Phytochemical Changes in Wheat (*Triticum aestivum* L.) by Arbuscular Mycorrhizal Fungi Under Water Stress Conditions

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ABSTRACT: : *Considering the importance of arbuscular mycorrhizal fungi (AMF) in sustainable agriculture the present investigation was aimed to study the Phytochemical changes in wheat (Triticum aestivum L.)during AM fungi treatment, water stress (Field Capacity %) treatment and combination of AM fungi and water stress (FC %) at the seedling as well as anthesis stage. The pot culture experiment was carried out in three replications by using four mycorrhizal treatments along with control, five water stress treatments and combination of AM fungi and water stress treatments. Theeffect of these treatments on wheat was assessed for Phytochemicals like reducing sugar and phenolcontent at seedling as well as anthesis stage.Results revealed that the phytochemicals such as reducing sugar and phenol content was increased in all mycorrhizal soil treatment. Also, the amount ofreducing sugars and phenol content increased in combine treatment of water stress and mycorrhiza in comparison to individual water stress treatments. But under water stress conditions, mycorrhizal plants accumulated more phytochemical metabolites than nonmycorrhizal plants. As a conclusion, Triticumaestivuminpresence of mycorrhiza accumulate more metabolites to tolerate water stress by improving the osmotic adjustment.*

Key Words: *Water stress, AM fungi, Triticum aestivum, Phytochemical*

INTRODUCTION

Wheat is the most important agricultural good in international market and also it is one of the strategic agricultural productions which have daily and universal consumption [1]. Wheat is the staple food for more than 35% of world population.Its grain is the main source of protein and carbohydrates. In developing countries, almost 32% of wheat crop face various types of drought stress during the growth season[2].

Among different environmental abiotic stresses, drought is one the most important environmental stress, severely impairs plant growth and developmentand the performance of crop plants [3]. Drought affects the quantity and quality of the grains produced [4]. Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. Available water resources for successful crop production have been decreasing in recent years. Furthermore, scientists suggested that in many regions of world, crop losses due to increasing water shortage will further intensify its impacts [5]. Plants respond to water stress atmorphological, anatomical, cellular and physiologicallevels with modifications that allow the plants to avoidstress and increase their tolerance.

Contributions of AM fungi to agriculture arewell known. Mycorrhizas were involved in protectionagainst drought stress through improved nutritional status and osmotic adjustments. In addition to the inherent response system of plants against stress, a number of soil microorganisms have been proved to be able to alleviate the stress symptoms.Mycorrhizal plants show better survival than non–mycorrhizal plants in extreme dry conditions.AM fungi are knownto enhance the adoption ability of host plants underwater stress conditions and help the host plants tocope up with situations of drought. Plants species inoculatedwith AM fungi are known to exhibit considerablephysiological responses. AMfungi can influence the host plants against water stress[6]. AM fungi help the hostplants to increase uptake of nutrients and tolerance toabiotic and biotic stresses [7]. Mycorrhizal plants enhance the photosynthesisand assimilation of carbohydrates more thanthose in non mycorrhizal plants [8]. The accumulation of metabolic substances maysuggest that AM colonization could improve osmoticadjustment originating not only from proline but alsofrom carbohydrates and proteins resulting in the enhancementof water stress tolerance. Arbuscular mycorrhizal (AM) symbiosis isthe most important mutualistic association betweenAM from soil and plant roots [9]. Symbiotic association between AMF andplants is based on the exchange ofcarbohydrates and other nutrients betweenboth the partners. Plants roots become astrong sink for sugars duringmycorrhization which in turn increases thephotosynthethic ability of the phototroph tocompensate this usage of sugars [10]. Phenols are important components functioning asdefense mechanisms against pathogenattack. Phenols occur naturally in plants andthey do have antimicrobial properties whichprevent fungal spore germination and toxinproduction [11].Theobjective of this study was to evaluate the effect of arbuscular mycorrhizal fungi (AMF) on phytochemical changes in *Triticum aestivum*L. under water stress conditions.

MATERIAL AND METHODS

The authentic seeds of wheat cultivar variety GW 496 were procured from the Mahatma Phule Agricultural University, Rahuri, Dist. Ahmednagar, (MS) for experimental work.

Mycorrhizal fungus inoculum

The pure culture of AM fungus, *Glomus mosseae*was procured from the Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India. Pure culture of AM fungi was multiplied in earthen pots using *Zea mays* in sterilized sand soil (1:1 v/v) mixture [12]. After 90 days under green house conditions a density of 20-25 spores per gram soil inoculum was attained. The fungal spores, hyphae and colonized root pieces were used as the source of inoculums for further experiment.

Experimental design for AM fungi

The pot culture experiment was carried out in three replications by using four mycorrhizal treatments as 25g, 50g, 75g and 100g of mycorrhizal soil. Five plastic buckets each of 30 X 30 X 27 cm size were serially numbered and weighed (0.5 kg). At the bottom of each bucket small holes were made to drain excess water. In each bucket 16kg (garden soil and well-decomposed compost in 3:1 proportion) was filled. The weights of all buckets along with soil were recorded (16.5kg). AM inoculum were placed 3 cm below the seeds at the time of sowing. The non-mycorrhizal treatment had equal amount of sterilized soil to provide the same microflora without mycorrhizal fungi treated as control. Plants were watered as required.

