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Effect of Salt and Acid Stress on *Triticum aestivum* L. Var. Inoculated with *Glomus fasciculatum*

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Abstract: Arbuscular mycorrhizal (AM) symbiosis favors increased resistance to various abiotic stresses, such as drought stress, acid and salt stress. The effect of salt and acid stress on *Triticum aestivum* L. varieties was studied with and without mycorrhizal inoculation. Four *Triticum aestivum* L. varieties were subjected to salt stress by supplementing 1N NaCl and acid stress by 1N HCl under control and inoculated conditions. Plant growth was reduced in control plants under salt and acid stress. AM inoculation helps the plants to withstand acid and salt stress. An inoculated plant shows better growth under salt and acid stress than control plants. Acid and salt treatments were found to be inhibitory for growth and development of *Triticum aestivum* L. varieties. Acid stress was found to be more inhibitory than salt stress. Less growth was noticed in plants subjected to acid stress.

Key words: Arbuscular mycorrhizal fungi % *Triticum aestivum* L. varieties % HCL and NaCl

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are known to facilitate water and mineral uptake by the host plants under normal and stress conditions [1, 2]. Several studies have demonstrated that AM symbiosis can improve resistance to various abiotic stresses [3, 4]. AM fungi help to overcome resistance to various salt stresses by increasing the water and nutrient uptake from the soil. Salt and acid tolerance of plants is a complex phenomenon that involves physiological, biochemical and molecular changes. The reduction in growth and yield are undoubtedly the most important physiological response of plants to the excess salt in the media. Salt resistance was improved by AM colonization in Maize [5], Mungbean and Clover with the AM fungal effect correlated with improving osmoregulation or proline accumulation. AM colonization also improved NaCl resistance in Tomato, with extent of improvement related to salt sensitivity of the cultivar [6]. There is considerable evidence to suggest that AM fungi are able to increase the host plant's tolerance to water stress [7, 8, 9], including that caused by high salinity [5, 6]. Soil salinity affects crop plants in three major ways, osmotic stress results in decreasing water availability, ionic stress and changes in the cellular ionic balance. Physiologically many processes are affected but notably these are reduced cell growth, decreased leaf area, biomass and yield. Wheat has a moderate tolerance to salinity.

The objective of this study is to elucidate how salt and acid stress influences the growth of AM fungus and the host plants. An attempt has been made to study the stress tolerance of indigenous AM fungus and its impact on the growth and development of *Triticum aestivum* L. var.

MATERIALS AND METHODS

Source of AM Inoculum: The AM fungus *Glomus fasciculatum* was isolated according to Gerdmann and Nicolson, [10] method. This AM fungus was mass multiplied by using S*orghum vulgare* L. grown on sterile soil. Finally three month old multiplied AM inoculum was used for the experiment.

Experimental Design: Experiments were conducted in earthen pots measuring 20cm diameter. The sterilized soil and sand was mixed in 1:1 ratio and filled in the experimental pots. Grains of four *Triticum aestivum* L. var. DWR 162, DWR 195, DWR 225 and NI 5439 were selected for the experiments. The germ plasm of these varieties was collected from University of Agricultural Sciences Dharwad. Grains were sterilized with 2% sodium hypo chloride solution. To remove the traces of sodium

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hypochloride grains were washed with distilled water 4 times. About 10 grains were placed in each pot. Control pots were not added with AM fungal inoculum. Plants were inoculated with AM fungus, before sowing the grains, inoculum of *Glomus fasciculatum* was placed 2 cm below the soil. Experiments were conducted in six groups, each group was maintained in triplicates as follows.

Group 1: Control plants were grown in pots containing sterilized soil and sand mix without inoculum. Plants were regularly watered on alternate days.

Group 2: Plants were grown in pots containing sterilized soil and sand mix with AM Fungal inoculum. Plants were regularly watered on alternate days.

Group 3: Plants were grown in pots containing sterilized soil and sand mix without inoculum. Plants were regularly watered on alternate days and are treated with 25 ml of NaCl (3%) solution per pot once in a week.

