GROWTH, NODULATION AND N₂ FIXATION IN DALBERGIA SISSOO INOCULATED BY RHIZOBIUM (COWPEA MISCELLANY) AND GLOMUS FASCICULATUM UNDER DIFFERENT MOISTURE LEVELS

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The effect of various levels of moisture on growth, nodulation and N₂ fixation of single (Rhizobium or AM alone) and dual inoculated (Rhizobium + AM) Dalbergia sissoo, has been studied in a pot culture experiment using sterilized soil. The parameters, growth, nodulation, nitrogen fixation and % AM colonization of roots were considerably influenced with the increase in moisture stress from -0.3 MPa to -1.8 MPa. At -0.3 MPa level, the growth of plants, total N content, percentage of AM colonization, nodulation and nitrogenase activity were found to be maximum in plants raised after dual inoculation with Rhizobium and Glomus fasciculatum. In other watering regimes, though the values of all the above parameters decreased with increase in moisture stress, the plants with dual inoculation performed better than single inoculation. These observations suggested a protective role played by AM in providing resistance to Dalbergia sissoo Roxb. against injurious effects of moisture stress.

Keywords: Dalbergia sissoo; Glomus fasciculatum; Moisture stress; Nodulation; Rhizobium.

Introduction
Mycorrhizal fungi occur in nearly all soils throughout the world and form symbiotic relationship with the roots of most terrestrial plants. They exist in the soil as spores or as vegetative propagates in root fragments unlike other soil borne and most root-inhabiting fungi. Vesicular Arbuscular Mycorrhizal fungi tolerate a wide range of soil water regimes and are found in habitats as diverse as arid deserts and aquatic environments. Moisture stress can have profound metabolic influence on plant, resulting not only in impaired gas exchange but also in considerable alteration of physiological processes. VAM association in plants contributes towards the development of resistance to water stress. Available reports indicate that plants infected with mycorrhizal fungi grow better even under stress conditions. The requirement of P is high in legumes and therefore leguminous plants respond more to mycorrhizal infection than cereals, which indirectly enhances the biological nitrogen fixation through increased P availability specially in soils with low P content. In this paper, the effect of inoculation of Rhizobium alone and in combination with Glomus fasciculatum on growth and nitrogen fixation in Dalbergia sissoo, a tree legume, under different moisture stress levels has been discussed.

Materials and Methods
Role of most preferred AM Glomus fasciculatum on resistance and recovery from moisture stress of test plants inoculated with most efficient Rhizobium was studied by conducting a pot culture experiment using sterilized soil for six months. The surface sterilized seeds pelleted with rhizobial isolates were sown in pots on AM inoculum pad containing 250 spores/50 g soil. Three seedlings were maintained in each of the five replicates for each treatment. Each of these treatments consisted of uninoculated control, Rhizobium alone, AM alone and Rhizobium + AM inoculated sets. These were separately maintained at four different soil moisture levels. Soil moisture content was determined in terms of MPa (matric potential) level (1MPa = 10 bars) by using thermocouple psychrometer. The four watering regimes were (i) watering once to the field capacity in 24h (-0.3 MPa), (ii) 48h (-0.6 MPa), (iii) 72h (-1.4 MPa) and (iv) 96h (-1.8 MPa). The plants were subjected to above moisture regimes after two month of their establishment. The above mentioned matric potential were approximately equal to soil moisture levels.

Following parameters were selected for the study: shoot-root length, shoot and root dry weight, total plant protein, total chlorophyll, total nitrogen and phosphorus content, nodule number, nodule dry weight and maximum nodule size, nitrogenase activity of root nodules and percentage AM colonization by roots.

