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ORIGINAL PAPER

Development of co-dominant SCAR markers linked to resistant gene against the *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Nedim Mutlu[1] · Aylin Demirelli[2] · Hülya Ilbi[3] · Cengiz Ikten[4]

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Abstract

Key message **We developed highly reliable co-dominant SCAR markers linked to the Frl gene. FORL testing is difficult. The marker is expected to be quickly adapted for MAS by tomato breeders.**

Abstract *Fusarium oxysporum* f. sp. *radicis-lycopersici* causes *Fusarium* crown and root rot (FCR), an economically important soil-borne disease of tomato. The resistance against FCR is conferred by a single dominant gene (Frl) located on chromosome 9. The aim of this study was to develop molecular markers linked to the Frl gene for use in marker-assisted breeding (MAS) programs. The FCR-resistant 'Fla. 7781' and susceptible 'B560' lines were crossed, and F_1 was both selfed and backcrossed to 'B560' to generate segregating F_2 and BC_1 populations. The two conserved set II (COSII) markers were found linked to the Frl gene, one co-segregated with FCR resistance in both F_2 and BC_1 populations and the other was 8.5 cM away. Both COSII markers were converted into co-dominant SCAR markers. $SCAR_{Frl}$ marker produced a 950 and a 1000 bp fragments for resistant and susceptible alleles, respectively. The linkage of $SCAR_{Frl}$ marker was confirmed in BC_2F_3 populations developed by backcrossing the resistant 'Fla. 7781' to five different susceptible lines. The $SCAR_{Frl}$ marker has been in use in the tomato breeding programs in BATEM, Antalya, Turkey, since 2012 and has proved highly reliable. The $SCAR_{Frl}$ marker is expected to aid in the development of FCR-resistant lines via marker-assisted selection (MAS).

Introduction

Tomato (*Solanum esculentum* Mill.) is attacked by many pest and diseases, reducing yield and quality of the crop. Fusarium has a broad range of host species, but *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) are the two *forma specialis* (f. sp.) causing disease in tomato (Armstrong and Armstrong, 1981; Steinkellner et al. 2005; Attitalla et al. 2004). FORL causes *Fusarium* crown and root rot (FCR), an important soil-borne disease of tomato (Fazio et al. 1999). The pathogen has spread all over the world since it was first reported in Japan in the 1960s and leads to substantial yield losses in both greenhouse and field production systems (Roberts et al. 2000; Omar et al. 2006).

The fungus infects susceptible plants through wounds and natural openings created by newly emerging roots. After infection by FORL, early symptoms in tomato seedlings include stunting, yellowing, and premature loss of cotyledons and lower leaves. A pronounced brown lesion, root rot, wilting and death are advanced symptoms (Roberts et al. 2000). Although some biocontrol agents (Liu et al. 2010; Xu and Kim 2014) and soil solarization (Sivan and Chet 1993) showed certain level of protection against the pathogen, currently there are no effective

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N. Mutlu and A. Demirelli contributed equally to this work.

✉ Nedim Mutlu
severmutlu@hotmail.com; nedimmutlu@akdeniz.edu.tr

[1] Department of Agricultural Biotechnology, Faculty of Agriculture, Akdeniz University, Antalya 07058, Turkey

[2] West Mediterranean Agricultural Research Institute (BATEM), Antalya, Turkey

[3] Department of Horticulture, Faculty of Agriculture, Ege University, Bornova, Izmir, Turkey

[4] Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Antalya 07058, Turkey

control methods against FORL, because it rapidly colonizes sterilized soil and persists for long periods (Roberts et al. 2000). Thus, the use of resistant cultivars is the most acceptable, environmental friendly and economic method of disease control.

The resistance to FORL was independently introduced into *Solanum lycopersicum* from three different *S. peruvianum* sources. Resistance for tobacco mosaic virus (Tm-2) and FORL were introgressed from PI126944 into *S. lycopersicum* line IRB♯301 in Japan (Yamakawa and Nagata 1975), from PI126926 in France (Elkind et al. 1988), and from PI128650 in Ohio, USA (Alexander 1963). Using the line IRB♯301, Scott and Farley (1983) developed Tm-2 and FORL-resistant tomato line Ohio 89-1. Then, Scott and Jones (2000) later developed FORL-resistant Fla. 7781 without Tm-2 resistance. The resistance against FCR is conferred by a single dominant gene (Frl) (Vakalounakis 1988), and allelic test using the three sources of resistance suggested that all three lines carried the same Frl allele (Laterrot and Moretti 1991). The Frl gene is located on the long arm of chromosome 9 close to the centromere. Vakalounakis et al. (1997) reported a tight linkage between *Frl* and *Tm-2* (5.1 ± 1.07 cM). Three RAPD markers (UBC#116, 194 and 655) were found linked to Frl using a combination of various resistant lines and near isogenic lines (NILs) (Fazio et al. 1999).

