RESEARCH PAPER

The symbiotic response of three millet varieties to Arbuscular mycorrhizal fungal (*Glomus* spp.) inoculation in marginal soil: implication in bio-fertilizer

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**Abstract**

The effect of three *Glomus* species on growth parameters in terms of shoot length, root length, dry weight proximate composition and quantitative evaluation of mycorrhizal dependency of three millet (*Pennisetum glaucum* L.) varieties i.e. Bajra super 1, PARC-MS 2, PARC-MS 3 were evaluated. Thirty earthen pots were filled with 8kg of soil. Each millet variety was represented by ten replicates i.e. five control and five test pots. The test pots were inoculated with soil containing spores of *Glomus* spp. The *Arbuscular mycorrhizal* (AM) inoculated plants showed significantly better performance than the non-inoculated plants in terms of plant height, number and length of leaves, root length, number of seminal roots and dry weight. Proximate analysis showed enhancement in crude protein, fat, moisture and ash content in mycorrhizal plants, except carbohydrate and crude fibers. Regarding mycorrhizal dependency (M.D), maximum value was noted in PV2 (71.67%) variety while least was observed in PV1 (55.91%). This study clearly indicates the potential of using indigenous AM fungi (*Glomus* species) as bio fertilizer in millet crop in low fertility soils. The use of AM fungi as bio fertilizer will not only reduce demand for chemical fertilizer, but will diminish chemical pollution.

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Introduction

Mycorrhizas are universal mutualistic associations between soil fungi and vascular plants and are essential in improving plant fitness and soil quality. It improves the resilience of plant communities against environmental, nutritional and drought stresses (Barea et al., 2011).

Mycorrhizae mean fungus roots. In this mutually beneficial partnerships, root of the host plant provide a convenient substrate for the fungus and also supply food in the form of simple carbohydrates. In exchange for this free room and board, the mycorrhizal fungus provides several benefits to the host plant (Wilkinson, 2008). Arbuscular mycorrhiza (AM) is a root endosymbiosis between plants and glomeromycete fungi. It is the most ubiquitous terrestrial plant symbiosis, improving plant growth, plant protection, soil quality, plant uptake of water and mineral nutrients (Mahmood and Rizvi, 2010). AM fungi are known to be of great importance due to their great capability to increase growth, yield and crop quality through efficient nutrient acquisition in infertile soils & therefore lessen the prerequisite for phosphate-based fertilizers (Sawers et al., 2008; Roy-Bolduc & Hijri, 2010). In turn, the fungi get carbon from the host plant. AM fungi are able to absorb and transfer all of the 15 major, macro and micro nutrients essential for plant growth (Lester, 2009).

Millet (Pennisetum glaucum L.) locally known as “Bajra” is also a coarse grain cereal crop which ranks fifth in Pakistan. It is grown on 34.6 million hectar area in the world with the production of 28.8 million tons per annum. In Pakistan, it is grown on 0.3 million hectare area with total production of 0.20 million tons and per unit yield of 563 kg ha⁻¹ which is very low than the world average (831 kg ha⁻¹) (FAO, 2005).

Soils of Pakistan like most of the arid and semiarid soils of world are mostly Phosphorus (P) deficient due to their alkaline and calcareous nature, affecting the maize crop adversely (Memon et al., 1992; NFDC, 2001).

Throughout the world, scientists are now focused on developing alternative technology to minimize the dependence on chemical fertilizers. Although remarkable research work has been done on various aspects of AM, but the issues of Asian countries including Pakistan such as nutrient deficiency and host growth responses of various crops are least addressed.

Keeping the importance of AM fungi as bio-fertilizers present investigations was carried to find out the feasibility of inoculation of some high yielding tropical millet varieties with indigenous tropical AM fungi.

Material and methods

The study was conducted at the Department of Botany, University of Peshawar. Seeds of the three millet varieties i.e. Bajra super 1 (PV1), PARC-MS 2 (PV2), PARC-MS 3 (PV3) were obtained from National Agricultural Research Centre NARC, Islamabad, Pakistan. The soil used was sandy loam with pH 7.8, electric conductivity 0.675 ds/m², Nitrogen 0.032% and Phosphorus 0.8mg/kg with low organic matter 0.6%.

