



Inoculation of drought-stressed wheat plant (*Triticum aestivum* L.) with single and combined inoculants of Arbuscular mycorrhizal fungi

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Received: 2 September, 2020; Accepted: 6 October, 2020; Published online: 12 October, 2020

Abstract

The current study aimed to compare between inoculation of wheat plant with a single and combinations of several species of Arbuscular mycorrhizal fungi (AMF), and promoting its growth under water stress conditions. In a greenhouse experiment, the effect of three AMF species on wheat plant growth was studied using single-inoculations with *Glomus monosporum*, *G. mosseae* and *Gigaspora gigantean*, and mixtures of various AMF species. Moreover, inoculation of wheat plant with pair of AM fungi composed mainly of; SN (*G. monosporum* and *Gigaspora gigantean*) and AA (*G. monosporum* and *Glomus mosseae*) were investigated. Current findings indicate that inoculation with AMF belonging to different families was more effective in improving plant growth than AMF from the same genus and with mono-inoculation. Results showed that plant height inoculated with SN and AA increased by 27.30% and 24.95%; respectively, while inoculation with *Gigaspora gigantean* (GG), *G. mosseae* (GS) and *G. monosporum* (GM) increased by 16.01%, 14.6% and 6.83%, respectively. Number of spikes/plant, spike length, number of spikelet/spike, 1000-grain weight and grain yield/plant of wheat plants inoculated with AMF increased significantly, compared with the non-inoculated control plants under water stress. In addition, current results demonstrated that inoculation with SN had relatively higher impact on promoting the growth of wheat plants followed by AA and GG, suggesting that inoculation with AMF consisting of different species may have a better effect than single inoculations. Furthermore, it is observed also that SN; which are combination from different genera have better effect on wheat plant growth, compared to that of AA from the same genus but of different species.

Keywords: Mycorrhiza, Wheat, Water stress, Yield

1. Introduction

Drought has negative effects on crop yield production worldwide. Condon *et al.*, (2004) revealed that plants response to water deficiency is complex

and involves molecular and biochemical mechanisms. Later, Mirzaee *et al.*, (2013); Hameed *et al.*, (2014) reported that drought stress limits root growth that

results in reduction of water, change chlorophyll contents and nutrients uptake, which are all down-regulated by water deficiency in crop plants. Furthermore, the function of root-associated microbial populations that are capable of improving plant drought tolerance has been explored in several studies conducted by Timmusk *et al.*, (2014); Coleman-Derr and Tringe, (2014); Nadeem *et al.*, (2014); Rolli *et al.*, (2015).

Wheat (*Triticum aestivum* L.) is regarded as an essential grain crop, a permanent nutrient source in most developing countries, and the world's third largest cereal crop following maize and rice from a output perspective (Pocketbook, 2015). The nutritional value of wheat is extremely important, as it plays a major role among the few crop species that are widely cultivated as staple food sources (Šramková *et al.*, 2009). At the same time, Sommer *et al.*, (2013); Borlu *et al.*, (2018) demonstrated that the productivity of wheat worldwide is affected by many abiotic stresses (i.e., heat, drought and salinity); among them soil salinity is the most important one.

Smith and Read, (2010); Berruti *et al.*, (2016) reported that vesicular arbuscular mycorrhizal (VAM) fungi form a symbiotic relationship with 80% of all the terrestrial plant species, and have tremendous potential to act as plant biostimulants. Spatafora *et al.*, (2016) added that Arbuscular mycorrhizal fungi (AMF) have been known as one of the most important beneficial microorganisms of the sub-phylum Glomeromycotina, of the phylum Mucoromycota.

