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EFFECTS OF *FUNNELIFORMIS MOSSEAE* INOCULATION ON CHILI PEPPER GROWTH UNDER REPEATED DROUGHT STRESS

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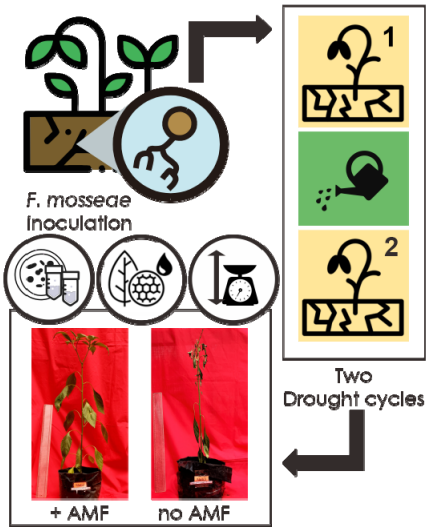
Graphical abstract



Abstract

Chili pepper (*Capsicum frutescens* L.) is a common commodity used as spice and pharmaceutical uses around the world. However, chili pepper cultivation failure often occurs due to drought exposure. The inoculation of arbuscular mycorrhizal fungi (AMF), such as *Funneliformis mosseae,* has the potential to induce defense against drought stress through symbiotic association with plant roots. The aim of this research was to investigate the effects of *F. mosseae* inoculation on the growth of chili pepper under repeated drought stress. Chili pepper plants were exposed to three drought regimes for two cycles, with one rewatering event between the cycles. The plant agronomic variables, physiological performance, and microorganism parameters were observed. The results showed that the plant height, fresh and dry shoot weight, along with fresh and dry root weight increased significantly with *F. mosseae* inoculation under repeated drought stress. The *F. mosseae* treatment also increased water relative content and decreased proline and lipid peroxidation significantly. Although drought exposure decreased the AMF root colonization rate, the total microbial activity and glomalin-related soil protein were still increased by the *F. mosseae* inoculation. However, *F. mosseae* inoculation was negatively correlated to the abundance of phosphate solubilizing microorganisms. The results suggested that *F. mosseae* gave positive effects on *C. frutescens* L. growth under repeated drought stress through induced morphological and physiological responses.

Keywords: Arbuscular mycorrhizae, *Capsicum frutescens* L., drought stress, *Funneliformis mosseae*, symbiotic association

1.0 INTRODUCTION

Chili pepper (*Capsicum frutescens* L.) is one of the everyday used spices around the world. It is commonly used as food additive due to its spicy taste from capsaicinoids [1]. Its high content of carotenoid, ascorbic acid, and flavonoid also increase chili pepper's economic value in the pharmaceutical aspect [2]. However, chili pepper stock shortage often occurs in certain periods due to

water scarcity after prolonged drought, recurring dry periods, or no access to water irrigation [3, 4].

Agricultural water shortage may cause stress to plants which in turn causes growth disturbance and impaired physiology [4, 5]. Drought stress causes damage to plant tissues due to the generation of reactive oxygen species (ROS) such as hydrogen peroxide, malondialdehyde (MDA), and singlet oxygen [6]. Reduced soil moisture by drought also altered soil microbial community, which in turn affects the biogeochemical cycles [7, 8]. Disruption of the cycles may lead to long-term soil degradation [9].

Plants can adapt to drought stress through several mechanisms, such as morphological and physiological responses. Drought stimulates plants to increase root growth to reach water source while shoot growth is delayed to reduce water loss [10, 11]. Some of the plant physiological reponses to drought stress include stomatal conductance change, osmotic adjustment, and increasing antioxidant activities [12]. However, the climate change can increase the severity of drought effects on agriculture [13].

Arbuscular mycorrhizal fungi (AMF) are a group of symbiotic microorganisms that are capable of forming colonies in plant root. AMF colonization has beneficial effects on plants, including drought stress tolerance [13]. The association allows the host plant to increase water and nutrient uptake, enhance the antioxidant system and osmotic adjustment towards water stress, and promote plant growth [4, 5, 14]. The promotion of water and nutrient uptake involves AMF hyphae extension and AMF-induced host plant genetic mechanisms that promote root growth [13, 15]. AMF colonization induces biosynthesis of antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) to ROS accumulation [15]. The glomalin glycoprotein in AMF hyphae and spores also increases soil aggregation to maintain soil moisture [16]. AMF also interacts with other beneficial microorganisms for plants to increase nutrient availability as well as protect plants from pathogen infections [14].

One of the dominant AMF species found in terrestrial plants is *Funneliformis mosseae*. Previous researches showed that *F. mosseae* induces drought resistance in tomato, sunchoke, and orange [14, 17, 18]. Therefore, the present work was done to unveil the effects of *F. mosseae* inoculation on the growth and physiological performance of chili pepper (*C.frutescens*) under repeated drought stress.

