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Mycorrhizal contribution to water economy in agriculture under hot and dry climate: The case study of water melon in Algeria

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Climatic warming and drought affect watermelon yields in Algeria, an important and water-demanding crop. This study aimed to test arbuscular mycorhizal (AM) fungi as an alternative to manage water deficit on watermelon culture. We experiment a watering regime gradient of 100%, 50%, 25 % and 5% of soil field capacity (FC) on mycorhized and non-mycorhized seedlings in greenhouse.

The results showed that limited water irrigation reduced mycorrhizal colonization by 50.01% at the severe water stress (5%FC). However, the mycorrhizal dependency of watermelon plants to AM fungi increased with increasing water deficit and improved significantly growth parameter, among them flowering and root/shoot ratio, wich are indicators of a good yields. Thanks to AM fungi, shoot biomass of inoculated plants watering with only (5%FC) was similar to those obtain with non-mycorrhized plants more irrigated at 50%FC and higher than irrigated at 25%FC. Roots biomass of mycorrhized plant under water stress of 50%FC and 25%FC was similar to those obtained with the irrigation of 100% FC for the controls. Mycorrhizal inoculation can be used as a sustainable solution to manage the water resources, improve drought tolerance and growth of watermelon culture in areas with limited water availability.

Keywords: AM Inoculum, mycorrhizal colonization, mycorrhizal dependence, watermelon (*Citrullus lanatus*), water stress, growth.

INTRODUCTION

Drought is considered as the most important abiotic factor limiting plant growth and yield in many areas (Bray, 2004, Trenberth et al., 2014) a problem accentuated by the global warming. Drought affects many aspects of plants, inhibiting photosynthesis, limiting water uptake, damaging plasma membranes, and ultimately resulting in decreased growth (Talbi et al., 2015). Plants have the ability to resist to drought conditions through various mechanisms including increased root growth, but this capacity remains limited (Mo et al., 2016). Plants have another drought adaptation strategy thanks to their ability to associate symbiotically with arbuscular mycorrhizal fungi (AMF) (Gianinazzi et al., 2010). Arbuscular mycorhizal fungi associate with plants and extend their network of hyphae beyond the root absorption zone, promoting the exploration of a greater volume of soil than the root system alone (Mohammadi et al., 2011 Cavagnaro et al., 2015), improving plant nutrient uptake, protecting plants from pathogens (Borowicz 2001, Ismail and Hijri 2012, Ren et al., 2013) and buffer against adverse environmental conditions, especially drought (Smith et al., 2010).

Watermelon (*Citrullus lanatus*) is an important summer crop in Algeria, but its cultivation is water demanding, especially during the fruit development stage (Kaya et al., 2003). According to FAO, Algeria is the sixth major producer of watermelon in the world with of 1,895,074.00 T/ha in 2017. Algeria, as a Mediterranean basin country is also impacted by the climate changes and scarcity of water resource. The climate data indicate that Algeria will know a declining precipitation of 13% and increasing temperatures of 1.1 ° C by 2020, until 2°C by 2050. These changes will lead to a frequent droughts and a growing irrigation demand by an agriculture sector already using 59% (or 4.99 billion m^3/year) of all freshwater consumption (FAOSTAT, 2016).

According to global climate change projections, the pressure of environmental stressors, especially drought on crop productivity is increasing and is challenging global food security. The application strategy of beneficial microorganisms can be an ecological means to improve crop performance and yield stability (Bernardo et al., 2019). The objective of this study is to test the efficiency of AM fungi application as an ecological and sustainable strategy to overcome water stress, to develop a better water management and to improve drought tolerance of watermelon.

MATERIALS AND METHODS

Plants and arbuscular mycorrhizal fungal inoculum

Watermelon seeds of the variety Meziane were germinated in alveolar plates filled with peat. Half of the plants were inoculated with 10g/plant of Symbivit®, an inoculum in granular form made of 6 species of AMF (*Claroideoglomus etunicatum, C. claroideum, Glomus microaggregatum, Rhizophagus intraradices, Funneliformis mosseae, F. geosporum*). After three weeks, the seedlings obtained were transplanted into planting bags containing 2000 g of the sterilized soil.

