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Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw-5* (*Tospovirus* resistance) gene cluster in a wide range of tomato accessions

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Abstract The best levels of broad-spectrum *Tospovirus* resistance reported in tomatoes thus far are conferred by the *Sw-5* locus. This locus contains at least five paralogues (denoted *Sw-5a* through *Sw-5e*), of which *Sw-5b* represents the actual resistance gene. Here we evaluated a panel of seven PCR primer pairs matching different sequences within a genomic region spanning the *Sw-5a* and *Sw-5b* gene cluster. Primer efficiency evaluation was done employing tomato isolines with and without the *Sw-5* locus. One primer pair produced a single and co-dominant polymorphism between susceptible and resistant isolines. Sequence analysis of these amplicons indicated that they were specific for the *Sw-5* locus and their differences were due to insertions/deletions. The polymorphic SCAR amplicon encompass a conserved sequence of the promoter region of the functional *Sw-5b* gene, being located in the position −31 from its open reading frame. This primer pair was also evaluated in field assays and with a collection of accessions known to be either susceptible or resistant to tospoviruses. An almost complete correlation was found between resistance under greenhouse/field conditions and the presence of the marker. Therefore, this primer pair is a very useful tool in marker-assisted selection systems in a large range of tomato accessions.

Keywords *Sw-5* · *Tospovirus* · Resistance · Marker · PCR · Selection

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Introduction

A number of related *Tospovirus* species (family Bunyaviridae) are responsible for the disease known as “spotted wilt”, “peste negra” (Williams et al. 2001), and “vira-cabeça” (Silberschmidt 1937), that causes severe annual yield losses of fresh-market and processing tomatoes (*Solanum lycopersicum* L.) around the world (Gordillo et al. 2008). These viruses are transmitted by thrips (Thysanoptera: Thripidae) in a circulative-propagative manner (Wijkamp et al. 1995). Susceptible plants show a wide range of symptoms, including chlorotic ring spots on leaves, petiole necrosis, chlorotic and necrotic rings on fruits, apical necrosis and browning, generalized necrosis,

and wilting. Early infection of highly susceptible cultivars often leads to complete decay and death. *Tomato spotted wilt virus* (TSWV), *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV) and *Chrysanthemum stem necrosis virus* (CSNV) are the four prevalent *Tospovirus* species infecting tomatoes in tropical and sub-tropical areas of South America (de Ávila et al. 1993; Giordano et al. 2000; Williams et al. 2001).

Sources of genetic resistance to tospoviruses have been found in domesticated and wild *Solanum* (*Lycopersicon*) accessions (for review see Soler et al. 2003). The wild tomato species are the best sources of resistance with many accessions being considered as either tolerant or immune to distinct *tospovirus* isolates and species (Stevens et al. 1994; Roselló et al. 1998; Gordillo et al. 2008). So far, the best levels of broad-spectrum resistance to tospoviruses are conferred by the *Sw-5* gene from *S. peruvianum*, which was introgressed into the tomato cultivar 'Stevens' (Van Zijl et al. 1986; Stevens et al. 1992). Plants carrying the *Sw-5* gene are able to restrict the systemic spread of the virus, showing only localized symptoms represented as tiny local lesions caused by a hypersensitive reaction. The *Sw*-5 gene is located on the telomeric region of the long arm of chromosome 9 and it was isolated via positional cloning (Brommonschenkel et al. 2000; Spassova et al. 2001). The *Sw-5* belongs to the same class of resistance genes as the *Mi*, which confers resistance to *Meloidogyne* species; *RPM1* (resistance gene to *Pseudomonas syringae* pv. *maculicola*) and many others R-genes (Brommonschenkel et al. 2000). This class of resistance genes is named CC-(NB-ARC)-LRR, containing leucine-rich repeats and a highly conserved nucleotide binding site (Martin et al. 2003). In addition, it was found that the *Sw-5* gene resides within a complex cluster comprising five linked gene copies, named *Sw-5a* through *Sw-5e* (Spassova et al. 2001). The efficiency of each copy in conferring resistance to *tospovirus* species is not yet completely clear, but separate analysis of the copies in transgenic plants indicated that the *Sw-5b* is the single gene for the expression of the resistance phenotype (Spassova et al. 2001).

Due to the economic losses caused by tospoviruses in many areas of the world, genetic resistance became the major research focus related to the disease management (Soler et al. 2003; Gordillo et al. 2008). The dominant nature of the *Sw-5* allowed a broad use of this locus in the development of hybrid cultivars. It was found that cultivars carrying the *Sw-5* locus had broad-spectrum resistance with high stability to distinct TSWV isolates (Stevens et al. 1992; Roselló et al. 1998) and also to the related *Tospovirus* species GRSV and TCSV (Boiteux and Giordano 1993; Boiteux et al. 1993). Therefore, the incorporation of this locus in commercial cultivars led to a considerable reduction on economic losses, even though this resistance can be broken by some *Tospovirus* species, by some TSWV isolates, by high inoculum pressure, or by drastic temperature variation (Lathan and Jones 1998; Roselló et al. 1998; McMichael et al. 2002; Aramburu and Marti 2003; Ciuffo et al. 2005).

