MARKER ASSISTED IDENTIFICATION OF TOSPOVIRUS RESISTANT TOMATO GENOTYPES IN SEGREGATING PROGENIES

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ABSTRACT: The SCAR (Sequence Characterized Amplified Region) 'Sw-421' molecular marker is located at 1.0 cM from the Sw-5 allele, originated from *Lycopersicon peruvianum* (L.), which confers resistance to the tomato spotted wilt virus (TSWV). However, it had not been tested yet in advanced tomato populations. The goal of this study was to distinguish resistant homozygotes (Sw-5/Sw-5) and heterozygotes (Sw-5/Sw-5$^+$) from susceptible (Sw-5$^+$/Sw-5$^+$) plants in crossing populations with the Stevens cultivar and advanced backcrossing populations by using 'Sw421' SCAR marker. The amplification of 940 bp and 900 bp bands characterized the resistant homozygotes and susceptible controls, respectively. A two band pattern (900 bp and 940 bp) was observed in heterozygote genotypes (Sw-5/Sw-5$^+$), which confirmed the co-dominant inheritance mechanism of the marker. Fifty seven plants from the isogenic progenies were characterized based on bands pattern: 18 plants (31.6%) were identified as resistant homozygotes, 8 plants (14.0%) as resistant heterozygotes and 31 plants (54.4%) were characterized as susceptible. The SCAR 'Sw-421' marker is an important tool for selection and pyramid resistance alleles, mainly when other resistance sources to the TSWV are available, such as the Rey de los Tempranos source.
Key words: *Lycopersicon esculentum,* Sw-5, vírus resistance, molecular marker

IDENTIFICAÇÃO DE GENÓTIPOS DE TOMATEIRO RESISTENTES A TOSPOVIRUS EM PROGÊNIES SEGREGANTES COM MARCADOR MOLECULAR

RESUMO: O marcador molecular SCAR 'Sw-421' é localizado a 1,0 cM do alelo Sw-5, proveniente de *Lycopersicon peruvianum* (L.), que confere resistência ao vira-çabeça em tomateiro. Contudo, o mesmo ainda não havia sido testado em populações avançadas de tomateiro. O objetivo do trabalho foi distinguir plantas resistentes homozigotas (Sw-5/Sw-5), resistentes heterozigotas (Sw-5/Sw-5$^+$) e suscetíveis (Sw-5$^+$/Sw-5$^+$) pelo marcador SCAR 'Sw-421'. As amplificações de bandas de 940 pb e 900 pb caracterizaram os genótipos resistentes homozigotos e suscetíveis, respectivamente. Duas bandas (900 pb e 940 pb) foram observadas nos genótipos heterozigotos, confirmando a herança codominante do marcador. De 57 plantas das progênies isogênicas avaliadas 18 (31,6%) plantas foram caracterizadas como resistentes, 8 (14,0%) como heterozigotas e 31 (54,4%) plantas suscetíveis. O marcador molecular SCAR 'Sw-421' constitui importante ferramenta para seleção e piramidação de alelos de resistência, especialmente quando se utilizam outras fontes de resistência ao vira-cabeça, como por exemplo, a fonte Rey de los Tempranos.
Palavras-chave: *Lycopersicon esculentum,* Sw-5, resistência a virus, marcador molecular

INTRODUCTION

Virus-induced diseases are considered an obstacle for tomato crop with significant losses in production. In Brazil, *Tomato spotted wilt virus* - TSWV, *Tomato chlorotic spot virus* - TCSV, *Crysanthemum stem necrosis virus* - CSNV - and *Groundnut ringspot virus* - GRSV are the most important viruses caused by Tospovirus in tomato crop (Lima et al., 2002; Ferraz et al., 2004; Lau et al., 2006).

The genetic resistance to the TSWV in tomato plants was reported in several accesses of the *Lycopersicon* genus (Rosello et al., 1998). Some resistance alleles were introduced in commercial cultivars, like the Rey de los Tempranos (resistance from *L. esculentum*) and the Stevens (resistance from *L. peruvianum*) cultivars. These cultivars are the two main resistance sources used in the genetic control of the TSWV in the breeding programs in progress in Brazil (Juliatti & Maluf, 1995; Lourenção et al., 1999; Ferraz et al., 2004). Resistance in the cultivar Stevens is controlled by one gene, denominated Sw-5 with dominant allelic interaction (Stevens et al., 1992; Juliatti & Maluf, 1995), whereas for the cultivar Rey de los Tempranos resistance is controlled by at least 1 to 3 genes with semi-dominant allelic interaction (Juliatti & Maluf, 1995).

