Infection of *Ulmus glabra*, *Acer velutinum* and *Taxus baccata* with endomycorrhizal fungi in Vaz Forest

Teimouri, M., Korori, S.A.A. and Matinizadeh, M.  
1- Ecophysiology and biotechnology group, Forest division, Research Institute of Forests and Rangelands, Tehran, Iran. Phone: +0098-0261-6619517. E-mail: mteimouri@rifr-ac.ir, korori@rifr-ac.ir, matini@rifr-ac.ir

Abstract
Mycorrhiza is a sustainable symbiosis between plant root and fungus. Mycorrhiza is divided to two main groups of ectomycorrhiza and endomycorrhiza. Different studies have shown the effect of endomycorrhizal symbiosis on physiology of forest trees. This study was done in a part of hycanian region in north Alborz in Vaz forest. Studies were done on three important forest trees including *Ulmus glabra*, *Acer velutinum* and *Taxus baccata*. Two first species are important industrial species in forest of Iran and located in hyrcanian regions where growth condition is suitable. *Taxus baccata* is one of the extincting conifers in world and used for wood production and secondary metabolites such as taxol. Samplings was done from hair roots and rhizosphere (in four replications) for each species in spring and autumn in two consecutive years. Roots were stained by Philips and Hayman method. Spores of mycorrhizal fungi were isolated by wet sieving method and identified by different keys. All of tree species showed infection by mycorrhizal fungi. The results indicated the more number of fungal spores in rhizosphere in autumn in compared to spring. Number of spores in rhizosphere of *Taxus* was more than two other species in both seasons. Three different fungal genera were found in each species rhizosphere. In *Acer velutinum* and *Taxus baccata* rhizosphere were identified four species of *Glomus*, one species of *Gigaspora* and one species of *Aculospora* whereas in *Ulmus glabra* three species of *Glomus*, two species of *Gigaspora* and one species of *Aculospora* were isolated.

Key words: Symbiosis, *Ulmus glabra*, *Acer velutinum*, *Taxus baccata*, Vesicular-arbuscular

Introduction
The hycanian forest at the northern slopes of the Alborz Mountains facing to the Caspian sea are important forests of Iran. They provide the country with valuable wood products. Their history dates back to the Tertiary period and they have not been heavily disturbed by the ice ages and as such they are rich in species compared to other temperate forest ecosystems in West Asia and Europe. There is a general need for more and well documented knowledge about the forests, their dynamic, functions and biodiversity to support a future sustainable utilization of this unique and valuable forest resource.

The mycorrhiza abundance, distribution and relation to various species are example of this incomplete knowledge. Mycorrhiza is a sustainable symbiosis between plants root and fungus. German botanist Albert Bernard Frank in 1885 introduced the Greek word mycorrhiza, which literally means fungus root to scientific terminology Mycorrhiza is divided to two main groups endomycorrhiza and ectomycorrhiza with some other alternative groups such as ericoides, orchidaceous, monotropoides according to symbiosis with specific hosts. Vesicular-arbuscular have the widest host range and distribution of all mycorrhizal association. It is established that about 90% of vascular plant normally mutalistic relationship with VA mycorrhiza. (1). VA mycorrhizal fungi, obligate biotrophes, do not grow on synthetic media and hence are classified according to morphological characteristic of the spore formed in soil. They are all grouped in the class zygomycetes in the subdivision zygomycotina. The well-known genera are called *Glomus*, *Sclerocystis*, *Enterophospora*, *Aculospora*, *Scutelospora* and *Gigaspora* (1).

Studies of mycorrhiza in other forest ecosystems have shown that fungi are important for the trees and soil. Gerdemann showed mycorrhizal fungi are a part of soil flora and act as inoculum (5). In 1974 khan reported the occurrence of mycorrhiza in halophytes, hydrophytes and xerophytes and
also spores of mycorrhizal fungi in soil. He observed the frequency of spores decreased with depth increasing (10). Boullard in 1986 studied the symbiont mycorrhizal fungi with Juniperus (3). Miller showed a direct connection between soil bulk and root system of plants by external hypha of mycorrhizal fungi that can improve water and nutrient uptake by plants (11). Mycorrhizal fungi also interface with the surrounding soil and contribute greatly to the improvement of soil texture for better aeration and water percolation (4). Establishment of VA mycorrhizal in plants usually confers resistance to nematode parasitism or adversely affects nematode reproduction (9). Gemma et al studied the responses of Taxus baccata to inoculation with Glomus intradices and Gigaspora gigantea. The root thickness and length were 1.3-1.4 and 1.7-2.1 times more than uninoculated ones, respectively (5). Griffith and Chadwick investigated the mycorrhizal symbiosis in understory trees such as Taxus and distribution and frequency of mycorrhizal fungi spores in coniferous forest. Results indicated that frequency of spores increased in rhizosphere with increasing the height of tree (8).

