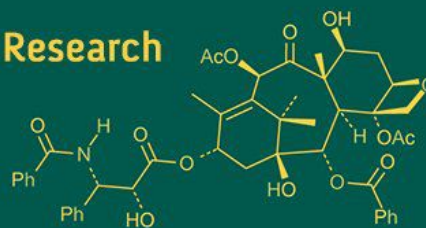
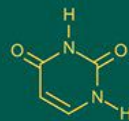
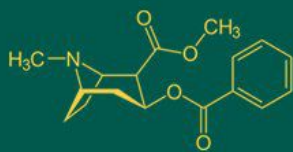


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Arbuscular mycorrhizal fungi isolated from saline soil alleviated the salinity induced stress and physiological parameters of wheat (*Triticum aestivum* L.)

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Abstract

The present experiment was conducted to assess the response of wheat to different salinity levels (EC ranging 4 dS m⁻¹, 6 dS m⁻¹ and 8 dS m⁻¹) and to determine the possible role of arbuscular mycorrhizal fungi (AMF) in enhancing salt tolerance. Increasing salinity induces hyperosmotic and hyperionic stress in plants due to the accumulation of Na⁺ ions, which disturbs the normal metabolism of plants. Tolerance mechanisms like enhanced activities of antioxidative enzymes and accumulation of osmolytes are usually considered as potent defense mechanisms against salt-induced changes. The results of the pot culture studies indicated that the total chlorophyll content of wheat plants decreased with increasing NaCl stress. However, AMF inoculated plants *viz.*, UASDAMF SL consortium and UASD consortium significantly increased the relative chlorophyll content, proline and glycine-betaine over uninoculated control. In addition, activities of antioxidant enzymes were increased by salt stress and further enhanced by AMF inoculation. Increase in antioxidant activities in AMF inoculated plants justifies the key role of AMF in promoting the growth of plants under salinity stress. Thus our study indicated AMF had positive impact on wheat plants subjected to salt stress, which suggested a potential possibility of application of AMF for ecological stability in saline areas.

Keywords: Salinity, AMF, wheat, antioxidants, Proline

Introduction

Food security is one of the essential human needs that can never be ignored by any society. Today, the food security for the world's growing population is threatened by environmental degradation and global climate change. Among cereals, wheat (*Triticum aestivum* L.) is one of the most important foods for 40% of the world's population. About 20% of calories and 55% of carbohydrates are being provided by wheat across the globe. Currently, it is dominating the most of arable land (38.8%), with relatively higher grain protein (12-15%) than other cereals, but the productivity remains low (FAO, 2016). In future, this productivity can further decrease, because wheat is highly sensitive to environmental conditions, *i.e.* temperature, water reduction and saline soils (Porter and Semenov, 2005) [29].

Among these stresses, salinity is one of the important and adverse environmental constraint restricting growth and development of wheat (Alqarawi *et al.* 2014) [3]. It has been reported that more than 20 and 33% of the total cultivated and irrigated agricultural lands worldwide are extremely affected by salinity, respectively (Kumar *et al.* 2019) [23]. Increasing industrialization, anthropogenic activities and excessive use of saline water for crop irrigation resulted in conversion of fertile soils into salt affected soils making the situation much graver. Excessive salts limit the soil water available for seedlings, causes osmotic imbalance, oxidative stress, nutrient imbalance, reduced nutrient uptake including P and ion toxicity because of high Na⁺ and Cl⁻ concentrations (Arif *et al.* 2020) [4]. This stress causes symptoms to appear in plants *viz.*, decrement of leaf area, leaf thickness and succulence, abscission of leaves, necrosis of root and shoot, and reduction of internode lengths (Rahneshan *et al.* 2018) [31]. Wheat is classified as a moderately salt tolerant plant with a saturated paste EC threshold value of 6.0 dS/m. Maas-Hoffman model reported that wheat will have a 7.1% yield loss with each 1 dS/m increase above 6 dS/m, by affecting almost all aspects of plant development such as germination, vegetative growth, and reproductive development. To counter the problems of salinity, plants exhibit growth plasticity

(morphological and developmental pattern change), accumulation of compatible osmolytes to maintain turgor as well as prevent ultrastructural damage, ion-homeostasis, regulation of water uptake and enhanced water use efficiency, enhanced photosynthesis, detoxification of ROS through antioxidant enzymes and molecules and induction of phytohormones (Augé *et al.*). However, these adaptive strategies become inefficient to cope with the rapidly increasing salinity. Hence new methods are necessary to increase plant tolerance under salinity. One of the ways to decrease the unfavorable effects of salinity on plant growth is to produce inbred crop plants, which are highly tolerant to salinity stress (Miransari and Smith, 2007) ^[25], but these methods requires high capital for their research.

In this respect, recently there has been a great interest in mitigating salt stress by the use of microorganisms. Plants, in their natural environment are colonized both by external and internal microorganisms. In particular, plant species are commonly associated with fungal symbionts such as mycorrhizal fungi and endophytes (within plants), which may influence their responses to environmental stimuli, including salinity stress. Accumulating evidence suggests that AMF colonization in roots can help improve plant tolerance to salinity stress in crops such as tomato, wheat, soybean and alfalfa (Begum *et al.* 2019; Evelin *et al.* 2019) ^[10, 14]. AMF employ various mechanisms to mitigate plant salinity stress. For instance, AMF can augment nutrient uptake, increase water uptake, increase the photosynthetic rate and regulate hormonal levels to abate the harmful effects of salts on plant growth and development.