Experimental design and water treatments

The experiment was conducted in a greenhouse located in northeast of Algeria at the University of Skikda. The average daily temperature was 18-25 °C, the relative humidity was of 60-70% and about 16 hours of normal daylight. The treatments applied were the level of irrigation and inoculation, with: (i) Four levels of irrigation regimen: 100% field capacity (FC), 50% FC, 25% FC, 5% FC and (ii) Two inoculation status: mycorrhized and non-mycorrhized plants (control). The totally randomized experimental design counted 8 treatments repeated 8 times, for a total of 64 plants. The treatments applied to the watermelon plants in a completely random manner were: (I) 100% FC irrigation without inoculation, (II) irrigation limited to 50% FC without inoculation, (III) irrigation limited to 25% FC without inoculation; (IV) 5% FC irrigation without inoculation; (V) 100% FC irrigation with inoculation; (VI) irrigation limited to 50% FC with inoculation; (VII) limited irrigation with inoculation at 25% FC and finally (VIII) irrigation at 5% FC with inoculation. The plants were maintained under greenhouse conditions for a period of one month and daily irrigated with sterile water at the needed level of each treatment.

The experiment required the preparation of a sterile culture medium from sandy-clay soil collected from an agricultural field suitable to the culture of water melon at the commune of Larbi Ben M'hidi in Skikda province, Algeria (36°,50',53''N 6°, 58'06''E). Soil sample was sterilized three times in an autoclave, 60 minutes at 120°C with 24 hour between each autoclave. The field capacity (FC) of the soil was calculated as the difference between the volume of water poured on 1 kilogram (kg) of dried soil and the volume of water that has drained. The field capacity thus corresponds to the volume of water retained, which was 106 ml for 1 kg of this soil, the irrigation levels applied was therefore: (i) 106 ml or 100% FC, (ii) 53 ml or 50% FC, (iii) 26.5 ml or 25% FC is, (iv) 5.3 ml or 5% FC.

Arbuscular mycorrhizal development, mycorrhizal dependence and measurement of plant growth parameters

After 30 days of greenhouse culture, mycorrhizal colonization, growth parameters and water content were evaluated. Root samples of the watermelon plants from each treatment were collected and stained according to the technique of Phillips and Hayman (1970). Root observations were made on 3 repetitions of 30 root fragments of 1 cm placed between slide and cover slip in a drop of glycerol. Estimation of the Frequency of mycorrhizal root colonisation (F%) (Percentage of the number of mycorrhizal root fragments, reflecting the importance of the penetration points of the colonized root system), was made according to the method described by Trouvelot et al. (1986), using MYCOCALC software (http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Mycorrhizal dependency is calculated as follows: (Dry weight of mycorrhized plants - Dry mass of control plants) / Dry mass of mycorrhized plants ×100 (Plenchette et al., 1983).

The decrease in loss of biomass was calculated by the difference between dry biomass loss of mycorhized plant and dry biomass loss of non mycorhized plant under different level of water stress. Dry biomass loss for mycorhized plant (%) = [(Dry biomass of mycorrhized plant watering at 100%FC – Dry biomass of mycorrhized plants stressed at 50%FC, 25%FC and 5%FC) / Dry biomass of mycorrhized plants watering at 100%FC] × 100 and the loss of dry biomass for non mycorhized plant (%) = [(Dry biomass of non mycorrhized plant watering at 100%FC – Dry biomass of non mycorrhized plants stressed at 50%FC, 25%FC and 5%FC) / Dry biomass of non mycorrhized plants watering at 100%FC] × 100. The leaves and flowers were enumerated. Shoot and root fresh and dry weight (after drying at 70°C for 72 hours), the length of the aerial part of the plant (cm) were measured and the ratio root on shoot have been calculated. The water content determined by the difference between the mass of fresh biomass (FB) and that of dry biomass (DB), expressed in gram of water per gram of dry biomass (g / g DB).

Statistical analysis

Differences between treatments were analysed for irrigation and mycorhization factors, as well as the interaction of these factors by a two-way ANOVA performed with the R software, with a general linear model (GLM). Treatment effects were considered significant at $P < 0.05$. Comparisons between means were performed with Tukey’s test Honestly Significant Difference (HSD) at the significance level $P<0.05$**.** Simple linear regression (SLR) were applied to evaluate the relationship between the mycorrhizal frequency (F%) and mycorrhizal dependency(%).

