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Races of *Colletotrichum graminicola* pathogenic to maize in Brazil

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ABSTRACT

Anthracnose caused by the fungus *Colletotrichum graminicola* (Ces.) Wilson is the most important disease of maize in Brazil, especially in no-tillage without crop rotation. In this system, maize debris from earlier plantings increases the fungal inoculum potential over time. The use of genetic resistance is the most appropriate and advantageous strategy for anthracnose control. However, the effectiveness and durability of this practice depends on knowledge concerning the genetic variability of *C. graminicola*. In this study, fifteen maize genotypes were tested against 190 single spore of *C. graminicola* collected from infected leaves of maize plants cultivated in seven different Brazilian ecogeographic areas. Five races of *C. graminicola* were identified, and eleven maize genotypes were susceptible to all isolates. Results indicated that both the number of pathogen isolates and the number of genotypes to be tested are pivotal for an accurate identification of *C. graminicola* races. This is the first study showing the occurrence of *C. graminicola* races in Brazil.

1. Introduction

Anthracnose caused by the fungus *Colletotrichum graminicola* (Ces.) Wilson is the most important disease of maize (*Zea mays*) in Brazil (Cota et al., 2012) as well as in other parts of the world (Perkins and Hooker, 1979; Pupipat and Mehta, 1969; Warren et al., 1973; Leonard, 1974). The main effect of this disease is the reduction in grain yield due to low kernel weight and lodging (Dodd, 1980). Although the occurrence of the disease in all plant parts, the leaves and stems are the most affected (Bergstrom and Nicholson, 1999). In the leaf stage, yellow to necrotic lesions with different shapes, can appear at any time during the development of the maize plant, and may cause extensive leaf blight (Bergstrom and Nicholson, 1999). In the ribs, elliptical lesions with acervuli of the pathogen are common. During severe infections, the decreased photosynthetic area on maize leaves can result in early leaf senescence (Jirak-Peterson and Esker, 2011). The top dieback symptoms occur due premature death of the upper leaves and stem internodes during the early stages of grain formation, and also results in reduction of yields (Bergstrom and Nicholson, 1999). Narrow and elliptical lesions in the bark characterize the stalk rot phase of the disease, which turn dark brown to black, with the development of the pathogen acervuli. The rind and pith tissues of the stalk exhibit a dark brown to black color, and the pith tissue may disintegrate, typically starting at the lowest internodes, leading to premature desiccation and tilting of plants (Jirak-Peterson and Esker, 2011). Although the primary source of inoculum for anthracnose stalk rot remains to be determined, many studies have shown that the stalk infection may occur directly by conidia produced in the leaf lesions, inoculum preservation in crop residues, through feeding damages caused by insect pests, or abrasions and wounds in the stalk and root system (Bergstrom and Nicholson, 1999; Jirak-Peterson and Esker, 2011; Lipps, 1983, 1985; Mims and Vaillancourt, 2002; Venard and Vaillancourt, 2007a, 2007b; Sukno et al., 2008; White and Humy, 1976). Some studies reported the lesion expansion in the stalk due to systemic movement of pathogen spores through the vascular tissues (Bergstrom and Nicholson, 1999; Panaccione et al., 1989; Sukno et al., 2008). However, Venard and Vaillancourt (2007a, 2007b) have not found any evidence that *C. graminicola* can enter through maize roots and cause a systemic infection.

In Brazil, maize is planted in two distinct growing seasons with the first crop planted in the summer and the second crop during the winter. The expansion of cultivated land area and the adoption of no-tillage without crop rotation resulted in increased incidence and severity of anthracnose in USA (Bergstrom and Nicholson, 1999; Jirak and Esker, 2009; Jirak-Peterson and Esker, 2011; Lipps, 1985) and Brazil (Casela et al., 2006; Coêlho et al., 2001; Costa et al., 2010; Cota et al., 2012; Cruz et al., 1996; Fernandes and Balmer, 1990).

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The use of genetic resistance is the most efficient and profitable strategy for anthracnose disease control. Badu-Apraku et al. (1987a, 1987b) found a single dominant gene, designated *CgL*, controlling the resistance at both the seedling and mature stages of development and two codominant genes for anthracnose leaf blight (ALB) resistance. Coêlho et al. (2001) reported a monogenic and dominant control of resistance to anthracnose. Rezende et al. (2004) observed that resistance to leaf anthracnose in maize was controlled by a major gene in all crosses and trials, and by polygenes in at least one trial. Thus, the inheritance of resistance to anthracnose in maize remains controversial. In one study, the role of additive effects were the most significant for resistance (Carson and Hooker, 1981) while additive, dominance and epistatic effects were important in other study (Badu-Apraku et al., 1987b). However, the effectiveness and durability of plant resistance as management strategy against anthracnose depends on knowledge about the pathogenic variability of *C. graminicola* in tropical conditions. Harris and Johnson (1967) were the first to suggest the possible existence of physiological races of *C. graminicola* in USA. However, the first report of races of *C. graminicola* was performed by Forgey et al. (1978) who identified eight physiological races from 10 isolates pathogenic to maize. Nevertheless, Nicholson and Warren (1981) using seven of those 10 isolates identified by Forgey et al. (1978) concluded that they did not produce symptoms allowing a clear differentiation of races. Other authors also concluded about the nonexistence of races in this pathosystem (Coêlho et al., 2001; Bergstrom and Nicholson, 1999).

