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Transmission of maize bushy stunt phytoplasma by *Dalbulus maidis* leafhopper

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Abstract

The maize bushy stunt phytoplasma (MBS) transmission by *Dalbulus maidis* was studied using two colonies of leafhoppers caged for five days in two MBS symptomatic plants. After the latent period, one leafhopper was confined per seedling in two different maize genotypes (popcorn hybrid and L22 inbred line). Leafhoppers samples were submitted to PCR analyzes before and after inoculation. Colony 1 and 2 had 27.5% and 3.3% of infected leafhoppers, respectively. Maize bushy stunt phytoplasma transmission to popcorn was almost 100% and around 30% to L22.

Key words: MBS, molicutes, *Dalbulus maidis*, *Zea mays*.

Introduction

The corn leafhopper *Dalbulus maidis* transmits molicutes maize bushy stunt phytoplasma (MBS) and corn stunt spiroplasma (CSS) in a persistent manner (Nault, 1980). Studies about MBS transmission by *D. maidis* show influence of the MBS strains from different regions, temperature, and spiroplasma acquisition (Moya-Raygosa and Nault, 1998; Legrand and Power, 1994; Oliveira *et al*., 2007). Understanding of the best condition for maximum transmission efficiency of MBS can contribute to improve the methodology for its inoculation. In this study the MBS transmission by infected leafhopper *D. maidis* was studied by PCR analyzes.

Materials and methods

Two experiments were carried out in screen house conditions. Two colonies of young adults of *D. maidis* (around 300 individuals/colony) were given a 5-day acquisition access period (AAP) on two MBS infected plants, that presented typical symptoms, followed by 39 and 44 days of incubation period on healthy plants (Nault, 1980), for colonies 1 and 2, respectively. Each experiment was carried out with 60 plants of a popcorn maize hybrid and 60 plants of susceptible maize L22 inbred line. It was used one leafhopper per plant with an inoculation access period (IAP) of six days in eight days old seedlings. Colony 1 was used in experiment 1 and colony 2 in experiment 2. The control consisted of six popcorn and six L22 plants with healthy leafhoppers. The MBS strain used in the experiments was obtained from popcorn plants. It has been cultivated for six years, in the same popcorn. A total of 132 pots with 5 kg of substrate and one plant per pot, cultivated for 60 days, were used in each experiment. Plant symptoms were evaluated weekly. The temperatures and relative humidity data were registered at the meteorological station of Sete Lagoas. Samples of 10 leafhoppers before testing, 10 leafhoppers after IAP from popcorn and 10 from L22 were tested by PCR (Lee *et al*., 1993) using primers R16F2 and R16R2 for phytoplasma detection. DNA from plants infected with MBS was used as positive control.

Results

PCR analyzes showed weak bands of 1.2 kb and non-specific bands in 21 leafhoppers samples from colony 1 and one specific 1.2 kb band in eight leafhoppers samples from colony 2 (table1). The MBS transmission was confirmed by plant symptoms only for leafhoppers samples that presented strong bands. Based on strong band percentage, leafhoppers infection was estimated in 27.5 and 3.3% in colonies 1 and 2, respectively. The MBS transmission to popcorn from infected leafhoppers was almost 100%, and around 30% for L22 inbred line (table 2). The environmental climatic variables are presented in table 3.

Discussion

MBS acquisition and transmission was dependent on the plant source and on the environmental temperature condition for AAP. The low MBS acquisition under minimum temperature of 17°C might be responsible for low MBS concentration in leafhoppers that was not enough for transmission and resulted on PCR with weak bands. Results suggest that could be convenient to use at least three leafhoppers for inoculation, after AAP and latent period. Popcorn maize showed to be more susceptible to the MBS than L22. The variability of MBS geographic isolates (Moya-Raygosa and Nault, 1998) and temperature adaptation might help MBS to survive in areas with different maize cultivars and environmental conditions, supporting this emergent disease spreading. PCR analyzes of leafhoppers can help on the decision of how many individuals are needed for an efficient inoculation, since confirmed their presence only when observed a 1.2 kb strong band as a PCR product.

Table 1. Results for PCR analyzes and transmission by infected leafhoppers.

Leafhoppers samples	Experiment 1 (colony 1) PCR product weak[1]	strong	Symptomatic plants
10 leafhoppers before IAP test	7	0	Not tested
10 leafhoppers after IAP in popcorn	7	0	0
10 leafhoppers after IAP in L22	5	0	0
Leafhoppers samples	**Experiment 2 (colony 2) PCR product weak[1]**	**strong**	**Symptomatic plants**
10 leafhoppers before IAP test	2	3	Not tested
10 leafhoppers after IAP in popcorn	0	3	3
10 leafhoppers after IAP in L22	0	2	2

[1] Band of 1.2 kb and non-specific bands; IAP, Inoculation access period.

Table 2. Results of transmission by leafhopper colonies 1 and 2.

	Experiment 1 (colony 1)		Experiment 2 (colony 2)	
	Popcorn	L22	Popcorn	L22
Number of MBS symptomatic plants	2	0	15	5
Percentage of MBS symptomatic plants	3.3	0	25	8.3
Percentage of MBS transmission (from strong band percentage 27.5)	-	-	92.5	30.7

Table 3. Average and standard deviation of maximum and minimum temperatures (Tmax, Tmin) and relative air humidity (RH) at Embrapa Maize and Sorghum (meteorological station of Sete Lagoas - INMET), during MBS acquisition access period (AAP), latent period (LP) and inoculation access period (IAP) by leafhoppers colonies (experiments 1 and 2).

Period	Experiment 1			Experiment 2		
	Tmax (°C)	Tmin (°C)	RH (%)	Tmax (°C)	Tmin (°C)	RH (%)
AAP	32.1 ± 1.2	17.4 ± 0.9	57.7 ± 4.4	30.4 ± 3.7	20.4 ± 0.5	75.2 ± 17.4
PL	29.6 ± 2.1	19.5 ± 1	75.1 ± 14.5	30.1 ± 3.3	19.2 ± 1	72.1 ± 14.5
IAP	32.7 ± 0.6	18.5 ± 0.3	58.9 ± 2.4	32.8 ± 0.8	18.8 ± 0.7	60.2 ± 4.19

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