SHORT COMMUNICATION

GENETIC CHARACTERIZATION BY REP-PCR OF MYANMAR ISOLATES OF RHIZOCTONIA spp., CAUSAL AGENTS OF RICE SHEATH DISEASES

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SUMMARY

Sheath disease of rice is one of the major constraints of rice production in Myanmar. Forty-four isolates of Rhizoctonia solani, 30 isolates of R. oryzae and 29 isolates of R. oryzae-sativae were recovered from diseased samples collected from three different regions of Myanmar, namely, Mandalay, Pyinmana and Hmawbe. Repetitive-element Polymerase Chain Reaction (Rep-PCR) was conducted using the BOXA1R and ERIC2 primers separately and a combined dendrogram was constructed for the isolates on the basis of the different fingerprint patterns generated by each primer. Two types of R. solani AG1, two types of R. oryzae and three types of R. oryzae-sativae were differentiated by rep-PCR amongst the analyzed isolates. The results indicate the presence of genetically diverse populations of R. solani, R. oryzae and R. oryzae-sativae in Myanmar.

Key words: Rhizoctonia solani, R. oryzae, R. oryzae-sativae, sheath disease of rice, detection, survey.
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Diseased rice sheath tissues were isolated in water agar (WA) media and identified according to morphological characteristics of the *Rhizoctonia* species as described by Sneh *et al.* (1991). The cultured fungal mass on potato dextrose agar (PDA) plates was then used for DNA extraction. Lyophilized fungal mycelia (100 mg) was ground in liquid nitrogen, transferred into a microtube containing 600 µl of extraction buffer (10 mM Tris-HCl, pH 7.5, 100 mM EDTA, 0.5% SDS, 100 mM LiCl) and incubated at 65°C for 30 min. After vortexing for 2 min, the samples were centrifuged at 11,000 g for 15 min and the supernatant was transferred into a new tube and extracted with 600 µl of phenol-chloroform-isooamyl alcohol (25:24:1) by centrifugation at 11,000 g for 15 min. DNA was precipitated with an equal volume of isopropyl alcohol followed by centrifugation at 11,000 g for 15 min., washed with 70% ethanol, dried in an evaporator for 30 min and dissolved in 100 µl of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). Two µl of RNase (10 mg/ml) were added and the solution was incubated at 37°C for 1 h. DNA was extracted sequentially with Tris-saturated phenol, phenol-chloroform-isooamyl alcohol, chloroform-isooamyl alcohol (24:1) and diethyl ether by centrifugation at 11,000 g for 15 min. The supernatant was precipitated with a half volume of isopropyl alcohol at -20ºC for 30 min, and the precipitated DNA was washed with 70% ethanol and dissolved in 200 µl of TE buffer.

Primers, BOXA1R (5’-CTACGGCAAGGCGACGCTGACG-3’) and ERIC2 (5’-CTACGGCAAGGGCGACGCTGACG-3’) were used separately for Rep-PCR analysis. Amplifications were done in a 25 µl reaction volume containing 2 µl of template DNA, 2.5 µl of 10 x reaction buffer, 2 µl of dNTPs (2 mM), 1.5 µl of the primer (100 pM), 0.25 µl *Taq* DNA polymerase (5 units/sample) and 16.75 µl of MillQ water. Amplifications were performed in a Thermal cycler with slight modifications to the temperature profiles reported by Toda *et al.* (1999), i.e., one cycle of initial denaturation (95°C, 7 min), followed by 30 cycles of denaturation (94°C, 1 min), annealing (52°C for 1 min) and extension (72°C for 8 min) with a final extension at 72°C for 16 min. PCR products were electrophoresed in 1.0% agarose gels stained with ethidium bromide and the gels were visualized under UV light. Based on the finger-

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**Table 1.** Population composition of each type of *Rhizoctonia* spp. at three different sampling sites in Myanmar.

