RESEARCH ARTICLE

Arbuscular mycorrhizal symbiosis alters morphological and biochemical indices in hot pepper (*Capsicum annuum* L.) under drought stress

M. S. R. Krishna, U. Lakshmisahitya, Lakshmi Sravani Ch, N. Sandhya Rani, K. Tabitha, V. Bhavani Akshitha

Department of Biotechnology, Koneru Lakshmaiah University, Guntur, Andhra Pradesh, India

Abstract

Introduction: Plants are persistently exposed to series of varied unfavorable conditions in natural environments. Around the world, drought is one of the major concerns negatively effecting crop yield. Decreased soil water availability has become more prevalent in several parts of the world limiting arable land for agriculture. Enhancement of drought tolerance in plants mediated by arbuscular mycorrhizal fungi (AMF) is one of the notable strategies that could be persuaded to achieve sustainable agriculture. *Capsicum annuum* is one of the important horticultural crops. Shallow roots render the crop more prone to water stress. This study was performed to evaluate the impact of AMF on four hot pepper cultivars in pot culture. **Materials and Methods:** Four cultivars of hot pepper plantlets, namely US-341, VNR-314, VNR-145, and Indam-05 were subjected to three water regimes - control (100% field capacity [FC]), moderate stress (60% FC), and severe stress (40% FC) and five treatments - control, 60% FC with AMF, 60% FC without AMF, 40% FC with AMF, and 40% FC without AMF. Plant growth attributes, namely shoot fresh weight, shoot dry weight, root fresh weight, and root dry mass were measured. Biochemical traits such as relative water content, chlorophyll content, chlorophyll stability index, and ion leakage were quantified. **Results:** The applied drought stress levels reduced plant growth (shoot biomass and root biomass) of mycorrhizal and non-mycorrhizal plants in comparison to control plants (100% FC). However, the association of mycorrhizal fungus with the plant stimulated growth by increasing the absorption of water from roots and maintaining the same in leaves comparing to AMF non-treated plants. Furthermore, mycorrhizal symbiosis allowed the plants to maintain membrane integrity, thereby reducing the deleterious effects of drought. Drought-stressed mycorrhizal plants showed significantly better performance than non-mycorrhizal plants. Besides, four pepper cultivars responded differently to same AMF emphasizing specific varietal sensitivity to AMF. Among the four cultivars used for the study, VNR-145 showed better performance in both mycorrhizal and non-mycorrhizal treatments. **Conclusion:** The results highlight that AMF alleviate drought by maintaining relatively higher water status in hot pepper plants. Furthermore, the association of *Glomus coronatum* AMF and *C. annuum var.* VNR-145 ecotype is advocated to determine the extent to which these results can be manifold under field conditions.

Key words: Arbuscular mycorrhizal fungi, drought, field capacity, pot culture, symbiosis

INTRODUCTION

Limited water availability has been considered as prevalent abiotic factor restraining agricultural productivity worldwide. Current situation of global environmental change probably makes water scarcity; even greater due to increased precipitation resulted from increased evapotranspiration.[1] Chili (*Capsicum annuum* L.) is widely cultivated for its pungent fruits. It is considered as a significant crop due to its enriched antioxidants, high pungency, rich flavor, and vitamins. It also comprehends many plant-derived

Address for correspondence:
Dr. M. S. R. Krishna, Department of Biotechnology, Koneru Lakshmaiah. University, Vaddeswaram, Guntur, Andhra Pradesh, India.
E-mail: msrkrishna_bt@kluniversity.in

Received: 22-11-2017
Revised: 09-04-2018
Accepted: 28-04-2018

compounds exhibiting anti-inflammatory, antiallergic, anticarcinogenic, and antioxidative properties.[2] Cultivation of pepper is mainly confined to warm and semi-arid regions where irrigation becomes limiting factor often resulting in decreasing the yield.[3] Several studies reported that chili yield was drastically reduced due to water stress.[4]

Arbuscular mycorrhizal fungi (AMF) are heterotrophic obligate soil fungi that establish symbiotic association with the roots. AMF were reported to provide broad spectrum of resistance to host plants against stress conditions.[5] Drought stress mitigation by symbiotic association between AMF and plant roots is one of the widespread and most ancient strategies adopted by the plant to augment water and nutrient acquisition. Drought alleviation of mycorrhizal plants could be possible due to improved hydraulic conductivity,[6] increased water uptake.[7] Variations in the ability and responsiveness of mycorrhizal association among different cultivars within a same plant species are one of the increasing interests. Such variations have been reported from several medicinal plants,[8] Coriander spp.[9] Hence, the objective of the present study is to investigate the effects of AMF on morphological, physiological, and biochemical indices of four different chili cultivars subjected to drought stress.

