

Influence of Different Encapsulation Types of Arbuscular Mycorrhizal Fungi on Physiological Adaptation and Growth Promotion of Maize (*Zea mays* L.) Subjected to Water Deficit

Suravoot YOOYONGWECH¹, Suriyan CHA-UM^{2*}, Rujira TISARUM², Cattarin THERAWITAYA², Thapanee SAMPHUMPHUNG², Supatida AUMTONG³, Jeerun KINGKAEW¹, Muenduen PHISALAPHONG⁴

¹Mahidol University, Kanchanaburi Campus, School of Interdisciplinary Studies, Kanchanaburi 71150, Thailand; suravoot@gmail.com; atomic_jeerun@yahoo.com

²National Science and Technology Development Agency (NSTDA), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani 12120, Thailand; suriyanc@biotec.or.th (*corresponding author); rujira.tis@biotec.or.th; cattarin.the@biotec.or.th; thapanee@biotec.or.th

³Maejo University, Faculty of Agricultural Production, Chiang Mai 50290, Thailand; suphathidaaumtong@yahoo.com

⁴Chulalongkorn University, Faculty of Engineering, Department of Chemical Engineering, Bangkok 10330, Thailand; Muenduen.p@chula.ac.th

Abstract

Under drought environment, arbuscular mycorrhizal fungi (AMF) can serve as a long-term biofertilizer to sustain the water and nutrient availability for the host plants. A study was conducted to check the effect of AMF and the encapsulations of the AMF and an organic fertilizer (Fer) with alginate (Al-FA) and agar-agar (Ag-FA) on maize (*Zea mays* L.) in response to water deficit conditions. The maximum quantum efficiency of PS II (F_v/F_m) of the maize inoculated with Al-FA and Ag-FA under the water deficit was recorded to be 0.70 and 0.50, respectively. Shoot and root water content of the Al-FA plants were found to be maintained under the water deficit and were better than Ag-FA. Besides, phosphorus content in the root tissues of the Al-FA plants grown under the water deficit stress was 1.56-folds greater than in the Ag-FA plants, thereby promoting the photosynthetic abilities and plant height in the former case. The study indicated that the Al-FA type of encapsulation may perform better than the Ag-FA in case of maize plants, leading to its better development under water limited conditions.

Keywords: arbuscular mycorrhiza; encapsulation; maize; organic fertilizer; water deficit

Abbreviations: Ag-FA: AMF plus Fer coating by agar-agar; AMF: arbuscular mycorrhizal fungi; Al-FA: AMF plus Fer coating by alginate; F_v/F_m : maximum quantum efficiency of PS II; Fer: organic fertilizer

Introduction

In the scenario of climate change, drought has been reported as one of the major environmental concerns (Wheeler and von Braun, 2013; Coletto *et al.*, 2014; Gauthier *et al.*, 2014; Daryanto *et al.*, 2017). Evidence of water deficit or drought stress are indicated by plant responses such as changes in water uptake, photosynthetic efficiency, and morphological adaptations (Rampino *et al.*, 2006; Suzuki *et al.*, 2014; Rigano *et al.*, 2016). During drought, there is a decrease in the capacity of nutrient uptake due to reduce on uptake rates and fine root biomass (Kreuzwieser and Gessler, 2010).

Microbial assistance in rhizosphere may help to improve the adaptability of crop plants during severe stress conditions (Barea *et al.*, 2005). In case of an association of AMF and plant, the AMF develops into the root cortical cell and participates in the nutrient exchange that is reflected through the enhanced plant productivity (Smith and Read, 2008; Park *et al.*, 2015). On the other hand, AMF inoculation can reduce the water and fertilization requirements and restore leaf hydration under drought, hence making its production more profitable (Ruiz-Lozano, 2003; Mena-Violante *et al.*, 2006). For field applications, use of gel encapsulation procedures for AMF isolated vesicles and internal hyphae or spores are proved to preserve an ability of AMF to regrow hyphae, especially under stressful environment conditions (Plenchette and Strullu,

2003; John *et al.*, 2011; Herrmann and Lesueur, 2013). The encapsulation methods used in practice include thermal gelation, ionic gelation (beads and hollow beads), spray drying and complex coacervation (Vemmer and Patel, 2013). In general, there is no report regarding the comparative efficacy of different encapsulation methods. Only the effects of with or without Na-alginate encapsulated AMF inoculation in the soil has been widely studied (Vassilev *et al.*, 2001; de Jaeger *et al.*, 2011). Furthermore, a part of the encapsulation expected for friendly farmer users and effectively reflected for healthy plants and growth stimulation (Vassilev *et al.*, 2015). By the way, several studies have demonstrated that AMF inoculation acts as long-term fertilizers for maize growth (Toljander *et al.*, 2008; Borriello *et al.*, 2012). Besides, AMF inoculation together with fertilizer application can save an enormous amount of fertilizers apart from increasing the vegetative biomass and yield in wheat (Sharma *et al.*, 2011).

