RESEARCH ARTICLE

COMPARATIVE STUDY ON VAM COLONIZATION IN HEDYCHIUM CORONARIUM KOEN. AND HEDYCHIUM FLAVESCENS CAREY EX ROSCOE

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ARTICLE INFO

Article History:
Received 18th, December, 2013
Received in revised form 28th, December, 2013
Accepted 11th, January, 2014
Published online 28th, January, 2014

Key words:
Mycorrhizae, symbiotic association, rhizospheric soil, vesicles, Glomus, Hedychium

ABSTRACT

Mycorrhiza infects the plant roots to form symbiotic associations whereby the fungi supply nutrients, water and protection to the plant in exchange for food in the form of carbon. The present investigation was carried out to study the prevalence of VAM fungi in Hedychium coronarium Koen. and Hedychium flavescens Carey ex Roscoe for determining the root colonization and spore density in the rhizospheric soil. Presence of mycelium and vesicles were identified using tripan blue staining technique. Hedychium flavescens shows high adaptability to mycorrhizal association. The colonization was observed in the form of mycelium and vesicles. VAM spores of Glomus were predominant in soil samples. The spore density and population was high in Hedychium flavescens than Hedychium coronarium. The physicochemical properties of the soil were also estimated, which determined the mycorrhizal association.

INTRODUCTION

Mycorrhiza a symbiotic association is essential for one or both partners, and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. This association is believed to be as ancient as land plants (Pirozynski and Malloch, 1975). Mycorrhizae occur in specialised plant organs where intimate contact results from synchronised plant-fungus development (Brundrett, 2004). It is estimated that 95% of the world’s present species of vascular plants are mycorrhizal (Quilambo, 2003). Arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread (Brundrett, 2002). Paleobotanical and molecular sequence data suggest that the first land plants formed associations with Glomalean fungi from the Glomeromycota about 460 million years ago. They are obligate mutualists and have a ubiquitous distribution in global ecosystems (Redecker et al., 2000). Mycorrhizae formed by fungi of the order Glomales sensu (Morton and Benny, 1990) generally are known as vesicular arbuscular mycorrhizae. VAM fungi are acidophilic with their pH ranging from 4.0 to 6.0. The mycelia penetrate into the root and form morphologically distinct structures as vesicles and arbuscules. Hyphae, arbuscules and vesicles together with large spores, constitute the diagnostic features of VA mycorrhizae. Vesicles serve as storage structures, and are generally produced in the older region of infection (Pawaar and Kakde, 2012). These mycelia interconnected between different groups of plants under one ecological condition serve in exchange of mineral and nutritional resources (Simard et al., 1997). VAM fungi have potential to influence the ecosystem processes, their potential to determine the plant communities and ability to induce a wide variety of growth responses in coexisting plant species (Klironomos et al., 2000). VAM has a range of effects which contribute to the amelioration of different types of stress experienced by their plant hosts, including metal toxicity, oxidative stress, water stress, and effects of soil acidification (Finlay et al., 2008). It has substantial role to maintain the ecology of the soil, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava et al., 1996).

Zingiberaceae, the largest family in the Zingiberales, are sources of valuable spices, dyes, perfumes, medicines and ornamentals. They occur chiefly in the tropics with about 53 genera and 1200 species and with abundant variation and diversity in the Indo-Malesian region of Asia. (Kress et al., 2002). About 70 species are endemic to India and several among them are rare and threatened; some of these are now in the vulnerable category. The genus Hedychium is the largest, with 39 species and 4 varieties in India. Taber and Trappe (1982) recorded association of vesicular arbuscular mycorrhizal fungi with ginger for the first time. These plants are heavily mycorrhizal and the percentage of VAM colonization intensely varied with locality (Chandra and Kehri, 2006). The presence of VAM colonization of Zingiberaceae was reported in Alpinia galanga, Curcuma aromatica and Hedychium coronarium (Mathew and Malathy, 2006). Hedychium coronarium Koen., (white ginger) and Hedychium flavescens Carey ex Roscoe (yellow ginger) are economically, medicinally and ecologically valuable. H. coronarium is known for its strong aromatic odour and used as hot natured drug in traditional Chinese medicine. The present investigation was carried out to study the prevalence of VAM fungi in two accessions each of Hedychium coronarium and Hedychium flavescens growing in different climatic regions, determining the extent of root colonization and spore density in the rhizospheric soil associated with the host plant.
MATERIALS AND METHODS

Site description and field sampling
The present study was conducted on two populations of *Hedychium coronarium* and *H. flavescens* growing at different altitudes in south Western Ghats of Kerala. The samples of *Hedychium coronarium* were collected from Munnan (1480 m asl; RHT 65120) and Vallakkadavu (874 m asl; RHT 65140), that of *H. flavescens* from Kanthalloor (1502 m asl; RHT 65189) and Marayoor (993 m asl; RHT 65137). Roots and soil (100 g) of three samples of each species from two locations were collected randomly, and brought to the laboratory for estimation of VAM association. The root samples were washed thoroughly in tap water and fixed in formalin - acetic acid - alcohol (FAA) and soil samples were stored at 4°C for further analysis.

