CHAPTER - 6

Effect of AM fungus *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe emend. Walker and Koske. on the accumulation of metabolites in four *Triticum aestivum* L. varieties under short-term water stress

INTRODUCTION

Drought is a world wide problem seriously affecting the productivity of many crop plants. Wheat is a staple food for more than 40% of the world's population. Drought affects the quantity and quality of the grains produced (Shao *et al.*, 2005). Water stress is considered to be the main environmental factor limiting growth and development of crop plants (Krammer and Boyer, 1997). In nature plants are frequently exposed to adverse environmental conditions that have a negative effect on plant survival, development and productivity. Drought and the salinity are considered the most important abiotic factors limiting plant growth and yield. Plants respond to water stress at morphological, anatomical, cellular and physiological levels with modifications that allow the plants to avoid stress and increase their tolerance (Bray, 1997).

Contributions of AM fungi to agriculture are well known. Mycorrhizas were involved in protection against drought stress through improved nutritional status and osmotic adjustments. AM fungi are known to enhance the adoption ability of host plants under water stress conditions and help the host plants to cope up with situations of drought. Plants species inoculated with AM fungi
are known to exhibit considerable physiological responses. The mechanisms by which mycorrhizal plants exhibit physiological response is not yet clearly studied (Auge, 2001). AM fungi can influence the host plants against water stress (Schellembaum et al., 1998). AM fungi help the host plants to increase uptake of nutrients and tolerance to abiotic and biotic stresses (Ruiz-Lozano et al., 1995).

Drought causes an oxidative stress and many other degenerative processes mediated by reduced oxygen species such as super oxide radicals or Hydrogen peroxide (Smirnoff, 1993). There is evidence that production of reactive oxygen species (ROS) is a major damaging factor in plants exposed to different environmental stresses including drought (Hoekstra et al., 2001). Plants have evolved specific protective mechanisms involving antioxidant molecules and enzymes in order to depend themselves against oxidants (Jiang and Zhang, 2002). Little attention has been paid to the role of antioxidant enzymes such as catalase and peroxidase. Water stress can cause an oxidative stress in plants. Degenerative reactions due to abiotic stress are mediated by reduced oxygen species, such as superoxide radicals (O2) or hydrogen peroxide (H2O2). They can cause damage to lipids and proteins. The catalase converts H2O2 to water and molecular oxygen in peroxisomes. Peroxidase found in the cell has much higher affinity than catalase to degrade H2O2. AM Fungi are well known to bring about physiological changes in plants by increasing various enzymatic activities. There is little information on drought stress effect on peroxidase enzyme activity in Triticum aestivum L. varieties using mycorrhizal
inoculation. Therefore in this study effort was made to assess the influence of AM fungal inoculation on peroxidase under water stress. Peroxidase enzyme quantification was studied under well watered as well as water stress conditions in mycorrhizal and non-mycorrhizal plants.

AM Fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots thus providing stimulus for greater photosynthetic activity (Bevege, 1975). Mycorrhizal plants enhance the photosynthesis and assimilation of carbohydrates more than those in non-mycorrhizal plants (Ghorbanli et al., 2004). The accumulation of metabolic substances may suggest that AM colonization could improve osmotic adjustment originating not only from proline but also from carbohydrates and proteins resulting in the enhancement of water stress tolerance.

The objective of this study was to evaluate the relative physiological indices in mycorrhizal and non-mycorrhizal Triticum aestivum L varieties in response to water stress applied at 60 and 90 DAS stages. Metabolites quantification was studied under well watered as well as water stress conditions in mycorrhizal and non-mycorrhizal plants at 60 and 90 DAS stages.
REVIEW OF LITERATURE

Accumulation of plant metabolites have been reported by the earlier workers. Water and salt stress induces the accumulation of these metabolites.