Furthermore, up-regulation of the antioxidant system and the efficient compartmentalization of excessive toxic ions are the important salinity tolerance strategies employed by AMF (Hashem *et al.* 2016; Rasool *et al.* 2023) ^[21, 32].

However, the magnitude of the effect of AMF on plant salinity tolerance differs greatly among various studies (Porcel *et al.* 2012; Chandrasekaran *et al.* 2019) ^[28, 13]. These differences can be attributed to various factors, such as the level of salinity, types of hosts and mycorrhizal partners, environmental conditions and their complex interactions. Henceforth, in the recent years, considerable progress has been made to understand these mechanisms. Therefore, present experiment was intended to analyze effect of different AMF isolates on the growth and stress associated parameters (physiological and biochemical parameters) of wheat plants under different salinity gradients, in order to further elucidate stress tolerance mechanisms in AM fungi.

Materials and methods

Treatments and experimental design

This experiment was determined using two factor factorial - completely randomized design (6*3) with three salinity levels [low salinity (4 dS m⁻¹), medium salinity (6 dS m⁻¹), and high salinity (8 dS m⁻¹)] combined five mycorrhizal inoculations [UASDAMF SL 11 (*Glomus leptotichum*), UASDAMF SL 27 (*Glomus phansihalose*), Reference strain (*Glomus fasciculatum*), UASDAMF consortium (*Glomus macrocarpum*, *Gigaspora margarita* and *Acaulospora laevis*), UASDAMF saline consortium (UASDAMF SL 11 and UASDAMF SL 27)] and control.

	I - Factor: AMF		II- Factor: salinity level
M1	UASDAMF SL 11 (<i>Glomus leptotichum</i>)	S1	Low saline soil (4 EC)
M2	UASDAMF SL 27 (<i>Glomus phansihalose</i>)	S2	Moderate saline soil (6EC)
M3	UASDAMF consortium <i>Glomus macrocarpum</i> , <i>Gigaspora margarita</i> and <i>Acaulospora laevis</i>	S3	High saline soil (8 EC)
M4	Reference strain (<i>G. fasciculatum</i>)		
M5	UASDAMF SL consortium UASDAMF SL 11 and UASDAMF SL 27		
M6	Control		

Preparation of arbuscular mycorrhizal inoculum

The AMF isolates viz., *Glomus leptotichum*, *Glomus phansihalose* used in the current experiment was isolated previously from the salt affected soils of Karnataka and screened for their salt tolerance and mitigation capacity *in vivo* (Abhinandana *et al.*, 2025) ^[11]. Reference strain (*Glomus fasciculatum*) and UASDAMF consortium (*Glomus macrocarpum*, *Gigaspora margarita* and *Acaulospora laevis*) was collected from the Department of Agricultural microbiology, University of Agricultural Sciences, Dharwad. The propagation of these AMF inocula was carried out by the trap culture protocol using corn plants (*Zea mays* L.) as the host. In this protocol, single spores of each AMF isolate were inoculated on autoclaved vermiculite (121°C for 3 separated times) as the culture bed in plastic pots (10 cm diameter), and maize seeds (0.5% [v/v] NaOCl used for seed surface sterilization) were sown in the pots (five seeds/pot). The pots were incubated at 27 ± 1 °C with 70-75% relative humidity for 3 months. The mycorrhizal inoculum needed in the experimental soil was 40 g of trap soil culture (approximately 100 spores/g trap soil)/pot (4 kg) and the inocula used in this study consisting of soil including spores, hyphae, and infected root fragments of corn plants (*Zea mays* L.).

Plant and growth conditions with salt and AMF treatments

Wheat (*Triticum aestivum* L.) var. DWR 162 was obtained from All India Coordinated Wheat Improvement Project, UAS, Dharwad). The seeds were surface sterilized with sodium hypochlorite (0.5%, v/v) for 3 min, washed thoroughly with distilled water before germination on blotter. Healthy and uniformly sized seedlings were selected and transplanted into plastic pots (30 cm diameter, six seedling/pot) containing different gradients of saline soil.

Different gradients of saline soils used in pot experiment was topsoil i.e. 0-20cm (salinity gradient - 4EC, 6EC and 8 EC) collected from sites of AICRP on management of salt affected soils and use of saline water in agriculture, Gangavathi. The site falls under the Northern Dry Zone of Karnataka state (semi-arid eco-sub region) lying between 15°35'07" N latitude and 76°15'47" E longitude at an altitude of 419 m above mean sea level with an average annual rainfall of around 542 mm. The characteristic of these soil used in the study was clay loamy soil with pH 8.1±0.3, ECe varying from 0.25 - 64 dS m⁻¹. Soils with different salinity levels (4EC, 6EC and 8 EC) were filled into respective treatment pots (4kg). Thereafter, prepared mycorrhizal inoculum [40 g of trap soil culture, approximately 100 spores/g trap soil/pot (4 kg)] according to treatment was placed 3 cm below the germinated wheat

grains at sowing to promote fungal inoculation of plant roots. Non mycorrhizal soil was used as control. Six plants were maintained in the greenhouse during the experiment, watered every alternate day as well as received every ten days with standard Hoagland's solution (Graham and Fardelmann, 1986) [18]. Water application to the pots was calculated depending on the soil field capacity.