Although UBC#116 was recently converted to a co-dominant SCAR marker (Truong et al. 2011), it was not informative for resistance evaluation (Truong et al. 2011) due to a 7 cM distance from the gene (Fazio et al. 1999). Recently, Staniaszek et al. (2014) developed a CAPS marker from a conserved ortholog set II (COSII) sequence C2_At2g38025 about 3 cM from *Frl* in an F_2 population. In addition, artificial inoculation, the only means of selection in breeding for resistance against FCR, is costly, time consuming and unreliable due to inconsistent virulence of FORL (Jones et al. 1990). Thus, identification of closely linked markers will make marker-assisted breeding programs more efficient. The objective of this study was to develop breeder-friendly molecular markers tightly linked to the Frl gene for use in marker-assisted selection (MAS) against FCR resistance in molecular breeding programs.

Materials and methods

Plant materials

The resistant source for FCR 'Fla. 7781' is an advanced breeding line developed by Scott and Jones (2000). The line was developed from Fla. 7440 and Fla. 7464 cross. The FCR resistance of Fla. 7781 traces back to Ohio 89-1 (Scott and Farley 1983). Fla. 7781 does not carry Tm-2 and *ah* genes (Scott and Jones 2000) and hence probably carries the smallest introgression from IRB#301 derived from *S. peruvianum* PI128650 with FCR resistance. Susceptible tomato lines B560, A1, A2, A3, A4 and A5 were developed by Bati Akdeniz Agricultural Research Institute (BATEM), Antalya, Turkey.

Fla. 7781 and B560 was crossed, F_1 plants selfed to promote F_2 seeds and backcrossed to B560. The 493 F_2 and 476 BC_1 plants were phenotyped (see below) for FCR resistance. The resistant F_2 plants were grown to maturity and selfed to create F_3 families. The 20 or 25 plants from each of the 304 F_3 families were root-dip inoculated and phenotyped to identify homozygous vs heterozygous resistant F_2 plants. The BC_1 population was used for testing the candidate molecular markers.

For confirmation of marker/Frl gene linkage, Fla. 7781 was separately backcrossed twice to five different susceptible lines A1, A2, A3, A4 and A5, and selfed twice to obtain BC_2F_3 populations. Single plant selections for FCR resistance were made with root-dip inoculation method at BC_1F_1, BC_2F_1 and BC_2F_2 generations. The homozygosity/heterozygosity of BC_2F_2 lines for resistance against FCR was confirmed in two different experiments where about 20 plants from each line along with the resistant and susceptible parents were tested. The BC_2F_3 plants were then used to confirm the linkage relationship of markers with FCR resistance by genotyping with SCAR markers.

Disease screening

The FORL isolates were collected from a tomato greenhouse in Adana, Turkey (Can et al. 2004). Cultures were grown in potato-dextrose broth in a rotary shaker for 6 days at room temperature. Then they were filtered through cheesecloth, the mycelial mat was washed with sterile water and the suspension centrifuged at 4000 rpm for 10 min. Spores were resuspended in water and the concentration was adjusted to 10^7 to 10^8 spores/ml (Gordon et al. 1989; Korolev et al. 2000). Seedlings were grown in sterile peat moss. The experiment was conducted in a completely randomized block with three replicates. Twenty plants each of F_1, Fla. 7781, B560 and A1–A5 were inoculated and replicated when phenotyping the segregating populations. A total of 493 F_2, 476 BC_1, 307 F_3 families (25 plants each of 107 F_3 families and 20 plants each of 197 F_3 families originating from resistant F_2 plants) and BC_2F_3 families obtained by backrossing Fla. 7781 to five different susceptible lines (A1–A5) were inoculated. The 20 plants each of BC_2F_3 lines were

phenotyped along with F_1 commercial hybrids in April 2014 and October 2014. The seedlings were grown in equal volume of steam-sterilized perlite:peat mix. The roots of the seedlings were washed off under tap water and dipped into FORL suspension at the five-leaf stage. After inoculation, the seedlings were transplanted into 2 L pots containing a mixture of sterile perlite and peat under a climate-controlled greenhouse, with temperature ranging from 14.3 to 23.7 °C and relative humidity from 54.6 to 86.6 %. The seedlings and plants were fertilized with a modified Hoagland solution, adjusting the final EC to 1.5 mmhos/cm (Hoagland and Arnon 1950). Four weeks after inoculation, the plants were scored as resistant (0) or susceptible (1) based on wilting and FCR symptoms on the roots and crown.

