Diversity of Mycorrhizal fungi isolated from the rhizospheric soils of various Chilli plants

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Article history:
Received: 17 March, 2014
Accepted: 27 March, 2014
Available online: 28 June, 2014

Keywords:
Chilli plants, mycorrhizal fungal diversity, rhizosphere

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Abstract
Numerous species of soil fungi which flourish in the rhizosphere of plants have shown great potential as biocontrol agents and a few also promote the growth of plants by an improved nutrient supply. The present study focuses on the diversity and dominance of mycorrhizal fungi in rhizosphere of chilli plants. Rhizospheric soil samples were collected from different locations of Mysore, Hassan and Mandya in Karnataka, India during the period extended from February, 2013 to October, 2013. Mycorrhizal fungi were successfully isolated and identified Penicillium sp., Fusarium sp., Pythium sp., Cladosporium sp., Tricoderma sp., Stachybotrys, Aspergillus sp., and Aspergillus niger were of common occurrence. Data obtained from all the soil samples were subjected to Pearson’s Correlation statistical analysis. On the basis of result obtained, Fusarium, Cladosporium, Tricoderma, Penicillium and Aspergillus were found to be highly significant to one or few other rhizospheric fungal microorganism.

Citation:

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Photon Ignitor: ISJN53497294673428062014

1. Introduction

The term rhizosphere is defined as the soil volume adjacent to the roots (Metting, 1993) and represents an area of intense microorganisms and its activity (Warcup, 1950), whose development is favoured by the exudates/organic nutrients exerted through the roots (Lynch, 1990). For sustained agricultural production, use of efficient fertilizer to maintain the soil and plant quality is critical. The application of organic fertilizer has been practiced for more than thousand years in many countries since it provides essential nutrients to plants, fixes nitrogen, improves soil structure, helps in the moisture retaining capacity in various soils and increases microbial activities(Chen et al., 2006; Mokula et al., 2012; Marcia et al., 2010)

Plant growth promoting rhizobacteria have been identified in influencing the growth and yield of many plants by the production of IAA, Phosphate solubilization (Reena et al., 2013; Difluza 2007; Mansoureh et al., 2012). The effects of PGPR on plant growth can be mediated by direct or indirect mechanisms (Sajani et al., 2010). The direct effects have been most commonly attributed to the production of plant hormones such as auxins, gibberellins and fix nitrogen (Manivannan et al., 2012; Shakilabanu et al., 2012). These PGPR also affect growth by indirect mechanisms such as suppression of bacterial, fungal and nematode pathogens by production of siderophores, ACC deaminase activity, catalase, HCN, ammonia, antibiotics, and volatile metabolites etc by competition with the pathogen for nutrients or colonization space (Ashrafuzzaman 2006; Ajay et al., 2012). Enlarged understanding of PGPR genetic diversity will expand the knowledge base regarding beneficial plant-microbe interactions (Kim et al., 2011) and functional analyses of the differential proteins are reported to be directly or indirectly involved in growth promotion in plants (Saveetha et al., 2009). Induce systemic resistance is a plant-mediated mechanism which resembles classic
pathogen-induced resistance, in which non-infected parts of previously pathogen-infected plants become more resistant to further infection (Shakilabanu et al., 2012). They also induce systemic resistance (ISR) against various pests and diseases. Plant growth promoting rhizobacteria (PGPR) are being exploited commercially (Ramamoorthy et al., 2001). Use of both living mycelium of *Penicillium chrysogenum* and its culture filtrate induces resistance independently in model plant *Arabidopsis* (Motahar et al., 2012). Effects of Plant growth promoting rhizobacteria and mycorrhizal fungi on citrus nurseries (Amelia et al., 1996; Abbott et al., 1985), sunflower, potato (Otroshy et al.), kiwifruit (Actinidia delicosa) have also been studied (Yasar et al., 2010). They also destroy nematodes continuously in virtually all soils because of their constant association with nematodes in the rhizosphere (Ambreen et al., 2010; Cristina et al., 2013).

The significance of rhizosphere microorganisms, especially mycorrhizal fungi and bacteria, in polluted soils can be enormous, since they are able to increase the tolerance of plants against abiotic stress, e.g. by an improved nutrient supply or by detoxification of pollutants (Katarzyna, 2011).

### 3. Methodology

#### 3.1 Soil analysis

The rhizosphere soil samples were collected from 3 different chilli plant cultivating areas: Mysore, Hassan and Pandavapura regions of Karnataka, India during the period extended from February, 2013 to October, 2013. Soil samples were collected from chilli plant rhizosphere in order to investigate the diversity of fungi associated with the roots of chilli plants. Top soil was removed and soil at 15 cms depth was collected. Roots were pulled out soil surrounding the roots were collected in sterile plastic bags and preserved at lower temperature. Analysis for rhizosphere microorganisms was done within 2 days of soil sample collection.