According to several studies conducted by Kiers *et al.*, (2011); Schausberger *et al.*, (2012); Bowles *et al.*, (2018), AMF can play an important role in promoting plant growth through numerous benefits including; absorption of nutrients and supply of water to their host plants by the establishment a huge hyphal network in their rhizosphere, protect their hosts from pathogenic microorganisms (Ismail and Hijri, 2012; Ren *et al.*, 2013), and ameliorate drought stress (Baum *et al.*, 2015; Zhao *et al.*, 2015). Moreover, Yang *et al.*, (2015) added that under natural and stressful

conditions many plants are thought to benefit from the mycorrhizal symbiosis.

In comparison with several previous studies between AM plants and non-AM plants, they showed the importance of VAM fungi for enhancement of the plant drought resistance (Bárcana *et al.*, 2012; Ruiz-Lozano *et al.*, 2012; Sánchez-Romera *et al.*, 2016). However, Mena-Violante *et al.*, (2006); Yooyongwech *et al.*, (2016); Moradtalab *et al.*, (2019) revealed that AMF regulate plant growth under drought stress in several crops such as; wheat, maize, barley, strawberry, soybean and onion. A previous study of Al-Karaki *et al.*, (2004) recorded the improvement of growth, yield of grains and absorption of nutrients in AM inoculated wheat cultivars under drought stress. In reference to Miransari *et al.*, (2008), the AM fungus was more successful under higher levels of drought stress in improving the resistance of wheat and maize to stress.

Very few researches studied the different effects of VAM monocultures and poly-cultures on the same plant species (Kiers *et al.*, 2011; Thonar *et al.*, 2014; Gosling *et al.*, 2016). Otherwise, functional differentiation between VAM families increased their positive effects on the plant to complement one another. This hypothesis has been supported by many studies, showing that different families have different functional capacities (Thonar *et al.*, 2014; Yang *et al.*, 2016). Functional complementarity, whereby more than one VAM species synergistically colonize a root, has been observed by Alkan, (2006); Jansa *et al.*, (2008); Jin *et al.*, (2013). For example, Koide, (2000) study proposed that plants co-colonization by two or more AMF species could allow for a broader range of advantages and thus be more beneficial to the host plants. Conversely, other studies conducted by Edathil *et al.*, (1996); Hart *et al.*, (2013) indicated that a single successful AMF species will provide full benefits to the host plant, and no more profits are gained on combination of species. Recently, Chen *et al.*, (2017) investigated the effects of three AMF compositions, a)-namely VT, which is a mixture of different genera including; *Claroideoglossus* sp., *Funneliformis* sp.,

Diversispora sp., *Glomus* sp. and *Rhizophagus* sp., b)-BF, which is a mixture of AMF of the same genera but of different species such as; *G. intraradices*, *G. microageregatum* and *G. Claroideum* BEG 210., and c)-*Funneliformis mosseae* (Fm) as a single species of AMF, on the growth parameters, exchange of gases and concentration of nutrients in cucumber seedlings. They found that VT had the greatest advantages followed by BF and FM, as shown by the different morphological, physiological and molecular parameters.

The objective of the present work was to test the existence of any difference between single and combined inoculations of AMF on the wheat plant growth, under drought stress conditions.

2. Material and methods

2.1. Mycorrhizal inocula

The mycorrhizal inocula used include; *G. monosporum* (GM), *G. mosseae* (GS) and *Gigaspora gigantean* (GG). The two tested isolates (GM and GS) belong to order Glomerales and family Glomeraceae, while *Gigaspora gigantean* belongs to order Diversisporales and family Gigasporaceae. Pure cultures of these inocula were supplied by the

Microbiological Resource Center, Ain Shams University, Cairo, Egypt. In total, there were six AMF inoculation treatments; inoculation with the three individual species separately, mixed inoculation with the same genera GM+ GS (AA), mixed inoculation with different genera GM+GG (SN), and an uninoculated control. In the mixed inoculation treatments, each genus or species was added at a rate of 50 % from each single pure culture.

2.2. Host plant, and soil substrate

The wheat (*Triticum aestivum* L.) plant variety Sakha 93 was obtained from the Agricultural Research Centre, Giza, Egypt. The seeds were surface-disinfected with a 2% sodium hypochlorite solution for 6 min., and then washed 3 times with deionized sterilized water (Hua and Höfte, 2011).