Barnet and Naylar (1966), reported that accumulation of proline was occurred when the plants are subjected to water stress. Stewart and Lee (1974), reported that many plants accumulate proline as non toxic and protective osmolyte to maintain osmotic balance under water stress conditions. Bevege et al., (1975), reported that AM fungi may act as metabolic sink causing basipetal mobilization of photosynthetase to roots, thus providing a stimulus for greater photosynthetic activity. Boggess et al., (1976), reported that accumulation of proline when the plants are subjected to water stress. Wright et al., (1998), reported the accumulation of more carbohydrates in mycorrhizal roots than non-mycorrhizal ones.

Jacobson and others (1990), reported that phosphorous is very much necessary in the synthesis of starch and the glucose. AM fungi increase the synthesis of carbon compounds by the P-uptake. Charest et al.,(1990), reported that soluble proteins were increased with AM fungal inoculation in maize. Carbcellar et al., (1991), reported that under water stress the free amino acids such as proline and glycinebetaine are strongly influenced and consequently quickly accumulated. Jindal et al., (1993), reported mycorrhizal mung bean. (Vigna radiata) plants were reported to have higher proline content than non-mycorrhizal plants under stress conditions. Amijee et al., (1993), reported that AM fungal colonization stimulated the rate of photosynthesis to
compensate carbon requirement of the fungus. Kameti and Losel (1993),
reported that glucose accumulation in wheat under drought taken place was
more rapid in mycorrhizal plants than non-mycorrhizal ones. Arines et al.,
(1993), reported a two to six fold increase in soluble protein content in
mycorrhizal clover roots. Kizhaerel et al., (1995), explained that under drought
conditions, AM fungal colonization of maize assists in the accumulation of
sugars and nitrogenous compounds, which contribute to drought tolerance in
the host plant. Ruiz-Lozano et al., (1995), reported that the total protein
concentrations were higher in mycorrhizal than in non-mycorrhizal plants
during drought.

Zhu (2002), reported the accumulation of organic solutes such as
carbohydrates and proline under stress conditions. Asha and Rao, (2002),
working with Arachis hypogea L. under water deficit found the same results.
Ghorbani et al., (2004), have shown higher peroxidase activities in mycorrhizal
soybean plants than in non mycorrhizal plants. Mahajan and Tuteja (2005),
explained that plants tend to adjust with water stress through osmotic
adjustment, this includes the synthesis and accumulation of non toxic solutes
such as proline. Turkan et al., (2005), showed that proline content in the leaves
under drought conditions were higher in a tolerant bean cultivar than in a
sensitive cultivar. Drought stress induced a similar level of proline
accumulation in both chickpea cultivars, throughout the experiment. Turkan et
al., (2005), showed that proline content in the leaves under drought conditions
were higher in a tolerant bean cultivar than in a sensitive cultivar. Drought
stress induced a similar level of proline accumulation in both chickpea cultivars, throughout the experiment. Ashraf and Foolad, (2007), explained that proline acts as free radical scavenger, a sub cellular structure stabilizer, a redox potential buffer. Sharifi et al., (2007), also reported higher proline concentration in AM Soya bean than non AM plants under stress conditions. Wu et al., (2008), explained that AM fungi increase the synthesis of enzymatic antioxidants to protect host plants against oxidative damage. Aboghalia (2008), suggested that the mycorrhizal plants accumulated more proline than those of non-mycorrhizal ones when they were subjected to water stress. Kafefetoghu Macar and Ekmekci (2009), reported accumulation of proline content is chickplants subjected to drought stress. Praba et al., (2009), demonstrated that drought stress induces numerous metabolic, biochemical and physiological changes in plants. These include water status, growth, membrane integrity, pigment content, osmotic adjustment and photosynthetic activity
MATERIALS AND METHODS

Grains of four *Triticum aestivum* L varieties commonly cultivated in Karnataka state of India DWR-162, DWR-195, DWR-225 and NI 5439 were procured from Wheat Research station, University of Agricultural Science, Dharwad, India. Grains were surface sterilized by placing them in 2% sodium hypo chloride and were washed thoroughly with distill water to remove the traces of sodium hypo chloride.