MATERIALS AND METHODS

Plant Cultivars and AMF

Popularly grown hot pepper cultivars, namely VNR-145, VNR-314, US-341, and Indam-05 were used for this study. AMF, *Glomus coronatum* was collected from Acharya NG Ranga Agricultural University, Guntur. The present work was carried out at greenhouse of K. L. University, Vaddeswaram, Andhra Pradesh, with a temperature of 27/18°C day/night during the year 2016–2017. Seeds were sown in black trays.

AMF Treatment and Drought Stress Imposition

After 45 days of sowing, seedlings were transplanted into pots containing autoclaved soil to ensure similar microflora in non-mycorrhizal and mycorrhizal treatments. Mycorrhizal treatment was given according to the method given by Wu *et al*.[10] Approximately 2000 spores/pot of AMF, fungal inoculum was applied to AMF-treated pots at the time of transplanting. Moisture stress was imposed 20 days after transplantation.

Experimental Design

A completely randomized block design was performed with three replications. Seedlings of each cultivar were assigned to three water regimes (100% field capacity [FC], 60% FC, and 40% FC) and five treatments (control - 100% FC, 60% FC without mycorrhiza, 60% FC with mycorrhiza, 40% FC without mycorrhiza, and 40% FC with mycorrhiza) independently for 1 week according the gravimetric method described by Hugh.[11]

Plant Growth Parameters

At the end of stress period, plant shoots and roots were harvested without any damage. Shoot fresh weight (SFW) and root fresh weight were measured. Roots and shoots were dried separately at 80°C for 48 h followed by the determination of shoot dry weight and root dry weight. Root mass and shoot mass were obtained from the difference between respective fresh weights and dry weights.

Leaf Relative Water Content (RWC)

Fresh weight of excised leaves was recorded and was soaked for 4 h in distilled water at room temperature followed by recording its turgid weight. Dry weight of leaves was taken after drying the leaves at 80°C for 24 h. RWC of leaves was calculated using method described by Soltys-Kalina *et al*.[12]

Quantification of Percent Ion Leakage

In this method, leaf discs of plants were transferred into 5 ml of distilled water. Solutions were incubated for 4 h at room temperature followed by measuring conductivity, which was considered as reading 1. Later, solutions containing leaf discs were autoclaved. Conductivity was measured after the solutions were cooled to room temperature and was taken as reading 2. Membrane stability index is calculated according to the method described by Bansal *et al*.[13]

Chlorophyll Content

Leaf discs were incubated in acetone and dimethyl sulfoxide (DMSO) in 1:1 proportion for 6–8 h. After incubation, optical density of the extract is measured at 645 nm and 663 nm using ultraviolet-visible spectrophotometer. Total chlorophyll content was derived according to the method described by Mafakheri *et al*.[14]

Determination of Chlorophyll Stability Index (CSI)

Briefly, in this method, 1 g of leaf tissue (control) was collected and stored at room temperature while another 1 gram of leaf tissue (test) was allowed to boil in water bath for 30 min at 60°C. Total chlorophyll contents of both the samples were estimated using DMSO-acetone method as described by Mafakheri *et al*.[14] CSI is calculated according to the formula described by Bajji *et al*.[15]

RESULTS

Plant Biomass

At 60% FC, the reduction in shoot mass was 38%, 28%, 12%, and 34% in AMF-treated US-341, VNR-314, VNR-145, and

Indam-05, respectively, in comparison to plants at 100% FC [Figure 1]. In non-AMF-treated plants, the reduction was more pronounced in US-341 (74%), VNR-314 (66%), VNR-145 (67%), and Indam-05 (74%) [Figure 1]. The alleviation of drought by AMF treatment at 40% FC was not significant in comparison to non-AMF-treated plants [Figure 1]. When plants are rendered to 60% FC, root biomass was reduced to 31%, 41%, 63%, and 42% in non-AMF-treated plants and 15%, 27%, 8%, and 13% in AMF-treated plants of US-341, VNR-314, VNR-145, and Indam-05, respectively [Figure 2]. At 40% FC, unlike to shoot biomass, there was significant increase in root biomass in AMF-treated plants than that of non-AMF-treated plants. Reduction in the root biomass ranged from 79 to 85% in non-AMF-treated plants, and it ranged between 47 and 64% under AMF treatment [Figure 2].