RESULTS

Root mycorrhizal colonization and dependency

Water stress caused a significant decrease of AM colonization frequency (F%) in roots of inoculated watermelon plants. This decrease was proportional to water availability, from a frequency of 46.67% at normal watering (100% FC) to 36.33% and 33.33% at reduced irrigation of the field capacity (50% and 25%, respectively), and up to 23.33% for severely stressed plants (5% FC).

The highest mycorrhizal dependency was recorded for stress at 5% FC followed by stressed plants at 50% FC and 25% FC with a mycorrhizal dependence of respectively: 48.38%, 35.54% and 27.29%. A very low rate was recorded for plants irrigated at 100% FC with 6.39% of mycorrhizal dependency.

A linear regression to highlight the relationships between the frequency and mycorrhizal dependency (%) according to different irrigation levels, showed the influence of water stress on these parameters. When irrigation was optimal at 100%FC, the roots were relatively well mycorrhized, however, their dependency to this colonization decreased. More the water lacked more the frequency decreases and the mycorrhizal dependency increases until the severe stress of 5%FC, where the mycorrhizal dependency was the highest (Figure 1).

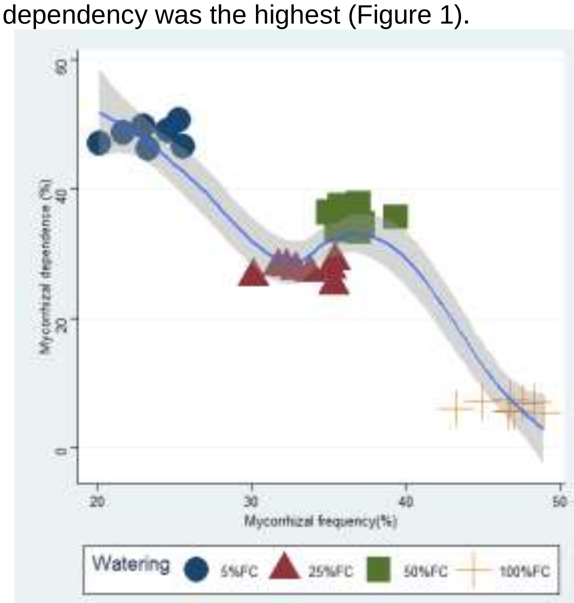


Figure 1; Effect of different level of stress water on the relation between mycorrhizal frequency and mycorrhizal dependence

Plant growth

Drought stress significantly decreased growth parameters. However, independently of water stress level, AM fungi significantly increased in watermelon plants the fresh and dry biomass production, length of aerial parts, as well as on the number of leaves and flowers and the water content which was greater compared to non-mycorhized plants. The interaction "water stress x inoculation" was significant for dry biomass, number of leaves and flowers and the ratio dry root biomass / dry shoot biomass (Table1).

Decrease in loss of biomass

Regardless of the water constraint applied, the inoculation decreased by half the biomass loss of water melon plants. For the mycorhized plant the biomass loss noted for plant subjected to water stress of 50%FC, 25%FC and 5%FC was respectively 40.42%, 97.11% et 141.30 %. The non mycorhized plants recorded a biomass loss of 104.56%, 155.25% and 338.25% respectively at the water stress of 50%FC, 25%FC and 50%FC.

Flower and leave enumeration and stem length

Flowering was significantly improved only for mycorrhized plants irrigated at 100% FC with a average number of flower/plant of (1.87±0.83), the non mycorhized plants had (0.50±0.75). No flowering occurred from 25% FC stress for mycorrhized or non-mycorrhized plants (Figure 2).

At 100% FC and 50% FC irrigation, the number of leaves of mycorrhized plants was respectively of (10.75 ± 0.70) and (8.75 ± 0.88). These values are significantly higher compared to non-mycorrhized plants with (7.75 ± 0.88) for plants irrigated at 100% FC and (7.12 ± 0.99) for plants irrigated at 50% FC. At 25% FC and 5% FC irrigation levels, whether they were mycorrhized or not, there was no significant difference in the number of leaves per plant.

Regardless of the water stress level, the total length of mycorhized watermelon plants was significantly greater (16.43 cm plant^{-1}) compared to non-mycorrhized plants (13.82 cm plant^{-1}), the gain in length was of 18.88%.

