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The role of arbuscular mycorrhizal fungi in refining plant photosynthesis and water status under drought stress: a meta-analysis

*Murugesan Chandrasekaran**

Department of Food Science and Biotechnology, Sejong University, Gwangjin-gu, Seoul, South Korea
**Corresponding author: chandrubdubio@gmail.com*

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Abstract: Due to increased climate change, crop productivity worldwide is in danger. Drought stress is considered one of the major environmental factors in relation to world food security. Previous studies showed that arbuscular mycorrhizal fungi (AMF) inoculation alleviates drought stress in various plants. However, whether AMF inoculation efficiency is based on gas exchange or water status and whether the effects differ among plants and AMF species remain unclear. To evaluate the effect of AMF on drought stress alleviation, a meta-analysis was conducted based on random-effect models accounting for effect size variation. Results revealed that photosynthetic rate had the highest effect size among gas exchange traits compared to stomatal conductance and transpiration rate. Our results also showed a significant positive impact on relative water content, water potential, and water use efficiency in AMF-inoculated plants compared to non-inoculated plants. Furthermore, among AMF species, *Funneliformis mosseae*, followed by *Rhizophagus irrgularis*, was an efficient AM fungi for drought stress alleviation. Therefore, this study suggests that a higher water use efficiency supports water transport to the leaf surface and keeps the stomatal opening, enhancing photosynthetic responses.

Keywords: arbuscular mycorrhizal fungi; water stress; gas exchange; stress alleviation; plant growth

Drought is one of the most important environmental factors limiting agricultural productivity and food security (Sheffield et al. 2012, Dietz et al. 2021). The drought stress induced the sequence of negative responses *via* ionic and osmotic imbalances in plants (Bahadur et al. 2019). Drought remains the primary abiotic stress factor limiting sustainable agriculture considerably. This leads to a series of changes in the plants, including closure of stomata, decreased carbon dioxide influx, and minimised photosynthesis. The sequence of physiological mechanisms mentioned above leads to decreased plant productivity and yield. For alleviation of drought stress, stomatal conductance (g) is combating water deficiency in leaves, thereby regulating the water potential (WP) and maintaining relative water content (RWC) in plants (Hura et al. 2007). Furthermore, physiological responses permit plants to regulate and augment CO_2 assimilation (photosynthetic rate (A)) *v*. evaporative water loss (transpiration rate (E)), which depends on g. Thus, drought stress limits stomatal closing, photosynthesis, and carbon sequestration (Osakabe et al. 2014, Dietz et al. 2021). Various practices actively fortify drought stress tolerance (Cattivelli et al. 2008, Hu and Xiong 2014). In addition, several studies have revealed the importance of photosynthetic abilities and maintenance of water status in alleviating drought stress globally (Yan et al. 2016).

As an alternative method, applying root-associated microorganisms like arbuscular mycorrhizal fungi (AMF) will extend the stress tolerance and alleviate the drought stress (Boutasknit et al. 2020, Begum et al. 2019, 2022). Due to its mutualistic symbiotic relationships with plant roots, AMF is a bio-fertilising microorganism for sustainable agriculture (Berruti et al. 2015). The hyphal network of AMF reaches

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the distantly located soil pores, which enhance the effective absorption of water, which is usually not accessible for non-mycorrhizal plants. Thus, AMF symbiosis is useful for better water acquisition abilities and g for heightened relationships between the host plant and soil for water uptake (Ruiz-Lozano 2003, Augé et al. 2015). Previous studies revealed that AMF symbiosis, attributed to changes in root morphology, growth, nutrient uptake, photosynthesis, water status, antioxidants, and osmolytes, alleviates drought stresses in various plant species (Augé 2001, Ruiz-Lozano 2003, Augé et al. 2015). However, a clear understanding of these interrelated physiological and AMF-mediated responses in drought stress alleviation and sustainable agricultural proposals needs more advanced strategies.