To obtain more information about the AMF applications, the effect of different types of encapsulations of AMF plus organic fertilizer on responses of chlorophyll content and maximum quantum efficiency of PS II (F_v/F_m), total phosphorus content, free proline content and growth characters of maize grown under water deficit was investigated.

Materials and Methods

Encapsulated fertilizer materials

Co-encapsulated beads consisted of AMF and sterilized organic fertilizer (Donk Bua[®]), were totally used 2.5 g of the fertilizer and 4 g of AMF powder (100 spores pot^{-1}). The encapsulated beads were coated by calcium alginate (Al-FA) or agar-agar (Ag-FA). In addition, the AMF powder (Micoriza[®], 25 living spores g^{-1} of *Glomus* sp. and *Acaulospora* sp.) procured from Department of Agriculture, Ministry of Agriculture and Cooperative (Thailand) was used for inoculation as per the method of Yooyongwech *et al.* (2016).

Plant materials and experimental treatments

Maize cv. 'Supreme' ('Kreu-ang Bin[®]') seeds were purchased and germinated in plastic pots containing soil under controlled greenhouse conditions with average temperature of 28.7 °C and 51.2% relative humidity (RH). Irrigation was provided with droplet system at a flow rate of 2 L h^{-1} for 5 min interval time. Plastic pots (25 cm in diameter) were prepared using autoclaved soil (2 kg pot^{-1}) composing of 0.3% nitrogen, 0.12% phosphorus, 0.56% potassium, and 11.19% organic matter with EC 1.08 dS m^{-1} and pH 5.58. In the experiment, six treatments were divided and represented by (1) 2.5 g pot^{-1} of the organic fertilizer (Fer), (2) 4 g pot^{-1} AMF powder (100 spores) (AMF), (3) 2.5 g pot^{-1} of the organic fertilizer plus 4 g pot^{-1} AMF powder (100 spores) (FA), (4) without AMF and fertilizer (Con). Other two treatments were the encapsulated beads of the (5) Al-FA and (6) the Ag-FA. Well irrigation (WW) was provided at $55.14 \pm 0.52\%$ soil water content (SWC). For a water deficit (WD) condition, one-month old seedlings of maize were withheld from irrigation (16 days, WD; $34.80 \pm 2.20\%$ SWC) and then physiological and biochemical changes were recorded.

AMF colonization

Roots of one-month-old seedlings were collected and stored in 60% alcohol. In the colonization analysis, the root sample was macerated using 10% KOH for 30 min at 95°C and rinsed three times with distilled water. The softened root was incubated in 5% HCl for 5 min and then stained with 0.05% (weight per volume) tryptan blue for 15 min. The AMF colonization was observed and calculated at 10× under light microscope (Zeiss, Germany), following the method of Brundrett *et al.* (1996).

Plant physiological and biochemical analysis

Total chlorophyll content (TChl) in the second fully expanded leaf from the shoot tip was evaluated by acetone extraction analysis (Shabala *et al.*, 1998). Photosynthesis efficiency or the maximum quantum efficiency of PS II (F_v/F_m), was measured from the surface of second fully expanded leaf following the method of Yooyongwech *et al.* (2016).

Phosphorus content in dried leaf and root tissues was measured in terms of blue molybdate-phosphate complexes, read at 420 nm using spectrophotometer [DR/4000; Model 48000, (HACH[®])], according to the method given by Jackson (1958). It was expressed as the relative phosphorus (P) content. Analysis of free proline content in the second fully expanded leaf was done according to Bates *et al.* (1973) using proline as standard. The reaction mixture was measured by colorimetric method using spectrophotometer [DR/4000; Model 48000, (HACH[®])]. Osmolarity of leaf tissues was measured as per the method given by Lanfermeijer *et al.* (1991).

