DEVELOPING OF DNA-MARKER TO THE *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* RESISTANCE GENES OF TOMATO

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Abstract: Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is a destructive disease of tomato crops worldwide. The use of resistant varieties is the best strategy for disease control. In the present study we analyze eight tomato lines and hybrids for Fusarium wilt disease resistance by polymerase chain reaction. Total genomic DNA was extracted from young leaves of three-week-old plants of tomato. Results of PCR of eight tomato lines and hybrids indicated that there are one dominant heterozygote, two recessive homozygotes and five dominant homozygotes. Also, results of polymerase chain reaction showed that it needs less time and is cheaper. Also by using this method, it is possible to determine genotype of plant (homozygote or heterozygote) without presence of the pathogen. Therefore, PCR technique was used in the identification gene *I2* conferring resistance to *F. oxysporum* f. sp. *lycopersici*.

Key words: tomato, Fusarium wilt, PCR

INTRODUCTION

Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. sp *lycopersici* (Sacc.) Snyder & Hans. and tomato (*Lycopersicon esculentum* Mill.) is the only host for forma specials (f.sp.) lycopersici. Three different races (1, 2, and 3) are distinguished by their differential virulence on tomato cultivars containing different dominant resistance genes (Mes *et al.* 1999). Based on the existence of monogenic dominant resistance traits, a gene-for-gene relationship between *F. oxysporum* f. sp. *lycopersici* and tomato is generally assumed. Fungal races contain dominant avirulence genes that correspond to dominant resistance genes in some plant cultivars are unable to infect them (Flor 1971). The primary control strategy of pathogen consists of breeding wilt-resistant cultivars (Beckman 1987). To identify the dominant resistance genes it is essential to understand the interaction between pathogen and tomato cultivars on a molecular level. To reach this aim, molecular markers are being widely used as a principal tool for breeding of many crops, e.g. tomato. In particular, a great work has been realized to find molecular markers linked to disease resistance genes (Grube *et al.* 2000; Bai *et al.* 2003). Today there is an increasing interest in the application of polymerase chain reaction (PCR) technology for the identification of plant pathogenic fungi.

The dominant I2 gene in tomato confers resistance against race 1 and 2 of the pathogen. The gene has been introgressed from a wild tomato species *Lycopersicon pimpinellifolium* (Stall and Walter 1965) and has been mapped genetically to chromosome 11 (Laterrot 1976). The I2 locus on chromosome 11 is composed of seven homologues. Recently, three members of this gene cluster have been cloned and characterized (Ori *et al.* 1997). The I3 locus from *L. pennellii* confers resistance to race 1, 2 and 3 and is mapped on chromosome 7 (Bournival *et al.* 1989, 1990).

They have shown a structural similarity to resistance gene (R genes) group that encode cytoplasmic proteins containing a nucleotide binding site motif (NBS) and leucine-rich repeats (LRRs). The R genes of this group are involved in resistance process characterized by a hypersensitive response. The members of the I2C family were mapped to five genomic positions. Two of these are clusters of several genes, both located on chromosome 11. Only circumstantial evidence was provided for the involvement of the cloned I2C-1 and I2C-2 genes in Fusarium resistance (Guus *et al.* 1998). The I2 gene is a member of I2 cluster that confers full resistance to *F. o.* f. sp *lycopersici race 2*. They show 82 and 88% similarity to the I2C-1 and I2C-2, respectively (Fig. 1). The I2C-1 and I2C-2 did not confer complete resistance that would be expected for a dominant resistance gene. Only partial resistance was accomplished by the I2C-1 gene.

The aim of our study was developing a DNA-based marker of *Fusarium oxysporum* f. sp. *lycopersici* that allows identifying resistant and sensitive genotypes of tomato.

MATERIALS AND METHODS

Plant material

Tomato seeds of several lines and hybrids were planted in the soil mixture with sand (80 : 20) and grown in seedling plug trays (plug size 3.4 by 3.4 by 5 cm, 64 plug/tray). Plug trays were kept at 22 to 28°C, 60–75% relative humidity, and 14-h light.