Molecular marker development

DNA extraction

The DNA of parents and segregating populations was extracted from young leaves using a modified CTAB extraction protocol (Doyle and Doyle 1990). For each sample, 0.2 g of fresh tissue was ground in 0.6 mL of extraction buffer [1.4 M of NaCl, 20 mM of EDTA, 100 mM of Tris–HCL (pH 8), 2 % CTAB, and 0.2 % of beta-mercaptoethanol]. The suspension was mixed well, incubated at 60 °C for an hour followed by chloroform–isoamyl alcohol (24:1) extraction and precipitation with 2/3 volume of isopropanol at −20 °C for 2 h. The pellet formed after centrifugation at 13,100g_n for 10 min was washed twice with 0.75 mL of 76 % ethanol and 10 mM of ammonium acetate, and then resuspended in sterile distilled water. The DNA concentration was calibrated on 1 % agarose gel using lambda DNA as standard. The DNA was stored at −20 °C until use.

PCR amplification

PCR reactions were performed in 15-µL aliquots containing 1× buffer, 2 mM MgCl2, 0.1 mM dNTPs, 0.5 U Taq polymerase (Fermentas, Waltham, MA, USA), 3–10 µM primer and 10 ng DNA in an MJ research PTC-200 thermocycler (Bio-Rad, Hercules, CA). All PCR products were separated on a 1.5 % agarose gel (Sigma, St. Louis, MO), visualized with ethidium bromide staining under ultraviolet light and photographed with a Kodak Gel Logic 200 system (Carestream Health, Rochester, NY).

SRAP (sequence-related amplified polymorphism) analysis

The SRAP analysis was carried out using 29 primers including 13 forward (Me1-13) and 19 reverse (Em1-16) SRAP primers, for a total of 208 primer combinations. SRAP primers target coding sequences in the plant genome. The PCR amplification conditions were as reported by Li and Quiros (2001).

RGA (resistance gene analog polymorphism) analysis

RGA markers are useful for tagging resistance genes. The 48 RGA primers designed from conserved regions of nucleotide binding site–leucine-rich repeat (NBS–LRR) resistance gene family (Mutlu et al. 2006) were used for a total 419 RGA primer combinations. The PCR amplification conditions were as reported by Mutlu et al. (2006).

Conserved ortholog set II (COS II) analysis

The 18 COS II loci located between RFLP marker T1177 and COSII marker C2_At3g63200 were used in combination with 12 restriction enzymes, for a total of 216 combinations. PCR amplification involved an initial denaturing at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 53–57 °C for 59 s, and 72 °C for 59 s and a final elongation at 72 °C for 10 min.

SCAR design

The 15 SCAR primers were designed using EST markers located between the RFLP marker T1177 and COSII marker C2_At3g63200. The PCR amplifications were carried out at the same conditions as the COSII analysis described above.

Polymorphism analysis

Parental polymorphism analysis was performed for COSII and SCAR markers. Then, bulk DNAs were prepared from equal quantities of DNA of ten resistant and ten susceptible F_2 plants. COSII and SCAR markers that yielded polymorphism between the parents were used to screen resistant and susceptible bulks. The polymorphic markers were subsequently tested on F_2 individuals and confirmed on BC_2F_3 population.

Segregation and linkage analysis

Segregation ratios for resistant and susceptible individuals for both F_2 and BC populations were checked for expected ratios with Chi-square goodness of fit test. The linkage analysis was performed in F_2 population using Mapmaker 3.0 software (Lincoln et al. 1992), using a minimum LOD score of 5 and the Kosambi mapping function (Kosambi 1944).