Fresh shoot and root was dried in a hot-air oven at 80 °C for three days and water content in shoot and root was calculated using the equation: %Water content = $[(\text{FW} - \text{DW}) \times 100]/\text{FW}$, where FW is fresh weight and DW is dry weight (after oven drying at 70 °C for 48 h).

Statistical analysis

The experiment consisted of an arrangement of 2×6 factorial (two types of irrigation conditions and six types of inoculations) in a completely randomized block design with 6 replications. The data were analyzed by analysis of variance (ANOVA) using SPSS software (SPSS ver. 11.5). The means (\pm SE) were compared using Tukey's HSD ($p \leq 0.05$). The physiological and biochemical values were represented by four replications ($n = 4$). AMF-colonization percentage was measured and represented by eight replications ($n = 8$).

Results

AMF root colonization

Root colonization in the AMF-inoculated seedlings (mycorrhizal treated-plants by FA, AMF, Al-FA, and Ag-FA) was evidently detected (>70% colonization). In the organic fertilizer (Fer), AMF root colonization was observed, whereas it was absent in the control seedlings. Colonization percentages in the Al-FA and Ag-FA encapsulated treated-plants under well-watered condition were 74.16% and 79.60%, respectively (Fig. 1).

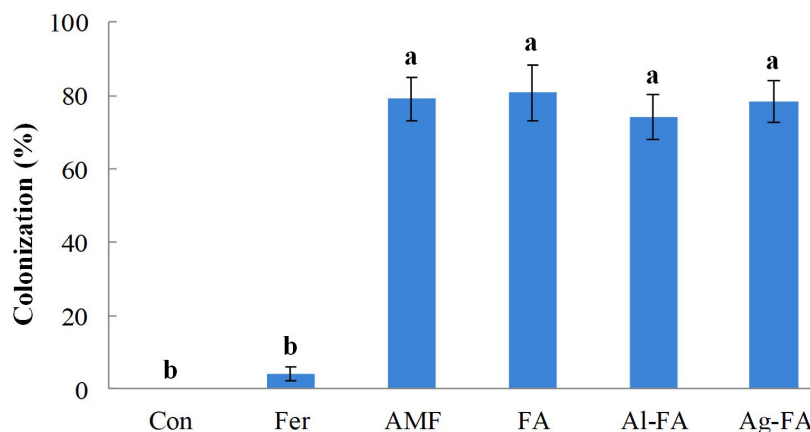


Fig. 1. Root colonization in control (Con), organic fertilizer (Fer), inoculated conditions of arbuscular mycorrhiza (AMF), organic fertilizer plus AMF (FA), FA encapsulated alginate (Al-FA), and FA encapsulated agar (Ag-FA). Mean values (\pm SE) with the letter are significant different at $p \leq 0.05$

Phosphorus content in maize under water deficit

Total phosphorus content in the leaf tissues of maize seedlings grown under well-watered conditions was unchanged when compared with controlled seedlings, except in the organic fertilizer-treated seedlings (Fer), where it showed an increasing trend (Fig. 2).

In water deficit, the P content in the leaf tissues was decreased in the Fer treatment (76.3% reduction compared to corresponding WW condition), AMF (63.3% reduction compared to corresponding WW condition) and control (46.0% reduction compared to corresponding WW condition). Interestingly, the P content in FA, Al-FA and Ag-FA was maintained when seedlings were subjected to water deficit conditions (Fig. 2). The P content in the root tissues of the AMF, FA and Al-FA treated seedlings grown under WW conditions were greater than the control seedlings. Under water deficiency, the P content in control seedlings declined by 47% over corresponding WW condition. In addition, the P content in all AMF treatments (AMF, FA, Al-FA and Ag-FA) under water deficit stress was maintained when compared with control (Fig. 2).

Physiology and growth parameters under water deficit

Free proline content in the leaf tissues of maize seedlings grown under WW was similar for all the treatments except for the Al-FA treated seedlings which had slightly lower content than all the other treatments ($17.49 \mu\text{mol g FW}^{-1}$). In the WD, the free proline level in the Al-FA plant was unchanged compared to other treatments. In the control plants, the maximum free proline content was observed to

be $24.75 \mu\text{mol g}^{-1}$ FW under well irrigated conditions (Fig. 3). Consequently, free proline enrichment in the controlled seedlings under the WD play a major role in osmotic adjustment to control the leaf osmotic potential. In the WD, the osmotic potential in the leaf tissues of control seedlings was sharply declined by 39.9% over WW seedlings, whereas the value of the Al-FA seedlings was closely maintained in the AMF-treated seedlings (Table 1). Shoot and root relative water content in AMF treated seedlings under the WD were maintained (Fig. 4). In control seedlings, shoot and root relative water content declined by 4.8% and 10.6%, respectively, when plants were exposed to the WD. Similar trend was observed in Fer treatment, where the free proline content was significantly dropped in the plant grown under the WD.