The complex nature of the *Tospovirus* species might lead to the occurrence of escapes (false resistant plants) in screening systems of breeding programs. These viruses are quite unstable in sap; symptom expression is highly influenced by environmental conditions and the use of fresh isolates is required in order to avoid the accumulation of defective (more attenuated) variants of the virus (Nagata et al. 2000). Therefore, large-scale selection systems for *Tospovirus* resistance in tomatoes based on the *Sw-5* locus would greatly benefit by the use of molecular markers.

Until now, the marker-assisted systems available for monitoring the incorporation of the *Sw-5* locus into susceptible lines are RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), RAPD-derived SCAR (sequence characterized amplified regions), and cleaved amplified polymorphic sequence (CAPS) markers (Stevens et al. 1995; Chagué et al. 1996; Smiech et al. 2000; Langella et al. 2004). These are closely linked markers, which might occasionally be separated from the gene of interest through genetic recombination (crossing-over) events. In this scenario, markers derived from the resistant locus itself, capable of differentiating among susceptible and resistant plants, would be the ideal ones (Folkertsma et al. 1999; Garland et al. 2005). Although sequences of the *Sw-5* locus are available at public databases, there are few reports of molecular markers derived from them. The work reported by Garland et al. (2005) is one of the few exceptions, where a dominant marker derived from the *Sw-5* locus combined with a closely linked

CAPS marker were employed to fingerprint *Tospovirus* susceptible and resistant lines.

Here we evaluated a panel of PCR primers spanning part of the *Sw-5* locus aiming to establish a fingerprinting system for this genomic region. The objectives of this research were the following: (a) to develop a locus-specific marker system for the *Sw-5* gene cluster that could be easily used in simple PCR assays; and (b) to validate this system through the evaluation of near-isogenic lines and by screening of a range of accessions contrasting for resistance to *Tospovirus* in order to establish their usefulness to monitor the incorporation of the *Sw-5* gene in elite tomato cultivars. In addition, we also characterized (via sequence analysis) the genomic region comprising the most promising polymorphic amplicon in a set of *Tospovirus* resistant and susceptible *Solanum* (*Lycopersicon*) accessions of distinct genetic backgrounds in order to evaluate how broad could be the application of this genetic information in marker-assisted tomato breeding programs.

Materials and methods

Design of the primers and their location within the *Sw-5* locus

Seven primer pairs (Table 1) able to amplify fragments of approximately 400 to 575 bp within the *Sw-5* locus were designed based upon the sequence of the *Sw-5a* and *Sw-5b* genes (GenBank accession AY007366). Primer pairs were designed using the Lasergene Package (Lasergene, Madison, WI). According to Spassova et al. (2001) the *Sw-5* gene has five paralogue copies along the chromosome 9, named *Sw-5a* through *Sw-5e*. The part of the sequence selected for amplification encompassed an area with high identity between the *Sw-5a* and *Sw-5b* copies, covering a region of approximately 3 kb. This region corresponds to the sequence of a TSWV-resistant tomato plant (Genbank accession AY007366), which supposedly expresses a template directly related to *Tospovirus* resistance (Spassova et al. 2001).

DNA extraction, PCR cycles, and agarose-gel analysis

Genomic DNA of tomato plants was purified as described (Boiteux et al. 1999) using a modified 2× CTAB buffer and additional purification steps with organic solvents. PCR reactions were as follows: ca. 1 μg of the DNA, 1 μl of 10× PCR buffer (Invitrogen), 0.3 μl of 50 mM $MgCl_2$, 0.7 μl of 2.5 mM dNTP, 100 ng of each primer, 0.5 U of Taq DNA polymerase (Invitrogen) and water in a final volume of 10 μl. The Mastercycler (Eppendorf) thermocycler program steps were: denaturation at 94°C for 2 min, followed by 29 cycles of 94°C for 30 s, 50°C for 1 min and 72°C for 30 s and final extension at 72°C

Table 1 Codes, sequences, and expected PCR amplicon sizes of a panel of primer pairs derived from the tomato *Sw-5* locus sequence (accession AY 007366). The *Sw-5* gene is known to confer broad-spectrum resistance to *Tospovirus*

Primer pair code	Primer pair sequence	Amplicon size (bp)
'Sw-5–1'	F = 5′-TTTGGAGGTTTGGAGGAAGAAGAG-3′	409
	R = 5′-TCATGTCCGACCCAATACCTAACT-3′	
'Sw-5–2'	F = 5′-AATTAGGTTCTTGAAGCCCATCT-3′	574
	R = 5′-TTCCGCATCAGCCAATAGTGT-3′	
'Sw-5–3'	F = 5′-GAATCTGTTTATGAGTGCGACCTT-3′	418
	R = 5′-ACACCCTCTTCTTCCTCCAAACCT-3′	
'Sw-5–4'	F = 5′-TTTTGCAAGAACATCATCAGTA-3′	538
	R = 5′-GGCGAAAATCCCAACAAG-3′	
'Sw-5–5'	F = 5′-AGTCTCCAAACATTCCTGCTTCTC-3′	416
	R = 5'-TTCTTTTGCCTGATTCCTTAGTCG-3'	
'Sw-5–6'	F = 5′-AATTCTTTCGGTTACAA-3′	496
	R = 5′-CCGCCTCTGAATACAAAGTCGTCT-3′	
'Sw-5–7'	F = 5′-TCGCAACGTAATAAAGTCCCACAT-3′	436
	R = 5′-ACATACTTCAACAACCTAAACTCC-3′	

for 5 min. PCR products were separated using 1% agarose gel in TBE buffer.

