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Development of novel specific molecular markers for the *Sw-5b* gene to assist with tomato spotted wilt virus-resistant tomato breeding

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Abstract

Tomato spotted wilt virus (TSWV) is a plant pathogen that causes devastating tomato yield losses worldwide. The *Sw-5b* gene is one of the most effective resistance genes for TSWV control in tomato plants, and has been widely used in resistance breeding. Molecular markers are specific DNA sequences with known locations on the chromosome; they are indispensable tools in marker-assisted selection, which detects the presence of target genes to expedite breeding. We developed gene-specific molecular markers for *Sw-5b* to facilitate the accurate distinction of resistance (*Sw-5b*^{R}) and susceptibility (*Sw-5b*^{S}) alleles of *Sw-5b*. Using these markers, we successfully detected *Sw-5b* and determined its genotype (homozygous *Sw-5b*^{R}, heterozygous *Sw-5b*$^{R/S}$, or homozygous *Sw-5b*^{S}) in six tomato varieties. Then we successfully applied these markers to 46 commercial tomato cultivars to detect and determine the genotype of *Sw-5b*. The results revealed a striking absence of the *Sw-5b*^{R} gene and high TSWV susceptibility among most of the analyzed commercial cultivars. With the assistance of the novel *Sw-5b*-specific molecular markers, we generated a TSWV-resistant and homozygous *Sw-5b*^{R} Micro-Tom tomato line, demonstrating the practical application of these markers in plant breeding. In summary, we developed novel gene-specific molecular markers for *Sw-5b*, and applied them to distinguish *Sw-5b* alleles for TSWV resistance or susceptibility. This marker set provides a valuable tool for breeding TSWV-resistant tomato varieties.

Keywords *Sw-5b*, TSWV, Gene-specific molecular marker, Marker-assisted selection, Breeding, Tomato

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Background

Tomato spotted wilt virus (TSWV) belongs to the genus *Orthotospovirus*. It exhibits a remarkably broad host range, with more than 1000 plant species spanning more than 100 families. TSWV causes devastating yield losses in many crops and ornamental plants worldwide, with an estimated annual global economic impact of approximately 1 billion USD (Parrella et al. 2003; Oliver and Whitfield 2016; Turina et al. 2016; Zhu et al. 2019). Consequently, TSWV is considered as the second most important plant virus in the top ten well-studied plant viruses (Scholthof et al. 2011). In nature, thrips, especially the Western flower thrip (*Frankliniella occidentalis*), transmit TSWV persistently in a circulative-propagative way (Whitfield et al. 2005; Gilbertson et al. 2015; Rotenberg et al. 2015). The TSWV particles harbor three single-stranded genomic RNAs designated L, M, and S RNA. RNA L encodes RNA-dependent RNA polymerase (RdRp) through negative coding strategy (Dehaan et al. 1991; Adkins et al. 1995; van Knippenberg et al. 2002), while the M RNA encodes the movement protein NSm and glycoproteins Gn and Gc (Kormelink et al. 1994; Nagata et al. 2000). The S RNA encodes nonstructural protein NSs and nucleocapsid protein N (Kormelink et al. 1991; Bucher et al. 2003; Snippe et al. 2007; Schnettler et al. 2010).

In response to the widespread devastation caused by TSWV globally, breeders have made great efforts to identify tomato varieties with natural resistance to TSWV. This endeavor has led to the discovery of multiple resistance sources in various tomato lines and cultivars. Among these sources, eight loci designated *Sw-1a*, *Sw-1b*, *Sw-2* to *Sw-7* have been reported to provide TSWV resistance (Turina et al. 2016; Qi et al. 2021). However, the resistance conferred by *Sw-1a*, *Sw-1b*, *Sw-2*, *Sw-3*, and *Sw-4* has been overcome by TSWV and other orthotospoviruses (Stevens et al. 1991; Saidi and Warade 2008). In contrast, *Sw-6* and *Sw-7* confer resistance to multiple TSWV isolates, but they have yet to be cloned (Rosello et al. 1998; Canady et al. 2001; Rosello et al. 2001; Qi et al. 2021). *Sw-5,* which was originally found in *Solanum peruvianum*, is a gene cluster located on the telomeric region of the long arm of chromosome 9, it encompasses six homologous paralogs, which were designated *Sw-5a* to *Sw-5f* (Stevens et al. 1991; Stevens et al. 1995b; de Oliveira et al. 2018). However, only *Sw-5b* is responsible for broad-spectrum resistance against TSWV and other orthotospoviruses (Rosello et al. 1998; Spassova et al. 2001; Peiro et al. 2014). *Sw-5b* is a classical resistance gene that encodes a nucleotide-binding leucine-rich repeat (NLR) immune receptor. This receptor comprises an extended N-terminal Solanaceae domain (SD), a coiled-coil (CC) domain, a central nucleotide-binding adaptor shared by ApaF-1, resistance proteins, a CED-4 (NB-ARC) domain, and a C-terminal leucine-rich repeat (LRR) domain (Brommonschenkel et al. 2000; Chen et al. 2016; De Oliveira et al. 2016). Sw-5b triggers plant immunity through the recognition of the viral movement protein NSm and provides broad-spectrum resistance to various orthotospoviruses by recognizing a conserved 21-amino acid peptide within NSm (Lopez et al. 2011; Hallwass et al. 2014; Zhao et al. 2016; Leastro et al. 2017; Zhu et al. 2017). Despite the discovery of resistance-breaking (RB) TSWV isolates, *Sw-5b* has been widely used as a resistance source against orthotospoviruses due to its durability and consistent broad-spectrum resistance (Turina et al. 2016).

The advent of molecular markers has substantially improved the efficiency and precision of trait selection, thereby expediting plant breeding efforts. These markers are closely linked to genes that determine specific traits, marker-assisted selection (MAS) can be applied to determine the presence and absence of a target gene by detecting linked molecular markers. MAS has been employed to assist the selection and identification of TSWV-resistant materials and breeding for commercial tomato cultivars. The development of molecular linkage markers for TSWV resistance genes has primarily focused on *Sw-5* and *Sw-7* (Qi et al. 2021). Several markers, including the restriction fragment length polymorphism (RFLP) markers CT71 and CT220, have been linked to *Sw-5* (Stevens et al. 1995a, 1995b; Garland et al. 2005). Concurrently, gene-specific markers directly targeting the *Sw-5b* gene have been established. In 2005, a dominant marker representing the *Sw-5b* gene sequence, combined with an improved cleaved amplified polymorphic sequence marker, were used to form a new marker system for *Sw-5* (Garland et al. 2005). Subsequently, *Sw-5b*-specific single nucleotide polymorphism (SNP) and sequence-characterized amplified regions (SCAR) markers were developed (Shi et al. 2011; Panthee and Ibrahem 2013; Lee et al. 2015). However, only a few of these markers have been found to distinguish homozygous resistant, heterozygous resistant, or homozygous susceptible varieties (Garland et al. 2005).

In this study, we developed novel gene-specific SCAR markers for the tomato *Sw-5b* gene, which confers broad-spectrum resistance to multiple orthotospoviruses including TSWV. These markers were validated through testing on an array of tomato varieties and cultivars. Our findings may contribute to the development of TSWV-resistant tomato cultivars.