Results and Discussion
The effect of Arbuscular mycorrhizal inoculation on plant growth (shoot and root length), plant dry matter production (shoot and root dry weight) and nutrient uptake level under different levels of moisture stress condition are given in Table-1. The highest value of growth in terms of shoot length, shoot dry weight, root length and root dry weight was observed in Rhizobium isolate DS Rhz-9 + Glomus fasciculatum inoculated plants at the first level of moisture.
<table>
<thead>
<tr>
<th>Interval of watering</th>
<th>MPa levels</th>
<th>Treatments</th>
<th>Shoot length (cm)</th>
<th>Shoot dry weight (g)</th>
<th>Root length (cm)</th>
<th>Root dry weight (g)</th>
<th>Total plant protein (mg/g)</th>
<th>Total plant chlorophyll (mg/L)</th>
<th>N-content (%) (dry weight)</th>
<th>P-content (%) (dry weight)</th>
<th>AM root colonization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once in 24 h</td>
<td>(-0.3 MPa)</td>
<td><strong>Glomus fasciculatum</strong></td>
<td>12.83±0.93</td>
<td>0.61±0.06</td>
<td>20.87±2.50</td>
<td>0.68±0.04</td>
<td>87.50±1.13</td>
<td>0.83±0.08</td>
<td>0.99±0.04</td>
<td>0.11±0.04</td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>28.50±2.24</td>
<td>1.23±0.04</td>
<td>32.45±4.73</td>
<td>0.93±0.05</td>
<td>117.33±1.35</td>
<td>1.16±0.10</td>
<td>1.05±0.07</td>
<td>0.1±0.05</td>
<td>28.30±3.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>DS Rhz-9</strong></td>
<td>30.81±2.87</td>
<td>1.56±0.08</td>
<td>36.08±4.55</td>
<td>1.73±0.08</td>
<td>119.33±1.57</td>
<td>1.39±0.12</td>
<td>1.02±0.07</td>
<td>0.12±0.04</td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>DS Rhz-9+Glomus fasciculatum</strong></td>
<td>34.75±2.51</td>
<td>1.67±0.09</td>
<td>41.13±4.75</td>
<td>1.89±0.09</td>
<td>196.67±1.85</td>
<td>1.96±0.16</td>
<td>1.25±0.08</td>
<td>0.20±0.06</td>
<td>32.97±5.73</td>
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<tr>
<td>Once in 48 h</td>
<td>(-0.6 MPa)</td>
<td><strong>Uninoculated</strong></td>
<td>9.38±0.86</td>
<td>0.56±0.04</td>
<td>18.23±2.34</td>
<td>0.61±0.05</td>
<td>62.67±8.22</td>
<td>0.78±0.08</td>
<td>0.91±0.04</td>
<td>0.09±0.02</td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>22.50±2.50</td>
<td>0.99±0.05</td>
<td>31.51±2.57</td>
<td>0.86±0.04</td>
<td>103.15±1.14</td>
<td>1.02±0.10</td>
<td>1.01±0.06</td>
<td>0.14±0.04</td>
<td>32.63±3.79</td>
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<tr>
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<td><strong>Glomus fasciculatum</strong></td>
<td>24.67±2.55</td>
<td>1.17±0.05</td>
<td>34.47±4.32</td>
<td>1.63±0.03</td>
<td>103.33±1.48</td>
<td>1.18±0.11</td>
<td>1.01±0.07</td>
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<td><strong>DS Rhz-9</strong></td>
<td>28.55±2.45</td>
<td>1.24±0.08</td>
<td>36.15±4.55</td>
<td>1.78±0.06</td>
<td>189.56±1.67</td>
<td>1.69±0.14</td>
<td>1.16±0.08</td>
<td>0.21±0.06</td>
<td>34.57±6.13</td>
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<tr>
<td>Once in 72 h</td>
<td>(-1.4 MPa)</td>
<td><strong>Uninoculated</strong></td>
<td>7.89±1.73</td>
<td>0.45±0.02</td>
<td>14.79±2.46</td>
<td>0.51±0.03</td>
<td>51.34±1.63</td>
<td>0.73±0.09</td>
<td>0.81±0.03</td>
<td>0.08±0.01</td>
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<tr>
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<td>Control</td>
<td>14.25±2.32</td>
<td>0.68±0.03</td>
<td>29.15±3.16</td>
<td>0.78±0.04</td>
<td>96.17±0.85</td>
<td>0.89±0.09</td>
<td>0.97±0.05</td>
<td>0.15±0.05</td>
<td>32.35±4.22</td>
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<tr>
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<td><strong>Glomus fasciculatum</strong></td>
<td>17.76±2.46</td>
<td>1.08±0.03</td>
<td>32.78±4.25</td>
<td>1.34±0.06</td>
<td>100.56±0.97</td>
<td>0.98±0.10</td>
<td>0.98±0.06</td>
<td>0.09±0.02</td>
<td>Zero</td>
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<tr>
<td></td>
<td></td>
<td><strong>DS Rhz-9</strong></td>
<td>18.95±2.53</td>
<td>1.18±0.03</td>
<td>34.34±4.58</td>
<td>1.46±0.08</td>
<td>181.78±1.25</td>
<td>1.46±0.14</td>
<td>1.11±0.07</td>
<td>0.23±0.06</td>
<td>37.45±6.08</td>
</tr>
<tr>
<td>Once in 96 h</td>
<td>(-1.8 MPa)</td>
<td><strong>Uninoculated</strong></td>
<td>6.63±0.64</td>
<td>0.28±0.02</td>
<td>13.85±2.53</td>
<td>0.41±0.04</td>
<td>48.12±0.58</td>
<td>0.71±0.08</td>
<td>0.67±0.03</td>
<td>0.06±0.00</td>
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<tr>
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<td>Control</td>
<td>12.74±1.87</td>
<td>0.51±0.01</td>
<td>27.53±3.51</td>
<td>0.61±0.04</td>
<td>82.89±0.75</td>
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<td>0.15±0.06</td>
<td>38.15±4.54</td>
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<td></td>
<td><strong>Glomus fasciculatum</strong></td>
<td>14.77±1.53</td>
<td>0.73±0.03</td>
<td>31.36±4.13</td>
<td>1.08±0.05</td>
<td>86.17±0.78</td>
<td>0.91±0.09</td>
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<td><strong>DS Rhz-9</strong></td>
<td>18.35±2.35</td>
<td>0.78±0.02</td>
<td>34.10±4.52</td>
<td>1.22±0.05</td>
<td>131.27±1.01</td>
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<td>0.96±0.06</td>
<td>0.24±0.07</td>
<td>43.68±5.93</td>
</tr>
</tbody>
</table>