TChl in the leaf tissues of Fer and AMF inoculated seedlings was maintained when exposed to water deficit conditions. Interestingly, TChl content in control seedlings under the WD was reduced by 31.3% than in the WW seedlings (Table 2).

Maximum quantum efficiency of PS II (F_v/F_m), a sensitive chlorophyll fluorescence parameter in plants treated with the Fer, AMF, FA, and Al-FA under the WD was maintained, leading to retained plant growth, as represented by plant height (Table 2). In contrast, the F_v/F_m in Ag-FA treated seedlings under the WD was significantly declined by 27.5% over the WW seedlings (Table 2). In addition, plant height of Ag-FA treated seedlings was evidently retarded in both with or without irrigation conditions.

Table 1. Leaf osmotic potential in control (Con), organic fertilizer (Fer), inoculated conditions of arbuscular mycorrhiza (AMF), organic fertilizer plus AMF (FA), FA encapsulated alginate (Al-FA), and FA encapsulated agar (Ag-FA) subsequently grown under well-watering (WW) or water-deficit (WD) conditions

Treatment	Osmotic potential (MPa)		Osmotic potential $_{WD-AMF}$ (MPa)
	WW	WD	
Con	-0.814 ± 0.06^a	-1.139 ± 0.08^b (28.71%)	-0.061
Fer	-1.086 ± 0.05^b	-1.214 ± 0.09^b (10.54%)	-0.136
AMF	-1.193 ± 0.17^b	-1.078 ± 0.07^b (0%)	0
FA	-0.938 ± 0.15^b	-1.133 ± 0.19^b (17.21%)	-0.054
Al-FA	-1.189 ± 0.10^b	-1.100 ± 0.08^b (0%)	-0.022
Ag-FA	-1.207 ± 0.11^b	-1.136 ± 0.08^b (0%)	-0.058

Mean values (\pm SE) with the letter are significant different at $p \leq 0.05$.

The osmotic potential $_{WD-AMF}$ is determined to compare the osmotic potential under water-deficit condition of AMF plants with other treatments (Con, Fer, FA, Al-FA, and Ag-FA) by osmotic potential $_{WD}$ of interest - osmotic potential $_{WD}$ of AMF. The value in parenthesis represents the % reduction between WD and WW conditions

Table 2. Total chlorophyll content (TChl), maximum quantum efficiency of PS II (F_v/F_m) and plant height in control (Con), organic fertilizer (Fer), inoculated conditions of arbuscular mycorrhiza (AMF), organic fertilizer plus AMF (FA), FA encapsulated alginate (Al-FA), and FA encapsulated agar (Ag-FA) subsequently grown under well-watering (WW) or water-deficit (WD) conditions

Treatment	TChl ($\mu\text{g g}^{-1}$ FW)		F_v/F_m		Plant height (cm)	
	WW	WD	WW	WD	WW	WD
Con	14.4 ± 1.2 ^a	9.9 ± 0.6 ^b (31.25%)	0.69 ± 0.009 ^a	0.66 ± 0.005 ^a (4.35%)	111.1 ± 5.9 ^{ab}	108.2 ± 3.6 ^{ab} (2.61%)
Fer	16.4 ± 1.6 ^a	13.8 ± 1.9 ^{ab} (15.85%)	0.73 ± 0.001 ^a	0.72 ± 0.003 ^a (1.37%)	110.2 ± 5.4 ^{ab}	112.3 ± 4.4 ^{ab} (0%)
AMF	14.4 ± 1.0 ^a	12.8 ± 1.5 ^{ab} (11.11%)	0.70 ± 0.004 ^a	0.69 ± 0.011 ^a (1.43%)	114.0 ± 3.4 ^{ab}	116.8 ± 4.0 ^a (0%)
FA	17.3 ± 1.5 ^a	12.7 ± 0.1 ^{ab} (26.59%)	0.73 ± 0.014 ^a	0.70 ± 0.009 ^a (4.11%)	112.1 ± 6.0 ^{ab}	111.0 ± 4.5 ^{ab} (1.00%)
Al-FA	17.6 ± 1.8 ^a	12.1 ± 0.6 ^{ab} (31.25%)	0.72 ± 0.007 ^a	0.70 ± 0.009 ^a (2.78%)	103.9 ± 4.1 ^{ab}	110.1 ± 3.2 ^{ab} (0%)
Ag-FA	11.9 ± 0.3 ^{ab}	10.8 ± 1.7 ^{ab} (9.24%)	0.69 ± 0.010 ^a	0.50 ± 0.080 ^b (27.54%)	99.2 ± 2.5 ^b	95.3 ± 3.8 ^b (3.93%)