2.0 METHODOLOGY

2.1 Materials and Study Area

The experiment was conducted at Research Center for Biology, Indonesian Institute of Sciences (LIPI) from February to May 2021. The chili pepper plantation was performed at a rain shelter (Figure 1) with an average temperature of 36.01 ± 4.61°C and relative humidity of 25 ± 10%. The mycorrhizal inoculum (*F. mosseae*) was obtained from Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). The inoculum was enumerated with *Sorghum bicolor* L. on zeolite carrier prior to the experiment. Each 100 g of zeolite contained approximately 700 spores of *F. mosseae*. Chili pepper *(C. annuum* L.) seeds were obtained from Bogor Agricultural University. The seeds were surface sterilized with 0.05% sodium hypochlorite for 8 min and germinated for 24 h before being sown on polyethylene trays. The soil used for plantation was obtained from empty land around the Institute. The soil was then air-dried for 5 days and pasteurized for 3 h to inactivate the indigenous mycorrhizal spore from the soil [19]. The soil were then assigned for AMF spore count measurement according to Brundrett *et al*. (1996) [18x]. No AMF spores were found on the soil before the experiment when examined under stereomicroscope (Olympus SZ61). The soil pH and amount of total N, available P, and K in the soil were 6.23, 0.09%, 49.54 $mg.kg^{-1}$, and 7.39 $mg.kg^{-1}$, respectively.

Figure 1 Rain shelter used for the experiment

2.2 Experimental Design

The experiment used a completely randomized design with two factors, namely AMF inoculation and drought stress. AMF treatments include seedlings with *F. mosseae* inoculation (M) and those without AMF inoculation (N). The drought stress was divided into three water regimes: 70% field capacity/well-watered (ww), 50% field capacity/moderate drought (md), and 30% field capacity/extreme drought (ed). Each seed was sown for 30 days on 25 g of sterilized compost with the addition of 5 g of zeolite containing AMF inoculum or sterilized zeolite. Diluted (10%) Hoagland's solution was applied once a week as a basic nutrient supply for plant growth. Each seedling was then transferred for plantation in polyethylene bags containing 3 kg of soil. Regular water supply was given to all seedlings for 35 days before drought treatment was performed. The drought treatment

was divided into two cycles. Each drought cycle lasted for 14 days with one week of regular water supply (70% field capacity) between the cycles. Water regimes were maintained using a daily weighing method to retain water content on each polyethylene bag.

2.3 Measurement of AMF Colonization Rate

AMF colonization rates on chili pepper roots were measured at the end of the drought cycles. Briefly, root samples were washed carefully and soaked in 10% KOH at 80°C (30 min). The roots were washed and acidified with 1 % HCl (30 min), then replaced in a new tube containing 0.05% trypan blue in lactoglycerol (w/v) for overnight staining [20]. Stained root tissues were cut into small pieces and examined under a light microscope (Olympus, Japan) [21]. Root colonization rate was measured according to slide method [22] and estimated using a scoring system (score 0 = no AMF present, score 1 = <1% colonization, score 2 = 1–10% colonization, score 3 =11–50% colonization, score 4 = 51–90% colonization, and score 5 = >90% colonization). The scores were then calculated according to Eq 1 [23].

$$RC\ (\%) = (90n_5 + 70\ n_4 + 30\ n_3 + 5n_2 + n_1)/N \quad (1)$$

Whereas RC is the AMF colonization rate, n_5, n_4, n_3, n_2, and n_1 are the number of root pieces obtained a score of 5, 4, 3, 2, and 1, respectively, and N is the total number of observed root pieces.

2.4 Determination of Plant Growth Parameters

Plant growth parameters measured include stem height, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. Stem height was measured using tape measure. Shoot and root dry weight were oven-dried separately at 70 °C for 48 h [24]. Plant growth parameters were recorded at the end of the drought cycles.

2.5 Measurement of Relative Water Content

Leaf relative water content (RWC) was measured according to Krishna *et al*. (2018) [25]. Excised leaves (1 x 1 cm) weight were recorded as fresh weight (FW) and soaked for 4 h for turgid weight (TW) measurement. The leaves pieces were then oven-dried at 80 °C (24 h) for dry weight (DW) measurement. The leaf RWC was calculated according to Eq. 2 [24].

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100 \quad (2)$$

Measurements of RWC were done in day 7 and 14 of the first drought cycle, 4 h after the rewatering event, and day 14 of the second drought cycle.

2.6 Estimation of Proline and Lipid Peroxidation

Measurement of proline content and lipid peroxidation were carried according to [26] with some modifications. Both measurements were done day 7 and 14 of the first drought cycle, 4 h after rewatering event, and day 14 of the second drought cycle. For proline measurement, 25 mg of fresh leaves were ground, mixed with 500 µL of 70% (v/v) ethanol, and heated at 85 °C (20 min). The samples were then cooled at room temperature and centrifuged at 5000 rpm (10 min). The supernatant (100 µL) was mixed with 200 µL of ninhydrin reaction mix (ninhydrin 1% (w/v) in acetic acid 60%, ethanol 20% (v/v)) and heated at 85 °C (20 min). The mixtures were then cooled at room temperature and spun down at 2500 rpm (1 min). Absorbance at 520 nm was recorded and calculated against a standard curve of L-proline 0.04–1 mM (Sigma-Aldrich, Germany). For lipid peroxidation measurement, 0.5 g of fresh leaves were mixed with 4 mL of TCA 1% (w/v) and ground in an ice bath. The samples were centrifuged at 5000 rpm (10 min). The supernatant (1.5 mL) was added with 0.5% (w/v) TBA in 20% (w/v) TCA and heated at 95°C (30 min). The tubes were then quickly cooled in an ice bath and centrifuged at 5000 rpm (5 min). The absorbance of the supernatant at 440, 532, and 600 nm were recorded. Lipid peroxidation measurement was based on the concentration of TBA reactive substances (nmol.g^{-1} DW), calculated using extinction coefficient of 155 mM^{-1}.cm^{-1}. The results expressed as MDA (nmol.g^{-1}).

