Gene Based Markers in Marker-Assisted Selection to Screen Tomato Genotypes Resistant to Fusarium Wilt, Late Blight, Verticillium Wilt, Leaf Mold, Bacterial Spot and Bacterial Speck

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Research Article

Keywords: Allele-specific markers, SCAR, SNP, InDel, CAPS.

Posted Date: April 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-404832/v1

Abstract

The tomato crop is exposed to serious losses due to infection with several diseases and pests, which threaten tomato production in Egypt and worldwide. Therefore, selecting the tomato germplasm resistant or tolerant to a specific pathogen by molecular markers closely linked to resistance loci is a desirable goal of this study. In this work, seven co-dominant markers targeting six resistance genes (*I-1, Ve, Ph3, Cf-9*/*Cf-4*, *Rx4*, and *Pto*) for six main diseases [fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), verticillium wilt (*Verticillium dahliae* and *V. alboatrum*), late blight (*Phytophthora infestans*), leaf mold (*Cladosporium fulvum*), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*)], respectively were determined. Theses molecular markers differentiated among 19 tomato genotypes resistant (homozygote/heterozygote) and susceptible (homozygote) to the pathogens. Therefore, this study supplied us with novel tomato lines with resistance to multiple diseases, and their pyramiding inside domesticated tomato cultivars are suggested to apply in the tomato breeding programs of resistance against fungal and bacterial diseases.

Introduction

Breeding for biotic stress resistance in the plants is considered one of the most crucial ways in the breeding programs. However, selecting the germplasm resistant or tolerant to a specific pathogen is more difficult (Peries 1971). Furthermore, the use of molecular markers in the identification and characterization of resistance genes has become an important tool, because they are not affected by environmental conditions. Besides, molecular markers supply a unique chance to select a big number of germplasms in a short time. Up to date, a big number of gene-based markers have been identified in various crops, including tomato (Foolad 2007).

Tomato (*Solanum lycopersicum* L.), one of the most important horticulture crops in Egypt and worldwide. It is infected with many fungal and bacterial diseases e.g., wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici*, verticillium wilt (*Verticillium dahliae* and *V. alboatrum*), late blight (*Phytophthora infestans*), leaf mold (*Cladosporium fulvum*), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*) are a dangerous threat to tomato farming (Lee et al. 2015). There are a big number of tomato germplasms, many resistance loci for various diseases have been reported (Van Ooijen et al. 2007). Hence, molecular markers become an important tool in the tomato breeding programs for the detection of resistance genes of the above-mentioned diseases (Arens et al. 2010; Shi et al. 2011).

Fusarium wilt disease in tomato is caused by fungus *F. oxysporum* f. sp. *lycopersici* (*Fol*). Three races of *Fusarium* fungus were known (1, 2, and 3) (Grattidge and O'Brien 1982). Resistance to *Fol* has been reported in multiple wild tomato species. The resistance genes *I-1*, *I-2* and *I-3* have been indicated in the wild tomato *S. pimpinellifolium* accession "PI79532", *S. lycopersicum* × *S. pimpinellifolium* hybrid "PI126915" and *S. pennellii* "LA716" respectively, which give resistance to *Fol* race 1, 2, and 3, respectively (Bohn and Tucker 1939; Simons et al. 1998; Scott and Jones 1989a). Besides, the single dominant gene (*I-7*) has been recorded in *S. pennellii* "PI414773" that confers resistance to *Fol* races 1, 2, and 3 (Gonzalez-Cendales et al. 2015).

Vascular wilt or verticillium wilt disease in tomato is a soil-born fungal pathogen caused by *Verticillium dahliae* and *V. alboatrum* (Fradin and Thomma 2006). The resistance gene (*Ve*) located on chromosome 9 (chr 9), which confers resistance to *V. alboatrum* race 1 (Diwan et al. 1999).

Late blight (LB) disease of tomato is caused by fungus *Phytophthora infestans* (Rodewald and Trognitz 2013); a few main resistance genes to LB in tomato have been reported. Three resistance loci to LB, *Ph1*, *Ph2* and *Ph3* from wild tomato *S. pimpinellifolium* have been located on chr 7, chr 10 and chr 9, respectively. The latter refers to incomplete resistance to *P. infestans* races (Foolad et al. 2008; Kim and Mutschler 2006; Zhang et al. 2013). Furthermore, the resistance gene (*Ph4*) in accession *S. habrochaites* LA1033 on chr 2 has been identified (Kole et al. 2006), and *Ph5-1* and *Ph5-2*, which have been found in *S. pimpinellifolium* "PSLP153", are mapped at chr 1 and chr 10, respectively (Merk et al. 2012; Merk and Foolad 2012).

