Novel Research in Microbiology Journal (2020), 4(5): 992-1004
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/nrmj.2020.118449

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

Alaa Fathalla Mohammed

Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

*Corresponding author E-mail: Alaa.fathalla@yahoo.com

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 vasicular arbuscular mycorrhizal (VAM) fungi form a symbiotic relationship with 80% of all the terrestrial plant species, and have tremendous potential to act as plant bio-stimulants. 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 (Bárzana *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; *Claroideoglomus* 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 un-inoculated 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:

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

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**
Mycorrihzal 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 mycorrihzal 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).

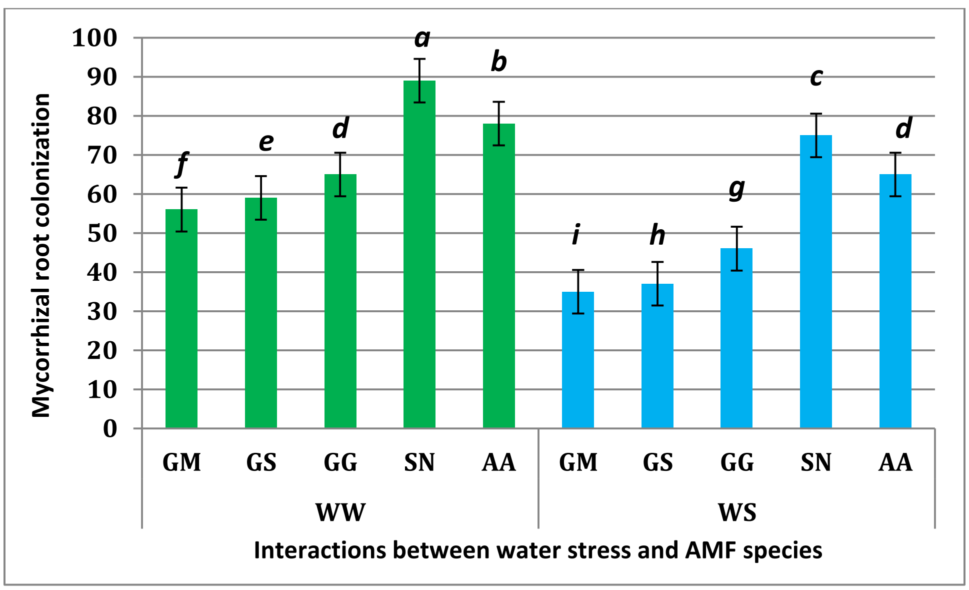

Fig. 1: Mycorrhizal colonization of wheat roots after 4 months under well watered (WW) and water stress (WS) conditions. Where; *G. monosporum* (GM), *G. mosseae* (GS), *Gigaspora gigantean* (GG), SN (*G. monosporum* and *Gigaspora gigantean*) and AA (*G. monosporum* and *Glomus mosseae*). The error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