Results

Development of gene-specific molecular markers for *Sw-5b*

To develop gene-specific molecular markers that can distinguish *Sw-5b* alleles for TSWV resistance or susceptibility, we analyzed nucleotide sequences of *Sw-5b* from the resistant tomato cultivar *Solanum peruvianum* cv. G17-60 (*Sw-5b*^{R}), and its susceptibility allele in the cultivar *S. lycopersicum* cv. Heinz1706 (*Sw-5b*^{S}). The sequence alignment result showed that the LRR region exhibited the highest level of polymorphism (Fig. 1a). We designed two primer pairs, Sw-5b-F1/R1 and Sw-5b-F2/R1, to specifically amplify the *Sw-5b*^{R} and *Sw-5b*^{S} fragments, respectively, based on nucleotide sequence alignment of the LRR regions of *Sw-5b*^{R} and *Sw-5b*^{S} (Fig. 1b and Table 1). Both primer pairs share a common reverse primer, Sw-5b-R1, which targets both *Sw-5b*^{R} and *Sw-5b*^{S}. However, the forward primers Sw-5b-F1 and Sw-5b-F2 were designed to match *Sw-5b*^{R} and *Sw-5b*^{S}, respectively (Fig. 1b, c). The primer pair Sw-5b-F1/R1 was designed to amplify *Sw-5b*^{R} exclusively, producing a 660-bp fragment, and Sw-5b-F2/R1 was designed to amplify *Sw-5b*^{S} exclusively, resulting in a 459-bp fragment (Fig. 1c). To ascertain the specificity of these primers, genomic DNA was isolated from the homozygous *Sw-5b*^{R} tomato cultivar G17-60, heterozygous *Sw-5b*$^{R/S}$ hybrid tomato line IVF3545, and homozygous *Sw-5b*^{S} tomato cultivar Heinz1706. These DNA samples were used as templates and amplified using the primer pairs Sw-5b-F1/R1 and Sw-5b-F2/R1, respectively. The results showed that Sw-5b-F1/R1 produced a 660-bp product from DNA obtained from homozygous *Sw-5b*^{R} and heterozygous *Sw-5b*$^{R/S}$ tomato plants, whereas Sw-5b-F2/R1 generated a 459-bp product from the DNA of homozygous *Sw-5b*^{S} and heterozygous *Sw-5b*$^{R/S}$ tomato plants (Fig. 1d). To confirm the above results, we employed the previously reported Sw-5 SNP marker Sw5-f2/r2 as the control marker. We conducted PCR amplifications by using genomic DNA extracted from tomato cultivars G17-60, IVF3545, and Heinz1706 as templates. The marker successfully amplified *Sw-5b*^{R} from G17-60 and IVF3545 (Additional file 1: Figure S1), which validated the results from the two primer pairs. Together, these results indicated that the primer pairs Sw-5b-F1/R1 and Sw-5b-F2/R1 are effective *Sw-5b*-specific molecular markers for distinguishing the resistance allele *Sw-5b*^{R} from the susceptibility allele *Sw-5b*^{S}. In addition, combining these two markers, we can identify the genotype of *Sw-5b* in tomato plants.

Detection of the *Sw-5b* gene and genotype determination of tomato varieties using *Sw-5b*-specific molecular markers

Next, we used the *Sw-5b*-specific molecular markers to detect the *Sw-5b* gene in six distinct tomato varieties (Table 2). The cultivars G17-60, IVF3545, and Heinz1706 were used as controls representing the *Sw-5b*^{R} homozygote, *Sw-5b*$^{R/S}$ heterozygote, and *Sw-5b*^{S} homozygote, respectively. Polymerase chain reaction (PCR) analysis detected a 660-bp product from DNA extracted from LA3667, Paronset, and G17-60; a 459-bp product form DNA extracted from D17-6, D17-18, 365, Heinz1706, and Micro-Tom; and 660-bp and 459-bp products from DNA extracted from IVF3545 (Fig. 2a). These results indicated that, similar to G17-60, the varieties LA3667 and Paronset were homozygous for *Sw-5b*^{R}. By contrast, D17-6, D17-18, 365, and Micro-Tom were homozygous for *Sw-5b*^{S}, mirroring Heinz1706. To determine whether these plants were able to confer resistance against TSWV, each tomato variety was mechanically inoculated with TSWV-infected crude leaf extracts. A dot-enzyme-linked immunosorbent assay (Dot-ELISA) and reverse transcription (RT)-PCR assay showed that LA3667, Paronset, IVF3545, and G17-60 were not infected by TSWV, whereas the remaining tomato plants were infected (Fig. 2b, c and Table 2). These results suggest that the *Sw-5b*^{R} homozygote varieties LA3667, Paronset, and G17-60 and the *Sw-5b*$^{R/S}$ heterozygote variety IVF3545 exhibit resistance to TSWV, whereas the *Sw-5b*^{S} homozygote varieties D17-6, D17-18, 365, Heinz1706, and Micro-Tom are susceptible to TSWV. Thus, the *Sw-5b*-specific molecular markers were applied successfully to determine whether tomato varieties carry *Sw-5b*^{R}, *Sw-5b*^{S}, or both.

Application of *Sw-5b*-specific molecular makers to detect *Sw-5b* in commercial tomato cultivars

The use of resistance genes in crop breeding is one of the most efficient approaches to mitigating viral diseases. To determine whether commercial hybrid tomato cultivars contain the *Sw-5b* gene and/or confer TSWV resistance, we collected samples from 46 cultivars (Additional file 2: Table S1) and applied the *Sw-5b*-specific molecular markers for gene detection. Only 1 of the 46 cultivars (ANX) harbored the *Sw-5b*^{R} gene, which is the *Sw-5b*$^{R/S}$ heterozygotes. The vast majority of the commercial cultivars were identified as *Sw-5b*^{S} homozygotes, and the remaining three cultivars (GQ6, JP1, and ZZ202) did not carry the *Sw-5b* gene (Fig. 3). To exclude the possibility of DNA sample quality issues from the three cultivars, we evaluated the DNA quality by amplifying the tomato internal gene *Actin* using the DNA from these cultivars as templates. The DNA extracted from cultivars G17-60, IVF3545, and Heinz1706, representing the *Sw-5b*^{R} homozygote, *Sw-5b*$^{R/S}$ heterozygote, and *Sw-5b*^{S} homozygote, respectively, were used as controls. The PCR results confirmed the successful amplification of the *Actin* gene from all DNA samples (Additional file 1: Figure S2a), affirming the quality of DNA extracted from