RWC

RWC among the cultivars when grown under 100% FC ranged from 83 to 85% while at mycorrhizal plants growing at 60% FC had RWC within the range of 81–85% [Figure 3]. There is no effect of drought stress in AMF-treated plants at 60% FC in pepper cultivars. VNR-145 (85%) retained relatively higher water content at 60% FC in comparison to 100% FC under the influence of AMF association. RWC drastically decreased in non-AMF-treated plants situated in water deficits. At both the water stress intensities, VNR-145

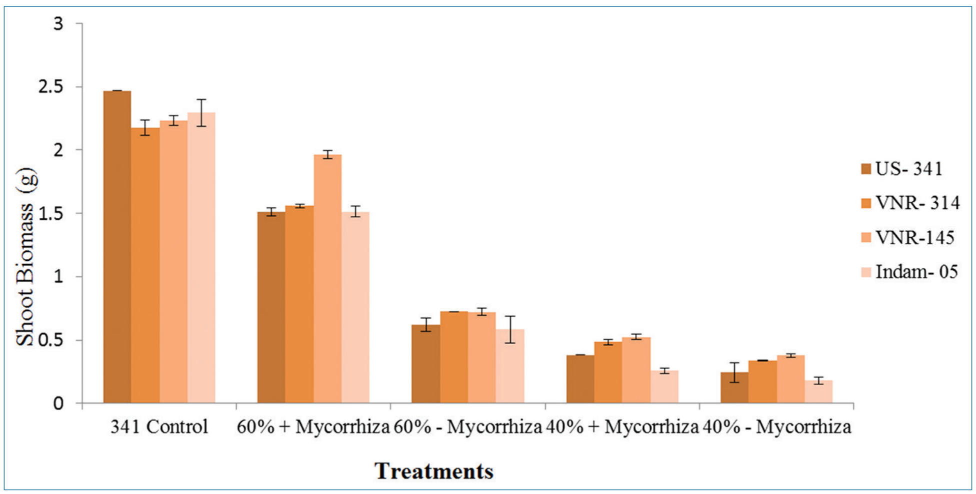

Figure 1: The effect of mycorrhiza on shoot biomass in different pepper cultivars at different water deficit intensities. Values are mean of three replicates with standard deviation

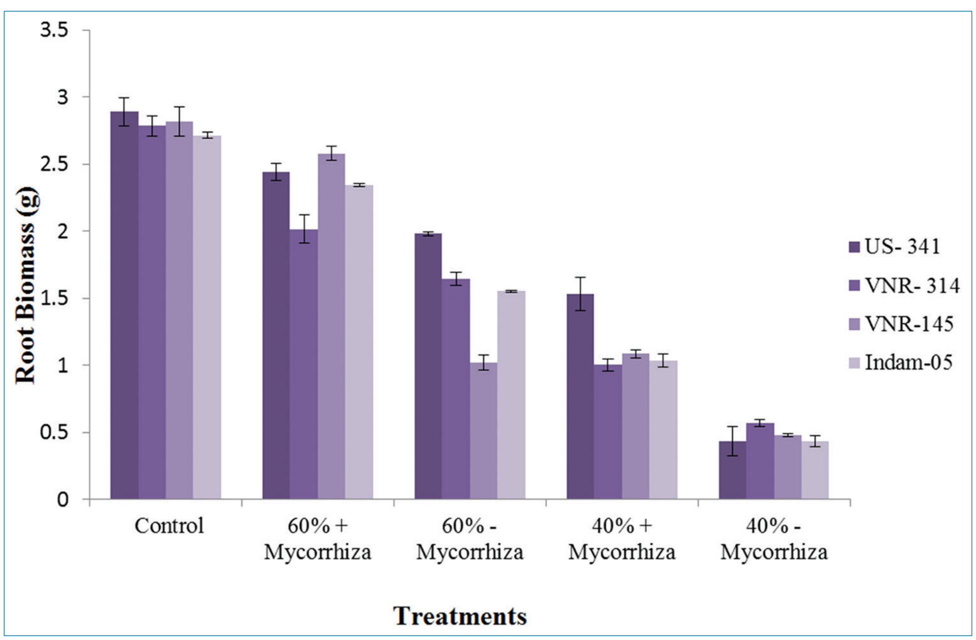

Figure 2: Effect of arbuscular mycorrhizal fungi treatment on root biomass at different water deficit conditions. Represented values are means of three replicates ± standard deviation