<table>
<thead>
<tr>
<th>Origin/Type</th>
<th>RS Type 1</th>
<th>RS Type 2</th>
<th>Totala (RS)</th>
<th>RO Type 1</th>
<th>RO Type 2</th>
<th>Totalb (RO)</th>
<th>ROS Type 1</th>
<th>ROS Type 2</th>
<th>ROS Type 3</th>
<th>Totalb (ROS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hmawbe</td>
<td>17</td>
<td>8</td>
<td>25 (56.8)</td>
<td>3</td>
<td>1</td>
<td>4 (13.3)</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5 (17.2)</td>
</tr>
<tr>
<td>Pyinmana</td>
<td>10</td>
<td>5</td>
<td>15 (34.1)</td>
<td>22</td>
<td>4</td>
<td>26 (86.7)</td>
<td>12</td>
<td>8</td>
<td>3</td>
<td>23 (79.3)</td>
</tr>
<tr>
<td>Mandalay</td>
<td>3</td>
<td>1</td>
<td>4 (9.1)</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Totalb</td>
<td>30 (68.1)</td>
<td>14 (31.8)</td>
<td>44</td>
<td>25 (83.4)</td>
<td>5 (16.7)</td>
<td>30</td>
<td>15 (57.7)</td>
<td>11 (37.9)</td>
<td>3 (10.3)</td>
<td>29</td>
</tr>
</tbody>
</table>

a Total number of each species at respective area; numbers in parentheses in the same column represent the percentage of each species at the respective area.

b Total number of each type from each species; numbers in parentheses in the same row represent the percentage of each type of the respective species.
prints, a total of 10 distinct bands generated by the BOXA1R primer and 12 distinct bands generated by the ERIC2 primer were used for similarity comparison tests. The polymorphic bands were scored as 1 and 0 according to the presence or absence of a band at a certain position for each isolate. The resulting binomial matrix data was analysed by statisXL software and a combined dendrogram was constructed by hierarchical clustering of the variance using the furthest neighbour method.

Fifteen isolates of *R. solani*, 26 isolates of *R. oryzae* and 23 isolates of *R. oryzae-sativae* were obtained from Pyinmana samples; four isolates of *R. solani* and 1 isolate of *R. oryzae-sativae* were recovered from Mandalay; and 25 isolates of *R. solani*, 4 isolates of *R. oryzae* and 5 isolates of *R. oryzae-sativae* came from Hmawbe. The total number of isolates of *R. solani*, *R. oryzae* and *R. oryzae-sativae* was 44, 30 and 29, respectively.

Based on the dendrogram, the Myanmar isolates of *R. solani* AG-1 IA were clustered into two groups and denoted RS Type 1 and RS Type 2 (Fig. 2). Examples of the ERIC2 and BOXA1R generated fingerprints for each RS Type are shown in Fig. 3. A total of 30 isolates were classified as RS Type 1 and 14 as RS Type 2 at the variance value of 0.21 (Fig. 2). Isolates of the RS Type 1 formed the major population type of *R. solani* AG-1 IA in Myanmar (Table 1).

Populations of RS Type 1 and RS Type 2 were found in all the sampled locations (Table 1). For example, isolates such as M1 (Hmawbe) and M58 (Mandalay), clustered closely in the RS Type 1. In addition, different types were found at the same location. For example, isolates from the same area (Hmawbe) showed different banding patterns and clustered in different RS Type 1 and RS Type 2 (Fig. 3) subgroups. Most isolates of *R. solani* AG-1 IA in Myanmar were distributed predominantly in the Hmawbe area, which is more humid than the other sampling sites.

Thirty isolates of *R. oryzae* from Myanmar were characterized by the rep-PCR assay and variations were observed in the fingerprints generated. The dendrogram showed that two different population structures existed, which were named RO Type 1 and RO Type 2, based on the banding patterns that were generated by the BOXA1R and ERIC2 primers. Twentyfive isolates of RO Type 1 and 5 isolates of RO Type 2 were classified at a variance value of 0.19 (Fig. 4). The RO Type 1 populations prevailed as they comprised 83.4% of the total isolates (Table 1). This RO Type 1 was mainly present in the Pyinmana area, with only a few isolates (3) found in

Fig. 2. Dendogram produced from polymorphic fingerprint patterns observed for 44 Myanmar isolates of *R. solani*. Numbers in parentheses indicate the total number of isolates classified into each population type.