Greenhouse assays with near-isogenic lines to evaluate the association between primer product profile and *Tospovirus* resistance

Plants of *S. lycopersicum* cultivars 'IPA-5' (susceptible to TSWV) and 'Viradoro' (a F_5BC_4 near-isogenic line of 'IPA-5' resistant to TSWV due to the presence of the *Sw-5* locus) (Giordano et al. 2000) were firstly used as DNA sources for evaluation of the primer pair panel. The accessions were sown in Styrofoam trays with 128 cells, filled with sterile substrate Plantmax® and maintained in a greenhouse. Mechanical inoculation with TSWV (BR01 isolate) was done in 20 plants of each line following standard procedures (Boiteux and Giordano 1993). Similar mechanical greenhouse inoculation assays were also done with 20 'Stevens' (resistant to TSWV and source of the *Sw-5* gene) (Van Zijl et al. 1986; Stevens et al. 1992) plants and 20 'Moneymaker' (susceptible to TSWV) plants. Genomic DNA samples were extracted from individual 'Stevens' and 'Moneymaker' plants and were amplified to verify the efficiency of the selected set of *Sw-5*-derived primers in revealing polymorphisms.

Field assays with near-isogenic lines to evaluate the association between 'Sw-5–2' primer amplicon and *Tospovirus* resistance

Field assays were also conducted for additional confirmation of the most promising primer pair for marker-assisted selection ('Sw-5–2'). The susceptible Brazilian cultivar 'Santa Clara' (Nagai 1993) and a group of F_5BC_5 'Santa Clara'-derived lines (near-isogenic *Tospovirus* resistant inbred lines) using 'Santa Clara' as recurrent parent and one inbred line produced at CNPH (Boiteux et al. 1993) as donor of the *Sw-5* locus were simultaneously analyzed via molecular markers and field tests with high inoculation pressure of GRSV. The experiment was conducted at the CNPH experimental station during the dry season (May-August, 2008) in Brasília-DF, Brazil. Field plots were composed by the original 'Santa Clara' (three replications of eight plants each) and 24 backcross inbred lines carrying the *Sw-5* locus (eight plants per each inbred line).

Entire sequence analysis of the cloned amplicons obtained with the 'Sw-5–2' primer pair in two near-isogenic lines

PCR products obtained from the near-isogenic lines 'Viradoro' (carrying the *Sw-5* locus) and 'IPA-5' (susceptible to *Tospovirus*) were size separated on 1.0% agarose gel. The single individual bands obtained from each line were excised from the gel under UV light and purified using QIAquick Gel extraction kits (QIAGEN Inc., Valencia-CA). Purified bands were cloned into the pGEM-T Easy® vector (Promega, Madison, WI, USA) and sequenced using SP6 and T7 primers. The sequences obtained for these near-isogenic lines were aligned in the region defined by the 'Sw-5–2' primer pair using the Clustal method available in the Megalign program (Lasergene, Madison, WI, USA).

Direct sequencing of the 'Sw-5–2' PCR amplicons from 14 tomato accessions

PCR amplicons were also obtained using the 'Sw-5–2' primer pair with genomic DNA extracted from 'Viradoro' and 'IPA-5'and other 12 accessions of distinct genetic backgrounds (Fig. 1). All 14 accessions produced single amplicons with varying sizes when analyzed by agarose-gel electrophoresis. In this assay, all the amplicons of the 14 accessions were directly sequenced from the PCR products using the 'Sw-5–2' forward and reverse primers. The sequencing reaction was done using the protocol of the BigDye® terminator cycle sequencing version 3.1 (Applied Biosystems of Brazil, São Paulo) with 2 µl of the amplicons as template. DNA was analyzed in an automatic ABI Prism sequencer model 3100 (Applied Biosystems of Brazil, São Paulo) at the CNPH's Genomic Analysis Laboratory. The quality of the sequences obtained in the trace files was screened using the SeqMan program (Allex 1999). The sequences obtained for the 14 accessions were aligned using the Clustal method available in the Megalign program (Lasergene, Madison, WI, USA).

Results and discussion

For monitoring of the genomic region encoding a given phenotype the so-called gene-derived (Varshney et al.