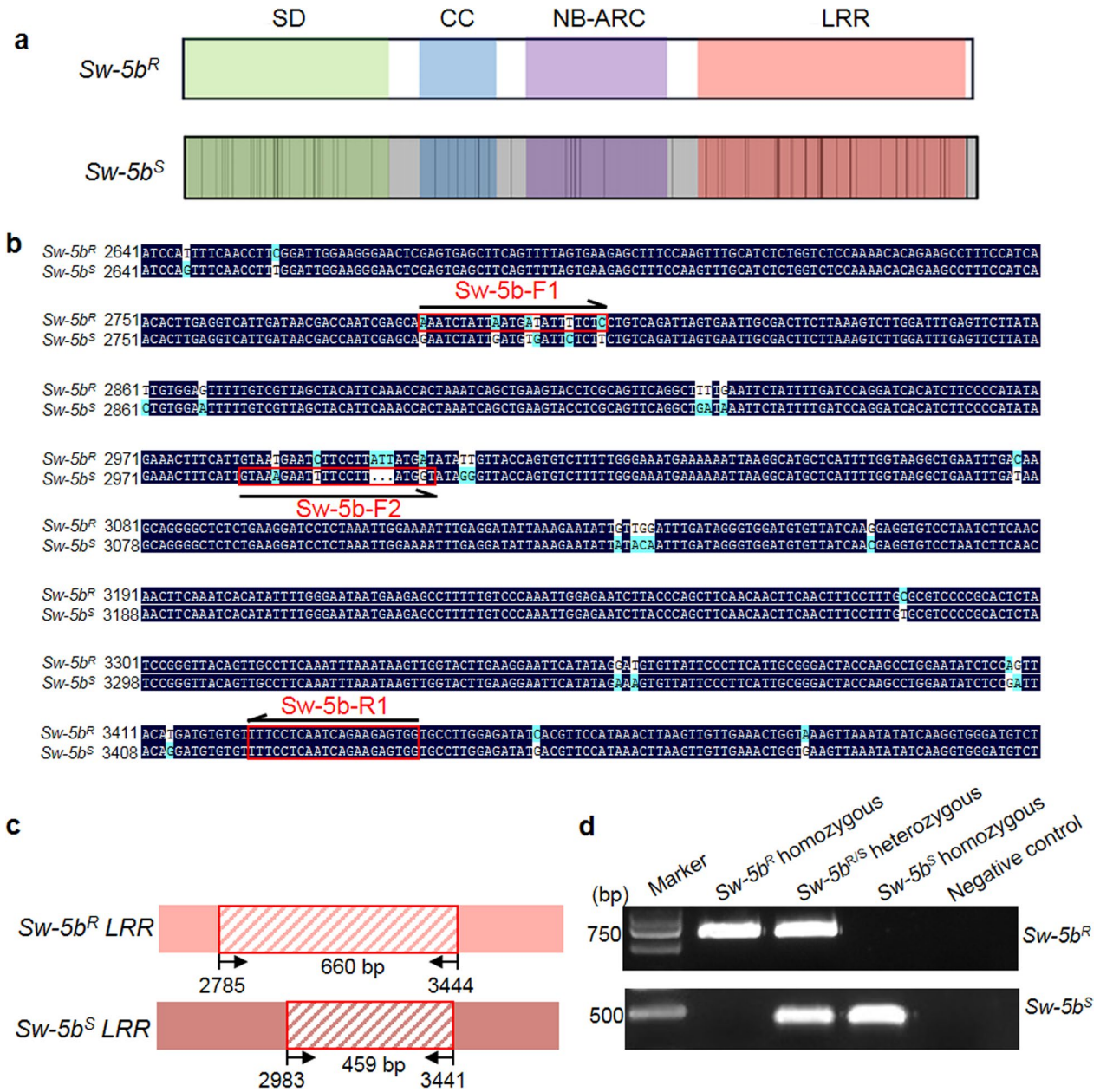

Fig. 1 Design of *Sw-5b* specific molecular markers. **a** Nucleotide sequence alignment between the resistance allele *Sw-5b*^{R} and the susceptibility allele *Sw-5b*^{S}. The alignment was used to generate a heatmap using the *ggplot2*, R package, with black marks indicating positions diverging from the reference *Sw-5b*^{R} sequence. Different colors distinguish the SD, CC, NB-ARC, and LRR regions of *Sw-5b*^{R} and *Sw-5b*^{S}. **b** Nucleotide sequence alignment between the LRR regions of *Sw-5b*^{R} and *Sw-5b*^{S}, highlighting the location of the *Sw-5b*-specific primers Sw-5b-F1, F2, and R1. **c** Positions of the *Sw-5b*-specific primer pairs, Sw-5b-F1/R1 and Sw-5b-F2/R1, within the *Sw-5b* gene. The expected PCR products sizes are indicated. Sw-5b-F1/R1 was designed to amplify *Sw-5b*^{R} exclusively, producing a 660-bp fragment, and Sw-5b-F2/R1 to amplify *Sw-5b*^{S} exclusively, resulting in a 459-bp fragment. **d** Validation of the specificity of the *Sw-5b*-specific molecular markers. Genomic DNA extracted from the tomato cultivar G17-60, IVF3545, and Heinz1706, representing the *Sw-5b*^{R} homozygote, *Sw-5b*$^{R/S}$ heterozygote, and *Sw-5b*^{S} homozygote, respectively, was used as template for PCR amplification. Genomic DNA from *Nicotiana benthamiana* was used as the negative control

Table 1 *Sw-5b*-specific primers

Primer name	Primer sequence (5′–3′)	Expected product size (bp)	
		Resistant	Susceptible
Sw-5b-F1	AAATCTATTAATGATATTTTCTC	660	–
Sw-5b-R1	CCACTCTTCTGATTGAGGAAA		
Sw-5b-F2	GTAAAGAATTTTCCTTATGGT	–	459
Sw-5b-R1	CCACTCTTCTGATTGAGGAAA		

Table 2 Evaluation of TSWV resistance in six tomato varieties through the *Sw-5b*-specific molecular markers and virus inoculation experiments

Lines	*Sw-5b*^{R}	*Sw-5b*^{S}	Genotype	TSWV
LA3667	+	–	*Sw-5b*^{R} homozygous	Resistant
Paronset	+	–	*Sw-5b*^{R} homozygous	Resistant
IVF3545	+	+	*Sw-5b*$^{R/S}$ heterozygous	Resistant
G17-60	+	–	*Sw-5b*^{R} homozygous	Resistant
D17-6	–	+	*Sw-5b*^{S} homozygous	Susceptible
D17-18	–	+	*Sw-5b*^{S} homozygous	Susceptible
365	–	+	*Sw-5b*^{S} homozygous	Susceptible
Heinz1706	–	+	*Sw-5b*^{S} homozygous	Susceptible
Micro-Tom	–	+	*Sw-5b*^{S} homozygous	Susceptible

these cultivars. Next, we inoculated these cultivars with TSWV and tested the plants for virus infection at 14 days post inoculation (dpi). The Dot-ELISA results showed that, only 1 of the 46 cultivars (ANX) was not infected by TSWV (Additional file 1: Figure S2b, c). These findings were summarized in Table 3, which reveals a noticeable lack of the *Sw-5b*^{R} gene and high susceptibility to TSWV among the 46 tested cultivars.

Breeding of TSWV-resistant tomato lines with the assistance of *Sw-5b*-specific molecular markers

In marker-assisted selection, molecular markers are applied directly to screen for plants carrying a target resistance gene in every generation during the breeding process, as well as to determine its genotype (Qi et al. 2021). The tomato variety Micro-Tom is characterized by its smaller size, shorter lifecycle, and amenability to efficient transformation. These traits have led to the widespread use of Micro-Tom in tomato functional genomic research (Okabe et al. 2013; Shikata and Ezura 2016). Using the *Sw-5b*-specific molecular markers, we identified Micro-Tom as an *Sw-5b*^{S} homozygote (Fig. 2a) and cultivated an *Sw-5b*^{R} homozygous Micro-Tom line (Fig. 4). Using G17-60 as the paternal line and Micro-Tom as the maternal line, we developed the F_1 generation. In examining

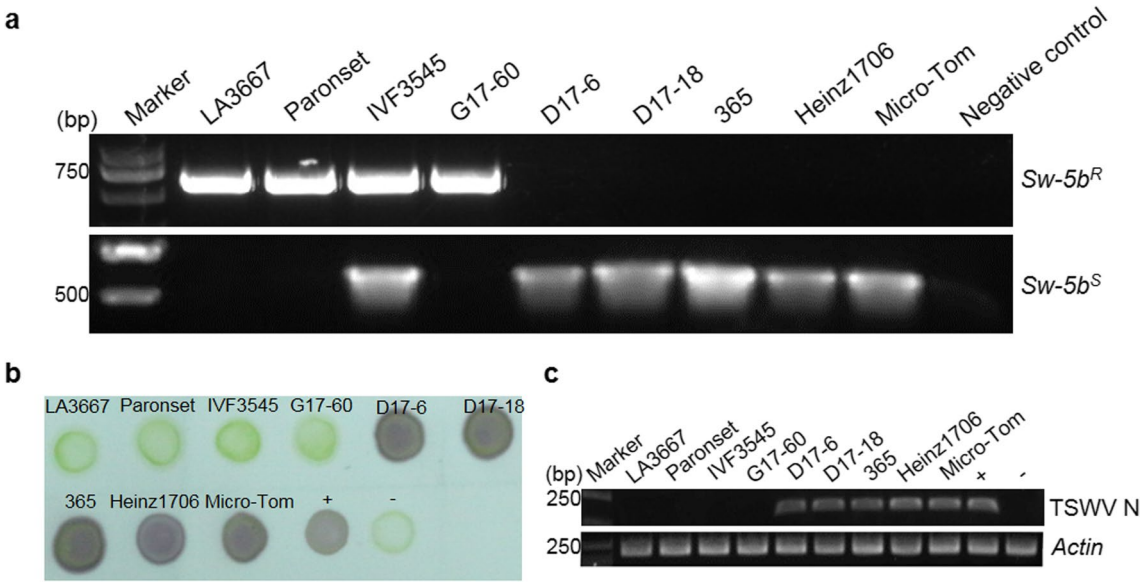

Fig. 2 Detection of the *Sw-5b* gene and genotype determination of tomato varieties using *Sw-5b*-specific molecular markers. **a** Detection and genotyping of *Sw-5b* gene in six tomato varieties using PCR with *Sw-5b*-specific molecular markers. Genomic DNA from *Nicotiana benthamiana* was used as the negative control. **b** Dot-ELISA to detect TSWV infection in tomato plants. Tomato plants were inoculated with TSWV and uninoculated upper leaves were collected and subjected to TSWV infection detection at 14 dpi. **c** RT-PCR to confirm TSWV infection in tomato plants. Uninoculated upper leaves of TSWV-infected tomato plants were subjected to RT-PCR analysis at 14 dpi. TSWV-infected tomato leaves were used as positive control (+), while leaves from uninfected healthy tomato plants were used as negative control (–)