Mean values (± SE) with the letter are significant different at $p \leq 0.05$. The value in parenthesis represents the % reduction between WD and WW conditions

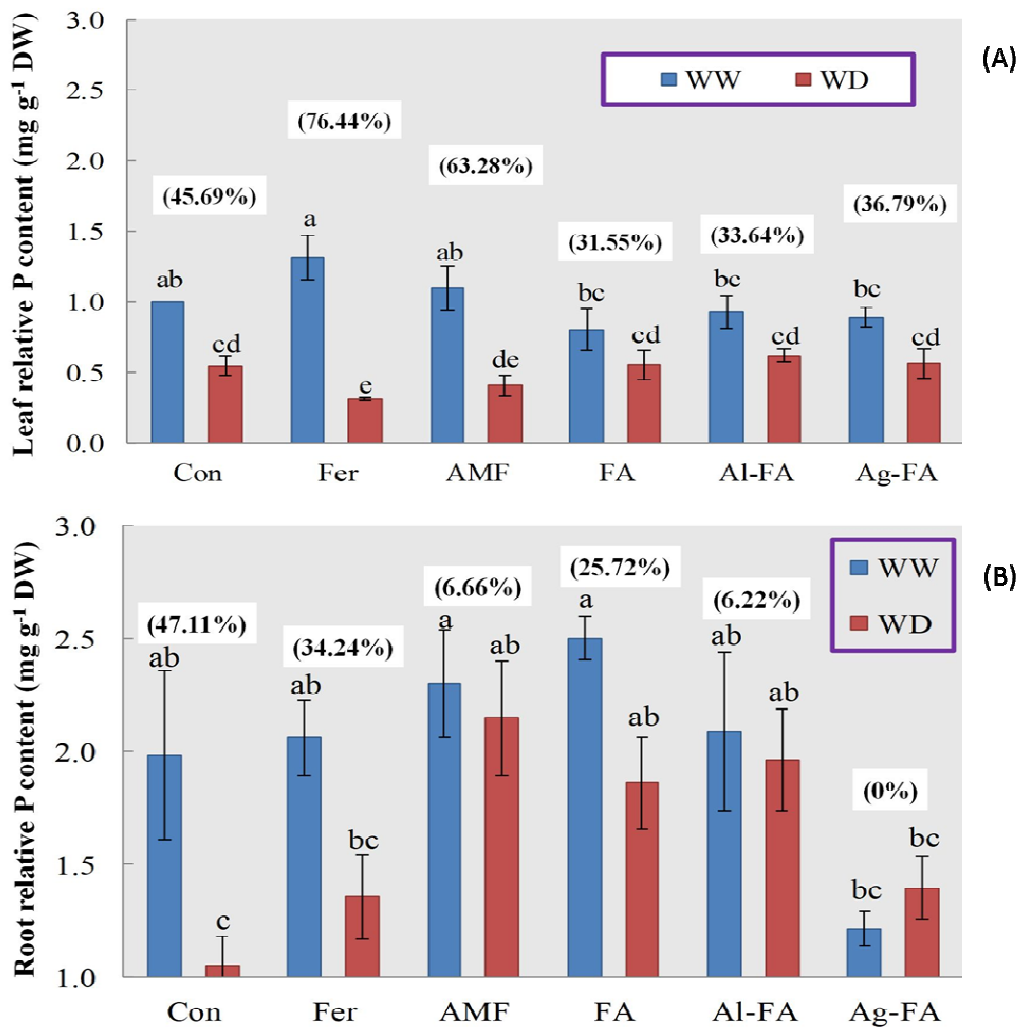


Fig. 2. Relative phosphorus content in leaf (A) and root tissues (B) of control (Con), organic fertilizer (Fer), inoculated conditions of arbuscular mycorrhiza (AMF), organic fertilizer plus AMF (FA), FA encapsulated alginate (Al-FA), and FA encapsulated agar (Ag-FA) subsequently grown under well-watering (WW) or water deficit (WD) conditions. Mean values (± SE) with the letter are significant different at $p \leq 0.05$. The value in parenthesis represents the % reduction between WD and WW conditions