Leaves of wheat plants were collected at 45 DAS and stored at 80 °C until further use for biochemical analysis. These leaf samples were used for estimation of osmolytes, anti-oxidative enzymes and relative chlorophyll content. At the end of pot experiment (12 weeks), the plants were harvested carefully, washed in distilled water, separated into shoots and roots. The samples were dried at 70 °C for 48 h and dry weight was recorded. Fresh root samples and soil samples were used for observing mycorrhizal studies (root colonization and spore count).

Relative chlorophyll estimation

The relative chlorophyll content in leaves of wheat plant was measured at 30 DAS using a SPAD (Soil Plant Analysis Development) meter (SPAD-502 KONICA Japan). The reading was taken between 10.00 am to 12.00 noon of the day.

Estimation of antioxidant enzymes

Peroxidase activity

Peroxidase activity was done by the following the procedure given by Maehly and Chance (1954) [11]. 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 40C. The clear extract was used as enzyme source. 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

$$\mu\text{moles per gram tissue} = \frac{\mu\text{g proline} \times \text{ml toluene/ml} \times 5 \times \text{sample}}{115.5 \times \text{g sample}}$$

Where 115.5 is the molecular weight of the proline

Estimation of Glycine-Betaine

Glycine-Betaine content in plant was estimated by following the procedure of Greive and Grattan (1983) [19]. The dry leaf sample (0.5 g) was shaken in 10 mL toluene (0.5%) and kept at 4°C overnight. After centrifugation, 1 mL of the filtrate was mixed with 1 mL of sulfuric acid (2N). 0.5 mL of solution was taken and then 200 µL of potassium tri-iodide (KI3) solution (containing 7.5 g Iodine and 10 g Potassium iodide in 100 mL of 1 N HCl) was added to the tubes. The content was cooled in a chiller. Afterwards 2.8 mL of ice cooled deionized H₂O and 5 mL of 1-2 di-chloroethane was added to the samples. The absorbance of the organic layer (lower layer) was recorded spectrophotometrically at 365 nm.

$$\% \text{AMF Colonization} = \frac{\text{Total numbers of AM positive segments}}{\text{Total number of segments studied}} \times 100$$

average difference of O. D. (optical density) between two consecutive time intervals and enzyme activity was expressed in terms of OD sec⁻¹ mg⁻¹ protein (*i.e.* specific activity).

Superoxide dismutase

Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) adopting the method of Giannopolitis and Ries (1977) [16]. The reaction mixture contained 500 µL phosphate buffer (pH 7.8), 0.5 mL distilled H₂O, 100 µL methionine (13 mM), 50 µL NBT (63 µM) and 50 µL enzyme extract and was exposed to light for 20 min. The OD of the irradiated aliquot was read at 560 nm. One enzyme unit was defined as the amount of enzyme that inhibits the nitro blue tetrazolium photoreduction by 50%. Per cent inhibition was calculated by following formula of Asada *et al.* (1974) [6].

Catalase activity

Catalase activity was determined by following method of Chance and Maehly (1955) [12]. The reaction mixture contain 1.9 mL potassium phosphate buffer (50 mM; pH 7.0) and 1 mL H₂O₂ (5.9 mM) and 100 µL of the enzyme extract. A control was run under similar conditions where enzyme extract was added after stopping the reaction. Decrease in OD will be observed at 240 nm for 2 min. One enzyme unit is defined as the amount of enzyme which catalyzed the oxidation of 1.0 µ mole H₂O₂ per minute under assay conditions.

Estimation of Proline

For estimation of proline, 0.5 g of plant tissue was extracted in 3% (w/v) sulfosalicylic acid, and the homogenate was subjected to centrifugation at 3,000 rpm for 20 min. The supernatant was reacted with acetic acid and ninhydrin, the mixture was boiled for 1 h, and the absorbance at 520 nm was read to quantify the proline content (Bates *et al.* 1973). The proline content on fresh weight basis is expressed as follows:

Determination of arbuscular mycorrhizal colonization and spore count

Mycorrhizal colonization was estimated by the method of Phillips and Hayman (1970) [27]. Randomly sampled roots were cleared with running tap water immediately after harvest. The roots were then fixed by FAA, cleared with 10% KOH and stained with 0.05% trypan blue in lacto glycerol. The stained root segments (one cm in length, 50 segments used as replicate for each sample) were observed under a digital computerized microscope (model DP-72, Olympus) at 20 magnification. The presence of mycelia, vesicles and arbuscules was recorded and analysed to assess the structural colonization as described by Giovannetti and Mosse (1980) [17] according to the following formula:

AM Spore Count

The chlamydospores in rhizosphere of wheat were determined by wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963) [15]. AM spores were isolated using wet sieving and decantation method.

The supernatant from the above protocol was separately filtered through Whatman No. 1 filter paper and the content of the filter papers were examined for spores under a stereo

zoom microscope (Olympus).

Statistical Analysis

Data were statistically analyzed with a analysis of variance (ANOVA). Means were separated by least significant difference (LSD, P 0.05). All of the measurements were performed three times for each treatment, and the calculated means and standard errors (SE) were mentioned.

