INFLUENCE OF MYCORRHIZAL FUNGI AND CUTTING TYPE ON ROOTING OF CUTTINGS IN ROSMARINUS OFFICINALIS L. PLANTS

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ABSTRACT

Rosmarinus officinalis is a member of the family Lamiaceae. This trial was performed in the greenhouse of research Institute of Zabol University on June 2013 with 2 factors (mycorrhizal fungi and type of cutting) randomized block design with 9 replication. Three sorts of cutting were used with different types of cutting (D1:1-2 mm, D2:3-4 mm and D3:5-7 mm). 3 species of Glomus as mycorrhizal fungi and their blend add to rooting media. The result of this trial showed the effect of cutting type was significant on all parameters (number of rooted cuttings, number of root in each cutting and total lengths of roots); whiles the effect of mycorrhizal fungi was significant only on total length of roots. Interaction of mycorrhizal fungi and type of cutting was significant only on rooting percentages. The most rooting percentages were measured in cuttings with 1-2 mm diameter; cuttings with thicker diameter were shown less rooting percentages. Number of roots in each cutting and roots length showed softwood cuttings were better than the other. Application of mycorrhizal fungi had significant effect against control treatment, but there weren’t any significant effect between different species of Glomus on roots length. Eless G. intraradices was better than the other species of Glomus. Interaction of mycorrhizal fungi and type of cuttings on rooting percentages showed the best treatments were cuttings with 1-2 mm in G. intraradices. So this trial eventuate that softwood cuttings and application of mycorrhizal fungi on rooting of rosemary can produce better cuttings with better quality.

Keywords: Rooting, Cutting Type, Mycorrhizal Fungi, Softwood Cutting, Rosemary Plant

INTRODUCTION

Rosemary, Rosmarinus officinalis L, is a member of Decreased growth is an adaptation for survival of plant in mint family Lamiaceae. It is endemic to the Mediterranean stress condition; As far as climate and geographical regions and commonly grown in many parts of Iran (Zargari, 1990). Success in the production of medicinal plants Rosemary lies in selecting appropriate substrate and condition. In addition to having desired physical, chemical and biological properties, a suitable substrate should be available and relatively inexpensive, stable and light enough to be easy to use and its transport is affordable (Isalm et al., 2002).

The mycorrhizal symbiosis is a natural relationship between plants roots and fungi that can enhance plant growth reduce plant nutrient requirements, increase survival and development of micropropagated plants, enhance crop uniformity and rooting of cuttings, and increase fruit production (Garmendia et al., 2004, 2005).

Characteristics of different materials used as substrates have the direct and effects on plant growth and crop production (Verdonk et al., 1982). Result of Seyedi et al., (2012) indicated that flowering time of cultivated flowers in Lilium was significantly sooner of other treatments at the medium containing 70% cocopeat and 30% perlite. Increasing the percentages of coco peat substrates in media caused to increase all growth indices particularly plant height, stem diameter, flower diameter and buds number. The highest number of suckers in Kakoei and Salehi (2013) trials was obtained in equal leaf-mold: sand mixture. It is concluded that these differences represent a direct effect on the rooting process and that substrate characteristics are of the utmost importance for the quality of rooted plants.

Nair et al., (2008) recommend rooting Stewartia in media that has good aeration and moderate water-holding capacity and over wintering them at 5 °c. According to Shadparvar et al., (2011) experiment, treatment of 4000 mg l^{-1} was better treatment in increasing percentage of rooting and other affecting
factors on quality of cuttings in both mixture beds (sand-perlite and peat-perlite). Shirzad et al., (2012) indicated that rooting percent was maximized in combined treatment of “4000 ppm IBA +perlite” and “6000 ppm IBA+ sand”. The high number of roots was gained in “6000 ppm IBA+ sand”.

Cutting position within the mother plant and stem length affected the rooting of leaf-bud cuttings of Schefflera arboricola L. Results showed that cuttings from sub-apical positions rooted more slowly produced fewer roots and had a lower rooting percentage than cuttings from the more basal regions. Furthermore, the number of roots and rooting percentage increased with the length of the stem below the node. The subsequent axillary bud break and shoot growth was improved considerably in cuttings from sub-apical to basal positions, and by increasing the length of the stem below the node (Hansen, 1986).

The effects of toophysis on the number of roots per plantlet and on root fresh weight were inconsistent. Double node cuttings against single node cuttings showed a delayed but more uniform bud break and a result a reduced heterogeneity in shoot growth. Double node cuttings of Schefflera were shorter in length, had fewer leaves, a smaller leaf area and lower dry weight at harvest. Double node cuttings with a larger leaf area produced larger plants (Vander et al., 1990).