Tomato leaf mold, which is caused by the fungus *Cladosporium fulvum*, causes significant yield loss in glasshouse-grown tomatoes (Rivas and Thomas 2005). Multiple resistance genes (*Cf*) to leaf mold have been recognized in wild tomato types namely, *Cf-2*, *Cf-4*, *Cf-4E*, *Cf-5* and *Cf-9* (Dixon et al. 1996; 1998; Takken et al. 1999). Both *Cf-4* and *Cf-9* originated from *S. habrochaites* and *S. pimpinellifolium*, respectively. They are mapped at the same locus on chr 1 (Parniske et al. 1997). *Cf-2* and *Cf-5* indicated in *S. pimpinellifolium* and *L. esculentum var. cerasiforme*, respectively. Both *Cf-2* and *Cf-5* are mapped at chr 6 (Dixon et al. 1998).

Bacterial spot disease in tomato, which is caused by a gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), is a constant threat to the tomato grown in both the greenhouse and the field (Jones et al. 1998). Five races of *Xcv* (T1 to T5) are identified by various tomato germplasms. Resistance genes, involving *Xv3* and *Xv4*, are responsible for mechanisms of hypersensitivity reaction (HR) resistance. *Xv3* discovered in *S. lycopersicum* "H7981" and *S. pimpinellifolium* (accessions "PI126932" and "PI128216") confers resistance against T3 races (Wang et al. 2011). Besides, resistance locus *Rx-4* located on chr 11 (accession "PI128216") also refers to resistance against T3 races (Robbins et al. 2009). A dominant resistance locus *Xv4* on chr 3 has been found in *S. pennellii* LA716, which confers resistance to T4 strains (Astua-Monge et al. 2000). Both *Rx-1* and *Rx-2* are mapped at chr 1, while *Rx-3* is located on chr 5, has been recognized in *S. lycopersicum* (accession "H7998"), which gives HR resistance to T1 strains (Scott and Jones 1989b).

Bacterial speck disease in tomato is caused by a gram-negative bacterium *Pseudomonas syringae* pv. *tomato*. The single dominant gene, *Pto* has been located on chr 5, which confers resistance to the bacterial speck in *S. pimpinellifolium* (Salmeron et al. 1996; Jia et al. 1997). The other genes originated from wild tomato *S. habrochites* "LA1777" are included in resistance against bacterial speck e.g., *bsRr1-1*, *bsRr1-2* and *bsRr1-12* are located on chr 1, chr 2 and chr 12, respectively (Thapa et al. 2015).

The purpose of this study was to identify the resistance alleles corresponding to fusarium wilt, verticillium wilt, late blight, leaf mold, bacterial spot, and bacterial speck of 19 tomato genotypes by molecular markers, which will be used as marker-assisted selection (MAS) in tomato breeding programs.

Materials And Methods

Plant materials

A total of 19 tomato genotypes, including accessions and commercial cultivars, were used in this study. The name and source of these genotypes were mentioned in **Table (1)**. Ten tomato seeds from each of the genotype have been sown in a greenhouse at 27°C:16°C (Light:Dark), a photoperiod of L16:D8 h and relative humidity of 68–75%. Seedlings were planted in peat moss: sand (2:1) in pots (Mahfouze and Mahfouze 2019).

Isolation Of DNA

DNA was isolated from fresh tomato leaves for each genotype. 30 mg of tissue was ground in liquid nitrogen and extracted with the DNA purification Kit (Bio Basic, Inc., Markham, Canada) following the manufacturer's instructions. DNA quality and quantity were determined by agarose gel electrophoresis and Spectrophotometer. DNA concentrations were adjusted to 50 ng/µl and extracts were frozen at -20^{o}C.

PCR amplification of resistance alleles

PCR with a gene-based marker was performed in 25 µl reactions containing 2.5 µl 2.5 mM dNTPs, 5 µl 5X buffer, 2.5 µl 2.5 mM $MgCl_2$, 0.1 µl (0.5 units) *Taq* DNA polymerase (Promega Corp., Madison, WI), 2.5 µl each forward and reverse primer at 10 µM, 1 µl of DNA extract and 8.9 µl dsH_2O. PCR cycles were 94°C for 4 min, 35 cycles of 94°C for 30 sec, annealing temperature (Table 2) for 1 min and 72°C for 1.5 min. These cycles were followed by 72°C for 10 min and then the reaction was held at 4°C. PCR reactions were performed in the Thermocycler (Biometra, biomedizinische Analytik GmbH). For CAPS markers, PCR products were digested by the restriction enzyme *Rsa*I (Table 2). 25 µl reaction mixture containing 10.75 µl dsH_2O, 3 µl buffer, 0.25 µl BSA (Bovine serum albumin), 1 µl restriction enzyme (*Rsa*I) 10 U/µl (Promega Corp.) and 10 µl PCR reaction mixture. The reaction mixture was placed in a 65°C water bath for about 2 h according to the manufacturer's instructions.