Table 1: Relative chlorophyll content of wheat as influenced by selected AM fungal isolates at different salinity levels (30 DAS)

Treatment	Soil Plant Analysis Development (SPAD) values			
	S1	S2	S3	Mean of A
AMF				
UASDAMF SL 11	38.16	37.50	36.20	37.28
UASDAMF SL 27	36.16	34.43	34.14	34.94
UASDAMF Consortium	42.63	41.96	39.33	41.84
<i>G. fasciculatum</i> (reference strain)	35.06	35.10	32.63	34.26
UASDAMF SL consortium	42.36	41.93	39.00	41.10
UIC	34.13	33.16	28.16	34.61
Mean of B	37.87	37.00	34.61	
	S.Em. $\pm$		C.D. (p = 0.01)	
C.D. of M (AMF)	0.23		0.32	
C.D. of S (Salinity)	0.32		0.46	
C.D. of A*B (AM fungi * Different salinity levels)	0.57		0.80	

Note: Salinity level 1 (S1) = 4 EC

Salinity level 2 (S2) = 6 EC

Salinity level 3 (S3) = 8 EC

UASDAMF SL consortium = UASDAMF SL 11 + UASDAMF SL 27

Table 2: Influence of selected AM fungal isolates on proline and glycine-betaine of wheat at different salinity levels (45 DAS)

Treatment	Proline ($\mu\text{mol g}^{-1}\text{FW}$)				Glycine betaine ($\mu\text{mol g}^{-1}\text{FW}$)			
	S1	S2	S3	Mean of A	S1	S2	S3	Mean of A
AMF								
UASDAMF SL 11	5.16	5.86	9.05	6.69	3.38	5.46	7.76	5.53
UASDAMF SL 27	4.96	5.63	8.89	6.59	3.14	5.06	7.58	5.26
UASDAMF Consortium	5.83	6.56	9.68	7.32	3.82	6.46	8.53	6.27
<i>G. fasciculatum</i> (reference strain)	4.42	5.60	8.55	6.19	2.93	5.26	7.46	5.22
UASDAMF SL consortium	5.91	6.88	10.23	7.66	3.96	6.66	8.63	6.42
UIC	3.86	5.09	7.83	5.59	2.83	4.16	6.99	4.66
Mean of B	4.99	5.91	9.04		3.34	5.51	7.82	
	S.Em. $\pm$		C.D. (p = 0.01)		S.Em. $\pm$		C.D. (p = 0.01)	
C.D. of M (AMF)	0.04		0.11		0.03		0.09	
C.D. of S (Salinity)	0.05		0.16		0.04		0.12	
C.D. of A*B (AM fungi * Different salinity levels)	0.09		0.27		0.07		0.22	

Note: Salinity level 1 (S1) = 4 EC

Salinity level 2 (S2) = 6 EC

Salinity level 3 (S3) = 8 EC

UASDAMF SL consortium = UASDAMF SL 11 + UASDAMF SL 27

Table 3: Influence of selected AM fungal isolates on peroxidase, catalase and superoxide dismutase activities at different salinity levels (45 DAS)

Treatment	Peroxidase (U $\text{mg}^{-1}\text{protein}$)				Catalase (U $\text{mg}^{-1}\text{protein}$)				Superoxide dismutase (U $\text{mg}^{-1}\text{protein}$)			
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
AMF												
UASDAMF SL 11	2.76	2.80	3.26	2.94	36.20	37.50	38.16	37.28	104.50	117.00	131.46	118.32
UASDAMF SL 27	2.30	2.33	2.66	2.44	34.03	34.33	36.16	34.94	101.73	115.83	129.16	115.57
UASDAMF Consortium	3.40	3.73	4.53	3.88	37.33	39.96	41.33	39.54	108.26	120.10	138.76	122.37
<i>G. fasciculatum</i> (reference strain)	2.30	3.00	2.43	2.57	32.63	35.10	35.06	34.26	98.36	113.50	127.33	113.06
UASDAMF SL consortium	3.80	4.56	4.80	4.38	39.00	41.93	42.36	41.00	113.40	123.06	144.26	126.91
UIC	1.46	1.93	2.03	1.81	28.16	33.16	34.13	31.82	93.76	107.30	120.83	107.30
Mean of B	2.67	3.06	3.28		34.61	37.00	37.87		103.33	116.13	132.30	
	S.Em. $\pm$		C.D. (p = 0.01)		S.Em. $\pm$		C.D. (p = 0.01)		S.Em. $\pm$		C.D. (p = 0.01)	
C.D. of M (AMF)	0.16		0.48		0.23		0.67		0.18		0.53	
C.D. of S (Salinity)	0.23		0.68		0.32		0.94		0.26		0.75	
C.D. of A*B (AM fungi * Different salinity levels)	0.41		1.23		0.57		1.64		0.45		1.30	

Note: Salinity level 1 (S1) = 4 EC

Salinity level 2 (S2) = 6 EC

Salinity level 3 (S3) = 8 EC

UASDAMF SL consortium = UASDAMF SL 11 + UASDAMF SL 27

Table 4: Mycorrhizal parameters as influenced by selected AM fungal isolates in wheat rhizosphere at different salinity levels (60 DAS)

