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The evidence of mycorrhizal fungi and dark septate endophytes in roots of *Chlorophytum borivillianum*

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Mycorrhizal and dark septate endophyte in *Chlorophytum borivillianum* cultivated in different parts of south- west India was found. Dark septate fungi with melanized hyphae and microsclerotia were documented. During culturing of the microsclerotia-containing roots, fungi with '*Rhizoctonia*-like' characters developed. The fungi showed reluctance in sporulation but produced microsclerotia in the medium. Specific taxa and their ecological role can be attributed only after detailed studies as different strains of single taxa may vary greatly in their physiology.

Key words : Mycorrhiza, Dark septate endophytes, *Rhizoctonia*, microsclerotia, sporulation

Abbreviations: VAM – Vesicular arbuscular mycorrhiza, DSE – Dark septate endophyte

Introduction

Mycorrhizal fungi form mutualistic associations with the roots of terrestrial plants, providing them with phosphorous and other nutrients in exchange for photosynthate (ALLEN 1991, SMITH and READ 1997). It is estimated that about 87% of terrestrial plants form mycorrhizal associations (STOYKE and CURRAH 1993). Mycotrophy is considered ancestral feeding in vascular plants. Vesicular arbuscular mycorrhiza (VAM) fungi are the most ancient and widespread obligate symbionts (MILLER et al. 1999, MUKERJI 1996). Mycorrhizal fungi are well adapted for nutrient acquisition, their small size allowing them to act as microscopic pipelines that can transport carbon and minerals to and away from the plant (BARROW 2004). They are well adapted to function in lower water potentials and hence can alleviate drought stress even in arid ecosystems (BARROW and AALTONEN 2001, GRIFFIN 1979). In addition to mycorrhiza, the roots of herbaceous plants may get frequently colonized by fungi with dematiaceous septate hyphae, which are often sterile in culture (NEWSHAM 1999). Such fungi are collectively called dark septate endophytes (DSE). Dark septate endophytes are a miscellaneous group of ascomycetous anamorphic fungi that colonize root tissues intra- or intercellularly (JUMPONEN 2001). They may have important functional relationship with host plants, but these functions and colonization processes remain unknown (OHKI et al. 2002). The fungi may act as a weak pathogen, a saprotroph on senescent root

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tissues or a mutualist (ADDY et al. 2005). They produce extensive wefts on the root surface and intra-corticular sclerotia consisting of darkly pigmented and irregularly lobed, thick walled hyphae (STOYKE and CURRAH 1993).

Chlorophytum borivillianum Sant. et F. is an important medicinal plant, widely cultivated in different parts of India, especially in the northern region. The plant, belonging to the Liliaceae family, is an annual herb with tuberous roots. The tuberous roots have aphrodisiac properties (DAVE et al. 2003) and are used in the treatment of arthritis, diabetes and against general debility (LAVANIA et al. 2005). Saponins of the plant are gaining popularity as a substitute for Viagra. The saponin called chloromaloside – A has cytotoxicity against cancer cell lines (KAUSHIK 2005). Due to the immense economic importance, the plant is now widely cultivated in the state of Kerala. Indigenous medicinal plants show varied mycorrhizal colonizations. With the growing importance of natural drugs, overexploitation of the plant in its natural habitat takes place and hence it is being cultivated. However the use of pesticides and fertilizers for enhancing the growth affect the quality of the medicine and this can be mitigated by inducing mycorrhizal associations (MATHEW and MALATHY 2007). The present study focuses on the natural colonization of VAM fungi and dark septate endophytes in roots of *Chlorophytum borivillianum.*

Materials and method

Five distinct areas in the state of Kerala differing in their microclimates were selected. The plant was introduced in these areas a decade ago and is now widely cultivated. From each area, 20 plants in their flowering stage were randomly collected. The mycorrhizal colonization percentage in the roots samples was estimated following the procedure of PHILIPS and HAYMAN (1970). The roots were cleaned free of soil particles, cut in to 1cm bits and were fixed in FAA for 24h. Root segments were cleared by autoclaving with 10% KOH solution (Merck, Mumbai, India). The alkalinity of samples was then neutralized with 1% HCl (Merck, Mumbai, India). For staining, the root segments were heated with 0.05% trypan blue (Loba chemie, Mumbai, India) in lactophenol reagent. Twenty-four root segments from each plant were scanned for the presence of mycelium, vesicles, arbuscules of the mycorrhizal fungi. The nature of vesicles, arbuscules and intercellular hyphae were studied to determine the taxa. The root segments were also examined for septate hyphae and microsclerotia characteristic of dark septate endophytes.

The roots of the plant showing mature microsclerotia (seen as black spots with naked eyes) were surface sterilized and were cultured in potato dextrose agar (Merck, Mumbai, India). The hyphal nature of the septate fungi, similar to those seen in root bits, was studied. The culture was screened for spores and microsclerotia at regular periods of time.

Results

Mycorrhizal associations were found in all the samples studied. Vesicles typical of VAM fungi were seen (Fig. 1). In all the samples, the colonization percentage was above 90%. The aseptate colonization hyphae grew parallel to each other and to the root axis and were interconnected through right angles (H connections). The vesicles were large, round and oval in shape.

The samples studied also showed dark septate endophytes in the roots. The hyphae stained well, with septation clear even with low percentage trypan blue. The samples showed a colonization percentage above 80%, the average number of microsclerotia per root bit being six (Tab. 1). In the early stages of development, microsclerotia were stained dark blue (Fig. 2, 3). However with development, cell wall became thick with high melanization (Fig. 4–6). Mature microsclerotia were seen with the naked eye as dark spots in the root bits. On culturing, septate hyphae developed with branching angle of 90° and small constriction at branch points, indicating a '*Rhizoctonia-like*' nature. No sporulation was noticed even after three months of culture. However microsclerotia were found to occur after 8 days of culture.