Group 4: Plants were grown in pots containing sterilized soil and sand mix with AM inoculum. Plants were regularly watered on alternate days and are treated with 25ml of NaCl (3%) solution per pot once in a week.

Group 5: Plants were grown in pots containing sterilized soil and sand mix without AM inoculum. Plants were regularly watered on alternate days and are treated with 25ml of 0.5% HCl per pot once in a week.

Group 6: Plants were grown in pots containing sterilized soil and sand mix with AM inoculum. Plants were regularly watered on alternate days and are treated with 25ml of 0.5% HCl per pot once in a week.

Pots belonging to four *Triticum aestivum* L. Var. DWR 162, DWR 195, DWR 225 and NI 5439 were maintained in triplicates with above mentioned treatments. Altogether 72 pots were maintained and are watered on alternate days to maintain sufficient moisture. Acid and salt stress was induced by treating the plants with NaCl and HCl respectively after 15 days from the date of sowing.

Growth Parameters: Growth parameters of *Triticum aestivum* L. var. like plant height, girth of stem, shoot biomass, root biomass, leaf number and leaf length were measured at 60 days old plants.

Determination of Percentage Root Colonization: AM fungal colonization in the roots of *Triticum aestivum* L. var. grown under different treatments were determined by Philips and Hayman, [11] method. Roots are washed with 10% KOH solution and stained with 0.05% (V/V) tryphan blue in lactophenol. 30 randomly choosen root fragments of 1cm length were mounted on slide and examined microscopically. Per cent of mycorrhizal colonization was determined using following formula.

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \text{ X } 100$$

Determination of Spore Count: Total spore count was determined by wet sieving and decanting method [10].

Statistical Analyses: The data were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated by Duncan's Multiple Range Test (DMRT) using SPSS 7.5 [12].

RESULTS

Mycorrhizal Colonization: None of the non inoculated plants grown with and without salt and acid stress show mycorrhizal colonization. Varied degree of colonization was found in inoculated plants grown with and without salt and acid stress. Very high per cent of root colonization was observed in inoculated plants which are not subjected to any stress. In all the four *Triticum aestivum* L. var. 80-85% of root colonization was observed in inoculated plants grown without salt and acid stress. Inoculated plants grown under salt stress show lesser colonization, which is of about 60-70%. In inoculated plants treated with acid exhibit very least per cent of root colonization than the plants grown without stress and with salt stress. From these experiments it is evident that salt stress resulted in the decrease of mycorrhizal colonization to moderate extent. But the acid stress was found to be lethal for mycorrhizal colonization, it has resulted in significant decrease of mycorrhizal colonization.

Number of Spores and Vesicles: Number of spores present in the rhizosphere was found proportional to the extent of mycorrhizal colonization. Spore number was counted per 100gm soil. Maximum spore number was observed in stress free plants, which are not subjected to acid or salt stress. The spore number is found to be around 150 per 100gm soil. In the rhizosphere of plants

Table 1: Showing the effect of salt and acid stress on *Triticum aestivum* L. var. NI 5439 inoculated with *Glomus fasciculatum*

Treatments	Plant height	Stem diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	4.66±0.088^{b}	1.00±0.152^{b}	2.62±0.095c	0.245±0.007^{b}	4.33±0.333a^{b}	9.6±0.296^{b}	0.0±0.00d	0.0±0.00d
Inoculated untreated	5.93±0.208^{a}	1.433±0.120^{a}	4.43±0.133^{a}	0.402±0.016^{a}	5.0±0.000^{a}	12.63±0.463^{a}	81.20±10.30^{a}	159±20.56^{a}
Salt stress without inoculation	3.13±0.218^{c}	0.633±0.066^{c}	1.96±0.175^{d}	0.226±0.004^{b}	4.33±0.333^{ab}	8.06±0.284^{b}	0.0±0.00^{d}	0.0±0.00^{d}
Salt stress with inoculation	4.93±0.240^{b}	1.00±0.057^{b}	3.56±0.159^{b}	0.262±0.029^{b}	4.66±0.333^{ab}	8.766±0.233^{b}	60.40±9.53^{b}	54.00±4.00^{b}
Acid stress without inoculation	3.166±0.284^{c}	0.600±0.054^{c}	1.516±0.090^{e}	0.211±0.019^{c}	4.33±0.333^{ab}	6.46±0.567^{d}	0.0±0.00^{d}	0.0±0.00^{d}
Acid stress with inoculation	3.466±0.338^{c}	0.733±0.30^{bc}	1.723±0.128^{de}	0.224±0.004^{c}	4.66±0.333^{ab}	6.66±0.504^{c}	14.66±1.32^{c}	17.33±1.85^{c}