Preparation of different moisture regimes (FC %)

The pot culture experiment was laid out in three replications and five treatments of moisture regimes e. g. 100% FC, 80% FC, 60% FC, 40% FC and 20% FC. For making different moisture regimes gravimetric method was followed with some modifications for which garden soil was used after determining its water holding capacity[13].

Experimental design for combinational treatment of AM fungi and water stress

The above prepared FC (100% FC, 80% FC, 60% FC, 40% FC and 20% FC) set and one AM fungi treatment (75g) i.e. (control + 100% FC, 75g + 80% FC, 75g + 60% FC, 75g + 40% FC, 75g + 20% FC) were used for the combinational experiments. A uniform and healthy seeds of wheat cultivar (variety GW 496) was selected, seeds were surface sterilized with 0.1 % $HgCl_2$, washed thoroughly 3-4 times in sterilized distilled water and then soaked in distilled water for 12 hours. Hundred well-imbibed seeds were sown in each bucket for water stress and for combinational (AM and water stress) treatment. The said experiment was replicated three times.
The methodology used for analyses is briefly described below,

Estimation of reducing sugars

Reducing sugars was estimated according to the method [14]. The leaves of treated and control seedlings were cut into small pieces and one g tissue was homogenized in 10 ml 80 % alcohol. This extract was condensed on hot water bath to approximately 1.0 ml and centrifuged at 5000 rpm for 15 minutes; the volume of the supernatant was adjusted to 10 ml. This extract was used for estimation of reducing sugars.

Estimation of total phenols

Total phenols were estimated as per the method of [15]. The leaves of treated and control seedlings were cut into small pieces and one gram tissue was homogenized in 10 ml (80 %) alcohol. The extract was condensed on hot water bath to approximately 1.0 ml; centrifuged at 5000 rpm for 15 min. Volume of the supernatant was adjusted to 10 ml with distilled water.

Statistical analysis

The data obtained from reducing sugar and phenol content was analyzed statistically for mean, standard error (SE), critical difference (CD), and correlation coefficient. Standard statistical methods were followed for estimating correlation coefficients [16]. CD was calculated at 5% probability and correlation coefficient was calculated at 5% and 1% probability. The correlation between agronomic characters was estimated by using software SPSS 9.0.

RESULTS AND DISCUSSION

REDUCING SUGARS

Sugars with reducing property (arising out of the presence of a potential aldehyde or keto groups) are called reducing sugars. Some of the reducing sugars are glucose, galactose, lactose and maltose.

Effect of mycorrhizal treatment on reducing sugars

In the present study, the obtained results showed that, the amount of reducing sugars increase with increase in mycorrhizal soil treatments at both seedling and anthesis stage. Maximum increase in reducing sugar content was 7.75 and 10.18 mg/gm fresh weight in 100 gm mycorrhizal soil treatment at seedling and anthesis stage respectively (Table 1). AM fungi always serve as a storage sink for sugars [17]. Our results are in agreement with the previous reports in which the various researchers recorded increase in reducing sugar content as an effect of mycorrhizal inoculation in *Ziziphus*[18] and tomato plants [19]

Effect of water stress treatment on reducing sugars content

The obtained results revealed that, the reducing sugars gradually accumulated as the level of water stress increased at seedling and anthesis stage.

Table 1: Effect of AM fungi on Reducing Sugars and Phenols content in leaves of wheat

AM Soil (gm)	Reducing Sugars (mg g^{-1} F.W.)		Phenols (mg g^{-1} F.W.)	
	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage
Control	6.52	8.16	1.50	1.26
25	6.71	8.78	2.29	1.53
50	7.04	9.05	2.66	2.07
75	7.33	9.90	2.90	2.28
100	7.75	10.18	3.07	2.73
SE	**0.22**	**0.37**	**0.28**	**0.26**
CD at 5%	**0.61**	**1.03**	**0.77**	**0.73**

Table 2: Effect of Water Stress on Reducing Sugars and Phenols contentin leaves of wheat

Field Capacity %	Reducing Sugars (mg g^{-1} F.W.)		Phenols (mg g^{-1} F.W.)	
	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage
100	6.50	8.34	1.48	1.24
80	7.80	10.94	2.02	1.37
60	8.19	11.04	2.58	1.98
40	9.36	12.51	2.72	2.12
20	10.14	12.66	2.98	2.53
SE	**0.63**	**0.78**	**0.27**	**0.24**
CD at 5%	**1.75**	**2.16**	**0.75**	**0.67**

Table 3: Effect of AM fungi and Water Stress on Reducing Sugars and Phenols contentinwheat