Stevens et al. (1995) developed the UBC 421 marker from a RAPD (Random amplified polymorphic DNA) decamer primer (ACG GCC CAC C), which shows a 940 bp band (421R band) in resistant genotypes and a 900 bp band (421S band) in susceptible materials at a distance smaller than 1.0 cM from the Sw-5 gene, with band polymorphism of the co-dominance type; that is, two bands at the allegedly heterozygote genotypes (Stevens et al., 1996). The 'UBC 421' marker was converted in another co-dominant SCAR marker type (20 pb primers 'Sw-421-1 and Sw-421-2'), but its efficiency is still to be proved in advanced segregating populations of tomato plants. Like Sw-421 many others SCAR type molecular markers were developed but their efficiency still needs to be tested in tomato breeding programs and in related species (Foolad, 2007; Moon & Nicholson., 2007). Converting specific RAPD markers into single locus markers such as SCAR type is desirable because markers based on PCR are easy to use, less laborious, inexpensive for simple locus assays and more reproducible (Moon, 2006).

The objective of this study was to evaluate the percentage of resistant and susceptible plants in crossing populations with the Stevens cultivar and advanced through backcrossing, by phenotypical evaluation and molecular pattern through SCAR markers distinguishing the resistant homozygotes (Sw-5/Sw-5), heterozygotes (Sw-5/Sw-5$^+$) from susceptible (Sw-5$^+$/Sw-5$^+$) plants.

MATERIAL AND METHODS

Genetic material and experimental details **-** seeds from tomato genotypes with different constitution in the Sw-5 locus were sowed in plastic trays and later transferred to polystyrene trays of 128 speedling type cells with commercial substrate and carbonized rice hulls in similar proportions. For phenotypic evaluations, the experiment was designed in randomized blocks, with four replications and eight plants per plot. The treatments were a control group [TSWV resistant homozygotes genotypes (Stevens, BPX339G-004, TOM-585, TOM-584, BPX339F-07 and BPX363C-18-01); heterozygotes (BPX339G-045-01 and BPX362C) and susceptible (BPX339E-050-01, BPX339-065-01, Santa Clara, BPX339E-050-02, BPX339F-06 and BPX339-065-02)] and isogenic segregating progenies for the Sw-5 allele (BPX385Epl#15, BPX385Epl#23, BPX385Epl#08, BPX385Epl#28 and BPX385Epl#04).

DNA isolation **-** individual leaves of all plants were collected for DNA micro-extraction. Total genomic DNA was extracted from young leaflets according to the procedure of Gawal & Jarret (1991). The reactions for the control groups were performed with a DNA bulk of plants and in isogenic segregating progenies by individual reactions for each selected plant.

DNA amplification **-** the PCR reactions were performed in a total volume of 15 µL – 1.5 µL PCR 10X Buffer; 1.5 µL $MgCl_2$ 25 µM; 3.0 µL dNTP 2 mM; 1.5 µL from each Primer 10 µM (Sw-421-1 and Sw-421-2); 0.75 µL Taq Polymerase 1 unit; 1.0 µL DNA 20–50 ng; 4.25 µL ddH_2O. The PCR was initially carried out for 30 s/94ºC, followed by 35 cycles of 20 s/94ºC; 20 s/55ºC; 2 min/72ºC. The final elongation reaction was 6 min/72ºC. Fragment separation was performed in agarose gel at 1.5% and a 5X TAE buffer. The fragment pattern of a known size (900 bp for susceptible genotypes; 940 bp for resistant genotypes) was dyed in ethidium bromide and visualized in ultra-violet light at 260 nm. Plants with only the 900 bp band corresponded to the susceptible genotype (S) to the TSWV (Sw-5$^+$/Sw-5$^+$); plants with only the 940 bp band corresponded to the resistant genotype (R) homozygote (Sw-5/Sw-5), whereas plants with both bands corresponded to the resistant genotype heterozygote (H) (Sw-5/Sw-5$^+$).