The soil used in the greenhouse experiments was a sandy soil with the following chemical properties (Table 1). This substrate was autoclaved three times at 120 °C for 1 h, with an interval of 24 h, in reference to (Hua and Höfte, 2011). Wheat grain (25 grains in each pot) were planted in plastic pots (30×50 cm) pre-filled with 15 kg of the autoclaved soil.

Table 1. Soil chemical properties

CaCO ₃ %	Electric conductivity (Ec) dsm ⁻¹	pH	Cations (meq/l)				Anions (meq/l)		
			Ca ⁺	Mg ⁺	Na ⁺	K ⁺	Cl ⁻	SO ₄ ⁻²	HCO ₃ ⁻
0.52	3.91	7.45	5.2	3.9	19.6	0.72	15	8.1	2.1

2.3. Mycorrhizal inoculation and drought treatment

The greenhouse experiment was conducted in the winter season 2019/2020 at Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Moreover; the AM inocula were applied at the rate of 10 ml/ plant (containing 500± 20 spores) after two weeks of planting, where the inocula were placed close to the plants roots. Drought stress was initiated 4 weeks after planting to allow successful establishment of the AM fungal inoculum. Two irrigation periods were used; well watered (WW) after 4 d, and water-stressed (WS) after 8 d of planting. Thus, it was a factorial design with six treatments and four replicates per each treatment. The experiment was carried out to study the effect of single and mixed inoculations with mycorrhiza on the growth of wheat under WW and WS conditions. About 60 d after sowing date, the chlorophyll (SPAD) value and relative water content (RWC) was recorded as follow:

The chlorophyll SPAD values were estimated using a SPAD-502 chlorophyll meter (Minolta Co., Ltd., Japan), which is a portable, self-calibrating, convenient, and non-destructive device that can be used for measuring the amount of chlorophyll in plant leaves at the flowering stage (Minolta, 1989). To determine the relative water content (RWC): A fresh leaf (100 mg) sample was submerged in 10 ml dist. water till saturation and then left overnight. Surface water of the leaves was blotted off without posing any pressure and then weighed (Turgid weight). The dry weight of the leaves was recorded after incubation at 70°C for 72 h. The RWC was calculated according to the method of Barr and Weatherley, (1962) using the following equation:

$$\text{RWC \%} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

At grain maturity, different growth parameters including; plant length (cm), number of spikes/ plant,

spike length (cm), number of spikelet/spike, 1000-grain weight (g) and grain yield/ plant were recorded.

2.4. Quantification of AMF root colonization

About 0.5 g of wheat roots from each treatment were washed carefully using dist. water and then chopped using a scalpel into 1 cm fragments. Root segments were soaked in 10 % KOH at 90°C for 30 min., and then rinsed with dist. water carefully, followed by neutralization with 1 % HCl for 30 min. The root samples were stained using trypan blue for 24 h at room temperature, according to the method of conducted by Brundrett *et al.*, (1984). Finally, the roots were rinsed with dist. water and then de-stained in 50% glycerol (Koske, 1989). The AMF colonization rate was quantified using a compound microscope in reference to Brundrett *et al.*, (1996).

2.5. Statistical analysis

The collected data for each quantitative treatment was subjected to two-way analysis of variance (ANOVA) for the randomized complete block (RCB) design with 4 replications. Variance analysis was performed using Co-stat software version 6.311. Duncan`s post hoc tests was performed for evaluation of the statistical significances among the main factors and their interactions. P value ≤ 0.05 was considered to be significant statistically.

3. Results

3.1. Analysis of variances

The analysis of variance showed significant difference ($p \leq 0.05$) among various treatments (well water and water stress) and the mycorrhizal species in all the studied traits (Table 2). The interaction between drought stress and mycorrhizal application is significant for all of the measured traits, except for the number of 1000-grain weight, chlorophyll SPAD value and relative water content (RWC).

