Artículo Original

Primer informe de caracterización molecular de of Septoria glycines de soja en Argentina y Uruguay

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Resumen

La mancha marrón en soja es causada por *Septoria glycines Hemmi*. El objetivo del estudio fue analizar molecularmente los aislamientos de *Septoria glycines* en Argentina y en Uruguay. Estos aislamientos fueron incubados a 24 ± 2 °C en agar papa dextrosa (PDA) durante 15 días. Cada uno de los 12 aislamientos fueron analizados mediante amplificación por PCR y secuenciación de fragmentos de la región espaciadora transcrita interna (ITS) y del gen de actina (ACT) con los primers: ITS1 / ITS4, ACT-512F / ACT-783R. Los resultados indican que fue posible identificar 7 y 8 haplotipos diferentes con las secuencias diana ITS y actina, respectivamente. Las secuencias ITS, bajo los números de acceso de Genbank MT162722-MT162733, mostraron homologías del 98,54 % al 99,38 % con las secuencias ITS de S. glycines KU195826.1 de China y AY826767.1 de EE. UU. Las secuencias de actina MT180168.1-MT180179.1 mostraron homologías del 97,35 % al 100 % con KF253733.1, previamente reportada como S. glycines en Japón. También se encontraron altas homologías con ITS y secuencias de actina de otras especies de China (S. tormentillae -MN150507.1), Japón (S. menthae-KF253780.1), Corea del Norte (S. glycinicola- KF253734.1, S. codonopsidis - KF253689.1) e Irán (S. sonchi-JQ308342.1). Sin embargo, ninguna de estas especies fue reportada como agente etiológico de la enfermedad de la mancha marrón en soja. Se concluye que la información genética confirmó que los aislamientos pertenecían *a Septoria glycines*. Esta es la primera descripción molecular del patógeno en Sudamérica.

Palabras clave: Septoria glycines, Soja, ITS, Actina, Haplotipos.

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Primer informe de caracterización molecular de of Septoria glycines de soja en Argentina y Uruguay

Brown spot on soybeans is caused by Septoria glycines Hemmi. The objective of the study was to molecularly analyze the isolates of Septoria glycines in Argentina and Uruguay. These isolates were incubated at 24 ± 2 °C in potato dextrose agar (PDA) for 15 days. Each of the 12 isolates were analyzed by PCR amplification and sequencing of fragments of the internal transcribed spacer region (ITS) and the actin gene (ACT) with the primers: ITS1 / ITS4, ACT-512F / ACT-783R. The results indicate that it was possible to identify 7 and 8 different haplotypes with the target sequences ITS and actin, respectively. The ITS sequences, under the Genbank accession numbers MT162722-MT162733, showed homologies of 98.54 % to 99.38 % with the ITS sequences of S. glycines KU195826.1 from China and AY826767.1 from the USA. Actin sequences MT180168.1-MT180179.1 showed homologies of 97.35 % to 100 % with KF253733.1, previously reported as S. glycines in Japan. High homologies with STIs and actin sequences from other species were also found from China (S. tormentillae-MN150507.1), Japan (S. menthae-KF253780.1), North Korea (S. glycinicola-KF253734.1, S. codonopsidis -KF253689.1) and Iran (S. sonchi-JQ308342.1). However, none of these species was reported as an etiologic agent of soybean brown spot disease. It is concluded that the genetic information confirmed that the isolates belonged to Septoria glycines. This is the first molecular description of the pathogen in South America..

Keywords: Septoria glycines, Soybean, ITS, Actin, Haplotype.

Introduction

Brown spot, caused by the fungus Septoria glycines, is one of the most common foliar diseases of soybean (Glycine max).

Lesions begin as dark brown and irregularly shaped, with the surrounding tissue turning yellow or chlorotic, and affected leaves may drop prematurely (Young, 1979).

Although disease susceptibility varies among lines, there are no sources of resistance or genes for this disease (Brogin et al., 2003). The fungal pathogen, S. glycines, overwinters in soybean debris forming pycnidia. Pycnidiospores produced in the following growing season are spread by rain splash to seedlings and initiate initial outbreaks from the lower canopy. Typical symptoms of this disease are irregular brown necrotic lesions surrounded by an extensive chlorotic zone. Vertical progression of disease development in the later stages of soybean maturity is positively associated with yield loss. We previously reported that when disease symptoms reach up to 30 % of plant height at the R6 stage, it leads to an estimated 10 % yield loss, and if it reaches 80 % of plant height, it leads to an estimated 27 % yield loss (Carmona, 2014).