Table 2: Showing the effect of salt and acid stress on *Triticum aestivum* L. var. DWR 162 inoculated with *Glomus fasciculatum*

Treatments	Plant height	Stem diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	4.66±0.088^{b}	0.866±0.033^{b}	2.80±0.30^{bc}	0.311±0.013^{b}	4.0±0.00^{a}	4.0±0.00^{a}	0.0±0.00^{d}	0.0±0.00^{d}
Inoculated untreated	5.93±0.208^{a}	1.300±0.115^{a}	3.69±0.349^{a}	0.427±0.043^{a}	4.0±0.00^{a}	10.83±0.28^{a}	91.03±3.80^{a}	150.33±9.7^{a}
Salt stress without inoculation	3.13±0.218^{c}	0.766±0.033^{b}	2.63±0.318^{bc}	0.270±0.022^{c}	3.33±0.33^{b}	7.53±0.145^{c}	0.0±0.00^{d}	0.0±0.00^{d}
Salt stress with inoculation	4.93±0.240^{b}	0.866±0.033^{b}	3.43±0.120^{ab}	0.320±0.002^{b}	4.0±0.00^{a}	9.03±0.233^{b}	64.0±5.56^{b}	86.0±2.08^{b}
Acid stress without inoculation	3.166±0.284^{c}	0.633±0.033^{c}	2.40±0.300^{c}	0.253±0.012^{c}	4.0±0.00^{a}	7.73±0.338^{c}	0.0±0.00^{d}	0.0±0.00^{d}
Acid stress with inoculation	3.466±0.338^{c}	0.667±0.033^{c}	2.89±0.261^{b}	0.289±0.019^{c}	4.0± 0.00^{a}	7.83±0.338^{c}	14.66±1.26^{c}	23.66±4.25^{c}

Table 3: Showing the effect of salt and acid stress on *Triticum aestivum* L. var. DWR 195 inoculated with *Glomus fasciculatum*

Treatments	Plant height	Stem diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	3.30±0.115^{bc}	1.13±0.066^{b}	3.386±0.095^{b}	0.287±0.027^{bc}	4.33±0.333^{ab}	9.06±0.338^{b}	0.0±0.00^{d}	0.0±0.00^{d}
Inoculated untreated	4.93±0.650^{a}	1.80±0.057^{a}	4.62±0.298^{a}	0.386±0.019^{a}	5.00±0.57^{a}	11.23±0.218^{a}	83.41±5.46^{a}	158.66±4.19^{a}
Salt stress without inoculation	3.03±0.404^{c}	0.633±0.088^{c}	3.06±0.147^{b}	0.273±0.022^{bc}	3.66±0.33^{b}	7.43±0.448^{cd}	0.0±0.00^{d}	0.0±0.00^{d}
Salt stress with inoculation	3.90±0.360^{b}	1.133±0.120^{b}	3.28±0.339^{b}	0.322±0.015^{ab}	4.00±0.00^{ab}	8.43±0.786^{bc}	68.00±7.54^{b}	98±9.20^{b}
Acid stress without inoculation	2.80±0.366^{c}	0.666±0.033^{c}	3.02±0.148^{b}	0.225±0.020^{c}	3.33±0.333^{b}	6.80±0.503^{d}	0.0±0.00^{d}	0.0±0.00^{d}
Acid stress with inoculation	2.83±0.305^{c}	0.766±0.088^{c}	2.99±0.291^{b}	0.251±0.030^{b}	3.33±0.333^{b}	7.60±0.47^{bcd}	16.33±2.43^{c}	23±4.50^{c}

