Full Length Research Paper

Screening of resistance genes to fusarium root rot and fusarium wilt diseases in F_3 family lines of tomato (Lycopersicon esculentum) using RAPD and CAPs markers

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Fusarium diseases constitute most of the loss in tomato production worldwide, because it spreads on all geographic fields that it is so hard to find a place without fusarium infestation. Thus, the best way to produce tomato is developing resistant cultivars against Fusarium species. In cultivar developing, molecular marker assisted techniques replaced traditional breeding techniques which are high cost and time consuming for breeders. In this study RAPD and CAPS markers were used to screen tomato (Lycopersicon esculentum) lines against resistance genes Frl and I-2, respectively. Results showed that out of 115 plants, 42 were homozygous resistant, 38 were heterozygous resistant and 35 were homozygous susceptible. Under the light of this information, the forthcoming cultivar development studies will be carried out.

Key words: Fusarium oxysporum, marker assisted selection, tomato root rot, CAPs, RAPD.

INTRODUCTION

Cultivated tomato (Lycopersicon esculentum Mill.) is one of the world’s most important crops due to the high value of its fruits both for fresh market consumption and in numerous types of processed products (Giovanni et al., 2004). World volume of production has increased approximately 10% since 1985, reflecting a substantial increase in dietary use of the tomato. One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production (Barone et al., 2007). The soil-borne fungus Fusarium oxysporum f. sp. radicis-lycopersici (FORL) causes fusarium crown and root rot of tomato (Lycopersicon esculentum Mill.), often referred to as ‘crown rot’ (Fazio et al., 1998). Fusarium oxysporum f. sp. lycopersici inhabits most tomato-growing regions worldwide, causing tomato production yield losses (Staniak et al., 2007). Today, it has an extensive presence in all continents (Menzies and Jarvis, 1994; Brayford, 1996). Crown rot develops primarily in cool climates in both field and greenhouse tomatoes. Substantial crop losses in infected fields have given the disease international attention. The host range of this pathogen comprises at least 36 other species (Menzies et al., 1990). The first symptom of fusarium wilt in gardens and fields is usually the golden yellowing of a single leaflet or shoot, or a slight wilting and drooping of the lower leaves on a single stem. Yellowed and wilted leaflets drop early. Affected plants turn to bright yellow, wilt, dry up, and usually die before maturity, producing few, if any, fruit.

The control of the pathogen spread mainly in three strategies: husbandry practices, application of agrochemicals and use of resistant varieties (Barone and Frusciante, 2007). Resistant varieties are mostly produced by crossing resistant wild types and existing cultivars.

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Abbreviations: RAPD, Randomly amplified polymorphic DNA; CAPS, cleaved amplified polymorphic sequences; FORL, Fusarium oxysporum f. sp. radicis-lycopersici; dNTP, deoxynucleoside 5’-triphosphate; PCR, polymerase chain reaction; bp, nucleotide base pair.
developed for their properties like good taste, shape and color. A molecular marker linked to resistance would be useful for tomato improvement programmes (Staniazsek et al., 2007).

The virulence profile of *F. oxysporum* f. sp. *lycopersici* isolates affecting tomatoes has been grouped into three races according to their ability to infect a set of differential cultivars carrying distinct resistance loci. Three fusarium wilt resistance loci have been genetically characterized in *Lycopersicon* species. The locus I, from *L. pimpinellifolium* (Just) Mill. ‘PI 79532’ (Bohn and Tucker, 1940) controls resistance to race 1. Isolates capable of infecting cultivars with the locus I were shortly identified (Alexander and Tucker, 1945) and a new disease resistance locus (I-2) was characterized in the accession ‘PI 126915’, which is a natural hybrid between *L. esculentum* and *L. pimpinellifolium* (Alexander and Hoover, 1955). A third race able to infect cultivars carrying both I and I-2 loci was reported first in Australia (Grattidge and O’Brien, 1982) and a new resistance locus (named I-3) was identified in the wild species *L. pennelli* (Corr.) D’Arcy. Races 1 and 2 are distributed throughout the world whereas race 3 has a more limited geographic distribution (Reis et al., 2005). Staniazsek et al. (2007) developed a marker, TAO1, to identify tomato genotypes possessing the I-2 gene, which confers resistance to *F. lycopersici* race 2. Fazio et al. (1998) found the marker UBC194, a 10-mer primer, to be closely linked to the disease resistance gene by TAO1 marker and those plants, derived from 10 F_{2} plants, were used for DNA extraction. Fresh young leaves of the lines were stained with ethidium bromide for 30 min and visualized under UV light using Kodak 2D Imaging System.