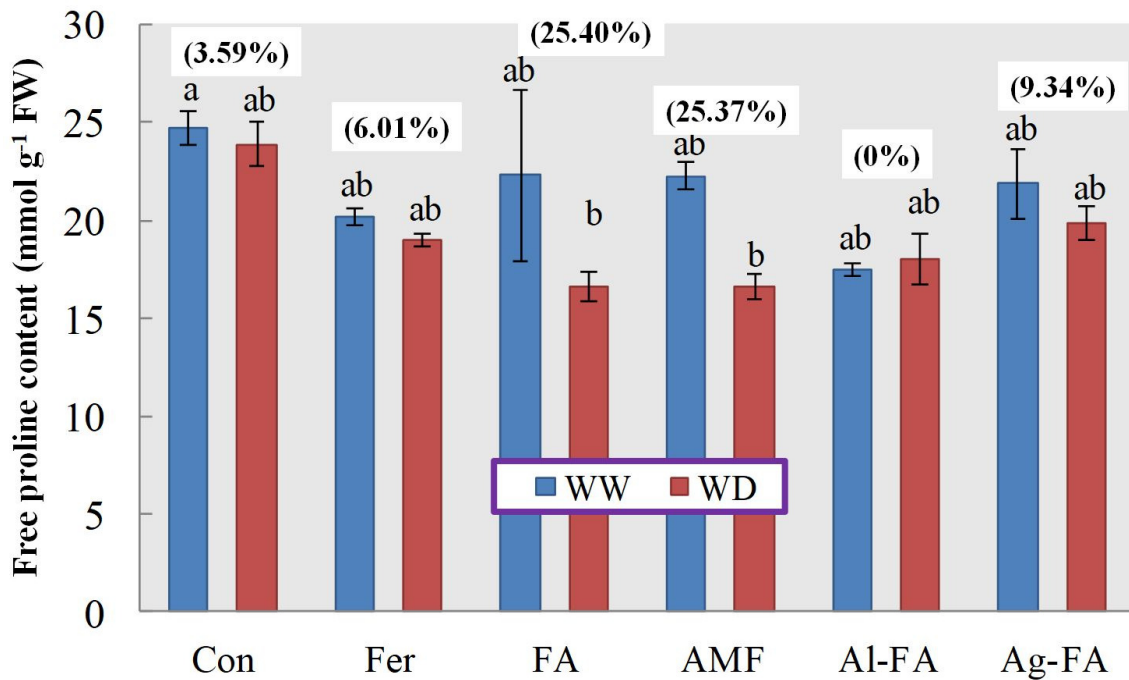


Fig. 3. Free proline content in leaf tissues of control (Con), organic fertilizer (Fer), inoculated conditions of arbuscular mycorrhiza (AMF), organic fertilizer plus AMF (FA), FA encapsulated alginate (Al-FA), and FA encapsulated agar (Ag-FA) subsequently grown under well-watering (WW) or water deficit (WD) conditions. Mean values (\pm SE) with the letter are significant different at $p \leq 0.05$. The value in parenthesis represents the % reduction between WD and WW conditions

Discussion

Under water deficit, the inoculation of the Al-FA encapsulation type seemed to maintain the chlorophyll content and the F_v/F_m in the maize plants, as same as the FA, and AMF plants, indicating that the mycorrhiza in the encapsulation type may able to be involved the photosynthetic system and more effective in these plants. Mirshad and Puthur (2016) supported that reduction in the chlorophyll content was high in non-AMF plants under drought conditions, which might be due to the suppression of enzymes involved in process of chlorophyll biosynthesis. Some reports of drought stress mentioned that AMF symbiosis assisted in reducing the chlorophyll loss and positively affected the photochemistry of PSII in watermelon (Mo *et al.*, 2016). In agreement with the previous observations of Yooyongwech *et al.* (2016), photosynthesis efficiency in the present study was enhanced upon AMF application in the target plant under water deficit stress. A decrease in the expression of *PAO* and *PPH*, the key genes in chlorophyll-breakdown process in non-inoculated plants, and an increase in the expression of *RBCS* and *RBCL* genes involved in initial Rubisco activity have been observed under water deficit conditions upon AMF-inoculation (Mo *et al.*, 2016). The chlorophyll content and photosynthesis efficiency, F_v/F_m , relative to the control, were also improved under the water deficit, even treating the plants with fertilizer alone. Previous studies have also reported that the fertilized plants had improved chlorophyll content compared to the non-fertilized plants (Fan *et al.*, 2014).

