Molecular Plant Pathology

Use of Random Amplified Polymorphic DNA (RAPD) to Characterize Race 2 of *Fusarium oxysporum* f. sp. *pisi*

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ABSTRACT


Genetic variability within four races of *Fusarium oxysporum* f. sp. *pisi* was assessed by random amplified polymorphic DNA (RAPD). Banding patterns generated from isolates of race 2 by 14 selected primers were uniform relative to patterns generated from races 1, 5, and 6. Isolates of races 1, 5, and 6 showed much greater variability within each race than did isolates of race 2. The high level of genetic variability among and within races of *F. o. pisi* allowed for fingerprinting of most of the isolates by examining patterns produced by several primers. Generalized race-specific patterns were not found for races 1, 5, and 6. The results suggested that races 1, 5, and 6 be grouped separately from race 2. Similarities and differences in banding patterns obtained by RAPD could be a useful molecular tool in evolutionary studies of the origins of different races.

Additional keywords: *Fusarium* wilt of peas.

*Rusarium* is a heterogeneous genus with a worldwide distribution that includes many species. A common characteristic of *Fusarium* spp. is the production of distinctive sickle-shaped macroconidia (27). A sexual stage has been identified for some *Fusarium* spp. Other than morphological and pathological traits, very little is known about the genus and, more specifically, about the species *Fusarium oxysporum*.

The high host specificity found among plant-pathogenic strains of *Fusarium* led Snyder and Hansen (19) to create a new taxa of the species based on this specificity. Isolates that cause disease in members of a particular family, genus, or species are forma specialis. Race characterizes isolates by resistance genes found in particular cultivars of a specific host species.

Identifying *Fusarium* at the species, forma specialis, and race levels is difficult (12) because many variables must be considered (27). Different methods have been used to assess genetic variability in *Fusarium* spp. and to identify the species, forma specialis, and race levels. Studies on genetic relationships and physiology among *Fusarium* spp. have been conducted at the protein (15,16) and DNA levels (9,10,12,17,20).

Another approach to identifying different forma specialis and races resulted from studies of vegetative incompatibility (18). Puhalla (18) found a relationship between vegetative compatibility groups (VCGs) and forma specialis in *F. oxysporum*. Puhalla stated that without sexual reproduction, genes that determine vegetative compatibility and genes that determine pathogenicity and host specificity became fixed together because the only possible genetic exchange was through heterokaryon production among strains with the same *vc* genes (8,18). Although clear relationships between pathogenicity and VCGs have been found in several instances (1,4,8,10), more complex associations were found in other instances (2,5,7). No relationship was found between VCGs and pathogenicity in other instances (6). Even in instances in which clear associations between VCGs and pathogenicity existed, there were several limitations to broad application of the technique (2,3).

Random amplified polymorphic DNA (RAPD) (26) offers several advantages that may be useful in studying forma specialis and races of *F. oxysporum*, to identify RAPD markers for forma specialis and race identification. RAPD reduces the time needed for race identification in diseased plants, and provides genetic information on isolates studied, allowing for fingerprinting of isolates (24,25). RAPD has several advantages over other polymorphic DNA-detecting techniques, including RFLP (restriction fragment length polymorphism). These advantages include quickness, small amounts of template DNA needed, no requirement of DNA sequence information, and use of fluorescence (22). Applications of RAPD include development of genetic maps, targeting genetic markers, pooling techniques (13), and phylogenetic information (21).

This research was conducted to study different isolates and races of *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *pisi* (J.C. Hall) W.C. Snyder & H.N. Hans. present in Washington, where *Fusarium* wilt of peas is a predominant disease, by means of RAPD. RAPD markers were found that identify race 2 isolates from isolates of races 1, 5, and 6. RAPD markers have been used to evaluate genetic diversity among isolates of races 1, 2, 5, and 6 of *F. o. pisi* and to fingerprint different isolates.

MATERIAL AND METHODS

Isolates of *F. o. pisi* were maintained as chlamydospores in autoclaved soil (11). The isolates of race 1 used in this study were taken from three areas of Washington; isolates of race 2 were taken from two areas of Washington and one isolate was taken from England. The isolates of race 5 were found in three areas of Washington, and the isolates of race 6 came only from the western part of Washington. All isolates used in this study are readily available through F. J. Muehlbauer (Pullman, WA), J. M. Kraft (Prosser, WA), and W. H. Haglund (Mt. Vernon, WA).