AM Soil (gm) and Field Capacity %	Reducing Sugars (mg g^{-1} F.W.)		Phenols (mg g^{-1} F.W.)	
	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage
Control+100	6.50	8.28	1.45	1.27
75 +80	8.90	11.73	2.19	1.47
75+60	9.33	12.14	2.75	2.00
75+40	10.66	13.28	2.94	2.17
75+20	11.23	13.61	3.01	2.66
SE	**0.82**	**0.95**	**0.29**	**0.25**
CD at 5%	**2.29**	**2.64**	**0.81**	**0.69**

The reducing sugars content was maximum i.e. 10.14 and 12.66 mg/gm fresh weight at 20 % FC water stress treatment over control (6.50 and 8.34 mg/gm fresh weight) at seedling and anthesis stage respectively(Table 2).Like proline and glycine betaine, reducing sugars also acts as compatible osmolyte during moisture stress. Our results are agreement with the previous resultsthat increase in soluble sugar content in durum wheat plants under water stress condition[20]. Similarly, increase in reducing sugars in six different cultivars of sorghum under PEG induced water stress[21]. The authors had suggested that the involvement of accumulation of soluble sugars in osmotic adjustments under stress condition.

Combine effect of AM fungi and water stress treatment on reducing sugars content

Like individual water stress treatment, the combination treatments showedsteady increase in reducing sugars content with the increase in mycorrhizal treatment along with water stress. The reducing sugars content was found maximum i.e. 11.23 and 13.61 mg/gm fresh weight over control (6.50 and 8.28 mg/gm fresh weight) at 75 gm mycorrhizal soil and 20 % FC water stress treatment at seedling and anthesis stage respectively (Table 3).Several physiological studies suggested that under stress conditions nonstructural carbohydrates (sucrose, hexoses, and sugar alcohols) accumulate in different plant species. The current hypothesis is that, sugars are either act as osmotica and/or protect specific macromolecules and contribute to the stabilization of membrane structures. Sugars may protect cells during desiccation (22). The results of present study arein agreement with the previous results that increase in soluble and insoluble sugar concentrations in mycorrhizal wheat plants subjected to water stress[23]. Similarly, maximum accumulation of reducing sugar content in the mycorrhiza inoculated *Origanum* plants under water stress condition[24].

PHENOLS

Phenol is one of the important phytochemical which play role in plant defense mechanism during abiotic and biotic stress condition.

Effect of mycorrhizal treatment on phenols content

Plants phenolic are the most widespread classes of secondary metabolites known to be involved in the plant microbe interaction (25). In the present investigation we observed that, the plants treated with mycorrhizal treatment showed increased phenol content than non mycorrhizal plants. The maximum phenol (3.07 and 2.73 mg/gm fresh weight) was recorded in 100 gm mycorrhizal soil treated plants in comparison to control plants (1.50 and 1.26 mg/gm fresh weight) at seedling and anthesis stage respectively (Table 1).Such increase in phenol content due to mycorrhizal symbiosis in treated plants could be due to the reaction of wheat plants to the mycorrhizal colonization. Phenols are responsible for providing barriers to pathogen attack and helps in building mechanical strength to cell wall (26). Similar observation recorded that increased concentration of total phenols in roots and leaves of cotton plants with higher colonization levels[27]. Similarly, increase in total phenol content in tomato plantsby AM amendment in soil [19]. AM adaptation to soil significantly increased the phenol content of wheat grains[28].

Effect of water stress treatment on phenols content

In the present study, the obtained results clearly indicate that, the amount of phenols slowly increased with decrease in percent field capacity 80, 60, 40 and 20 % (or increase water stress) at seedling and anthesis stage. The highest amount of total phenols (2.98 and 2.53 mg/gm fresh weight) was recorded in 20 % field capacity wheat plants at seedling and anthesis stage respectively (Table 2). The phenolic compounds are important protective components of plants cells which synthesis is generally affected in response to different biotic and abiotic stress. The increase in polyphenol contents by drought in selected genotypes of cotton[29].The results of present study arein agreement with the previous results that increase in phenolic content in horse gram plants under water stress condition[30]. Accumulation of phenol under stress condition indicates that the selected variety may be tolerant to water stress.

Combine effect of AM fungi and water stress treatment on phenol content

Similar to individual water stress treatment, phenol content also increased with increase in combination treatment of AM fungi and water stress. But the combine effect of AM and water stress was more effectual on phenol content than individual water stress. The highest amount of total phenol content was exhibited in 20 % field capacity with 75 gm mycorrhizal soil treated plants at both stages. At this treatment, seedling stage plant showed 3.01 mg/g fresh weight and anthesis stage showed 2.66 mg/g fresh weight phenol content (Table 3). The mycorrhizal colonization would initially be perceived by the plant as a stress or an attack at the location of colonization by endomycorhiza[31].

CONCLUSION

The results from this study showed that the phytochemicals such as reducing sugars and phenolscontent was increased in mycorrhizal soil treatment.Compare to control, reducing sugars and phenolscontent was increased in the water stress treatments. Under water stress conditions, mycorrhizal plants accumulated more metabolites than nonmycorrhizal plants. As a conclusion, *Triticumaestivum*inpresence of mycorrhiza accumulate more phytochemicals to tolerate water stress by improving osmotic adjustment.

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