

Proceedings of the
International Forestry
and Environment
Symposium
Volume 26
(2022)

**TWENTY SIXTH
INTERNATIONAL
FORESTRY AND
ENVIRONMENT**

**Symposium
2022**

Developments in Forestry and Environmental Science in 2021



**20th & 21st January 2022 at
University of Sri Jayewardenepura**



Symposium Organizing Committee
**Department of Forestry and Environmental Science,
University of Sri Jayewardenepura,
Nugegoda, Sri Lanka.**

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Diversity of the Endophytic Fungal spp. in Selected Rice (*Oryza sativa* L) Varieties of Sri Lanka and their Hydrolytic Enzyme Producing Abilities

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Abstract

Rice (*Oryza sativa* L.) is the staple food in Sri Lanka and with increases in population, the demand for rice has also increased requiring higher yields. The use of endophytic fungal (EF) assemblages has been successful in increasing the yields in a number of crops including rice. However, the existent knowledge on the diversity among the species of EF assemblages associated with different rice varieties is hardly sufficient for this purpose. Therefore, this study was aimed at identifying and assessing the diversity of EF present in three newly improved rice varieties At 362, Bg 352, Bw 367 and one traditional variety i.e. Suwandel grown in different geographical locations/climatic zones of Sri Lanka. Healthy plant samples of each rice variety was collected during the Maha and Yala seasons in 2018/2019 from Anuradhapura, Kurunegala, Gampaha and Kalutara districts in Sri Lanka. Endophytic fungi were isolated from leaves, stems and roots of the four rice varieties using previously optimized protocols. Identifications of the isolated fungal spp. was carried out using morphological and molecular characteristics. Species were identified by PCR amplification of the Internal Transcriber Spacer (ITS) regions and comparing their sequences with those of well characterized/type strains in the National Centre for Biotechnology Information (NCBI) database. 1,920 plant segments used for isolations yielded 26 fungal genera and 39 fungal spp. The most frequently isolated and dominant species among all rice varieties were *Microdochium fisheri*, *Dendryphiella* sp and *Penicillium oxalicum*. Species diversity was analyzed using Shannon Wiener's (H') and Simpson's dominance (1-D) indices and a high diversity of different fungal spp. were observed in Bg 352 collected from Kalutara during the Yala season. As EF have been reported to produce extra cellular enzymes as a means of showing mycoparasitic activity, selected EF isolates were screened in enriched media for production of Chitinase and Protease enzymes using standard plate assays and Glucanase enzyme production was analyzed by Dinitro Salicylic Acid assay. Out of the tested isolates, *Rhizopus microsporus* produced a significantly high ($p \leq 0.05$) level of chitinase, while *Aspergillus fisheri* showed a significantly high ($p \leq 0.05$) protease production. *Penicillium oxalicum* showed a significantly high ($p \leq 0.05$) glucanase production. Thus, findings of this study postulate rich EF assemblages in Sri Lankan rice varieties which could potentially be a foundation for studies on novel models of rice-fungal mutualism. Rice endophytes also produce extra-cellular hydrolytic enzymes that may contribute towards controlling rice pathogens.

Keywords: Endophytes, Fungal diversity, Rice varieties, ITS region