Several publications over the decades about the effect of AMF on gas exchange and water status acquisition necessitate further research (He et al. 2017, Essahibi et al. 2018, Boutasknit et al. 2020, Langeroodi et al. 2020). Plenty of publications in random experimental conditions have left us weak in finding the degree of efficiency, i.e., whether AMF symbiosis accounts for photosynthesis or water status-related traits. The lack of quantitative studies makes us incapable of progressing in efficient, sustainable agriculture and its comparative impacts on AMF symbiosis. Therefore, meta-analysis is used for quantitative analysis and new hypotheses for future studies. Meta-analysis is a statistical method for the comprehensive analysis of published data on a given topic. Meta-analysis is a helpful tool for analysing potential factors causing variation among studies. Our previous studies, Chandrasekaran et al. (2014, 2016) and Chandrasekaran (2020), evaluated the effect of AMF on plant biomass and nutrient uptake under salt stress and normal conditions, respectively. Moreover, our previous study showed the positive effects on photosynthesis and the water status of plants under salt stress in AMF-inoculated plants (Chandrasekaran et al. 2019). Nevertheless, meta-analysis studies on drought stress abatement through photosynthesis and water status have not been documented earlier. Therefore, the present meta-analysis study aims to evaluate the ameliorative efficiency of the AMF inoculation on gas exchange and water status traits under different levels of drought stress conditions.

MATERIAL AND METHODS

Literature search and inclusion criteria. Web of Science, Google Scholar, Science Direct, Springer,

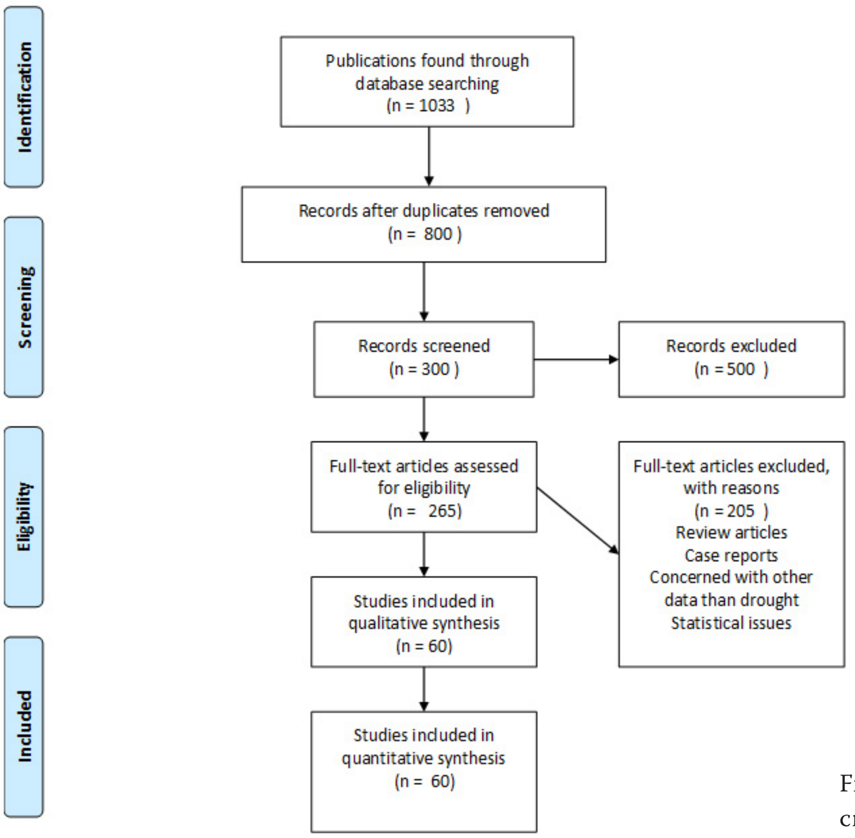

Figure 1. Prisma flow chart selection criteria

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Table 1. Arbuscular mycorrhizal fungi (AMF) inoculation effect on gas exchange and water status

