Short communication

Virulence of Colletotrichum gloeosporioides causing strawberry anthracnose disease in Tucumán, Argentina

Virulencia de Colletotrichum gloeosporioides causante de la enfermedad de la antracnosis en frutilla en Tucumán, Argentina

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Abstract

A fungal strain with phytopathological behaviour was isolated from strawberry leaves and fruits with symptoms of anthracnose disease in Tucumán province (north-west Argentina), and it was identified as strain L9 of Colletotrichum gloeosporioides. Inoculation experiments on different strawberry cultivars using the isolated strain revealed dissimilar phytopathological responses. Whereas the cultivars ‘Pájaro’ (accession Mendoza), ‘Milsei’, ‘Selva’ and ‘Seascape’ triggered a typical response compatible with strain L9, the cv. ‘Pájaro’ (accession Cafayate) and ‘Gaviota’ showed a typically incompatible response.

Keywords: Anthracnose; Colletotrichum spp.; Fragaria ananassa.

Resumen

Una cepa fúngica que mostró un comportamiento fitopatológico fue aislada de hojas y frutos de frutilla que presentaban síntomas de la enfermedad antracnosis en la provincia de Tucumán (Noroeste de Argentina), y fue caracterizada como cepa L9 de Colletotrichum gloeosporioides. Los experimentos de desafío llevados a cabo con diferentes cultivares de frutilla frente a la cepa aislada revelaron respuestas fitopatológicas diferentes. Mientras que el cultivar ‘Pájaro’ accesión Mendoza, ‘Milsei’, ‘Selva’ y ‘Seascape’ desencadenaron una respuesta compatible con la cepa L9, la respuesta fue incompatible en los cultivares ‘Pájaro’ (accesión Cafayate) y ‘Gaviota’.

Palabras clave: Antracnosis; Colletotrichum spp.; Fragaria ananassa.

Strawberry (Fragaria ananassa Duch.) anthracnose, also called black spot, fruit rot, or crown rot, is one of the most important diseases affecting the crop (Freeman and Katan, 1997). The term “anthracnose”, introduced by Brooks in 1931, was initially used to describe a new disease of strawberry caused by Colletotrichum fragariae (Brooks, 1931), but the term was later generalized and used to refer to all diseases caused by fungi that belong to the genus Colletotrichum (Delp and Milholland, 1980; Smith and Black, 1990). These pathogens can attack crowns, leaves (petioles and leaflets), peduncles, pedicels, fruits, flowers, buds, runners and roots (Howard et al., 1992; Freeman and Katan, 1997). Three species of Colletotrichum are known to be etiological agents of anthracnose disease in strawberry: C. acutatum J.H.Simmonds, C. fragariae Brooks and C. gloeosporioides (Penz.) Penz. and Sacc. in Penz. (teleomorph Glomerella cingulata (Stoneman) Spauld. Schrenk) (Smith, 1986; Smith and Black, 1990; Howard et al., 1992; Adaskaveg and Hartin, 1997; Nam et al., 2006). Colletotrichum dematium (Pers.) Grove (Beraha et al., 1973) and species of Gloeosporium were also initially included as anthracnose agents, but they were later determined to be C. acutatum (Howard et al., 1992). Traditionally, the characterization of these fungal pathogens has been mainly based on classical microbiological methods, which consider colony color and morphology, conidium size and shape, presence or absence of setae and the existence of the teleomorph G. ciculata/G. cingulata (Smith and Black, 1990).
Colletotrichum gloeosporioides has been reported in a large number of host species, but its occurrence in strawberry in the EU is only occasional (Garrido et al., 2008). When plants and fruit samples with anthracnose symptoms collected in the crop area of northwestern Argentina were studied in detail, results revealed that the etiological agents corresponded to C. fragariae, C. acutatum and, to a much lesser extent, C. gloeosporioides (Mena et al., 1974; Mónaco et al., 2000; Ramallo et al., 2000). Since tolerance or disease symptoms in a specific strawberry genotype depends not only on the particular pathogen strain, but also on plant tissue and inoculation procedure (Smith and Black, 1990; Mertely and Legard, 2004), this study aimed to perform a phytopathological evaluation with all the pathogens isolated. The trial consisted in spraying conidia on unwounded plants and analyzing disease symptoms on leaves (petioles and leaflets) at different times.