Thirty earthen pots were filled with 8kg of soil. Each millet variety was represented by ten replicates i.e. five control pots and five test pots arranged in RCBD having factorial arrangement with two treatments (controlled and uncontrolled). The test pots were inoculated with soil containing spores of Glomus spp. Harvesting of millet plants was done after 80 days. After harvesting different growth parameters were taken including plant height, number & length of leaves, root length and number of seminal roots. In the laboratory dry weight of plants was taken by drying the plants in the oven at 65°C for 72 hours. Mycorrhizal inoculum preparation, placement and application were done by the method given by Gaur and Adholeya (2002). Mycorrhizal dependency was also calculated by the following formula.

Experimental data was statistically analyzed by applying ANOVA test; the means were subjected to LSD test.
While, proximate analysis (moisture content, ash, protein, fats and crude fibers) were determined by standard methods of AOAC (2006).

**Result and discussion**

**Growth Parameters and Mycorrhizal dependency**

In the present study, all measured parameters of mycorrhizal plants showed significant differences (P < 0.05) as compared to control. It is evident from mean data (Table 1, Fig. 1) that MPV2 responded better followed by MPV1 and then MPV3 in terms of plant height in mycorrhizal plants as compared to control. Our results are in opposite to the work of (Shrestha et al., 2009).

**Table 1.** Plant height, number & length of leaves, root length, number of seminal roots and dry weight (g.) of mycorrhizal (M) and non mycorrhizal (NM) millet varieties. Each value is a mean of five replicates. Values followed by different letters are significantly different (p < 0.05).

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>No. of leaves</th>
<th>Leaves length (cm)</th>
<th>Root length (mm)</th>
<th>No. of seminal roots</th>
<th>Dry weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV1</td>
<td>M</td>
<td>112.8</td>
<td>11.00</td>
<td>64.82</td>
<td>113.8</td>
<td>19.00</td>
<td>9.394</td>
</tr>
<tr>
<td></td>
<td>NM</td>
<td>66.10</td>
<td>10.00</td>
<td>48.96</td>
<td>84.28</td>
<td>11.00</td>
<td>4.140</td>
</tr>
<tr>
<td>PV2</td>
<td>M</td>
<td>120.5</td>
<td>13.00</td>
<td>78.50</td>
<td>143.0</td>
<td>24.00</td>
<td>6.850</td>
</tr>
<tr>
<td></td>
<td>NM</td>
<td>87.02</td>
<td>10.00</td>
<td>62.72</td>
<td>104.0</td>
<td>14.00</td>
<td>1.940</td>
</tr>
<tr>
<td>PV3</td>
<td>M</td>
<td>77.64</td>
<td>13.00</td>
<td>57.16</td>
<td>140.4</td>
<td>21.00</td>
<td>5.260</td>
</tr>
<tr>
<td></td>
<td>NM</td>
<td>54.22</td>
<td>8.000</td>
<td>45.32</td>
<td>69.20</td>
<td>12.00</td>
<td>1.540</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td></td>
<td>34.48</td>
<td>0.3801</td>
<td>22.84</td>
<td>55.55</td>
<td>4.723</td>
<td>3.410</td>
</tr>
</tbody>
</table>

**Table 2.** Effect of AM on proximate analysis of millet varieties.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percent on dry matter basis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture</td>
</tr>
<tr>
<td>MPV1</td>
<td>9.28</td>
</tr>
<tr>
<td>NMPV1</td>
<td>9.22</td>
</tr>
<tr>
<td>MPV2</td>
<td>10.11</td>
</tr>
<tr>
<td>NMPV2</td>
<td>7.51</td>
</tr>
<tr>
<td>MPV3</td>
<td>12.46</td>
</tr>
<tr>
<td>NMPV3</td>
<td>9.77</td>
</tr>
</tbody>
</table>

It has been suggested that in VAM inoculated plants the mitotic activity of stem cells may enhance, resulting in taller plants (Tarafdar & Marschner, 1995). This may be because of extra-radical mycelium (being smaller in diameter than roots) better penetrates beyond the depletion zone for better acquisition of nutrients (Sylvia et al., 1993; Mengel and Kirkby, 2001).

