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Role of AM Fungi in Mitigating Moisture Stress in Soybean (*Glycine max* L. Merill)

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A B S T R A C T

An attempt was made to screen forty native AM fungal isolates from the soybean rhizosphere of Belagavi and Dharwad districts based on their peroxidise activity and total dry matter accumulation in soybean under the restricted water regime of -500 kPa. Top three fungal isolates viz., UASDAMFS15, UASDAMFS25 and UASDAMFS36 tentatively identified as *G. maculosum*, *G. manihotis* and *G. globiferum* respectively. For pot culture studies three irrigation regimes were maintained *viz.*, -33 kPa -500 kPa and -1000 kPa. An experiment was laid out in completely randomized design with factorial concept. There were twenty one treatments and four replications. In general, AMF inoculations reduced the moisture stress in plants significantly. Among them, UASDAMF consortium and UASDAMF soybean consortium significantly increased the stomatal conductance, photosynthetic activity, transpiration rate, ACC deaminase activity and leghaemoglobin content in the plants. Further, UASDAMF consortium and UASDAMF soybean consortium greatly reduced the leaf temperature, proline, glycine betaine content, peroxidase and PALase activity in the plants compared to single inoculants and UIC. Thus, our preliminary findings are indicative of the effectiveness of AMF in protecting plants from water stress and hence can be a promising strategy to develop a biological tool for mitigation of moisture stress.

Introduction

It is generally known that soil productivity depends on its physical and chemical properties. Water is an essential parameter for plant growth and development as well as much concern has been expressed in the recent year towards increase in the area of arid regions of the world. Crop productivity in arid regions depends on the length, magnitude and stage of the plant that is affected by moisture stress.

Drought is a serious problem in the state, with about two-thirds having 750 mm or less annual rainfall. The severity and the extent of drought not only depend on low rainfall but also on the other hydro-meteorological factors like soil moisture, infiltration and moisture retention capacity of the soil.

To ameliorate the adverse affects of moisture stress, microorganisms could play an important role in adaptation strategies and increase of tolerance to abiotic stresses in agricultural plants, such as plant growth promoting microorganisms which colonizes the portion of soil attached to the root surface and influenced by root exudates termed as rhizosphere soil (Bent, 2006; Lugtenberg and Kamilova, 2009). Some of the beneficial microorganisms can also enter into the root system of their hosts and enhance their beneficial effects with an endophytic lifestyle like plant growth promoting fungi (PGPF) such as arbuscular mycorrhizal fungi (AMF) as well as plant growth promoting rhizobacteria (Das and Varma, 2009).

Among them mycorrhizal association is emerging as one of the efficient ways to combat stress effects and thereby making plants to grow better under drought environment (Gianinazzi *et al.,* 2010). The AMF hyphae can absorb water, as well as improve the capacity of the root system to extract soil water by giving it access to enter pores that are too small for root hairs to access. Furthermore, AMF regulate the plant nutrient uptake, stomatal conductance, leaf water potential, photosynthesis and transpiration rate (Li *et al.,* 2014). Besides this, symbiotic association could also induce production of growth promoting hormones; antioxidative enzymes improve plant root architecture (Wu *et al.,* 2012) and soil properties. Taken together, all these events help the plants to produce significantly higher yields under stressful condition.

Materials and Methods

Experimental design and statistical analysis

The data were subjected to analysis following Completely Randomised Design (CRD) as defined by Gomez and Gomez (1984) with two factorials. There were twenty one treatments and each treatment was replicated four times. First factor was six AM fungus treatments, one uninoculated control and second factor was three irrigation regimes i.e, I_1 (-33 kPa), I_2 (-500 kPa) and I_3 (-1000 kPa). This was measured by using Pressure plate and membrane apparatus. Moisture in the pots was maintained by measuring daily evapotranspiration rate. Pot trials were conducted in the poly house at department of agricultural microbiology, University of Agricultural Sciences, Dharwad.

Soil and biological material

The soil which used for the experiment was Black soil and it was collected from University of Agricultural Sciences, Dharwad farm. The AM fungal species used were UASDAMFS15, UASDAMFS25 and UASDAMFS36 which were isolated from the Dharwad and Belgaum districts. UASDAMF soybean consortium was prepared by mixing of all three native AM fungal isolates. Reference strain and UASDAMF consortium AM fungal inoculations were collected from the Department of Agricultural Microbiology, UAS, Dharwad. The pots were filled with three kg of black soil and AMF inoculum @ 50 g pot^{-1} was mixed thoroughly with the top 10 to 15 cm of the soil.