However, low water availability and total P content in both shoot and root of the Fer treated plants may imply that the fertilizer alone may not be beneficial enough to maintain plants under the WD. Whereas, in case of the AMF-related plants (Al-FA, FA, and AMF), these parameters were successfully maintained. Sajedi *et al.* (2010) supported the view that AMF enhanced the water efficiency in maize plants subjected to drought conditions. This could be due to an activated extraradical mycelium (ERM) of AMF in the soil as reported by Derelle *et al.* (2012), Zouet *et al.* (2015). The ERM are hydrophilic in nature and arrange water in the rhizosphere of the host plants across mycelium tips, even during drought conditions (Derelle *et al.*, 2012; Zou *et al.*, 2015). Particularly, the interaction of AMF mycelium or hyphae in host plants (symbiont) might be helpful in retaining the water availability in the soil as well as in contributing phosphorus as macronutrient for plant growth and development (Neumann *et al.*, 2009; Hodge *et al.*, 2010).

In the same way, free proline content in case of Al-FA FA, and the AMF treated plants under the WD condition was comparatively low unlike in non-mycorrhizal plants (Con and Fer), and Ag-FA treated plants. Under drought conditions, the lower levels of free proline content in AMF treated *Populus* spp. were observed (Liu *et al.*, 2016). Proline is known to play a key role in plant osmoregulation (Jinyou *et al.*, 2004; Hayat *et al.*, 2012) and the lower accumulation of free proline was performed to maintain osmotic pressure in the AMF plants to avoid drought stress (Porcel and Ruiz-Lozano, 2004; Liu *et al.*, 2016). In addition, the calcium