Each cutting should have one or two nodes. One of the nodes should be in soil. One leaf must be there in each cutting. Since the leaf is responsible for photosynthesis and transpiration the area of the leaf may be kept in a comfortable size by trimming to maintain a balance between transpiration, photosynthesis and respiration (Behera et al., 2009). Golden pothos nodal position and length of cuttings also determine the root retention for in vivo establishment (Wang and Boogher, 1998).

Garmendia and Mangas (2012) concluded that both inocula tested caused low levels of mycorrhizal root colonization, with higher percentages in Rosa associated with Glomus mossaea significant improvements of plants biomass, leaf nutritional status or flower quality was not detected in inoculated plants probably due to the low symbiosis establishments.

The aim of the study was the evaluation of the effect of the substrate and the cutting type on the rooting process of Rosmarinus plant.

MATERIALS AND METHODS
This experiment was carried out in the greenhouse of Research Institute of University of Zabol in 2013. On June 2013 cuttings were taken from insect- and disease-free plants with 12-15 cm length from rosemary plants with gardening secateurs, and 2/3 of bottom leaves is deleted. For investigation of type of cutting on rooting of rosemary cuttings, Three types of cuttings were prepared i.e. hard wood, semi hardwood and soft wood with 3 different diameters (D₁: 1-2 mm as softwood cuttings, D₂: 3-4 mm as semi hard-wood cuttings and D₃: 5-7 mm as hard-wood cuttings) were selected. The hard wood cutting was prepared from basal portion of the branches, whiles the semi hard wood cutting was prepared from the mid portion, and the soft wood cuttings was prepared from terminal portion of current growth. Before transplanting cuttings were dipped for 5-10 s in 4000 mg.L⁻¹ rooting hormone solution Indol-3-Butyric Acid (IBA).

Cuttings were placed under mist (each 1 hour, 1 minute mist) and watered as needed in a greenhouse with no bottom heat. Parameters to be measured were number of rooted cuttings (rooting percentages); number of roots in each cutting and total roots length. A 5x3 factorial experiment in randomized block design with 9 replications was initiated (each replication had 12 cuttings). Analysis of variance was conducted using the Statistical Analysis software SPSS with significance at P≤ 0.05 and average comparison had done by Duncan method.

RESULTS AND DISCUSSION
Approximately 2 months after transplanting the cuttings into the substrate, the measurements of parameters were commenced.

Rooting percentage (number of rooted cuttings): Data analyzed showed that the effect of mycorrhizal fungi wasn’t significant on rooting percentages in rosemary cuttings, But the effect of cuttings type was
significant (P≤0.01) on number of rooted cuttings (rooting percentages). The application of various type of cuttings on the number of rooted cuttings (figure 1) showed that softwood cuttings with less diameter had the most rooting percentage and hardwood cuttings had the least rooting percentages and were the worst treatment.

**Figure 1:** Effect of different type of cuttings on the number of rooted in rosemary

**Figure 2:** Interaction between different cutting type and rooting substrates on the number of rooted cuttings
According to this trial (figure 1) effect of cuttings type was significant (P≤0.01) on rooting percentages. The best treatment was softwood cuttings with 74% rooting, after that, semi-hardwood cuttings (with 37% rooting) were better than hardwood cuttings (with 4% rooting).

Mardani et al., (2011) discussed that concurrent usage IBA (2500 ppm) and high diameter (wood cuttings) had significant effect on increscent weight and roots length in *Populus deltoids*.

Interaction effect of mycorrhizal fungi and cutting type was significant (P≤0.05). According figure 2 the result of this trial showed in application of all mycorrhizal fungi cuttings with 1-2 mm (softwood cutting) had the most rooting percentages than the other cutting type. The best treatments were softwood cuttings in *G. intraradices*, *G. hawaii*, *G. mossaea*, mycorrhizal mixing and control respectively. In semi hard-wood cuttings control treatments was better than the others. After that *G. mossaea*, mycorrhizal mixing, *G. hawaii* and *G. intraradices* were the best, respectively. But in hardwood cuttings, *G. intraradices* was better than control, mycorrhizal mixing, *G. mossaea* and *G. hawaii* respectively. So these results showed application of each cutting type in each mycorrhizal fungi had specific output finally. If there are softwood cuttings and cuttings producers want to increase rooting of rosemary by application of mycorrhizal fungi, they can use *G. intraradices*, *G. hawaii* and *G. mossaea* due to the better result in this trial. But in semi hard-wood cuttings control treatments was better than cuttings with mycorrhizal fungi, so application of mycorrhizal fungi in this cutting type don't need. As via as if mycorrhizal fungi be accessible, it is better to producers use softwood cuttings instead of semi hard-wood and hardwood cuttings due to better rooting percentage in semi hard-wood cuttings with less diameter (1-2 mm).

**Number of roots:** The result of this trial showed the effect of cutting type was significant on the number of roots in rosemary cuttings. But the effect of mycorrhizal fungi and interaction of cutting diameter and mycorrhizal fungi were not significant.