#### 3.2 Sample processing

The soil samples were desiccated, compacted and sieved, from which 1gm soil was suspended in 9ml saline and swen at 150 (rpm) for 20 minutes at 37°C. Supernatant was serially diluted (10^{-1} to 10^{-10} in triplicates) and inoculated in Modified Rose Bengal Agar (MRSA). Using spread plate method; soil suspensions were spread on MRSA plates and incubated for 3-5 days at room temperature for the growth of rhizospheric fungi (Aneja, 2001).

#### 3.3 Microscopic analysis

After the incubation period, plates were checked for the growth of fungi. Fungal identification was done by observing the macroscopic and microscopic structures (Bajjal et al., 1980).

#### 3.4 Statistical analysis

The diversity of fungal organisms that occurred in different samples was subjected to Pearson’s Correlation matrix.

### 4. Justification of Research

Mycorrhizal fungi colonizing the roots of plants can enhance the growth of plant directly or indirectly. Therefore, they are environmental friendly and offer sustainable approach to increase production of crops and health. Hence, it is a need to isolate and identify the mycorrhizal fungi.

### 5. Results

#### 5.1 Identification

In the present study, Mycorrhizal fungi were successfully isolated and identified as *Penicillium sp.*, *Fusarium sp.*, *Pythium sp.*, *Cladosporium sp.*, *Tricoderma sp.*, *Stachybotrys*, *Aspergillus sp.*, and *Aspergillus niger* (Plate 1). Diversity of Mycorrhizal fungi in rhizospheric chilli soils collected from various places is shown in Table 1 (3 trails).

#### 5.2 Comparison using Statistical analysis

Diversity of Mycorrhizal fungi in rhizospheric chilli soils collected from various places is compared using Pearson’s Correlation statistical analysis (Table 2).
In Table 2 (h - horizontal, v - vertical) the diversity of *Stachybotrys* (6v) is positively significant to the diversity of same organism (5h) in Mysore region, but *Stachybotrys* of Mysore and Mandya region is negatively significant to *Aspergillus* (18v). The diversity of *Aspergillus niger* (8v) and *Tricoderma* (13v) of Mysore region is negatively significant to *Fusarium* (2h) of Mysore region. *Tricoderma* sp. diversity of Hassan (13v) and Mysore (14v) region is positively significant to the diversity of

**Table 1 – Diversity of Rhizospheric fungi**

<table>
<thead>
<tr>
<th>Location</th>
<th><em>Fusarium</em> sp.</th>
<th><em>Stachybotrys</em></th>
<th><em>Aspergillus</em> niger</th>
<th><em>Pythium</em> sp.</th>
<th><em>Tricoderma</em> a sp.</th>
<th><em>Aspergillus</em> sp.</th>
<th><em>Penicillium</em> sp.</th>
<th><em>Cladosporium</em> sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hassan</td>
<td>2 1 6</td>
<td>2 9</td>
<td>6 2 3</td>
<td>6 4 5</td>
<td>4 1 2</td>
<td>9 5 4</td>
<td>3 1 1</td>
<td>2 1 3 0 0 0</td>
</tr>
<tr>
<td>Mysore</td>
<td>1 6 1 0</td>
<td>1 0 0 0</td>
<td>7 3 2</td>
<td>4 5 3</td>
<td>5 1 3</td>
<td>0 2 0</td>
<td>3 2 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>Mandya</td>
<td>1 4 1 9</td>
<td>1 1 5 2 4</td>
<td>5 2 4</td>
<td>5 2 6</td>
<td>6 5 6</td>
<td>2 0 0 1</td>
<td>2 4 0 3 1 2</td>
<td></td>
</tr>
</tbody>
</table>

*Aspergillus niger* of Mysore (8h) and Hassan (7h) region.

*Cladosporium* diversity of Hassan (22v) region is positively significant to *Aspergillus sp.* (18h) diversity of Mandya region and *Stachybotrys* diversity of Mysore (5h) and Mandya (6h) region. *Cladosporium* (24v) diversity of Mandya region is positively significant to *Aspergillus niger* (7h) diversity of Hassan and *Tricoderma* (14h) diversity of Mysore region. *Penicillium* (19v) diversity of Hassan is negatively significant to *Pythium* (11h) diversity of Mysore; the same (20v) of Mandya

Plate 1 - Microscopic view (10X and 40X) of various rhizospheric fungi isolated from chilli plant. 1. *Aspergillus* sp. 2. *Fusarium* sp. 3. *Pythium* sp.

