of the average crop-yield [3,4,5] and it becomes increasingly severe in some regions due to the changes in the global climate [6]. This water deficit affects a vast range of plant characteristics including plant morphology, physiology and biochemistry [7] and reduces growth and productivity [8,9,10,11].

Maize (Zea mays L.), an important cereal crop grown all over the world [12], is sensitive to drought stress. Limited water availability negatively affects the performance of maize at almost all growth stages [13] and productivity of maize [13,14]. According to [15], its average annual yield loss due to drought is around 15% of its potential yield. Study of 14 tropical-adapted, registered maize hybrid conducted by [16] showed that grain yield of water-deficit plants were also approximately 62% lower than in well-watered plants.

There may be opportunities to improve plant productivities under water stress. Application of beneficial microorganisms could be among sustainable mitigation practices but there is a lack of information on their soil microorganism functionalities in relation to water deficit. Amongst these microorganisms, arbuscular mycorrhizal (AM) fungi are received increasing attention because of their numerous benefits for their host plants. These fungi are key components of soil microbiota forming symbiotic relationships with the roots most terrestrial plants, including maize [17,18].

Mycorrhizal colonization of the roots has been shown to increase drought tolerance in some plants [19-22]. Recently published reports have indicated that AM fungi ameliorate plant tolerance to
drought stresses through a variety of mechanisms such as enlargement of root areas and stomatal regulation [23-25], improvement of nutritional, physiological, and genetical plant [26,27], and changes in soil structure in terms of the quantity and quality of aggregate stability [28]. Their effect of mycorrhization on plant adaptation to water stress depends on the intensity of the stress and the type of the fungal isolate [29,30,31]. Besides, it is well known that drought adapted and tolerant microbial ecotypes native to a particular soil or environment are the most efficient [32-34] Even, [35] showed that agroforestry trees can serve as a source of AM inoculants to intercropped annuals such maize.

Some soil microorganisms are known to enhance the AMF symbiosis with vascular plants [36-38] but studies about the effect AM and cellulolytic fungi is still rarely reported especially under water stress. Fungi such as Neurospora crassa, Talaromyces cellulolyticos, T. pinophilus, Trichoderma asperelium, T. virens, and T. harzianum had cellulolytic ability [39-43]. The experiment conducted by [43] indicated that dual treatment of native AMF and T. harzianum was better for biomass production of Ricinus communis L. than dual treatment of AMF and Aspergillus niger or mycorrhiza alone. Contrary to previous results, [44] found that co-inoculation of plants with AMF and T. harzianum did not result in an additive effect on plant growth and nutritional status. Even, [45] exhibited negative effect of this CF on the development of AM fungi. Because of these unconcistency results, this study was aimed to test the hypothesis that both native AM and cellulolytic fungi act synergistically to alleviate drought stress on maize by increasing the growth, yield, mycorrhizal colonization, and the phosphorous (P) and reducing proline contents of maize under drought.

2. Materials and methods

2.1. Experimental Design

This experiment was conducted on the basis of a factorial experiment in a randomized complete block design (RCBD) with three replications. The first factor was application of native AM fungi including without AM fungal inoculation, Acaulospora tuberculata, and Gigaspora cf. gigantea. The second factor was cellulolytic fungi (CF) consisting of without CF, Talaromyces pinophilus strain MR107 and T. pinophilus isolate OK3SP103P.

2.2. Soil collection and preparation

Soils (0–20 cm) were collected from Aceh Besar (05°34′25″N, 095°32′1,2″E. 47 m a.s.l). The soils were classified as Typic Dystrudepts according to USDA soil classification (Soil Survey Staff, 2010). The main soil characteristics were pH 5.77 (1:1 soil:water), 1.81% organic carbon (Walkley and Black method), 0.15% total nitrogen (Kjeldhal method), 4.68 ppm (Bray 1 method), 1.92 cmol (+) kg⁻¹ exch. K, 11.94 cmol (+) kg⁻¹ exchangeable (exch.) Ca, and 4.75 cmol (+) kg⁻¹ exch. Mg.

