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Fingerprinting the rice isolates of *R. solani* Kuhn using RAPD markers

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The genetic differences underlying *Rhizoctonia solani* populations provide a useful means for examining the nature and spread of the population within the rice system. So far, no attempt has been made to define variability in relation to spatial distribution and no information is available on the amount of variability in *R. solani* within the field. Many anastomosis groups are subdivided on the basis of the cultural, virulence, molecular, biochemical, immunological, and other characteristics into intraspecific groups (ISGs) (Ogoshi 1987). The most convincing validation of AG and ISG concepts has come from molecular systematic studies (Vilgalys and Cubeta 1994). Our study was undertaken to (1) analyze the interfield variability within 46 Indian rice isolates of *R. solani* collected from two fields, one each at Seola-Kala, Dehradun District (hilly region, 24 isolates), and Nagina, Bijnore District (plain region, 22 isolates), for cultural and morphological characteristics, aggressiveness, anastomosis behavior, nuclear staining, and molecular characterization by RAPD analysis; (2) assess the agreement among five methods in differentiating the isolates; and (3) study the extent and possible factors responsible for intrafield variability in rice. This is the first attempt to define intrafield variability among *R. solani* isolates that cause sheath blight in rice.

Of 22 primers that were screened, the following eight were selected for amplifying DNA of all the isolates: T11 = 5′-GTCCATTCAGTCGGTGCT-3′; T13 = 5′-GAATGCCTTC CAAGCCGGT-3′; C15 = 5′-GGTGCCACGAGTAATC-3′; G16 = 5′-CCAGTCTTC GTAGAGAATCG-3′; T19 = 5′-GTAAAACGACGGCCAGT-3′; C20 = 5′-ATGGATCCGC-3′; G21 =5′-GAGTACGTGC TCGCTCG ATG-3′; and C22 = 5′-TGGGTCGAGGGGTTC-3′. Amplified polymerase chain reaction products were separated on agarose gel (Fig. 1). A dendrogram was constructed using Jaccard's similarity coefficient and UPGMA clustering using NTSYS-PC version 2.11a (Rohlf 2000). The cut-off point to decide the number of clusters was determined using canonical discriminant function analysis by SPSS Software Version 10.

All isolates were multinucleate and shared typical characteristics with *R. solani*. They exhibited varying degrees of virulence on Pusa Basmati 1 and, on the basis of the disease severity (lesion length), could be classified as highly virulent (2), moderately virulent (7), less virulent (33), and avirulent (40). All isolates gave two (incompatible fusion) or three types of anastomosis reaction (compatible fusion) with the tester isolate of *R. solani* (N15) belonging to AG-1 IA.

The dendrogram, constructed using 88 polymorphic bands obtained with eight primers and 41 isolates, was divided into seven clusters at 0.125 similarity level (Fig. 2). No amplification was obtained with isolates N3, D5, D22, D23, and D24. The isolates N18 (crushed and mycelial aggregate-type sclerotia) and N16 (macro sclerotia) were present independently at the 12.5% similarity level. Among isolates of cluster 1 (N1, N9, N13, N14, N15, N20, N21, N22, and D12), similarity values ranged from 13.7% to 32.5%, whereas, among isolates of cluster 2 (D1, D3, D6, D7, D10, D11, D15, D16, D17, D18, D19, D20, and N17), cluster 3 (N8, N19, D2, and D13), cluster 4 (N6, N11, and N12), cluster 5 (D9, D14, and D21), cluster 6 (N2, D4, and D8), and cluster 7