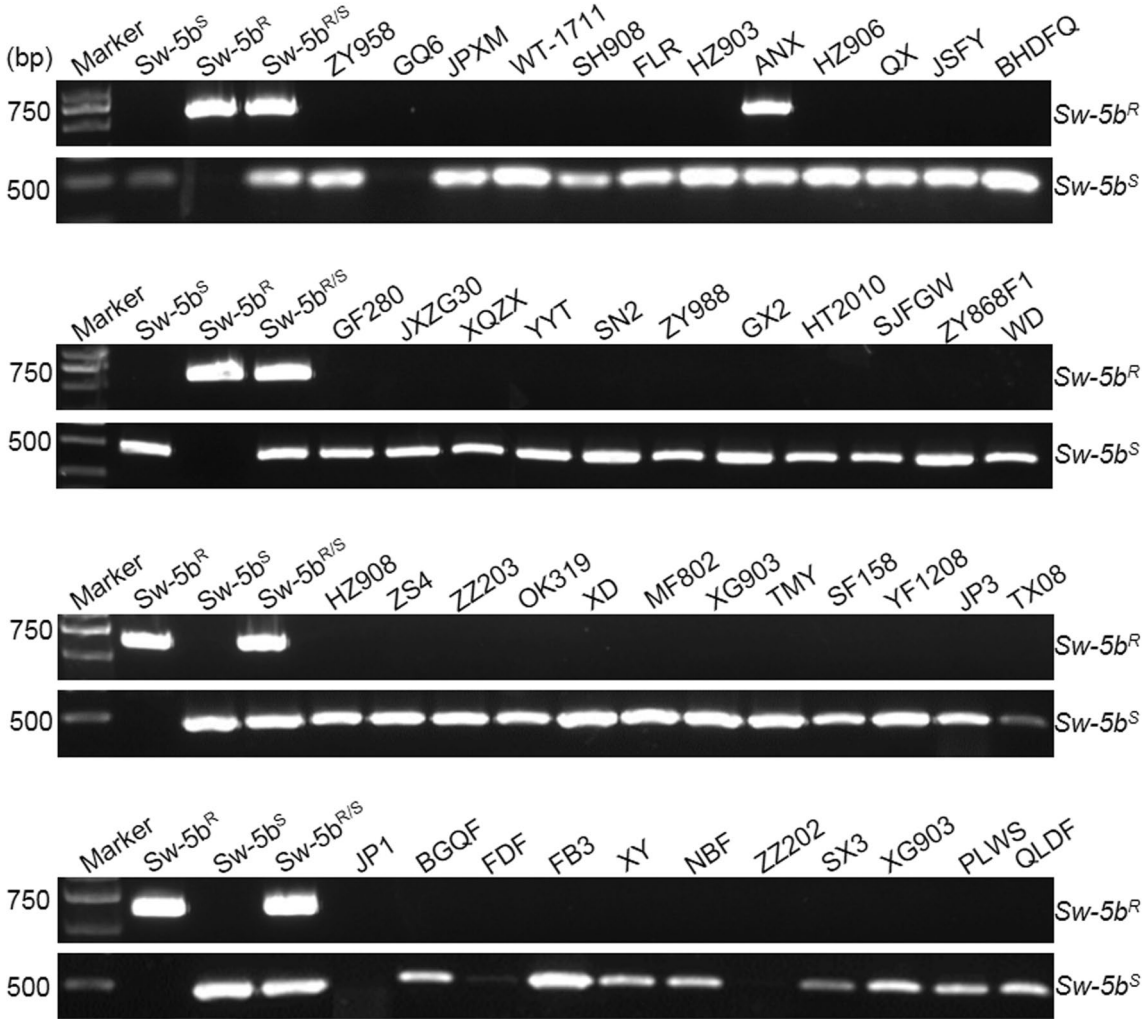

Fig. 3 Detection and genotyping of the *Sw-5b* gene in commercial tomato cultivars. Detection of the *Sw-5b* gene in 46 commercial tomato cultivars through PCR amplification using *Sw-5b*-specific molecular markers. Genomic DNA obtained from tomato cultivars G17-60, IVF3545, and Heinz1706 was used as template for PCR amplification, representing the *Sw-5b*^{R} homozygote, *Sw-5b*$^{R/S}$ heterozygote, and *Sw-5b*^{S} homozygote, respectively

the resultant F_1 generation, we discovered that all 12 grown plants exhibited faster growth than G17-60. Genomic DNA extractions followed by *Sw-5b*-specific molecular markers assays showed that all these plants carried *Sw-5b*^{R}. Next, we repeatedly backcrossed the F_1 generation with Micro-Tom as the recurrent parent. We used the *Sw-5b*-specific molecular markers to determine the genotypes of the resulting plants of each round of backcrossing. Tomato plants carrying *Sw-5b*^{R} and that were similar in size to Micro-Tom were selected for subsequent backcrossing. Following five rounds of backcrossing, the resultant BC_5 plants were uniformly small, with rapid growth similar to Micro-Tom. Detection using the *Sw-5b*-specific molecular markers showed that 33% of these plants carried the *Sw-5b*^{R} gene, and these plants were used for subsequent self-crossing. The obtained BC_5F_1 plants were tested with *Sw-5b*-specific molecular markers; the results showed that only BC_5F_1-3 was an *Sw-5b*^{R} homozygote. Next, the BC_5F_1-3 plant was used for self-crossing, and generated BC_5F_2. All BC_5F_2 plants showed the small, rapid-growth phenotype; and PCR using *Sw-5b*-specific molecular markers confirmed that their genotype was *Sw-5b*^{R} homozygous. Next, we inoculated these plants with TSWV and found that they were all resistant to viral infection (Additional file 1: Figure S3). The proportions of plants carrying *Sw-5b*^{R} and size phenotypes produced throughout the

Table 3 Evaluation of TSWV resistance in 46 commercial tomato cultivars through the *Sw-5b*-specific molecular markers and virus inoculation experiments

Number	Cultivars	*Sw-5b*^{R}	*Sw-5b*^{S}	Genotype	TSWV
1	BHDFQ	–	+	*Sw-5b*^{S} homozygous	Susceptible
2	JSFY	–	+	*Sw-5b*^{S} homozygous	Susceptible
3	QX	–	+	*Sw-5b*^{S} homozygous	Susceptible
4	HZ906	–	+	*Sw-5b*^{S} homozygous	Susceptible
5	ANX	+	+	*Sw-5b*$^{R/S}$ heterozygous	Resistant
6	HZ903	–	+	*Sw-5b*^{S} homozygous	Susceptible
7	FLR	–	+	*Sw-5b*^{S} homozygous	Susceptible
8	SH908	–	+	*Sw-5b*^{S} homozygous	Susceptible
9	WT-1711	–	+	*Sw-5b*^{S} homozygous	Susceptible
10	JPXM	–	+	*Sw-5b*^{S} homozygous	Susceptible
11	GQ6	–	+	*Sw-5b*^{S} homozygous	Susceptible
12	ZY958	–	+	*Sw-5b*^{S} homozygous	Susceptible
13	WD	–	+	*Sw-5b*^{S} homozygous	Susceptible
14	ZY868F1	–	+	*Sw-5b*^{S} homozygous	Susceptible
15	SJFGW	–	+	*Sw-5b*^{S} homozygous	Susceptible
16	HT2010	–	+	*Sw-5b*^{S} homozygous	Susceptible
17	GX2	–	+	*Sw-5b*^{S} homozygous	Susceptible
18	ZY988	–	+	*Sw-5b*^{S} homozygous	Susceptible
19	SN2	–	+	*Sw-5b*^{S} homozygous	Susceptible
20	YYT	–	+	*Sw-5b*^{S} homozygous	Susceptible
21	XQZX	–	+	*Sw-5b*^{S} homozygous	Susceptible
22	JXZG30	–	+	*Sw-5b*^{S} homozygous	Susceptible
23	GF280	–	+	*Sw-5b*^{S} homozygous	Susceptible
24	HZ908	–	+	*Sw-5b*^{S} homozygous	Susceptible
25	ZS4	–	+	*Sw-5b*^{S} homozygous	Susceptible
26	ZZ203	–	+	*Sw-5b*^{S} homozygous	Susceptible
27	OK319	–	+	*Sw-5b*^{S} homozygous	Susceptible
28	XD	–	+	*Sw-5b*^{S} homozygous	Susceptible
29	MF802	–	+	*Sw-5b*^{S} homozygous	Susceptible
30	XG903	–	+	*Sw-5b*^{S} homozygous	Susceptible
31	TMY	–	+	*Sw-5b*^{S} homozygous	Susceptible
32	SF158	–	+	*Sw-5b*^{S} homozygous	Susceptible
33	YF1208	–	+	*Sw-5b*^{S} homozygous	Susceptible
34	JP3	–	+	*Sw-5b*^{S} homozygous	Susceptible
35	TX08	–	+	*Sw-5b*^{S} homozygous	Susceptible
36	JP1	–	+	*Sw-5b*^{S} homozygous	Susceptible
37	BGQF	–	+	*Sw-5b*^{S} homozygous	Susceptible
38	FDF	–	+	*Sw-5b*^{S} homozygous	Susceptible
39	FB3	–	+	*Sw-5b*^{S} homozygous	Susceptible
40	XY	–	+	*Sw-5b*^{S} homozygous	Susceptible
41	NBF	–	+	*Sw-5b*^{S} homozygous	Susceptible
42	ZZ202	–	+	*Sw-5b*^{S} homozygous	Susceptible
43	SX3	–	+	*Sw-5b*^{S} homozygous	Susceptible
44	XG903	–	+	*Sw-5b*^{S} homozygous	Susceptible
45	PLWS	–	+	*Sw-5b*^{S} homozygous	Susceptible
46	QLDF	–	+	*Sw-5b*^{S} homozygous	Susceptible