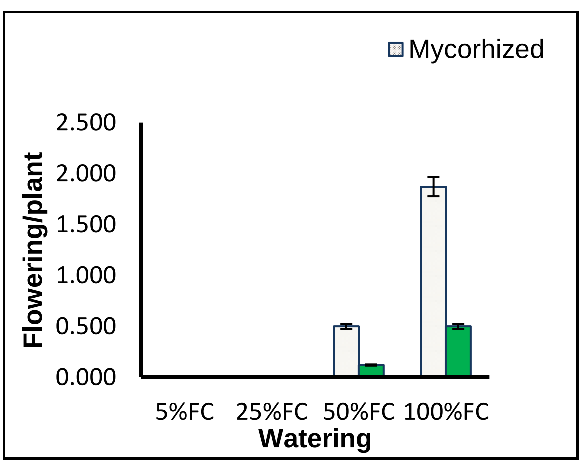


Figure 2; Flowering/plant of Mycorhized and non-Mycorrhized plants of watermelon at water stress of (5% FC, 25% FC, 50% FC and 100% FC)

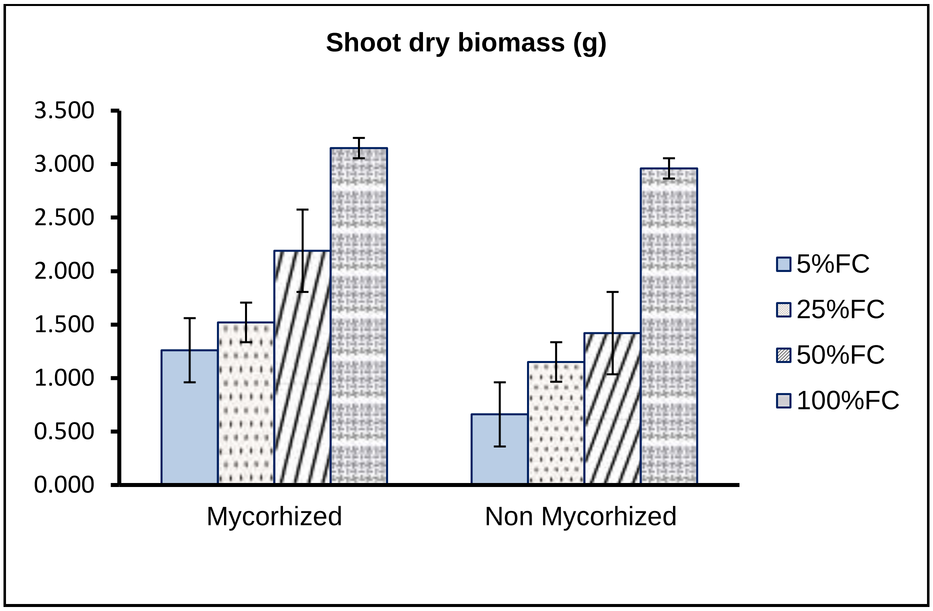
Biomass and root/shoot ratio

The optimal irrigation (100%FC), gave the highest shoot and root biomass but no significant differences were observed between mycorrhized and non-mycorrhized plants.

The non-mycorrhized and severely stressed plants (5%FC) had the lowest fresh and dry shoot biomass with (4.51g ± 0.50 g) and (0.66 ± 0.06 g), respectively. On the other hand, the mycorrhized plants subjected to the same stress level had fresh and dry shoot biomass of (7.53 g ± 0.74 g) and (1.26 ± 0.13 g), respectively, which is significantly higher compared to non-mycorhized and more irrigated plants (25% FC) with (5.56± 0,54 g) for the fresh biomass and (1,15 ± 0,04 g) for the dry biomass. They were also statistically similar to those obtained by non-mycorrhized plants and irrigated at 50% FC with fresh shoot weight of (7.36 ± 0.61 g) and dry weight of (1.42 ± 0.12 g).

Fresh roots of the mycorrhized plants subjected to a water stress of 50% FC and 25% FC gave biomasses respectively of (2.40 ± 0.31 g) and (2.22 ± 0.22 g). These results are significantly similar to those obtained with non mycorhized plants at the normal irrigation of 100% FC with (2.40 ± 0.28 g). Mycorrhized plants grown with 50% FC and 25% FC had (0.23 ± 0.04 g) and (0.23 ± 0.03 g) respectively and non-mycorrhized plants irrigated at 100% FC had the similar root dry biomass. Mycorrhized plants gave at the severe stress of 5% FC a root fresh biomass of (1.59 ± 0.24g) and a dry biomass of (0.16g ± 0.04), which is higher than those obtained with the non mycorhized plants at 25% FC (1,56 ± 0,25 g) and (0,09 ± 0,01 g), respectively). Mycorrhized plants at severe stress of (5% FC) had also fresh and dry root biomass equal to those obtained by controls at 50% FC with (1.87 ± 0.21 g) and (0.13 ± 0.02 g), respectively. Non-mycorrhized plants treated with 5% FC, 25% FC and 50% FC did not give the same performance (Figure 3).