Soils were air-dried after collection, crushed and passed through a 5 mm sieve to remove coarse organic matter and stones. Then, 10 kg soil samples were placed in each plastic pots, 26 cm high and 35 cm in diameter. At each pot, 50 g sterilized siam weed compost and the soil were mixed throughoutly and incubated during seven days. Pots were covered by black plastic and the water status of growth medium was maintained at 100% of field capacity (FC) before water stress treatment. The pots were placed on a screenhouse with the diurnal temperature range between 26°C and 34°C (night and day, respectively).

2.3. Preparation and multiplication of native mycorrhizal and cellulolytic fungi inoculum

These AM and cellulolytic fungi, obtained from rhizosphere of some plant at which soil sampling area, were selected inoculants from previous studies. These native spores of AM fungi (A. tuberculata and G. cf gigantea) were multipliclated in an open pot culture of Z. mays as host and sterile zeolite medium. After two months of plant growth the shoots were eliminated and the cultures were dried gradually for a month. The under-grown part (mycorrhizal roots, fungal spores and mycelium) and the medium was used as a stock culture.
The native cellulolytic fungi isolates (T. pinophilus strain MR107 and T. pinophilus isolate OK3SP103P) used were first grown on Mandel medium in petri plate and were further multiplicated in 250-mL flasks containing 50 mL of Mandel medium in shake culture for 48 h at 28 °C. The Mandel medium contained (NH$_4$)$_2$SO$_4$ 1.4; urea 0.3; KH$_2$PO$_4$ 2.0; CaCl$_2$ 0.3; MgCl$_2$•H$_2$O 0.3; FeSO$_4$•7H$_2$O 0.005; MnSO$_4$•H$_2$O 0.016; ZnCl$_2$•2H$_2$O 0.014; CoCl$_2$•2H$_2$O 0.002; Tween 80 0.2 % v/v. This was prepared following standard procedure (Azcón, 1993).

2.4. Inoculation trials and growing conditions
Ten gram of AM inoculum (having an average of 100 spores and 70% of AM colonization of root) from the stock culture was inoculated at the appropriate pots immediately at sowing time just below the maize seeds whereas the cellulolytic inoculum was applied with 10 mL of the suitable fungal culture ($10^8$) at the soil surface of each pot around the seeds. Three seeds of maize (variety Hybride Bisi 2) previously surface sterilized were sown 2 cm deep into each pots which was covered by the black plastic with a small growing hole at the middle of the pot to reduce evaporation. The plants were thinned to one per pot after seven days.

Plants were maintained under natural light and temperature conditions, and were watered daily to keep soil moisture at 100% of field capacity (FC) until water stress treatment. At the sowing time, amounts of basal nutrient were added i.e. 250, 75 and 100 kg·ha$^{-1}$ as urea, TSP and KCl to keep the water condition.

2.5. Parameters

2.5.1. Plant growth and yield
Plant growth parameter measured consisted of plant height and stem diameter. Plant samples were collected respectively. Half doses of urea and full doses of TSP and KCl were applied during the early stage of planting before the initiation of the drought stress while another part of urea was given 14 days after planting.

2.5.2. Procedure for water stress application
The crops were watered at 100 % of field capacity (FC) until the water stress treatments (30 days after sowing). The methodology for the application of water stress is described by [46, 47] To determine 100% FC, we weighed pots containing 10 kg of dry soil (P1 = 10 kg dry soil) and these pots were then watered until soil saturation (flow of excess water under gravity). The watered pots were covered with black plastic bag to avoid the evaporation and they were soaked for 48 hours in screenhouse. Pots were weighed again in order to determine P2, which was considered as saturated soils. The difference between P2 and P1 (P2-P1) provided the amount of water that was required to saturate the soil, which represents 100% of field capacity. In order to determine the watering volumes for 50% FC, we added to the pots containing dry soil, water volumes corresponding to 0.5 X (P2-P1). Pot cultures containing maize seeds were watered regularly using tap water at 50% FC. Lost water was replaced with fresh distilled water each day at 18:00, at 45 days after sowing (DAS). Plant height was also measured from the ground level to the point of the plant from where the tassel emerges using a steel ruler while stem diameter was measured using Vernier calipers. Maize yields (100 grain) was measured at crop maturity (100 DAS) and presented at 14% moisture.