Table 1
Tomato genotypes used in this study.

No.	Genotype	Source	No.	Genotype	Source
1	*Solanum hirsutum* 24036	CGN*	11	*S. chilense* 56139	CGN
2	*S. galapagense* 0317	TGRC**	12	*S. lycopersicon* cv. Super Marmande	Egypt***
3	*S. neoricki* 0247	TGRC	13	*S. lycopersicon* cv. Strain B F1	Egypt
4	*S. arcanum* 1346	TGRC	14	*S. corneliomulleri* 1283	TGRC
5	*S. corneliomulleri* 1274	TGRC	15	*S. habrochaites* 1739	TGRC
6	*S. pennellii* 1733	TGRC	16	*S. pimpinellifolium* 1279	TGRC
7	*S. huaylasense* 1358	TGRC	17	*S. pimpinellifolium* 1332	TGRC
8	*S. pimpinellifolium* 1342	TGRC	18	*S. pennellii* 2963	TGRC
9	*S. peruvianum* 1333	TGRC	19	*S. pennellii* 1942	TGRC
10	*S. habrochaites* 1352	TGRC			

CGN*= Centre for Genetic Resources, Netherlands (http://www.wur.nl).

TGRC**= Tomato Genetics Resource Center (TGRC), Department of Plant Sciences, University of California, Davis, CA 95616 (http://tgrc.ucdavis.edu).

***Two commercial cultivars were purchased from Egyptian Company for Seeds, Oils and Chemicals, Egypt.

Table 2
Primers sequences used in this study.

Primer name	Marker name[a]	Disease name	*R*-gene[b]	Chromosome No	Single nucleotide sequence (5′-3′)	Annealing temperature (AT)°C	Restriction enzyme	Molecular size of PCR product (bp)	Referenc
SCAR I1F	SCAR	Fusarium wilt	*I-1*	11	CGAATCTGTATATTACATCCGTCGT	55	-	R = 130	Scott et a (2004)
SCAR I1R					GGTGAATACCGATCATAGTCGAG			Other = 92	
SCAR I1 86.1 F	SCAR	Fusarium wilt	*I-1*	11	TGTTGGCGGTAGTGATGAGA	52	-	R = 314, S = 583	Gonzalez Cendales et al.
SCAR I1 86.1 R					TCACCAATATTAGGCCCCTTT			H = 314 and 583	(2014)
Ve SNP F	SNP	Verticillium wilt	*Ve*	9	CCTTGATGGGGTTGATCTTTCGT	57	-	R = 476, S = 158	Kawchuk et al.
Ve SNP R					GTAGGTGAGTTTCTTGGACAGTCGA			Other = 580	(2001)
SCAR Ph3 F	SCAR	Late blight	*Ph3*	9	CTACTCGTGCAAGAAGGTAC	50	-	S = 154, R = 176	Jung et a 2015
SCAR Ph3 R					TCCACATCACCTGCCAGTTG				
InDel2_Cf-9/Cf-4 F	InDel	Leaf mold	*Cf-9/Cf-4*	1	TCCTAAACCTCTATGGAATCTCAC	55	-	R = 434 (*Cf-9*[c])	Kim et al (2017)
InDel2_Cf-9/Cf-4 R					GGAGTGAATTCGGAATACGACC			R = 297 (*Cf-4*)	
pcc12 Indel Rx4 F	InDel	Bacterial spot	*Rx4*	11	TCCACATCAAATGCGTTTCT	52	-	R = 113	Pei et al. (2012)
pcc12 Indel Rx4 R					TTCCAATCCTTTCCATTTCG			S = 119	
Pto CAPSf	CAPS	Bacterial speck	*Pto*	5	ATCTACCCACAATGAGCATGAGCTG	60	*Rsa*I	R = 552	Coaker and Francis, (2004)
Pto CAPS R					GTGCATACTCCAGTTTCCAC			S = 113 and 439	

[a]SCAR= Sequence characterized amplified region, SNP = Single nucleotide polymorphism, InDel = PCR based Insertion-deletions, CAPS = Cleaved amplified polymorphic sequences.

