Root fungal diversity associated with three Disa species

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Introduction

Orchid mycorrhizas are mutualistic interactions between fungi and members of the Orchidaceae (Dearnaley 2007).

Orchids have ‘dust seeds’, that are very small (0.3-14 µg) consisting of minute embryos that lack endosperm and have few reserves (Burgeff 1936; Arditti & Ghani 2000).

The presence of fungi assist in germination of seeds (Tsutsui & Tomita 1986; Clements 1988; Rasmussen et al 2009).

Orchids are highly depended on the provision of nutrients by mycorrhizal fungi during early seedling development (Smith & Read 2008).

In adult orchids, the mycorrhizal associations are important for mineral nutrition (Gebaur and Meyer 2003; Smith & Read 2008; Brundrett 2009).

Orchid mycorrhizal (OM) research in South Africa has received little attention.
Aims and Objectives

Objective:

– To identify the mycorrhizal fungi interacting with *Disa bracteata*, *D. cornuta* and *D. polygonoides*

Aims:

– Confirm mycorrhizal colonization of roots
– Isolate and identify associated root fungi (culture dependent approach)
– Assess root fungal biodiversity using culture independent approach
Mycorrhizal colonization

- Roots were cleared and stained, observed microscopically using light microscope (Kristiansen et al 2004)
- Mycorrhizas produce intracellular coils called pelotons within roots cells (Smith and Read 2008)
- All three Disa species were colonized by mycorrhizal fungi
Fungal isolates

- Root pieces were surface sterilized and plated on various media
- Single fungal colonies were sub-cultured and molecular identified
- PCR was conducted using ITS1F and ITS4 primers (Gardes and Bruns 1993)
- Purified PCR products were sent for Sanger sequencing
Fungal Isolates

- Chaetomium aureum strain 100%/96%/0.0
- Penicillium sp. 99%/98%/0.0
- Trichoderma sp. 100%/93%/0.0
- Talaromyces radicus 96%/98%/0.0
- Oidiodendron sp. 99%/99%/0.0
Diversity

- Roots were surface sterilized and stored in RNA later
- DNA was extracted, PCR was conducted using ITS1F and ITS4 primers (Gardes and Bruns 1993)
- Purified PCR products were cloned using pGEM T-Easy Vector
- Plasmids were sent for Sanger sequencing
- Sequences were aligned and submitted for comparison to GenBank (https://www.ncbi.nlm.nih.gov/Genomes/index.html) and UNITE (https://unite.ut.ee/analysis.php)
<table>
<thead>
<tr>
<th>Orchid species</th>
<th>Clones</th>
<th>Description</th>
<th>Query cover in Percentage (%)</th>
<th>Identification Percentage (%)</th>
<th>E-value</th>
<th>Accession Number</th>
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<tbody>
<tr>
<td><em>Disa cornuta</em></td>
<td>DC1</td>
<td>Epicoccum nigrum</td>
<td>93%</td>
<td>99%</td>
<td>0.0</td>
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<td></td>
<td>DC2</td>
<td><em>Tulasnella sp.</em></td>
<td>78%</td>
<td>96%</td>
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<td>JX514389.1</td>
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<td></td>
<td>DC3</td>
<td>Fungal sp. strain</td>
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<td>KU839098.1</td>
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<td>DC4</td>
<td>Helotiales sp.</td>
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<td>100%</td>
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<td>DC5</td>
<td>Uncultured fungus</td>
<td>89%</td>
<td>98%</td>
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<td><em>Disa polygoniodes</em></td>
<td>DP1</td>
<td>Terfezia boudieri</td>
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<td>100%</td>
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<td><em>Disa brecteata</em></td>
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<td>0.0</td>
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</table>
Conclusion

- *D. polygonoides* and *D. cornuta* are associated with *Tulasnella* (Basidiomycota) a known orchid mycorrhizal fungus
- *D. breceata* associates could not be sufficiently identified, but further cloning is being done using more orchid specific primers
- *Oidiodendron sp.* Isolated from roots is a known ericoid mycorrhizal fungus (Ascomycota) and may be associating with orchids, this requires further investigation.


Acknowledgments

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Thank you