The AM fungus *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe emend.Walker and Koske was isolated from the rhizospheric soil of *Triticum aestivum* L. by wet sieving and decanting technique (Gerdemann and Nicolson. 1963). Mass multiplication of *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe emend.Walker and Koske was done by using *Sorghum vulgare* L. as host plant. Soil used for the experiments was sterilized by autoclave method (121°C, 15 PSI, 15 min). Three kg sterilized soil was taken in each poly bag. 10 g AMF inoculum containing colonized root bits was placed 2cm below the soil. Non-mycorrhizal treatments were maintained without the addition of AM inoculum to the soil taken in polybags. In each poly bag 4 grains were sown. Plants were grown in open field conditions under natural photoperiods with following treatments.

**Treatment 1:** Plants grown in polybags without mycorrhizal inoculation (AM) watered on every alternate day till harvest.

**Treatment 2:** Plants grown in polybags without mycorrhizal (AM) inoculation watered on alternate day. It was just before 60th and 90th day plants were
subjected to water stress by withholding water for 10 days. After each stress period the plants were rewatered till harvest.

**Treatment 3:** Plants grown in polybags with mycorrhizal (AM) inoculation. Plants were watered regularly on alternate days till harvest.

**Treatment 4:** Plants grown in polybags with mycorrhizal (AM) inoculation. Plants were watered regularly on alternate days. Just before 60th and 90th day they were subjected to water stress by withholding water for 10 days. After each stress period the plants were rewatered till harvest.

Hoagland’s solution minus P was applied once in 15 days to all the plants grown in 4 treatments. Plants were maintained in triplicates and arranged in complete randomized design.

**Harvest:** Plants were harvested after 60 and 90 DAS (days after sowing). Plant materials are used for the determination of total carbohydrates, protein content, proline and peroxidases. Plant materials were dried in an oven at 80°C for 48 hrs and were then ground to a fine powder. Proline and peroxidase content were determined from fresh materials after ground in liquid nitrogen.

**Estimation of proline:**

0.5g of plant tissue from the experimental plants was extracted by homogenizing in 10mL of 3% aqueous Sulphosalicylic acid. The homogenate was filtered through Whatman's filter paper No. 2, and 2 mL of acid Ninhydrin was added. Samples were placed in boiling water bath for 1 hour. Reaction was terminated by placing samples in ice bath. 4 mL of Toluene was
added to the reaction mixture and stirred well for 20-30 minutes. Warm the sample to room temperature to separate Toluene layer. Red colour intensity was measured at 520 nm. Standard curve was prepared with series of pure proline taken at different concentrations. Amount of proline present in plant sample was measured by using standard curve.

Amount of proline in the samples was measured by using following equation.

\[
\text{Moles of proline /g fresh tissue} = \frac{\text{g proline / mL} \times \text{mL of Toluene}}{115.5} \times \frac{5}{\text{g sample}}
\]

Where 115.5 is the molecular weight of proline

**Estimation of Peroxidase:**

Guaiacol was used as substrate for the estimation of Peroxidase.

**Preparation of Enzyme extract:**

1 g fresh plant tissue was taken in 3mL of 0.1M phosphate buffer (pH=7) by grinding with pre cooled mortar and pestle. The obtained homogenate was centrifuged at 18,000 g for 15 minutes at 5°C. Supernatant was used as enzyme source.

**Preparation of Guaiacol solution**

240 mg Guaiacol was dissolved in 100 mL of distilled water.
Preparation of Hydrogen peroxide solution (0.042%)

0.14 mL of 30% H₂O₂ was diluted in to 100 mL of distilled water.

Procedure:

3 mL of phosphate buffer (0.1M), 0.5 mL guaiacol solution; 0.1 mL enzyme extract and 0.03 mL hydrogen peroxide solution were pipetted in cuvette. The solutions were mixed thoroughly, and then cuvette was placed in spectrophotometer. Wait until the absorbance has increased by 0.05. Start the stopwatch to note down there time required in minutes (t) to increase the absorbance by 0.1.