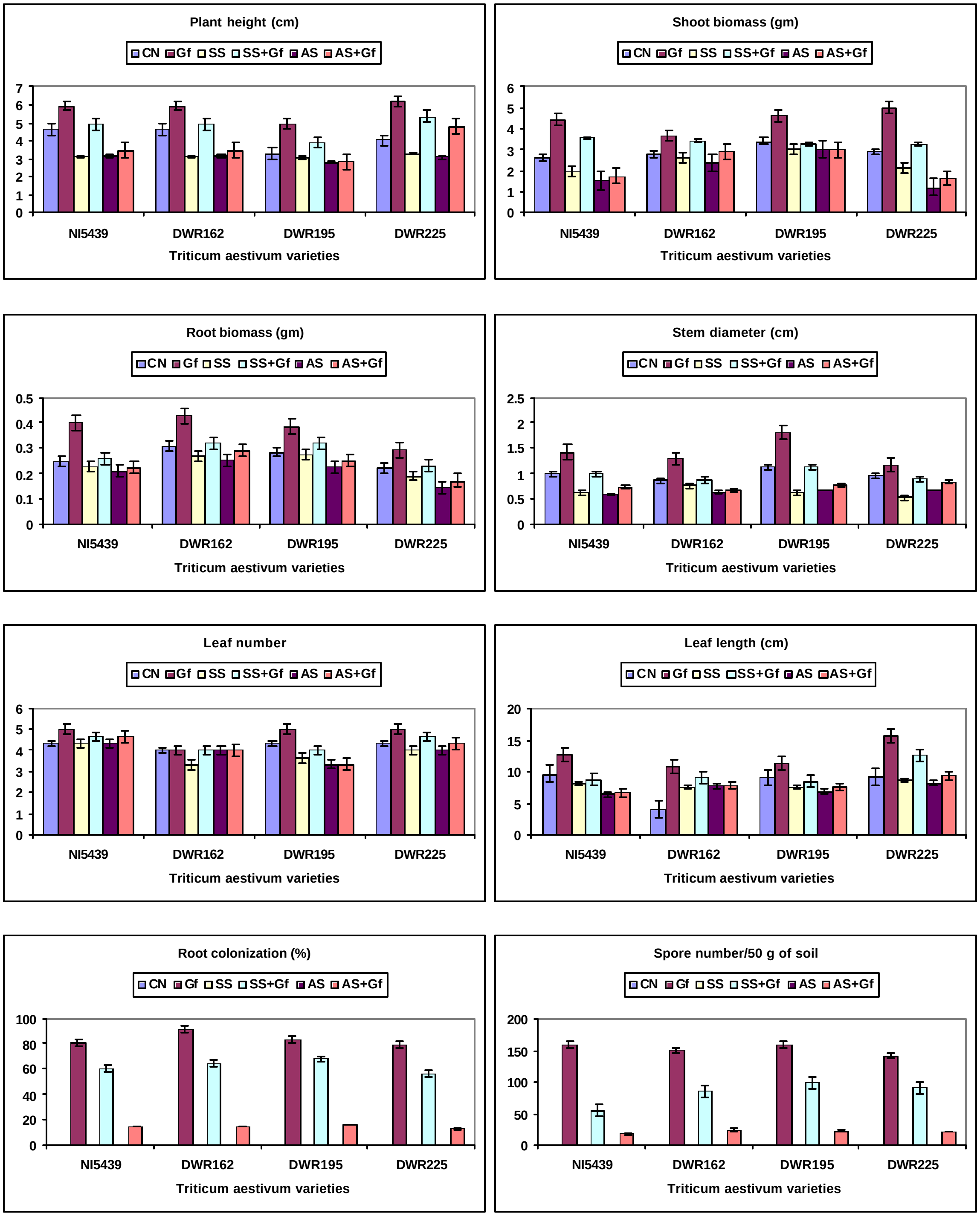
Table 4: Showing the effect of salt and acid stress on *Triticum aestivum* L. var. DWR 225 inoculated with *Glomus fasciculatum*

