Identification of a New Molecular Marker C2-25 Linked to the *Fusarium oxysporum* f.sp. *radicis-lycopersici* Resistance *Frl* Gene in Tomato

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Abstract

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Fusarium oxysporum Schlecht. f.sp. *radicis-lycopersici* Jarvis & Schoemaker (FORL) is a saprophytic fungus, responsible for the fusarium crown and root rot disease in tomato (*Solanum lycopersicum* L.). This is one of the most destructive pathogens of this species. A new cleaved amplified polymorphic sequence (CAPS) marker C2-25 was developed for the detection of the dominant gene *Frl*, which confers tomato resistance to FORL. C2-25 was amplified from a conserved ortholog set II (COSII) sequence C2_At2g38025. The *Xap*I-derived restriction product of 700 bp was informative for the identification of FORL resistant tomato genotypes and can be used as a diagnostic marker in tomato breeding programmes and hybrid seed production.

Keywords: CAPS marker; fusarium crown and root rot; marker assisted selection (MAS); *Solanum lycopersicum* L.

The disease fusarium crown and root rot of tomato, caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), occurs in the majority of the tomato growing regions worldwide, causing yield losses in commercial tomato production in open field and greenhouse even by 65% (Sonoda 1976; Jones *et al.* 1991; McGoveren *et al.* 1998; Ozbay & Newman 2004). The disease occurs at any stage of plant development and is manifested by the chlorosis and necrosis of lower leaves. The vascular tissue of roots has brown discoloration limited up to 10–30 cm above the ground. The tap root of infected plants often rots partially or completely, and brown cankers appear at the soil line. Infected plants can be stunted and withered. The infection can be spread by wounds and natural holes, contaminated seeds, microconidia from the air and infested soil or compost (Jarvis 1988; Di Primo *et al.* 2001; Steinkellner *et al.* 2005; Koike *et al.* 2006).

In traditional breeding practice, an artificial inoculation is used for the selection of *F. oxysporum* f.sp. *radicis-lycopersici* resistant lines. This procedure is time consuming, expensive and requires numerous repetitions. Breeding of resistant cultivars is an alternative approach to chemical treatments, limiting environmental and consumer risks. In tomato, the resistance to FORL is determined by the single dominant gene *Frl* which is derived from *Solanum peruvianum* (Yamakawa & Nagata 1975; Berry & Oakes 1987). This gene is located on the long arm of tomato chromosome 9, and is closely linked with the *Tm-2*^{2} gene (Vakalounakis 1988; Vakalounakis *et al.* 1997; Fazio *et al.* 1999). Fazio *et al.* (1999) indicated that the random amplified polymorphic DNA (RAPD) marker UBC 194, tightly linked to *Frl* gene, was useful for selecting the resistant tomato genotypes. However, Tanyolac and Akkale (2010) and Truong *et al.* (2011) reported that this amplicon was not informative for the resistance evaluation. Moreover, the RAPD technique produces dominant markers, therefore it is not possible to distinguish whether they are amplified from a locus that is heterozygous or homozygous.

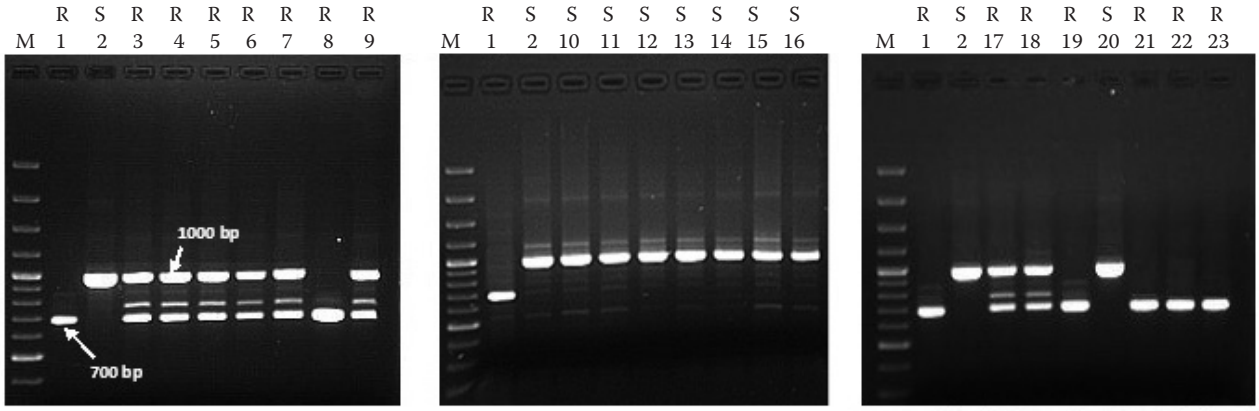

Figure 1. Electrophoretic patterns of the cleaved amplified polymorphic sequence marker C2-25 linked to the tomato gene *Frl*
Lanes: 1 – resistant parent 3070; 2 – susceptible parent A 100; 3–7 and 9 heterozygous F_2 resistant plants; 8 – homozygous F_2 resistant plant; 10–16 F_2 susceptible plants; 17–23 F_1 hybrids FR1/2/10, FR2/1/10, cvs Mospomor, Motelle, Mogeor, F_1 Blitz and cvs Momor, respectively; R – resistant; S – susceptible; M – DNA ladder, 100 bp