breeding process are listed in Table 4. These results demonstrate the successful generation of an *Sw-5b*^{R} homozygous Micro-Tom line with the assistance of the *Sw-5b*-specific molecular markers.

Discussion

The *Sw-5b* gene is the most widely applied and effective resistance gene against TSWV in tomato (Turina et al. 2016). Therefore, breeders are striving to cultivate tomato variants that both carry the *Sw-5b* gene and harbor excellent horticultural traits. MAS has played a significant role in hastening the breeding process, and the development of precise and highly specific molecular markers, particularly the gene-specific markers, has been critical to

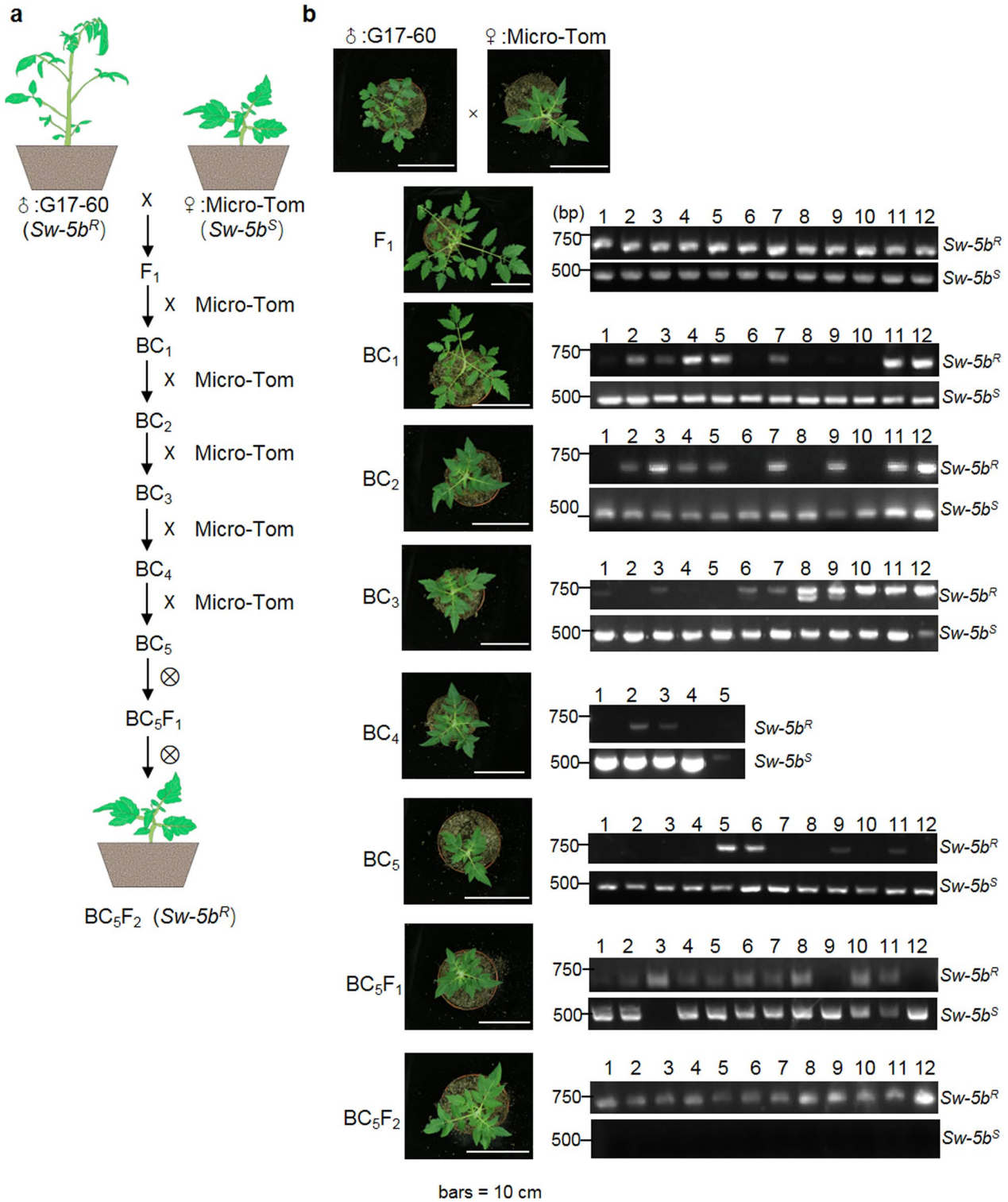

Fig. 4 Breeding TSWV-resistant tomato lines with the assistance of *Sw-5b*-specific molecular markers. **a** Flow chart illustrating the cultivation of *Sw-5b*^{R} homozygote Micro-Tom lines. **b** Phenotypes and genotypes of the plants obtained in each round of the breeding process of *Sw-5b*^{R} homozygous Micro-Tom lines. In each round, plant phenotypes were observed, and the genotypes were determined using the *Sw-5b*-specific molecular markers

Table 4 The proportions of plants carrying *Sw-5b*^{R} and size phenotypes produced in the breeding process of *Sw-5b*^{R} homozygous Micro-Tom lines

Generation	Carrying *Sw-5b*^{R} (%)	Tall plants (%)	Short plants (%)
F_1	100	100	0
BC_1	58.3	100	0
BC_2	66.7	75	25
BC_3	75	58.3	41.7
BC_4	40	0	100
BC_5	33	0	100
BC_5F_1	75	0	100
BC_5F_2	100	0	100

breeding success. Among the reported molecular markers for the *Sw-5b* gene, the SNP markers Sw-5b-SNP and Sw5-f2/r2 and the SCAR marker NCSW-012 have demonstrated the ability to effectively distinguish *Sw-5b*^{R} and *Sw-5b*^{S} in numerous tomato populations (Shi et al. 2011; Panthee and Ibrahem 2013; Lee et al. 2015). However, these markers can only detect the presence of *Sw-5b*^{R}, without providing information about its genotype, i.e., whether it is an *Sw-5b*^{R} homozygote or *Sw-5b*$^{R/S}$ heterozygote. Some researchers have combined the *Sw-5b*-specific molecular marker Sw-5b-LRR with the *Sw-5* locus molecular marker CT220 to create a marker system that can discern *Sw-5b*^{R} homozygotes from *Sw-5b*$^{R/S}$ heterozygotes. However, this marker system employs an RFLP marker CT220, which requires an enzyme digestion reaction following PCR (Garland et al. 2005). Previous studies revealed that the LRR domain of Sw-5b is responsible for recognizing the viral effector NSm. A comparison of the amino acid sequences of Sw-5b^R and Sw-5b^S showed that the LRR domain exhibited the most polymorphic sites (Zhu et al. 2017; Li et al. 2019). Among these polymorphic sites, four polymorphic ones, polymorphic sites 3, 4, 5, and 6 within the Sw-5b LRR domain, are critical for NSm binding and recognition, which subsequently determines the resistance or susceptibility of tomatoes to TSWV (Zhu et al. 2017). Furthermore, polymorphic sites 2 and 5 displayed the most significant polymorphisms at the nucleic acid level. Therefore, we designed the Sw-5b-F1, which corresponds to the region of polymorphic site 2 and specifically matches *Sw-5b*^{R}, as well as Sw-5b-F2, which corresponds to the region of polymorphic site 5 and exclusively matches *Sw-5b*^{S}. Both forward primers were utilized in combining with a shared reverse primer, Sw-5b-R1, targeting both *Sw-5b*^{R} and *Sw-5b*^{S}. These primer pairs were designed to amplify specific bands of different sizes. Testing across a range of tomato varieties and cultivars revealed that these primers could accurately differentiate *Sw-5b*^{R} from *Sw-5b*^{S}. Next, we combined these primers to develop a specific marker system for the *Sw-5b* gene that would accurately distinguish *Sw-5b*^{R} from *Sw-5b*^{S} and precisely identify the genotype of tomato plants carrying *Sw-5b*. This *Sw-5b*-specific marker system improves the detection and selection of tomato germplasm resources carrying the *Sw-5b* gene, thereby facilitating the process of breeding for TSWV-resistant tomatoes.

