

**ISOLATION AND MOLECULAR DETECTION OF *RHIZOCTONIA* AND *FUSARIUM* CAUSING ROOT ROT IN LEGUMES**

**P.H.S. Piyadigama<sup>1</sup>, W.A.R.T. Wickckramaarachchi<sup>2\*</sup> and V.A. Sumanasinghe<sup>1\*</sup>**

<sup>1</sup>*Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka*

<sup>2</sup>*Division of Plant Pathology, Horticultural Crops Research & Development Institute, Gannoruwa, Sri Lanka*

*\*wartwa@gmail.com*

Grain legumes alone contribute about 33 % of dietary protein nitrogen to our diet. The quality and quantity of legumes depend on both biotic and abiotic factors. Among the biotic factors, soil borne fungi are prevalent worldwide. Early detection of these fungi is required for minimizing crop losses. *Rhizoctonia solani* from “yard long bean”, *Fusarium oxysporum* from “yard long bean” and “winged bean” were isolated and used as positive control in PCR, for the detection of fungi based on ITS regions of disease affected root tissues. DNA was extracted from pure cultures of fungi and disease affected root tissues using the same modified CTAB extraction protocol. ITS-fu-F and ITS-fu-R primers gave a 389 bp product for *Fusarium* spp in yard long bean and winged bean. The same plants were positive with a 340 bp fragment for *F. oxysporum* when using FO-F and FO-R primer pair. For *R. solani* ITS1 and ITS4 primers were used and a 650 bp product was observed in bean and yard long bean. Yard long bean showed positive results for both *F. oxysporum* and *R. solani* proving mix infection of root rot. PCR product of *R. solani* was sequenced and based on BLAST analysis, the isolate exhibited a strong homology (97 %) with 38 hits of *R. solani* isolates out of 74 hits deposited in NCBI database. Moreover, they too are ITS and 5.8s regions. Accordingly, it can be safely concluded that isolates used in this study are precisely from *R. solani*. The findings of this study indicate that molecular methods using both DNA extracted from pure cultures and root tissues are equally effective for early diagnose of fungi, avoiding necessity of obtaining pure cultures.