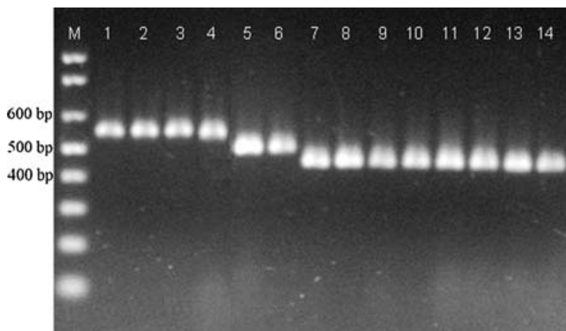

Fig. 1 PCR amplification profile (in 1% agarose gel in TBE buffer) obtained with the 'Sw-5–2' primer pair. PCR assays were carried out using as template genomic DNA extracted from 14 *Solanum* (*Lycopersicon*) accessions. *M* 1 kb ladder (Invitrogen); *1* 'Stevens' (breeding source of the *Sw-5 Tospovirus* resistance locus); *2* 'Viradoro' (contains the *Sw-5* locus and it is near-isogenic to 'IPA-5'); *3 Solanum peruvianum* 'PI 128660' (found to be resistant to *Tospovirus* isolates in the USA); *4* 'Santa Clara R' (Santa Cruz type inbred line with the *Sw-5* locus and near-isogenic to 'Santa Clara S'); *5* inbred line derived from the hybrid 'NemoNetta' (susceptible to *Tospovirus*); *6* 'Ohio 8245' (susceptible to *Tospovirus*); *7* 'Santa Clara S' (Traditional Brazilian Santa Cruz type cultivar and highly susceptible to *Tospovirus*); *8* 'Ponderosa' (very old obsolete fresh-tomato cultivar, susceptible to *Tospovirus*); *9* 'IPA-5' (processing type highly susceptible to *Tospovirus*); *10* inbred line derived from the hybrid 'Densus' (long-shelf life type susceptible to *Tospovirus*); *11* 'Moneymaker' (introgression-free obsolete tomato susceptible cultivar); *12* 'CNPH Tx-577' (susceptible cherry tomato type); *13* susceptible inbred line derived from the hybrid 'Alambra' (long-shelf life type); *14* susceptible inbred line derived from the hybrid 'Netta' (long-shelf life type). The first 'Sw-5–2' PCR pattern group (*lanes 1–4*) generated an amplicon of ca. 574 bp. The second group (*lanes 5–6*) displayed an amplicon of ca. 510 bp. The third phenotype group (*lanes 7–14*) displayed an amplicon of ca. 464 bp

2005) and/or locus-specific markers represent robust tools since they minimize the risk of accidental separation through genetic recombination (crossing-over) events as reported for closely linked DNA markers. To date, *Sw-5* locus-specific and co-dominant PCR markers are not publicly available, even though the entire locus has been already cloned and characterized (Brommonschenkel et al. 2000; Spassova et al. 2001).

In the present work, we evaluated a panel of seven PCR primer pairs (Table 1) derived from sequence information of the *Sw-5* locus aiming to establish a useful fingerprinting system for this genomic region. The primer pairs 'Sw-5–4' and 'Sw-5–7' displayed a complex amplicon profile with the first being monomorphic and the latter exhibiting a profile of intensity polymorphism, which was very difficult to score. The complex amplicon profiles (with several monomorphic bands) indicated that these primers were not *Sw-5* locus-specific. The other primer pairs ('Sw-5–1', 'Sw-5–3' and 'Sw-5–6') displayed either no reaction at all under the employed PCR conditions or gave a monomorphic pattern ('Sw-5–5'). For all these primers no further testing was carried out.

The PCR primer pair 'Sw-5–2' was the only one able to reveal a stable polymorphic pattern between the two near-isogenic lines 'Viradoro' and 'IPA-5' (Fig. 1, lanes 2 and 9). The PCR profile obtained with this primer pair was a single amplicon, which also provided a stable and reliable discrimination of the cultivars 'Stevens' and 'Moneymaker' (Fig. 1, lanes 1 and 11). 'Stevens' (breeding source of the *Sw-5* locus) had a resistant reaction to TSWV (isolate 'BR 01') after mechanical inoculation under greenhouse conditions. All plants of 'Moneymaker' were found to be susceptible to this virus in the greenhouse assay. In addition, the amplicon obtained with the primer 'Sw-5–2' was found to be co-dominant, indicating the existence of insertions and/or deletions (indels) of about 100 bp in this region.