Peroxidase activity was calculated as follows:

\[
\text{Enzyme activity (units/liter mg / g fresh wt)} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times t \times 0.1} \times \frac{500}{t}
\]

Estimation of total Carbohydrates:

The phenol sulphuric acid method was used to estimate total carbohydrate. Homogenize 500 mg of sample in hot 80% ethanol to remove sugars residue obtained through centrifugation. Residue was repeatedly washed with 80% hot ethanol till the washings did not give colour with anthrone reagent. Residue was dried over water bath. 5 mL of water and 6.5mL of 52% perchloric acid was added to the residue. Centrifugation was done and supernatant saved. Extraction using fresh perchloric acid was repeated. Centrifuge and supernatants were pooled; volume of the supernatants was making up to 10mL. Pipette out 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard in to a series of test tubes. Pipette out 0.1 and 0.2mL of the sample
solution in two separate test tubes. Made up the volume in each tube to 1mL with water (Blank is set by using 1 mL water). 1mL of phenol solution to each tube. 5 mL of 96% Sulphuric acid was added to each tube and shaken thoroughly. The contents in the tubes placed in water bath at 25°C-30°C. Read the colour at 490 nm, the amount of total carbohydrates present in the given sample solution was determined by using the standard graph. Pure glucose was used as standard.

**Calculation:**

Absorbance corresponds to 0.1 mL of the test = X mg of glucose

100mL of the sample solution contains

\[
\frac{X}{0.1} \times 100 \text{ mg of glucose} = mg/\text{g dry wt of sample.}
\]

**Estimation of crude Protein by Microkjeldhal method**

The crude protein was determined by using the microkjeldhal method which is described as follows.

100 mg sample was taken in 30 mL digestion flask. 1.9 mg potassium sulphate 80 mg mercuric oxide and 2 mL conc. H₂SO₄ was added to digestion flask. Digest the sample till the solution become colorless. Cool the digest, dilute it with distilled water and transfer to the distillation apparatus. The Kjeldhal flask was rinsed with successive small quantities of water. Place a 100 mL conical flask containing 5mL of boric acid solution with few drops of mixed indicator with the tip of the condenser dipping below the surface of
solution. 10mL of sodium hydroxide and sodium thiosulphate solution to the test solution in the apparatus was added. Ammonia was collected on boric acid. Tip of the condenser was rinsed and solution was titrated against standard acid until the first appearance of violet colour. Run a reagent blank with an equal volume of distilled water and subtract the titration volume from that of titre volume.

**Calculation:**

\[
N \text{ (mglg dry wt)} = \frac{(\text{mL HCl} - \text{mL blank}) \times \text{Normality} \times 14.01}{\text{Weight (g)}}
\]

Multiplying total nitrogen value with 6.25 will give the crude protein content.
**RESULTS**

**Proline**

Accumulation of proline was increased considerably in four *Triticum aestivum* L. varieties due to water stress and mycorrhizal inoculation (Figure 6.1). It was observed that mycorrhizal plants accumulated more proline than non mycorrhizal plants both under well watered as well as water stress conditions. More proline accumulation was observed under water stress conditions. It was also observed that plants accumulated more proline at 60 DAS stage than 90 DAS stage. Mycorrhizal plants grown under water stress conditions belong to DWR-225 have accumulated 240% more proline than mycorrhizal plants grown under well watered conditions. DWR-162 plants exhibited better response to water stress and resulted in more proline accumulation than the other two varieties DWR-195 and NI-5439 (Table 6.1 and 6.2).

**Total carbohydrate**

The total carbohydrate concentrations were observed to be more in mycorrhizal plants compared to nonmycorrhizal plants under well watered and water stress conditions (Figure 6.2). Plants of all the varieties have shown more accumulation of carbohydrates in well watered plants than the plants subjected to water stress. Results indicated water stress affects the carbohydrate accumulation and decreases the accumulation of total carbohydrates both in mycorrhizal and non-mycorrhizal plants. Relatively mycorrhizal plants showed more carbohydrates accumulation than non mycorrhizal plants (Table 6.1).
Table 6.1: Effect of *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe emend.Walker and Koske.on plant metabolites accumulation in *Triticum aestivum* L. varieties under well watered conditions.