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DNA extraction

DNA extraction followed the protocol outlined by Bernatzky and Tanksley (1986) with some modification from 41 plants of 8 tomato lines and hybrids. About three young leaves of three-week-old plants of tomato were ground in buffer (0.35 M sorbitol, 100 mM tris-HCL, 5 mM EDTA, PH 7), incubated at 3–5°C and the mixture was centrifuged at 14 000 rpm for 10 min. Then was added lysis buffer (1 M Tris-HCL, PH 7.5, 0.5 mM EDTA, 5 M Nacl, 2% CTAB and incubated at 65°C for one hour. Extraction was washed out with a mixture of chloroform and isoamyl alcohol (24 : 1, vol/vol) and centrifuged at 14 000 rpm for 10 min. DNA was precipitated by adding one-half volume isopropanol and centrifuged at 14 000 rpm for 10 min. Precipitant (DNA pellets) was dissolved in sterile water. Quantity and purity of isolated DNA was evaluated by electrophoresis in 1% agarose gel.

Polymerase chain reaction

The PCR reaction mixture (25 μl) contained the following components: 0.25 mM of the each dNTP, 70 mM Tris-HCl, PH 8.6, 0.001% Triton-X100, 16,6 mM $(NH)_4So_4$, 1,5 mM $Mgcl_2$, 0,3 μM of Fus1 and Fus2 primers, 100 ng DNA of template genomic DNA, 2.5 U DNA polymerase. Amplification reaction in a thermocycler were performed at 94°C for 2 min; 30 cycles – 94°C for 30 s, 65°C for 30 s, 72°C for 30 s; 72°C for 7 min.

Restriction was carried out in 10 μl restricted mix which consisted of 4 μg PCR- product, 20 unit restriction enzyme and 1μl suitable buffer and then added to solution 2 μl EDTA. DNA separated in 1.5% agarose gel.

RESULTS

One of the most effective methods for detection of DNA polymorphism is using PCR with the following restriction fragments of the PCR product (CAPS- marker). We used restriction analysis of known members of I2C cluster for detection of the resistant and sensitive genotypes (Fig. 1). We developed primers, that allow to distinguish resistant and susceptible plants without restriction of the PCR products (Fig. 2).

To develop co-dominant marker, we amplified LRR repeats of I2C genes with subsequent PAGE. One pair of primers allowed us to distinguish the dominant and recessive homozygote and also heterozygote (Fig. 3).

We used PCR and following restriction of PCR products with common gel electrophoresis in agarose. This CAPS marker is co-dominant. It allows distinguishing between homo- and heterozygote. The lane 1 shows dominant homozygote, the lane 2 and 3 shows heterozygote and recessive homozygote, respectively (Fig. 4).

Result of PCR analysis in our research showed that resistance to FOL race 1 was controlled by gene I and I2.

At least, results of PCR products of eight tomato lines and hybrids indicated that there are one dominant heterozygote, two recessive homozygotes and five dominant homozygotes (table 1).

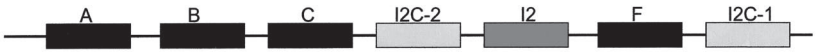

Fig. 1. The I2 locus span a region about 90 kb

Table 1. Result of polymerase chain reactions of eight lines and hybrid of tomato

No.	Samples	Recessive homo-zygote (i_2i_2)	Dominant homo-zygote (I_2I_2)	Dominant hetero-zygote (I_2i_2)
1–5	Beliy naliv-241	+		
6–10	Sultan F_1			+
11–15	Benito F1		+	
16–20	Blagovest F_1	+		
21–25	Ев-1 (F_2)		+	
26–30	YLF		+	
31–35	DRW-1		+	
36–40	01/01-1 (F_2)		+	

