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Isolation of mycorrhizal fungi in selected epiphytic orchids and investigation of the effect of potential host bark extracts on seed germination under *in vitro* condition

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Orchids (Family: Orchidaceae) are one of the largest, diverse plant groups in the world with high ornamental value. Orchid seed germination, in nature, is reported to be influenced by the mycorrhizal fungi associated in the bark of the host plants. These fungi penetrate the seeds by their hyphae and supply the nutrients for developing embryo resulting the formation of a globular shape structures referred as protocoms. Fungal hyphae infect the cortical cells of the orchids and forming pelotons, that are digested by the orchid cells for their nutrition. This study was focused on isolation of mycorrhizal fungi from six selected epiphytic orchids: *Dendrobium aphyllum*, *Dendrobium* sp., *Bulbophyllum* sp., *Eria* sp., *Coelogyne* sp., an unidentified species, and evaluation of *in vitro* seed germination using host bark extracts inoculated with isolated fungi from orchid roots. Specimens of wild orchids and the host plants were collected from their natural habitats in low-land wet zone and montane zone in Sri Lanka. The mycorrhizal fungi in roots of selected orchid species were isolated and identified as *Trichoderma* sp. and *Rhizoctonia* sp. by comparing with the authenticated morphological characters. Preliminary phytochemical screening was performed for each bark extract of host plants using TLC. Presence of secondary metabolites including alkaloids, flavonoids, terpenoids and phenolic were observed in all the bark extracts. According to the spot observations of TLC, although similar type of secondary metabolites was present in the bark extracts of hosts plants, intensity of the secondary metabolites was detected as different. The TLC analysis revealed the presence of high concentration of chemical compounds in methanolic and aqueous extracts while less amounts were in dichloromethane and hexane extracts. *In vitro* seed germination of the orchid species was assessed using the V/V basis of host bark extracts with the inoculation of isolated fungi from orchid roots. Effect of host bark extracts with mycorrhizal fungi inoculum on seed germination of orchid seeds was analyzed by performing two-way ANOVA using Minitab statistical software. Comparatively high percentage of seeds were germinated in the aqueous extracts (~30%) and hexane extracts (~25%) while very low percentage was in methanolic (0%) and dichloromethane (~0%) extracts of host barks. The findings suggest that secondary metabolites present in the barks of the plants influence the promotion or inhibition of the orchid seed germination whereas fungal inoculation too has a significant effect on orchid seed germination.

Keywords: Secondary metabolites, Phytochemical, Fungal hyphae, Protocoms