<table>
<thead>
<tr>
<th>Name of metabolite</th>
<th>DWR-162</th>
<th>DWR-195</th>
<th>DWR-225</th>
<th>NI5439</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline (μmol/g fresh tissue)</td>
<td>1.83</td>
<td>2.37</td>
<td>1.77</td>
<td>2.15</td>
</tr>
<tr>
<td>Days</td>
<td>±0.52</td>
<td>±0.40</td>
<td>±0.074</td>
<td>±0.093</td>
</tr>
<tr>
<td>Total (mg/g dry wt)</td>
<td>283.5</td>
<td>291.00</td>
<td>236.90</td>
<td>272.66</td>
</tr>
<tr>
<td>Days</td>
<td>±6.5</td>
<td>±5.27</td>
<td>±7.00</td>
<td>±7.30</td>
</tr>
<tr>
<td>Carbohydrates (mg/g dry wt)</td>
<td>250.73</td>
<td>264.00</td>
<td>214.24</td>
<td>223.0</td>
</tr>
<tr>
<td>Days</td>
<td>±3.66</td>
<td>±4.05</td>
<td>±6.40</td>
<td>±10.31</td>
</tr>
<tr>
<td>Proteins (mg/g dry wt)</td>
<td>21.20</td>
<td>24.28</td>
<td>20.95</td>
<td>22.56</td>
</tr>
<tr>
<td>Days</td>
<td>±0.55</td>
<td>±0.39</td>
<td>±0.85</td>
<td>±1.21</td>
</tr>
<tr>
<td>Peroxidase (mg/g fresh wt)</td>
<td>1.52</td>
<td>1.74</td>
<td>1.250</td>
<td>1.40</td>
</tr>
<tr>
<td>Days</td>
<td>±0.15</td>
<td>±0.092</td>
<td>±0.186</td>
<td>±0.091</td>
</tr>
</tbody>
</table>
| CN=Control; IN=Inoculated. All the values are means of three replicates ± standard error.
Table 6.2: Effect of *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe 
emend.Walker and Koske.on plant metabolites accumulation in 
*Triticum aestivum* L. varieties under water stress conditions

<table>
<thead>
<tr>
<th>Name of metabolite</th>
<th>DWR-162</th>
<th>DWR-195</th>
<th>DWR-225</th>
<th>NI5439</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>μmol/g fresh tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td>±0.294</td>
<td>±0.355</td>
<td>±0.132</td>
<td>±0.19</td>
</tr>
<tr>
<td>Days</td>
<td>±0.269</td>
<td>±0.426</td>
<td>±0.266</td>
<td>±0.313</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>90</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>mg/g dry wt</strong></td>
<td>±9.8</td>
<td>±8.63</td>
<td>±2.36</td>
<td>±10.98</td>
</tr>
<tr>
<td>Days</td>
<td>±3.27</td>
<td>±10.26</td>
<td>±5.78</td>
<td>±2.07</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>mg/g dry wt</strong></td>
<td>±0.91</td>
<td>±1.22</td>
<td>±1.15</td>
<td>±1.58</td>
</tr>
<tr>
<td>Days</td>
<td>±0.88</td>
<td>±0.257</td>
<td>±0.60</td>
<td>±2.31</td>
</tr>
<tr>
<td><strong>Peroxidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>mg/g fresh wt</strong></td>
<td>±0.16</td>
<td>±0.092</td>
<td>±0.098</td>
<td>±0.077</td>
</tr>
<tr>
<td>Days</td>
<td>±0.16</td>
<td>±0.089</td>
<td>±0.24</td>
<td>±0.084</td>
</tr>
</tbody>
</table>
| CN=Control; IN=Inoculated. All the values are means of three replicates ± standard error.