Parameters measured

Biophysical parameters (Infra-Red Gas Analyser)

Measurement of photosynthetic rate, stomatal conductance, rate of transpiration and leaf temperature were made on the top fully expanded leaf at different locations by using a portable photosynthesis system (LI-6400 LICOR, Nebraska, Lincoln USA,). These measurements were made between 10.00 am to 12.00 noon of the day.

Proline

Estimation of Proline was done by following the procedure of Bates *et al.,* (1973).

Plant material of 0.5 g was homogenized in 10 ml of 3 per cent aqueous sulphosalicylic acid. Filtered the homogenate through Whatman No.2 filter paper and 2 ml of filtrate was taken in a test tube and 2 ml of glacial acetic acid and 2 ml acid ninhydrin was added. Heated it in the boiling water bath for 1 h. The reaction was terminated by placing the tube in ice bath. 4 ml toluene was added to the reaction mixture and stirred well for 20-30 sec. Toluene layer was seperated and warmed it to room temperature. The red colour intensity was measured at 520 nm. Series of standard with pure proline in a similar way was done and prepared a standard curve. Amount of proline in the test sample was found out from the standard curve.

Glycine- Betaine

Glycine-Betaine content in plant was estimated by following the procedure of Greive and Grattan (1983).

Estimation was done using dried leaf powder. Finely powdered plant material (0.5 g) was mechanically shaken with 20 ml of deionized water for 48 h at 25 °C. The samples were then filtered and the filtrate was stored in freezer until analysis. Thawed extracts was diluted 1:1 with 2 N sulfuric acid. Aliquot (0.5 ml) was measured into test tube and cooled in ice water for 1 h. Cold potassium iodide (0.2 ml) was added and the mixture was gently mixed with vortex mixture. The samples were stored at 0-4 °C for 16 h. After the expiring of the period samples were transferred to centrifuge tubes and then centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was carefully aspirated with 1 ml micropipette. As the solubility of the periodite complexes in the acid reaction mixture increases markedly with temperature it is important that the tubes be kept cold until the periodite complex is separated from acid media. The periodite crystals were dissolved in 9 ml of 1,2-dichloro ethane (reagent grade). Vigorous vortex mixing was done to effect complete solubility in developing solvent. After 2.0-2.5 h the absorbance was measured at 365 nm with UV/visible spectrophotometer. Reference standard of GB (50-200 mg ml^{-1}) was prepared in 2 N sulfuric acid and the procedure for sample estimation was followed.

Peroxidase activity

Peroxidase activity was done by the following the procedure given by Maehly and Chance (1954). A 0.5 g of fresh leaf sample was weighed and ground well in a mortar with little quantity of chilled phosphate buffer at pH 6.6 and filtered through a double layered muslin cloth to remove the pulp, made up to 25 ml and centrifuged for 30 minutes at 2,000 rpm at 4 ^{0}C. The clear extract was used as enzyme source. The same enzyme was used for the estimation of soluble protein. 3 ml of 0.05 M guaiacol solution was pipetted out into a test tube to which 0.1 ml of enzyme extract was added. Then 0.5 ml of 1 per cent hydrogen peroxidase was added, mixed the contents rapidly and the absorbance was measured in calorimeter at 470 nm at an interval of 20 seconds.

Enzyme activity was calculated by taking the average difference of O. D. (optical density) between two consecutive time intervals and enzyme activity was expressed in terms of ΔOD sec^{-1} mg^{-1} protein (*i.e*. specific activity).

ACC deaminase activity

ACC deaminase activity was estimated by the following the procedure of Penrose and Glick (2003).

ACC (1-Aminocyclopropane-1-carboxylate) deaminase activity was determined by measuring the production of α- ketobutyrate and ammonia generated by the cleavage of ACC deaminase (Penrose and Glick, 2003). The bacterial cells were harvested by centrifugation at 3000 rpm for 5 min, washed twice with 0.1 M Tris-HCl (pH 7.5) and resuspended in 200 µl of 0.1 M Tris-HCl (pH 8.5). The cells were labilized by adding 5 per cent toluene and then vortexed at the highest speed for 30 seconds. 50 µl of labilized cell suspension were incubated with 5 µl of 0.3 M ACC in an Eppendof tube at 28 ^{0}C for 30 min.