Fungal isolate L9 of *Colletotrichum gloeosporioides* was collected from symptomatic cv. ‘Chandler’ strawberry in Lules (Tucumán, Argentina) (Mónaco et al., 2000). Isolates were characterized based on several criteria. Potato dextrose agar (PDA) was used to evaluate cultural and morphological characteristics of the isolate. Isolates were single-spore propagated to obtain pure cultures, and maintained on PDA slants at 4 ºC. The isolate L9 was selected for phytopathological characterization. This strain was also sent to CABI BioScience (International Mycology Institute, UK Centre, Egham, England) for its molecular characterization.

Cultures were obtained by transferring a 4 mm-diameter mycelium plug from a PDA culture to newly prepared PDA in a 90 mm diameter Petri plate. For all the experiments, cultures were incubated at 28 ºC for 12 days, under continuous fluorescent light to induce conidium formation (Smith and Black, 1990; Sutton, 1992; Mertely and Legard, 2004). Three replicate plates were prepared, and growth rate was determined by measuring colony diameter after twelve days of incubation. Conidial suspensions prepared in sterile distilled water were observed under an Olympus BH-2 microscope, and their shape was determined by examining 100 randomly chosen samples. The isolate L9 was classified into the following category: straight cylindrical body with both ends rounded. aberrant and ambiguous conidial shapes represented less than 10 % of the population analyzed. Conidial size and perithecia were observed and photographed with a JEOL JSM35 CF scanning electron microscope, measuring the length and width of 100 conidia randomly chosen from the isolate. These measures were expressed as an average. In order to evaluate phytopathological responses, inoculation experiments were performed. The culture surface was scraped gently with a Pasteur pipette to remove conidia, and these were suspended in sterile distilled water. Conidial suspensions obtained were filtered through gauze to remove mycelial debris under axenic conditions. Suspensions were then diluted with sterile distilled water containing two drops of Tween 20 per liter to a final concentration of 1.5 x 10⁶ conidia per ml, and the plants were sprayed with this preparation until run-off (Smith and Black, 1990; Mertely and Legard, 2004; MacKenzie et al., 2009). The experimental design was randomized with eight plants per genotype and per experimental unit, four of which corresponded to the challenged plants, and four to the control plants. All experiments were repeated at least twice or more times under identical experimental conditions when results were not clear. Immediately after inoculation, plants were placed in a dew chamber with 100 % RH at 28-30 ºC (infection chamber), for 48 h in the dark. Then the plants were returned to the greenhouse (Smith and Black, 1990; Nam et al., 2006).

Strawberry plants of the cultivars ‘Pájaro’, accessions Mendoza and Cafayate (García et al., 2002), ‘Camarosa’, ‘Chandler’, ‘Milsei’, ‘Sweet Charlie’, ‘Selva’, ‘Seascape’ and ‘Gaviota’ were used in the experiments. Plants for phytopathological studies were obtained from runners of these cultivars, which were rooted in sterile substrate under axenic conditions. Plants were grown for 14 to 16 weeks and maintained in growth cabinets at 28 ºC, with 70 % RH and a light cycle of 16 hours per day. The ability of isolate L9 to infect different strawberry cultivars was tested by spraying the leaves and petioles of four plants of each cultivar with a suspension of 1.5 x 10⁶ conidia per milliliter. Four uninoculated plants were used as controls. Plants were placed in the infection chamber for 48 h, and then transferred to a growth cabinet bench for evaluating disease symptoms and severity. After 50 days incubation, pathogens were isolated from crowns of plants that developed the disease, and they were compared with the strains for inoculation to check pathogen identity. Disease severity rate (DSR) was evaluated and scored on a scale ranging from 1 to 5, using a
The phytopathological evaluation of the nine strawberry cultivars inoculated with strain L9 showed differences among cultivars. Plants of cv. ‘Pájaro’ (accession Mendoza) showed lesions and strangulation of petioles. DSR values of cvs. ‘Pájaro’ (accession Mendoza), ‘Milsei’, ‘Selva’ and ‘Seascape’ displayed a strong compatible interaction, with a DSR ≈ 5 (Table 1). In the case of cv. ‘Pájaro’ (accession Mendoza), the maximum DSR value (5.0) was obtained 10 days after inoculation, whereas cv. ‘Pájaro’ (accession Cafayate) plants showed minor lesions or no disease symptoms 50 days after inoculation.