Treatments	Plant height	Stem diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	4.03±0.152^{c}	0.966±0.208^{ab}	2.906±0.36^{b}	0.224±0.02^{bc}	4.33±0.57^{ab}	9.16±1.04^{c}	0.0±0.00^{d}	0.0±0.00^{d}
Inoculated untreated	6.23±0.208^{a}	1.166±0.152^{a}	5.00±0.264^{a}	0.293±0.03^{a}	5.0±0.00^{a}	15.76±0.208^{a}	79.60±5.98^{a}	140.66±15.94^{a}
Salt stress without inoculation	3.30±0.264^{d}	0.533±0.02^{d}	2.13±0.208^{c}	0.192±0.03^{d}	4.0±0.0^{b}	8.66±0.472^{c}	0.0±0.00^{d}	0.0±0.00^{d}
Salt stress with inoculation	5.36±0.680^{b}	0.900±0.02^{b}	3.26±0.251^{b}	0.230±0.03^{b}	4.66±0.57^{ab}	12.56±0.73^{b}	56.59±5.66^{b}	90.00±6.08^{b}
Acid stress without inoculation	3.10±0.100^{d}	0.666±0.02^{cd}	1.21±0.189^{d}	0.146±0.03^{e}	4.0±0.0^{b}	8.16±0.15^{c}	0.0±0.00^{d}	0.0±0.00^{d}
Acid stress with inoculation	4.80±0.264^{b}	0.833±0.23^{bc}	1.63±0.29^{d}	0.172±0.02^{de}	4.33±0.50^{b}	9.32±0.43^{c}	12.93±0.85^{c}	21.33±5.34^{c}

treated with NaCl and are subjected to salt stress show comparatively lesser spore number, which was found to be around 80 per 100 gm soil. In the rhizosphere of plants subjected to acid stress show least spore number, which was about 15-20 spores per 100gm soil.

Number of vesicles was found to be maximum in inoculated plants without stress. Plants treated with NaCl show less number of vesicles than stress free plants. Acid treatment results in least vesicle formation. The number of vesicles produced in acid treated plants is very least. Salt and acid treatments not only reduce the mycorrhizal colonization, spore number but also the number of vesicles.

Growth Parameters of *Triticum aestivum* L. Var.: The effect of *Glomus fasciculatum* on *Triticum aestivum* L. var. was measured with the consideration of morphological parameters like plant height, stem diameter, root biomass, shoot biomass leaf number and leaf length at 60 days old plants. Inoculated plants demonstrate better performance and growth parameters than uninoculated plants. Salt and acid stress resulted in reduced growth than untreated plants. Plants colonized by *Glomus fasciculatum* have shown increased parameters than uninoculated plants. Maximum plant height was observed in inoculated and stress free plants



CN=Control; Gf=*Glomus fasciculatum*; SS=Salt stress; AS=Acid stress;

Fig. 1: Showing the effect of salt and acid stress on *Triticum aestivum* L. var. under control and inoculated conditions

Picture 1: Showing the effect of *Glomus fasciculatum* on NI 5439 var. under control and inoculated conditions without stress

Picture 2: Showing the effect of *Glomus fasciculatum* on DWR 225 var. under control and inoculated conditions with salt stress

Picture 3: Showing the effect of *Glomus fasciculatum* on DWR 195 var. under control and inoculated conditions with acid stress

belong to DWR 225 (6.233±0.120) and DWR 162 (6.40±0.05). Very least plant growth was observed in acid treated uninoculated plants. Among acid treated uninoculated plants DWR 195 and DWR 162 have shown least plant height (Table 2 and3). Plants subjected to salt stress have shown better plant height than plants subjected to acid stress. Plants treated with NaCl inoculated with *Glomus fasciculatum* have shown more plant height than uninoculated plants (Picture 2). The results revealed that AM fungal inoculation minimized the effect of salt and acid stress in all the four *Triticum aestivum* L. var. (Figure 1).

