RUST-SUGAR CANE INTERACTION - POSSIBLE APPLICATION OF SEROLOGICAL METHODS FOR EARLY SELECTION TO RUST DISEASE

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Key words: Sugar cane, rust, P. melanocephala, serological techniques, indirect ELISA, early selection

ABSTRACT

Antigens and antisera from in vitro cultures of Puccinia melanocephala Sydow, uredospores collected from the field and sugar cane varieties were obtained. Cross-reactions were detected between the fungi and plants by counterimmunoelectrophoresis, immunoelectrophoresis and indirect ELISA. The last technique detected more antigenic relationships with the susceptible plants. This result may be used to study the role of these antigens and their possible application in selection for resistance.

INTRODUCTION

Serological relationships between organisms of diverse phylogeny such as plants fungi are not normally expected, since even highly conserved molecules have undergone considerable changes in the course of evolution. However, there is evidence that such relationships do occur between some plants and their parasites, and these observations have been considered important in the understanding of host-parasite systems (Charudattan and De Vay1,2, De Vay et al3 and Heide and Smedegaard-Peterson10). In addition strong serological relationships have been detected in some systems with susceptible varieties compared with those for resistant ones (Doubly et al4, Fedotova5, and Palmerley and Callow6). These differences might be useful when selecting varieties or confirming the resistance of varieties to some diseases.

Consequently, different serological methods to detect common antigens in the rust-sugar cane system were evaluated to find their possible usefulness for the early selection of resistant varieties.

MATERIALS AND METHODS

Extraction of antigens

Fungi

Liquid cultures of Puccinia melanocephala Sydow were grown in Czapek Dox Medium as described by Melián and Ojeda13. The mycelial mats were harvested after eight days, thoroughly washed with cold water and stored at −20°C. The antigen was extracted by homogenization of mycelium in a Waring Blender with bicarbonate buffer (0.004 M) and centrifugation at 4,000 g for 20 min. Supernatants were adjusted to a final concentration of 200-400 µg/ml and used for immunization purposes.

Uredospores of P. melanocephala were collected from naturally infected leaves of the susceptible sugar cane variety B4362. Protein extraction was performed according to Diaz et al17. Uredospores were washed with cold water and centrifuged three times at 4,000 g for 20 min. The pellet was resuspended in cold water (pH approximately 7) and sonicated at maximum intensity for 1 h in a MSE sonicator mounted over an ice
bath. The suspension was centrifuged at 4 000 g for 20 min and the supernatant was
concentrated to 200-400 µg/ml.

Plants
Healthy sugar cane plants of two varieties with different degrees of resistance to rust
disease, B4362 (susceptible) and PR 980 (resistant), were grown in a glasshouse for 3-4
months. Antigens were obtained as described by Hernandez and Diaz11. Leaves were
collected and macerated in saline solution (0.9%) for 1 h; this was filtered, centrifuged
at 4 000 g for 20 min, precipitated with ammonium sulfate solution (95%), left over-
night at −4°C, centrifuged at 4 000 g for 10 min, and the pellet was resuspended in
1/5 of the initial volume of saline and dialysed against distilled water. The final con-
centration was adjusted to 200-400 µg/ml. Antigens from leaves of maize plants grown
under the same conditions were obtained by the same method, to be used as negative
plant antigens.

Protein determinations were made by the method of Bradford1.

Production of antisera
Antisera of P melanocephala and leaf antigens were produced in male rabbits of ap-
proximately 2 kg weight. Pre-bleeding was performed before immunization to obtain
control sera. Each rabbit received an intraplantal injection of 1 ml 1:1 mixture of antigen
and Freund's Complete Adjuvant (0.2ml in each leg). After 3 weeks, they were injected intramuscularly with Freund's Incomplete Adjuvant
into the hind leg with the same dose. On day 30, the animals were bled from the eyes
with capillary tubes. Antisera were stored at −20°C without preservatives.

Seraological test
All antigens were adjusted to 0.5 mg for serological tests. Rust antigens were treated
with 0.1% SDS and heated in a water bath at 100°C for 3 min before use in precipitation
tests. Homologous and heterologous reactions were evaluated by the following tests:
(i) counterimmunoelectrophoresis (CIET). Slides were prepared with agarose 1% in
borate buffer (pH = 8.6). The same buffer was used as running buffer for 60
min and 4 mA/slide.
(ii) immunoelectrophoresis (IET). This was performed using slides prepared as
above, but using veronal buffer 0.01 M for 90 min and 4 mA/slide.
(iii) indirect ELISA. This serological test was carried out as reported by Hernández et
al12. Specific IgG purification, conjugated production and other solutions were
prepared as reported by Clark and Adams4. Only the mycelial antigen and antiserum were used with this technique. Statistical significance of ELISA values
was determined using standard analysis of variance and Duncan's test.

RESULTS

CIET
Cross-reactions were observed in all heterologous reactions (Figs 1 and 2). No differ-
ence was found between susceptible and resistant varieties with mycelium or uredospore antigens and antisera. Table 1 shows antigen dilutions reacting with homolo-
gous and heterologous antisera. Control tests involving control sera and maize antigen
were all negative.

IET
Figs 3 and 4 show homologous and heterologous reactions. Common antigens were
demonstrated between plants and pathogens, with mycelium and uredospores. Control tests were all negative.