Phenotypic evaluation **-** the same plants sampled for molecular investigation were artificially inoculated (stage of two to three definitive leaves) with a local isolate of TSWV obtained from tobacco plant cultivar TNN with systemic symptoms of TSWV (Paterson et al., 1989). Three inoculations were made in weekly intervals, and on the 12th day of the last inoculation the evaluation of the individual plants were performed according to the score scale proposed by Juliatti et al. (1994). Based on the symptoms, some plants of the control group and the isogenic segregating progenies were chosen for molecular evaluations.

RESULTS AND DISCUSSION

The efficiency of the SCAR 'Sw-421' marker was verified in preliminary tests using contrasting genotypes for the presence of Sw-5 gene (cultivars Stevens and Santa Clara and the F_1 generation from the crossing between them). Three patterns of polymorphism had been observed (Figure 1), showing the codominant inheritance of the marker. In Stevens cultivar a 940 bp band characterized the resistant homozygote genotype. The presence of two bands (900 bp and 940 bp) characterized the heterozygote F_1 (Stevens × Santa Clara) and the molecular characterization of the susceptible genotype Santa Clara (band of 900 bp) indicated absence of Sw-5 allele.

The results of phenotypical evaluations confirmed the efficiency in the inoculation process and showed the differential reaction of the genotypes according to the presence or absence of the Sw-5 allele. The molecular marker allowed the identification of homozygotes and heterozygotes among the resistant plants, both in the control group and isogenic segregating progenies (Table 1).

The amplification of a 940 bp DNA fragment in the resistant patterns (Stevens, TOM-584 and TOM-585) characterized the homozygote genotypes (Sw-5/Sw-5), similar to that observed by Stevens et al.

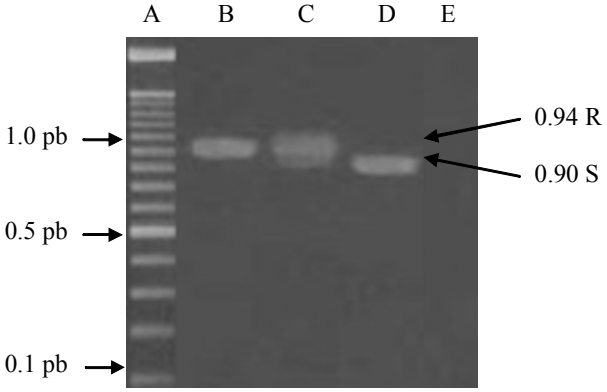

Figure 1 - Amplification result of the PCR using molecular marker SCAR 'Sw-421' in tomato genotypes. (A) Molecular marker of 100 bp DNA (Gibico). (B) Standard band of the resistant genotype cultivar Stevens (Sw-5/Sw-5). (C) Standard bands of the heterozygotes genotypes (Sw-5/Sw-5$^+$). (D) Standard band of the susceptible homozygous genotype cultivar Santa Clara (Sw-5$^+$/Sw-5$^+$). (E) Control without DNA.

Table 1 - Phenotypical reaction and results of PCR for the marker SCAR 'Sw-421' and test reaction to the segregation tospovirus into genotypes of tomatoes.

Genotype	Number of evaluated plants	Reaction[1]		Band paterns[2]		
		S	R	0.90	0.90/0.94	0.94
1 - Stevens	32	0	32	–	–	+
2 - BPX339G-04	32	0	32	–	–	+
3 - BPX 339G-045-01	32	0	32	–	+	–
4 - BPX339F-050-01	32	32	0	+	–	–
5 - BPX339G-065-01	32	32	0	+	–	–
6 - TOM 585	32	0	32	–	–	+
7 - TOM 584	31	0	32	–	–	+
8 - Santa Clara	32	32	0	+	–	–
9 - BPX339E-050-02	32	32	0	+	–	–
10 - BPX339F-06	32	32	0	+	–	–
11 - BPX339F-07	32	0	32	+	–	+
12 - BPX339E-065-02	28	28	0	+	–	–
13 - BPX363C-18-01	32	0	32	–	–	+
14 - BPX363C-04-02	32	32	0	+	–	–
15 - BPX362C-18-02	32	0	32	–	+	–
	Phenotypic evaluation of segregant progenies			Observed bands pattern		
16 - BPX385Epl#15	32	7	25	+(4)[3]	+(7)	+(6)
17 - BPX385Epl#23	32	26	6	+(22)	+(1)	–
18 - BPX385Epl#08	32	0	32	–	–	+(11)
19 - BPX385Epl#28	32	32	0	+(4)	–	–
20 - BPX385Epl#04	32	16	16	+(1)	–	+(1)