remained to poses high RWC than other cultivars. At 40% FC, RWC was 42–57% and 37–43% in mycorrhizal and non-mycorrhizal-treated plants, respectively [Figure 3].

Ion Leakage

At 60% FC, ion leakage ranged from 42 to 52% [Figure 3] in non-AMF-treated plants, whereas ion leakage was only at the range of 20–25% [Figure 3] in AMF-treated plants. At 40% FC, electrolyte leakage increased up to 79% [Figure 3] in non-AMF-treated plants, whereas it reached up to 65% in AMF-treated plants. Furthermore, cultivars (US-341 and VNR-314) which maintained less leaf water content at 40% FC with AMF treatment and non-treatment were seen to lose membrane integrity, leading to higher electrolyte leakage.

Chlorophyll Content

The contents of photosynthetic pigments, total chlorophyll was presented in Figure 4. Among all the treatments, content of chlorophyll in mycorrhizal plants remained higher than those of non -mycorrhizal plants at both water stress intensities. Chlorophyll content ranged from 15 to 19 mg/g FWt at 100% FC while it got reduced to 10–12 mg/g FWt and 6.2–1.6 mg/g FWt in plants subjected to 60% and 40% stress without mycorrhizal treatment, respectively [Figure 4]. Chlorophyll content decreases by 4%, 11%, 12%, and 10% in AMF-treated plants, and it was further reduced by 39.14%, 34.49%, 33%, and 19.59% in AMF non-treated US-341, VNR-314, VNR-145, and Indam-05 plants at 60%, respectively. Average chlorophyll content in AMF non-treated plants was observed to be 6.38 mg/g FWt while it was

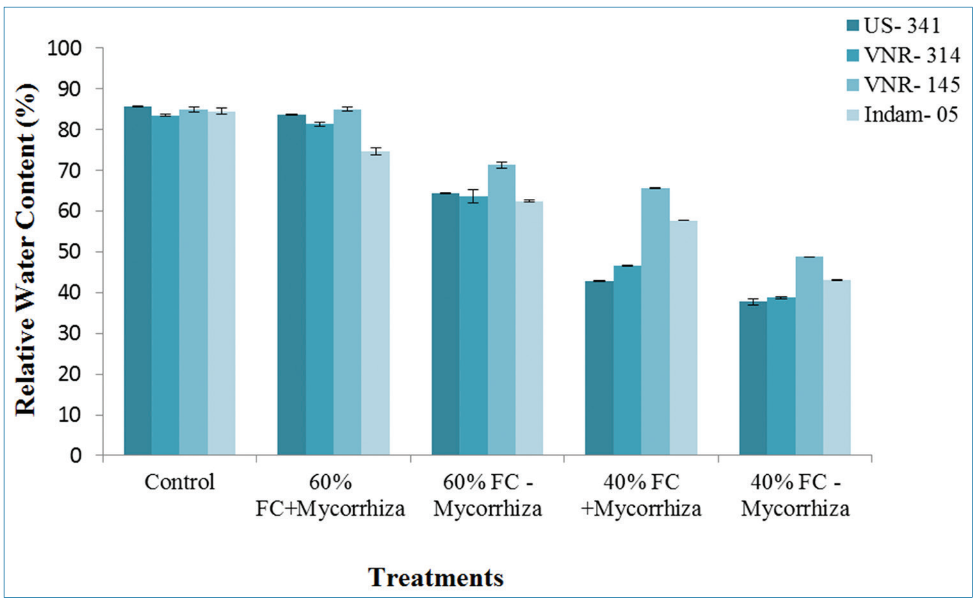

Figure 3: Effect of arbuscular mycorrhizal fungi treatments on relative water content in hot pepper cultivars at 60% and 40% field capacity. Values are means of three replicates ± standard deviation