Mycorrhizal and non-mycorrhizal plants have shown maximum total carbohydrates at 60 DAS stage under well watered as well as water stress conditions. The carbohydrate concentration in 90 DAS old plants was found to be lesser than those at 60 DAS old plants. Plants belong to DWR-225 have shown maximum accumulation of carbohydrates compared to other three
varieties. It was observed that more accumulation of carbohydrates could be observed in mycorrhizal plants compared to non-mycorrhizal plants under water stress conditions (Table 6.2). The variety DWR-162 showed higher accumulations of carbohydrates, but comparatively lesser than DWR-225. The variety NI-5439 showed lesser quantity of total carbohydrates than other three varieties.

**Crude protein content**

The crude protein content was found to be more in mycorrhizal plants than non-mycorrhizal plants both under well watered and water stress conditions at 60 DAS and 90 DAS stage (Figure 6.3). The crude protein content was high in 90 DAS stage than 60 DAS stage. Mycorrhizal inoculation has increased the crude protein content, but the increase is comparatively higher in DWR-225 and DWR-162 under water stress conditions (Table 6.2). Accumulation of crude protein was more in plants grown under water stress conditions. The plants belong to DWR-195 and NI-5439 accumulated comparatively lesser amount of crude protein than other two varieties.

**Peroxidase content**

In the present investigations peroxidase activity was examined at 60 and 90 DAS in mycorrhizal and nonmycorrhizal plants grown under water stress and well watered conditions (Figure 6.4). The results showed that the concentration of peroxidase enzyme was more in mycorrhizal plants than non mycorrhizal plants under well watered as well as water stress conditions. All the varieties accumulated more peroxidase at 60 DAS in comparison to those at
90 DAS stage. Peroxidase content was more in mycorrhizal plants subjected to water stress.

Plants belonging to DWR-225 and DWR-162 have shown more peroxidase content than DWR-195 and NI-5439. Mycorrhizal plants belonging to DWR-225 have shown more peroxidase than non mycorrhizal plants under water stress conditions (Table 6.2). It was observed that mycorrhizal plants belonging to DWR-162 have shown moderate peroxidase content grown under water stress, DWR-195 and NI-5439 have shown lesser accumulation in peroxidase under water stress conditions. Plants belonging to these four varieties were known to accumulate more metabolites at 60 DAS stage than 90 DAS except for protein content.
Fig. 6.1: Proline content in *Triticum aestivum* L. varieties at 60 and 90 DAS under well watered and water stress conditions with and without AM fungus (*Glomus fasciculatum*) inoculation.

Fig. 6.2: Total carbohydrates content in *Triticum aestivum* L. varieties at 60 and 90 DAS under well watered and water stress conditions with and without AM fungus (*Glomus fasciculatum*) inoculation.
Fig. 6.3: Protein content in *Triticum aestivum* L. varieties at 60 and 90 DAS under well watered and water stress conditions with and without AM fungus (*Glomus fasciculatum*) inoculation.

![Protein content graph](image)

WW-Well watered; WS-Water stress; CN=Control; IN=Inoculated

Fig. 6.4: Peroxidase content in *Triticum aestivum* L. varieties at 60 and 90 DAS under well watered and water stress conditions with and without AM fungus (*Glomus fasciculatum*) inoculation.

![Peroxidase content graph](image)

WW-Well watered; WS-Water stress; CN=Control; IN=Inoculated
DISCUSSION

In all varieties of *Triticum aestivum* L., more proline accumulation was observed in mycorrhizal plants subjected to water stress. It has been shown that mycorrhizal colonization and drought interact in modifying free amino acid and sugar pools in *Rosa* roots (Auge *et al.*, 1992). Proline is a non protein amino acid formed in tissues under water stress conditions (Barnett and Naylor, 1966; Wu *et al.*, 2008). The proline together with sugar is readily metabolized upon recovery from the drought in white clover leaves. Proline serves as a sink for energy to regulate redox potentials, such as hydroxy radical scavenger, as a solute that protects macromolecules against denaturation (Shao *et al.*, 2005).