China is a preeminent producer and exporter of tomato products, with a crop area of more than 1.1 million ha and the highest total tomato yield worldwide, producing more than 66.2 million tons in 2022. However, viral diseases pose a significant threat to tomato yield and quality (Qi et al. 2021; Tettey et al. 2023), among which TSWV is particularly destructive. The most effective control strategy is the use of disease-resistant genes in cultivar breeding (Turina et al. 2016; Liu and Tang 2023). To date, the *Sw-5b* is the most effective resistance gene against TSWV in tomato. In this study, we examined a broad range of commercial tomato cultivars from China for their resistance to TSWV, using our novel *Sw-5b*-specific molecular markers, followed by TSWV inoculation. Among the tested 46 cultivars, only one carried the *Sw-5b*^{R} gene, which was *Sw-5b*$^{R/S}$ heterozygotes. After TSWV inoculation, this cultivar was found to be resistant to the virus. This suggests that very few commercial tomato cultivars carry the *Sw-5b*^{R} gene and possess TSWV resistance. Therefore, more effort is needed to breed and popularize TSWV-resistant tomato plants. Notably, the resistance spectrum of *Sw-5b* was recently been expanded via stepwise artificial evolution, thereby enabling it to defend against RB TSWV isolates. Ongoing advancements in genome editing technology are anticipated to lead to the emergence of innovative methods surpassing traditional disease-resistant breeding to develop new TSWV-resistant materials.

Micro-Tom is a model tomato variety used in functional genomics studies for its small size, short lifecycle, and adaptability to greenhouses (Shikata and Ezura 2016). Our previous studies have revealed the mechanisms underlying the broad-spectrum resistance conferred by Sw-5b against orthotospoviruses, and illustrated how Sw-5b recognizes NSm to trigger plant immunity (Chen et al. 2016; Zhu et al. 2017; Li et al. 2019). To gain deeper insights into the signaling pathways activated by Sw-5b post-activation, we cultivated an *Sw-5b*^{R} homozygous Micro-Tom line. Throughout this breeding process, we applied our novel *Sw-5b*-specific markers to test the plants obtained in each generation. This procedure allowed the rapid identification of plants carrying *Sw-5b*^{R} during the seedling stage, which could then be used in the next round of backcrossing or crossbreeding. The

selection of resistant plants using *Sw-5b*-specific markers requires only basic PCR to detect and genotype the *Sw-5b* gene, bypassing the need for TSWV inoculation in resistance determination. This approach reduces the costs associated with breeding and accelerates the breeding process. The *Sw-5b*^{R} homozygous Micro-Tom line generated in this study provides an invaluable resource for scientific research and can be used as a TSWV-resistant tomato germplasm resource.

Conclusions

We developed gene-specific molecular markers for the tomato resistance gene *Sw-5b* that were able to distinguish its resistance (*Sw-5b*^{R}) and susceptibility (*Sw-5b*^{S}) alleles. We successfully detected the gene and determined its genotype in six tomato varieties. Our screening of 46 commercial tomato cultivars using these markers revealed a striking absence of the *Sw-5b*^{R} gene and high TSWV susceptibility among the majority of the analyzed cultivars. With the assistance of the *Sw-5b*-specific molecular markers, we generated a TSWV-resistant, *Sw-5b*^{R} homozygous Micro-Tom tomato line. The *Sw-5b*-specific markers will be valuable tools for breeding TSWV-resistant tomato varieties and mitigating viral diseases.

Methods

Plant materials and growth conditions

Tomato seeds were subjected to surface sterilization and then germinated in Petri dishes in an incubator under controlled conditions (25 °C, 60% humidity, and a 16 h light/8 h dark photoperiod). Once the seedlings produced two true leaves, they were transplanted into soil and transferred to a greenhouse with a 16 h light/8 h dark photoperiod.

Sequence alignment

The nucleotide sequences of the resistance (*Sw-5b*^{R}) and susceptibility (*Sw-5b*^{S}) alleles of the *Sw-5b* gene were aligned to generate a difference matrix consisting of two rows, the first representing *Sw-5b*^{R} and the second representing *Sw-5b*^{S}. A heatmap was generated using the *ggplot2* package in the software R (R Core Team, Vienna, Austria).

Extraction of tomato genomic DNA

For genomic DNA extraction, 0.1 g 5-week-old tomato leaves was ground to a fine powder using liquid nitrogen. The powder was mixed with 300 µL extraction buffer (1.5 M Tris-HCI pH 8.0, 5 M NaCl, 0.5 M EDTA, 2% CTAB) and incubated at 65 °C for 10 min. After cooling, 300 µL chloroform was added, and the mixture was vigorously shaken and centrifuged at 13,200×*g* for 5 min. Then the supernatant was mixed with 300 µL isopropanol, followed by centrifugation at 13,200×*g* for 5 min. The supernatant was discarded, and the precipitate was washed with 500 µL 70% ethanol. After centrifugation at 13,200×*g* for 3 min, the precipitate was dried at room temperature and suspended in 100 µL water.

PCR-based detection and genotyping of *Sw-5b*

To detect *Sw-5b* and determine its genotype in different tomato varieties, two pairs of primers were designed separately. The primer pair Sw-5b-F1/R1 was designed to amplify *Sw-5b*^{R} exclusively, producing a 660-bp fragment, and Sw-5b-F2/R1 was designed to amplify *Sw-5b*^{S} specifically, generating a 459-bp fragment (Table 1). The optimum annealing temperature for PCR amplification with the Sw-5b-F1/R1 primer pair is 52 °C, whereas for the Sw-5b-F2/R1 primer pair, it is 56 °C. Genomic DNA extracted from tomato samples was used as template and subjected to PCR amplification using the primer pairs Sw-5b-F1/R1 and Sw-5b-F2/R1, respectively, with Max Super-Fidelity DNA Polymerase (Vazyme Biotech, Nanjing, China). The resulting PCR products were visualized through agarose gel electrophoresis.

TSWV inoculation

Virus inoculation assays were performed as described previously (Zhu et al. 2017). TSWV was isolated from local diseased plants in Yunnan Province, China, and propagated on *Nicotiana benthamiana* plants. For TSWV inoculation, leaf tissues infected with TSWV were collected and ground in 1×phosphate buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.4). The resulting crude leaf extracts were gently rubbed onto the leaves of 6-week-old tomato plants. After inoculation, the plants were grown in a growth chamber with 16 h light /8 h dark photoperiod.

Dot-ELISA

Dot-ELISA was performed to detect TSWV in tomato plants. At 14 dpi, uninoculated systemic leaves were collected and placed in a centrifuge tube. Then, 100 µL of encapsulating solution (0.5 M carbonate buffer) was added to the tube, and the leaf tissues were ground with a grinding rod. The resulting mixture was centrifuged at 1467×*g* for 4 min. From the supernatant, 3 µL was pipetted onto nitrocellulose membranes. The resulting blots were probed using an anti-TSWV N (1:5000; produced in our laboratory) and detected using alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (1:10,000; no. A3687, Sigma-Aldrich, St. Louis, MO, USA), followed by staining with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (Sangon Biotech, Shanghai, China).