2.5.3. Proline analysis
Proline was analysed by High-Performance Liquid Chromatographic (HPLC) method. This amino acid was analysed at Environmental Biotechnology Laboratory (EBL), Indonesian Center for Biodiversity and Biotechnology (ICBB), Bogor.
2.5.4. Root colonization
Root colonization by AM fungi was measured after 45 DAS. The subsample of roots for determination of AM colonisation was cleared in potassium hydroxide (KOH, 10% W/V) then stained with ink and vinegar using a modification of the method of Vierheilig et al. (1998) and using a modified line-intersection method for 100 intersections (McGonigle et al., 1990).

2.5.5. P concentration analysis
Tissue phosphorous status was analysed after wet digestion with HNO$_3$ + H$_2$O$_2$ by molybdate blue method.

2.5.6. Data analysis
All data was analysed by analyzed by variance analysis using SPSS statistical software version 22.0. Means were compared by one-way analysis of variance and Duncan's multiple range test at the 5% level of significance.

3. Results and Discussion

3.1. Plant growth and yield
Plant growth as indicated in plant height and diameter under water stress was significantly affected by native AM fungi and maize inoculated with Gi. cf gigantea presented better plant growth (Table 1). Compared to A. tuberculata, this AM fungi improved plant height significantly and plant diameter not significantly. Contrary to the plant growth, there were no significant differences in dry weight of 100 maize grain due to AM fungi inoculation. This study also showed that native cellulolytic fungi significantly improved plant growth and yield under water stress (Table 1). Native cellulolytic fungi (T. pinophilus strain MR107 and T. pinophilus isolate OK3SP103P) led to a significant increase in plant height, plant diameter, and dry weight of 100 grain in comparison to non-inoculated plant under water stress.

Maize growth under water stress was significantly improved by native AM fungi, indicating this inoculant could improve unfavourable conditions caused by water deficit stress and make the plants more tolerant to this stress. These results were in line with [48,49]. Contrary to expectations, there were no statistical differences detected on maize yield, indicating that the grain yield of maize was similar for either AM fungi inoculated or uninoculated treatments. Similar to this result was also reported by [50].

In addition to the intensity of the stress, the effect of mycorrhization on plant adaptation to water stress also depends on the type of the fungal isolate [51] AM fungi species even within the same genus have different effects on plant response to drought stress [52]. This study indicated that Gi. cf gigantea was superior to increased plant growth than A. tuberculata. This result was in agreement with several previous studies [50,52,34] and confirms the results of [53]. Gigasporaceae species having a larger soil mycelium may be better at nutrient uptake than AMF with small mycelia (i.e. Acaulosporaceae) [54].

Inoculation with cellulolytic fungi could also increase significantly maize growth and yield. Both cellulolytic inoculants had similar effects on those parameters. Applying cellulase/cellulolytic fungi to crops has shown to promote plant growth performance [55]. Among cellulolytic fungi studied with regarding to plant productivity, Trichoderma was the most popular. For examples, [56] reported that seeds among three varieties of maize treated with T.harzianum showed a significant higher germination than untreated seeds grown under water stress. A study carried out by [40] suggested that inoculation of T. asperellum not only enhanced the growth and yield of rice, but also significantly increased the number of healthy seed and reduced the percentage of dirty panicle infected seed and empty (unfertile, undeveloped) seed. Another study on clover showed that T. hammatum enhanced plant height and growth rate [57].
3.2. Proline content

Proline content of maize leaf was significantly affected by inoculation of native AM fungi inoculation. Maize inoculated by *Gi. cf gigantea* accumulated lowest proline content (Table 1) and the level of this amino acid at this plant was 14.59% lower than non-inoculated plant. Otherwise, inoculation of native cellulolytic fungi did not affected significantly the proline content of maize leaf (Table 1). Nevertheless, cellulolytic fungi slightly decreased the proline content by 11% dan 4.52% by *T. pinophilus* isolate OK3SP103P and *T. pinophilus* strain MR107 respectively.