Table 1 The segregation analysis of reaction of F_2 and BC_1 generations derived from the cross of Fla. 7781 (resistant) × B560 (susceptible) to *Fusarium oxysporium form. sp. radicis* isolate

Population	Observed resistant plants (no)	Observed susceptible plants (no)	Estimated segregation ratio *R*:*S*	X^2	Probability (*P*)
F_2	375	118	3:1	0.298	0.585
BC_1	252	224	1:1	1.64	0.199

Results

Genetic basis of FCR resistance in Fla. 7781

Of the 493 F_2 and 476 BC_1 plants phenotyped for reaction against FCR, 375 and 252 plants were resistant, respectively (Table 1). The susceptible parent (B560) showed brown lesions a week after inoculation and wilted and died completely 2 weeks after the first lesions occurred, while the resistant parent (Fla. 7781) and F_1 plants did not show any symptoms 4 weeks after inoculation. Two separate experiments were carried out to determine Frl genotypes (homozygous vs heterozygous) of the F_2 plants. In the first experiment, 25 F_2 progeny of each of the 107 resistant F_2 plant were inoculated along with parents and F_1 with the FORL isolate. In the second experiment, 20 F_3 plants each of 197 resistant families were phenotyped along with parents and F_1s. A total of 6615 F_3 plants were tested with the FORL isolate. The genotypes of F_2 plants for resistance against FCR were confirmed by phenotyping the 304 F_3 families. However, the five (1.6 %) F_3 families were fully susceptible due to escapes in F_2 phenotyping. The resistant vs susceptible ratio fit a 3:1 segregation ratio ($X^2 = 0.298$, $P = 0.585$) for F_2, while there was an acceptable fit to a 1:1 ratio ($X^2 = 1.64$, $P = 0.199$) for the BC_1 population, confirming control of resistance by the involvement of a single dominant gene (Table 1).

Identification of Frl gene-linked marker

A total of 1491 primers and primer combinations were screened in DNA bulks of FCR resistant and susceptible F_2 plants, and the parents 'Fla. 7781' and 'B560'. Of the 247 SRAP primer combinations, 161 primers yielded a total of 644 bands, 25 polymorphic between parents and 3 between bulks. But polymorphic SRAP markers did not co-segregate with the resistance. The COSII markers located around centromeric region of chromosome 9 were tested for polymorphism using the parents and bulks. The C2_At3g63200 COSII marker cut with *Taq*I, *Asu*II, *Rsa*I, *Hha*I restriction enzymes was polymorphic between the parents.

Development of SCAR markers

The result of the work with the CAPS marker confirmed that the C2_At3g63200 marker was near the *Frl* locus on chromosome 9. First, the fragment of C2_At3g63200 locus was amplified using the primers given in the solgenomics database. Then, a co-dominant SCAR marker, $SCAR_{200}$, was developed by utilizing indel differences between the sequences of resistant and susceptible parents. A number of primers were designed flanking a 5 Mb region around the C2_At3g63200 locus, to find indels to identify co-dominant SCAR markers. One co-dominant InDel SCAR marker, C2_At4g28660 (SL2.40ch09:57371789–57373287), was polymorphic between the parents and the bulks. This SCAR marker has been named $SCAR_{Frl}$ and produced a 950 bp fragment in the resistant parent and a 1000 bp fragment in the susceptible parent (Fig. 1, Table 2). The segregations of the two markers were evaluated both in F_2 (493 plants) and BC_1 (476 plants) populations. Linkage analysis was carried out using 304 F_2 plants, genotypes of which were confirmed in the F_3 families. The $SCAR_{Frl}$ marker C2_At4g28660 co-segregated with resistance, while the CAPS marker C2_At3g63200 was at a distance of 8.5 cM from the *Frl* gene in the F_2 population.

Confirmation of linkage between SCAR markers and Frl gene

The $SCAR_{Frl}$ and $SCAR_{200}$ markers were also tested using the BC_2F_3 population and commercial F_1 hybrids (Table 3). The 60 BC_2F_3 lines were either homozygous or heterozygous resistant against FCR. The $SCAR_{Frl}$ marker predicted the FCR resistance of the F_1 hybrids and 59 of the 60 lines correctly (Table 3). $SCAR_{200}$ marker showed 8 recombinants among 60 BC_2F_3 lines (Table 3). The probability of breaking linkage was determined by using the formula: $1-(1-r)^{n+1}$, where r is the recombination fraction and n is the number of backcrosses (Allard, 1999). The probability of breaking linkage between the Frl gene and $SCAR_{Frl}$ marker would be ≤ 0.05, and $SCAR_{200}$ marker ≤ 0.35 as calculated in BC_2F_3 population. It was determined that the mapping order was Frl gene-$SCAR_{Frl}$ marker (C2_At4g28660)- and $SCAR_{200}$ (C2_At3g63200) marker, because the $SCAR_{Frl}$ and $SCAR_{200}$ markers carried the same recombination event in the BC_2F_3 population (Table 3).