2.7 Estimation of Glomalin-Related Soil Protein (GRSP)

Glomalin content from AMF was measured at the end of the second drought cycle according to [27] with some modifications. The glomalin quantification was divided into *easily extractable*-GRSP (EE-GRSP) and total-GRSP (T-GRSP) based on the difference of extraction conditions. For EE-GRSP extraction, 1 g of soil samples were mixed with 8 mL of sodium citrate (20 mmol.L^{-1}, pH 7.4), autoclaved (121°C, 30 min). For T-GRSP extraction, 1 g of soil samples were mixed with 8 mL of sodium citrate (20 mmol.L^{-1}, pH 8.0). The mixtures were autoclaved multiple times until the samples were straw-colored. The yield of each autoclavation cycle was pooled for quantification. All fractions were centrifuged at 5000 rpm (15 min). The supernatants were subjected to Bradford protein quantification [28].

2.8 Measurement of Soil Microbial Activity

Soil microbial activity was measured using fluorescein diacetate (FDA) assay according to [29] with some modifications. Briefly, 2 g of soil sample was added with 15 mL of potassium dihydrogen phosphate buffer (pH 7.6) and 200 µL of FDA (1000 µg/mL). The mixture was incubated in a shaker at 120 rpm for (30°C, 60 min). The suspension (950 µL) was transferred to new tube with 950 µL chloroform:methanol (2:1 (v/v)) to stop the reaction and centrifuged at 6000 rpm (6 min). Absorbance

measurement was performed for the supernatant using 490 nm wavelength. The concentration of the fluorescein of each sample was calculated using 0–5 $\mu g.mL^{-1}$ of fluorescein as the standard. The analysis was performed at the end of each drought cycle.

2.9 Measurement of Nitrogen-Fixing Bacteria Abundance and Phosphate-Solubilizing Bacteria

The abundance of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) which are beneficial for plants were measured using total plate count analysis. Suspended soil samples (0.1 mL) from each treatment were subjected to the analysis at the end of each drought cycle. The medium used for microbial enumeration was NFB agar for nitrogen-fixing bacteria and Pikovskaya's agar for phosphate-solubilizing bacteria and incubated for 96 h. NFB colonies show blue color [30]. PSB bacteria are indicated by clear zones around the colonies [31].

2.10 Statistical Analysis

All data were subjected to statistical analysis using SPSS 22 software. The differences among treatments were compared using one-way analysis of variance (ANOVA) followed by Duncan multiple range test post hoc test. Two-way ANOVA was used to determine the significance of the water and AMF treatments and their interaction. Pearson correlation coefficients were determined to assess the relationship between AMF colonization and other variables.

3.0 RESULTS AND DISCUSSION

3.1 AMF Colonization Rate

Figure 2 shows that AMF colonization was found in all treatments. The significantly higher colonization rate in the AMF treatments than the non-AMF treatments indicated that *F. mosseae* was compatible with chili pepper as its host [5]. AMF root colonization can occur in non-AMF treatments due to indigenous spore from the soil [18] or airborne spores contamination [32]. A previous study with air-dried soil showed a possibility of indigenous AMF colonization in roots at a low level [18]. However, no AMF spores were found on the soil before the plantation in this study after the soil pasteurization. Therefore, the slight colonization might originate from the land around the rain shelter [32].

Decreasing the water supply decreased AMF colonization rate on both AMF and non-AMF treatments. Previous studies [13, 24] reported that drought stress might disrupt AMF spore germination, reduce hyphal growth, and limit carbon supply from plants to AMF due to the decrease in photosynthetic rate [13]. However, AMF establishment before drought stress may show plant alleviation to drought stress better than the non-inoculated ones [24]. Therefore, early AMF inoculation was important to be established to influence plant growth and drought stress tolerance [34].