Analysis	Response variable	Number of observations (n)	Effect size	Prob (Chi-Square)
Overall analysis	overall studies	473	1.41	0.0000
	gas exchange	243	1.54	0.0000
	water status	230	1.27	0.0001
Gas exchange	photosynthetic rate (A)	71	2.05	0.0005
	stomatal conductance (g)	105	1.29	0.0005
	transpiration rate (E)	67	1.46	0.1100
Water status	relative water content	97	1.30	0.1600
	water potential	63	0.98	0.0020
	water use efficiency	14	1.42	0.0400

Taylor, and Francis, Wiley, Springer, Nature, and Science were used in the literature survey. The keywords used in the search are mycorrhiza, photosynthesis, arbuscular water status drought AM fungi gas exchange, or mycorrhizal inoculation. To avoid bias in publications, we used the following inclusion criteria for our analysis: (1) plants exposed to drought; (2) should contain both control (without AMF inoculation) and treatments (with AMF inoculation); (3) included drought intensity; (4) a minimum of two extractable studies from each publication; (5) results were published international journals. In total, 60 of 1 033 publications meet these criteria (Figure 1). The collected means, standard error/deviations, and sample size were estimated from each publication. The standard deviation was calculated from standard error using a meta-win statistical calculator. The data was extracted from graphs using WebPlotDigitizer v. 2.4 (Rohatgi 2021).

Meta-analysis Meta-Win v2.1 statistical software was used to calculate Hedges' d+ effect size based on random-effects models (Rosenberg et al. 2000). Hedges' d+ effect sizes are used to estimate the magnitude of effect and to compare the results of multiple studies and in the present study, calculated the effect sizes of a plant grown with AMF (X^E) (treatment) relative to a plant grown without AMF (X^C) (control) under drought stress. Positive Hedges' d+ effect size indicates increased levels of response variables, and negative effect size indicates decreased effects of the particular response variable under drought stress in AMF-inoculated plants. Hedges' d+ statistic is useful for calculating effect size when the sample size is low and avoiding small sample size bias (Gurevitch et al. 2001, Delavaux et al. 2017). The effect size was calculated with a 95% bootstrap confidence interval (BS CI). The effect size of a particular study is statistically significant at 95% BS, where CI does not overlap with zero.

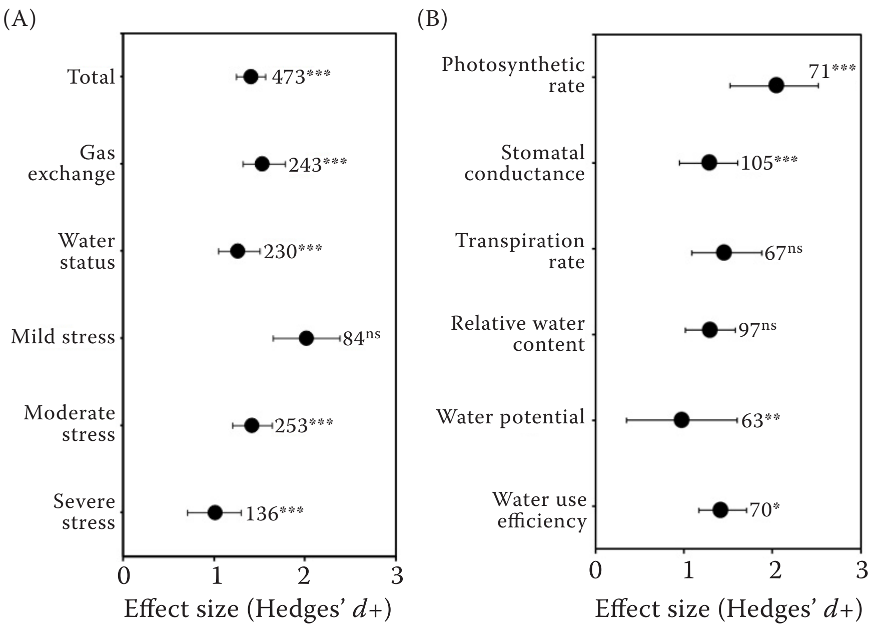

Figure 2. (A) Overall arbuscular mycorrhizal fungi (AMF) inoculation efficiency under different levels of drought stress (B) AMF inoculation on gas exchange and water status under drought stress. *P < 0.05; **P < 0.01; ***P < 0.001; ns – not significant