Figure 1. Detection of common antigens by CIET with mycelium antisera of _P. melanocephala_ (RM = rust mycelium; B = B4362 variety; PR = PR 980 variety)

Figure 2. Detection of common antigens by CIET with uredospore antisera of _P. melanocephala_ (RU = rust uredospore; B = B4362 variety; PR = PR 980 variety)
Table 1: Antigen dilutions reacting with homologous and heterologous antisera by CIET

<table>
<thead>
<tr>
<th>Item</th>
<th>Antigen</th>
<th>Mycelium</th>
<th>Uredospores</th>
<th>B4362</th>
<th>PR980</th>
</tr>
</thead>
<tbody>
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<td>Mycelium rust</td>
<td>1:16</td>
<td>—</td>
<td>1:4</td>
<td>1:4</td>
<td></td>
</tr>
<tr>
<td>Uredospores rust</td>
<td>—</td>
<td>1:32</td>
<td>1:2</td>
<td>1:2</td>
<td></td>
</tr>
<tr>
<td>B4362</td>
<td>1:4</td>
<td>1:2</td>
<td>1:16</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>PR 980</td>
<td>1:4</td>
<td>1:2</td>
<td>—</td>
<td>1:64</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Common antigens detection by IET with mycelium antisera of *P. melanocephala* (RM = rust mycelium; B = B4362 variety; PR = PR 980 variety)

Figure 4. Common antigens detection by IET with rust uredospore antisera (RU = rust uredospore; B = B4362 variety; PR = PR 980 variety)
Indirect ELISA

Mycelium antiserum showed positive reactions with antigens of both cane varieties, but the value with $10^{-3}$ rust antiserum dilution with B4362 antigen was significantly higher ($P<0.001$) than for the same dilution with PR 980 and maize antigens (Fig 5). There was no significant difference between PR 980 and maize antigens in their reactions with rust antiserum. Variability of the ELISA test was between 1.4% and 22%, with 90% of the values less than 10%.

The heterologous reactions between mycelium antigen and plant antiserum confirmed the presence of common antigens in this host-parasite system. Variability was between 0.56 and 25%, with 88% of values less than 10% (Fig 6).

Figure 5. Common antigens detection by indirect ELISA test with *P. melanocephala* antiserum.

Figure 6. Common antigens detection by indirect ELISA test with sugar cane antiserum.
DISCUSSION

The results of the present work indicate the presence of common antigens in sugar cane and P. melanocephala. These common antigens were demonstrated by CIEF, IET and indirect ELISA tests, but only using this last technique could differences be detected between susceptible and resistant varieties. Precipitation and ELISA tests may recognise different kinds of antigens, so it is possible that common antigens related to susceptibility might be recognised only by ELISA. Dazzo and Hubbell\(^\text{5}\) and Palmerley and Callow\(^\text{14}\) postulated that not all common antigens necessarily contribute towards determining host-parasite compatibility, but rather that only certain “key” common antigens are important.

The function of common antigens for the plant-host-parasite interaction is not well known. Their possible role in the recognition mechanisms has been postulated by Dazzo and Hubbell\(^\text{5}\), and it is possible that an immune response may be an underlying basis for the success or failure of a parasitic relationship. However, its role could be subjected to modification by some environmental conditions and possibly other factors.

Confirmation of the presence of common antigens related to susceptibility in the rust-sugar cane system, and the sensitivity of the ELISA test in the detection of them, might offer a method of selecting resistant varieties.

REFERENCES


L'INTERACTION ROUille/CANNE A SUCRE
L'UTILISATION POSSIBLE DE METHODES SEROLOGIQUES POUR LA SELECTION PRECOCE DE LA RESISTANCE A LA ROUille

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EXTRAIT

Des antigènes et des antisérums issus de cultures in vitro de Puccinia melanocephala Sydow, et des uredospores provenant des champs et de variétés de cannes à sucre furent recueillis. Des interactions entre les champignons et les plantes furent observées par les méthodes de contre-immunoélectrophorèse et de l'ELISA. La dernière méthode permet de découvrir une relation plus étroite entre l'antigène et les plantes sensibles. Ce résultat peut être utilisé pour étudier le rôle de ces antigènes et pour la sélection lors des tests de résistance.

INTERACCION ROYA-CÁÑA DE AZÚCAR. POSIBLE APLICACION DE METodos SEROLOGICOS EN LA SELECCION PRECOZ DE VARIEDADES RESISTENTES A LA ROYA

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Palabras claves: Caña de azúcar, roya, P melanocephala, técnicas serológicas, ELISA indirecto, selección precoz.

RESUMEN

Se obtuvieron antígenos y anticuerpos del cultivo in vitro de Puccinia melanocephala Sydow, de uredosporas colectadas en el campo y de variedades de caña de azúcar con diferente grado de resistencia a la roya, detectándose antígenos comunes en la relación planta-patógeno mediante contraimunoelectroforesis, inmunoelectroforesis y ELISA indirecto. Esta última técnica detectó mayor relación serológica con la variedad susceptible, lo que posibilita profundizar en el estudio del papel de estos antígenos y su posible aplicación en la selección de variedades resistentes a la enfermedad.