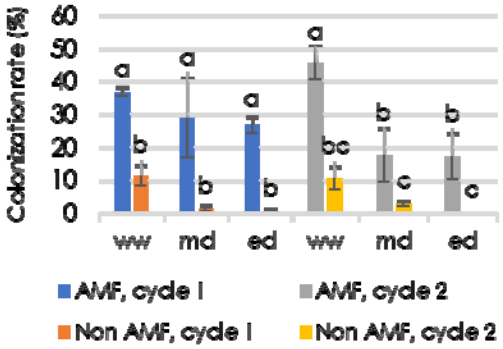


Figure 2 AMF colonization rate (ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters indicate significant differences according to DMRT ($p < 0.05$)

3.2 Plant Growth Parameters

The inoculation of *F. mosseae* did not show any difference in chili pepper shoot height during the first drought cycle. However, significant difference was found on the second drought cycle (Figure 3). AMF colonization showed significant positive correlation with chili pepper shoot height ($r = 0.436$, $p < 0.01$). The inoculation of *F. mosseae* showed an increase in shoot fresh and dry weight compared to those without AMF inoculation. However, no interaction was found between AMF and drought treatment (Table 1). Similar results were also found for the root fresh and dry weight. A significant positive correlation was also found between AMF colonization and SFW ($r = 0.883$), RFW ($r = 0.580$), SDW ($r = 0.876$), and RDW ($r = 0.735$) ($p < 0.01$).

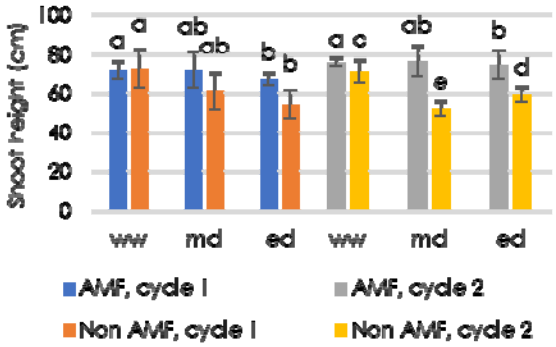


Figure 3 Chili pepper shoot height (ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters indicate significant differences according to DMRT ($p < 0.05$)

Table 1 Biomass comparison (mean ± SD) of chili pepper among treatments (M: AMF-inoculated; N: non-AMF; ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters in each column indicate significant differences according to DMRT. Two-way ANOVA output: ns, not significant; *, p<0.05

Treatment	Shoot Fresh Weight		Shoot Dry Weight		Root Fresh Weight		Root Dry Weight	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Mww	38.38 ± 1.35^{a}	45.69 ± 6.17^{a}	8.56 ± 0.74^{a}	11.23 ± 1.20^{a}	18.22 ± 4.36^{a}	15.19 ± 3.07^{a}	3.45 ± 0.52^{a}	3.77 ± 0.64^{a}
Mmd	38.35 ± 4.94^{a}	31.77 ± 9.21^{b}	8.13 ± 0.88^{a}	7.48 ± 2.05^{b}	7.95 ± 1.54^{b}	10.99 ± 1.66^{b}	2.17 ± 0.22^{b}	2.64 ± 0.47^{b}
Med	23.27 ± 3.77^{b}	25.22 ± 4.56^{bc}	5.47 ± 0.74^{b}	6.04 ± 1.08^{bc}	3.62 ± 0.72^{b}	9.90 ± 2.33^{bc}	1.12 ± 0.20^{cd}	2.20 ± 0.42^{bc}
Nww	25.67 ± 1.22^{b}	21.66 ± 2.43^{bc}	5.31 ± 0.61^{b}	5.35 ± 0.46^{bc}	9.67 ± 4.12^{b}	7.26 ± 1.21^{bc}	1.72 ± 0.37^{bc}	1.78 ± 0.24^{bcd}
Nmd	19.14 ± 2.59^{bc}	18.83 ± 3.76^{c}	4.07 ± 0.37^{bc}	4.49 ± 0.93^{c}	9.47 ± 2.26^{b}	6.10 ± 1.44^{cd}	1.73 ± 0.40^{bc}	1.58 ± 0.37^{cd}
Ned	13.87 ± 2.19^{c}	13.43 ± 2.34^{c}	3.10 ± 0.58^{c}	4.23 ± 1.07^{c}	4.26 ± 0.85^{b}	4.60 ± 0.69^{d}	0.90 ± 0.07^{d}	1.17 ± 0.26^{d}
Significance								
AMF	*	*	*	*	ns	*	*	*
Drought (Dr)	*	*	*	*	*	*	*	*
AMF x Dr	ns	ns	ns	ns	*	ns	ns	ns

Shoot height is essential for plants to acquire optimum sunlight for photosynthesis [34]. Optimum photosynthesis, along with a well-developed root system, can help plants to increase their biomass and productivity. One cycle and repeated drought events reduced plant biomass. Drought reduced the expression of genes that encode tubulin and cyclin, proteins involved in cell division [24]. Water deficit causes disruption in nutrient and water transport from root to shoot and reduces the availability of some nutrients [4, 35].

F. mosseae inoculation in this experiment increased chili pepper shoot height and plant biomass in repeated drought stress as in Figure 4, similar to the result obtained by [4]. The extraradical hyphae of AMF can release lipid droplets to maintain soil moisture and microbial activities [4]. AMF also increased essential nutrients acquirement from soil regardless of the water status [13]. Furthermore, AMF colonization also induced the biosynthesis of growth hormones such as IAA and GA_3 which promote cell elongation and tissue development [36]. AMF colonization increased the uptake of N, P, and K which are essential for plant growth and development [24]. However, AMF-plant interaction is complex and involves dynamics in indigenous microbial communities that may further affect nutrient availability [37].