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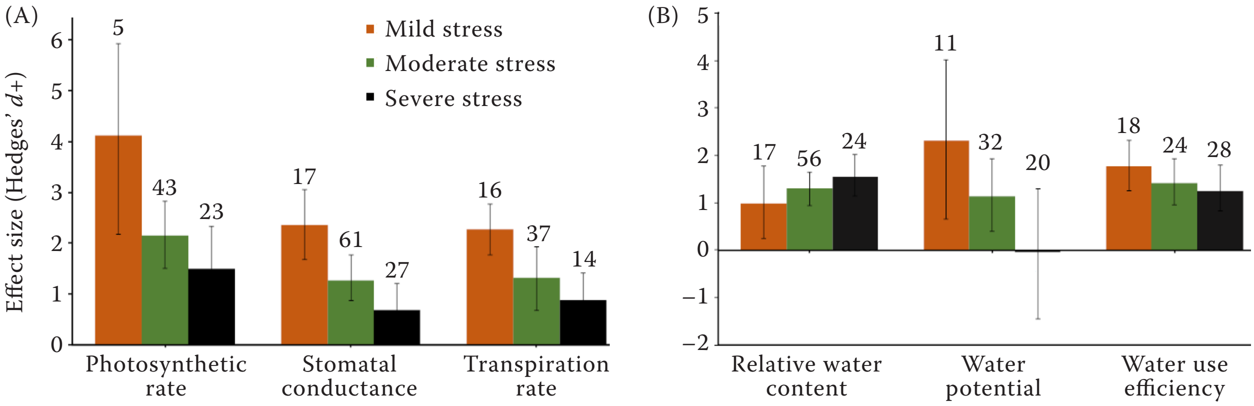

Figure 3. Effect of drought intensities on (A) gas exchange and (B) water status. When confidence intervals overlap zero, the effect sizes are not significantly different from zero

RESULTS

AMF inoculation on gas exchange under drought stress Overall analysis showed that AMF-inoculated plants significantly positively affected gas exchange and water status traits under drought stress (Table 1, Figure 2). Among gas exchange traits, A had the highest effect size compared to those of non-mycorrhizal plants (Hedges' $d+ = 2.05$, $P < 0.0001$) (Figure 2B). Moreover, as the level of drought increases from mild to severe, the effect size gradually decreases for A (6.72, 2.37, and 1.49 for mild, moderate, and severe drought, respectively) (Figure 3A). g also showed a positive impact on AMF-inoculated plants than those of non-mycorrhizal plants (Hedges' $d+ = 1.29$, $P < 0.0001$). Our results also showed a positive effect size for E in AMF-inoculated plants (Hedges' $d+ = 1.46$, $P = 0.01$).

Among plant species (herbaceous, woody, and grass), herbaceous and grass showed higher and lower effect sizes for A. Meanwhile, g and E had the highest and lowest effect sizes for woody and herbaceous plants. When the two life cycles (annual and perennial) were considered, the effect sizes of A, g, and E in annual plants were lower than those in perennial plants. The perennial plants performed better in the context of drought stress alleviation in AMF-inoculated plants than those in the control group. When the two plant groups (monocot and dicot) were considered, it was found that dicot had the highest effect sizes for A, g, and E. The results