[1]According to the scale proposed by Juliatti et al. (1994) (R = Resistant; S = Susceptible); [2]Pattern of bands observed for the label SCAR 'Sw-421'; [3]Number of evaluated plants by molecular marker.

(1995). The bands of 900 bp observed in susceptible control groups BPX339E-050-01, BPX339-065-01, Santa Clara, BPX339E-050-02, BPX339F-06 and BPX339-065-02, distinguished the Sw-5^+/Sw-5^+ genotypes (Figure 2).

A two bands pattern (900 bp and 940 bp) were observed in heterozygote genotypes (Sw-5/Sw-5^+) BPX339G-045-01 and BPX362C-18-01 from control groups (Figure 2), which confirmed the co-dominant inheritance mechanism of the marker, similar to RAPD 'UBC 421' marker developed by Stevens et al. (1995). In this case, the co-dominance showed by the marker allows the identification of resistant heterozygote plants in advanced generations without the need of progeny testing. The molecular marker-assisted backcrossing approach has the potential to increase the chance to develop plants with TSWV resistance by rapid identification of the desired plants and the conversion procedure of a RAPD marker into a SCAR type will expand the usefulness of the technique (Moon, 2006).

Sixty plants from the isogenic progenies were evaluated and fifty seven plants were characterized based on the molecular approach: 18 plants (31.6%) were identified as resistant homozygotes, 8 plants (14.0%) as resistant heterozygotes and 31 plants (54.4%) as susceptible (Figure 3).

Susceptible genotypes expressed typical symptoms of tospovirus (necrotic ring spots, line patterns, wilting, stunting, mottling, chlorosis and necrosis) since the first phenotypic evaluation. Some Stevens cultivar plants and other resistant lines showed necrosis restrict to local lesions on inoculated leaflets, as a characteristic symptom of the genotypes with the Sw-5 allele (Lau et al., 2006).

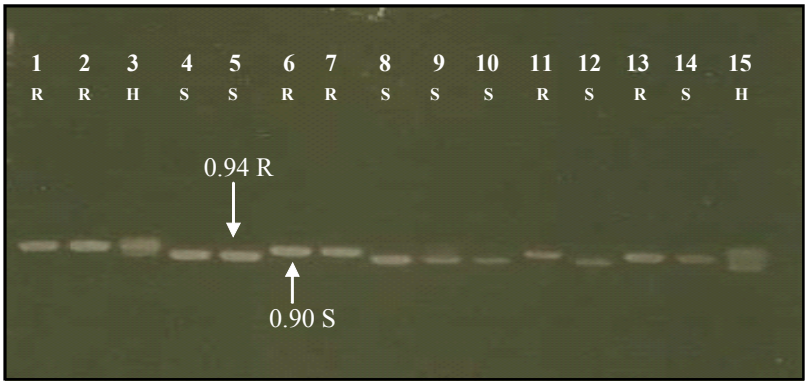

Figure 2 - Electrophoretic pattern of amplified DNA fragments for the Sw-421 marker in tomato plants. L – Ladder marker; 1 – Stevens; 2 – BPX339G-004; 3 – BPX339G-045-01; 4 – BPX339F-050-01; 5 – BPX339G-065-01; 6 – TOM 585; 7 – TOM 584; 8 – Santa Clara; 9 – BPX339E-050-02; 10 – BPX339F-06; 11 – BPX339F-07; 12 – BPX339E-O65-02; 13 – BPX363C-18-01; 14 – BPX363C-04-02; 15 – BPX362C-18-02. R = resistant; H = heterozygote; S = susceptible.