The negative control for this assay was included of 50 µl of 0.1 M Tris-HCl (pH 8.5) with 5 µl of 0.3 M ACC. The samples were mixed thoroughly with 500 µl of 0.56 N HCl by vortexing and the cell debris were removed by centrifugation at 10,000 rpm for 5 min. A 500 µl aliquot of the supernatant was transferred to a glass test tube and mixed with 400 µl of 0.56 N HCl and 150 µl of DNF solution (0.1 g 2,4-dinitrophenylhydrazine in 100 ml of 2 N HCl) and the mixture was incubated at 28 ^{0}C for 30 min. One ml of 2 N NaOH was added to the sample before measuring the absorbance at 540 nm.

The concentration of α-ketobutyrate in each sample was determined by comparison with a standard curve generated as follows: 500 µl α-ketobutyrate solutions of 0, 0.01, 0.05, 0.1, 0.2, 0.5, 0.75 and 1 mM was mixed respectively with 400 µl of 0.56 N HCl and 150 µl DNF solution. One ml of 2 N NaOH was added and the absorbance versus α-ketobutyrate concentration (mM) was used to construct a standard curve.

PALase activity (Phenylalanine Ammonia Lyase)

PALase activity was done by following the procedure of Dickerson *et al.,* (1984).

Plant sample of one g was homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble poly vinyl pyrrolidone (PVP). The resulting extract was filtered through cheese cloth and the filtrate was centrifuged at 20,000 rpm for 15 min at 4 ºC and the supernatant was used as the enzyme source.

PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.5 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer pH 8.8 and 9.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 ºC. The amount of trans-cinnamic acid synthesized was calculated. Enzyme activity was expressed on fresh weight basis as n mol trans-cinnamic acid min^{-1} mg^{-1} of sample.

Leghaemoglobin content

Leghaemoglobin content was estimated by the following the procedure of Wilson and Reisenauer (1963).

Nodules of 0.2 g was taken in 600 µl (1 g nodules in 3 ml) Drabkin solution in a micro centrifuge tube and crushed properly. The suspension was centrifuged @ 1,500 rpm for 15 minutes. The supernatant was collected in fresh micro centrifuge tube. The pellet was similarly reextracted two or more times while collecting the supernatant in the same tube everytime.

Then, the volume of the supernatant was made up to 2 ml and centrifuged again @ 20,000 rpm for 20 minutes. Absorbance of the supernatant was recorded at 540 nm. The concentration of legheamoglobin was calculated according to the standard curve (obtained using Bovine Serum Haemoglobin as standard).

Results and Discussion

Screening of forty native isolates

Forty native isolates were screened for peroxidase activity and total dry matter accumulation under -500 kPa water regime. Among forty native isolates, UASDAMFS15, UASDAMFS25 and UASDAMFS36 recorded low peroxidase activity and higher dry matter accumulation. Based on these parameters top three isolates i.e., UASDAMFS15, UASDAMFS25 and UASDAMFS36 were taken for further pot culture experiment.

Pot culture studies

Biophysical parameters of soybean as influenced by AMF

The data on biophysical parameters of soybean plants as influenced by different AM fungal isolates are presented in Table 1.

Stomatal conductance, photosynthetic rate and transpiration rate were maximum in UASDAMF soybean consortium, UASDAMF consortium, UASDAMFS25, UASDAMFS36, *G. fasciculatum* (reference strain) and UASDAMFS15 over uninoculated control plants at all the moisture regimes. Leaf temperature was maximum in non mycorrhized plants. Low leaf temperature was recorded with mycorrhized plants UASDAMF soybean consortium, UASDAMF consortium, UASDAMFS25, UASDAMFS36, *G. fasciculatum* (reference strain) and UASDAMFS15.

Proline and glycine -betain content

UASDAMF consortium and UASDAMF soybean consortium, single isolates *viz.*, UASDAMFS25, UASDAMFS36, *G. fasciculatum*, UASDAMFS15 decreased the proline and glycine –betaine content in plants, over non mycorrhized soybean plants at at all the moisture regimes (Table 2).

Antioxidant activities

Peroxidase and PALase activites were decreased upon inoculation of mycorrhiza. Non mycorrhized plants showed high peroxidase and PALase activity at all water regimes. ACC deaminase activity was highest in mycorrhized plants compared to non mycorrhized plants at all the water regimes (Table 3).

Leghaemoglobin content

Leghaemoglobin content was maximum in the plants inoculated with UASDAMF soybean consortium, UASDAMF consortium, UASDAMFS25, UASDAMFS36, *G. fasciculatum* (reference strain) and UASDAMFS15 over non mycorrhized plants at all the moisture regimes (Table 4).