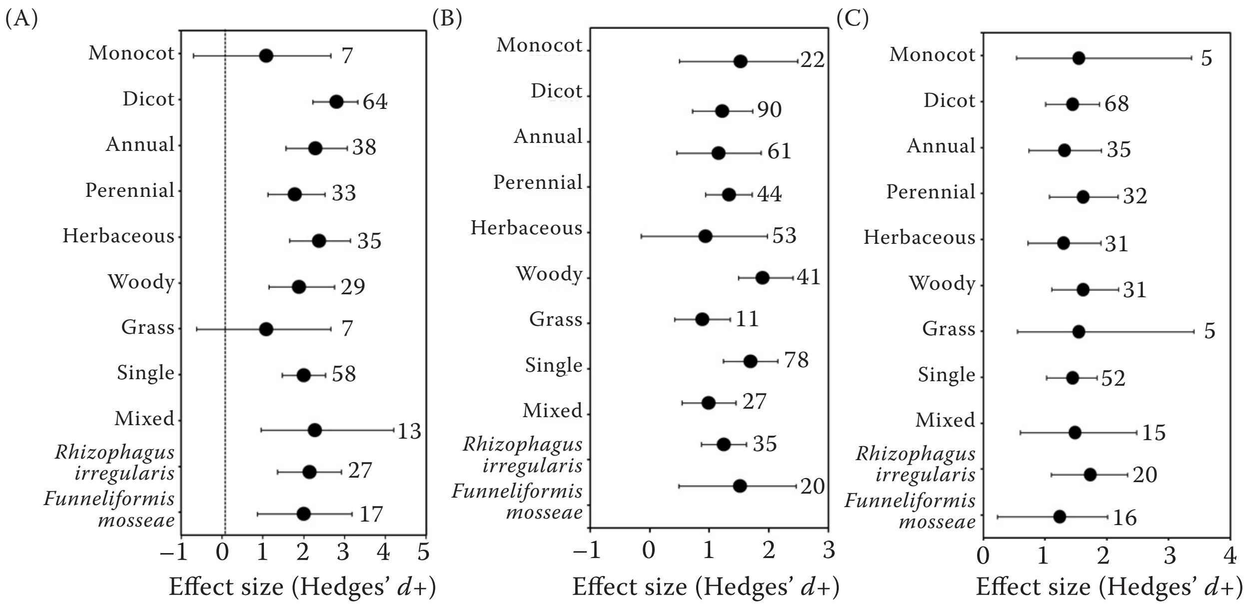

Figure 4. Categorical analysis of gas exchanges (A) photosynthetic rate; (B) stomatal conductance, and (C) transpiration rate. When confidence intervals overlap zero, the effect sizes are not significantly different from zero

of the monocot were dealt with with caution owing to the lower sample size (Figure 4).

There was a significant difference in the mean effect depending on the plant species (P = 0.04) in AMF inoculation under drought stress. For A, among the most studied AMF species, *Rhizophagus irregularis* (Hedges' d+ = 2.71) indicated a higher effect size than *Funneliformis mosseae* (Hedges' $d+$ = 2.42) (Figure 4). G and E had the highest effect sizes for *F. mosseae* (1.85 and 1.55, respectively) than those of *R. irregularis* (1.44 and 1.50, respectively). For AMF richness, single species showed the highest effect sizes for A, whereas mixed species showed the highest effect sizes for Gs and E.

AMF inoculation on water status change under drought stress. Relative water content, water potential, and water use efficiency were considered important factors for variations in the water status of plants under drought stress. Our results showed a positive impact on relative water content, water potential, and water use efficiency in AMF-inoculated plants compared to non-inoculated plants (Figure 2B). Also, there is variation in stress levels for relative water content; as stress increases (mild to severe), effect size also increases for AMF-inoculated plants (Figure 3B). For water potential, mild stress conditions were more efficient and positive than moderate stress, but severe stress had negative and non-significant effects. For water use efficiency, as the level of stress increases, the effect size also decreases but is found to be positive (Figure 3B).