The genus *Colletotrichum* shows great phenotypic plasticity generating conflicting results often difficult to interpret (Crouch et al., 2009a, 2009b, 2009c; Hyde et al., 2009). Consequently, this complicate efforts to understand host relationships, diagnose disease accurately, and the development of effective control strategies through genetic resistance (Hyde et al., 2009; TeBeest et al., 1997).

In *Colletotrichum sublineolum*, which is closely related to *C. graminicola*, races, pathotypes, and haplotypes have been extensively identified in many countries over the last fifty years (Ali and Warren, 1987, 1992; Cardwell et al., 1989; Casela and Ferreira, 1987; Pastor-Corrales and Frederiksen, 1979; Ferreira and Casela, 1986; Frederiksen and Rosenow, 1971; Harris and Johnson, 1967; Harris and Sowell, 1970; King and Frederiksen, 1976; Nakamura, 1982; Nicholson and Warren, 1981; Rosewich et al., 1998; Ozolua et al., 1986; Pande et al., 1991; Thomas et al., 1995). Although is reasonable to expect that a similar diversity of races also may occurs *C. graminicola* due its high genetic similarity with *C. sublineolum*, the existence of races in *C. graminicola* is uncertain.

In this paper, results based on greenhouse experiments concerning the identification of *C. graminicola* races occurring in different Brazilian maize field areas, and a discussion of some key points for disease management strategies and deployment of host resistance against maize stalk rot.

2. Materials and methods

2.1. Differential host genotypes

This study was performed in the Plant Pathology laboratory and in the greenhouse at the National Maize and Sorghum Research Center – CNPMS – EMBRAPA, Sete Lagoas – MG, Brazil. Fifteen maize genotypes were used as differential hosts. Due to scarce information on maize resistance to anthracnose leaf blight in Brazil, the 15 maize genotypes were selected based on both their diverse genetic origin and because they are planted in the main maize production areas. The genotypes were also chosen because they have contrasting reactions to other important foliar diseases like Cercospora leaf spot, Maize White Spot disease, and Polysora rust. These included six inbred lines and five one-way hybrids derived from these lines from Embrapa maize breeding program. The other genotypes were: one one-way hybrid from Pioneer and two from Dow AgroSciences, and one one-way hybrid from Agroeste Company (Table 1).

2.2. Collection of samples, production and maintenance of single spore isolates of C. graminicola

A total of 190 single spore isolates of *C. graminicola* were obtained from 11 Brazilian localities corresponding to different ecological conditions (Uberlândia, Uberaba, Iraí de Minas, and Sete Lagoas, in Minas Gerais state; Jaguarão, Londrina, and Ponta Grossa, in Paraná state; Goianésia, Inhumas, and Rio Verde, in Goiás state; and Passo Fundo, in Rio Grande do Sul) and represent a broad geographical distribution of the pathogen. A single spore isolate was obtained from fragments removed from the border of the lesions and surface sterilized for 2 min in 0.5% sodium hypochlorite. Samples were plated on oat meal agar medium (OMA) with tetracycline and incubated under intermittent fluorescent light at 25 °C for seven days to induce sporulation. Afterward, plates were submerged with 10 ml of sterile distilled water and scraped with a scalpel to remove the conidia. Each sample was passed through a serial dilution to obtain spore suspensions at a concentration ranging from 50 to 100 conidia per ml. An aliquot of 1 ml was transferred to petri dish containing 2% agar and incubated in growth chambers under fluorescent light flashing at 25 °C for 12 h for conidial germination. A single conidium was transferred to culture tubes containing OMA, and after the fungal development, cultures were submerged with 10 ml sterile mineral oil for maintenance.

2.3. Inoculum production

For inoculum production, an aliquot of each culture was spread on OMA medium and incubated for 7 days at 25 °C under continuous fluorescent light to induce sporulation. Thereafter, individual plates were submerged with 10 ml of sterile water, and superficially scraped to remove the conidia. Spore suspensions were filtered through a double layer of cheesecloth, and its concentration was adjusted to 10^6 spores per ml.

2.4. Inoculation

Each genotype (Table 1) was planted in four plastic pots with three plants each. The spore suspension was applied (10 ml per pot) on both

Table 1
Maize genotypes used to test virulence of *C. graminicola* in the greenhouse.

Number	Origin	Genotype	Type	Maturity
1	Embrapa	BRS1001	OWH	SS
2	Embrapa	BRS1010	OWH	SS
3	Embrapa	BRS1030	OWH	SS
4	Embrapa	BRS1031	OWH	SS
5	Embrapa	BRS1035	OWH	SS
6	Embrapa	L3	IL	–
7	Embrapa	L2841	IL	–
8	Embrapa	L2283	IL	–
9	Embrapa	L112	IL	–
10	Embrapa	L141	IL	–
11	Embrapa	L182	IL	–
12	Agroeste	AS1570	OWH	SS
13	Pioneer	30F35	OWH	SS
14	Dow agroscience	2B710	OWH	SS
15	Dow agroscience	2B587	OWH	SS

OWH – one-way hybrid; IL – inbred line; SS – short season.