Table 2. Significance of mean squares from analysis of variance (ANOVA) for yield and its attributes in wheat plant

Degrees of freedom (D.f)	1	4	4
Characters	Drought (D)	AMF species (S)	D×S
Plant height	**	**	**
No. of spike/ plant	**	*	*
Spike length	**	**	*
No. of spikelet/ spike	**	**	*
1000-grain weight	**	**	ns
Grain yield/ plant	**	*	**
Chlorophyll SPAD value	**	**	ns
Relative water content (RWC)	*	*	ns

Where; ns= not significant, *=significant, **= highly significant

3.2. Plant growth parameters

Results of this study showed that when water stress is increased, plant growth parameters are significantly reduced (Table 3). In contrast to the AMF plants, plant growth parameters are promoted by the mycorrhizal treatment under WW and WS conditions. In comparison to the non-inoculated control plants under stress condition, the recorded increase in plant height of wheat inoculated with SN and AA are by 27.30% and 24.95%; respectively, whereas GG, GS and GM treatments increased plant height by 16.01%, 14.6% and 6.83%, respectively (Table 3). The number of spikes/plant, spike length, number of spikelet/ spike, 1000-grain weight and grain yield/ plant are recorded by SN at 20.65%, 72.86%, 82.16%, 29.45% and 57.07%; respectively,

followed by AA inoculation demonstrating increase by 17.65, 59.97%, 70.78%, 24.44% and 46.51%, respectively. At the end of this greenhouse assay, all VAM-inoculated plants are significantly improved compared to the non-treated control plants.

3.3. Effects of different AMF species on chlorophyll contents of wheat plants

Table (3) shows a notable increase in chlorophyll contents which is observed in AMF-inoculated plants, compared to the non-AMF control. The overall amounts of chlorophyll in SN inoculated wheat plants is increased by 28.63%, followed by AA inoculated plants (21.42%). Meanwhile, the total chlorophyll contents of GG, GS and GM inoculated wheat plants are increased by 13.44% 11.03% and 7.12 %, respectively.

Table 3. Promotion of wheat plant yield and its attributes under normal and water stress conditions

		Plant height (cm)	No. of spikes	Spike length (cm)	No. of spikelet's	1000-grain weight (g)	Grain yield/plant (g)	Chlorophyll SPAD value	Relative water content (RWC)
Main Effects									
Water stress	WW	97.89	18.43	14.45	22.55	53.38	10.57	57.63	68.63
	WS	81.42	12.33	9.31	14.51	40.49	7.71	45.13	72.68
Mycorrhizal sp.	Control	82.08	13.00	9.67	15.44	41.10	8.05	47.01	73.64
	GM	84.34	14.50	11.00	17.06	44.34	8.22	49.97	70.92
	GS	87.59	13.83	11.00	17.12	46.60	8.74	48.46	71.50
	GG	91.42	13.78	12.17	19.21	47.45	9.24	51.58	71.69
	AA	95.25	17.83	13.50	20.77	48.75	9.90	54.43	69.14
	SN	97.25	19.34	13.93	21.60	53.37	10.71	56.83	67.05
L.S.D 0.05% for water stress		2.71	1.06	0.79	0.73	2.37	1.63	2.47	3
L.S.D 0.05% for mycorrhizal sp.		4.7	1.83	1.37	1.27	4.11	2.83	4.28	5.2
Interaction									
Well watered (WW)	Control	93.33	14.67	12.67	20.33	47.67	10.23	54.30	71.37
	GM	93.00	16.67	13.67	22.67	48.40	9.23	55.83	69.84
	GS	94.00	15.33	13.67	22.23	53.70	10.10	54.37	69.20
	GG	100.67	16.56	14.00	22.54	53.93	10.47	58.10	71.15
	AA	102.00	22.33	16.33	23.54	54.53	11.20	60.63	66.94
	SN	104.33	25.00	16.33	24.00	62.03	12.20	62.57	63.30
Water stress (WS)	Control	70.83	11.33	6.67	10.54	34.53	5.87	39.72	75.91
	GM	75.67	12.33	8.33	11.44	40.27	7.20	44.10	72.00
	GS	81.17	12.33	8.33	12.00	39.50	7.37	42.55	73.80
	GG	82.17	11.00	10.33	15.88	40.97	8.00	45.06	72.22
	AA	88.50	13.33	10.67	18.00	42.97	8.60	48.23	71.34
	SN	90.17	13.67	11.53	19.20	44.70	9.22	51.09	70.80
L.S.D 0.05% for W×S interaction		3.32	1.29	0.97	0.89	2.9	2	3.03	3.68

