Studies on dynamics of AM fungal association and spore density in
*Elettaria cardamomum* Maton. (small cardamom)

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Abstract

Relationship between environmental factors and AM mycorrhizal variation in the rhizosphere of the *E. cardamomum* was studied. The soils were acidic in nature and organic carbon ranged between 2.2 to 4.6 %. The extent of root colonization by AM fungi in *E. cardamomum* ranged between 19.0 to 86.1 and was found to be maximum during February and minimum during July. The total AMF spore number varied from 18.66 to 67.00 g⁻¹ soil. The number of spores in *E. cardamomum* was maximum during June and minimum during September. *Acaulospora* sp., *Gigaspora* sp., *Glomus aggregatum*, *G. fasciculatum*, *G. intraradices* were the spore types recorded from the rhizosphere of *E. cardamomum* at Thadiyankudisu. Temperature was significantly and positively correlated with EC and negatively with soil N. Rainfall was correlated significantly to soil P and negatively to temperature. Soil moisture was correlated positively to %RLH and %RLC pertaining to mycorrhizae. Rainfall also significantly correlated with %RLA and negatively with spore density. Percent root length hyphae (RLH) were significantly positively correlated with %RLC.

Introduction

Mycorrhizal fungi are important in sustainable agriculture due to their role in plant and soil nutrition, acting as agents in transporting mineral nutrients to the plant and C compounds to the soil (Reide, 1990). These fungi are known to occur in all soil types and enhance the uptake of diffusion-limited nutrients such as phosphorus (P) in soils having with low available P in spite of their high P retention capacity. These fungi are also reported to be useful as biocontrol agents against root rot pathogens and nematodes (Thomas and Ghai, 1989). Arbuscular mycorrhiza (AM) fungi are not host specific. Differents host plants and soil fertility stimulate differential sporulation by AM fungi species in the field (Hayman, 1975) and spore production is seasonal in several habitats (Brundett, 1991). However, the VAM status of a crop is decided by interplay of various edaphic and climatic factors. The present study was therefore undertaken to find out the influence of edaphic and climatic factors on the quantitative and qualitative aspects of AM fungal association in *E. cardamomum* at a given time at Thadiyankudisu.

Materials and methods

Study area

A survey was carried out to study the dynamics of AMF in *E. cardamomum* in the Western Ghats ecosystem, Southern India. A seven-year-old plantation was selected for the study. The root and root-zone soil samples were collected at Indian Cardamom Research Institute, Regional Research Station, Thadiyankudisu located at Lower Pulney hills in Kodaikanal, Tamil Nadu.

Weather data

Weather data [minimum and maximum temperature, relative humidity (RH %) and rainfall] were obtained from Indian Cardamom Research Institute, Regional
Dynamics of AM fungal association in small cardamom

Research Station, Thadiankudisai.

Sampling

Root and root-zone (up to 30 cm soil depth) soil samples were collected from

_E. cardamomum_ plantations. The samples were collected at monthly intervals for a period of 12 months from November-2000 to October - 2001. For each sampling, five individuals were selected. The roots were dug out, washed free of soil and fixed in formalin-acetic acid-alcohol. The root-zone (1 Kg) from the individuals was mixed to form a composite soil sample. These were air-dried, packed in polythene bags and kept at room temperature (20-30°C) until further analysis.

pH

Ten gram of dry soil was taken in a beaker and 100 ml of water added to make a suspension of 1:10 (w/v) dilution and the pH was determined with a digital pH meter (Systronics-335).

Electrical conductivity

Ten gram of dry soil was taken in a beaker and 100 ml of water was added to make a suspension of 1:10 (W/V) dilution and the electrical conductivity was measured with a digital electric conductivity meter (DEC-1-USA).

Analysis of soil nutrient content

The total nitrogen (N) and available phosphorus (P) were determined respectively by micro-Kjeldahl and molybdenum blue methods of Jackson (1973). Exchangeable K was extracted from the soil in ammonium acetate solution (pH 7) and measured with a digital flame photometer (IM-Sambhavi-impex,India) (Jackson, 1973). Soil organic carbon was determined according to Piper (1966).