This study showed that both AM fungi inoculants significantly decreased proline content in maize under drought. A number of previous studies also show similar results [58-61]. The amount of proline content increased with increase in stress [62] but the lower content of this amino acid in inoculated plant may reflect an increased drought tolerance [36]. Accumulation of proline is an inevitable physiological response of plants experiencing severe water stress [14]. Proline not only acts as cytoplasmic osmoticum but also serves as a reservoir of carbon and nitrogen sources for post stress growth [63]. Contrary to AM fungi, the effect of cellulolytic inoculation on proline content was rarely reported. This study indicated that the proline content in maize inoculated by native cellulolytic microorganisms was lower than uninoculated plant exposed to water stress. This result was in contrast to the experiment by [39] on tomato inoculated with *T. Harzianum*.

3.3. Mycorrhizal colonization

Mycorrhizal colonization of maize root was affected significantly by interaction between myorrhizal and cellulolytic fungi. This interactive effect is presented in Figure 1. Root colonization level of maize varied between 37% and 77%. The best AM fungi inoculant for mycorrhizal colonization was *G. cf gigantea* (Figure 1a). This AM fungi inoculation treatment gave similar effect on mycorrhizal colonization to *A. tuberculata* inoculation treatment regardless of cellulolytic fungi inoculation treatment. Mycorrhizal colonization was least in uninoculated plant either by AM fungi or cellulolytic fungi (as control) but the colonization was increased differently after being inoculated by each cellulolytic fungi.

Table 1. Effect AM and cellulolytic fungi inoculation on plant height, plant diameter, dry weight of 100 grain and proline content of maize

| Treatments | Plant height (cm) | Plant diameter (cm) | Dry weight of 100 grain (g) | Proline content (ppm) |
|------------|-------------------|---------------------|----------------------------|-----------------------|
| AM inoculation |                   |                     |                            |                       |
| Non- AM      | 171.35 ab         | 2.71 a              | 23.64 a                    | 7.62 b                |
| *A. tuberculata* | 168.40 a         | 2.81 ab             | 24.47 a                    | 7.15 ab               |
| *G. cf gigantea* | 174.97 b         | 2.88 b              | 24.42 a                    | 6.51 a                |
| CF inoculation |                   |                     |                            |                       |
| Non- CF      | 167.99 a          | 2.70 a              | 30.28 a                    | 7.52 a                |
| *T. pinophilus* strain MR107 | 175.92 b | 2.81 b              | 33.23 b                    | 7.05 a                |
| *T. pinophilus* isolate OK3SP103P | 170.81 ab | 2.89 b              | 33.21 b                    | 6.71 a                |

Significance

|                      | AM inoculation | CF inoculation | AM x CF |
|----------------------|---------------|---------------|---------|
| *                    | *             | *             | *       |
| **                   |               | **            | ns      |
| ns                   |               | ns            |         |

AM, arbuscular mycorrhizal; CF, cellulolytic fungi; ns, not significant
The effect of cellulolytic fungi inoculation on mycorrhizal was varied depending on AM fungi species (Figure 1b). Mycorrhizal colonization was highest in the uninoculated plant by cellulolytic fungi but AM fungi treatment. At plant inoculated by *T. pinophilus* strain MR107, its coinoculation with both species of native AM fungi gave better effect on mycorrhizal colonization. The different result was indicated by plant inoculated by *T. pinophilus* isolate OK3SP103P where inoculation of this cellulolytic fungi alone or coinoculation with each AM fungi species gave similar effect to mycorrhizal colonization.

The mycorrhizal colonization of maize was successful both for plants inoculated with AM fungi and for those inoculated with cellulolytic fungi and the high rate of root colonization of maize confirmed the compatibility between the plants and the AM fungi. Both AM species had similar effect on mycorrhizal colonization whether the the plants were colonized with cellulolytic fungi or not. Eventhough, *G. cf gigantea* more success (77%) than *A. tuberculata* (62.33%) in colonizing maize root in non-cellulolytic treatments and well cooperate with *T. pinophilus* MR107. The tendency of plants to be colonized by mycorrhizal fungi depends on the strain of mycorrhizal fungi [64]. Besides, both cellulolytic microorganisms also enhance effectively AM colonization either combined with *G. cf. gigantea* or *A. tuberculata*. Therefore, *G. cf gigantea* could be as the best native inoculant AM fungi to improve mycorrhizal colonization in maize root under water stress alone and/or in combination with *Talaromyces pinophilus* MR107. The synergistically effects on this parameter were also observed between AM fungi with other beneficial microorganisms such as *Thiobacillus* [65], *Bradyrhizobium* [66], *Paenibacillus mucilaginosus* [67], and yeast [68].