Stem diameter, leaf number, leaf length, shoot and root biomass were found to be higher in inoculated stress free plants. The uninoculated stress free plants show lesser parameters than inoculated plants. DWR 225 shows maximum shoot biomass (Table 4) and DWR 162 shows maximum root biomass under inoculated conditions without stress (Table 2). Lesser root and shoot biomass is observed in uninoculated plants without stress. Plants subjected to salt and acid stress exhibit lesser root and shoot biomass than stress free plants both under inoculated and uninoculated plants (Figure 1). Plants inoculated with AM fungus (*Glomus fasciculatum*) show comparatively higher root and shoot biomass than uninoculated plants under salt and acid stress conditions (Figure 1). Acid stress is found to be more deleterious than salt stress plants show lesser growth parameters in presence of acid stress (Picture 3). AM inoculation do not help to improve the growth of plants treated with acid, there is no much difference in the growth parameters of inoculated and uninoculated plants treated with acid. Over all plant growth promoted was the least in inoculated plants treated with acid. AM fungal inoculation promotes overall growth of plants to the extent of 30 to 50% in salt treated plants. Leaf number do not exhibit much difference in plants subjected to various treatments. Leaf length was found to be minimum in uninoculated acid treated plants and is found to be maximum in AM fungal inoculated stress free plants (Figure 1).

DISCUSSION

Earlier workers reported better growth performance of AM fungal inoculated plants to salt and acid stress. Salt resistance was improved by AM fungal colonization in Maize [5]. NaCl and HCl treatments were known to reduce Mycorrhizal colonization in Maize [13]. AM fungi were tested to protect Cucumber plants from NaCl stress compared to similar sized non AM plants. Alfalfa was also more effectively protected against salinity stress by AM symbiosis than by P supplementation [14] and improvement of NaCl resistance in lettuce plants.

Soil salinity affects the crop plants in three ways through osmotic stress, ionic stress and changes in cellular ionic balance, which ultimately decreases the water availability to the host plants resulting in restricting plant growth. Physiologically many processes are affected due to physiological water stress, such as decreased cell growth, stomatal conductance, photosynthetic rate, biomass and yield. AM fungi are known to reduce the salt and acid stress and helping the host plants to produce more biomass and yield than non mycorrhizal plants. The mycorrhizal colonization was found to be more in untreated inoculated plants than plants treated with NaCl and HCl high salt concentration may affect mycorrhizal colonization and hyphal growth in plants. Vesicle formation is greatly reduced in stress induced plants in particular in acid treated plants. This is probably because the contents of AM fungi are absorbed by the host plants under stress conditions [15]. The decrease in the number of spores in the rhizosphere of NaCl treated plants supports the view that vesicles are certainly related to spore formation. Plants treated with acid show poor mycorrhizal colonization, spore and vesicle number. The AM fungus *Glomus fasciculatum* was found to be sensitive to acid stress. However AM fungi are known to increase phosphorus availability in acid soils. AM fungi may increase the uptake of phosphorus and promote growth. This is the reason for better growth of inoculated plants than uninoculated plants [16].

Mycorrhizal symbiosis could enhance the plant growth and stress conditions through inducting metabolic changes. Mathur and Vyas [17] reported that mycorrhizal symbiosis is resulted in significant increase in protein, chlorophyll, reducing sugars, free amino acids under stress conditions as compared with non mycorrhizal plants. Crude protein content is reported to be higher in mycorrhizal plants than non mycorrhizal plants [18]. AM symbiosis led to enhanced growth, nutrition, productivity and improved yield in Wheat plants [19]. Plants colonized by mycorrhizal fungi have shown to absorb water more thoroughly than non mycorrhizal plants [20]. This is the reason for higher shoot and root biomass in AM inoculated plants than control plants [21]. It was reported that inoculation with AM fungi brought about an important increase in biomass production which might be attributable to increased dependence of Wheat on AM fungi for water uptake [22]. The AM fungus *Glomus fasciculatum* helps the host plants to maintain higher Relative water content than uninoculated plants, thus enabling the mycorrhizal plants to carry out metabolic function even under stress situations without any inhibitory effect of stress [23]. Dry weights of AM plants were moderately greater than nonmycorrhizal plants when subjected to salt stress [24].

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