Where; *Glomus monosporum* (GM), *Glomus mosseae* (GS), *Gigaspora gigantean* (GG), SN (*G. monosporum* and *Gigaspora gigantean*) and AA (*G. monosporum* and *Glomus mosseae*)

3.4. Effects of different AMF species on the relative water content (RWC)

The relative water content (RWC) of the wheat plant has been estimated under drought stress conditions. The RWC of AMF fungi under normal and stress conditions appeared high values compared to the control; however, it decreased significantly in AMF -inoculated plants at both conditions (Table. 3).

3.5. Root colonization

The recorded percentage of AMF colonization on wheat roots are presented in Fig. (1). All plants

except the non-inoculated ones are colonized by AMF. Mycorrhizal root colonization under well watered treatment is significantly greater than for their corresponding water stress treatments. The lowest colonization is observed in *G. monosporum* (GM), *G. mosseae* (GS) and *Gigaspora gigantean* (GG) recording 35%, 37% and 42%, respectively, under water stress. However, mycorrhizal colonization by more than 60% is clearly visible for both AMF mixture treatments under the water stress conditions. Mycorrhizal colonization of inoculated wheat roots are demonstrated in Fig. (2).

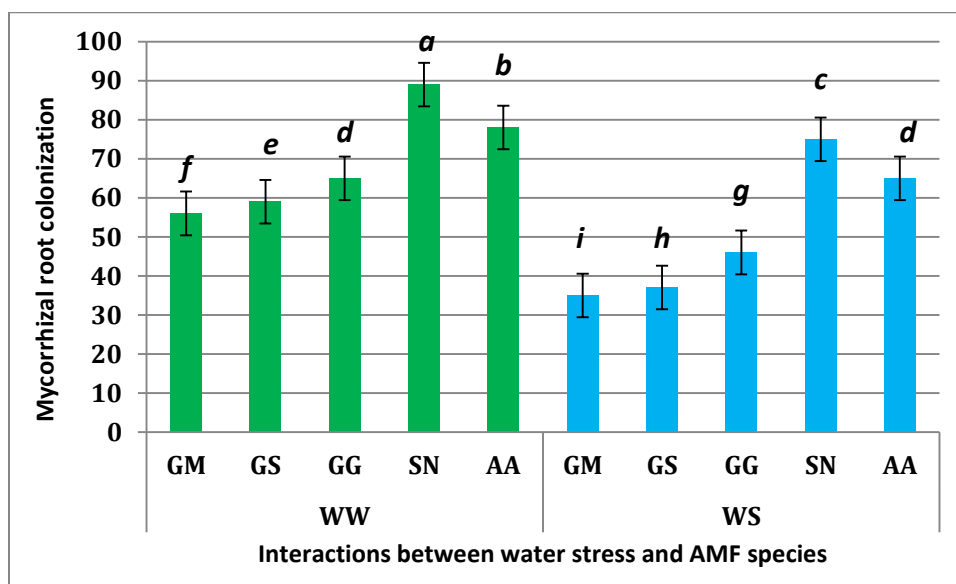


Fig. 1: Mycorrhizal colonization of wheat roots after 4 months under well watered (WW) and water stress (WS) conditions. Where; *G. monosporum* (GM), *G. mosseae* (GS), *Gigaspora gigantean* (GG), SN (*G. monosporum* and *Gigaspora gigantean*) and AA (*G. monosporum* and *Glomus mosseae*). The error bars represent standard deviations (SD). Columns headed by the same letter are not significantly different according to Duncan’s multiple range test ($p < 0.05$).