When ‘Camarosa’, ‘Chandler’, ‘Sweet Charlie’ and ‘Gaviota’ plants were infected, they showed no significant differences regarding disease symptoms and evolution (Table 1). Only few lesions and strangulation of petioles were observed. It is noteworthy that cv. ‘Pájaro’ (accession Mendoza), ‘Milsei’, ‘Selva’ and ‘Seascape’ reached the maximum DSR value 9-21 days after infection with strain L9, whereas cv. ‘Pájaro’ (accession Cafayate) and ‘Gaviota’ showed an incompatible interaction with minor lesions or no disease symptoms (even many months after the inoculation) and petiole strangulation was not observed in these cultivars (Table 1).

The strain L9 presented white to pale beige colonies with abundant aerial mycelium, and the underside of the colony was gray to dark-olive near the center, and white at the edges of the colony (Figure 1A-B). Growth rate analysis showed that after 12 days of incubation on PDA at 28 ºC and under continuous fluorescent light, average colony diameter was 8.3 cm for the isolate L9 of *C. gloeosporioides* (standard deviation σ_{n,1} = 0.5 cm).

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### Table 1. Disease severity rates (DSR) of strawberry cultivars infected with L9 *Colletotrichum gloeosporioides* strain.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>DSR</th>
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<tbody>
<tr>
<td>‘Pájaro’ acc. Mendoza</td>
<td>5.00 ± 0.25 a</td>
</tr>
<tr>
<td>‘Pájaro’ acc. Cafayate</td>
<td>1.10 ± 0.06 b</td>
</tr>
<tr>
<td>‘Camarosa’</td>
<td>1.85 ± 0.11 b</td>
</tr>
<tr>
<td>‘Chandler’</td>
<td>2.20 ± 0.15 b</td>
</tr>
<tr>
<td>‘Milsei’</td>
<td>4.95 ± 0.45 a</td>
</tr>
<tr>
<td>‘Sweet Charlie’</td>
<td>1.90 ± 0.15 b</td>
</tr>
<tr>
<td>‘Selva’</td>
<td>4.75 ± 0.33 a</td>
</tr>
<tr>
<td>‘Seascape’</td>
<td>4.74 ± 0.24 a</td>
</tr>
<tr>
<td>‘Gaviota’</td>
<td>1.35 ± 0.07 b</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences (LSD Fisher test, P < 0.05).

Results of cultural and morphological studies also showed that isolate L9 presents traits typical of *C. gloeosporioides* (Smith and Black, 1990; Mónaco et al., 2000; Garrido et al., 2008; Nguyen et al., 2010). Comparative phytopathological analysis showed that regardless of disease severity degree, the nine strawberry genotypes used in this study presented qualitatively similar symptoms. Our results showed that whereas cv. ‘Pájaro’ (accession Mendoza), ‘Milsei’, ‘Selva’ and ‘Seascape’ displayed the highest susceptibility toward the isolate L9, cv. ‘Pájaro’ (accession Cafayate) and ‘Gaviota’ showed a totally different behavior. These results also revealed that
each strawberry cultivar can respond differently when challenged with the same pathogen isolate. This suggests that the strawberry-\textit{Colletotrichum} interaction has to be considered on a genotype basis, as each cultivar may respond with a different tolerance degree depending on the particular dominant pathogen present in the fungal complex at the moment of infection (Smith and Black, 1990; Denoys Rothan et al., 1999; MacKenzie et al., 2009). Hence, it becomes important to undertake thorough assessment of the pathogen populations present in an agroecological system, and to perform a correct phytopathological evaluation of these genotypes with the strawberry cultivars used in that particular system.

Taking into account these results, we may conclude that in the case of strawberry, it is more important to assess the fraction of virulent pathogen genotypes present in a specific crop area (in relation to the principal cultivar grown there) than the composition of the population of \textit{Colletotrichum} species present in that area. Therefore, the main pathogen population exchange factor operating in the crop area after some time would be the particular cultivar used by farmers.

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**References**


Ramallo C.J., Ploper L.D., Ontivero M., Filippone M.P.,