3.3 Leaf Relative Water Content

Figure 5 displays the RWC of chili pepper leaves. The RWC in all treatments decreased significantly due to the drought stress treatment. On the first 7 days, AMF treatments showed lower RWC than the non-AMF treatments. The lower RWC may be caused by the larger foliar area on AMF-inoculated seedlings that led to ahigher transpiration rate [24].

The longer period of drought stress caused a shift in chili pepper leaf RWC. On day 14 of the first drought cycle, AMF-inoculated seedlings showed slightly higher RWC than the non-inoculated ones. The same pattern can be seen in the rewatering event (Figure 5). RWC is related to many factors, such as stomatal conductance, transpiration rate, osmotic adjustment, and root conductance [15]. Root conductance can change with the increase of aquaporin-coding genes, which play a role in water transport. *F. mosseae* increased the expression of *LeNIP3;1* gene during water stress conditions which encodes constituent of aquaporin [18]. The increased water absorption range from AMF hyphae and AMF-induced root growth also played a role in increasing leaf RWC [15].

At the end of the second drought cycle (Figure 5), significantly higher RWC was found on AMF with extreme drought. No significant differences were found on the other two water regimes with the addition of AMF. Plants can adapt to drought stress to certain conditions with increasing osmolyte synthesis [24]. However, under extreme drought, AMF showed higher RWC than the non-inoculated seedlings due to the additive effects of AMF induced-drought tolerance and increased water absorption area [3]. AMF colonization showed significant positive correlation with RWC ($r = 0.335$; $p < 0.05$). This confirms that AMF colonization played an essential role in improving chili pepper RWC.