Plants face various abiotic stresses in adverse environmental conditions. Abiotic stress is a complex process against which signals the cells to adapt themselves at the molecular, biochemical and physiological levels. Drought, induced by climate change or depletion of ground water, was arguably the major agent limiting the crop growth and productivity and poses biggest challenge for the global food security (Rajkumar *et al.*, 2013). Drought tolerance in crop plants is multidimensional ranging from morphological, physiological to molecular level (Lopes *et al.*, 2011). Although plants could adapt a number of strategies to alleviate stress effects, mycorrhizal association is emerging as one of the efficient ways to combat stress effects in plants (Gianinazzi *et al.*, 2010). In fact, several studies have shown the importance of use of microbial inoculants, especially the AMF in alleviating drought stress in crop plants (Zoppellari *et al.*, 2014).

Table.1 Biophysical parameters as influenced by selected AM fungal isolates at different moisture levels (Infra-Red Gas Analyser) at 30 DAS

Treatment	Stomatal Conductance (µmol/CO_2/m^2/sec)				Photosynthetic rate (µmol/m^2/ sec)				Leaf temperature (^{0}C)				Transpiration rate (mmol/m^2/sec)			
AMF	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**
UASDAMFS15	0.22	0.16	0.15	**0.18**	27.00	24.00	22.61	**24.53**	27.67	29.38	29.91	**28.98**	4.23	4.22	4.16	**4.20**
UASDAMFS25	0.24	0.20	0.18	**0.20**	27.81	26.33	23.64	**25.92**	25.89	27.61	29.83	**27.78**	4.36	4.28	4.21	**4.28**
UASDAMFS36	0.23	0.19	0.16	**0.19**	27.60	25.93	22.94	**25.49**	26.14	27.75	29.85	**27.91**	4.30	4.24	4.12	**4.22**
UASDAMF Soybean Consortium	0.47	0.45	0.35	**0.42**	29.09	27.82	27.04	**27.98**	24.87	26.53	28.73	**26.71**	4.63	4.60	4.28	**4.50**
***G. fasciculatum* (reference strain)**	0.23	0.17	0.15	**0.18**	27.25	25.79	22.74	**25.26**	26.74	28.13	29.88	**28.25**	4.27	4.23	4.15	**4.21**
UASDAMF Consortium	0.38	0.37	0.26	**0.33**	28.24	27.59	26.58	**27.47**	25.35	27.10	29.53	**27.32**	4.48	4.34	4.28	**4.36**
UIC	0.20	0.15	0.11	**0.15**	24.60	22.67	21.32	**22.86**	28.50	30.49	30.95	**29.98**	4.21	4.19	4.14	**4.18**
Mean of B	**0.28**	**0.24**	**0.19**		**27.37**	**25.73**	**23.83**		**26.45**	**28.14**	**29.81**		**4.34**	**4.30**	**4.19**	
	S.Em.±		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**	
C.D. of M (AMF)	0.01		0.29		0.22		0.67		0.19		0.57		0.01		0.04	
C.D. of S (Moisture)	0.06		0.18		0.09		0.28		0.16		0.48		0.02		0.09	
C.D. of A × B (AM fungi × Different moisture levels)	0.01		0.05		0.25		0.74		0.44		1.20		0.02		0.07	

Note - I_1- Irrigation regime 1 (-33 kPa)
I_2-Irrigation regime 2 (-500 kPa)
I_3-Irrigation regime 3 (-1000 kPa)
UASDAMF soybean Consortium (UASDAMFS15 + UASDAMFS25 + UASDAMFS36)

Table.2 Proline and Glycine-Betaine content of soybean at different moisture levels at 30 DAS

Treatment	Proline (µmol g^{-1} FW)				Glycine-Betaine (µmol g^{-1} FW)			
AMF	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**
UASDAMFS15	5.39	6.92	7.83	**6.71**	3.40	7.82	10.50	**7.24**
UASDAMFS25	4.40	6.00	6.65	**5.68**	2.52	5.65	8.70	**5.62**
UASDAMFS36	4.75	6.29	6.94	**5.99**	2.82	6.62	9.05	**6.16**
UASDAMFsoybean Consortium	4.04	5.51	6.49	**5.35**	2.11	5.12	8.05	**5.09**
***G. fasciculatum* (reference strain)**	5.13	6.48	7.41	**6.34**	2.87	7.37	9.67	**6.63**
UASDAMF Consortium	3.68	5.12	6.03	**4.94**	1.62	4.45	7.25	**4.44**
UIC	5.66	7.40	8.27	**7.11**	3.52	8.85	10.77	**7.71**
Mean of B	**4.72**	**6.24**	**7.09**		**2.69**	**6.55**	**9.14**	
	S.Em. ±		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**	
C.D. of M (AMF)	0.08		0.20		0.09		0.29	
C.D. of S (Moisture)	0.51		1.55		0.07		0.21	
C.D. of A × B (AM fungi × Different moisture levels)	0.41		1.25		0.20		0.57	