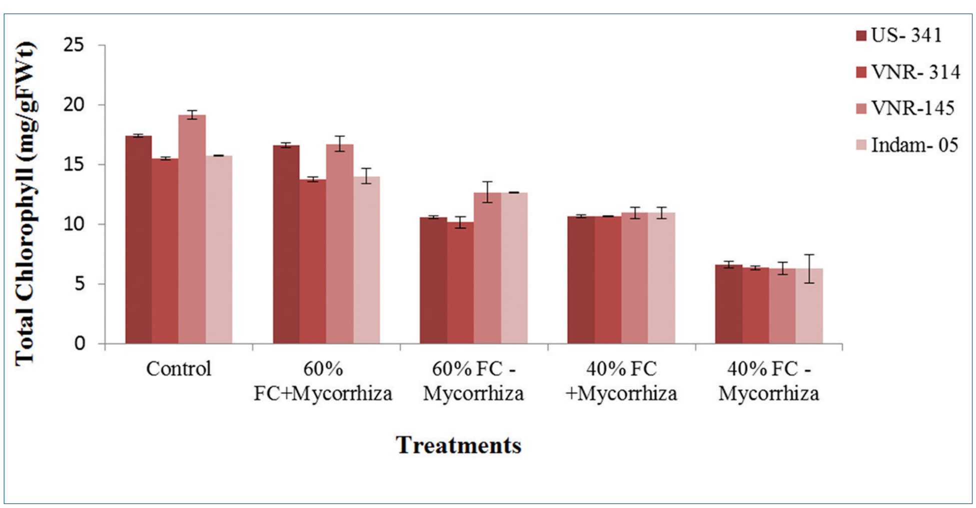

Figure 4: Total chlorophyll content among four different hot pepper cultivars in mycorrhizal and non-mycorrhizal-treated plants. Values are means of three replicates ± standard deviation

increased to 10.81 mg/g FWt in mycorrhizal-treated plants at severe stress [Figure 4]. US-341 (16.60 mg/g FWt) and VNR-145 (16.68 mg/g FWt) exhibited high chlorophyll content at 60% FC with AMF treatment. VNR-145 (10.96 mg/g FWt) and Indam-05 (10.93 mg/g FWt) possessed high chlorophyll at 40% with AMF treatment [Figure 4].

CSI

In AMF non-treated plants at 60% FC, CSI was observed to be 64.05%, 63.12%, 65.17%, and 51.15% in US-341, VNR-314, VNR-145, and Indam-05, respectively [Figure 5]. Among AMF-treated plants at the same stress conditions, CSI was ranged between 81 and 83% [Figure 5]. Average CSI was found to be 45.54% in AMF-treated plants, and it reduced to 40.22% in non-AMF-treated plants at 40% FC [Figure 5]. US-341 and VNR-145 cultivars displayed higher degree of CSI under stress even without mycorrhizal treatment.

DISCUSSION

In the present investigation, two different drought intensities were imposed on four hot pepper cultivars, and we determined the effect of AMF association on plant tolerance toward drought stress. Drought evidently reduced plant biomass in all the cultivars and reduction was prominent with the increase in the intensity of stress. Such detrimental effects on growth by drought have been reported in many plants.[16,17] Irrespective to the AMF treatment pepper cultivars showed decreased growth, but the reduction was less in AMF-treated plants. This is because of increase in root surface area, thereby intensifying water and nutrient uptake from soil due to AMF.[18] Soil microbiota plays pivotal role in plant growth by increasing the uptake of nutrients and soil.[19]

Results revealed increased plant biomass under water stress with AMF symbiosis. Our results are in accordance with Fouad *et al.*[20] The present work represented greater accumulation of root biomass than that of shoot biomass in AMF-treated and non-AMF-treated plants at both water stress intensities [Figure 2]. This negative correlation is because plants tend to allocate more biomass to roots preferentially when subjected to limited water and nutrient availability.[21] Interestingly, VNR-145 accumulated considerable biomass in AMF treatment while failed to accumulate the same in the absence of AMF. There is no significant effect of AMF on root biomass in VNR-314 at 60% FC [Figure 1]. Different varieties responded differently to water stress. Our results signify cultivar-specific responsiveness to AMF treatment. Such varying responses of cultivars among same plant species were reported in marigold[22] and onions.[23]