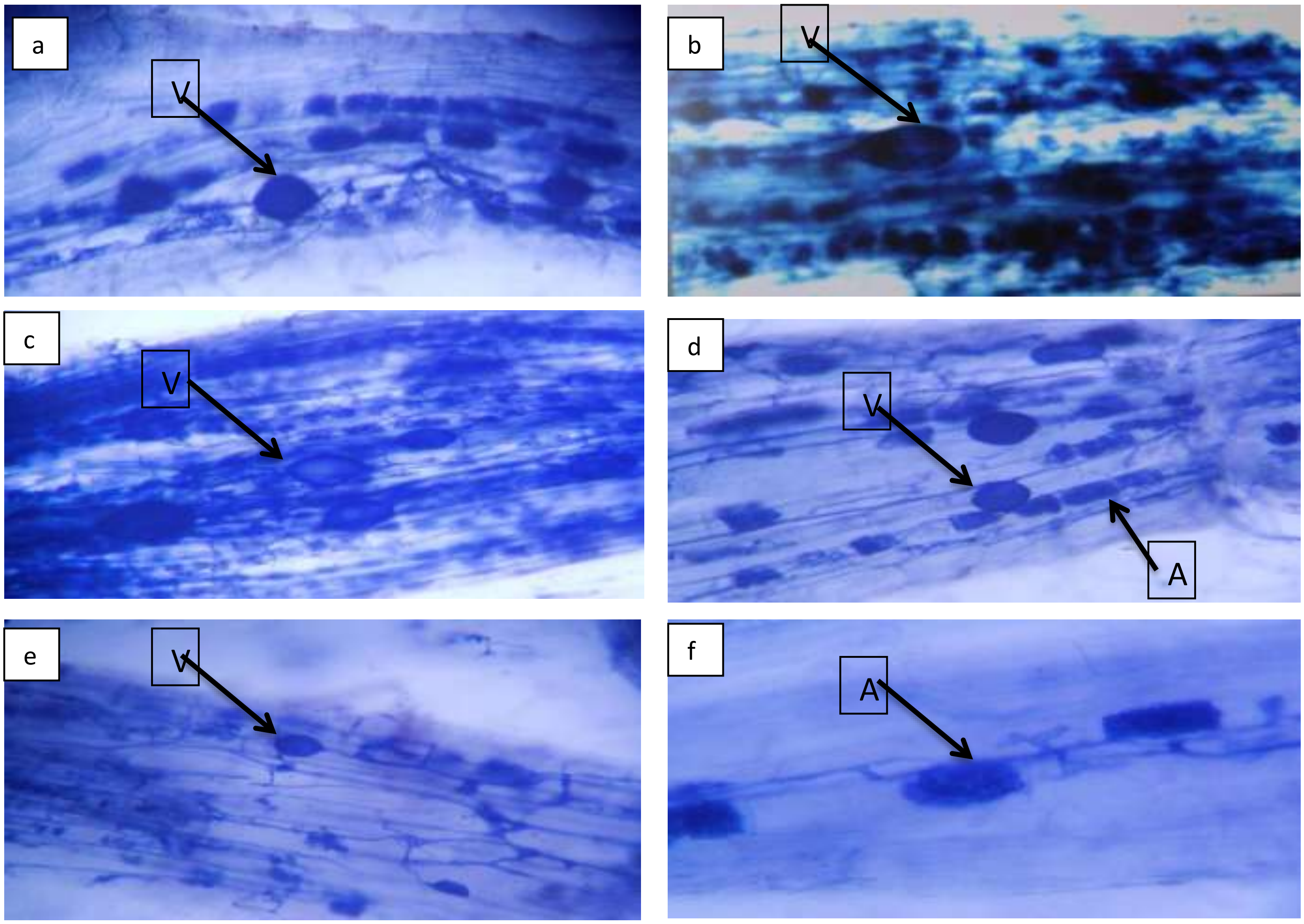

Fig. 2: Photomicrographs of mycorrhizal colonization of wheat plant roots after clearing and staining (200×). Where; (V): typical vesicle and (A): arbuscular, formed by AMF in the root cortex of the wheat samples

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 nom-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 Maherali and Klironomos, (2007), 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 Maherali 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; Maherali 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.

Ethical approval

Non-applicable.

5. References

Alkan, N. (2006). Analysis of quantitative interactions between two species of Arbuscular mycorrhizal fungi, *Glomus mosseae* and *G. intraradices,* by real-time PCR. Applied and Environmental Microbiology. 72: 4192-4199.

Al-Karaki, G.; McMichael, B. and Zak, J. (2004). Field response of wheat to Arbuscular mycorrhizal fungi and drought stress. Mycorrhiza. 14: 263-269.

Amir, H.; Cavaloc, Y.; Laurent, A.; Pagand, P.; Gunkel, P.; Lemestre, M.; Médevielle, V.; Pain, A. and McCoy, S. (2019). Arbuscular mycorrhizal fungi and sewage sludge enhance growth and adaptation of *Metrosideros laurifolia* on ultramafic soil in New Caledonia: A field experiment. Science of the Total Environment. 651: 334-343.