Treatment	Spore count / 50 g ⁻¹ of soil				Percent root colonization (%)			
	S1	S2	S3	Mean of A	S1	S2	S3	Mean of A
UASDAMF SL 11	178	203	219	200.00	68.03	51.53	37.16	52.23
UASDAMF SL 27	144	166	189	166.34	64.33	42.31	32.34	46.33
UASDAMF Consortium	198	216	259	224.33	75.76	67.33	48.00	63.70
<i>G. fasciculatum</i> (reference strain)	163	178	204	181.88	49.66	35.30	26.66	38.87
UASDAMF SL consortium	226	264	283	257.39	81.50	74.30	56.40	70.73
UIC	57	61	80	67	16.83	14.28	9.49	13.53
Mean of B	161.61	181.16	205.66		58.35	47.51	34.34	
	S.Em. $\pm$		C.D. (p = 0.01)		S.Em. $\pm$		C.D. (p = 0.01)	
C.D. of M (AMF)	1.38		3.99		0.32		0.92	
C.D. of S (Salinity)	1.96		5.64		0.45		1.30	
C.D. of A*B (AM fungi * Different salinity levels)	3.39		9.77		0.78		2.25	

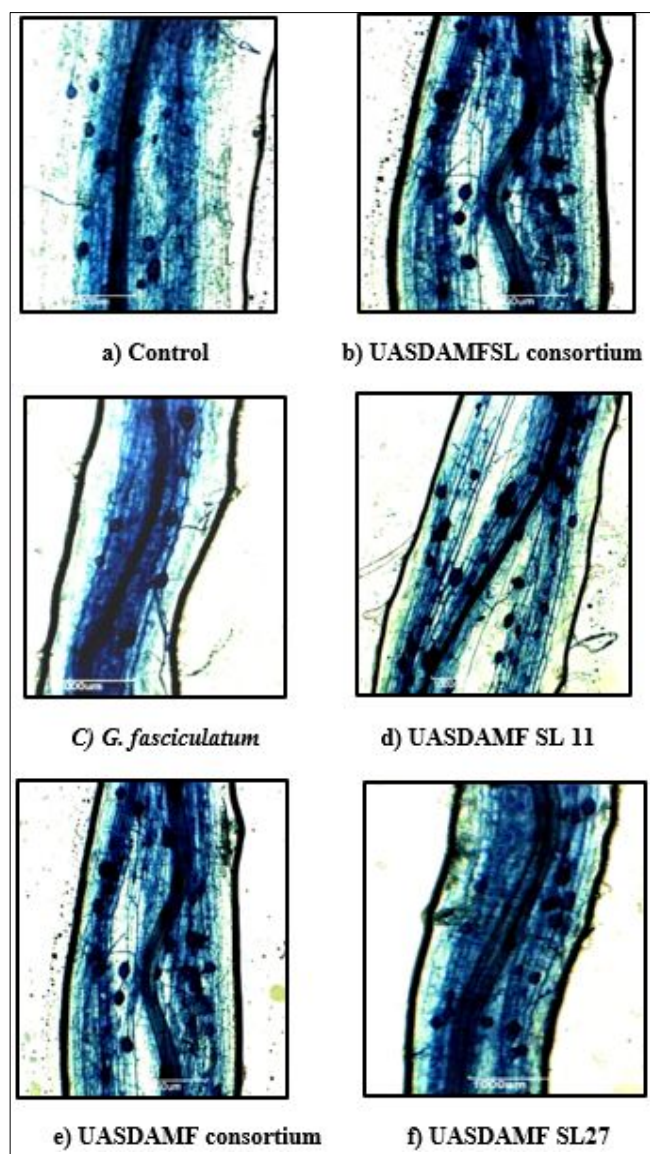


Plate 1a: Influence of AM fungi on root colonization of wheat under low salt stress 4 dS m⁻¹