Genotypes adapt to drought environment by retaining higher water contents in leaves at given water potential. As RWC being one among the indicators of degrees of drought stress, present work was focused on estimation of RWC in the leaves of hot pepper genotypes at different stress intensities and effect of AMF on RWC. No variations in RWC among the genotypes were observed when grown at control conditions. These results are in accordance with that of Rampino *et al.*[24] Cultivars which accumulated higher root biomass were observed to poses superior RWC. Enhancement of biomass accumulation and retention of RWC is due to superior water uptake assisted by mycorrhizal association. Furthermore, reports suggest that AMF symbiosis postpones the decline in RWC.[25]

Under stress conditions, plant cell membranes become more permeable, leading to the loss of cell membrane integrity. When the severity of the damage increases, concurrently, there is increased electrolyte leakage.[26] As this parameter indicates the degree of injury caused by stress, the present work aimed at investigating the percent ion leakage in both AMF-treated and non-treated plants. When plants are under 100% FC, electrolyte leakage in

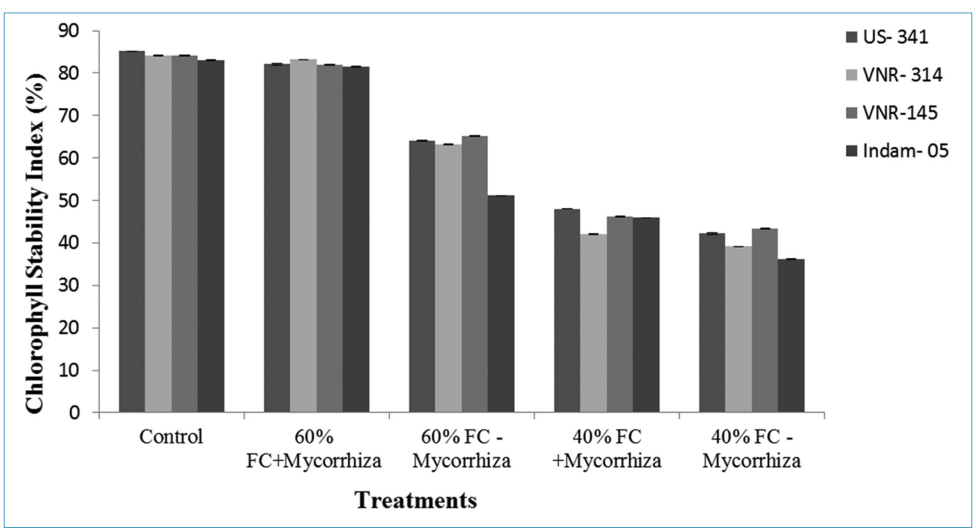

Figure 5: Chlorophyll stability index at 60% and 40% field capacities. Values are means of three replicates ± standard deviation

