Molecular Detection of *Stenocarpella maydis* in Corn and Biochemical Changes Induced by Diplodiatoxin

Premila N. Achar, Zhengjun Xia, M.F. Rahman

I. Department of Biological and Physical Sciences, Kennesaw State University, Kennesaw, GA 30144. 2. Laboratory of Plant Breeding and Genetics, Faculty of Horticulture, Chiba University, Chiba, Japan. 3. Department of Biology, Indian Institute of Chemical Toxicology, Hyderabad, India.

**INTRODUCTION**

Corn is one of the major economic crops all over the world. Corn infected by *S. maydis* causes field outbreak of a mycotoxicosis known as diplodiosis, which is characterised by ataxia, pararalysis, and paralysis and can also cause liver damage to animals fed on infected crops (Kellerman et al., 1985). Molecular techniques allow more specific and sensitive identification and detection of pathogens, although serological and conventional methods are often used in routine detection of micro-organisms from plants or the environment. Random amplified polymorphic DNA (RAPD) markers have been widely used for genetic diversity of many fungal species. The ribosomal DNA (rDNA) of fungal genome is also useful for detection using PCR (Toth et al., 1996). The main aim of this study was to design specific PCR primers for detection of *S. maydis* on the basis of sequence information of the ITS region of rDNA. Further, the toxicity of diplodiatoxin was determined in relation to changes in serum, brain, liver, and kidney of male and female rats.

**MATERIALS AND METHODS**

**RAPD and PCR Markers for the Differentiation and Detection of *Stenocarpella maydis***

- PCR amplification was performed in 50 μl reaction volumes containing 1× Taq incubation buffer, 10 mM each of dNTPs, 0.1 mM primer, 100 ng of genomic DNA, 1U Taq DNA polymerase (Gibco, BRL) and sterilized deionized water.
- A hot-start PCR procedure was used for amplification: Denaturation at 96°C for 5 min followed by a hold step at 96°C to allow addition of Taq DNA polymerase, 35 cycles of 1 min at 34°C; extension for 2 min at 72°C; and denaturation for 15 sec at 92°C. A final cycle of 1 min at 34°C followed by 5 min at 72°C.
- PCR and RAPD fragments were cloned with PCR-Script Cloning Kits (Stratagene). Plasmid DNAs were purified either with High Pure Plasmid Isolation Kit (Boehringer) or conventional Alkaline lysis method.
- Fluorescence-based DNA sequencing was conducted at Medical School, University of Cape Town.
- Searches for the most similar sequences in the sequence databases were performed by the BLAST search algorithm.

**RESULTS**

- Results of RAPD and RFLP showed that two distinct genetic groups exist among *S. maydis* isolates.
- Sequence of ITS1 and ITS2 region of rDNA of *S. maydis* revealed higher homogeneity to *Phomopsis* spp. with a maximum similarity of 93%.
- Primers P1/P2 and P3/P4 permit a sensitive and specific detection of *S. maydis* in the field.

**DISCUSSION**

- Male and female rats were given single dose of 5.7 mg/kg diplodiatoxin mixed in coconut oil through oral intubation.
- For sub-acute toxicity study, the rats were orally treated with 0.27 mg/kg/day for 21 days.
- In liver, ACP increased while AKP decreased in both male and female treated rats.
- Diplodiatoxin causes lacunae formation in liver. Diplodiatoxin increased aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels in serum and acetylcholinesterase (ACHE) in RBC's but the membrane bound enzymes decreased in liver in both male and female treated rats.

**LITERATURE CITED**


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**Fig. 1** Electrophoresis of random amplified polymorphic DNA fragments with primer UBC 220 on a 2.5% agarose gel. Lane 1 to 5: *S. maydis* isolates. Lane 6: M DNA digested with Hind III (Boehringer-Mannheim).

**Fig. 2** Electrophoresis of random amplified polymorphic DNA fragments with primer UBC 220 on a 2.5% agarose gel. Lane 1 to 5: *S. maydis* isolates. Lane 6: M DNA digested with Hind III (Boehringer-Mannheim).

**Fig. 3** Alignment of DNA partial sequences of the internal transcribed spacer region of *S. maydis* from isolates of *S. maydis*. Lane 1: through 9: U7B, U6H2, U2, U3H, S6, Y4, Y10, respectively. Lane 6: PCR product without restriction.

**Fig. 4** Alignment of DNA partial sequences of RAPD fragments of random DNA of *S. maydis*. The location of primers P1 and P2 are indicated. The sequence of P1 is complementary to those indicated.

**Fig. 5** Phenogram showing relationships among 34 *S. maydis* isolates. Genetic distances were obtained by random amplified polymorphic DNA analysis using 5 primers, UBC220, UBC233, and UBC248.

**Fig. 6** Alignment of DNA partial sequences of the internal transcribed spacer region of *S. maydis* from isolates of *S. maydis*. Lane 1: through 9: U7B, U6H2, U2, U3H, S6, Y4, Y10, respectively. Lane 6: PCR product without restriction.

**Fig. 7** Alignment of DNA partial sequences of the internal transcribed spacer region of *S. maydis* from isolates of *S. maydis*. Lane 1: through 9: U7B, U6H2, U2, U3H, S6, Y4, Y10, respectively. Lane 6: PCR product without restriction.

**Fig. 8** Alignment of DNA partial sequences of the internal transcribed spacer region of *S. maydis* from isolates of *S. maydis*. Lane 1: through 9: U7B, U6H2, U2, U3H, S6, Y4, Y10, respectively. Lane 6: PCR product without restriction.

**Fig. 9** Alignment of DNA partial sequences of the internal transcribed spacer region of *S. maydis* from isolates of *S. maydis*. Lane 1: through 9: U7B, U6H2, U2, U3H, S6, Y4, Y10, respectively. Lane 6: PCR product without restriction.