The ratio between root and shoot biomass was influenced by the water condition, this ratio increased when water availability decreased. However the root/shoot ratio of mycorhized plants (0,11 ± 0,03) was significantly greater compared to the non mycorhized plants (0,08 ± 0,02) under the stress water.



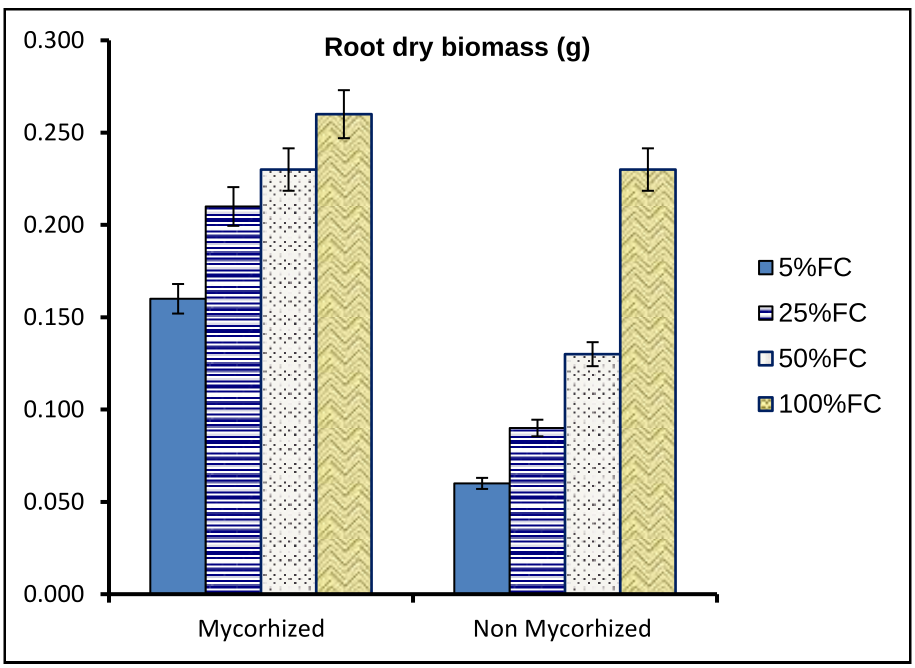


Figure 3; Shoot and root dry biomass of Mycorhized and non-Mycorrhized plants at various levels of water stress (5% FC, 25% FC, 50% FC and 100% FC)

Table 1. **Dry biomass (DB), number of leaves, number of flowers, root dry biomass ratio on shoot dry biomass (RDB/SDB) of watermelon plants mycorhized (M) or non mycorhized (NM) with a commercial inoculum and subjected to various stresses water**

Growth parameter	Water condition					Inoculation			interaction water stress inoculation
	5%FC	25%FC	50%FC	100%FC	Significance	M	NM	Significance	
DB (g plant^{-1})	1.07±0,37A	1,49±0,28 B	1,99±0,47C	3,30±0,24D	***	2,25±0,80A	1,68±0,94B	***	***
Leaf number	5,62±0,88A	6,37±0,80A	7,94±1,23B	9,25±1,73C	***	8,09±1,98A	6,50±1,29B	***	**
Flower number	0,00±0,00A	0,00±0,00A	0,31±0,48A	1,18±1,04B	***	0,60±0,91A	0,16±0,45B	*	***
RDB/SDB	0,11±0,03B	0,11±0,03B	0,10±0,02AB	0,08±0,01A	**	0,11±0,03A	0,08±0,02B	***	***

The averages followed by different letters are significantly different. * P <0.05, ** p <0.01, *** p <0.001