Preparation of roots and AM assessment

Fixed roots were washed free of FAA, and observed under a dissection microscope (X 20) for AM fungal spores attached to them. After examination, the roots were cut into 1 cm bits, cleared in 2.5% KOH (Koske and Gemma, 1989), acidified with 5 N HCl and stained with trypan blue (0.5% in lacto glycerol). The roots were kept immersed in stain overnight. The stained roots were examined with a compound microscope (X 200 – 400)(Bio-Lab, Newzealand) for AM fungal structures and the percentage of root length colonization was estimated according to magnified intersection method (McGonigle et al., 1990).

Enumeration and isolation of AM fungal spores

One hundred gram soil was dispersed in 1L water and the suspension was decanted through 710- to 38-

mm sieves. The residues in the sieves were washed into beakers. The sievings were dispersed in water and filtered through grided filter papers. Each filter paper was spread on a petridish and scanned under a dissection microscope X 40 magnification and all intact spores were counted. Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were transferred using a wet needle to polyvinyl alcohol-lacto glycerol with or without Melzers reagent on a glass slide for identification. Spores were identified based on spore morphology and sub-cellular characters and compared with original descriptions (Schenck and Perez, 1990). Spore morphology was also compared with the culture database established by INVAM (http://invam.cag.wvu.edu).

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA) and the means were separated using Duncan’s Multiple Range Test (DMRT). Data on AM colonization and spore numbers were arcsine and log transformed [ln (1 + x)] respectively prior to statistical analysis. Pearson’s correlation analysis was used to assess the relationships between edaphic-climatic factors, spore number and root colonization (Zar, 1984).

Results and Discussions

The edaphic factors showed that the soils were acidic in nature (5.4 – 6.9) and organic carbon ranged between 2.2 to 4.6 %. The percent root colonization by AM fungi in _E. cardamomum_ ranged between 19.90 to 86.10. It was recorded maximum during February and minimum during July. The population of AMF spores was calculated. The total AMF spore number varied from 18.66 to 67.00 g\(^{-1}\) and number of spores in _E. cardamomum_ was maximum during June and minimum during September. AMF spores belonging to five species such as _Acaulospora_ sp., _Gigaspora_ sp., _Glomus aggregatum_, _G. fasciculatum_, _G. intraradices_ were recorded from the rhizosphere of _E. cardamomum_ (Plate.2).

The relationship between climatic, edaphic and biotic factors in _E cardamomum_ was studied and is presented in Table.1. Temperature was significantly and positively correlated with EC and negatively with soil N. Rainfall was correlated significantly to soil P and negatively to temperature. Soil moisture was correlated positively to %RLH and % RLC. Rainfall also significantly correlated with %RLA and negatively with spore density. Percent root length hyphae (RLH) were significantly positively correlated with %RLC (Plate.1).

The variations in abundance of AM fungi as assessed by spore number and root length colonization throughout
Table 1. Correlation coefficients of climatic, edaphic and mycorrhizal variables in *Eleteria cardamomum*.

<table>
<thead>
<tr>
<th>Climatic factors *</th>
<th>Soil factors *</th>
<th>Arboreal mycorrhiza *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxi. Tem.</td>
<td>RF</td>
<td>RH</td>
</tr>
<tr>
<td>Arbuscular mycorrhiza *</td>
<td>0.127</td>
<td>0.283</td>
</tr>
<tr>
<td>%RLC</td>
<td>0.049</td>
<td>0.233</td>
</tr>
<tr>
<td>%RLH</td>
<td>0.487</td>
<td>0.375</td>
</tr>
<tr>
<td>%RLV</td>
<td>0.072</td>
<td>0.466</td>
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<td>%RLA</td>
<td>0.239</td>
<td>0.148</td>
</tr>
<tr>
<td>OC</td>
<td>0.457</td>
<td>-0.224</td>
</tr>
<tr>
<td>SK</td>
<td>-0.17</td>
<td>-0.188</td>
</tr>
<tr>
<td>SP</td>
<td>0.372</td>
<td>0.199</td>
</tr>
<tr>
<td>SN</td>
<td>-0.576*</td>
<td>-0.415</td>
</tr>
<tr>
<td>EC</td>
<td>0.830*</td>
<td>0.159</td>
</tr>
<tr>
<td>pH</td>
<td>-0.145</td>
<td>0.127</td>
</tr>
<tr>
<td>SM</td>
<td>-0.191</td>
<td>0.224</td>
</tr>
<tr>
<td>RH</td>
<td>-0.071</td>
<td>-0.267</td>
</tr>
<tr>
<td>RF</td>
<td>0.173</td>
<td>0.523</td>
</tr>
<tr>
<td>Mini.</td>
<td>0.344</td>
<td></td>
</tr>
</tbody>
</table>