Discussion

Fla 7781 carried a smaller introgression, because it lacks Tm-2 allele, than similar lines (Fla 7775) carrying FCR resistance. Thus, it is expected that the SCAR marker developed in this study would be useful for MAS for FCR

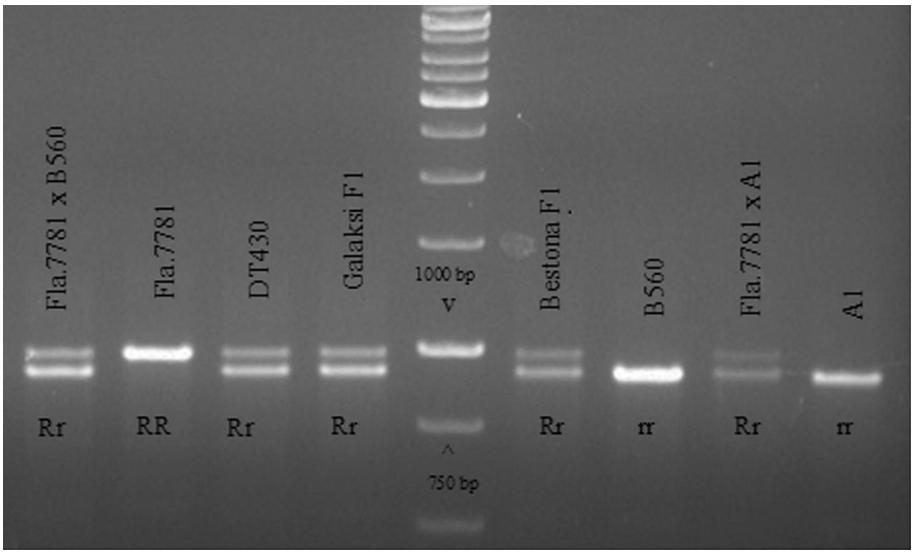

Fig. 1 The $SCAR_{Frl}$ marker located at the conserved ortholog II 'C2_At4g28660' marker locus. PCR fragments represent $SCAR_{Frl}$ marker amplified from *Fusarium* crown and root rot (FCR) resistant and susceptible genotypes, resolved in 1.5 % agarose gel, M: 1 Kb DNA ladder. *RR* resistant parent, *rr* susceptible parents, *Rr* heterozygote resistant genotypes

Table 2 The SCAR markers $SCAR_{200}$ and $SCAR_{Frl}$, their conserved ortholog II loci, chromosome location based on ITAG2.4 genomic annotations (www.solgenomics.net), forward and reverse primer sequences, allele sizes for resistant and susceptible reaction against *Fusarium* crown and root rot (FCR), type of polymorphism, and genetic distance to Frl gene, estimated in F_2 for $SCAR_{200}$ and in BC_3F_2 for $SCAR_{Frl}$, for resistance against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL)

Marker type	Locus	Primers (forward and reverse)	Resistant/ susceptible alleles	Polymorphism	Distance to Frl gene (cM)
$SCAR_{200}$	C2_At3g63200 (ch09:63298665–63299591)	F: tcggtccaaattcacttcaa R: actcctccacttgcataccc	290 R/310 S	InDel	8.5
$SCAR_{Frl}$	C2_At4g28660 (ch09:61774146–61775809)	F: CACATTCATCATCTGTTTTTAGTCTATTC R: CACAATCGTTGGCCATTGAATGAAGAAC	950 R/1000 S	InDel	0.016

resistance. The SSR and COSII marker loci were examined to identify linkage with the FCR resistance locus. The two SCAR markers were found to be linked to the Frl gene. The COSII marker C2_At3g63200 (63298665–63299591) located at 52 cM on chromosome 9 was converted into a co-dominant SCAR marker ($SCAR_{200}$). It produced a 290 bp fragment for resistant and a 310 bp for susceptible alleles. The map distance between the $SCAR_{200}$ marker and the Frl gene was 8.5 cM in the F_2 population. The co-dominant $SCAR_{Frl}$ marker located at 61774146–61775809 on chromosome 9 yielded 950 bp and 1000 bp fragments specific to resistant and susceptible alleles, respectively. The $SCAR_{Frl}$ marker is 121 Kb from the RFLP marker T1267 (61654854 bp) that is located at 51 cM on Tomato-EXPEN 2000 map, and at 53.1 cM on Kazusa F_2-2000 genetic map. Based on the data, the Frl gene is located at between 47.5 and 52 cM on chromosome 9, confirming previous results (Vakalounakis et al. 1997; Fazio et al. 1999).