RT-PCR to detect TSWV infection

To validate the Dot-ELISA results, RT-PCR was conducted as described previously (Wu et al. 2023). Total RNA was isolated from tomato leaves using the RNA Simple Rapid Isolation Kit (Tiangen, Beijing, China). Then, first-strand cDNA was synthesized using the HiScript III 1st Strand cDNA Synthesis Kit (Vazyme Biotech, Nanjing, China). *Phanta* Max Super-Fidelity DNA Polymerase (Vazyme Biotech, Nanjing, China) was used to perform PCR reactions to detect the *N* gene in tomato plants. The tomato *Actin* gene was used as an internal reference gene.

Breeding *Sw-5b*^{R} homozygous Micro-Tom lines

To cultivate *Sw-5b*^{R} homozygous Micro-Tom lines, the *Sw-5b*^{R} homozygous tomato cultivar G17-60 was used as the paternal parent and the *Sw-5b*^{S} homozygous tomato cultivar Micro-Tom was used as the maternal parent. A cross was made between these two parents to generate the F_1 generation. The F_1 plants were identified as *Sw-5b*$^{R/S}$ heterozygous using the *Sw-5b*-specific molecular markers. To continue the breeding process, the F_1 generation was repeatedly backcrossed with Micro-Tom as the recurrent parent. The resulting progeny, designated BC_1, were backcrossed again with Micro-Tom and the genotype of the resulting plants were determined using the *Sw-5b*-specific molecular markers. Then BC_1 plants carrying *Sw-5b*^{R} were backcrossed with Micro-Tom to obtain BC_2. After five rounds of backcrossing, the obtained BC_5 plants were found to exhibit the same rapid-growth phenotype as Micro-Tom, with *Sw-5b*$^{R/S}$ heterozygous genotype. BC_5F_1 plants were obtained from the BC_5 population through self-crossing. These plants were subsequently screened for the *Sw-5b*^{R} homozygous genotype using *Sw-5b*-specific molecular markers. The selected BC_5F_1 plants were further self-crossed and the resulting BC_5F_2 plants were all *Sw-5b*^{R} homozygous, conferred resistance to TSWV, and exhibited the same growth phenotype as Micro-Tom.

Abbreviations

CC	Coiled-coil
Dot-ELISA	Dot-enzyme-linked immunosorbent assay
LRR	Leucine-rich repeat
MAS	Marker assisted selection
NB-ARC	Central nucleotide-binding adaptor shared by ApaF-1, resistance proteins, CED-4
NLR	Nucleotide-binding leucine-rich repeat immune receptor
PCR	Polymerase chain reaction
RB	Resistance-breaking
RdRp	RNA-dependent RNA polymerase
RFLP	Restriction fragment length polymorphism
SCAR	Sequence characterized amplified regions
SD	Solanaceae domain
SNP	Single nucleotide polymorphisms
TSWV	Tomato spotted wilt virus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00214-9.

Additional file 1: Figure S1. Validation of the results of the *Sw-5b*-specific molecular markers through the reported Sw-5b SNP marker. **Figure S2**. Dot-ELISA detection of TSWV in tomato cultivars. **Figure S3**. Evaluation of TSWV resistance in *Sw-5b*^{R} homozygous Micro-Tom lines.

Additional file 1: Table S1. Source of tomato varieties used in this study.

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Author contributions

CT, SH, MZ, and XT conceived and designed the experiments. CT, SH, HY, RZ, MZ, and XT analyzed the data. QW, YJ, ZZ, and HZ performed technical supports. CT, SH, YS, LS, ZD, and YX performed the experiments. CT, MZ, and XT wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

Adkins S, Quadt R, Choi TJ, Ahlquist P, German T. An RNA-dependent RNA polymerase activity associated with virions of tomato spotted wilt virus, a plant-infecting and insect-infecting bunyavirus. Virology. 1995;207(1):308–11. https://doi.org/10.1006/viro.1995.1083.

Brommonschenkel SH, Frary A, Frary A, Tanksley SD. The broad-spectrum tospovirus resistance gene Sw-5 of tomato is a homolog of the root-knot nematode resistance gene Mi. Mol Plant Microbe Inter. 2000;13(10):1130–8. https://doi.org/10.1094/MPMI.2000.13.10.1130.

Bucher E, Sijen T, de Haan P, Goldbach R, Prins M. Negative-strand tospoviruses and tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. J Virol. 2003;77(2):1329–36. https://doi.org/10.1128/jvi.77.2.1329-1336.2003.

Canady MA, Stevens MR, Barineau MS, Scott JW. Tomato spotted wilt virus (TSWV) resistance in tomato derived from *Lycopersicon chilense* Dun. LA 1938. Euphytica. 2001;117:19–25. https://doi.org/10.1023/A:1004089504051.

Chen XJ, Zhu M, Jiang L, Zhao WY, Li J, Wu JY, et al. A multilayered regulatory mechanism for the autoinhibition and activation of a plant CC-NB-LRR resistance protein with an extra N-terminal domain. New Phytol. 2016;212(1):161–75. https://doi.org/10.1111/nph.14013.

De Oliveira AS, Koolhaas I, Boiteux LS, Caldararu OF, Petrescu AJ, Resende RO, et al. Cell death triggering and effector recognition by Sw-5 SD-CNL proteins from resistant and susceptible tomato isolines to tomato spotted wilt virus. Mol Plant Pathol. 2016;17(9):1442–54. https://doi.org/10.1111/mpp.12439.

de Oliveira AS, Boiteux LS, Kormelink R, Resende RO. The Sw-5 gene cluster: tomato breeding and research toward orthotospovirus disease control. Front Plant Sci. 2018;9:1055. https://doi.org/10.3389/fpls.2018.01055.

Dehaan P, Kormelink R, Resende RD, Vanpoelwijk F, Peters D, Goldbach R. Tomato spotted wilt virus L RNA encodes a putative RNA polymerase. J Gen Virol. 1991;72(9):2207–16. https://doi.org/10.1099/0022-1317-72-9-2207.

Garland S, Sharman M, Persley D, McGrath D. The development of an improved PCR-based marker system for Sw-5, an important TSWV resistance gene of tomato. Aust J Agric Res. 2005;56(3):285–9. https://doi.org/10.1071/AR04140.

Gilbertson RL, Batuman O, Webster CG, Adkins S. Role of the insect supervectors bemisia tabaci and frankliniella occidentalis in the emergence and global spread of plant viruses. Annu Rev Virol. 2015;2(1):67–93. https://doi.org/10.1146/annurev-virology-031413-085410.

Hallwass M, De Oliveira AS, Dianese ED, Lohuis D, Boiteux LS, Inoue-Nagata AK, et al. The Tomato spotted wilt virus cell-to-cell movement protein (NSM) triggers a hypersensitive response in Sw-5-containing resistant tomato lines and in *Nicotiana benthamiana* transformed with the functional Sw-5b resistance gene copy. Mol Plant Pathol. 2014;15(9):871–80. https://doi.org/10.1111/mpp.12144.

Kormelink R, Kitajima EW, Dehaan P, Zuidema D, Peters D, Goldbach R. The nonstructural protein (NSs) encoded by the ambisense S RNA segment of tomato spotted wilt virus is associated with fibrous structures in infected plant cells. Virology. 1991;181(2):459–68. https://doi.org/10.1016/0042-6822(91)90878-f.

Kormelink R, Storms M, Van Lent J, Peters D, Goldbach R. Expression and subcellular location of the NSM protein of tomato spotted wilt virus (TSWV), a putative viral movement protein. Virology. 1994;200(1):56–65. https://doi.org/10.1006/viro.1994.1162.

Leastro MO, De Oliveira AS, Pallas V, Sanchez-Navarro JA, Kormelink R, Resende RO. The NSm proteins of phylogenetically related tospoviruses trigger Sw-5b-mediated resistance dissociated of their cell-to-cell movement function. Virus Res. 2017;240:25–34. https://doi.org/10.1016/j.virusres.2017.07.019.

Lee HJ, Kim B, Bae C, Kang WH, Kang BC, Yeam I, et al. Development of a single-nucleotide polymorphism marker for the Sw-5b geneconferring disease resistance to tomato spotted wilt virus in tomato. Korean J Hortic Sci. 2015;33(5):730–6. https://doi.org/10.7235/hort.2015.15025.