Barr, H.D. and Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficit in leaves. Australian Journal of Biological Sciences. 15(3): 413-428.

Bárzana, G.; Aroca, R.; Paz, J.A.; Chaumont, F.; Martinez-Ballesta, M.C.; Carvajal, M. and Ruiz-Lozano, J.M. (2012). Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Annals of Botany. 109(5): 1009-1017.

Baum, C.; El-Tohamy, W. and Gruda, N. (2015). Increasing the productivity and product quality of vegetable crops using Arbuscular mycorrhizal fungi: A review. Scientia Horticulturae. 187: 131-141.

Berruti, A.; Lumini, E.; Balestrini, R. and Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Frontiers in Microbiology. 6: 1559.

Borlu, H.O.; Celiktas, V.; Duzenli, S.; Hossain, A. and El Sabagh, A. (2018). Germination and early seedling growth of five durum wheat cultivars (*Triticum durum* desf.) is affected by different levels of salinity. Fresenius Environmental Bulletin. 27: 7746-7757.

Bowles, T.M.; Jackson, L.E. and Cavagnaro, T.R. (2018). Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes. Global Change Biology. 24: e171-e182.

Brundrett, M.; Bougher, N.; Dell, B.; Grove, T. and Malajczuk, N. (1996). Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research. 374.

Brundrett, M.C.; Piché, Y. and Peterson, R.L. (1984). A new method for observing the morphology of vesicular–arbuscular mycorrhizae. Canadian Journal of Botany. 62: 2128-2134.

Cavagnaro, T.R.; Bender, S.F.; Asghari, H.R. and Heijden, M.G.A. (2015). The role of Arbuscular mycorrhizas in reducing soil nutrient loss. Trends in Plant Science. 20: 283-290.

Chen, S.; Zhao, H.; Zou, C.; Li, Y.; Chen, Y.; Wang, Z.; Jiang, Y.; Liu, A.; Zhao, P. and Wang, M. (2017). Combined Inoculation with Multiple Arbuscular Mycorrhizal Fungi Improves Growth, Nutrient Uptake and Photosynthesis in Cucumber Seedlings. Frontiers in Microbiology. 8: 2516.

Coleman-Derr, D. and Tringe, S.G. (2014). Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. Frontiers in Microbiology. 5: 283.

Condon, A.G.; Richards, R.A.; Rebetzke, G.J. and Farquhar, G.D. (2004). Breeding for high water-use efficiency. Journal of Experimental Botany. 55: 2447-2460.

Edathil, T.T.; Manian, S. and Udaiyan, K. (1996). Interaction of multiple VAM fungal species on root colonization, plant growth and nutrient status of tomato seedlings (*Lycopersicon esculentum* Mill.). Agriculture, Ecosystems and Environment. 59: 63-68.

Gosling, P.; Jones, J. and Bending, G.D. (2016). Evidence for functional redundancy in arbuscular mycorrhizal fungi and implications for agroecosystem management. Mycorrhiza. 26: 77-83.

Hameed, A.; Wu, Q.S.; Abd-Allah, E.F.; Hashem, A.; Kumar, A.; Lone, H.A. and Ahmad, P. (2014). Role of AM fungi in alleviating drought stress in plants. In Use of Microbes for the Alleviation of Soil Stresses. 55-75.

Hart, M.M.; Forsythe, J.; Oshowski, B.; Bücking, H. and Jansa, J. (2013). Hiding in a crowd—does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis?. Symbiosis. 59: 47-56.

Hua, G.K.H. and Höfte, M. (2011). The use of coconut fiber and antagonistic *Pseudomonas* CMR12a for biological control of *Rhizoctonia* damping-off on Chinese cabbage (*Brassica rapa*). Plant growth-promoting rhizobacteria (PGPR) for sustainable agriculture. In: Proceedings of the 2nd Asian PGPR Conference, Beijing, China. pp. 233-239.