However, more specific knowledge on the mycorrhiza and the interaction with different tree species in the Iranian hyrcanian forests is needed. In this study, three tree species (Ulmus glabra, Acer velutinum and Taxus baccata), were selected and studied the occurrence of VA mycorrhizal on their roots. Ulmus and Acer were primarily chosen because of their importance as timber species, whereas Taxus baccata is a protected species that is threatened by extinction. The aim of this study was to gain more fundamental knowledge about the occurrence of VA mycorrhizal with Ulmus glabra, Acer velutinum and Taxus baccata in Vaz forest. The ultimate goal is to contribute a generally improved knowledge and understanding of the hyrcanian forest ecosystems and thereby contribute to the scientific base for a future sustainable forest management, seedling production in nurseries and protection of these forests.

Methods and material
Vaz forest which has an area of 14102 hectares is located in the southern slopes of Chamestan in Mazandaran province between 250 to 33350 meter from sea level. It is located between eastern altitude 51º, 55´, 15´´- 52º, 12´ ,15´´ and northern latitude 36º, 12´, 30´´ – 36º ,30´. Forests and rangelands associations are present in this area. 5 stands were selected in same ecological condition for Ulmus glabra, Acer velutinum and Taxus baccata in Vaz research forest in north of Iran.

A) Root Colonization
Fine terminal root (< .5-2 mm diameter ) were sampled in 0-30 cm soil depth in spring and autumn in two successive years. Roots were transferred at 4°C to the Laboratory in polyethylene bag (13). Roots were stained according to Philips and Haymann method (14) and examined under microscope at 10-40 X magnification. The percentage of root colonization was determined according to Giovanetti and Mosse method and categorized into several classes (7).

B) Extraction and Identification of spores of vesicular- arbuscular fungi
Samples of soil cores were collected from 5-10 different site around the root systems in the upper 0-30 cm of soil surface. The soils from all cores were mixed thoroughly. Roots were transferred at 4 °C. Soil samples were dried at room temperature. Some general soil characters were determined. The spores of vesicular- arbuscular mycorrhizal fungi were isolated by wet sieving and decanting method of Gerdemann (6). Isolated spores were mounted by Canada balsam and identified according their dimension, color, shape and presence or absence of sporocarps (12, 2 and 15).

Results and Discussion
Some soil characters are presented in table number 1. Results indicated that these Characters are different in rhizosphere of different species. Staining of roots showed the presence of VA mycorrhizal symbiosis in all of studied Species (Figures 1, 2,3,4). Root colonization percent showed Ulmus, Acer and Taxus belonged to 4, 4 and 5 class (7). Total number, frequency of VA
mycorrhizal fungi spores in spring and autumn in 1g of soil are presented in Table 2. Results indicated the frequency in autumn was more than spring. Condition in spring is more suitable for fungi growth then they are more active in symbiosis with plants but with beginning of autumn and reduction of plant physiologic activity, they tend to be inactive and produce spores. Total number of spores in Taxus baccata rhizosphere was more than others. It may be related to different factors such as ecological parameter and being soil poor or rich. The occurrence of VAM symbiosis in poor soil (for example soil with phosphorus deficiency) is more. Identification of isolated spores (figures 5 and table 3) did not show any specific fungus for colonization and production of VA symbiosis. Taxus baccata, Ulmus glabra and Acer velutinum are very important species in Iran forests. Their population in north of Iran has decreased because of human activity, harvesting and low regeneration of them. Then rehabilitation program is necessary for inhibiting their extinction for example production of potent seedling in nursery and use of them in reforestation and aforestration plans. In conclusion it seems these trees have a good potential for VAM symbiosis and then it can be in forest management and in forest nursery for production of more suitable seedlings.

References
Table 1- Some characters of soils

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>% Silt</th>
<th>% clay</th>
<th>% sand</th>
<th>Texture</th>
<th>pH</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulmus glabra</td>
<td>21</td>
<td>11.2</td>
<td>67.82</td>
<td>Loam-sandy</td>
<td>7</td>
<td>0.322</td>
</tr>
<tr>
<td>Acer velutinum</td>
<td>36.92</td>
<td>47.24</td>
<td>15.8</td>
<td>Loam-clay</td>
<td>6.75</td>
<td>0.312</td>
</tr>
<tr>
<td>Taxus baccata</td>
<td>26.34</td>
<td>15.74</td>
<td>57.92</td>
<td>Loam-sandy</td>
<td>7.385</td>
<td>0.499</td>
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</tbody>
</table>

Table 2- Frequency of VAMF spores

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Spring</th>
<th>Autumn</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulmus glabra</td>
<td>766</td>
<td>821</td>
<td>1487</td>
</tr>
<tr>
<td>Acer velutinum</td>
<td>581</td>
<td>856</td>
<td>1437</td>
</tr>
<tr>
<td>Taxus baccata</td>
<td>928</td>
<td>1924</td>
<td>2852</td>
</tr>
</tbody>
</table>

Table 3- Isolated and classified VA fungi spores

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Isolated VAMF spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulmus glabra</td>
<td>Glomus (3 species ), Gigaspora (2species ), Acaulospora (1species)</td>
</tr>
<tr>
<td>Acer velutinum</td>
<td>Glomus (4 species ), Gigaspora (1 species ), Acaulospora (1species)</td>
</tr>
<tr>
<td>Taxus baccata</td>
<td>Glomus (4 species ), Gigaspora (1 species ), Acaulospora (1species)</td>
</tr>
</tbody>
</table>