AFLP FINGERPRINTING OF FUNGI OF THE GENUS *VERTICILLIUM*

RADIŠEK S[1]., JAKŠE J[2]., JAVORNIK B[2].

[1]Institute of Hop Research and Brewing Žalec, Cesta Žalskega tabora 2, 3310 Žalec
[2]Centre for Plant Biotechnology & Breeding, Biotehnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana 1000, Slovenija

INTRODUCTION

The genus *Verticillium* Nees ex Link contains a numerous group of asexual fungi, of which the plant pathogen species *Verticillium alboatrum* Reinke et Barthold and *Verticillium dahliae* Klehban are economically important. Traditional detection and diagnosis of *Verticillium* species is primarily based on the use of semi-selective media, vegetative compatibility analysis, differences in virulence, cultural differences and biochemical characteristics (Arora et al. 1996). These methods are lengthy and laborious, but necessary for the identification of species and pathotypes for appropriate phytosanitary measures, for disease resistance breeding and crop selection for a particular field (Koike and Fujita.1996). Wider use of molecular methods in plant pathogen diagnostics have shown that classical methods can be successfully complemented with such methods because molecular analysis provides an accurate, faster and less resource demanding means of pathogen detection.

AFLP (amplified fragment length polymorphism) is a novel molecular technique with wide application in many different organisms, mainly due to the ability to detect a very high number of polymorphisms in a single assay, good repeatability and possibilities of automation (Vos et al. 1995). It is based on the selective PCR amplification of restriction fragments from total digestion of genomic DNA.

The purpose of the current study was to apply the AFLP technique to the phytopathogenic fungi *Verticillium alboatrum* and *Verticillium dahliae* and define the genetic relationship between them and between mild and lethal forms of *Verticillium alboatrum* in hop wilt.

MATERIAL AND METHODS

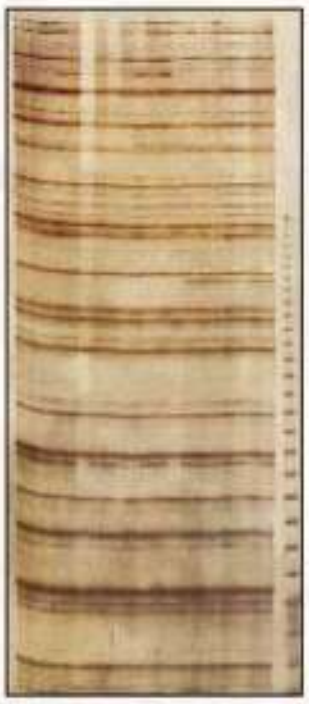
Twelve isolates of the lethal form and six isolates of the mild form of *Verticillium alboatrum* from hop, one from cucumber, one from petunia and two isolates of *Verticillium dahliae* from hop and one from pepper were analysed. The isolates were obtained from different areas in the Savinja valley and maintained as monosporic cultures on potato dextrose agar (PDA).

AFLP was performed using the protokol, which was similar to that reported by Vos et. al. (1995) and GIBCO BRL, AFLP™ Analysis System I, with modifications in detection techniques, whereby silver staining was used instead of radioactivity. The genomic DNA was extracted by SDS (Lee and Taylor, 1990) method and digested with two restricted enzymes *Eco* RI and *Msp* I. After digestion, the restriction fragments were ligated with enzyme specific adapters. Preamplification was performed with primers having no selective nucleotides and the products were used as a template for selective amplification using primers with two selective nucleotides. Two isolates of mild and lethal forms of *Verticillium alboatrum* from hop and two isolates of *Verticillium dahliae* from hop and one from red pepper were initially screened with 40 primer combinations. The amplified products were separated on 5 % denaturing polyacrylamid gels and visualised with silver staining.

RESULTS AND DISCUSSION

Generally, primer pairs produced 35 to 65 bands in a range of 50 to 800 base pairs. Based on initial screening, 8 primer pairs were chosen to analyse differences between all isolates. All primer combinations from screening clearly distinguished between *Verticillium alboatrum* and *Verticillium dahliae*, and 8 primer combinations showed polymorphisms between mild and lethal forms of *Verticillium alboatrum* from hop.

The AFLP technique was found to be useful in diagnostics and estimations of genetic polymorphisms of the fungi *Verticillium alboatrum* and *Verticillium dahliae*. This technique is objective, repeatable, and enables the identification of polymorphisms associated with host specificity, virulence and avirulence, which is very important for carrying out proper disease control.



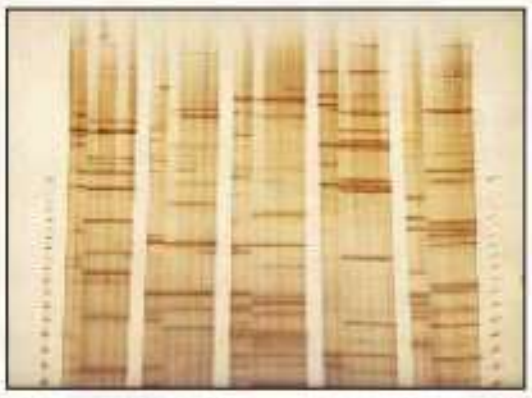


Figure 1: AFLP fingerprints of (from left) two isolates of *Verticillium dahliae* and five of *Verticillium alboatrum*, obtained with five primer combinations (from left) *Eco* RI-GT/*Msp* I-AG, *Eco* RI-GT/*Msp* I-GA, *Eco* RI-GT/*Msp* I-CG, *Eco* RI-GT/*Msp* I-TA and *Eco* RI-GT/*Msp* I-TG.

Figure 2: Example of AFLP fingerprints of *Verticillium alboatrum* isolates, using the primer combination *Eco* RI-GA/*Msp* I-AT.

Figure 3: Conidiophores with phialides and conidia of *Verticillium alboatrum* isolate from hop (200 x magnification).

Figure 4: Black resting mycelium of *Verticillium alboatrum* (200 x magnification).

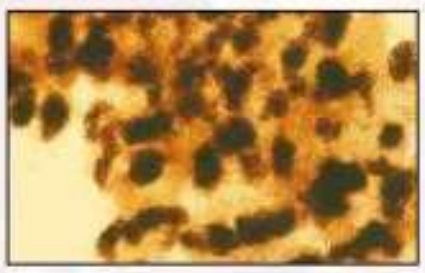


Figure 5: Black microsclerotium of *Verticillium dahliae* (200 x magnification).

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