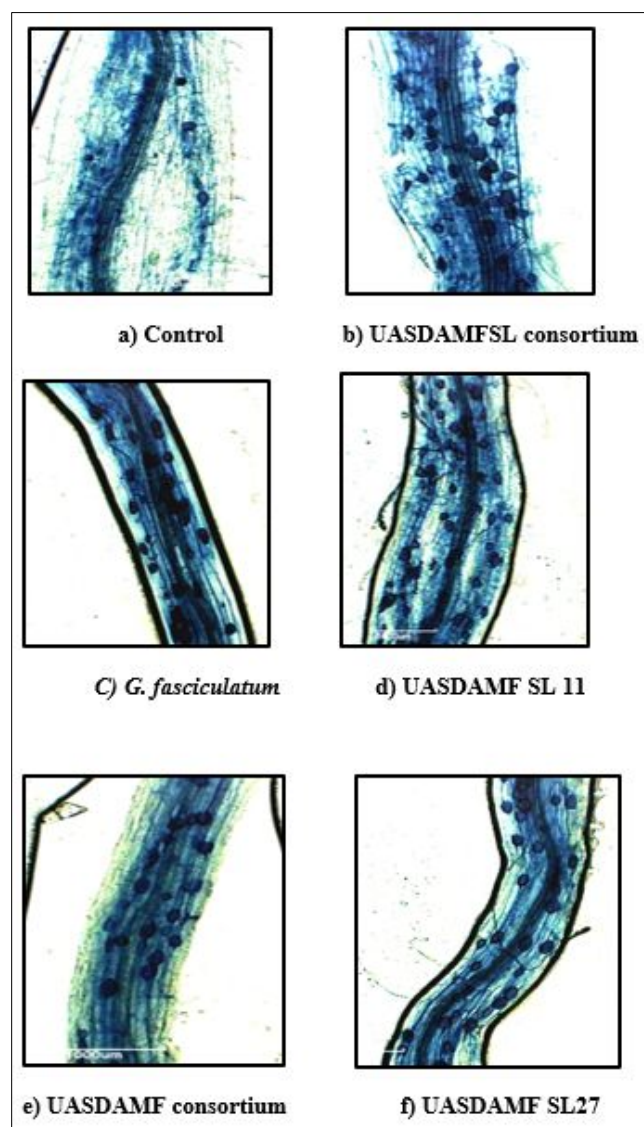


Plate 1b: Influence of AM fungi on root colonization of wheat under moderate salt stress 6 dS m⁻¹

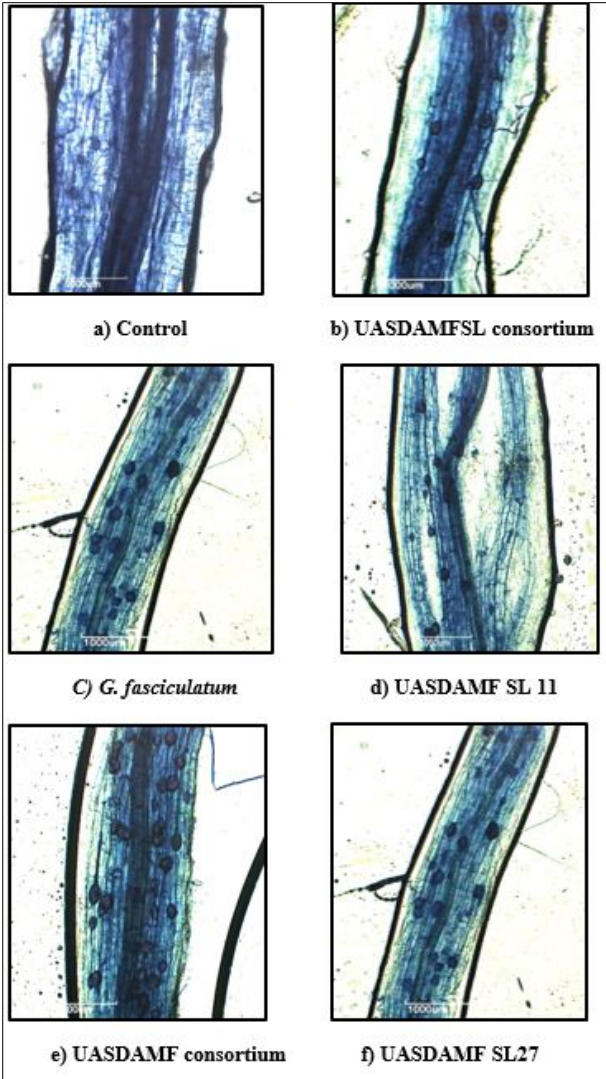


Plate 1c: Influence of AM fungi on root colonization of wheat under high salt stress 8 dS m-1

