Biocontrol potential of endophytic fungi associated with weedy and wild rice species in Sri Lanka

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Plant fungal endophytes are known to influence the ecology and fitness of host plants while enhancing their resistance against biotic and abiotic stress conditions. Fungal endophytes, amidst their other uses in agriculture, are considered as potential biocontrol agents to mitigate pests and diseases of crop plants. In views of this, this study was aimed at isolating fungal endophytes from weedy and wild rice species in Sri Lanka and investigating their antifungal activity against the fungal rice pathogen *Rhizoctoniasolani*. In addition, an attempt was made during this study to optimize a transformation technique to fluorescently label fungi with the long term aim of using the optimized technique to label fungal endophytes to study their compatibility and colonization patterns in different host plants. A total of 22 weedy (*Oryza sativa* f. *spontanea*) and wild rice (comprising of *O. granulate*, *O. rufipogan*, *O. rhizomartis*, *O. nivara* and *O. echingeri*) samples were collected from the Batalegoda Rice Research Institute and Gannoruwa Plant Genetic Resource Center respectively. Putative fungal endophyte isolation using standard procedure yielded 77 and 205 morphologically distinct isolates from the weedy and wild rice species respectively. Dual culture assay of 50 isolates (from the total of 282) revealed the ability of 5 isolates to inhibit the radial colony growth of *R. solani* on 1% PDA at room temperature by 80 to 95% compared to the control (*R. solani* alone). Ethyl acetate crude extracts of the two most promising isolates (according to dual culture assay), B.W.E.R.L 0005 and G.W.I.G.L 0001, tested using the well diffusion method demonstrated significant ($P<0.05$) inhibition of radial colony growth of *R. solani* at 50 µL of fungal extract (20 mg/mL) per well compared to the negative control (50 µL of DMSO), while inhibition of *R. solani* by the extract of isolate B.W.E.R.L 0005 was comparable with the positive control (20 mg/mL hygromycin). ITS sequences of B.W.E.R.L 0005 and G.W.I.G.L 0001 showed 99 and 98% identity with ITS sequences of *Xylariafeejeensis* (KY951907.1) and *Trichodermaharzianum* (KC561083.1) respectively. Polyethylene glycol (PEG) mediated transformation of Fusarium protoplast with pYH2a (harbor a hygromycin resistant hph gene and a histone H2A gene fused to yellow fluorescence protein gene) and pPN1688 (harbor hygromycin resistant hph gene) plasmids separately resulted in hygromycin resistant transformants on selective media and hyphae with fluorescently labelled globular structures (presumed to be nuclei) when observed under fluorescent microscope, confirming the successful transformation of Fusarium protoplasts and expression of histone H2A gene fused to yellow fluorescence protein.