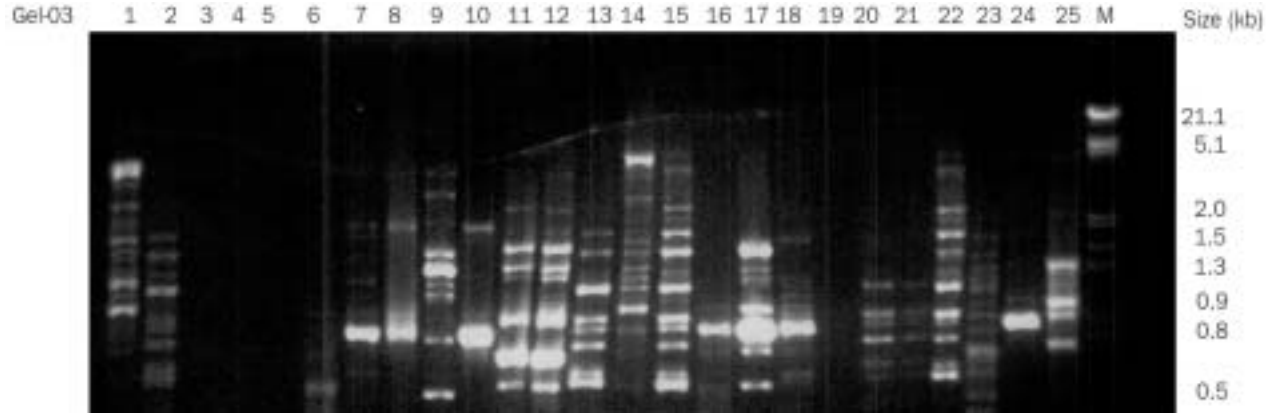

Fig. 1. Agarose gel showing PCR amplification products of 25 isolates obtained with the primer T13. Numbers on the right indicate band size in kilobase pairs. Lanes 1–22 = Nagina isolates (N1 to N22); lanes 23–25, Dehradun isolates (D1 to D3), and M = molecular weight marker, lambda *Hin*dIII/*Eco*RI double-digested.

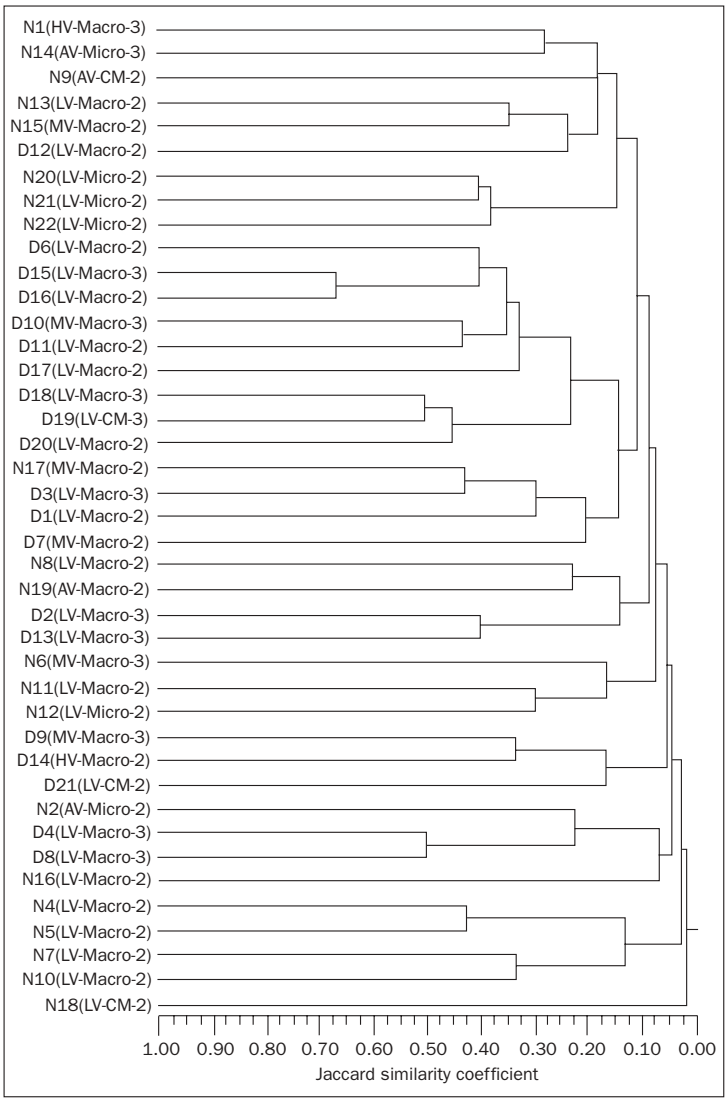

Fig. 2. Dendrogram of 41 rice isolates of *Rhizoctonia solani* revealed by UPGMA cluster analysis of genetic similarities based on RAPD data of 88 fragments amplified with 8 primers. N1 to N22 = Nagina isolates and D1 to D24 = Dehradun isolates. HV = highly virulent, MV = moderately virulent, LV = less virulent, and AV = avirulent. Macro = macro sclerotia, micro = micro sclerotia, and CM = crushed and mycelial aggregate type of sclerotia. 3 = 3-type anastomosis reaction (compatible fusion) and 2 = 2-type anastomosis reaction (incompatible fusion).