According to figure 3, the most number of roots were observed in cuttings with 1-2 mm diameter, whiles the lowest roots number were observed in cuttings with 5-7 mm diameter or hard-wood cuttings. There is not any significant difference between different mycorrhizal fungi Nonetheless *G. intraradices* and *G. mossaea* had more number of roots about 9.81 and 8.4 roots in each cutting against 7.77 roots in control treatments respectively.

![Figure 3: Effect of different cutting type on number of roots in rosemary cutting](image-url)
Length of root: The measuring of all the roots length in each cutting showed that the effect of stem diameter of cuttings and different mycorrhizal fungi were significant on roots length (P≤0.05). But the interaction effect of type of cutting and mycorrhizal fungi weren’t significant on roots length.

Figure 3: Effect of different type of cuttings on total length of roots in each rooted cuttings of rosemary plants

According to figure 3, softwood cuttings with 1-2 mm stem diameter had the most length of roots in each cutting (33.93 cm). While semi-hardwood cuttings with 3-4 mm diameter had 13.24 cm length in all roots that form in each cutting and hardwood cuttings with 5-7 mm diameter had 2.76 cm length. So cuttings with the most length are better than the others because of better absorption of water and nutrient from the culture medium. Again cuttings with more roots can fix in soil more easily than cuttings with lower roots that can’t maintain themselves in the culturing medium.

Figure 4: Effect of different mycorrhizal fungi on total length of roots in each rooted cuttings of rosemary plants
Using different mycorrhizal fungi in rooting medium had significant differences with control treatments. According to figure 4, the best treatments were cutting inoculated by *G. intraradices* that had significant differences to control only. Mixing of fungi had better effects against *G. mossaea* and *G. hawaii*.

Mean square of different characteristics and significance letters showed in table 1 and 2 for various stem diameters and mycorrhizal fungi treatments.

### Table 1: The effect of different cutting type on the Rosemary cutting characterization

<table>
<thead>
<tr>
<th>Stem diameter</th>
<th>Rooting (%)</th>
<th>Number of root</th>
<th>Total roots length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 mm</td>
<td>74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.93&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-4 mm</td>
<td>37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5-7 mm</td>
<td>4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table 2: The effect of different mycorrhizal fungi on rosemary cutting characterization

<table>
<thead>
<tr>
<th>Type of cutting</th>
<th>Rooting (%)</th>
<th>Number of root</th>
<th>Total roots length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. intraradices</em></td>
<td>41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. mossaea</em></td>
<td>39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. hawaii</em></td>
<td>37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mix of fungi</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Conclusion

Mycorrhizae can enhance efficiency of plants roots to absorb water and macro- and microelements from the soil or container media. This helps reduce fertility and irrigation requirements, increase drought resistance and plant resistance to pathogens (Davies *et al.*, 1993). In regards to adventitious root formation, both endo- and ectomycorrhiza can enhance adventitious root formation of cuttings (Hartmann *et al.*, 2002). There can be enhanced root initiation and root development. The ectomycorrhiza are capable of producing auxines, gibberellins and other phytohormones. With some of the more difficult-to-root plant species, it may be useful to try combinations of Mycorrhizae and auxines to stimulate better root formation (Fred and Davies, 2008). So cuttings inoculated with Mycorrhizae in this trial due to above reasons had better qualification than control treatments and these cuttings had more roots in each cutting, and these roots help cuttings for absorption of water and nutrients. The endo Mycorrhizae, Glomus intraradices, increased adventitious rooting of softwood cuttings and liner plant development of desert willow (*Chilopsislinearis*) that was similar to this trial result (Davies *et al.*, 2000). Ectomycorrhiza increased the rooting percentage or accelerated root formation of fascicular shoots of *Pinus sylvestris*; the ectomycorrhizal fungi *Pisolithustinctorius* produces auxines (IAA) which can enhance rooting (Neimi *et al.*, 2000). Salmanizadeh *et al.*, (2011) expressed type of cutting had significant effect on stem diameter characteristics, rooting percentages, root fresh and dry weight (P≤0.01) that was similar to these trial result. Rahman *et al.*, (2003) concluded that maximum number of roots (59.66) and lengthy shoot (8.24 cm) was recorded in softwood cuttings of Gauva that it is accordant to the result of this experiment on rosemary cuttings.

Thus according to result of this experiment and other authors that discussed in before paragraph, using of mycorrhizal fungi (in this experiments *G. intraradices* was the best for rosemary cuttings) can enhance qualification and quantification characteristics in cuttings, it was observed even in hardwood cuttings of rosemary; but softwood cuttings was the best. So producer can enhance their cuttings qualification by application of mycorrhizal fungi in rooting medium.
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