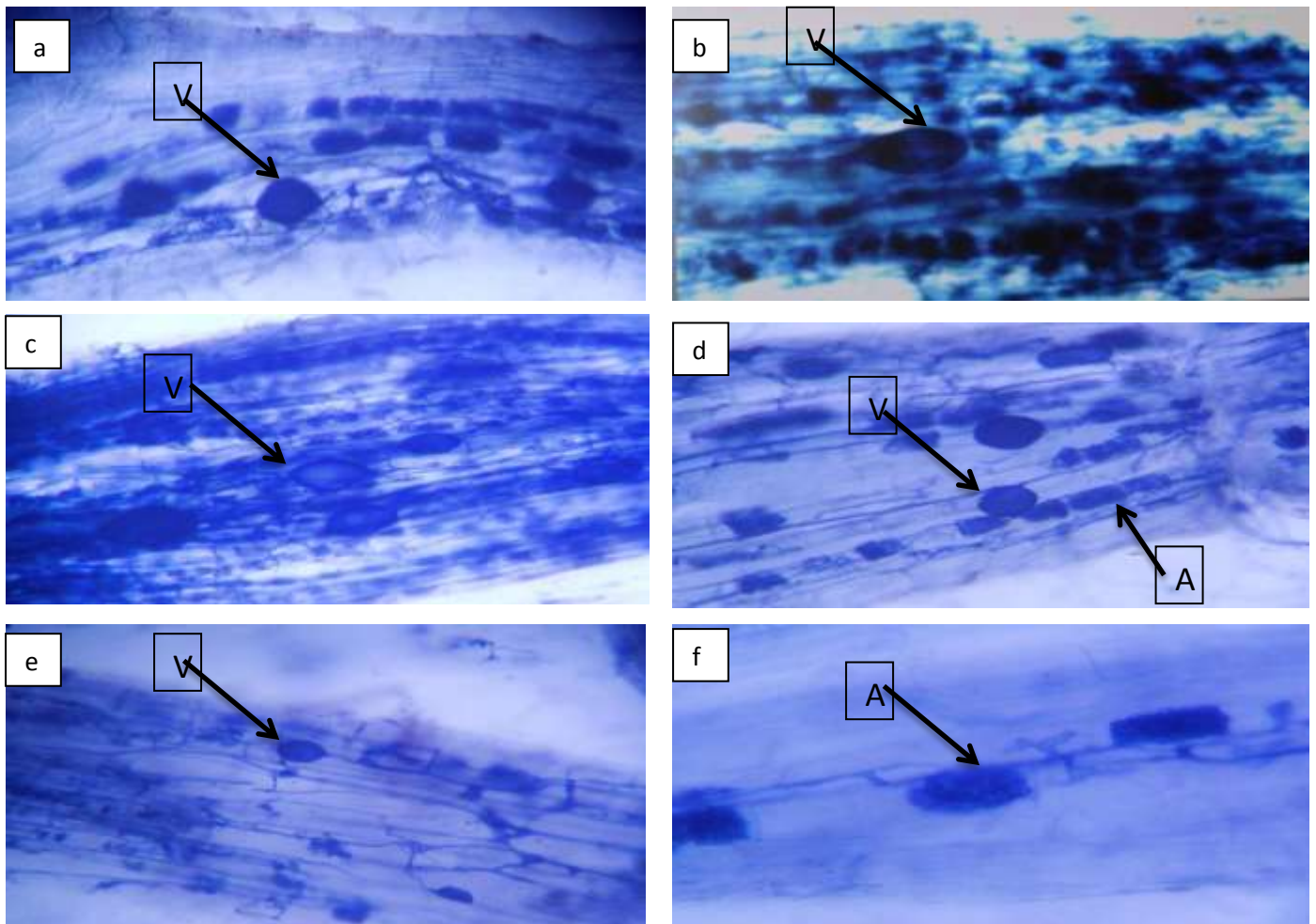


Fig. 2: Photomicrographs of mycorrhizal colonization of wheat plant roots after clearing and staining (200×). Where; (V): typical vesicle and (A): arbuscular, formed by AMF in the root cortex of the wheat samples