Every sample consisted of 5 replicates

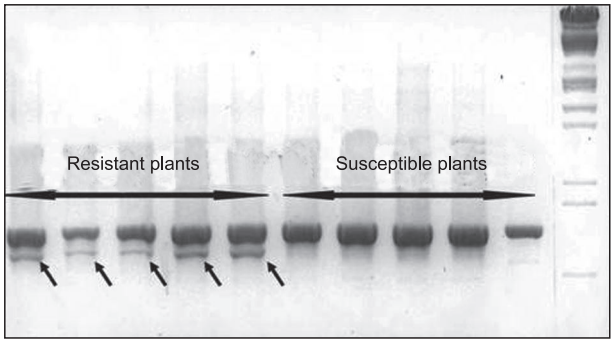

Fig. 2. The electrophoresis analysis of the PCR products

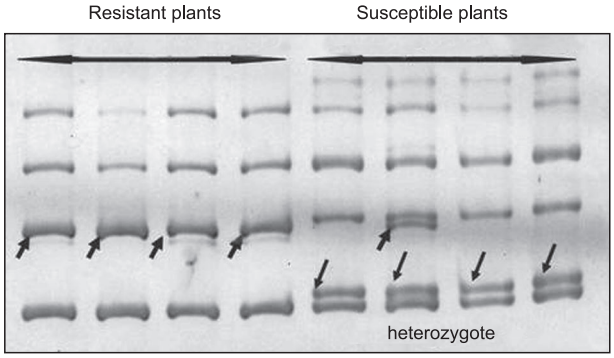

Fig. 3. The PAGE of the PCR products

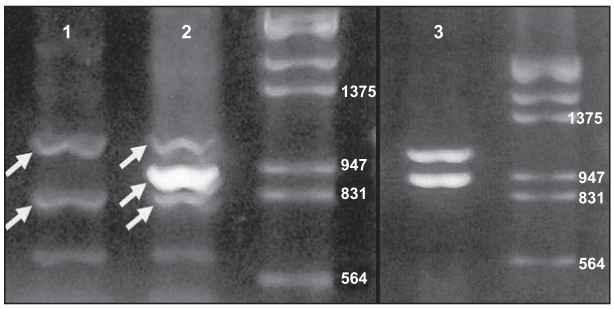

Fig. 4. The dominant homozygote, heterozygote and recessive homozygote

DISCUSSION

Results of the polymerase chain reaction showed that it needs little time and is cheaper in comparison with artificial inoculation of plants. (Sarfatti *et al*.1991). By using this method, it is also possible to determine genotype of plant (homozygote or heterozygote) without presence of the pathogen. Therefore, PCR technique was used in the identification the gene *I2* conferring resistance to *F. oxysporum* f. sp. *lycopersici*.

Markers linked to disease resistance loci can now be used for marker-assisted selection (MAS) programs, thus also allowing several resistance genes to be cumulated in the same genotype. The availability of PCR-based markers for many resistance genes allows the MAS for biotic resistance in tomato to be successfully applied in any laboratory without the need of high technology. In addition, the rapid development of new molecular techniques, combined with the increasing knowledge on structure and function of resistance genes, will help getting new molecular markers for MAS (Hulbert *et al*. 2001). Such methods offer the advantage of reducing or eliminating the need for lengthy culturing and difficult morphological identification procedures (Hamelin *et al*. 1993; Zhang *et al*. 1999). The potential benefits of this technology have been especially recognized in the regulatory field, where time and accuracy of identifications are crucial (Goodwin and Annis 1991; Smith *et al*. 1996).

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POLISH SUMMARY

OPRACOWANIE MARKERÓW DNA DLA GENÓW ODPORNOŚCI POMIDORA NA *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

Więdnięcie pomidora wywoływane przez *Fusarium oxysporum* f. sp. *lycopersici* jest destruktywną chorobą w skali światowej. Najlepszą straegią zwalczania choroby jest wykorzystywanie odpornych odmian. W pracy przeanalizowano osiem linii i mieszańców pomidora na odporność przeciwko więdnięciu fuzaryjnemu, wykorzystując łańcuchową reakcję polimerazy (PCR).