The result regarding length and number of leaves of millet (Table 1, Fig. 2, 3) showed that mycorrhizal plants shows better performance than non-mycorrhizal plants. The data shows that increase in number of leaves in mycorrhizal plants range from 11.11 - 62.5% in millet varieties. These results are also in agreement with the findings of Wu & Xia, (2006) in *Citrus tangerine* and Wu et al. (2008) in *Poncirus trifoliata* who reported that VAM inoculation resulted in greater number of leaves per plant as compared to the control plants.

The positive effect may be attributed to the enhancement of P nutrition (Henrike et al., 2007) and water uptake by hyphae (Faber et al., 1991).

Ghazi & Zak, (2003) investigated that improved plant growth in terms of leaf length, leaf water turgidity and stomatal activities might be due to enhanced uptake of water and nutrients like Zinc (Zn) and Copper (Cu).
Our present results (Table 1, Fig. 4) evidently showed that in millet varieties, the maximum root length (143.0mm) was recorded in MPV2, while least (113.8mm) was observed in MPV1. Our findings are in agreement with the work of Nzanza et al. (2011) and Ayoob et al. (2011).

The possible explanation may be that mycorrhizal inoculation stimulates rooting and growth (Kumar et al., 2007) and also reduces soil compaction which results in root development (Miransari, 2007).

Regarding number of seminal roots in, millet varieties mycorrhizal plants showed greater number than control plants. The number of seminal roots in millet varieties was increased by 75%, 72% and 71.42% in MPV3, MPV1 and MPV2 respectively.

The results (Table 1, Fig. 5) are in consistent with the fact that mycorrhizal inoculation changed the root morphology (Subramanian et al., 2008) and bring about stimulation for development of the root system, generally by increasing the formation of lateral roots (Berta et al., 2002).

Mycorrhizal dependency
In present research work three varieties of millet were investigated for mycorrhizal dependency (MD) (Fig. 7). The degree of mycorrhizal dependency was found a maximum in PV2 (71.67%) and least in PV1 (55.91%).
The results of our investigation have showed that cereals were more dependent on mycorrhiza association for better growth in nutrient deficient soil. Root branching determines plant dependence on symbiosis (Smith & Read, 1997; Barakh and Heggo, 1998). The results showed that all varieties were found to be differs in their mycorrhizal dependency as also shown by Xavier & Germida, (1998) and Sawers et al., (2008). They found that mycorrhizal dependency is not the same in plant species and even in their cultivars also. This difference in general is related to root geometry, soil type, soil phosphorus, plant growth rates and mycorrhizal species (Plenchette et al., 1983; Hatrick et al., 1993).

As evident from the result that amount of crude protein enhanced in mycorrhizal plants as compared to non-mycorrhizal plants.

The data revealed that increase of crude protein was 24.81% in MPV2, as compared to control. Our results are supported with the findings of Khalafallah & Abo-Ghalia, (2008) who reported that AM inoculation increased crude proteins contents of wheat plant by 6% than non-mycorrhizal. Our results are also in agreement with the finding of other workers (Wu et al., 2006b; Manoharan et al., 2008) who observed that the crude protein content is higher in AM than non-AM plants. Similarly, highest rate of increase of crude fat was 24% in MPV2. Our results agreed with the work of Cooper & Losel, (1978), according to them infected roots contained more total lipid than uninfected roots. Moreover, Omomowo et al. (2009) found that inoculation with Glomus mosseae has higher fat content of cowpea than un-inoculated control. An increase of 36.11% ash content was recorded in MPV1 of inoculated maize variety. According to Mehrvarz & Chaichi, (2008) inoculated plants of Barely (Hordeum vulgare L.) exhibited higher level of total ash (8.05%) than non-mycorrhizal (7.84%).
While the crude fiber and carbohydrate content showed a relative decrease of -7.50% (MPV3) and -14.81% (MPV2), respectively in mycorrhizal plants. Similar, decrease in crude fiber contents were also reported by Adewole & Ilesanmi, (2011), while Manoharan et al. (2008) reported contradictory results to our findings. The reason behind decrease in carbohydrate content is believed that carbohydrates are transferred from host to the fungal partner (Johnson et al., 1997). However, our results are contradictory to Khalafallah and Abo-Ghalia, (2008) who reported that mycorrhizal plants shows maximum amount of carbohydrates content of wheat plant than non-mycorrhizal under well watered conditions.

References


