Genetic Diversity of *Rhizoctonia solani* causing Sheath Bligth of Rice in the Philippines

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INTRODUCTION

The soilborne basidiomycete *Rhizoctonia solani* Kühn *(Thanatephorus cucumeris)* occurs world-wide and is a destructive plant pathogen with a wide host range (Adams, 1988). Isolates of *R. solani* are assigned to anastomosis groups (AGs) based on the occurrence of hyphal fusion within members of designated AGs. AG-1 is the *R. solani* group frequently associated with rice sheath bligth which become an increasing concern for rice production in South and Southeast Asia, especially in intensified production systems (Savary et *al.*, 1995).

Several reports indicated important genetic variations within and between anastomosis groups of *R. solani* based on biochemical and molecular techniques (Mordue et *al.*, 1989; Vilgalys and Gonzales, 1990; Cubeta et *al.*, 1991; Duncan et *al.*, 1993; Liu and Sinclair 1993; Balali et *al.*, 1996; Keijer et *al.*, 1996).

In this study, we used RFLPs and ITS-PCR to evaluate the amount the genetic diversity among populations of *R. solani* fom different regions of Philippines during different cultural seasons.

MATERIALS AND METHODS

Isolates: One hundred and twenty isolates of *R. solani* were collected from rice fields in different regions of Philippines. They were recovered from rice leaves, sclerotia and soil samples. Fungal mycelium was cultivated on potato dextrose broth (PDB; Difco) liquid medium for 4 days at 25°C in the dark.

RFLP analysis: For each isolate approximately 4 to 5 µg of total genomic DNA was digested overnight at 37°C with 20 to 30 U of the following restriction enzymes: *Eco*RI, *Hind* III, *Pst* I and *Xho* I (Eurogentec). The hybridization probes used were obtained from a *Pst* I library of anonymous DNA fragments of *R. solani* AG-1 (Table 1) (Pettway et *al.*, 1997).

Table 1: DNA probes tested

Probes	Characteristics
RS 5	1kb *Pst* I fragment in pUC18, Repetitive
RS12	4 kb " Single-copy
RS16	1.5 kb " Repetitive

ITS -PCR: The ITS (Internal Transcribed Spacer) region of the nuclear rDNA unit was amplified using PCR with primers ITS1F and ITS4 (White et *al.*, 1990). Amplification reactions were performed in a total volume of 25 µl, containing 10 mM Tris Hcl (pH8.3), 50 mM Kcl, 1.5 mM $MgCl_2$, 50 µM dNTP, 20 pmol for each primer, 25 ng of genomic DNA, and 1 U of *Taq* polymerase (Eurogentec). The parameters used for the amplifications were: 94°C for 5 min followed by 30 cycles of 1 min at 94°C, 1 min at 58°C and 2 min at 72°C. A cycle with 15 min at 72°C was conducted after the 30 cycles.

The PCR fragments were digested with 4-base recognition enzymes *Hae* III, *Hin6* I,*Msp* I and *Nde* II (Eurogentec, Belgium).

RESULTS AND DISCUSSION

1 - RFLPs

Among the 12 probe-enzyme combinations used, 1 detected RFLPs among the isolates and 4 allowed to establish repetitive DNA patterns.

1 - Using the probe RS12 for hybridization to Southern blots of *Hind* III digested total DNA, eleven different patterns (genotypes) were obtained for the 120 isolates tested (Fig. 1). In total, 9 different restriction fragments (alleles) were found ranging from 1.5 to 6 kb. Nine of these genotypes were found in the same

rice field (i.e., isolates from Pila area). Those results demonstrated the high level of polymorphism between *R. solani* isolates from the Philippines.
If we consider the RFLP bands as alleles, we found that probe RS12 revealed the presence of heterozygoty among the isolates of *R. solani*.

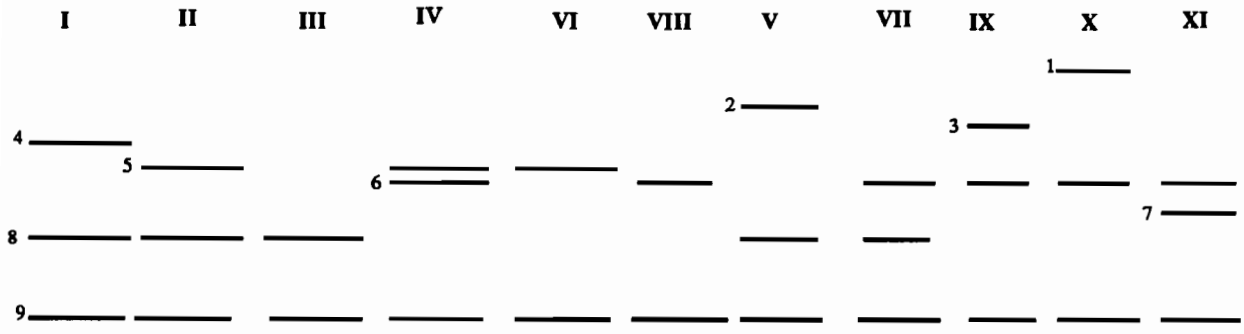

Fig.1: RFLPs revealed within isolates of *R. solani* with probe RS12.

2 - Hybridization profiles with the probes RS5 and RS16 on both *Hind* III and *Pst* I digested total DNA revealed repetitive DNA patterns among the isolates. Each of these probes detected 11 different genotypes. Isolates belonging to the same genotype with the probe RS12 have the same DNA fingerprints patterns. The genotypic diversity based on DNA fingerprints among the isolates was similar to that revealed by using the single-copy probe RS12.

2 - ITS-PCR

Amplification of the ITS region of the nuclear rDNA resulted in an approximately 750-bp fragment for all the isolates. DNA patterns generated by digestion of the PCR products with the 4 enzymes revealed the same banding patterns for all the isolates.

Conclusion: The PCR-RFLP analysis of the ITS rDNA amplification and showed an unique genetic grouping of *R. solani* isolates. In contrast, based on RFLPs results with anonymous probes, the Philippines populations of the rice pathogen appear to be genetically heterogeneous.
More studies are being undertaken to analyse the evolution of the *R. solani* populations at the field level.

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