Fig. 3. Fingerprint patterns generated by the ERIC2 primer and the BOXA1R primer for representative samples of each RS Type.
the Hmawbe area. *R. oryzae* isolates did not occur in the central dry Mandalay area, likely because of the parameters, including weather, cultural practices and epidemiological conditions that affect the dispersal and distribution of *R. oryzae* in Myanmar.

*R. oryzae-sativae* populations were differentiated into three different subgroups denoted ROS Type 1, ROS Type 2 and ROS Type 3 (Fig. 5). A total of 15 isolates were classified as ROS Type 1, 11 isolates as ROS Type 2, and 3 isolates as ROS Type 3 at a variance of 0.15 based on the fingerprints. The ROS Type 1 population was found to be predominant and was obtained from all sampling sites in Myanmar (Table 1). In this survey, the *R. oryzae-sativae* isolates prevailed in Pyinmana, which accounted for 23 out of the total of 29 *R. oryzae-sativae* isolates.

The prevalence of *Rhizoctonia* species can vary as a result of environmental conditions or geographical location. The highest percentage of *R. solani* was isolated from Mandalay where a total of 4 isolates of *R. solani*, no isolates of *R. oryzae* and 1 isolate of *R. oryzae-sativae* were recovered. There is the possibility of natural suppression of these pathogens in this area, and the influence of the environment on disease development is an interesting subject for future epidemiological studies. Datta (1981) reported that the occurrence and distribution of rice sheath disease is influenced by weather factors, physiological responses and agricultural practices. Our results revealed the presence of different population structures in the three different locations of sampling characterized by different environmental conditions. Nevertheless, further studies are required to provide a more extensive analysis of the existing population structures of the species at different rice growing areas in Myanmar.

Many researchers have used different methods to differentiate the *Rhizoctonia* isolates. Matsumoto et al. (1996) described a total cellular fatty acid protocol and successfully differentiated between isolates of *R. solani* Ag1-1A, AG2-IIIB, *R. oryzae*, *R. oryzae-sativae* and *R. fumigate*. Taheri et al. (2007) studied the genetic relationships among *Rhizoctonia* species by using fingerprint patterns generated by four primers. Matsumoto et al. (1997) reported the detection of *Rhizoctonia* from diseased plant tissue by PCR-RFLP analysis. In this present report, we successfully used Rep-PCR to characterize and differentiate among different types within the same species to identify different populations of three *Rhizoctonia* species.

Genetic relatedness among and within different *Rhizoctonia solani* anastomosis groups has been studied by Toda et al. (1999), who presented the polymorphic fingerprint patterns generated by the ERIC primers. In our research, the polymorphic fingerprint patterns generated by the ERIC2 and BOXA1R were detected in different PCR samples. Simple banding patterns were produced by ERIC2 and complex banding patterns by BOXA1R. According to Versalovic et al. (1994), the optimal number of bands for Rep-PCR is 8 to 15. Fingerprints generated by BOXA1R consisted of more than 15 bands some of which seemed to overlap adjacent bands. The overall results of BOXA1R and ERIC2 amplification indicate that the ERIC2 generates more efficient fingerprints for differentiating *Rhizoctonia* population types. So, for future studies we suggest that the ERIC2 primer is best to use for classification of *R. solani*, *R. oryzae* and *R. oryzae-sativae*.

Taheri et al. (2007) stated that geographic region is significant in determining the genetic structure of the *Rhizoctonia* population in India. In the present study, genetic structure was diverse even within the same geographical region, which agrees with the findings of Ducan et al. (1993). For example, isolates M10, M5 and
M79 were collected from the same region of Hmawbe, but these isolates showed diverse fingerprints and were identified as different types. Linde et al. (2005) described the possibility of gene flow by some *R. solani* genotypes up to a distance of 280 m. Our evidence supports this. In fact, the occurrence of isolate M89 at Mandalay and of several isolates at Hmawbe with the same RS Type 2 group suggests a possibility of gene flow of up to a distance of 716 km, highlighting the importance of extensive sampling of sheath diseases from different locations.

In summary, although our investigation disclosed the presence of genetically diverse populations of *R. solani*, *R. oryzae* and *R. oryzae-sativae* in Myanmar, further comparative studies for the grouping of such species by different methods seem advisable to help with the solution of the taxonomic problem of the *Rhizoctonia* genus in Myanmar.

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**REFERENCES**


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