Note - I_1- Irrigation regime 1 (-33 kPa)
I_2-Irrigation regime 2 (-500 kPa)
I_3-Irrigation regime 3 (-1000 kPa)
UASDAMF soybean Consortium (UASDAMFS15 + UASDAMFS25 + UASDAMFS36)

Table.3 Influenced of selected AM fungal isolates on Peroxidase, ACC deaminase and PAL activities at different moisture levels at 30 DAS

Treatment	Peroxidase (U mg^{-1} protein)				ACC deaminase (nmol α ketobutyrate g^{-1} biomass h^{-1})				PAL (Phenylalanine Ammonia Lyase) (n mol transcinnamic acid $min^{-1}mg^{-1}$ sample)			
AMF	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**
UASDAMFS15	1.42	4.51	8.44	**4.79**	6.07	9.38	10.40	**8.61**	1.90	3.95	4.26	**3.37**
UASDAMFS25	1.28	4.09	7.81	**4.39**	7.03	9.93	11.04	**9.33**	1.67	3.62	4.30	**3.19**
UASDAMFS36	1.35	4.14	7.95	**4.48**	6.92	9.76	11.03	**9.23**	1.68	3.56	4.50	**3.24**
UASDAMF soybean Consortium	1.27	3.79	7.09	**4.05**	8.06	10.09	12.18	**10.11**	1.59	2.80	3.65	**2.68**
***G. fasciculatum* (reference strain)**	1.41	4.25	8.29	**4.65**	6.23	9.71	10.93	**8.95**	1.95	3.65	4.31	**3.30**
UASDAMF Consortium	1.07	3.38	6.92	**3.79**	7.79	9.97	12.11	**9.95**	1.50	3.10	3.80	**2.80**
UIC	1.77	5.49	9.86	**5.71**	5.89	9.02	10.73	**8.54**	1.90	3.87	4.77	**3.51**
Mean of B	**1.36**	**4.23**	**8.05**		**6.85**	**9.69**	**11.20**		**1.74**	**3.50**	**4.22**	
	S.Em. ±		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**	
C.D. of M (AMF)	0.05		0.20		0.04		0.20		0.14		0.43	
C.D. of S (Moisture)	0.21		0.43		0.05		0.15		0.10		0.28	
C.D. of A × B (AM fungi × Different moisture levels)	0.12		0.38		0.01		0.04		0.26		0.76	

Note - I_1- Irrigation regime 1 (-33 kPa)
I_2-Irrigation regime 2 (-500 kPa)
I_3-Irrigation regime 3 (-1000 kPa)
UASDAMF soybean Consortium (UASDAMFS15 + UASDAMFS25 + UASDAMFS36)

Table.4 Nodule parameters as influenced by selected AM fungal isolates at different moisture levels at 45 DAS

Treatment	Nodule parameters											
	Nodule number				Nodule dry weight (g plant^{-1})				Leghaemoglobin content (mg g^{-1} FW nodules)			
AMF	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**	**I_1**	**I_2**	**I_3**	**Mean of A**
UASDAMFS15	26.84	21.27	13.92	**20.67**	1.74	0.71	0.51	**0.98**	0.50	0.19	0.18	**0.29**
UASDAMFS25	34.66	28.10	17.45	**26.73**	1.80	0.87	0.55	**1.07**	0.55	0.26	0.19	**0.33**
UASDAMFS36	30.89	24.81	16.22	**23.97**	1.80	0.84	0.52	**1.05**	0.54	0.23	0.19	**0.32**
UASDAMF soybean Consortium	36.44	28.59	17.48	**27.50**	2.12	1.13	0.54	**1.26**	0.69	0.36	0.24	**0.43**
***G. fasciculatum* (reference strain)**	29.61	22.79	15.19	**22.73**	1.76	0.81	0.51	**1.02**	0.51	0.22	0.17	**0.30**
UASDAMF Consortium	39.75	31.13	20.75	**30.54**	2.15	1.17	0.56	**1.29**	0.66	0.29	0.22	**0.39**
UIC	25.31	20.02	13.16	**19.49**	0.77	0.76	0.40	**0.64**	0.29	0.20	0.16	**0.22**
Mean of B	**31.93**	**25.24**	**16.31**		**1.73**	**0.90**	**0.51**		**0.53**	**0.25**	**0.19**	
	S.Em.±		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**		**S.Em. ±**		**C.D.(p=0.01)**	
C.D. of M (AMF)	0.43		1.28		0.02		0.07		0.12		0.38	
C.D. of S (Moisture)	0.38		1.09		0.01		0.04		0.07		0.22	
C.D. of A × B (AM fungi × Different moisture levels)	1.01		2.88		0.03		0.11		0.20		0.50	