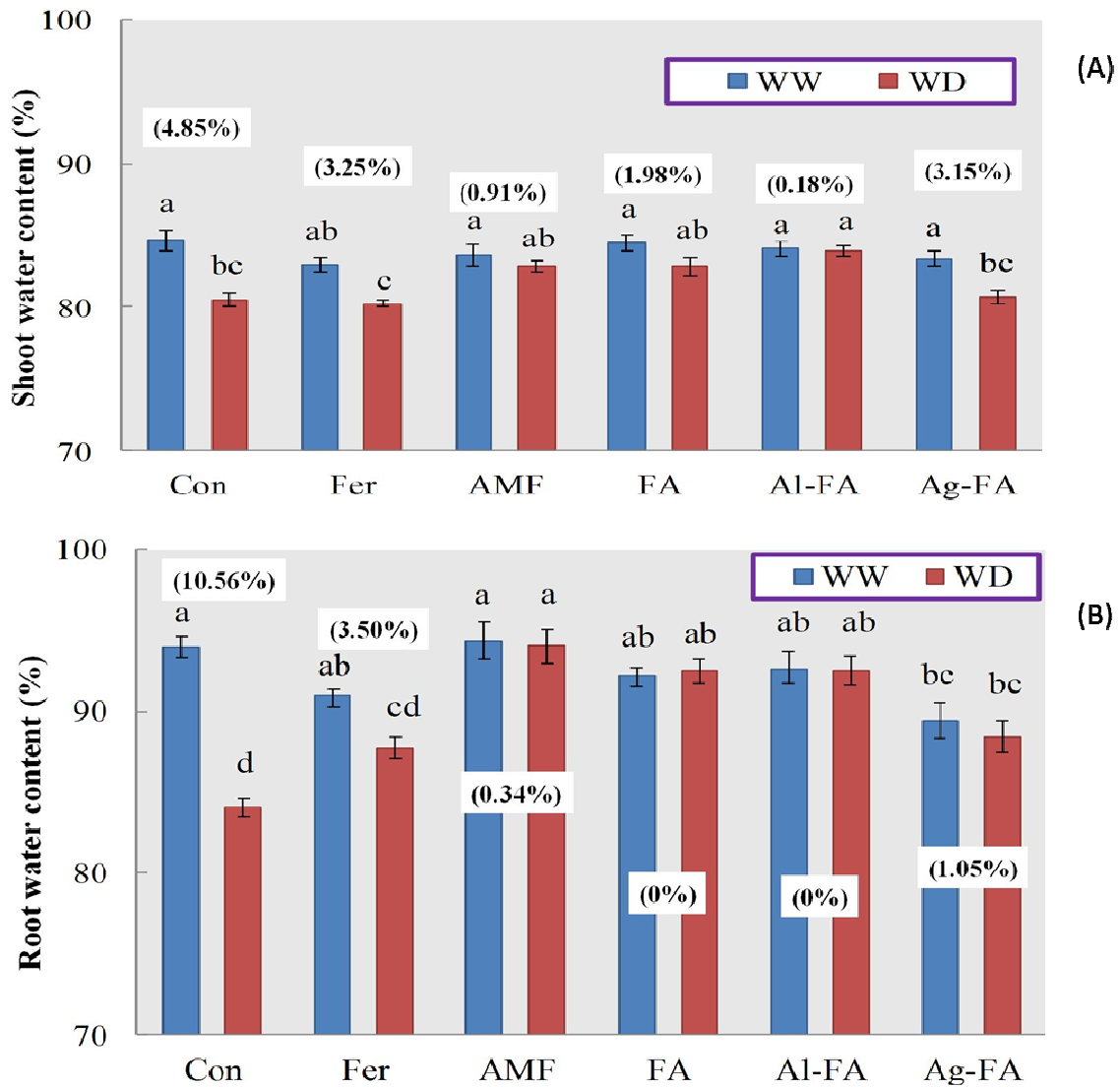


Fig. 4. Water content in shoot (A) and root tissues (B) of control (Con), organic fertilizer (Fer), inoculated conditions of arbuscular mycorrhiza (AMF), organic fertilizer plus AMF (FA), FA encapsulated alginate (Al-FA), and FA encapsulated agar (Ag-FA) subsequently grown under well-watering (WW) or water deficit (WD) conditions. Mean values (\pm SE) with the letter are significant different at $p \leq 0.05$. The value in parenthesis represents the % reduction between WD and WW conditions

compound of alginate coating agent in the Al-FA treatment may regulate the free proline accumulation in the treated plants under the two irrigated conditions that caused decrease in free proline content in Al-FA treated plants, even under the WW. Xu *et al.* (2013) proved that external application of the low doses of calcium chloride (5-10 mM) induced a reduction of proline content in *Zoysia* grass, especially under drought. Moreover, calcium is one of the important plant-nutrients and it acts as a secondary messenger in the cellular signaling pathways, participates in H₂O₂ perception and induces antioxidant genes (Rentel and Knight, 2004; Tuteja and Mahajan, 2007) in regard to drought avoidance and plant water use efficiency (Shao *et al.*, 2008).

In the Ag-FA plants, the water and P content appeared relatively lower than in Al-FA plant, despite the high percentage of root colonization in both the treatments. In the roots, the trend of water and P content was evidently

decreasing under both WW and WD (Fig. 2). This lower level of the water and P content may be due to the film segments of agar that were dried under water dehydration and turned thick when exposed to open conditions (Rigou *et al.*, 1995). It could also be possible that a portion of coated agar-agar film on the beads interfered the water movement through the external hyphae of mycorrhiza and/or root from the rhizosphere, leading to decrease in the efficiency of water adsorption and nutrient fixation. Besides, the reduction in water use efficiency directly affected the quantum efficiency of PSII in the plant cell (Ren *et al.*, 2015). This caused gradual reduction in the photosynthetic efficiency and plant height in case of Ag-FA treated plants (Table 2). Eventually, an encapsulated agar-agar film of the Ag-FA inhibits the maize growth and development under the WW and the WD conditions. It also depends on the softening coat beads, drying period and the type of microorganism (Vemmer and Patel, 2013).

Conclusions

The two types of encapsulations of the AMF and the organic fertilizer with the calcium-alginate (Al-FA) and the agar-agar (Ag-FA) responded differently on the maize plants. The plants treated with the Al-FA beads allowed the maintenance of maximum quantum efficiency of PS II (F_v/F_m), chlorophyll content, water content in shoot and root, and phosphorus content, especially in the root parts under the water deficit condition. In the water deficit, free proline content of the Al-FA treated plants remained low. Water availability and total phosphorus in the Ag-FA plant under WW and WD conditions were limited, leading to inhibited photosynthetic efficiency and growth performances. According to the results, Al-FA proved more reliable in alleviating the water deficit stress in maize. The Al-FA beads may provide an option for further research, development and practical applications for the improvement of maize growth under drought.

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