Parallel evaluation was conducted with both PCR assays with the primer 'Sw-5–2' and reaction to GRSV using the susceptible cultivar 'Santa Clara' ('Santa Clara S') as susceptible control and a group of 24 *Sw-5*-carrying backcross near-isogenic lines ('Santa Clara R' lines) at field conditions. All plants in all three 'Santa Clara S' plots displayed severe GRSV symptoms with the virus infection being confirmed by ELISA using a specific antiserum. These results indicated an adequate and uniform inoculum pressure across the experimental field. The *Sw-5*-derived amplicon of ca. 574 bp (Fig. 1, lane 4) was observed in all 192 plants of the 24 near-isogenic 'Santa Clara + *Sw-5*' inbred lines. On the other hand, all susceptible 'Santa Clara S' plants displayed only a smaller amplicon of around 464 bp (Fig. 1, lane 7). An almost complete correlation was observed between the *Sw-5*-derived marker result and resistance to GRSV under field conditions, except for two 'Santa Clara + *Sw-5*' plants that displayed either apical necrosis or ring spots on the fruits. The presence of a small fraction of symptomatic plants is commonly observed even in highly inbred lines carrying the *Sw-5* locus under field conditions in Brazil (e.g. Boiteux and Giordano 1993). This result

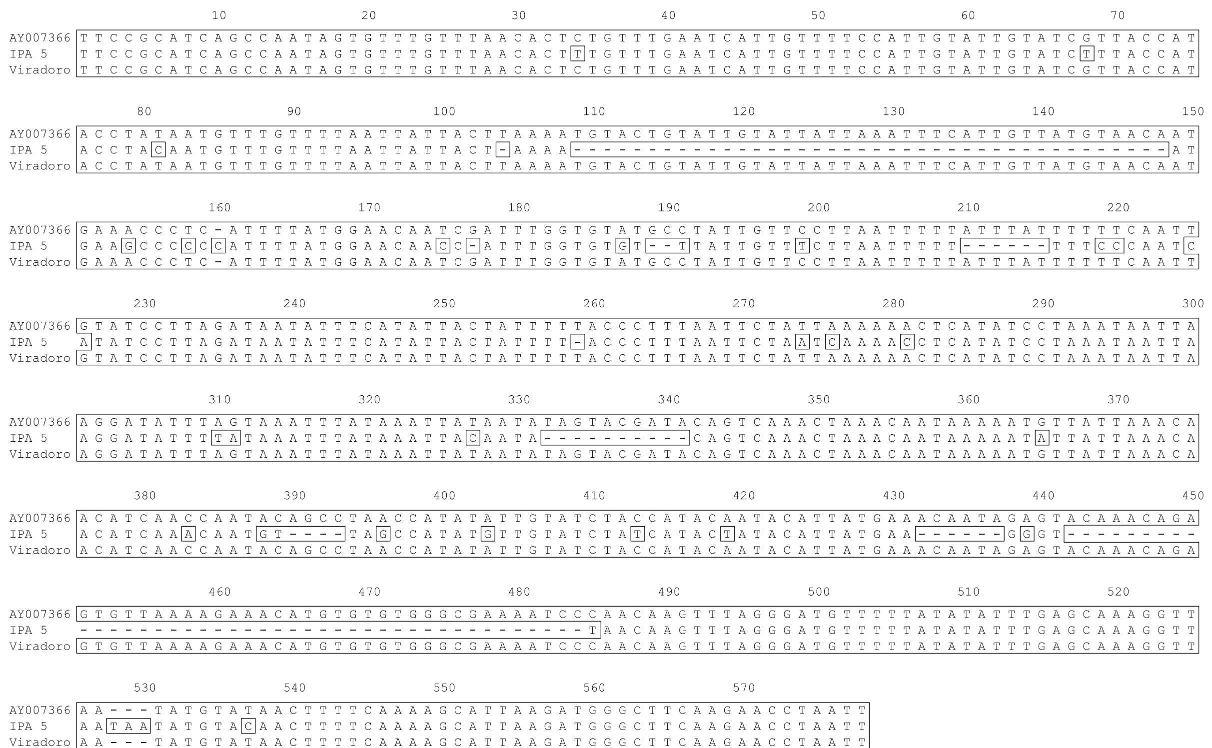

Fig. 2 Sequence comparison of the entire segment of the cloned 'Sw-5–2' PCR amplicons obtained from *Solanum lycopersicum* 'Viradoro' (574 bp) and 'IPA-5' (464 bp) genomic DNA. 'Viradoro' is a processing type cultivar resistant to *Tospovirus* species due to the presence of the *Sw-5* locus and 'IPA-5' is a near-isogenic line of 'Viradoro', which is highly susceptible to tospoviruses. The primer pair 'Sw-5–2' (flanking the sequences) is derived from the tomato 'Stevens' (GenBank AY 007366) that is the source of the *Sw-5* locus introgressed from *S. peruvianum*. The nucleotide position of the original AY 007366 sequence encompassing the 'Sw-5–2' amplicon ranged from nucleotides 33283 to 33851. The 'Sw-5–2' primer pair sequences (5′-AATTAGGTTCTTGAAGCC-CATCT-3′ and 5′-TCATGTCCGACCCAATACCTAACT-3′) are flanking the sequences

has been explained by the incomplete penetrance of the *Sw-5* gene (Stevens et al. 1992) rather than by the occurrence of distinct viral strains and/or environmental effects on gene expression.