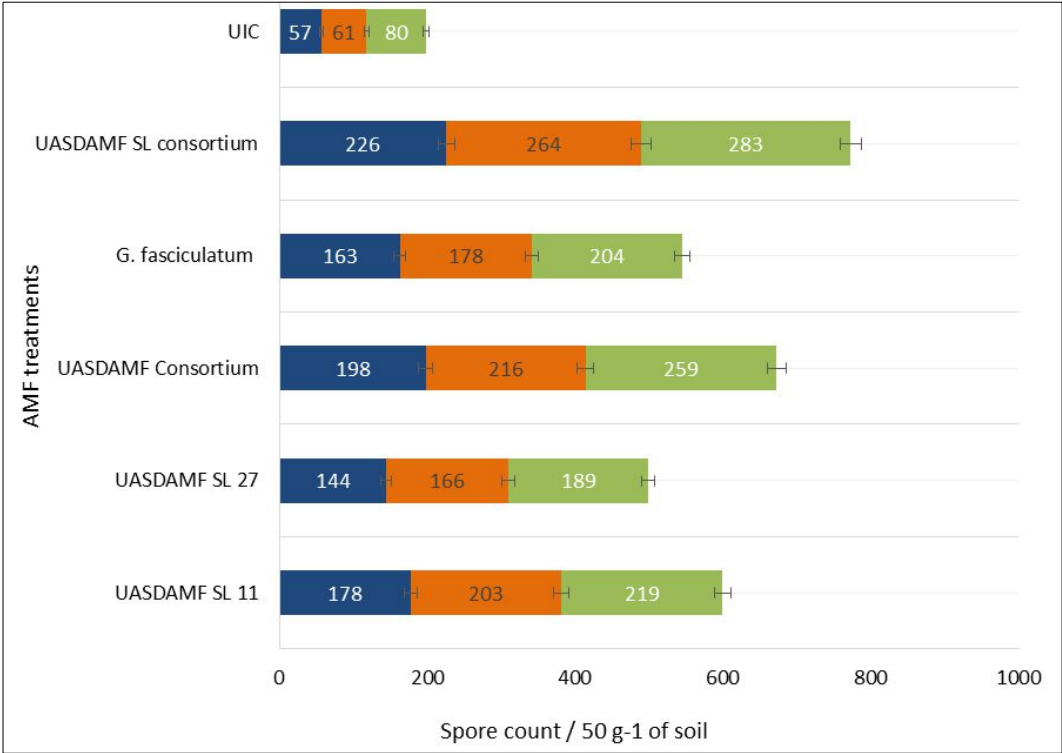


Fig 1: Spore count of different AMF treatments under varying salinity stress

Result and Discussion

Salinity is the most adversely affecting factor on productivity and quality of wheat through altering the physiological as well as biochemical activities in plants. Generation of ROS due to Na^+ toxicity, which damage biomolecules (e.g., lipids, proteins, and nucleic acids) on the cellular level and alters redox homeostasis, is a common phenomenon under salt stress. Therefore, present study discusses mycorrhiza fungi mediated regulation physiological and biochemical parameters under different range of salinity gradients.

AMF improved relative chlorophyll content in NaCl treated wheat plants

Salinity induced reduction in growth attributes has been reported by plethora researchers (Ahmad *et al.* 2015; Rasool *et al.* 2023) [12, 2]. In tomato, length as well as fresh and dry biomass of shoot and root declined with the increasing salinity (Rahman *et al.*, 2021) [30]. In the present study, wheat plants subjected to different concentrations of salt stress showed decreased growth and biomass at all NaCl concentrations examined (4EC, 6EC and 8 EC). This reduction in growth parameters may be due to the inhibition of cell division and reduced rate of cell elongation exerted by the high salinity levels (Baronova *et al.*). This could be due to positive interactions between these AMF isolates and also with host plants which helps in counteracting negative effects of salt stress.

Salinity caused considerable decline in chlorophyll pigments with the effect being more obvious under higher concentrations (8 EC) (Table 1). Our results of reduced chlorophyll content under NaCl stress corroborates with the reports of Taibi *et al.* (2016) [34] and Loudari *et al.* (2020) [24] for *Phaseolus vulgaris* L. and *Solanum lycopersicum* respectively. This reduced chlorophyll contents under stress can be attributed to the enhanced chlorophyllase activity which causes pigment degeneration. These degraded pigments cause reduction in photosynthesis and hence the growth is affected. Also reduction in the uptake of minerals (e.g., Mg) needed for chlorophyll biosynthesis reduces the chlorophyll content in the leaf (Sheng *et al.*). While in the presence of mycorrhiza, the antagonistic effect of Na^+ on Mg^{2+} uptake in plants is counter balanced and suppressed. Plants received UASDAMF SL consortium followed by UASDAMF consortium, isolates UASDAMF SL 11, UASDAMF SL 27 and *G. fasciculatum* significantly improved the chlorophyll content compared to uninoculated plants. This result is in accordance with Khalid *et al.* (2015) who demonstrated that in sweet basil inoculation of AMF increased chlorophyll content under normal as well as salt-stressed conditions and also observed enhanced mineral uptake especially magnesium, an important component of chlorophyll molecule. Hence, it is clear from the results that AMF inoculation enhances chlorophyll contents and thereby mitigates the negative impact of salinity to some extent.

AMF modulates the accumulation of proline and glycine betaine in wheat plants treated with NaCl

Osmolyte production during stress conditions is a plant protective mechanism against stress. Salinity enhanced the proline synthesis in both control and AMF treated plants. However, the role of AMF in proline accumulation in plants is not consistent: several studies reported a higher proline content, whereas others reported a lower proline content in AMF-colonized plants under stress (Hashem *et al.* 2016) [21].

In our study, it is evident that AMF inoculation increased the proline content in wheat plants compared to control plants and also higher proline accumulation observed with increasing salinity stress from 4 to 8 EC (Table 2). Among different salt stress conditions, the wheat plants grown at salinity level 8 dS m^{-1} recorded significantly higher proline content (9.04 $\mu\text{mol g}^{-1}$ FW) compared to 6 dS m^{-1} and 4 dS m^{-1} (5.91 and 4.99 $\mu\text{mol g}^{-1}$ FW respectively). The increased accumulation of proline in these studies because of AMF could be linked to AMF induced salinity resistance with proline acting as osmoprotectant. The enhanced proline accumulation in the severe salt stressed plants believed to increase the tolerance capacity of plants by stabilizing the osmotic homeostasis and quenching toxic radicals (Ahmad *et al.* 2015) [2]. Similarly, increased synthesis of glycine betaines in salt-stressed wheat seedlings inoculated with AMF support the findings. Syeed *et al.* (2021) [33]. GB is believed to stabilize the quaternary protein structures against the damaging effects of salt stress. In addition, GB protects several important components of photosynthetic apparatus including Rubisco and photosystem II from the deleterious impact of high salinity (Hossain & Fujita). High salinity causes dissociation and inactivation of important photosynthetic components like Rubisco and PSII into subunits, and hence causing obstructions in normal photosynthetic pathway (Syeed *et al.* 2021) [33]. Therefore, GB has been reported to maintain higher activities of antioxidant enzymes and hence lowering the levels of H_2O_2 .