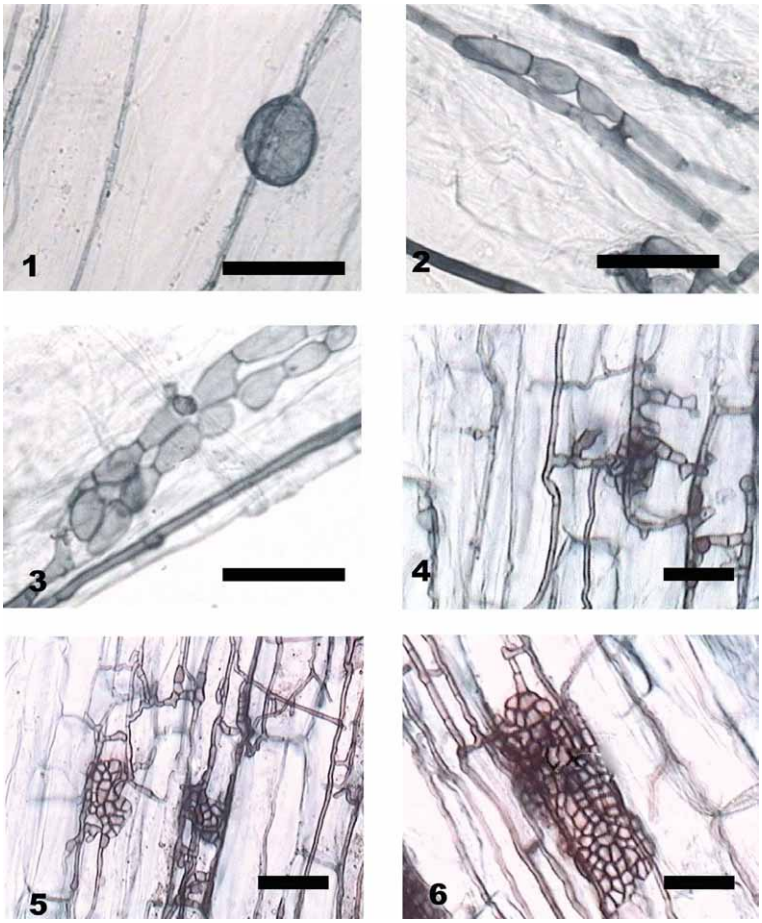

Fig. 1–6. Vesicular arbuscular mycorrhiza (VAM) with Dark septate endophyte. **1 –** vesicle of VAM fungi; **2–6 –** dark septate endophytes. **2–3 –** initiation of microsclerotia; **4–5 –** developing microsclerotia; **6 –** mature microsclerotia. Bars denote 10um.

Discussion

Mycorrhizal fungi occur in nearly all soils throughout the world and form symbiotic associations, the fungal symbionts inhabiting the root forming a second absorptive organ for the host (TRAPPE 1996). The presence of mycorrhizal endophytes in plants is influenced by factors such as species diversity, degree of stability of habitat, extent of fertilizer usage and edaphic factors like season, soil texture and moisture content (NICOLSON 1960, RAGHUPATHY and MAHADEVAN 1991). Even though *Chlorophytum borivillianum* is new to the locality and studies were conducted in non-inoculated plants, this high percentage of colonization implies suitable ecological factors in this area. Mycorrhizae are known to control the deleterious effects of pathogenic microbes by chemical, physiological & morphological alternations in the host. They provide long-term benefits by improving the fitness of the plant and quality of the seeds. This in turn will have a profound effect on offspring vigor (JUMPONEN 2001). With organic farming and the role of mycorrhiza as a biofertilizer gaining momentum, the high percentage of colonization implies the opportunity for large-scale cultivation of the plant in this area. The parallel growth of hyphae and 'H' connections indicate the mycorrhiza to be of the 'Arum series' and the oval shaped vesicles indicate the possibility of Glomales being the taxon.

Dark septate endophytes are conidial or sterile fungi, likely to be ascomycetous and to colonize plant roots. They occur from the tropics to Arctic and alpine habitats and comprise a heterogeneous group that functionally and ecologically overlaps with soil fungi, saprophytic rhizoplane-inhabiting fungi, obligately and facultatively pathogenic fungi and mycorrhizal fungi (JUMPPONEN and TRAPPE 1998). It is reported that many dark septate endophytes possess a wide range of enzymes, which enable them to utilize diverse organic matter (CALDWELL et al. 2000) This may play a great role in their ability to withstand drought, cold and nutrient stress. Septate endophytes like *Heteroconium chaetospira and Phialocephala fortinii* can be used as an effective control of Verticillium wilt in egg plant seedling (NARISAWA et al. 2002). However, in some experiments, *P. fortinii* showed pathogenic relations to the host plant (WILCOX and WANG 1987). The cultured strains showed characteristics similar to the genus *Rhizoctonia.* Many dark septate endophytes even though ascomycetous show '*Rhizoctonia*-like' features. The exact genus could not be attributed due to the reluctance in sporulation and frutification. Most DSE may get sporulated but require long duration and specialized conditions. Even after assigning the fungi to specific taxa, no clear generalization of their ecological role can be made, as different strains of a single anamorph taxon can vary greatly in their physiological characters. Thus the exact role of the fungi, stimulatory or inhibitory, on *Chlorophytum* cannot be assessed with out further work, which is in progress.

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