

# Reaction of maize inbred lines to the bacterium *Pantoea ananas* isolated from *Phaeosphaeria* leaf spot lesions

Luzia Doretto Paccola-Meirelles<sup>\*1</sup>; Walter Fernandes Meirelles<sup>2</sup>; Sidney Netto Parentoni<sup>2</sup>; Ivanildo Evaneo Marriel<sup>2</sup>; Alexandre Silva Ferreira<sup>2</sup> and Carlos Roberto Casela<sup>2</sup>

<sup>1</sup> Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Caixa Postal 6001, CEP 86051-990, Londrina, PR, Brazil, Fax: (43) 371-4079; <sup>2</sup> Embrapa Milho e Sorgo, Caixa Postal 151, CEP 35701-970 Sete Lagoas, MG, Brazil, Fax: (31) 3779-1088. (\* Corresponding Author. E-mail: paccola@uel.br)

## ABSTRACT

A leaf spot disease in maize has been reported throughout all corn producing areas in Brazil, and its incidence and severity have increased significantly. Initial symptoms are characterized by the development of dark green water-soaked leaf spots, which later turn into straw-colored necrotic lesions. Some authors associated the symptoms of this disease to those of the disease *Phaeosphaeria* Leaf Spot caused by fungus *Phaeosphaeria maydis* (f. imperf. *Phyllosticta* sp.). However, we reported in previous works the involvement of a bacterium, the *Pantoea ananas* (syn. *Erwinia ananas*) as the initiator agent of this disease. The aim of this work was to evaluate the behavior of corn genotypes when inoculated with *P. ananas* in the greenhouse. The results reinforced those previously obtained that showed the role of the bacterium as the initiator agent of the disease.

**KEY WORDS:** *Pantoea ananas*, *Erwinia ananas*, resistance.

## INTRODUCTION

Leaf spot disease in maize was first observed in Brazil in the 1980s and its incidence and severity have increased significantly since the 1990s (Fantin, 1994). This disease has been reported throughout all corn producing areas of the country. Initial symptoms are characterized by the development of dark green water-soaked leaf spots, which later turn into straw-colored necrotic lesions. Under favorable environmental conditions, the disease can lead to premature leaf death, resulting in a reduction in the plant cycle and in the weight and size of grain (Pinto et al., 1997). Yield reductions of up to 63% were observed by Pinto (1995) in severely infected plants.

The symptoms of this disease in Brazil have been associated to those of the disease *Phaeosphaeria* Leaf Spot caused by *Phaeosphaeria maydis* (P. Henn) Rane, Payak and Renfro (sin. *Sphaerulina maydis* = *Leptosphaeria zae maydis*), f. imperf. *Phyllosticta* sp. (Rane et al., 1966) (Fig. 1A). Recently, the involvement of the bacterium *Pantoea ananas* (syn. *Erwinia ananas*) with this disease was reported (Paccola-Meirelles et al., 2001).

The aim of this work was to evaluate the behavior of corn genotypes, previously known by their contrasting reactions to *Phaeosphaeria* Leaf Spot in field under natural infections, when inoculated with *P. ananas* in the greenhouse, and to verify if the

reactions are compatible with those observed in field conditions.

## MATERIAL AND METHODS

Six maize inbred lines from the Active Germplasm Bank of Embrapa Maize and Sorghum Center were used in this study: L26, L 404-1 and L11 (resistant) and L 942, L 724 and L 726 (highly susceptible).

The strain Reis 1 of *P. ananas* (Paccola-Meirelles et al., 1999) was grown for 12h in TSA (Tryptic Soy Agar) medium, at 30° C. Then 2mL was transferred to 50ml of TSB (Tryptic Soy Broth) medium, incubated for 6 h at room temperature., in a rotary shaker, and used as inoculum.

Leaves of 30 and 35-day-old maize plants were injured with a rough sponge and then sprayed with the inoculum containing about 10<sup>8</sup> CFU. Control plants were sprayed with sterilized water. Three pots with two plants per pot were used in each treatment. After inoculation plants were kept for 60h in a dew chamber with 100% relative humidity. Disease symptoms developed on inoculated plants within 5-7 days. Lesions developed initially as dark green areas with a swollen and water-soaked aspect, that later became grayish and finally turned into straw-colored lesions similar to those resulting from natural infections in the field.

Symptom severity was evaluated 10 days after inoculation, according to the following scale: 0 = no symptoms; 1 = < 2 lesions/ cm<sup>2</sup>; 2 = 2 – 3 lesions/ cm<sup>2</sup>; 3 = > 3 lesions/ cm<sup>2</sup>.

## RESULTS AND DISCUSSION

Susceptible plants inoculated with the bacterium under controlled conditions, showed symptoms similar to those of *Phaeosphaeria* Leaf Spot lesions. The results presented confirmed the reactions to *Phaeosphaeria* Leaf Spot previously observed in field. The inbreds L26, L404 -1 and L11 showed resistance reactions and the L942, L724 and L 726 genotypes showed susceptible reactions (Figure 1 and Table 1). The fact that the reactions obtained in the greenhouse tests are in agreement with those observed under natural infections, reinforce the results previously obtained by Paccola-Meirelles et al. (1999, 2001) that showed the role of the bacterium as the initiator agent of the disease. The methodology used here demonstrates that is possible select maize genotypes resistant to this disease.

## RESUMO

### Reação de linhagens de milho à bactéria *Pantoea ananas* isolada de lesões da mancha foliar de *Phaeosphaeria*

Uma doença causando mancha foliar em milho tem sido observada em todas as regiões produtoras de milho no Brasil, e sua incidência e severidade vem aumentando significativamente. Os Sintomas iniciais da doença se caracterizam pelo desenvolvimento de

**Table 1.** Reaction of maize genotypes to strain Reis 1 of *Pantoea ananas* (syn. *Erwinia ananas*) isolated from initial stage *Phaeosphaeria* Leaf Spot lesions of maize.

Inbred lines	Lesion Score (1 to 3 scale)	Lesion type
L11	2	Small <sup>1/</sup>
L 404-1	0	-
L26	1	Small <sup>1/</sup>
L724	3	Medium <sup>2/</sup>
L726	3	Medium <sup>2/</sup>
L 942	3	Medium <sup>2/</sup>

<sup>1/</sup> Small: 0.05-0.2 cm; <sup>2/</sup> Medium: 0.21- 0.6 cm.

manchas foliares oleosas de coloração verde escuras, as quais tornam-se necróticas de coloração palha. Alguns autores associaram os sintomas desta doença com aqueles da doença mancha foliar de *Phaeosphaeria* causada pelo fungo *Phaeosphaeria maydis* (f. imperf. *Phyllosticta* sp). Entretanto em trabalhos anteriores nos reportamos o envolvimento de uma bactéria, a *Pantoea ananas* (syn. *Erwinia ananas*) como agente iniciador da doença. O objetivo deste trabalho foi avaliar o comportamento de genótipos de milho quando inoculados com *P. ananas* em casa de vegetação. Os resultados reforçam aqueles descritos anteriormente que demonstram o papel da bactéria como agente iniciador da doença.



b1



b2

**Figure 1.** a) Maize plant showing the described disease as *Phaeosphaeria* Leaf Spot under natural infection conditions in the field; b) Maize plant, artificially inoculated with *Pantoea ananas* bacterium (syn. *Erwinia ananas*), showing characteristic symptoms of the disease described in Brazil as *Phaeosphaeria* Leaf Spot: b1- genotype showing sensitive reaction and b2- L26 genotype showing resistance reaction

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