Table 1. Effect of moisture stress on growth and AM root colonization of *Dalbergia sissoo* Roxb. inoculated with *Rhizobium* (DS Rhz-9) and AM (*Glomus fasciculatum*). Values are Mean ± Standard Deviation of 15 replicates.
(-0.3 MPa) in comparison to single inoculated (DS Rhz-9 alone or *Glomus fasciculatum* alone) plants. The un inoculated plants recorded the lowest growth. In other watering regimes, though, the values decreased with increase in moisture stress, the plants with dual inoculation performed better than single inoculation (Table 1).

The dual inoculated plants recorded maximum values of total protein, total chlorophyll and total N at the first level of moisture. The values decreased at the subsequent levels. Total P content per plant increased only in dual and AM alone inoculated plants with the increase in moisture stress. Therefore maximum total P content was observed in dual inoculated plants at fourth level of moisture (-1.8 MPa) (Table 1).

The nodulation (nodule number, nodule dry weight, maximum nodule size) and nitrogen fixation in terms of nitrogenase activity by nodules, were recorded maximum in plants inoculated with dual combination of *Rhizobium* (DS Rhz-9) + *Glomus fasciculatum* (Fig. 1). The maximum nitrogenase activity of nodulated roots was recorded 1.64 \( \mu \text{mol C}_2\text{H}_4\text{ g}^{-1}\text{ fresh nodule h}^{-1}\) in dual inoculated plants in comparison to the plant inoculated with *Rhizobium* (DS Rhz-9) alone, at the first level of moisture. The nodulation and nitrogen fixation were very poor and decrease drastically at the subsequent levels of moisture stress (Fig. 1).

The colonization percentage (Table 1) increased with higher levels of moisture stress. Maximum value of percentage AM root colonization was recorded at fourth level of moisture (-1.8 MPa) in dual inoculated plants as compared to only AM inoculated plants.

The result from the experiment clearly indicated that *Rhizobium* isolate DS Rhz-9 and *Glomus fasciculatum* formed an effective dual combination resulting in highest values of all the parameters studied. The percentage AM root colonization enhanced further when AM was associated with *Rhizobium*. These results were also supported by other workers\(^{14-17}\). In green house studies mycorrhizae have been shown to increase the drought resistance of cultivated crops such as wheat\(^{18,19}\), soybean\(^{20,21}\), onion\(^{22}\), pepper\(^{23}\) and red clover\(^{24}\) as well as several native plant species\(^{25-30}\). The observations presented in this paper are in agreement with the above findings.

Under moisture stress condition it was found that the growth of plants decreased according to the increase in moisture stress. Even though the plants had low growth compared to the non-stressed mycorrhizal plants, the treated plants showed more growth than their non-mycorrhizal counter parts\(^{31}\). The data presented suggested that inoculation of efficient AM fungi (*Glomus fasciculatum*) during the course of the study, prevented the injurious effects of moisture stress in the test plants.
due to enhanced water and nutrient uptake thereby promoting growth, nodulation and nitrogen fixation of the *Dahurica sissoo* under investigation.

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**References**