The first mapping study for Frl was carried out by Fazio et al. (1999), reporting RAPD markers with the closest being 5.1 cM to the gene. One of these markers was converted into a co-dominant SCAR marker (Truong et al. 2011). Based on the recombination frequency, $SCAR_{Frl}$ marker was found to be more tightly linked to Frl than RAPD marker determined by Fazio et al. (1999). Recenlty, Staniaszek et al. (2014) mapped the COSII marker C2_At2g38025 at 45 cM (Tomato-EXPEN 2000), about 3 cM distal to *Frl* in an F_2 population. However, the C2_At2g38025 locus is located at 5892090–5892355 bp in *S. pennelli* chromosome 9, close to T1212 and T1177 RFLP markers at 48 and 47.5 cM in Tomato-EXPEN 2000 map, respectively. There seems to be a highly significant discrepancy between the map location and genomic location of the C2_At2g38025 locus and its linkage to *Frl*. The genomic distance between C2_At2g38025 and C2_At4g28660 ($SCAR_{Frl}$ locus) is 55.88 Mb. It seems unlikely that the two loci would be linked in any segregating population. There might be: (1) mistakes in genome annotation, (2) genome re-arrangements on chromosome 9 among related species (*S. pennelli–S. peruvianum–S. lycopersicum*), (3) a second locus determining/effecting FCR resistance.

The co-dominant $SCAR_{Frl}$ marker co-segregated with resistance in F_2 and BC_1F_1 populations. There was one recombination event between the $SCAR_{Frl}$ marker in the BC_2F_3 population. Thus, the $SCAR_{Frl}$ marker can detect FCR resistance in segregating populations with very high

Table 3 The *Fusarium crown rot* (FCR) resistant line FR7781 was backcrossed with five susceptible *S. lycopersicum* lines differing for their fruit size. Then, F_1s were backcrossed to the susceptible parents. The BC_1F_1 populations were phenotyped with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) isolate, and resistant BC_1F_1 plants were backcrossed again to susceptible parents to obtain BC_2F_1 generations, which were phenotyped for FCR resistance. Then, resistant plants of each population were successively selfed, and phenotyped to obtain BC_2F_3 lines. The plants from each line along with the resistant and susceptible parents and commercial F_1 hybrids were tested twice with virulent FORL isolate at the BATEM Institute, Antalya, Turkey in spring and fall of 2014. The lines and hybrids were genotyped with the SCAR markers. Pedigrees of BC_2F_3 (FR7781 × A1–A5 S. *esculentum*) lines and commercial names of F1 hybrids, number of resistant and susceptible plants against FCR, marker genotypes of $SCAR_{Frl}$ and $SCAR_{200}$ of the lines and hybrids are presented

BC_2F_3 lines	Number of resistant:susceptible plants against FORL	Marker genotypes	
		$SCAR_{Frl}$ (C2_At4g28660)	$SCAR_{200}$ (C2_At3g63200)
A-401	40:0	RR	RR
A-402	39:0	RR	RR
A-404	39:0	RR	RR
B-407	40:0	rr[a]	rr[a]
B-413	40:0	RR	RR
B-414	40:0	RR	RR
C-415	40:0	RR	RR
C-419	39:0	RR	RR
C-422	40:0	RR	RR
C-424	40:0	RR	RR
C-427	40:0	RR	RR
D-431	40:0	RR	RR
D-433	40:0	RR	RR
D-434	40:0	RR	RR
D-438	40:0	RR	RR
D-441	40:0	RR	RR
D-442	40:0	RR	RR
D-443	40:0	RR	RR
D-444	40:0	RR	RR
D-445	40:0	RR	RR
D-446	40:0	RR	RR
D-449	39:0	RR	RR
D-450	39:0	RR	RR
D-451	40:0	RR	RR
A-452	40:0	RR	RR
A-453	40:0	RR	RR
A-454	40:0	RR	RR
A-455	40:0	RR	RR
A-457	40:0	RR	RR
A-459	40:0	RR	RR
A-461	40:0	RR	RR
A-462	40:0	RR	RR
A-463	40:0	RR	RR
C-470	40:0	RR	rr[a]
C-471	40:0	RR	rr[a]
E-472	40:0	RR	rr[a]
E-473	40:0	RR	rr[a]
E-474	40:0	RR	rr[a]
E-475	40:0	RR	RR
A-476	40:0	RR	RR
A-477	40:0	RR	RR
A-478	40:0	RR	rr[a]
A-479	40:0	RR	rr[a]
A-480	40:0	RR	RR
A-481	40:0	RR	RR
A-482	39:0	RR	RR
A-483	40:0	RR	RR