Ismail, Y. and Hijri, M. (2012). Arbuscular mycorrhisation with *Glomus irregulare* induces expression of potato PR homologues genes in response to infection by *Fusarium sambucinum*. Functional Plant Biology. 39: 236-245.

Jansa, J., Smith, F.A. and Smith, S.E. (2008). Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi?. New Phytologist. 177: 779-789.

Jin, H., Germida, J.J. and Walley, F.L. (2013). Impact of arbuscular mycorrhizal fungal inoculants on subsequent arbuscular mycorrhizal fungi colonization in pot-cultured field pea (*Pisum sativum* L.). Mycorrhiza. 23: 45-59.

Kiers, E.T.; Duhamel, M.; Beesetty, Y.; Mensah, J.A.; Franken, O.; Verbruggen, E.; Fellbaum, C.R.; Kowalchuk, G.A.; Hart, M.M. and Bago, A. (2011). Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science. 333: 880-882.

Koide, R.T. (2000). Functional complementarity in the Arbuscular mycorrhizal symbiosis. The New Phytologist. 147: 233-235.

Koske, R.E. (1989). A modified procedure for stainng roots to detect VA mycorrhizas. Mycological Research. 92: 486-505.

Liu, A.; Chen, S.; Wang, M.; Liu, D.; Chang, R.; Wang, Z.; Lin, X.; Bai, B. and Ahammed, G.J. (2016). Arbuscular Mycorrhizal Fungus Alleviates Chilling Stress by Boosting Redox Poise and Antioxidant Potential of Tomato Seedlings. Journal of Plant Growth Regulation. 35: 109-120.

Maherali, H. and Klironomos, J.N. (2012). Phylogenetic and Trait-Based Assembly of Arbuscular Mycorrhizal Fungal Communities. PLoS ONE. 7: e36695.

Maherali, H. and Klironomos, J.N. (2007). Influence of Phylogeny on Fungal Community Assembly and Ecosystem Functioning. Science. 316: 1746-1748.

Mena-Violante, H.G.; Ocampo-Jiménez, O.; Dendooven, L.; Martínez-Soto, G.; González-Castañeda, J.; Davies, F.T. and Olalde-Portugal, V. (2006). Arbuscular mycorrhizal fungi enhance fruit growth and quality of chile ancho (*Capsicum annuum* L. cv. *San Luis*) plants exposed to drought. Mycorrhiza. 16: 261-267.

Minolta, K. (1989). Chlorophyll meter SPAD-502 instruction manual. Minolta Co., Ltd., Radiometric Instruments Operations Osaka, Japan.

Miransari, M.; Bahrami, H.A.; Rejali, F. and Malakouti, M.J. (2008). Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biology and Biochemistry. 40: 1197-1206.

Mirzaee, M.; Moieni, A. and Ghanati, F. (2013). Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. Journal of Agricultural Science and Technology. 15: 593-602.

Moradtalab, N.; Hajiboland, R.; Aliasgharzad, N.; Hartmann, T.E. and Neumann, G. (2019). Silicon and the Association with an Arbuscular-Mycorrhizal Fungus (*Rhizophagus clarus*) Mitigate the Adverse Effects of Drought Stress on Strawberry. Agronomy. 9(1): 41.

Nadeem, S.M.; Ahmad, M.; Zahir, Z.A.; Javaid, A. and Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnology Advances. 32: 429-448.

Omirou, M.; Ioannides, I.M. and Ehaliotis, C. (2013). Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots, but leads to improved colonization and plant response under water stress only. Applied Soil Ecology. 63: 112-119.

Pocketbook, F.S. (2015). World Food and Agriculture. Food and Agriculture Organization: Rome, Italy.

Porcel, R.; Redondo-Gómez, S.; Mateos-Naranjo, E.; Aroca, R.; Garcia, R. and Ruiz-Lozano, J.M. (2015). Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. Journal of Plant Physiology. 185: 75-83.