Relative water content and water potential had the highest and lowest effect sizes for woody and herbaceous plants. However, herbaceous had the highest effect sizes for water use efficiency among the three growth forms. Perennial plants showed high RWC values with lower water potential and water use efficiency. In contrast, annual plants showed higher water potential and efficiency in water use but lower relative water content. Monocots exhibited a higher relative water content, water potential, and water use efficiency than dicot plants. For AMF richness, mixed species showed the highest effect size for relative water content, whereas single species exhibited a higher effect size for water potential and water use efficiency. When the most studied two AMF species (*R. irregularis* and *F. mosseae*) were considered, the effect sizes of water status traits in *R. irregularis* were lower than those in *F. mosseae*, indicating the importance of *F. mosseae* in drought stress alleviation (Figure 5).

DISCUSSION

The importance of AMF inoculation efficiency on plant physiological and biochemical mechanisms in plants has been systematically studied in various crops (Augé et al. 2015, He et al. 2017, Essahibi et al. 2018, Boutasknit et al. 2020, Langeroodi et al. 2020). AMF-inoculated help plants absorb more water and assist their transport from the root to shoot, resulting in increased gas exchange and water status under drought stress. Our analysis revealed that AMF-inoculated plants had significantly positive effects under drought stress across all studies (Figure 2). Similarly, previous research studies also positively impact AMF symbiosis

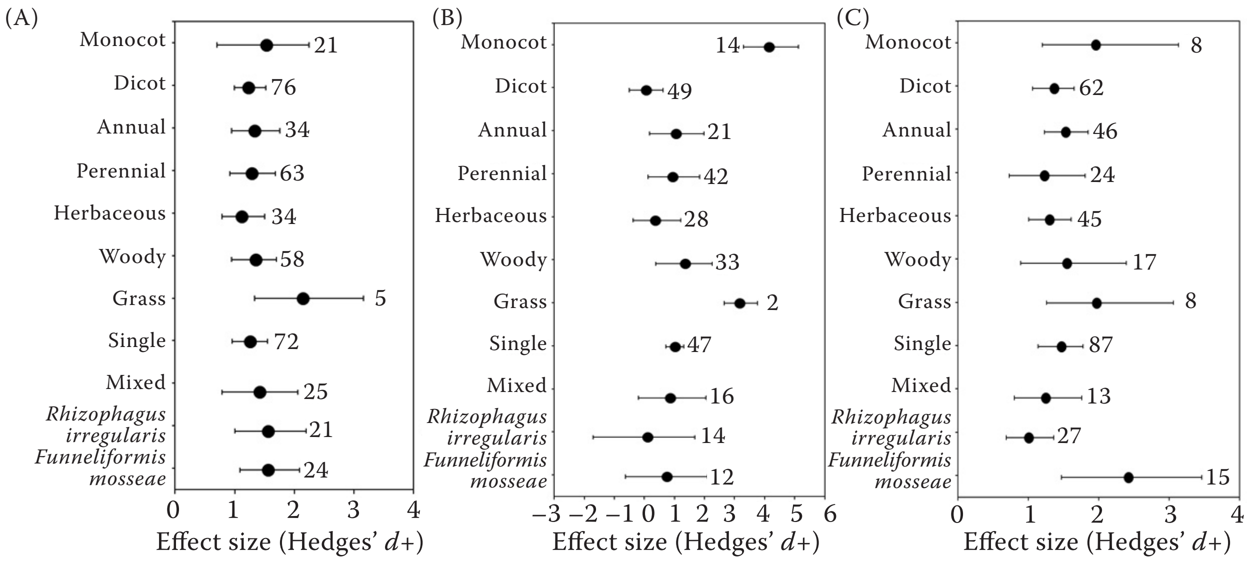

Figure 5. Categorical analysis of water status (A) relative water content; (B) water potential, and (C) water use efficiency. When confidence intervals overlap zero, the effect sizes are not significantly different from zero

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for gas exchange and water status (Zhu et al. 2012, 2015, Begum et al. 2022).