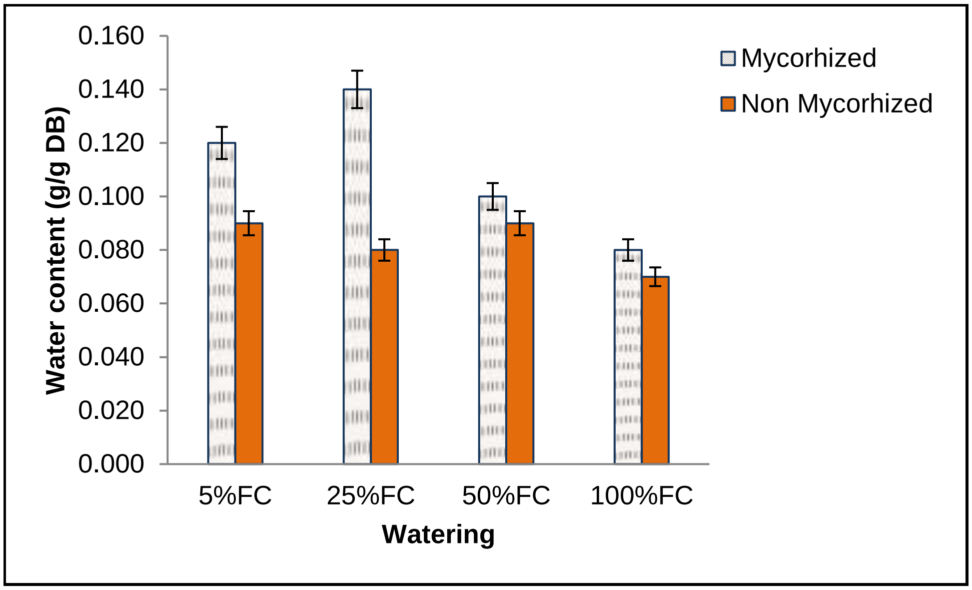


Figure 4; Water content (g/g DB) of Mycorhized and non-Mycorrhized plants of watermelon at various levels of water stress (5% FC, 25% FC, 50% FC and 100% FC)

Water content

With regard to the water content expressed in g of water/g of dry biomass, the mycorrhized plants had an average of water content of (0.11 ± 0.03), significantly greater than the water content of the control plants with (0.08 ± 0.02). (Figure 4).

DISCUSSION

Drought stress is a worldwide problem, which reduces plant growth, yield and other physiological processes of most field crops. The AM symbiosis can protect crop plants against the detrimental effects of water deficit. Several mechanisms have been proposed to explain how the AM symbiosis can alleviate plant drought stress, including physical, nutritional and cellular effects (Jeffries and Barea, 2001). The positive effects of AMF rely mainly on the uptake and transport of water and on an improved uptake of nutrients.

Water stress induced a slowdown and a significant drop in mycorrhizal colonization parameters, up to a 50% drop at 5% FC water stress. This result is consistent with the results obtained with Citrus (Wu and Xia, 2006), black locust (Yang et al., 2014) and soybean (Pavithra and Yapa, 2018).

The reduction of frequency of AMF colonization parameters under water stress conditions can be explained by physiological changes in the host plant (Juniper and Abbott, 1993). The fungus uses for its development carbon substrates that the plant gives through photosynthesis (Adib et al., 2015). The hydric stress induces in the roots the synthesis of abscisic acid which will cause the closure of stomata and reduces photosynthesis. This can explain the decrease in the rate of mycorrhizal development. Also the significant decreases in the AMF colonization in roots at the different level of water deficit in this study can probably be caused by drought stress that inhibited spore germination and hyphal growth (Huang et al., 2011, Gong et al., 2013). However, AMF remained able to improve the growth of watermelon seedling even at severe stress water. Similar results had been obtained by (Fakhech et al., 2019) on *Retama monosperma* and *Acacia gummifera.*

The mycorrhizal dependency increased with the increase of stress water. This correlation suggests that the watermelon becomes more dependent on the AM symbiosis that allowed them to adapt to difficult conditions of water stress, even if the limited watering decreases their frequency in roots. Similar results had been observed by Boyer et al. (2015) on strawberry plants and Damask rose (Abdel-salam et al., 2018).

In this study AMF inoculation enhanced the biomass production of watermelon and decreased the loss of biomass induced by the water stress by half. Previous studies showed that AM symbiosis with *Rhizophagus irregularis* of poplar showed enhanced growth and reduced loss of biomass during the drought stress compared with the non-mycorrhizal seedlings (Liu et al., 2015). Same result was found for mycorrhizal plants of White clover wich showed considerably greater biomass production than non-mycorrhizal plants, irrespective of soil water status (Tuo et al., 2017).