The objective was to molecularly analyze Septoria glycines isolates from Argentina and Uruguay.

Materials and methods

Isolations were made from leaves from Argentina and Uruguay under a vertical laminar flow chamber (Dahuerhaft by BIOBASE BIODUSTRY (SHANDONG) Co., Ltd.) with the burner lit under sterile conditions, in Petri dishes previously prepared using Potato Dextrose Agar (PDA) as the culture medium, with a pH of 5.5. The culture medium was incubated for 15 days in a growth chamber at 24°C ± 2°C, alternating between 12 hours of near-ultraviolet light and 12 hours of darkness. The isolates were described macroscopically and microscopically.

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Molecular Study of Septoria Isolates

To conduct the molecular study of the isolates, approximately 50 mg of fresh, actively growing mycelium was sampled. DNA was extracted, and its quality and concentration were estimated on 0,8 % agarose gels stained with ethidium bromide (5 µl/100 ml TAE) and compared with samples of known concentration. These DNAs were subsequently used to amplify two known partial sequences by polymerase chain reaction (PCR). The primers were named: actin and the internal transcribed spacer (ITS) region.

The samples were amplified in a 30 µl reaction volume. Each reaction used 1 µl of template DNA, 7.8 µl of reaction mix, and 0.23 µl of Taq.

The reaction mix was made with 500 µl of 10X Buffer, 150 µl of MgCl2 (50 mM), 10 µl of each of the dNTPs (100 mM), and adjusted to a final volume of 1300 µl with water.

The annealing temperature or TM was different for each sequence: act: 53 °C and ITS: 55°C.

The thermocycling program used was as follows: 5 min at 95 °C; 45 cycles of 45 seconds at 94 °C, 45 seconds at the annealing (melting) temperature (TM) corresponding to each primer pair, and 45 seconds at 72 °C. and a final extension step of 10 min at 72°C. The PCR amplification products were stored in a freezer at -20°C.

Before sending the samples for sequencing, the quality and quantity of amplified DNA were analyzed on agarose gels as previously described, using 3 µl of the PCR product, 10 µl of xylene glycol as a dye, and 5 µl of 100 bp marker. The PCR products were sent to an external sequencing service provider using the Sanger technique (Macrogen, Korea).

Results

Seven and eight different haplotypes were identified with the ITS and actin target sequences, respectively. The ITS sequences, under Genbank accession numbers MT162722-MT162733, showed 98,54 % to 99,38 % homologies with the ITS sequences of S. glycines KU195826.1 from China and AY826767.1 from the USA. The action sequences MT180168.1-MT180179.1 showed 97,35 % to 100 % homologies with KF253733.1, previously reported as S. glycines in Japan. High homologies with ITS and action sequences from other species from China (S. tormentillae -MN150507.1), Japan (S. menthae-KF253780.1), North Korea (S. glycinicola-KF253734.1, S. codonopsidis - KF253689.1) and Iran (S. sonchi -JQ308342.1) were also found; however, none of these species was reported as an etiological agent of soybean brown spot disease.

Discussion

It is considered an end-of-cycle disease, although it can manifest throughout the crop, but it becomes more widespread in advanced reproductive stages (R5-R6) (Carmona 2014).

The G143A mutation (Bolton et al., 2012) has been detected in the mitochondrial cytb gene of Septoria glycines, associated with resistance to QoI fungicides (quinone inhibitors). This mutation indicates the need to monitor the fungus' sensitivity to fungicides and the dynamics of these mutations at the population level.

The cytb gene is a key component of the mitochondrial respiratory chain and plays an important role in fungicide resistance in several fungi (Rivera et al., 2018).

The pathogen often infects plants at the V2 to V3 stage and remains in the lower canopy until the reproductive stage.

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The development of specific and sensitive molecular tools for the detection, quantification, and diagnosis of Septoria brown spot is necessary to manage fungicide applications, better understand epidemics caused by this disease, and characterize the pathogen's interactions with other late-season soybean diseases (White et al., 1990).

The Septoria glycines sequences used were identified for the species at NCBI

Conclusions

Genetic information confirmed that the isolates belonged to Septoria glycines. This is the first molecular description of the pathogen in South America.

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