Further analysis revealed that AMF inoculation had a positive effect size for A, g, and E under all levels of drought intensities compared to non-mycorrhizal plants. These results are similar to previous studies (Augé et al. 2015, Bahadur et al. 2019, Begum et al. 2022). Our results also showed that effect sizes of A were greater than those of other variables across all studies (Figures 2 and 3). The increased A of the AMF-inoculated plants showed an increase in g, signifying AMF-inoculated plants with the opening of stomata (Santander et al. 2017). According to Yan et al. (2016), stomatal behaviour corresponds to water and nutrient status and is in close association with soil and climate conditions of plants, affecting controlled water loss and CO_2 absorption. As a result, stomata act as a gate for plants and the external environment during photosynthesis and transpiration. We also found increased g and E in the AMF-inoculated plants compared to non-inoculated plants. Similarly, the study by He et al. (2017) also shows increased gas exchange traits due to AMF inoculation in peanuts and tomatoes.

Moreover, the level of drought stress remains the limiting factor for plant productivity and is mainly controlled by length and severity. Our results revealed relatively strong positive effects under mild drought stress followed by moderate and severe stress. It proved that AMF inoculation was more effective under mild stress but efficient under moderate levels of drought stress than those under severe stress. Drought stress varies from species to species and plant-fungal interactions. It was witnessed that the effect size of herbaceous species was the smallest, specifying that their aptitude to acclimatise to drought stress is comparatively reduced. Woody and grass plants responded more quickly to drought due to their improved E and root architecture. Thus, different plant growth forms and their root interaction with AMF were found to be vital factors that possibly influence the amelioration of drought. Furthermore, AMF species were evaluated as follows: *F. mosseae* > *R. irregularis* > *Diversispora versiformis* > mixed AMF species. A similar effect was found in previous studies, in which *F. mosseae* was found to be the most effective species (Augé et al. 2015, Chitarra et al. 2016).

Furthermore, the water status of plants is an essential trait for ameliorative responses of AMF symbiosis under drought stress. During plant-fungal interactions, mycorrhizal hyphae account for water transportation *via* plant root cells (Zhang et al. 2018). Our analysis showed that AMF plants had increased effect size for RWC, signifying the importance of RWC. Thus, increased RWC was found to be a good indicator of stress tolerance in the amelioration of drought stress in AMF. Our results are also similar to previously published research findings (He et al. 2017, Wu et al. 2017). WUE signifies a vital factor for drought stress, and increased levels of WUE are helpful for water movement to the leaf surface and stomatal opening (Osakabe et al. 2014, Yang et al. 2014). Moreover, increased levels of WUE in AMF-inoculated plants under drought were found to be an indicator of the level of photosynthesis (Essahibi et al. 2018). In our analysis, AMF inoculation significantly increased plants' WUE under all drought stress levels. Previous studies also showed AMF-inoculated plants had enhanced water use efficiency (Yang et al. 2014, Chitarra et al. 2016). The encouraging attributes of AMF on WUE can be recognised for the increase of root hydraulic conductivity and the absorption of water and nutrients, which in turn regulate the gas exchange such as A, g, and E (Yang et al. 2014, Zhu et al. 2015).

CONCLUSION AND PROSPECTS

Our meta-analysis study revealed that AMF inoculation had increased A, g, E, and WUE under different levels of drought stress than those of non-inoculated plants. However, the efficiency of AMF inoculation on drought stress alleviation depends on environmental factors (i.e., level of stress), physiological traits, type of plants, and AMF species. Hence, the influences of AM symbiosis might be determined by interaction between types of the plant and AM fungal species. This study suggests that an increase in WUE and RWC eventually caused increased A, g, and E, which are disposed of by plant and fungal species traits for sustainable agriculture. The above results signify the effective drought stress management elucidated by AMF-inoculated plants.

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