PCR assays were also conducted with primer pair 'Sw-5–2' using genomic DNA extracted from a collection of 14 accessions known to be either susceptible or resistant to *Tospovirus* isolates (Fig. 1). The analyses indicated three DNA patterns. The first group, displaying an amplicon of ca. 575 bp was exhibited by three *Tospovirus* resistant accessions carrying the *Sw-5* locus ('Stevens', 'Viradoro' and 'Santa Clara R') and one *S. peruvianum* 'PI 128660', which was found to be resistant to TSWV isolates in Australia and USA (Gordillo et al. 2008). The second group displayed an amplicon of ca. 510 bp and encompassed two susceptible accessions (an inbred line derived from the hybrid 'Nemonetta' and the cultivar 'Ohio 8245'). The third group displayed an amplicon of 464 bp and was composed by the two near-isogenic lines 'IPA-5' and 'Santa Clara S' and six other *Tospovirus*-susceptible accessions and inbred lines derived from commercial hybrids (Fig. 1). It is important to mention that, in all situations, heterozygous plants were able to produce amplicons with quite similar intensity allowing, therefore, a reliable co-dominant marker system, especially in gels with higher (1.4–1.8%) agarose concentration. However, the presence of more than one gel pattern for the susceptible accessions demands either the comparison with PCR samples from DNA template extracted from sources of the *Sw-5* locus or a careful estimation of the amplicon size. This procedure will avoid misclassification of "pseudo-resistant" plants, for example, in *Tospovirus* susceptible populations segregating for the gel patterns 2 and 3. In addition, it might be possible that novel susceptible gel

patterns might occur in tomato accessions with genetic background distinct from the accessions employed here.

The complete sequence analysis of the cloned 'Sw-5–2' amplicons obtained from 'Viradoro' (574 bp) and 'IPA-5' (464 bp) indicated an overall identity of 81.7% with the presence of indels and point mutations, but with no indication of sequence rearrangements (Fig. 2). The estimated 110 bp difference observed in the agarose-gel assays between the near isogenic lines 'Viradoro'/'IPA-5' and 'Santa Clara R'/'Santa Clara S' were caused by two large deletions (83 bp in total) plus eight single nucleotides or small deletions (31 bp in total), which were compensated in size by two insertions in the susceptible lines. Twenty-seven point mutations (21 transitions and six transversions) were observed along the analyzed *Sw-5* derived amplicons (Fig. 3).

Amplicons of 'Viradoro' and 'IPA-5' were also directly sequenced using the 'Sw-5–2' forward and reverse primers together with the amplicons obtained with other 12 tomato accessions (Fig. 1). For this reason, the sequence analysis was done with only 522 out of the 574 bp PCR amplicons (about 91% of the entire sequence, which did not include the 'Sw-5–2' primer pair). The co-dominant markers were found to be specific for the *Sw-5* locus. The polymorphic SCAR amplicon encompass a conserved sequence of the promoter region from the functional *Sw-5b* gene, being located in the position −31 from its open reading frame between the nucleotide sites 33283 and 33851 in the accession AY007366. The differences in the amplicon sizes were due to a number of insertions/deletions in this genomic region. A number of point mutations were also observed (Fig. 3). The sequences of the resistant lines 'Viradoro' and 'Santa Clara R' were 100% identical to that of 'Stevens' for this genomic region. It is interesting to note that the genomic region within the *Sw-5* locus corresponding to the 'Sw-5–2' amplicon was highly conserved across the two *S. peruvianum* loci analyzed here (i.e. the 'Stevens' locus and the 'PI 128660' locus). The sequences of 'Stevens' and 'PI 128660' are almost identical with only a single point transition mutation (T/C) in the position 141 (Fig. 3). This level of nucleotide identity between these two *S. peruvianum* accessions suggests that they might be closely related. However, the *S. peruvianum* accession used for the introgression of the *Sw-5* locus into the cultivar 'Stevens' has not been disclosed, making this hypothesis very difficult to be tested. Only one group of *Tospovirus*-susceptible accessions formed by 'Santa Clara S', 'IPA-5', 'CNPH Tx-557' and inbred lines from 'Densus' and 'Alambra', shared 100% sequence identity. The other susceptible accessions had identity levels ranging from 87.9 to 99.8%.

From the marker-assisted selection point of view, a reliable, fast, and simple PCR assay to screen for the presence of the *Sw-5* locus is highly desirable. RFLP, RAPD, SCAR, and CAPS markers linked to the *Sw-5* locus have been employed for marker-assisted selection in tomato breeding (Stevens et al. 1995; Chagué et al. 1996; Smiech et al. 2000; Langella et al. 2004). This collection of closely linked markers might occasionally be separated from the *Sw-5* locus by crossing-over events during the breeding process of generating populations and/or incorporating this gene cluster into elite tomato lines via backcrossing. In this context, the 'Sw-5–2' co-dominant, within-locus, SCAR marker system is more advantageous than the other closely linked markers reported thus far. In addition, the SCAR marker reported here is much simpler than the CAPS marker system reported by Garland et al. (2005), which requires the use of the closely linked CT220 marker as a positive control in order to discriminate susceptible plants from false negative PCR reactions (Garland et al. 2005). It is important to mention that the amount of sequence variation observed in the genomic region encompassing this polymorphic amplicon might allow the further development of even better markers potentially suitable for gel-free systems.