Table 3 continued

BC_2F_3 lines	Number of resistant:susceptible plants against FORL	Marker genotypes	
		$SCAR_{Frl}$ (C2_At4g28660)	$SCAR_{200}$ (C2_At3g63200)
A-484	40:0	RR	RR
A-485	40:0	RR	RR
A-486	40:0	RR	RR
B-491	40:0	RR	RR
C-492	40:0	RR	rr[a]
DT407	40:0	RR	RR
DT420	40:0	RR	RR
DT427	40:0	RR	RR
DT433	40:0	RR	RR
DT408	32:8	Rr	RR
DT430	26:14	Rr	Rr
DT463	29:11	Rr	Rr
DT422	0:40	rr	rr
Verty F1	40:0	RR	RR
Bestona F1	40:0	Rr	Rr
Çiğdem F1	40:0	Rr	Rr
Galaksi F1	40:0	Rr	RR
Anıt F1	0:40	rr	Rr[a]

[a] Recombinant gentoypes

accuracy. We have been using this marker in our tomato breeding programs for single plant selections and marker-assisted backcrossing at the institute (BATEM, Antalya) since 2012. The results of the breeding activities indicate that the recombination observed in the BC_2F_3 population may be a rare event. We are using this recombinant to fine map the Frl locus. Thus, the SCAR marker developed in this study can be useful for identifying FCR-resistant genotypes in MAS breeding programs. This would permit an early and accurate selection of resistant genotypes without inoculation and symptom detection. Furthermore, the co-dominant nature of the $SCAR_{Frl}$ marker makes it possible to discriminate homozygous vs heterozygous resistant genotypes.

The $SCAR_{Frl}$ marker C2_At4g28660 was identified to be tightly linked to the Frl gene. The marker was in complete linkage in F_2 and confirmed in the F_3 population. The co-dominant $SCAR_{Frl}$ marker can easily be resolved in a standard agarose gel and is expected to be adapted quickly by tomato breeders and breeding companies throughout the world.

Our marker codes for photosystem II reaction center Psb28 protein with gene symbol LOC101245880 and genomic location identifier NC_015446.2 (NCBI). The gene does not have disease resistance function and is therefore not the Frl gene itself. There are resistance gene candidates in this genomic region. The gene LOC101268425, receptor-like serine/threonine-protein kinase ALE2 (61438536–61449481) is located at 324 Kb distal to the $SCAR_{Frl}$ marker. Although PXL2 leucine-rich repeat receptor-like protein kinase gene LOC101246467 (61803995–61807505) is 30 Kb, and the putative disease resistance protein RGA4 (61926271–61930342) LOC101247854 is 152 Kb to the $SCAR_{Frl}$ marker and seemed to be more distal to Frl gene, both could yet be candidate resistance genes because local rearrangement may change the marker order. Based on the recombinants among the SCAR markers and Frl gene, we can suggest that the Frl gene is located below 61774146 bp, the SCARFrl locus on chromosome 9 of tomato.

Author contribution statement N.M. wrote the manuscript and developed the SCAR marker $SCAR_{Frl}$; A.D. developed the populations, conducted phenotyping and mapped the C2_At3g63200 locus; H.I. supervised A.D., designed the study and received the funding; C.I. developed the $SCAR_{200}$ and aided in the development of the $SCAR_{Frl}$ marker.

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Conflict of interest We (authors) have read and understood the Theoretical and Applied Genetics Journal policy on declaration of interests and the authors declare that they have no conflict of interest.

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