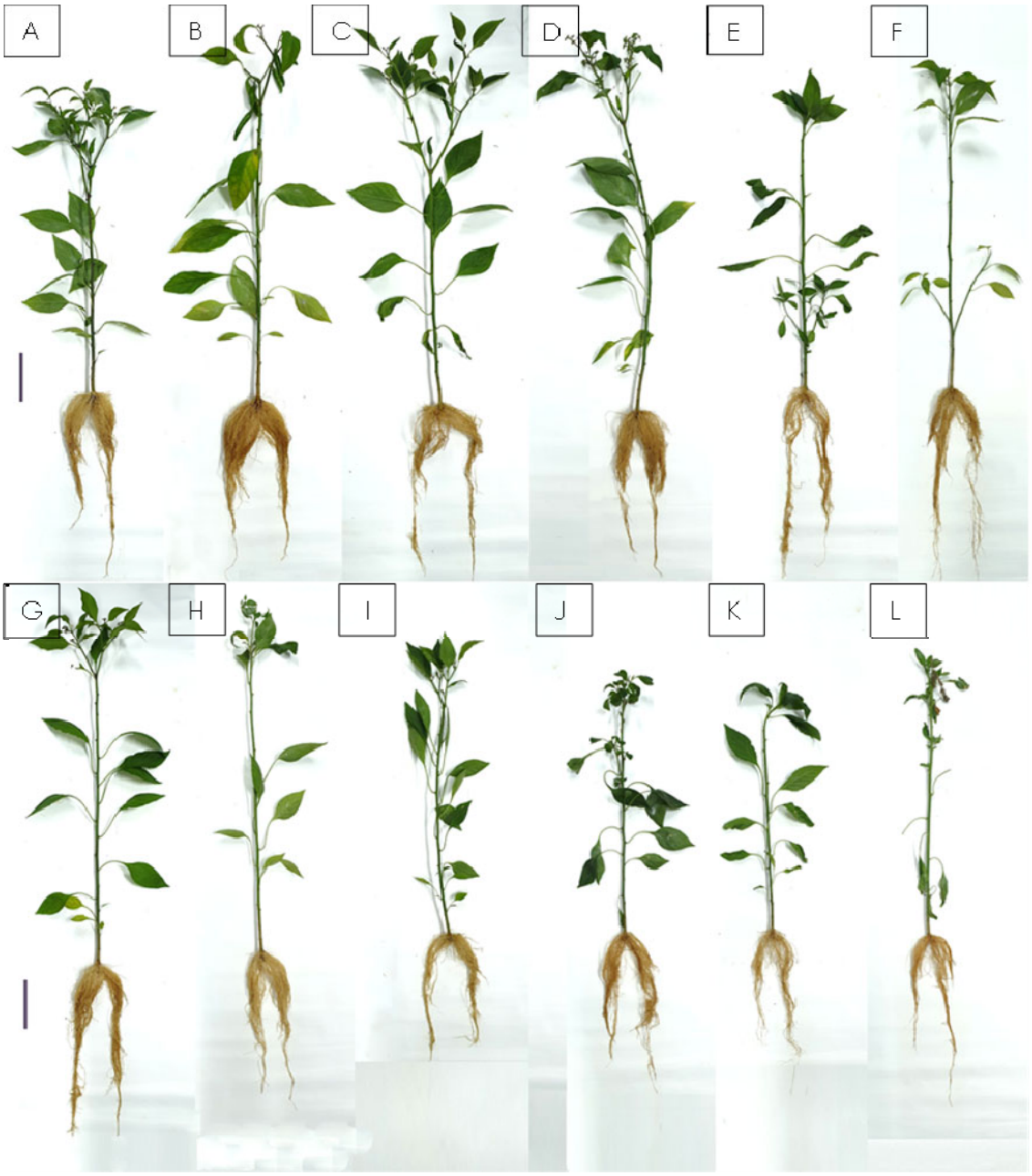


Figure 4 Chili pepper morphology comparison. A. AMF + ww (1 cycle), B. AMF + ww (2 cycle), C. AMF + md (1 cycle), D. AMF + md (2 cycle), E. AMF + ed (1 cycle), F. AMF + ed (2 cycle), G. non-AMF + ww (1 cycle), H. non-AMF + ww (2 cycle), I. non-AMF + md (1 cycle), J. non-AMF + md (2 cycle), K. non-AMF + ed (1 cycle), L. non-AMF + ed (2 cycle)

3.4 Proline Content and Lipid Peroxidation

Proline content of each treatment is displayed in Figure 6A. Proline is a common osmolyte used as a drought stress indicator in plants. Less watering often results in proline increase to adjust plant cell osmotic balance [14], similar to the results obtained in this experiment. No significant difference was found between AMF and non-AMF seedlings' proline content on day 7. However, AMF-inoculated seedlings showed lower proline content than the non-inoculated ones after 14 days of extreme drought. This result suggested that *F. mosseae* colonization may alleviate the drought stress effect on chili pepper.

Rewatering event lowered proline content of all treatments (Figure 6A). All treatments showed similar proline content, except for the non-AMF seedlings, which were previously treated with extreme drought. Rehydration can lower proline content [38]. This result confirms that *F. mosseae* colonization increase water absorption faster than the non-AMF ones.

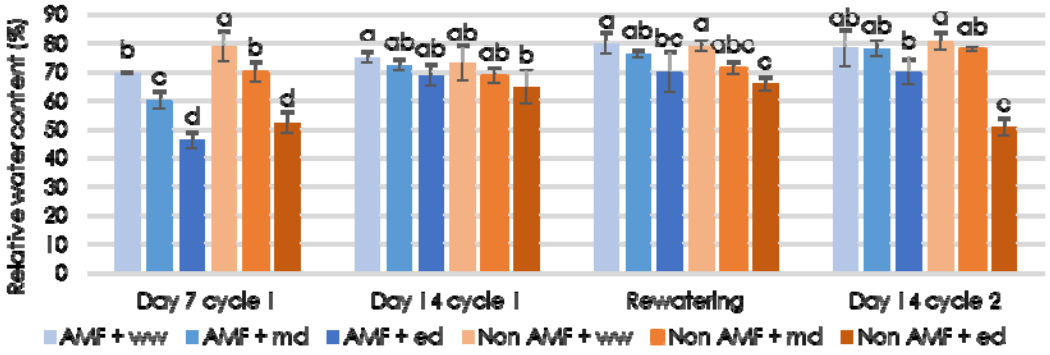


Figure 5 Leaf relative water content (ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters indicate significant differences according to DMRT ($p < 0.05$)

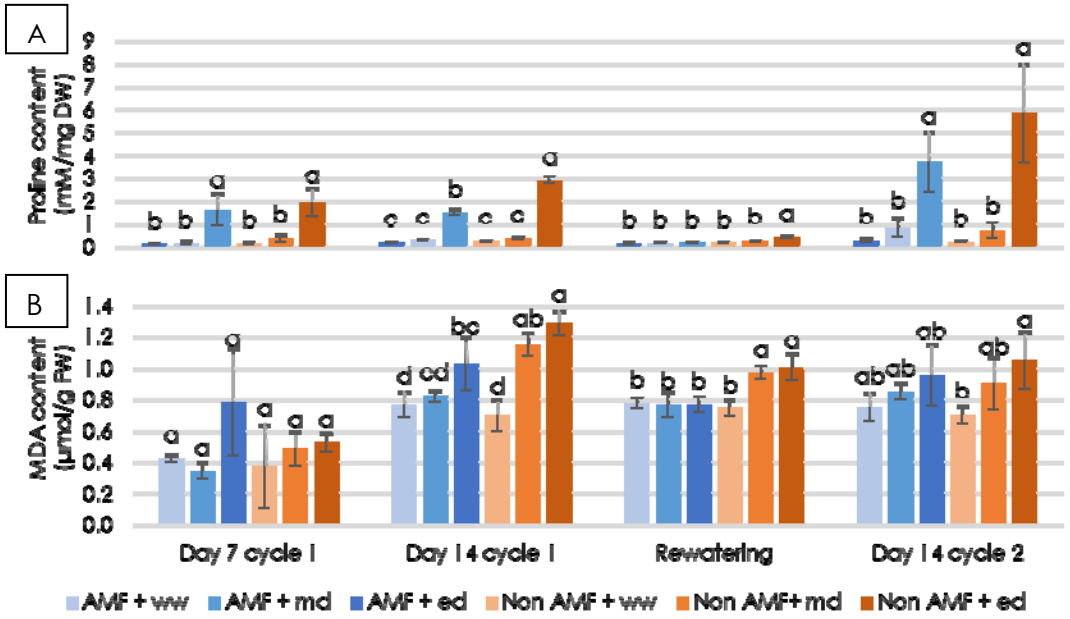


Figure 6 Proline and MDA content (mean ± SD) of chili pepper leaves (ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters indicate significant differences according to DMRT ($p < 0.05$)