4. Discussion

In recent decades, AMF has been involved in the improvement of plant growth, nutrient acquisition, photosynthesis, and tolerance to biotic and abiotic stresses (Cavagnaro *et al.*, 2015; Liu *et al.*, 2016). Furthermore, Porcel *et al.*, (2015) revealed that AMF colonization increased the plant growth as it may increase the area of nutrients absorption in AMF-colonized plants. A previous study conducted by Omirou *et al.*, (2013) reported that roots of the water-stressed strawberries

presented higher AMF colonization, indicating that under stress conditions; plants become increasingly dependent on the consistent improved productivity of water usage. However, the use of appropriate AMF, either singly or in combinations, remains a major challenge as the reciprocal benefits vary greatly depending on the AMF strains.

In the current work, combinations of multiple or single species of AMF were used to investigate their effects on wheat plant growth, RWC and its chlorophyll contents. Results demonstrated that plant

height, number of spikes, spike length, number of spikelet's, 1000-grain weight and grain yield/ plant, chlorophyll content, and RWC in wheat plants treated with AMF were substantially higher compared to the non-treated control plants. Moreover, we also observed that SN treatment, which is combination of different genera and species, had better effect on wheat growth compared to that of AA treatment, which is a combination of AMF from the same genus but of different species. Functional redundancy between closely related microorganisms might explain their lower competitive efficiency, as reported previously by Thonar *et al.*, (2014); Yang *et al.*, (2016); Gosling *et al.*, (2016).

In addition, both of SN and AA were more successful than GG, GS and GM treatment which were a single AMF species. These results are consistent with previous study of Chen *et al.*, (2017), who reported that combination of VAM from different genera and species had better effects on cucumber growth, and combination of VAM appeared to be more effective than a single AMF species. In a recent study of Amir *et al.*, (2019) on ultramafic soil in the field, treatment of the same plant species with mixture of three AMF isolates recorded four times higher biomass than the control plants. Our results are in accordance with those of Maherli and Klironomos, (2007a), who reported that tested AMF co-inoculations of four to eight species belonging to one, two or three different families were most efficient. Similarly, Koide, (2000) proposed that co-colonization of two or more AMF species could allow for a broader range of advantages and thus be more beneficial to the plants. Earlier research of Maherli and Klironomos, (2012) showed that distant AMF species offer greater promoting effects to the plant growth and coexist better more than the closely related AMF species. It is more likely that related species coexist phylogenetically better than similar ones. All of these findings indicate that when a plant faces multiple stress factors, the use of an AMF mixture

comprising various taxa is more effective than mono-inoculation to increase the plant biomass (Hart *et al.*, 2013; Maherli and Klironomos, 2007; Yang *et al.*, 2016). These findings also clearly emphasize the significance of mycorrhizal diversities in plant symbiosis especially under multi-stress conditions. Finally, AMF symbiosis can have a major impact on plant growth and adaptation when inoculated in combination with native AMF taxa, and this opens up a new viewpoint for the usage of AMF under the plant stress conditions.

Conclusion

The current findings clearly emphasize the significance of mycorrhizal diversities on plant growth and adaptation, especially when inoculated in combination with native AMF taxa under drought stress. This opens up a new viewpoint for the usage of AMF under the plant stress conditions.

Acknowledgment

The author would like to acknowledge the Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, for supporting the current work.

Funding source

No fund was received for this work from any organization.

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