Altogether, the results obtained with the primer pair 'Sw-5–2' indicate an array of applications as a robust selection tool to monitor the *Sw-5* locus introgression in tomato accessions. Since this marker is able to specifically target the locus, it might be useful to amplify analogous loci in other *Tospovirus* resistance sources in *Solanum* (*Lycopersicon*) aiming to isolate alleles with potentially distinct characteristics, including thermal stability, better expression of the resistance in fruits, and resistance to isolates able to overcome the *Sw-5*. Another possible use of this primer set would be chromosome landing on *Sw-5* gene orthologues that might be present in other crop species of the genus *Solanum*, enhancing its scope as a universal marker that would be useful not only for tomato breeding programs.

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Positions 1–75
AY007366     GTGTTTGTTTAACACTCTGTTTGAATCATTGTTTTCCATTGTATTGTATCGTTACCATACCTATAATGTTTGTTT
Viradoro     GTGTTTGTTTAACACTCTGTTTGAATCATTGTTTTCCATTGTATTGTATCGTTACCATACCTATAATGTTTGTTT
PI 128660    GTGTTTGTTTAACACTCTGTTTGAATCATTGTTTTCCATTGTATTGTATCGTTACCATACCTATAATGTTTGTTT
S Clara R    GTGTTTGTTTAACACTCTGTTTGAATCATTGTTTTCCATTGTATTGTATCGTTACCATACCTATAATGTTTGTTT
NemoNetta    GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
Ohio         GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
S Clara S    GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
Ponderosa    GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTC-AT-GTATTGTATCTTTACCATACCTACAATGTTTGTTT
IPA 5        GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
Densus       GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
Moneymaker   GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
CNPH Tx 577  GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
Alambra      GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT
Netta        GTGTTTGTTTAACACTTTGTTTGAATCATTGTTTTCCATTGTATTGTATCTTTACCATACCTACAATGTTTGTTT

Positions 76–150
AY007366     TAATTATTACTTAAAATGTACTGTATTGTATTATTAAATTTCATTGTTATGTAACAATGAAACCCTC-ATTTTAT
Viradoro     TAATTATTACTTAAAATGTACTGTATTGTATTATTAAATTTCATTGTTATGTAACAATGAAACCCTC-ATTTTAT
PI 128660    TAATTATTACTTAAAATGTACTGTATTGTATTATTAAATTTCATTGTTATGTAACAATGAAACCCCC-ATTTTAT
S Clara R    TAATTATTACTTAAAATGTACTGTATTGTATTATTAAATTTCATTGTTATGTAACAATGAAACCCTC-ATTTTAT
NemoNetta    TAATTATTACTAAAAATGTATTGTGTTATATTATTAAATTTCATTGTTATGTAACAATGAAGCCCCC-ATTTTAT
Ohio         TAATTATTACTAAAAATGTATTGTGTTATATTATTAAATTTCATTGTTATGTAACAATGAAGCCCCC-ATTTTAT
S Clara S    TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
Ponderosa    TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
IPA 5        TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
Densus       TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
Moneymaker   TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
CNPH Tx 577  TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
Alambra      TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT
Netta        TAATTATTACTAAAAATG-----------------------------------------AAGCCCCCCATTTTAT

Positions 151–225
AY007366     GGAACAATCGATTTGGTGTATGCCTATTGTTCCTTAATTTTTATTTATTTTTTCAATTGTATCCTTAGATAATAT
Viradoro     GGAACAATCGATTTGGTGTATGCCTATTGTTCCTTAATTTTTATTTATTTTTTCAATTGTATCCTTAGATAATAT
PI 128660    GGAACAATCGATTTGGTGTATGCCTATTGTTCCTTAATTTTTATTTATTTTTTCAATTGTATCCTTAGATAATAT
S Clara R    GGAACAATCGATTTGGTGTATGCCTATTGTTCCTTAATTTTTATTTATTTTTTCAATTGTATCCTTAGATAATAT
NemoNetta    GGAACAACCCATTTGGTGTGT--TTATTGTTCCTTAATTTTT-----TTTTCC-AATCATATCCTTAGATAATAT
Ohio         GGAACAACCCATTTGGTGTGT--TTATTGTTCCTTAATTTTT-----TTTTCC-AATCATATCCTTAGATAATAT
S Clara S    GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT
Ponderosa    GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT
IPA 5        GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT
Densus       GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT
Moneymaker   GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TTCTCCCAATCATATCCTTAGATAATAT
CNPH Tx 577  GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT
Alambra      GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT
Netta        GGAACAACC-ATTTGGTGTGT--TTATTGTTTCTTAATTTTT-----TT-TCCCAATCATATCCTTAGATAATAT