Analysis of soil physicochemical properties
Soil pH was determined using a digital pH meter. Electrical Conductivity was measured using Electrical conductivity meter. Organic carbon was analysed by colorimetric method (Anderson and Ingram, 1993) and available phosphorus by molybdenum blue method (Allen et al., 1974). Available potassium was measured by flame photometric method (Toth and Prince, 1949).

Processing of roots for assessing the existence of root colonization
Mycorrhizal infection in roots was determined following the method of Biermann and Liedermann (1981). The root samples stored in FAA were first washed with tap water repeatedly for complete removal of traces of FAA and 1 cm segments were cut from the fine roots. Thereafter, the samples were cleansed with 10% KOH, acidified with 1 N HCl, and stained in 0.05% trypan blue following the method of Phillips and Hayman (1970). Mycorrhizal infection in the roots was expressed as the percentage of segments containing fungal structures like mycelium/fungal hyphae, and vesicles (50 root segments per sample were evaluated for mycorrhizal infection). Percent root colonization was determined using formula as mentioned under:

\[
\text{% root colonization} = \left( \frac{\text{Number of positive segments}}{\text{Number of segments observed}} \right) \times 100
\]

The average number of vesicles per 1 cm root length was also determined by counting them in 50 root segment for each sample.

Isolation of VAM spores
The spores were isolated from rhizospheric soil by using wet sieving and decanting method (Gerdermann and Nicolson, 1963). The spores were quantitatively and qualitatively estimated from 100 g of air dried soil samples (Gaur and Adholeya, 1994). Sieves with various mesh size 2000 μm, 425 μm, 325 μm, 250 μm, 105 μm and 45 μm were used for isolation. The VAM fungal spores collected on filter paper (Whatman filter paper No.1) were observed under stereoscopic binocular microscope. Spores picked through needle were mounted on glass slide in polyvinyl alchol lactoglycerol (PVLG) mountant. VAM spores were identified following the "Manual for the identification of VA mycorrhizal fungi" of Schenck and Perez (1990).

Statistical analysis
Statistical analysis was performed using the SPSS windows v. 16.0 programs.

RESULTS AND DISCUSSION
In the present investigation most of the samples showed mycelial-root colonization and the formation of vesicles (Figure). *Hedychium flavescens* from higher altitude showed immense response to VAM mycorrhizal association. In *H. flavescens* colonization percentage was 80.67±1.15 and an average of 18.20±0.20 vesicles were observed in 1 cm of root bit. The same sample from the lower altitude showed 14.0±2.0% root colonization and 5.83±0.45 vesicles per root bit. *Hedychium coronarium* from higher and lower altitudes got somewhat the same result, with colonization percentage of 35.33±3.05 and 23.33±2.31 and the vesicle number of 5.53±0.50 and 3.40±0.60 respectively (Table 1). Songachan and Kayang (2011) reported the presence of VAM fungi in the roots of *H. coronarium* from pine forest of Meghalaya, North East India. Uma et al., (2010) also reported AMF colonization in *H. coronarium* (69.7%) from South India, which is slightly higher than our finding, suggesting that the intensity of AMF colonization could be influenced by specific habitat conditions. Mago et al., (1993) investigated the highest degree of colonization by VAM fungi of scale leaves of rhizome of *Zingiber officinale* with rare occurrence of arbuscles (Chandra and Kehri, 2006).

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Plant species</th>
<th>Altitude</th>
<th>*Mean total root colonization (%)</th>
<th>*Mean spore density 100g soil</th>
<th>No. of vesicles /1cm root</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Hedychium coronarium</em></td>
<td>1480m</td>
<td>35.33±3.05</td>
<td>38.66±10.06</td>
<td>5.53±0.50</td>
</tr>
<tr>
<td>2</td>
<td><em>Hedychium coronarium</em></td>
<td>874m</td>
<td>23.33±2.31</td>
<td>16.0±5.29</td>
<td>3.40±0.60</td>
</tr>
<tr>
<td>3</td>
<td><em>Hedychium flavescens</em></td>
<td>1502m</td>
<td>80.67±1.15</td>
<td>238.3±10.40</td>
<td>18.20±0.20</td>
</tr>
<tr>
<td>4</td>
<td><em>Hedychium flavescens</em></td>
<td>931m</td>
<td>14.0±2.0</td>
<td>63.3±7.57</td>
<td>5.83±0.45</td>
</tr>
</tbody>
</table>