Ren, L.; Lou, Y.; Zhang, N.; Zhu, X.; Hao, W.; Sun, S.; Shen, Q. and Xu, G. (2013). Role of arbuscular mycorrhizal network in carbon and phosphorus transfer between plants. Biology and Fertility of Soils. 49: 3-11.

Rolli, E.; Marasco, R.; Vigani, G.; Ettoumi, B.; Mapelli, F.; Deangelis, M.L.; Gandolfi, C.; Casati, E.; Previtali, F. and Gerbino, R. (2015). Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environmental Microbiology. 17: 316-331.

Ruiz-Lozano, J.M.; Porcel, R.; Azcon, C. and Aroca, R. (2012). Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. Journal of Experimental Botany. 63: 4033-4044.

Sánchez-Romera, B.; Ruiz-Lozano, J.M.; Zamarreño, Á.M.; García-Mina, J.M. and Aroca, R. (2016). Arbuscular mycorrhizal symbiosis and methyl jasmonate avoid the inhibition of root hydraulic conductivity caused by drought. Mycorrhiza. 26: 111-122.

Schausberger, P.; Peneder, S.; Jürschik, S. and Hoffmann, D. (2012). Mycorrhiza changes plant volatiles to attract spider mite enemies. Functional Ecology. 26: 441-449.

Smith, S.E. and Read, D.J. (2010). Mycorrhizal symbiosis. 3rd Edition, Academic press.

Sommer, R.; Glazirina, M.; Yuldashev, T.; Otarov, A.; Ibraeva, M.; Martynova, L.; Bekenov, M.; Kholov, B.; Ibragimov, N. and Kobilov, R. (2013). Impact of climate change on wheat productivity in Central Asia. Agriculture, Ecosystems and Environment. 178: 78-99.

Spatafora, J.W.; Benny, G.L.; Lazarus, K., Smith, M.E.; Berbee, M.L.; Bonito, G.; Corradi, N.; Grigoriev, I.; Gryganskyi, A. and James, T. Y. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia. 108: 1028-1046.

Šramková, Z.; Gregová, E. and Šturdík, E. (2009). Chemical composition and nutritional quality of wheat grain. Acta Chimica Slovaca. 2: 115–138.

Thonar, C.; Frossard, E.; Šmilauer, P. and Jansa, J. (2014). Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. Molecular Ecology. 23: 733-746.

Timmusk, S.; El-Daim, I.A.A.; Copolovici, L.; Tanilas, T.; Kännaste, A.; Behers, L.; Nevo, E.; Seisenbaeva, G.; Stenström, E. and Niinemets, Ü. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PloS one. 9: e96086.

Yang, Y.; Liang, Y.; Han, X.; Chiu, T.-Y.; Ghosh, A.; Chen, H. and Tang, M. (2016). The roles of arbuscular mycorrhizal fungi (AMF) in phytoremediation and tree-herb interactions in Pb contaminated soil. Scientific Reports. 6: 20469.

Yang, Y.; Han, X.; Liang, Y.; Ghosh, A.; Chen, J. and Tang, M. (2015). The Combined Effects of Arbuscular Mycorrhizal Fungi (AMF) and Lead (Pb) Stress on Pb Accumulation, Plant Growth Parameters, Photosynthesis, and Antioxidant Enzymes in *Robinia pseudoacacia* L. PLoS One. 10(12): e0145726.

Yooyongwech, S.; Samphumphuang, T.; Tisarum, R.; Theerawitaya, C. and Cha-um, S. (2016). Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. Scientia Horticulturae. 198: 107-117.

Zhao, R.; Guo, W.; Bi, N.; Guo, J.; Wang, L.; Zhao, J. and Zhang, J. (2015). Arbuscular mycorrhizal fungi affect the growth, nutrient uptake and water status of maize (*Zea mays* L.) grown in two types of coal mine spoils under drought stress. Applied Soil Ecology. 88: 41-49.