(N4, N5, N7, and N10), the similarity values ranged from 13.7% to 74.5%, 12.5% to 40%, 15% to 30%, 15% to 32.5%, 12.5% to 50%, and 13.80% to 42.5%, respectively. Distinct subclusters were detected within clusters 1 and 2, indicating a more complex pattern of genetic variation. In contrast, isolates of clusters 3, 4, 5, 6, and 7 were less differentiated into marked subclusters. The micro sclerotia-forming isolates (N20, N21, and N22) formed a distinct subcluster within cluster 1 at 35% similarity level. All isolates belonging to cluster 7, which were isolated from Nagina, were less virulent on rice and depicted two types of anastomosis reaction.

The lack of a perfect state, wide intrafield variability, and compatible fusion among morphologically and virulent-wise dissimilar isolates (D19 and N14), demonstrated for the first time during this investigation, indicate heterokaryosis as a major mechanism for creating intrafield variability. Isolate-specific primers open up the possibility to use RAPD to study population dynamics and survival of *R. solani* in the soil and polymorphism in the *R. solani* complex in rice.

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Is the trap barrier system with a rice trap crop a reservoir for rice insect pests?

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In the Banaue rice terraces, we monitored the rice arthropod dynamics in a rice trap crop planted inside (1) a trap barrier system (TBS + TC), (2) surrounding rice crops 25, 50, 100, 200, and 400 m away from the TBS + TC setup, and (3) those grown in areas farther away (> 1,000 m) and where the TBS + TC system was not introduced.

Rice is planted once a year in Banaue, with seeds sown on seedbeds in late November and December. The area is planted to Lacoop, which is a photoperiod-sensitive traditional rice variety with 6-mo maturity. Lacoop was also planted inside the TBS 1 mo in advance of the farmers' main crop. One of each TBS + TC setup was strategically established in terraced fields adjacent to the farmers' residences, creeks, and forest on 21 Dec 2002. This was done to lure rats from possible source habitats to the TBS + TC early in the rice cropping season, thereby reducing the number of breeding rats in the main season.

The main purpose of quantifying rice arthropod dynamics and diversity was to provide information on the TBS + TC technology to cooperating farmers, who need to validate its role in the development of an ecologically sustainable rodent management program. During farmers' meetings, concerns about TBS + TC serving as a reservoir for rice insect pests were raised. The farmers feared that this would cause greater damage to the surrounding rice crops.

Rice arthropods were sampled using standard insect sweep nets. Each sweep net sample was composed from 10 strokes. Each TBS + TC setup was sampled 12 times starting on 18 Feb 2003. The rice crops surrounding the TBS + TC and those in non-TBS+TC areas were sampled at various distances four times each, starting on 8 May 2003. In all these areas, samplings were done at weekly intervals with four samples on each sampling occasion; pooled values were presented for each sampling date.

Rice arthropods were sorted and identified on the basis of

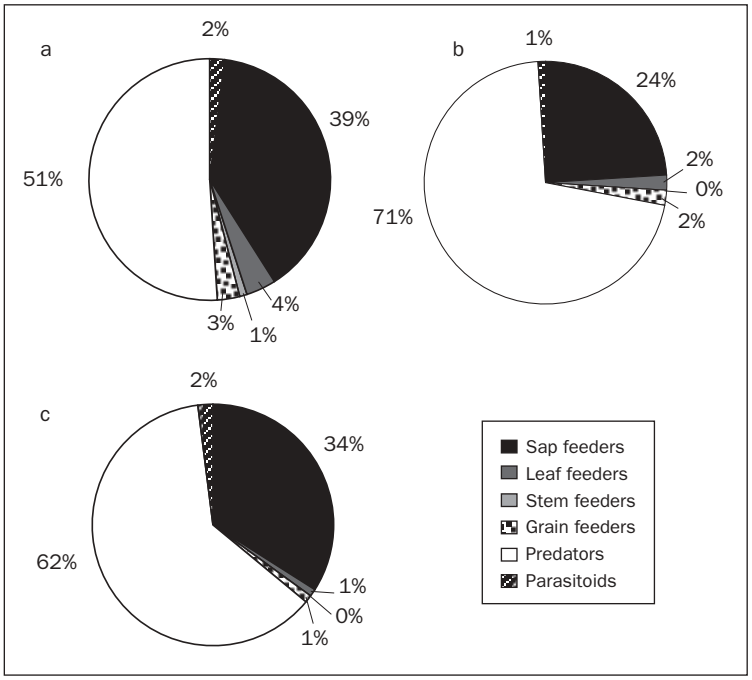

Fig. 1. Rice arthropod composition in (a) TBS + TC, (b) surrounding rice crops 25–400 m away from TBS + TC, and (c) in non-TBS + TC rice-cropped areas, Banaue rice terraces, Ifugao Province, Philippines.