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2  | .929|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3  | -.438| -.075| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4  | -.689| -.908| -.350| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5  | .887| .655| -.803| -.277| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6  | .887| .655| -.803| -.277| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 7  | .461| -.756| -.596| .961| .000| .000| .000| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 8  | .929| -.100| .075| .908| -.655| -.655| .756| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 9  | .283| -.619| -.737| -.891| .189| .189| .982| .619| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 10 | -.620| -.866| -.434| .966| -.189| -.189| .982| .866| .929| .1  |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 11 | -.538| -.189| .993| -.240| -.866| -.866| -.500| .189| -.655| -.327| .1  |     |     |     |     |     |     |     |     |     |     |     |
| 12 | .283| -.091| .986| .500| .693| .693| .721| .091| .839| .577| -.961| .1  |     |     |     |     |     |     |     |     |     |     |
| 13 | -.929| -.100| .075| .908| -.655| -.655| .756| .100| .961| .866| .189| .091| .1  |     |     |     |     |     |     |     |     |     |
| 14 | .461| -.756| -.596| .961| .000| .000| .100| .756| .982| .982| -.500| .721| .756| .1  |     |     |     |     |     |     |     |
| 15 | -.751| -.454| .923| .038| -.971| -.971| -.240| .454| -.419| -.052| .961| -.846| .454| -.240| .1  |     |     |     |     |     |     |
| 16 | -.843| -.982| -.115| .971| -.500| -.500| .866| .982| .756| .945| .000| .277| .982| .866| .277| .1  |     |     |     |     |     |
| 17 | -.046| .327| .918| -.693| -.500| -.500| -.866| -.327| .945| .756| .866| -.971| -.327| -.866| .693| -.500| .1  |     |     |     |     |
| 18 | .887| .655| -.803| -.277| .100| .100| .000| -.655| .189| -.189| -.866| .693| -.655| .000| -.971| -.500| -.500| .1  |     |     |
| 19 | .538| -.189| .993| .240| -.866| -.866| -.500| -.189| .655| .327| .100| `.961| -.189| .500| -.961| .000| -.866| .866| .1  |
| 20 | -.843| -.982| -.115| .971| -.500| -.500| .866| .982| .756| .945| .000| .277| .982| .866| .277| .100| -.500| -.500| .000| .1  |
| 21 | -.538| -.189| .993| .240| -.866| -.866| -.500| -.189| .655| .327| .100| `.961| -.189| .500| -.961| .000| -.866| .866| -.100| .000| .1  |
| 22 | .887| .655| -.803| -.277| .100| .100| .000| -.655| .189| -.189| -.866| .693| -.655| .000| -.971| -.500| -.500| .100| .866| -.500| -.866| .1  |
| 23 | .999| .945| -.397| .721| .866| .866| -.500| -.945| .327| -.655| -.500| .240| .945| -.500| .721| -.866| .000| -.866| .500| -.866| .500| .866| .1  |
| 24 | .461| -.756| -.596| .961| .000| .000| .100| .756| .982| .982| -.500| .721| .756| .100| .240| .866| .866| .000| .500| .866| .500| .500| .100| .1  |

region is positively significant to Pythium (11h) diversity of Mysore region. Penicillium (20v) diversity of Mysore region is positively significant to Aspergillus (16 h) diversity of Hassan region.

6. Discussion

The present investigation showed that the diversity of Fusarium is antagonistic to A. niger and Trichoderma. Cladosporium is antagonistic to Fusarium, Stachybotrys, Aspergillus and Trichoderma. Trichoderma and
Penicillium are antagonistic to Aspergillus. Aspergillus is antagonistic to Stachybotrys.

From the results we can infer that rhizospheric region of chilli plants harbors a wide variety of fungal microorganism and the interaction between them may be competitive or antagonistic (Niranjan et al., 2005), but at the same time a few among them may a positive role in supporting the growth of chilli plant. There are many papers related to the advantages and screening of such microorganisms from crop plants particularly rice, maize, potato and sugar cane.

Conclusion

Penicillium sp., Fusarium sp., Pythium sp., Cladosporium sp., Tricoderma sp., Stachybotrys, Aspergillus sp., and Aspergillus niger were found to be the most diverse rhizospheric fungi in the chilli soils.

These observations reserve the fact that the rhizospheric region of chilli plant harbors a wide range of mycorrhizal fungi are highly antagonistic to one or more rhizospheric fungal or bacterial microorganism, but sometimes show an effective promotion of chilli plant growth. Hence, the use of Mycorrhizal fungi to promote chilli plant growth offers an attractive way to replace chemical fertilizers and reduce the use of pest control agents.

Research Highlights

The present paper is an attempt to isolate, identify, determine the diversity and dominance of mycorrhizal fungi isolated from the rhizospheric soil of chilli plants from different locations.

Limitations

This project was limited by certain factors. One of the main limitations was to collect samples from various locations was a limitation to this project.

Acknowledgements

The author is grateful to Prof. Prabhakara, K.V., Principal and Dr. Shankar P. Hosmani, Convenor, Research Cell and HOD of Biotechnology department, for their encouragement and for providing financial assistance under the UGC – CPE minor research project.

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