**RESULTS**

Amplification with RAPD primer

According to Fazio et al. (1998), amplification with UBC 194 primer gives a marker (590 bp) linked to *Frl* gene. In this study, none of the 115 samples revealed the 590 bp marker (Figure 1) so they were determined to be *Frl* negative.

Amplification with CAPS primers

In contrast to RAPD analysis, amplification with TAO1 primers revealed the 902 bp fragment for 80 out of 115 plants (Figure 2). A size of 902-bp-long fragment of the TAO1 marker was found to be polymorphic in resistant tomato lines (Staniazsek et al., 2007). This shows that these plants have the resistance gene (*I*-2). But, to understand better the genotypic structure whether they are homozygous or heterozygous, further analysis was carried out by digestion of the PCR products with *FokI* restriction endonuclease (Figure 3).

Restriction with *FokI*

Restriction fragments of 390 and 410 bp with *FokI* digestion of TAO1902 revealed that in the homozygous-resistant parent lines were A241 and A238 (Staniazsek et al., 2007). After digestion with *FokI*, some of the samples...
revealed 390 and 410 bp fragments as reported by Staniazsek et al. (2007). These fragments show that both alleles from parents related to the I-2 gene are present in the sample, thus the lines were considered to be from a homozygous resistant plant. Forty-two (42) plants showed these restriction fragments. Full list of the lines was given in Table 1 showing the positive and negative results.
DISCUSSION

Tomato (Solanum lycopersicum, formerly L. esculentum) is one of the most widely grown vegetable crops in the world. It is used as a fresh vegetable and can also be processed and canned as a paste, juice sauce, powder or as a whole (Barone and Frusciante, 2007). One of the devastating diseases, is fusarium wilt, caused by three races of F. oxysporum f. sp. lycopersici, is one of the most important diseases of tomato (L. esculentum). Races 1 and 2 are distributed worldwide, whereas race 3 has a more limited geographic distribution (Reis et al., 2005). F. oxysporum f. sp. radicis-lycopersici (FORL) causes fusarium crown and root rot of tomato often referred to as ‘crown rot’ (Fazio et al., 1998) which also gives a substantial damage to crops.

A phenotypic selection for F. lycopersici resistance is a complex and time-consuming process in tomato (Lindhout, 2002). DNA marker technology has been used in commercial plant breeding programmes since the early 1990s, and has proved helpful for the rapid and efficient transfer of useful agronomically important traits into desirable varieties and hybrids (Lindhout, 2002; Tanksley et al., 1989). Rapid death of the infected plants means that each F₂ tomato seedling is a unique genotype for any resistance bioassay. Identification of homozygote for the I-2 gene is especially important when F₂ plants are to be used in further selection. Knowledge of the genetic state for I-2 in F₂ individuals via testing of F₃ seedlings involves much more extensive disease screening (Staniazsek et al., 2007).

In this study, F₃ segregation populations were screened for I-2 resistance gene by TAO1₉₀₂ CAPS marker and for Frl resistance gene by UBC 194 RAPD marker. These analyses revealed that none of the lines screened were resistant to the soil-borne fungus F. oxysporum f. sp. radicis-lycopersici (FORL) while most of them had the I-2 gene which confers resistance to F. lycopersici race 2. We found that not all of the lines which had the resistance gene were homozygous. Even, there came out polymorphisms among the lines in F₃ population. In lines 2, 3 and 12, most of the plants were homozygous resistant while the remaining were homozygous susceptible. In lines 4 and 6, all the plants were homozygous susceptible. The line number 1, 5, 9 and 10 were heterozygous resistant. These information show that, susceptible or resistant, these lines have homogenous genotypes among the plants. But lines 7, 8 and 11 show a more mixed structure. In line 7 and 11, 6 of the plants are homozygous resistant and the remaining are susceptible. In the line 8, 4 of the samples are homogygous resistant, 2 heterozygous resistant and 2 susceptible.

According to Table 1, homozygous resistant lines which show a homogenous structure, can be selected and used in further studies to combine various resistance genes against different pathogens like nematodes, viruses and bacteria in the same thus obtaining an optimum line for cultivating.

REFERENCES