In plants, proline is an important organic compound that participates in the osmotic adjustment. It has been shown that plants produce more proline when they are subjected to more stress. Greater accumulation of amino acids might also indicate plants more capably osmotically adjusted to water stress (Auge 2001); Mathur and Vyas, (2000), have shown that mycorrhizal colonization and drought interact in modifying free amino acids and sugar pools and proline contents in *Ziziphus mauritiana* Lam. plants under water stress conditions. In present investigation similar results were observed confirming the accumulation of proline due to water stress and mycorrhizal inoculation.

Accumulation of amino acid proline is one of the most frequently reported modifications induced by water stress in plants. As soil dries out the soil water potential becomes more negative and plants must decrease their water potentials to maintain water flow from soil in to the roots. To achieve such an
Effect, plants develop osmotic adjustment by active accumulation of organic ions or solutes as suggested by Morghan, (1984); Hoekstra et al., (2001). The increase showed in free amino acids is due high protein hydrolases. Sircelj et al., (2005), explained that under water stress the free amino acid proline is accumulated in apple trees to retain water through osmotic adjustment. Vendruscolo et al., (2007), reported that proline might confer drought stress tolerance to wheat plants by increasing anti oxidant system rather as an osmotic adjustment. It has also been shown that mycorrhizal colonization and drought stress interact in modifying free amino acids and sugar pools in roots (Auge 2001). In dry or saline environments osmotic pressure increases to protect cellular constituents. Their protective effects also extend to temperature extremes and other stresses.

AM fungal colonization stimulated the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and to eliminate growth reduction of the host plants. Morghan (1984), explained that in Cucumber, mycorrhizal plants have higher amount of total sugars than nonmycorrhizal plants. Al-Karaki and Al-Raddad, (1997), reported that mycorrhizal colonization is often thought to increase water stress tolerance in wheat genotypes mainly by enhancing nutrient acquisition. Porcel. et al., (2004), reported that the greater soluble sugar and carbohydrate concentration in AM inoculated soybean plants may be due to the sink effect of AM fungus demanding sugar from the leaves. Net accumulation of carbohydrates and sugars suggested that AM colonization enhanced host plant drought tolerance.
Increased peroxidase activity could be observed in experimental plants subjected to drought stress. Blilou et al., (2000), observed that transient increase in peroxidase activity in tobacco plants inoculated with AM fungi. Whereas Bartels (2001), suggested that prevention of oxidative stress and elimination of reactive oxygen species are the most effective approaches used by plant to gain toleration against abiotic stresses including drought.

Wu et al., (2006), noticed that the crude protein content has been consistently higher in AM than non AM plants during drought. Arines et al., (1993), reported that the AM association increases the protein content in red clover roots under water stress. Charest et al., (1993), reported that in Maize enhanced soluble proteins appear to be an indicator of stress tolerance. The accumulation of metabolic substance may suggest that AM colonization could improve osmotic adjustment originating not only from proline but also from carbohydrates and proteins resulting in the enhancement of water stress tolerance. Results obtained in the present study were strongly supported by earlier workers. However, in the present study more proline accumulation was observed in mycorrhizal plants subjected to water stress. This probably indicates the resistance to water stress through osmotic adjustment.
CONCLUSION

The present study showed that AM fungi led to enhancement of drought tolerance in experimental plants subjected to short term water stress at critical stages of plant growth followed by recovery. Increased accumulation of metabolites in presence of AM fungus *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe emend. Walker and Koske. was observed during water stress. This helps to improve osmotic adjustment of plants. Among four *Triticum aestivum* L. varieties, DWR-225 and DWR-162 have shown more accumulation of metabolites to tolerate water stress by improving osmotic adjustment. The results conclude that plants belonging to these two varieties are more drought tolerant.