The following drought cycle increased the proline content higher than before (Figure 6A). The highest proline content was found on the non-AMF seedlings with extreme drought treatment, but not significantly higher than the AMF-inoculated ones. AMF colonization in this experiment showed significant negative correlation with proline content ($r = -0.390$, $p < 0.01$). This was in line with a previous study [4] that reported significantly reduced proline content in AMF-inoculated bell pepper treated with two drought cycles. Lower proline content indicated a milder effect of water stress on plants [14].

The lipid peroxidation quantified by MDA is displayed in Figure 6B. Lipid peroxidation level indicates the severity of oxidative damage on plant cell due to the generation of ROS after certain stresses such as drought [39]. The experiment showed that the highest MDA content was found on AMF-inoculated seedlings with extreme drought treatment. However, the differences among all treatments were not statistically significant. The higher MDA content may be caused by the larger foliar area on the AMF-inoculated seedlings that can increase transpiration rate [13, 39]. The higher MDA content may also be caused by the relatively short duration of drought stress that *F. mosseae* had not successfully established effective root colonization to give significant effect on chili pepper [40].

MDA content on day 14 of drought cycle 1 was significantly higher on the non-AMF seedlings, except for the well-watered condition (Figure 6B). This result indicated the role of AMF colonization in lowering lipid peroxidation in chili pepper. The MDA content during rewatering was also found lower in all AMF-inoculated seedlings compared to the non-AMF seedlings, indicating that AMF can reduce water stress damage faster than the non-inoculated ones [26].

In the second drought cycle, MDA content of all seedlings increased (Figure 6B). No significant

difference was found on the moderate drought stress, but the extreme drought showed that AMF-inoculated seedlings had slightly lower MDA content than the non-inoculated ones. The result was also supported by the less severe wilting symptoms found on AMF-inoculated seedlings than the non-inoculated ones in this experiment (Figure 7). A strong negative correlation (r = - 0.447, p < 0.01) was found between AMF colonization and MDA content. Earlier studies [41, 42] reported that AMF decreases lipid peroxidation through increasing root membrane permeability and antioxidant activity.

Figure 7 Wilting comparison among treatments. A. AMF + ww, B. AM +md, C. AMF + ed, D. non-AMF + ww, E. non-AMF + md, F. non-AMF + ed

3.5 GRSP Content

Easily extracted (EE) and total (T) GRSP content from the soil of the well-watered and extreme drought treatment (second drought cycle) is shown in Figure 8. EE-GRSP and T-GRSP were found on the soil of all treatments. The plants inoculated with *F. mosseae* showed slightly higher EE-GRSP than those without such treatment. However, no significant difference was found in T-GRSP content. T-GRSP reflects the accumulation of GRSP for a long time that it bounds to the soil particles more intrinsically [27]. AMF colonization showed non-significant positive correlation with EE-GRSP content (r = 0.453) but significant positive correlation with T-GRP content (0.632, p < 0.05).

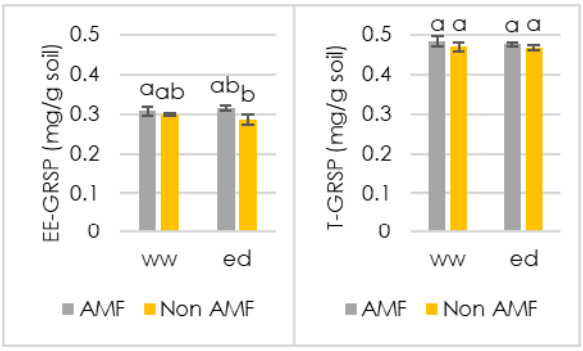


Figure 8 EE-GRSP and T-GRSP content from the second drought cycle (ww: well-watered; ed: extreme drought).. Different small letters indicate significant differences according to DMRT ($p < 0.05$)

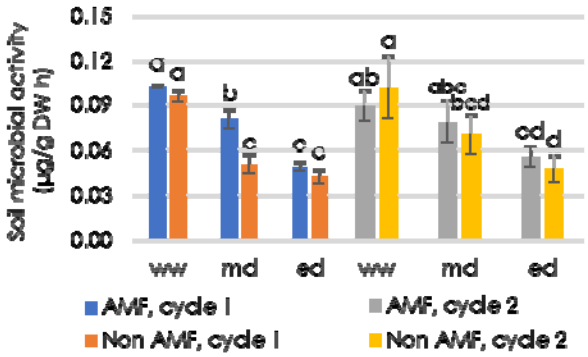


Figure 9 Soil microbial activity (ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters indicate significant differences according to DMRT ($p < 0.05$)

The result indicated that the newly deposited glomalin (quantified as EE-GRSP) belongs to *F. mosseae* inoculated in this experiment. Glomalin plays role in promoting soil aggregate stability, maintain soil moisture, and improved gas exchange for root growth [13]. Low soil water content can limit nutrient solubility and availability to be absorbed by plant root [43]. The result indicated that the glomalin released by *F. mosseae* took part in increasing soil moisture and therefore, increased soil nutrient availability for chili pepper plant growth in drought conditions.