A small amount of infested soil was sprinkled onto petri dishes containing Nash medium (1.5% Difco peptone, 0.1% KH₂PO₄, 0.5% MgSO₄.7H₂O, 2% agar, and 0.1% 75% wettability powder Terracol, pH adjusted to 5.5-6.5, that was autoclaved for 20 min, when the medium cooled, 0.01% streptomycin and 0.0012% neomycin were added) (12,14). The cultures were incubated for
5 days at 25 C in light to produce colonies (23). Resultant cultures were aseptically transferred to flasks of potato-dextrose broth. The flasks were placed on a rotary shaker (125 cycles per minute) and given 24 h of artificial light per day for 5 days at room temperature. The mycelia were filtered from the liquid medium through four cheesecloth layers. DNA was extracted from 1 g of mycelia by a slightly modified CTAB method (9).

Arbitrary random amplification of DNA sequences was performed with a set of 40 primers (each having 10 bases) obtained from Genosys Biotechnologies, Inc. (The Woodlands, TX). A modification of the RAPD method of Williams et al. (26) was used to perform the amplification. Each 25 µl of reaction contained 1 U of Taq polymerase (Promega, Madison, WI); 50 mM KCl; 10 mM Tris-Cl, pH 8.8; 1.5 mM MgCl2; 0.1% Triton X-100 (Promega 10X buffer); 0.1 mM of each dNTP (Promega); 2 µM primer; and approximately 50 ng of template DNA.

Forty amplification cycles were performed on a Perkin-Elmer/Cetus Gene Amp PCR system 9600 (Norwalk, CT). Each cycle consisted of denaturation at 94 C for 20 s, annealing at 36 C for 1 min, followed by a 3-min rise to 72 C and primer elongation at 72 C for 1 min. The final primer-elongation segment of the run was extended to 9 min. The reaction product was resolved in a 2% agarose gel (1% Seakem and 1% NuSieve [FMC Bio-products, Rockland, ME]) at 4 V/cm for 3 h. PCR product was visualized by UV-fluorescent staining with ethidium bromide.

The banding patterns produced by 40 primers on DNA from 23 isolates of F. o. pisi were analyzed. From these 40 primers, we selected the 14 primers that showed a greater number of polymorphisms (Table 1). The entire experiment, from spore growth to DNA extraction and amplification, was repeated to verify the results and to test the consistency of the method. Banding patterns were analyzed by scoring 1 for the presence of major bands and 0 for their absence.

The discrete-data set generated was analyzed by the MIX program of PHYLP (3.4) (developed by P. Felsenstein in 1991), using the Wagner parsimony method (changes from 0 to 1 are equally probable to changes from 1 to 0). This program generates an unrooted most parsimonious tree with the fewest possible stepwise changes.

**RESULTS AND DISCUSSION**

Many primers in this study clearly distinguished race 2 from races 1, 5, and 6 (Fig. 1). Isolate 167 was received as race 1 but yielded a banding pattern similar to that of race 2 isolates. Pathogenicity tests with a set of standard cultivars revealed that isolate 167 was a race 2 isolate.

All race 2 isolates tested showed a highly conserved and characteristic banding pattern (Fig. 1). The isolates of races 1, 5, and 6 presented more genetic variability than was apparent in race 2 isolates (Fig. 1).

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**TABLE 1. Source and sequence of the primers used in this study**

<table>
<thead>
<tr>
<th>Primer identification</th>
<th>Sequence</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS12</td>
<td>GCGACGCTA</td>
<td>Genosys</td>
</tr>
<tr>
<td>CS14</td>
<td>GTCAACCCGA</td>
<td>Genosys</td>
</tr>
<tr>
<td>CS15</td>
<td>AACATGCC</td>
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</tr>
<tr>
<td>CS16</td>
<td>CGTGTGATGC</td>
<td>Genosys</td>
</tr>
<tr>
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<td>TACCGTGGC</td>
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</tr>
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<td>CS22</td>
<td>CTTGTGGAAA</td>
<td>Genosys</td>
</tr>
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<td>CS24</td>
<td>GGCGATCCTT</td>
<td>Genosys</td>
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<tr>
<td>CS27</td>
<td>AGTGTGCGGC</td>
<td>Genosys</td>
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<tr>
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<tr>
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</tr>
</tbody>
</table>

*Primers are listed 5' to 3' end, from left to right.
Most isolates used in this study could be fingerprinted with the use of primers that differentiate the banding pattern of a particular isolate from other isolates (Fig. 3). This is of interest for culture storage and might be useful in studies of the evolution of new races.

Pathogenicity tests are the only means of determining the pathological classification of fungal strains present in diseased plants or in soil samples. RFLP and RAPD applied to fungal studies would be useful in providing markers for identification purposes, such as the RAPD markers found for race 2 isolates in this study. These techniques can reduce the time and greenhouse space needed for pathological classification. RFLP and RAPD also could provide an additional means of studying the genetic characteristics and evolution of *Fusarium* spp., formae specialis, and races. In this study, we found that *F. o. pisi* race 2 clearly differs from the other races.

**LITERATURE CITED**