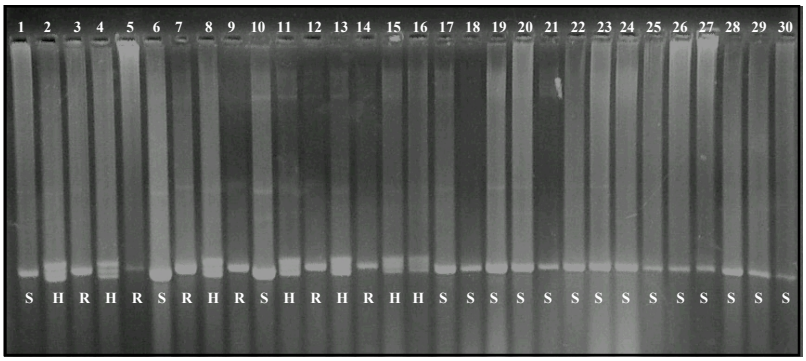

Figure 3 - Electrophoretic pattern of amplified DNA fragments for the Sw-421 marker in tomato plant progenies. 1 - BPX 385Epl#15-01; 2 - BPX 385Epl#15-02; 3 - BPX 385Epl#15-03; 4 - BPX 385Epl#15-04; 5 - BPX 385Epl#15-05; 6 - BPX 385Epl#15-06; 7 - BPX 385Epl#15-07; 8 - BPX 385Epl#15-08; 9 - BPX 385Epl#15-09; 10 - BPX 385Epl#15-10; 11 - BPX 385Epl#15-11; 12 - BPX 385Epl#15-12; 13 - BPX 385Epl#15-13; 14 - BPX 385Epl#15-14; 15 - BPX 385Epl#15-15; 16 - BPX 385Epl#15-16; 17 - BPX 385Epl#23-01; 18 - BPX 385Epl#23-02; 19 - BPX 385Epl#23-03; 20 - BPX 385Epl#23-04; 21 - BPX 385Epl#23-05; 22 - BPX 385Epl#23-06; 23 - BPX 385Epl#23-07; 4 - BPX 385Epl#23-08; 25 - BPX 385Epl#23-09; 26 - BPX 385Epl#23-10; 27 - BPX 385Epl#23-11; 28 - BPX 385Epl#23-12; 29 - BPX 385Epl#23-13; 30 - BPX 385Epl#23-14. R = resistant; H = heterozygote and S = susceptible.

The phenotypic reaction of 32 plants from each segregating progenie indicates close agreement with the hypothesis proposed by Stevens et al. (1995) (Table 1), proving the independence and co-segregation of the marker with the phenotypic reaction observed. These results reinforce the efficiency of the employment of the 'Sw-421' SCAR marker in evaluating different progenies allowing marker-assisted selection in several tomato breeding programs.

Many advantages were attributed to molecular marker-assisted selection (Foolad, 2007; Moon & Nicholson, 2007), as the absence of environmental effects and the independence of the developmental stage of the plant, which is considered by some authors as one of the main problems in selecting resistant or immune plants to viruses through phenotypic selection (Lanza et al., 2000; Ribeiro et al., 2006). In what concerns virus resistance it is possible to pyramid multiple resistance alleles. In the case of TSWV, other known resistance sources such as the one present in Rey de los Tempranos cultivar, the SCAR 'Sw-421' marker is an important tool for this purpose. Several molecular markers associated to virus-resistant genes have been identified, although their use has not been frequent when they are not closely associated to the gene(s) with an interest allele (s) (Yu et al., 1993; Webb et al., 1995; Silva et al., 2003; Santos, 2004; Ribeiro et al., 2006). The distance between the Sw-421 marker and the Sw-5 allele is smaller than 1.0 cM (Stevens et al., 1996), and this allows the selection of the Sw-5 allele with a good safety margin.

CONCLUSION

The 'Sw-421' SCAR marker band pattern was efficient for genetic characterization of the resistant tomato plants, with high reproducibility. The routinely employment of 'Sw-421' SCAR marker for assisted selection in breeding programs could contribute for the development of new tomato cultivars resistant to tospovirus. The codominant inheritance mechanism associated to this marker allowed the precise identification of heterozygote plants in segregant populations and could be a powerful tool for tomato breeding programs.

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