[b]Resistance genes of disease.

[c]*Cf-9* and its paralogs.

Gel electrophoresis

All the PCR and restriction-digested products were analyzed with 1% agarose gel electrophoresis in 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3). The genomic DNA was stained with RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr) and was visualized with UV light. The size of each band was estimated with reference to a size marker of 100 bp DNA ladder (BioRoN, Germany).

Results

Fungi-high-efficiency markers for marker-assisted selection (MAS) in tomato.

Five molecular markers linked with three fungal diseases were estimated to select tomato genotypes carrying resistance alleles for MAS programs. For Fusarium wilt, two markers SCAR I1 and SCAR 86.1 were applied to the target *I-1* gene. However, the SNP marker is associated with *Ve* gene, which gives resistance to verticillium wilt. Besides, the SCAR Ph3 marker linked to *Ph3* responsible for resistance to late blight. Finally, the InDel2_Cf-9/Cf-4 marker was used to detect resistance allele to leaf mold (*Cf*) disease.

Gene-based SCAR markers for I-1 resistance.

Two SCAR markers (SCAR I1 and SCAR I1 86.1) (Table 2) were used to detect resistant and susceptible tomato genotypes to fusarium wilt disease. The primer set SCAR I1 scored two bands of (130 and 92 bp) in all tested tomato genotypes, which refer to the presence of resistance allele *I-1* (Fig. 1 **and** Table 3). This result indicated that the primer SCAR I1 has not differentiated between the resistant and susceptible tomato lines to *F. oxysporum* f. sp. *lycopersici*, consequently this primer SCAR I1 cannot be applied in the tomato breeding programs for the selection of resistance allele *I-1* to fusarium wilt fungus.

Table 3
Tomato genotypes used to evaluate gene-based markers for resistances against tomato pathogens.

No.	Genotype	Resistance genes and DNA markers						
		Fusarium wilt (I-1)		Verticillium wilt (Ve)	Late blight (Ph3)	Leaf mold (Cf-9/Cf-4)	Bacterial spot (Rx4)	Bacterial speck (Pto)
		SCAR[a] I1	SCAR I1 86.1	Ve SNP[b]	SCAR Ph3	InDel2_Cf-9/Cf-4[c]	pcc12 Indel Rx4	Pto CAPS[d]
1	*Solanum hirsutum* 24036	RR[e]	-	-	RR	RR (*Cf-9*)	-	-
2	*S. galapagense* 0317	RR	rr[f]	-	RR	RR (*Cf-9*)	-	-
3	*S. neoricki* 0247	RR	-	-	Rr[g]	RR (*Cf-9*/ *Cf-4*)	rr	Rr
4	*S. arcanum* 1346	RR	-	-	Rr	RR (*Cf-9*/ *Cf-4*)	rr	RR
5	*S. corneliomulleri* 1274	RR	-	rr	-	RR (*Cf-9*/ *Cf-4*)	rr	RR
6	*S. pennellii* 1733	RR	-	-	RR	RR (*Cf-9*)	rr	RR
7	*S. huaylasense* 1358	RR	-	-	-	RR (*Cf-9*/ *Cf-4*)	rr	RR
8	*S. pimpinellifolium* 1342	RR	rr	-	RR	RR (*Cf-9*)	rr	RR
9	*S. peruvianum* 1333	RR	-	-	RR	RR (*Cf-9*/ *Cf-4*)	rr	RR
10	*S. habrochaites* 1352	RR	-	-	-	RR (*Cf-9*)	rr	RR
11	*S. chilense* 56139	RR	-	-	RR	RR (*Cf-9*)	rr	RR
12	*S. lycopersicon* cv. Super Marmande	RR	rr	-	-	RR (*Cf-9*)	rr	Rr
13	*S. lycopersicon* cv. Strain B F1	RR	rr	-	-	RR (*Cf-9*/ *Cf-4*)	rr	Rr
14	*S. corneliomulleri* 1283	RR	-	rr	-	RR (*Cf-9*/ *Cf-4*)	rr	RR
15	*S. habrochaites* 1739	RR	-	-	-	-	rr	RR
16	*S. pimpinellifolium* 1279	RR	RR	rr	-	RR (*Cf-9*)	rr	RR
17	*S. pimpinellifolium* 1332	RR	RR	-	-	RR (*Cf-9*)	rr	RR
18	*S. pennellii* 2963	RR	-	-	-	RR (*Cf-9*)	rr	RR
19	*S.pennellii* 1942	RR	-	rr	-	RR (*Cf-9*/ *Cf-4*)	rr	RR

SCAR[a] = Sequence characterized amplified region, SNP[b] =Single nucleotide polymorphism, InDel[c] = PCR based Insertion-deletions and CAPS[d] = Cleaved amplified polymorphic sequence.