AMF enhanced the activity of enzymatic antioxidants in wheat seedlings treated with NaCl

The results related to the effect of NaCl and AMF on enzymatic antioxidants of wheat plants were presented in the Table 3. The results showed the increased activities of superoxide oxide dismutase (SOD), peroxidase (POX) and catalase (CAT) in the AMF colonized plants as the salinity increased and thus increasing the ROS scavenging capacity. The wheat plants subjected to 8 dS m^{-1} salt stress showed highest peroxidase activity (3.28 U mg^{-1} protein) followed by 6 dS m^{-1} (3.06 U mg^{-1} protein). However lowest peroxidase activity was recorded in the plants grown at 4 dS m^{-1} salinity (2.67 U mg^{-1} protein). Among the AM fungal cultures, UASDAMF SL consortium recorded significantly highest peroxidase activity (4.38 U mg^{-1} protein) followed by UASDAMF consortium (3.88 U mg^{-1} protein) as compared to the treatments which received single inoculation of UASDAMF11, *G. fasciculatum* and UASDAMF27 (2.94, 2.57 and 2.44 U mg^{-1} protein). While uninoculated control plants recorded the lowest peroxidase activity (1.81 U mg^{-1} protein). It is evident from the results of the present study that application of AM fungi enhanced the activity of peroxidase in plants against the oxidative stress compared to control plants, thereby helping the plants to withstand stress condition. Peroxidase is the hydrogen peroxide scavenging enzyme which detoxifies the reactive oxygen species and protects cell against harmful concentration of hydroperoxides.

Superoxide dismutase activity was also higher in the wheat plants received AM fungi and their activity increased with increasing salinity stress. The results of our study are in conformity with the findings of Hajiboland (2012) [20] who reported that mycorrhizal application increased the SOD activity and restrain the adverse effects of salt stress in pea plants. Also higher SOD enables detoxification of more $\text{O}_2^{\cdot-}$

to H_2O_2 , which is further detoxified to H_2O . The conversion of H_2O_2 to H_2O can be catalyzed by enzymes, such as CAT, APX, and POX. In addition, Wei *et al.* (2018)^[37] reported that arbuscular mycorrhizal fungi possesses various *SOD* genes, which get up-regulated and provides tolerance to the colonized *Elaeagnus angustifolia* seedlings against oxidative damage. Mittal *et al.* (2012)^[26] have reported that the improved growth in *Brassica juncea* under high salinity accompanied by increased CAT activity. CAT mediates breakdown of H_2O_2 to H_2O . Mycorrhizal colonization further enhanced the activity of CAT and thereby strengthening the antioxidant defense system (Rasool *et al.* 2023). Higher CAT activity is associated with improved stress tolerance and reduces the oxidative stress induced deleterious change. The present experiment also demonstrates the highest catalase activity in salt stressed plants. At 45 DAS, the catalase activity was maximum in the treatment received UASDAMF SL consortium (41 U mg^{-1} protein) followed by UASDAMF consortium (39.54 U mg^{-1} protein). Among single isolates, UASDAMF SL 11 showed greater CAT activity compare to uninoculated control in all salinity levels.

Mycorrhizal parameters as influenced by different salinity stress

The mycorrhizal parameters like spore count and per cent root colonization varied due to AMF inoculation at different salt levels. Salinity, not only affects the host plant but also the AMF. It can hamper colonization capacity, spore germination and growth of hyphae of the fungus. Several researchers have reported the negative effects of salinity on the fungus. In our study, the treatments received UASDAMF SL consortium, UASDAMF consortium and individual application of isolates *viz.* UASDAMF SL11, UASDAMF SL27 and *G. fasciculatum* recorded highest number of spores and root colonization over UIC (Table 4). But spore number increased with increased salinity stress (Figure 1), in contrast root colonization declined with increased salinity stress (Plate 1). This is in agreement with the findings of Wang *et al.* (2022)^[36] where greater root colonization percentage was observed under mild salt stress condition and decreased with severe salt stress. Several studies found that the number of AM fungal spores did not significantly decrease with soil salinity and reported a relatively high spore number (mean of 100 per 10 g soil). The higher spore density in saline soils may be due to the fact that sporulation is stimulated under salt stress (Tressner and Hayes, 1971)^[35] which means that AMF may produce spores at low root-colonization levels in severe saline conditions. Reduced colonization as a result of salinity reported in our study is in confirmation with the findings of Aroca *et al.* (2013)^[5]. Wang *et al.* (2022)^[36] reported that colonization of plant roots by some AMF species is reduced in the presence of NaCl, probably due to the direct effect of NaCl on the AM fungi indicating that salinity can suppress the formation of AMF. However, colonization of mycorrhiza in such adverse salt range helps the plants to overcome the negative effects of salt.

Salinity tolerance is a complex phenomenon, which is usually an amalgamation of several adaptive attributes. Increased production of ROS as a result of salinity-induced oxidative stress results in membrane damage, reduced photosynthesis, and growth retardation. Tolerance mechanisms like enhanced activities of antioxidative

enzymes, accumulation of osmolytes in response to environmental extremes are usually considered as potent defense mechanisms against salt-induced changes. The present work suggests that AMF can be helpful in counteracting the saline stress and maintaining the plant growth and development. Increase in antioxidant enzyme activities (SOD, CAT), proline, glycine betaine content in AMF inoculated plants justifies the key role of AMF in promoting the growth of plants under salinity stress. Thus our study indicated AMF had positive impact on wheat plants subjected to salt stress, which suggested a potential possibility of application of AMF for ecological stability in saline areas.

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