Li J, Huang HN, Zhu M, Huang S, Zhang WH, Dinesh-Kumar SP, et al. A plant immune receptor adopts a two-step recognition mechanism to enhance viral effector perception. Mol Plant. 2019;12(2):248–62. https://doi.org/10.1016/j.molp.2019.01.005.

Liu J, Tang D. Plant immunity research in China. Phytopathol Res. 2023;5:37. https://doi.org/10.1186/s42483-023-00196-8.

Lopez C, Aramburu J, Galipienso L, Soler S, Nuez F, Rubio L. Evolutionary analysis of tomato Sw-5 resistance-breaking isolates of tomato spotted wilt virus. J Gen Virol. 2011;92(1):210–5. https://doi.org/10.1099/vir.0.026708-0.

Nagata T, Inoue-Nagata AK, Prins M, Goldbach R, Peters D. Impeded thrips transmission of defective tomato spotted wilt virus isolates. Phytopathology. 2000;90(5):454–9. https://doi.org/10.1094/PHYTO.2000.90.5.454.

Okabe Y, Ariizumi T, Ezura H. Updating the micro-Tom TILLING platform. Breed Sci. 2013;63(1):42–8. https://doi.org/10.1270/jsbbs.63.42.

Oliver JE, Whitfield AE. The genus tospovirus: emerging bunyaviruses that threaten food security. Annu Rev Virol. 2016;3(1):101–24. https://doi.org/10.1146/annurev-virology-100114-055036.

Panthee DR, Ibrahem R. New molecular markers associated with the Sw-5 gene conferring resistance to tomato spotted wilt virus in tomato. J Hortic Sci Biotech. 2013;88:129–34. https://doi.org/10.1080/14620316.2013.11512946.

Parrella G, Gognalons P, Gebre-Selassie K, Vovlas C, Marchoux G. An update of the host range of tomato spotted wilt virus. J Plant Pathol. 2003;85(4):227–64. https://doi.org/10.2307/41998156.

Peiro A, Canizares MC, Rubio L, Lopez C, Moriones E, Aramburu J, et al. The movement protein (NSm) of tomato spotted wilt virus is the avirulence determinant in the tomato Sw-5 gene-based resistance. Mol Plant Pathol. 2014;15(8):802–13. https://doi.org/10.1111/mpp.12142.

Qi S, Zhang S, Islam MM, El-Sappah AH, Zhang F, Liang Y. Natural resources resistance to tomato spotted wilt virus (TSWV) in tomato (*Solanum lycopersicum*). Int J Mol Sci. 2021;22(20):10978. https://doi.org/10.3390/ijms222010978.

Rosello S, Diez MJ, Nuez F. Genetics of tomato spotted wilt virus resistance coming from *Lycopersicon peruvianum*. Eur J Plant Pathol. 1998;104(5):499–509. https://doi.org/10.1023/a:1008622128504.

Rosello S, Ricarte B, Diez MJ, Nuez F. Resistance to Tomato spotted wilt virus introgressed from *Lycopersicon peruvianum* in line UPV 1 may be allelic to Sw-5 and can be used to enhance the resistance of hybrids cultivars. Euphytica. 2001;119:357–67. https://doi.org/10.1023/A:1017506213974.

Rotenberg D, Jacobson AL, Schneweis DJ, Whiffleld AE. Thrips transmission of tospoviruses. Curr Opin Virol. 2015;15:80–9. https://doi.org/10.1016/j.coviro.2015.08.003.

Saidi M, Warade SD. Tomato breeding for resistance to tomato spotted wilt virus (TSWV): an overview of conventional and molecular approaches. Czech J Genet Plant. 2008;44(3):83–92. https://doi.org/10.17221/47/2008-CJGPB.

Schnettler E, Hemmes H, Huismann R, Goldbach R, Prins M, Kormelink R. Diverging affinity of tospovirus RNA silencing suppressor proteins, NSs, for various RNA duplex molecules. J Virol. 2010;84(21):11542–54. https://doi.org/10.1128/JVI.00595-10.

Scholthof KBG, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, et al. Top 10 plant viruses in molecular plant pathology. Mol Plant Pathol. 2011;12(9):938–54. https://doi.org/10.1111/j.1364-3703.2011.00752.x.

Shi A, Vierling R, Grazzini R, Chen P, Caton H, Panthee D. Identification of molecular markers for Sw-5 gene of tomato spotted wilt virus resistance. Am J Biotechnol Mol Sci. 2011;1(1):8–16. https://doi.org/10.5251/ajbms.2011.1.1.8.16.

Shikata M, Ezura H. Micro-Tom tomato as an alternative plant model system: mutant collection and efficient transformation. Methods Mol Biol. 2016;1363:47–55. https://doi.org/10.1007/978-1-4939-3115-6_5.

Snippe M, Willem Borst J, Goldbach R, Kormelink R. Tomato spotted wilt virus Gc and N proteins interact in vivo. Virology. 2007;357(2):115–23. https://doi.org/10.1016/j.virol.2006.06.037.

Spassova MI, Prins TW, Folkertsma RT, Klein-Lankhorst RM, Hille J, Goldbach RW, et al. The tomato gene Sw5 is a member of the coiled coil, nucleotide binding, leucine-rich repeat class of plant resistance genes and confers resistance to TSWV in tobacco. Mol Breed. 2001;7(2):151–61. https://doi.org/10.1023/A:1011363119763.

Stevens MR, Scott SJ, Gergerich RC. Inheritance of a gene for resistance to tomato spotted wilt virus (Tswv) from *Lycopersicon-peruvianum* Mill. Euphytica. 1991;59:9–17. https://doi.org/10.1007/BF00025356.

Stevens MR, Heiny DK, Rhoads DD, Griffiths PD, Scott JW. A linkage map of the tomato spotted wilt virus resistance gene Sw-5 using near isogenic lines and an interspecific cross. Acta Hortic. 1995a. https://doi.org/10.17660/ActaHortic.1996.431.33.

Stevens MR, Lamb EM, Rhoads DD. Mapping the Sw-5 locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. Theor Appl Genet. 1995b;90:451–6. https://doi.org/10.1007/BF00221989.

Tettey CK, Mu XQ, Ma HY, Chen XY, Geng C, Tian YP, et al. The role of different innate and environmental factors in *Tm-2*^{2}-mediated resistance to tomato mottle mosaic virus. Phytopathol Res. 2023;5:8. https://doi.org/10.1186/s42483-023-00162-4.

Turina M, Kormelink R, Resende RO. Resistance to tospoviruses in vegetable crops: epidemiological and molecular aspects. Annu Rev Phytopathol. 2016;54:347–71. https://doi.org/10.1146/annurev-phyto-080615-095843.

van Knippenberg I, Goldbach R, Kormelink R. Purified tomato spotted wilt virus particles support both genome replication and transcription in vitro. Virology. 2002;303(2):278–86. https://doi.org/10.1006/viro.2002.1632.

Whitfield AE, Ullman DE, German TL. Tospovirus-thrips interactions. Annu Rev Phytopathol. 2005;43:459–89. https://doi.org/10.1146/annurev.phyto.43.040204.

Wu Q, Tong C, Chen Z, Huang S, Zhao X, Hong H, et al. NLRs derepress MED10b- and MED7-mediated repression of jasmonate-dependent

transcription to activate immunity. Proc Natl Acad Sci U S A. 2023;120(28): e2302226120. https://doi.org/10.1073/pnas.2302226120.
Zhao WY, Jiang L, Feng ZK, Chen XJ, Huang Y, Xue F, et al. Plasmodesmata targeting and intercellular trafficking of tomato spotted wilt tospovirus movement protein NSm is independent of its function in HR induction. J Gen Virol. 2016;97(8):1990–7. https://doi.org/10.1099/jgv.0.000496.
Zhu M, Jiang L, Bai BH, Zhao WY, Chen XJ, Li J, et al. The intracellular immune receptor Sw-5b confers broad-spectrum resistance to tospoviruses through recognition of aconserved 21-amino acid viral effector epitope. Plant Cell. 2017;29(9):2214–32. https://doi.org/10.1105/tpc.17.00180.
Zhu M, van Grinsven IL, Kormelink R, Tao X. Paving the way to tospovirus infection: multilined interplays with plant innate immunity. Annu Rev Phytopathol. 2019;57:41–62. https://doi.org/10.1146/annurev-phyto-082718-100309.