3.4. *P* content

This study indicated that phosphorus in maize under water stress was significantly affected by interaction between native AM and cellulolytic fungi (Figure 2). As at the mycorrhizal colonization, *G. cf gigantea* was superior to *A. tuberculata* on *P* content (Figure 2a). The highest of plant *P* content was shown at co-inoculation *G. cf gigantea* and *T. pinophilus* strain MR107 while its coinoculation with the other cellulolytic fungi has similar effect to the cellulolytic-uninoculated treatment. Maize inoculated with *A. tuberculata* alone had significant higher *P* content in its tissue than after being coinoculated by each cellulolytic fungi. Inoculation of both cellulolytic fungi at uninoculated plant by AM fungi indicated similar effect on *P* content.

![Figure 1](image-url)  
**Figure 1.** Effect of AM fungi inoculation at different cellulolytic fungi inoculation (a) and effect of cellulolytic fungi at different AM fungi (b) on mycorrhizal colonization of maize root. Means followed by different small letters above the bars (represent standard error) indicate a significant difference at *P* = 0.05. NM, non mycorrhiza; *At*, *A. tuberculata*; *Gg*, *G. cf gigantea*; NC, non cellulolytic fungi; T1, *T. pinophilus* strain MR107 and T2, *T. pinophilus* isolate OK3SP103P.
P content of maize inoculated *T. pinophilus* strain MR107 and *Gi. cf gigantea* had highest value and this value was significantly different from other AM fungi inoculation treatment in the cellulolytic fungi treatments. *T. pinophilus* isolate OK3SP103P also showed better cooperation with *Gi. cf gigantea* to improve P content while *A. tuberculata* increased significantly P content in cellulolytic-uninoculated plan.

AM contribution to plant P uptake has been long studied but there was a limited reports about their interaction with cellulolytic fungi. Many studies revealed improvement leaf P concentration due to the presence of AM fungi [21] and different AM fungi varied in their potential on nutrient acquisition [60]. Improved P uptake and leaf P concentration by AM fungi under water shortage has been postulated as a primary mechanism for the enhanced drought tolerance of the host plants. This study showed that interaction of native AM and cellulolytic fungi was beneficial (p≤0.05) for the P status of maize plants. *Gi. cf gigantea* showed its best effect on P content of maize after being coinoculating with cellulolytic inoculant *T. pinophilus* MR107. The positive effect of interaction between these microorganisms was also reported by Medina [51]. The ability of both microorganisms to improve P uptake was related to their potency as phosphate solubilizer. They secrete phosphatases and several organic acids having ability to solubilize insoluble phosphate.

![Figure 2](image-url)

**Figure 2.** Effect of AM fungi inoculation at different cellulolytic fungi inoculation (a) and effect of cellulolytic fungi at different AM fungi (b) on P content of maize leaf. Means followed by different small letters above the bars (represent standard error) indicate a significant difference at P = 0.05. NM, non mycorrhiza; At, *A. tuberculata*; Gg, *Gi. cf gigantea*; NC, non cellulolytic fungi; T1, *T. pinophilus* strain MR107 and T2, *T. pinophilus* isolate OK3SP103P

4. Conclusions
Coinoculation of native AM and cellulolytic fungi to manage water stress could improve mycorrhizal colonization and P content in maize. Increasing plant height and diameter, mycorrhizal colonization, and P content and decreasing proline content were significantly affected either by native AM or cellulolytic fungi, but significantly increased of dry weight of 100-grain was only by native cellulolytic fungi. *Gi. cf gigantea* and *T. pinophilus* strain MR107 were more potential native inoculants to eliminate negative effect of water stress on maize.

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