3.6 Soil Microbial Activity

Soil microbial activity that was quantified by FDA assay is displayed in Figure 9. Drought reduced soil microbial activity, regardless of the AMF status. In well-watered condition, the differences in soil microbial activity were not significant. However, *F. mosseae* inoculation showed significantly higher microbial activity in moderate drought and slightly higher microbial activity in extreme drought in both drought cycles. A similar result was found by a previous report [5] who reported that AMF promoted soil microbial activity. AMF colonization showed strong significant positive correlation with soil microbial activity ($r = 0.519$, $p < 0.01$).

Soil microbial activity can be used as a soil quality indicator based on beneficial microorganisms and organic material [44]. The higher microbial activity on the AMF treatments indicated that *F. mosseae* inoculation could maintain soil moisture and well-aggregated soil which was beneficial for microbial enzymatic activity. The glomalin produced by AMF may play role in promoting suitable condition for soil microbial activity [13]. In addition, AMF provided carbon stock in the soil that contributed to microbial biomass increase [45]. Exudates released by AMF are known to act as interactive agents for rhizosphere microbiota.

3.7 Soil NFB and PSB Abundance

NFB and PSB abundance in the soil are displayed in Table 2. Drought increased NFB and PSB abundance in the soil, regardless of the AMF status. AMF inoculation showed higher NFB abundance than those without such treatment. However, the value was not statistically significant. AMF colonization did not show significant positive correlation ($r = 0.263$) with soil NFB abundance. NFB abundance is affected by several factors, such as soil chemical elements and soil texture. Diazotrophs (free-living NFB) require an anaerobic environment to enable their nitrogenase activity. They are mainly concentrated in the clay part of soil particles [46]. This explains the higher abundance in the soil of AMF-inoculated plants which have higher soil moisture than the non-inoculated ones. AMF is also capable of forming synergistic association with certain types of NFB, such as *Azospirillum* spp. [47]. The increasing abundance of NFB on the less-watered treatments may be caused by aerobic NFB such as Actinobacteria as drought increases air exchange into the soil [48].

AMF inoculation decreased the abundance of PSB. The differences were not significant in the first drought cycle, but became significant in the second cycle. AMF colonization showed a strong significant negative correlation ($r = -0.456$, $p < 0.01$) with PSB abundance. This is in contrary to a previous study [49] that found synergistic interaction between AMF and PSB . The difference may be caused by the high level of available phosphorus (P) used at the beginning of the experiment. AMF might compete with PSB when soil available P is low and both performing synergistic interaction when the available P is increased [50]. More research about the interaction between AMF and PSB in high soil P level is required to understand their ecology in providing P for plants.

Table 2 Soil NFB and PSB abundance (mean ± SD) x 10^5 CFU per soil DW (M: AMF-inoculated; N: non-AMF; ww: well-watered; md: moderate drought; ed: extreme drought). Different small letters in each column indicate significant differences according to DMRT. Two-way ANOVA output: ns, not significant; *, p<0.05.

Treatment	Nitrogen-fixing bacteria (x10^5 CFU/g soil DW)		Phosphate-solubilizing bacteria (x10^5 CFU/g soil DW)	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Mww	29,24 ± 11,19^{ab}	49,76 ± 5,50^{a}	4,73 ± 2,80^{b}	3,52 ± 0,17^{d}
Mmd	51,23 ± 14,32^{a}	50,69 ± 5,89^{a}	12,63 ± 4,78^{ab}	9,17 ± 1,60^{c}
Med	54,91 ± 13,55^{a}	60,10 ± 9,47^{a}	21,29 ± 7,38^{ab}	23,02 ± 1,19^{b}
Nww	17,21 ± 11,64^{b}	33,05 ± 13,10^{a}	13,26 ± 7,22^{ab}	5,03 ± 1,61^{d}
Nmd	31,41 ± 15,16^{ab}	40,35 ± 18,55^{a}	22,20 ± 12,18^{ab}	9,82 ± 1,59^{c}
Ned	49,92 ± 8,26^{a}	46,11 ± 12,80^{a}	27,15 ± 13,27^{a}	41,14 ± 2,91^{a}
Significance				
AMF	ns	ns	ns	*
Drought (Dr)	*	ns	ns	*
AMF x Dr	ns	ns	ns	*

4.0 CONCLUSION

F. mosseae inoculation gave positive effects to the growth and physiological performance of chili pepper (*C. frutescens* L.) under repeated drought stress. The growth promotion can be observed from increased root biomass for improved water and nutrient uptake. In addition, *F. mosseae* maintains soil moisture and soil microbial activities which are beneficial for plant growth. The reduced proline and lipid peroxidation by *F. mosseae* inoculation indicates drought stress alleviation in chili pepper. The present study suggests that AMF application has the potential to mitigate crop failure due to drought stress. Further studies are required to investigate AMF role in mitigating drought stress effects on other agricultural species and under field conditions.

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