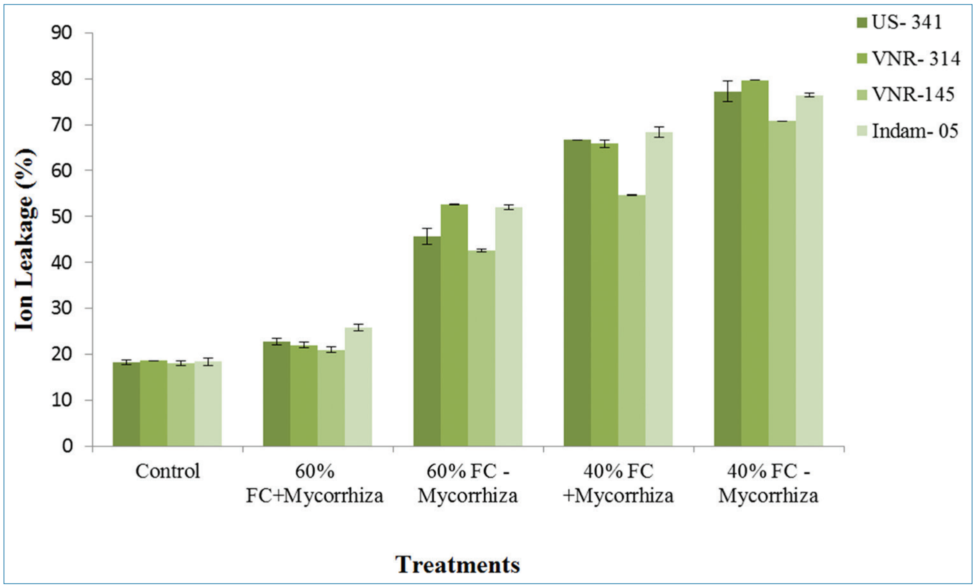

Figure 6: Ion leakage among pepper cultivars in water deficit conditions under the influence of arbuscular mycorrhizal fungi. Values are means±standard deviation

hot pepper cultivars was minimal [Figure 6]. With increase in stress intensity, electrolyte leakage was increased in non-AMF-treated plants. Our results are in consistent with Beltrano and Ronco.[27] Studies reported mycorrhizal inoculation significantly reduced the electrolyte leakage in chili under salinity.[26] With the increase in stress, decrease in chlorophyll contents was reported by several works.[28,29] Although there was decrease in chlorophyll content, the reduction was much less in mycorrhizal-treated plants. According to Wu *et al.*,[30] mycorrhizal colonization results in the increase of photosynthetic pigments. CSI has been drastically affected by drought in the present investigation. With the increase in the intensity of stress, stability index got reduced, but there was alleviation in the mycorrhizal-treated plants. The present results suggest that AMF association facilitated plants to counterattack photoinhibition of chlorophyll pigments. Results are in accordance with Asrar *et al.*[31] who reported increased photosynthetic pigments on AMF application. There was no major effect of mycorrhizal treatment on the CSI of plants at 40% FC. This may be due to irreversible damage of chloroplast. All the four pepper cultivars responded to same AMF differently and these varieties variations would help in choosing suitable cultivars for cultivation in association with AMF.

CONCLUSION

The results of the present work show that chili plants are affected drastically under water deficit conditions. Mycorrhizal colonization and association with the chili plant roots alleviate drought and confer tolerance toward drought stress among hot pepper cultivars. Increase in the root biomass further suggests that AMF mitigated water stress by increasing exchange between soil and roots of host plant. Further, drought alleviation may also be resulted from decrease in cellular damage by maintaining cell membrane integrity. Hence, inoculation of AMF to hot pepper cultivars ought to be considered as one of the most efficient methods for enhancement of drought tolerance, thereby achieving sustainability in agriculture.

ACKNOWLEDGMENT

Authors are thankful to University Grants Commission, Government of India (Project No. F. 30-10/2015/SA II), for providing financial assistance. We are also thankful to K. L. University for providing the laboratory facilities for carrying out this work.

REFERENCES

1. Yan W, Zhong Y, Shangguan Z. A meta-analysis of leaf gas exchange and water status responses to drought. Sci Reports 2016;6:20917.
2. Lee JJ, Crosby KM, Pike LM, Yoo KS, Leskovar DI. Impact of genetic and environmental variation on development of flavonoids and carotenoids in pepper (*Capsicum* spp.). Sci Hortic Amsterdam 2005;106:341-52.
3. Dorji K, Behboudian MH, Zegbe-Domi'ez JA. Water relations, growth, yield, and fruit quality of hot pepper under deficit irrigation and partial rootzone drying. Sci Hort 2005;104:137-49.
4. Sezen SM, Yazar A, Eker S. Effect of drip irrigation regimes on yield and quality of field grown bell pepper. Agric Water Manage 2006;81:115-31.

5. Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 2003;13:309-17.
6. Augé RM, Toler HD, Sams CE, Nasim G. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. Mycorrhiza 2008;18:115-21.
7. Fagbola O, Osonubi O, Mulongoy K, Odunfa S. Effects of drought stress and arbuscular mycorrhiza on the growth of *Gliricidia sepium* (Jacq). Walp, and *Leucaena leucocephala* (Lam.) de Wit. in simulated eroded soil conditions. Mycorrhiza 2001;11:215-23.
8. Farahani HA, Valadabadi SA, Daneshian J, Shiranirad AH, Khalvati MA. Medicinal and aromatic plants farming under drought conditions. J Hortic 2009;1:86-92.
9. Farahani A, Lebaschi H, Hussein M, Hussein SA, Reza VA, Jahanfar D. Effects of arbuscular mycorrhizal fungi, different levels of phosphorus and drought stress on water use efficiency, relative water content and proline accumulation rate of Coriander (*Coriandrum sativum* L.). J Med Plants Res 2013;2:125-31.
10. Wu HH, Zou YN, Rahman MM, Ni QD, Wu QS. Mycorrhizas alter sucrose and proline metabolism in trifoliate orange exposed to drought stress. Sci Reports 2017;7:42389.
11. Hugh JE. A precise gravimetric method for simulating drought stress in pot experiments. Crop Sci 2003;43:1868-73.
12. Soltys-Kalina D, Plich J, Strzelczyk-Żyta D, Śliwka J, Marczewski W. The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of ‘Katahdin’-derived potato cultivars. Breed Sci 2016;66:328-31.
13. Bansal R, Pradheep K, Kumari J, Kumar S, Yadav MC, Gurung B, Rana JC. Physiological and biochemical evaluation for drought tolerance in wheat germplasm collected from arid western plains of India. Indian J Biochem Biophys 2016;52:212-7.
14. Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Biol Sci 2010;4:580-5.
15. Bajji M, Kinet JM, Lutts S. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul 2002;36:61-70.
16. Kerbiriou PJ, Stomph TJ, Van Der Putten PE, Van Bueren EL, Struik PC. Shoot growth, root growth and resource capture under limiting water and N supply for two cultivars of lettuce (*Lactuca sativa* L.). Plant Soil 2013;371:281-97.
17. Xu W, Cui K, Xu A, Nie L, Huang J, Peng S. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. Acta Physiol Plant 2015;37:9.
18. Abdelmoneim TS, Moussa TA, Almaghrabi OA, Alzahrani HS, Abdelbagi I. Increasing plant tolerance to drought stress by inoculation with arbuscular mycorrhizal fungi. Life Sci J 2014;11:10-7.
19. Satyanarayana SD, Krishna MS, Kumar PP. Optimization of high-yielding protocol for DNA extraction from the forest rhizosphere microbes. 3 Biotech 2017;7:91.
20. Fouad MO, Essahibi A, Benhiba L, Qaddoury A. Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought. Span J Agric Res 2014;12:763-71.
21. Xue J, Zhou S, Wang W, Huo L, Zhang L, Fang X, *et al*. Water availability effects on plant growth, seed yield, seed quality in *Cassia obtusifolia* L., a medicinal plant. Agric Water Manag 2017;195:104-13.
22. Linderman RG, Davis EA. Varied response of marigold (*Tagetes* spp.) genotypes to inoculation with different arbuscular mycorrhizal fungi. Sci Hort 2004;99:67-78.
23. Tawaraya K, Tokairin K, Wagatsuma T. Dependence of *Allium fistulosum* cultivars on the arbuscular mycorrhizal fungus, *Glomus fasciculatum*. Appl Soil Ecol 2001;17:119-24.
24. Rampino P, Pataleo S, Gerardi C, Mita G, Perrotta C. Drought stress response in wheat: Physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell Environ 2006;29:2143-52.
25. Moucheshi A, Heidari B, Assad MT. Alleviation of drought stress effects on wheat using arbuscular mycorrhizal symbiosis. Int J Agric Sci 2012;2:35-47.
26. Selvakumar G, Thamizhiniyan P. The effect of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on the growth and yield of chilli (*Capsicum annuum* L.) under salinity stress. World Appl Sci J 2011;14:1209-14.
27. Beltrano J, Ronco MG. Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus *Glomus claroideum*: Effect on growth and cell membrane stability. Braz J Plant Physiol 2008;20:29-37.
28. Shao HB, Chu LY, Wu G, Zhang JH, Lu ZH, Hu YC. Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. Coll Surf B Biointerf 2007;54:143-9.
29. Dhanda SS, Sethi GS, Behl RK. Indices of drought tolerance in wheat genotypes at early stages of plant growth. J Agron Crop Sci 2004;190:6-12.
30. Wu QS, Xia RX, Zou YN. Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. Eur J Soil Biol 2008;44:122-8.
31. Asrar AW, Elhindi KM. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. Saudi J Biol Sci 2011;18:93-8.

Source of Support: Nil. **Conflict of Interest:** None declared.