*Maxi. Tem., Maximum Temperature; Mini. Tem., Minimum Temperature; RH, Relative humidity; RF, Rainfall.*

The year has attracted considerable attention (Abbot and Rahson 1991). The present investigation clearly indicates the periods and factors that favour root colonization and AMF associated with *E. cardamomum*. During the study period low spore count was recorded in the rhizosphere of this crop. Moreover the low spore density of AMF is due to the acidic nature (pH 5.4-6.9) of the soil. This above finding is supported by Udayian *et al.* (1996). The other reason for low spore number can be the presence of the AMF propagules like the intraradical structures persisting in root which are the main source of inoculum for perennial plants (Baylis, 1969).

During the study period the variation in AMF colonization and spore population in *E. cardamomum* may also be due to seasonal influence. The higher AMF colonization and low spore numbers observed is in agreement with Udayian *et al.* (1996) and Rajesh Kannan (2002). The reduction in spore numbers may result from spore germination and limited spore life span caused by the activity of antagonistic soil microorganisms, which may coincide with root growth (Moss and Brown, 1968; Sutton and Barron, 1972). Sporulation has also been reported to be depressed by hyperparasitic fungi (Steinhank and Nicolson, 1977). Seasonal variations in mycorrhizal spore number were studied previously (Ebbets *et al.*, 1987). In most cases, spores were less abundant during the periods of mycorrhizal formation and became more numerous during periods of roots senescence and or of the growing season (Brundrett, 1991). Higher AMF colonization and low spore number observed in host species, in the present study is in agreement with those noted by Udayian *et al.* (1996) in *Acacia farnesiana* and *A. planifrons* and Rajesh Kannan (2002) in *Casuarina equisetifolia* and *Dalbergia latifolia*.

The results revealed an inverse relationship between %RLC and spore number in *E. cardamomum* which agree with previous studies where higher AM fungal colonization and a low spore number have been noted (Udayian *et al.*, 1996). Under environmental conditions where root growth is continuous the vegetative phase of AM fungi may be actively involved in initiating infection and spreading onto new roots. Root colonization was highest during February in *E. cardamomum* when the soil moisture was moderate in this plant. The reduction in soil moisture level adversely affected the root colonization but enhanced sporulation. However, spore numbers also tended to reduce at very low soil moisture. The extrametrical phase of the AM fungi in the soil, its growth and development is influenced by the edaphic factors. A study on the effect of pH on AM fungi has showed that germination of AMF spores is sensitive to pH. However, the effect of pH on root colonization tends to vary with strains of AMF species (Medeiros *et al.*, 1994).
The %RLC was positively correlated with soil moisture, indicating the response of AMF colonization in roots of fluctuating soil moisture levels (Allen and Allen, 1984). Soil N was correlated positively with spore density and negatively to % RLC in *E. cardamomum*. Soil N plays an important role in influencing the mycorrhizal formation and function mainly through changes in soil pH. Soil N was positively correlated with spore number in *Acacia planifera* (Udayan et al., 1996), however, the effect of N on AM fungal spore abundance is related to other soil factors and to the host with which they are associated (Rohini Iyer et al., 1989). However, soil N was correlated negatively with EC and temperature and positively with spore population and % RLC in *D. latifolia* (Rajesh Kannan 2002).

In *E. cardamomum* spore density was negatively correlated with rainfall. These observations agree with those of Rajesh Kannan (2002) and emphasize that climatic factors can strongly influence AMF colonization (Furlan and Forti, 1973) The influence of soil P on AMF structures is in accordance with the observations of Udayan et al. (1996) where soil P tended to influence root colonization in *A. planifrons*. It is well established that increasing soil P can reduce AMF formation and the inhibition may be due to the direct effect of P on the external hyphal network development or indirectly associated with host P status (Sanders, 1975). However, the effect of P on spore density tends to vary with host species. Native soil often contains AMF spores of more than one species and spore belonging to three AMF genera were isolated from host species of rhizosphere soil. Five species of AMF were recorded in the rhizosphere of *E. cardamomum* during the study period. Generally, more than one AMF species are quite common in the rhizosphere of perennial hosts (Thapar and Khan, 1985), which is substantiated by the recovery of spores belonging to different species in each host.

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References