In this paper we report a codominant PCR marker C2-25 linked to the locus *Frl* and being useful for the selection of tomato lines resistant to FORL.

Two tomato parental lines were chosen for the study: line 3070, resistant to FORL and homozygous for *Frl* gene, and A100 line susceptible to FORL. These lines were received from Department of Genetics, Breeding and Biotechnology of Vegetable Plant, Research Institute of Horticulture, Skierniewice. A single F_1 plant derived from 3070 × A100 mating was self-pollinated to produce 98 F_2 progeny. In addition, three cultivars resistant to FORL: Momor, Mospomor, Mogeor, one susceptible to FORL: Motelle (pedigree information is available on http://tgrc.ucdavis.edu, seeds received from National Institute for Agricultural Research, INRA, Montfavet, France), two experimental F_1 hybrids: FR1/2/10, FR2/1/10 (derived from breeding lines resistant to FORL: 3070 and 3061 × A100, received from the collection of Research Institute of Horticulture, Skierniewice) and commercial hybrid Blitz F_1 (De Ruiter Seeds, Bergschenhoek, The Netherlands), were examined. The fungal strain *Fusarium oxysporum* f.sp. *radicis-lycopersici* was obtained from National Institute for Agricultural Research, INRA, Montfavet, France. The resistance tests were done according to Kozik (1999). Plants were classified as resistant when no disease symptoms were observed. Out of the 98 F_2 plants evaluated 71 were resistant and 27 were susceptible. DNA was isolated from freeze-dried leaves using a DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany).

A conserved ortholog set II (COSII) sequence C2_At2g38025 which was positioned at 45 cM on tomato chromosome 9 (Tomato-EXPEN 2000, www.solgenomics.net) was tested by PCR. Forward and reverse primer sequences were: f: 5'-ATGGGCGCTGCATGTTTCGTG-3', r: 5'- ACACCTTTGTTGAAAGCCATCCC-3'. DNA amplification was carried out in 20 µl. The reaction mixture contained: 1× reaction buffer, 0.1mM of each dNTP, 1.5mM $MgCl_2$, 0.4µM of each primer, 1 U Taq DNA polymerase (Invitrogen, Carlsbad, USA) and 30 ng genomic DNA. The PCR was performed on a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, USA) under the following thermal conditions: 94°C – 60 s, followed by 40 cycles of 94°C – 25 s, 55°C – 35 s, 72°C – 90 s and final extension of 5 min at 72°C. A 1100 bp PCR product was amplified in all parental lines and F_1 plants.

This PCR product was digested by twelve restriction enzymes: *Dde*I, *Rsa*I, *Hinf*I, *Dra*I, *Alu*I, *Hpa*I, *Hind*III, *Xap*I, *Mbo*I, *Hae*III, *Mun*I, *Eco*RI. Digestion of amplicons was carried out at 37°C for 3 h in a 20 µl mixture containing 5 U of restriction enzyme, 18 µl PCR product and 10x concentrated restriction enzyme buffer. The restriction products were visualized by electrophoresis in 1.4% agarose gel and ethidium bromide staining. Polymorphism was revealed after digestion of the amplicon with *Xap*I. The restriction fragment of 700 bp was observed in the resistant parent 3070. In the susceptible parent A100, only 1000 bp long band was found (Figure 1, lanes 1 and 2, respectively). The 700 bp fragment was detected in 69 resistant F_2 plants and was not identified in any of 29 susceptible F_2 plants, thereby confirming the linkage of the marker with the *Frl* locus. In the group of F_2 resistant plants 20 and 49 were homozygous and heterozygous for *Frl,* respec-

tively. Examples of the corresponding patterns are shown in Figure 1. In addition, the marker C2-25 was revealed in three cultivars resistant to FORL: Momor, Mospomor, Mogeor, two experimental F_1 hybrids: FR1/2/10, FR2/1/10 and Blitz F_1 (Figure 1, lanes 17–19 and 21–23) and was not observed in susceptible cultivar Motelle (Figure 1, lane 20). Therefore, we suggest that this marker can be used in tomato breeding programmes to select FORL resistant lines.

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