Positions 226–300
AY007366     TTCATATTACTATTTTTACCCTTTAATTCTATTAAAAAACTCATATCCTAAATAATTAAGGATATTTAGTAAATT
Viradoro     TTCATATTACTATTTTTACCCTTTAATTCTATTAAAAAACTCATATCCTAAATAATTAAGGATATTTAGTAAATT
PI 128660    TTCATATTACTATTTTTACCCTTTAATTCTATTAAAAAACTCATATCCTAAATAATTAAGGATATTTAGTAAATT
S Clara R    TTCATATTACTATTTTTACCCTTTAATTCTATTAAAAAACTCATATCCTAAATAATTAAGGATATTTAGTAAATT
NemoNetta    TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
Ohio         TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
S Clara S    TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
Ponderosa    TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
IPA 5        TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
Densus       TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
Moneymaker   TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
CNPH Tx 577  TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
Alambra      TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT
Netta        TTCATATTACTATTTT-ACCCTTTAATTCTAATCAAAACCTCATATCCTAAATAATTAAGGATATTTTATAAATT

Positions 301–375
AY007366     TATAAATTATAATATAGTACGATACAGTCAAACTAAACAATAAAAATGTTATTAAACAACATCAACCAATACAGC
Viradoro     TATAAATTATAATATAGTACGATACAGTCAAACTAAACAATAAAAATGTTATTAAACAACATCAACCAATACAGC
PI 128660    TATAAATTATAATATAGTACGATACAGTCAAACTAAACAATAAAAATGTTATTAAACAACATCAACCAATACAGC
S Clara R    TATAAATTATAATATAGTACGATACAGTCAAACTAAACAATAAAAATGTTATTAAACAACATCAACCAATACAGC
NemoNetta    TATAAATTACAAT----------ACAGTGAAACTAAATAATAAAAATATTATTAAACAACATCAAACAATGCAGT
Ohio         TATAAATTACAAT----------ACAGTGAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATGCAGT
S Clara S    TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
Ponderosa    TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
IPA 5        TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
Densus       TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
Moneymaker   TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
CNPH Tx 577  TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
Alambra      TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T
Netta        TATAAATTACAAT----------ACAGTCAAACTAAACAATAAAAATATTATTAAACAACATCAAACAATG---T

Positions 376–450
AY007366     CTAACCATATATT-GTATCTACCATACAATACATTATGAAACAATAGAGTACAAACAGAGTGTTAAAAGAAACAT
Viradoro     CTAACCATATATT-GTATCTACCATACAATACATTATGAAACAATAGAGTACAAACAGAGTGTTAAAAGAAACAT
PI 128660    CTAACCATATATT-GTATCTACCATACAATACATTATGAAACAATAGAGTACAAACAGAGTGTTAAAAGAAACAT
S Clara R    CTAACCATATATT-GTATCTACCATACAATACATTATGAAACAATAGAGTACAAACAGAGTGTTAAAAGAAACAT
NemoNetta    -TAGCCATATGTT-GTATCTATCATGCTATACATTATGAAACAA-----------CAGAGGGTTAA---------
Ohio         -TAGCCATATGTTTGTATCTATCATACTATACATTATGAAACAA-----------CAGAGGGTTAA---------
S Clara S    -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
Ponderosa    -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
IPA 5        -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
Densus       -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
Moneymaker   -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
CNPH Tx 577  -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
Alambra      -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------
Netta        -TAGCCATATGTT-GTATCTATCATACTATACATTATGAA-------------------GGGTTAA---------

Positions 451–522
AY007366     GTGTGTGGGCGAAAATCCCAACAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATA---TGTATA
Viradoro     GTGTGTGGGCGAAAATCCCAACAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATA---TGTATA
PI 128660    GTGTGTGGGCGAAAATCCCAACAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATA---TGTATA
S Clara R    GTGTGTGGGCGAAAATCCCAACAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATA---TGTATA
NemoNetta    ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTATA
Ohio         ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTATA
S Clara S    ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
Ponderosa    ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
IPA 5        ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
Densus       ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
Moneymaker   ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
CNPH Tx 577  ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
Alambra      ---------------------CAAGTTTAGGGATGTTTTTATATATTTGAGCAAAGGTTAATAATATGTACA
Netta        ---------------------CAAGTTTAGGGATGTTTTTATATATT-GAGCAAAGGTTAATAATATGTACA
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◀ **Fig. 3** Sequence comparison of segments of PCR amplicons obtained with the 'Sw-5–2' primer pair using as template genomic DNA extracted from 14 *Solanum* (*Lycopersicon*) accessions. The primer pair sequence is derived from the tomato 'Stevens', source of the *Sw-5* locus, which was introgressed from *S. peruvianum* (GenBank AY 007366). The following resistant (R) and susceptible (S) accessions were analyzed: 'Stevens' (R); 'Viradoro' (R); *S. peruvianum* 'PI 128660' (R); 'Santa Clara R' (R); one inbred line derived from the hybrid 'NemoNetta' (S); 'Ohio 8245' (S); 'Santa Clara S' (S); 'Ponderosa' (S); 'IPA-5' (S); one inbred line derived from the hybrid 'Densus' (S); 'Moneymaker' (S); 'CNPH Tx-577' (S); one inbred line derived from the hybrid 'Alambra' (S) and one inbred line derived from the hybrid 'Netta' (S)

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