Table 1 Vesicular arbuscular mycorrhizal status in two species of *Hedychium* from different altitudes of Western Ghats, Kerala

<table>
<thead>
<tr>
<th>SlNo</th>
<th>Parameters</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>5.23±0.02</td>
<td>4.81±0.05</td>
<td>5.45±0.05</td>
<td>6.51±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Electrical Conductivity (dS/m)</td>
<td>0.06±0.01</td>
<td>0.15±0.01</td>
<td>0.09±0.005</td>
<td>0.11±0.015</td>
</tr>
<tr>
<td>3</td>
<td>Organic carbon (%)</td>
<td>3.1±0.045**</td>
<td>3.7±0.05**</td>
<td>3.7±0.05**</td>
<td>0.11±0.015**</td>
</tr>
<tr>
<td>4</td>
<td>Available Phosphorous(kg/ha)</td>
<td>54.02±1.1**</td>
<td>1.0±0.15</td>
<td>4.02±0.09</td>
<td>150.9±0.25***</td>
</tr>
<tr>
<td>5</td>
<td>Available Potassium (kg/ha)</td>
<td>178.08±10.05**</td>
<td>244.09±1.1**</td>
<td>482.9±0.18***</td>
<td>345.09±0.08***</td>
</tr>
</tbody>
</table>

Table 2 The physico-chemical characteristics of the soil under two plant species from different altitudes

Values are mean±standard deviation; *F*-test significant at 0.05 level of probability.
Table 1 also depicts the mean spore density in 100 g soil assessed. The average of mycorrhizal spore density ranged from 16.0±5.29 to 238.3±10.40 spores per 100 g of dry rhizosphere soil in the collected plant species. *H. flavescens* from higher altitude showed the maximum spore density, followed by *H. flavescent* from lower altitude (63.3±7.57), *H. coronarium* from higher altitude (38.6±10.06) and *H. coronarium* from the lower altitude showed the least. Spore density was comparatively higher in *H. flavescent* than *H. coronarium*. It may be the first report in *H. flavescent* with VAM mycorrhizal association. VAM species was almost same in all the four sample types. *Glomus* species is the most dominant and frequently observed fungus in the soil samples. The VAM fungi (*Glomus*) also were earlier reported in the scale like leaves of *Hedyochium coronarium*, *Curcuma domestica*, *Alpinia purpurata* and *Costus spicatus* (Thomas and William, 1984). Philip and Iyer (1994) have observed *Glomus* as the VAM fungi associated with ginger.

The physicochemical properties of soil samples show variation reflecting variation in altitude and climatic regime (Table 2). Mycorrhizal colonization in roots occupying a defined volume of soil will depend on a balance between root and fungal activity (Koide, 1993) which is influenced by several factors including soil properties, root phenology, predation, local disturbance and propagule availability (Brundrett, 1991). The samples taken from all locations were more or less acidic (4.81-6.51) (Table 2). Stahl et al., (1988) considered mycorrhizae as a special manifestation of soil with poor nutrient content. Bjorkman (1970) recorded that N and P contents of soil influenced the intensity of mycorrhizal infection. This is also reflected in our investigation except in the case of *H. coronarium* from lower altitude. It is evident from the investigation that there is a direct relationship of VAM colonisation and/or sporulation with available phosphorus in the rhizospheric soil. Rhizospheric soil of *Hedychium flavescent* collected from higher altitude have lower amount of available phosphorus and shows higher rates of VAM colonisation. Rhizospheric soils of *H. flavescent* from lower altitude and *H. coronarium* from higher altitude have higher amounts of available phosphorus and lower rates of VAM colonisation. *H. coronarium* collected from lower altitude shows low VAM root colonisation though it has lower amount of phosphorus in the rhizospheric soil. This might be due to very strong acidic condition that prevails at that site (pH 4.81±0.05). Reduced soil pH increases the hydrogen and mineral ion concentration, depending on physical and chemical properties of soil (Abbott and Robson, 1985), which possibly inhibits spore germination (Hepper, 1979).

Harley (1989) suggested that the production of phosphatases by mycorrhizal fungi play an important role in the hydrolysis of organic and inorganic phosphate. Bjorkman (1970) had noted that the spore density was related to environmental parameters and soil properties and the strongest correlated factor was the soil pH. Many efforts have been made in recent years to know benefits derived from mycorrhizae for development of agriculture, horticulture, forestry, and site remediation. Mycorrhizae are essential below-ground components in the establishment and sustainability of plant communities, but a thorough knowledge is required to achieve maximum benefits from these microorganisms and their associations.

**Acknowledgements**

The authors are grateful to the Department of Microbiology, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala for providing laboratory facilities.

**References**


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