Note - I_1- Irrigation regime 1 (-33 kPa)
I_2-Irrigation regime 2 (-500 kPa)
I_3-Irrigation regime 3 (-1000 kPa)
UASDAMF soybean Consortium (UASDAMFS15 + UASDAMFS25 + UASDAMFS36)

The biophysical parameters recorded with the help of IRGA, such as photosynthetic rate and stomatal conductivity, transpiration rate of soybean in the present study have shown maximum values with AMF inoculated plants. This is in agreement with the findings of Ruiz-Lozano *et al.,* (2001). Wherein they have also reported an increased photosynthetic activity, stomatal conductance, transpiration rate and decreasing rate of leaf temperature due to AMF inoculated plants under drought stressed condition. Low leaf temperature was observed due to cooling effect by water which was provided by AMF to plants during stressed condition (Ruiz-Lozano and Azcon, 1995).

A lower concentration of proline may be attributed to either greater drought resistance AMF colonized plants under drought stress conditions (Wu and Xia, 2006). The amount of proline was found more in non mycorrhizal plants than mycorrhizal plants. This was due to AM fungi which helps the host plant during water stress condition. Mycorrhizal plants synthesize less amount of proline than non mycorrhizal plants. Proline is an important amino acid in plant under drought stress that prevents oxidation of cells from inside. It also regularizes osmotic pressure of plant under drought stress for absorbing water (Bhosale and Shinde, 2011).

Increased glycine betaine with water stress conditions was reduced by the application of AM fungi. This indicates that glycine betaine as an effective compatible solute enhanced by the drought stress. The major role of glycine betaine might be protection of the integrity of the cell membrane from drought stress damage and involvement in osmotic adjustment (Lv *et al.,* 2007).

The lower oxidative damage in the AM plants seems to be a consistent effect of AM symbiosis, regardless of the fungal species involved in the association (Ruiz-Lozano *et al.,* 2001 and Porcel *et al.,* 2003). AMF contribution to plant drought tolerance might also have occurred through drought avoidance mechanisms such as hyphal water uptake (Marulanda *et al.,* 2003) or increased water uptake related to mycorrhizal changes in root morphology (Kothari *et al.,* 1990) or soil structure (Auge *et al.,* 2001a). Such mycorrhizal effects could allow plants to remain more hydrated than non-AM plants as soil dries (Auge *et al.,* 2001b). AM formation contributes to the production of scavenging peroxyl radicals, buffering cellular free-radicals and producing a powerful ROS-scavenging system (Ashraf and Foolad, 2007).

Although ethylene is essential for normal growth and development in plants, at high concentration it can be harmful as it induces defoliation and other cellular processes that may lead to reduced crop performance. Using their 1-amino cyclopropane-1-carboxylic acid (ACC) deaminase activity, microorganisms can divert ACC from the ethylene biosynthesis pathway. Thus microorganisms assist in diminishing the accumulation of ethylene levels and reestablish a healthy root system needed to cope with environmental stress (Glick *et al.,* 2007).

The biosynthesis of PALase and have been reported up on pathogenic attack including viruses, tissue wounding, UV irradiation, low temperatures, drought condition, salinity, low levels of nitrogen, phosphate and iron (Ritter and Schulz, 2004; Gholizadeh *et al.,* 2004). Similar results were also found in our experiment where at -500 kPa and -1000 kPa irrigation regimes, an increased PALase activity was noticed compared to -33 kPa. Inoculation of AM fungi to plants decreased the PALase activity as plants are less susceptible to stress conditions. UASDAMF soybean consortium showed less PALase

activity followed by UASDAMF consortium compared to uninoculated control.

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