The limited availability of water causes the delay of stem elongation, leaf and flower formation. Nevertheless, mycorrhization has improved the tolerance of watermelon plants to the water deficit with a marked improvement of these parameters. Comparable results were observed in mycorrhized palm with significant leaf formation, which varied with water regime (Meddich et al., 2015) and an increase in aerial parts elongation, compared to non-mycorrhized plants. An improvement in the height of mycorrhizal *Lycium barbarum* plants subjected to a water deficit was observed by Hu et al., (2017). Abdel-salem et al., (2018) noted an increase in the number of flowers of inoculated damask rose compared to non-inoculated ones and submit to a drought stress. Water plays an essential role in physiological processes of plants (Gong et al., 2013) and the enhanced water status resulting from AM formation may assist the host plants in maintaining their normal physiological functions under drought conditions.

The comparison of measured parameters between mycorrhized and non mycorrhized watermelon plants showed the ability of AMF to mitigate the adverse effect of water stress as this stress increases, like observed in the case of mountain peanuts (Tian et al., 2013), cypress seedlings (Zarik et al., 2016) and soybean (Pavithra and Yapa, 2018). Moreover, AMF inoculation of plants subjected to reduced irrigation upgrade plant growth back to the same or higher values as the non-mycorrhized, moderated or optimally watered plants, as observed for lettuce (Baslam and Goicoechea, 2012) and strawberry (Boyer et al., 2015). The enhanced growth effects of mycorhized plants are often related to the improvement of P and other nutrients (N, K, Ca, Mg) acquisitions (Augé, 2001). AM fungi may provide host plants additional transport channels for improving the uptake of limited water and nutrients from the soil through external hyphae (Gong et al., 2013, Yang Y. et al., 2014). AMF colonization can, under different drought stress levels, improve plants-water relations and photosynthetic status (Abdel-salem et al., 2018). The photosynthesis is one of the most important physico-chemical processes that is directly linked to plant biomass production; however, it is very sensitive to drought stress (Yang P.M. et al., 2014).

The root/shoot ratio gives an idea about the plant stress status and resources availability (Harris, 1992). Roots are directly affected by soil conditions where water and/or nutrients unavailability causes greater root/shoot ratios (Munns et al., 2010). Mycorrhization has promoted the growth of root biomass relative to shoot biomass; this is confirmed by values of the ratio of root parts on the shoot parts which increases with the increasing of water stress. According to Mo et al. (2016), AM fungi appear to play a role in the allocation of biomass to root growth than shoot growth of watermelon plants. This was confirmed by the increased ratio root / shoot resulting from colonization by AM fungi, irrespective of water regime. It has been suggested that increasing the flow of photo-assimilates to the roots leads to a greater development of the root system compared to the surface components (Toscano et al., 2014) and could allow an increase in the volume of soil that they explore.

The water content of mycorhized plants was significantly higher compared to non Mycorhized, wich means that the AM fungi increased the water uptake. AMF colonization not only increases water uptake, but also results in the plant becoming more efficient in using available water (Kaya et al., 2003, Omirou et al., 2013). AM fungi improved the water parameters of the plant compared with non-mycorrhized plants, regardless of the hydric regime applied. Improving the hydrological status resulting from the formation of mycorrhizal symbiosis can help host plants maintain their normal physiological functions under drought conditions (Mo et al., 2016).

CONCLUSION

Mycorrhizal colonization, although slowed by limited water availibility, the mycorrhizal dependence of watermelon plants to AM fungi increased by water stress and improves the tolerance of watermelon plants to this stress.

The results showned the effectiveness of AMF inoculation against the deleterious effects of water stress by promoting flowering and decreasing the loss of biomass, the leaf formation, stem elongation, shoot and root biomass are also parameters enhanced by the AM fungi.

Application of AMF inoculation can be an effective biological means to exploit for a bio-protection in Algerian agro-ecosystems that are frequently under water stress and may contribute to a reduced need for irrigation without compromising plant growth or yield.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We are grateful to Skikda university to allowed us to realise our experiment in their greenhouse and laboratory.

AUTHOR CONTRIBUTIONS

NH designed and performed the experiments, laboratory tests, data collection, data analysis and writing manuscript, HZ contributed to data analysis and writing, AMH contributed to the design of the experiments, laboratory tests and writing. SG contributed to writing. All authors revised and approval the final version.

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