RR[e] = Resistance allele, homozygote, rr[f] = Susceptibility allele, homozygote, Rr[g] = Heterozygote, - = Absence of allele.

For SCAR I1 86.1, it scored one amplicon of 314 bp in two tomato accessions containing homozygous dominant allele *I-1* e.g., *S. pimpinellifolium* 1279 and 1332. Furthermore, SCAR I1 86.1 recorded one amplified fragment with a molecular size of 583 bp in four tomato germplasms may be susceptible to fusarium wilt disease such as *S. galapagense* 0317, *S. pimpinellifolium* 1342, *S. lycopersicon* cv. Super Marmande and *S. lycopersicon* cv. Strain B F1, which have a recessive allele with homozygous (Fig. 2 **and** Table 3).

Gene-based SNP marker for Ve1 resistance.

PCR amplification of DNA from 19 tested tomato accessions using primer set Ve SNP, gave a faint band of 158 bp in the four tomato genotypes expected to be susceptible to fungus verticillium wilt i.e., *S. corneliomulleri* 1274 and 1283, *S. pimpinellifolium* 1279 and *S. pennellii* 1942 (Fig. 3 **and** Table 3). Moreover, the other15 tomato genotypes have not shown any unique bands. Our results have not recorded any tomato genotypes resistant to verticillium wilt disease.

Gene-based SCAR marker for Ph3 resistance.

A PCR assay was used by a single pair of primer SCAR Ph3 to amplify the resistance gene to late blight (*Ph3*). Among the 19 studied tomato genotypes, six lines were homozygous for the *Ph3* allele, which gave a unique band of 176 bp like *S. hirsutum* 24036, *S. galapagense* 0317, *S. pennellii* 1733, *S. pimpinellifolium* 1342, *peruvianum* 1333 and *S. chilense* 56139 (Fig. 4 **and** Table 3). Three genotypes were heterozygous that scored two amplicons with molecular sizes of 154 and 176 bp e.g., *S. neoricki* 0247 and *S. arcanum* 1346 are expected to be *Ph3* resistant. In addition, the other tomato lines have not scored any products. On the other hand, none of the studied tomato lines were homozygous recessive for the *ph3* allele (Fig. 4 **and** Table 3).

Gene-based InDel marker for Cf-9/Cf-4 resistance.

The primer pair InDel2_Cf-9/Cf-4 was able to amplify a 434 bp PCR product from ten tomato genotypes have only the *Cf-9* resistance allele including *S. hirsutum* 24036, *S. galapagense* 0317, *S. pennellii* 1733 and 2963, *S. pimpinellifolium* 1342, 1279 and 1332, *S. habrochaites* 1352, *S. chilense* 56139 and *S. lycopersicon* cv. Super Marmande (Fig. 5 **and** Table 3). On the other hand, the primer set InDel2_Cf-9/Cf-4 gave two bands of 297 and 434 bp in eight wild type tomato species viz., *S. neoricki* 0247, *S. arcanum* 1346, *S. corneliomulleri* 1274 and 1283, *S. huaylasense* 1358, *S. peruvianum* 1333, *S. lycopersicon* cv. Strain B F1 and *S. pennellii* 1942 carrying both the *Cf-4* and *Cf-9* resistance alleles. In contrast, none of the examined tomato lines has only a *Cf-4* allele. Besides, *S. habrochaites* 1739 has not any *Cf-4* or *Cf-9* resistance loci (Fig. 5 **and** Table 3).

Bacteria-high-efficiency markers for MAS in tomato.

Two gene-based markers related to two bacterial diseases were examined to screen tomato lines carrying resistance alleles. For bacterial spot, pcc12 Indel Rx4 marker was used to the target *Rx4*. Besides, Pto CAPS markers associated with the *Pto* gene, responsible for resistance to bacterial speck disease.

Gene-based InDel marker for Rx4 resistance.

Genomic PCR using primer set pcc12 Indel yielded a single band of 119 bp for the recessive allele in all tested tomato genotypes, except *S. hirsutum* 24036 and *S. galapagense* 0317 have not recorded any products (Fig. 6 **and** Table 3). On the other hand, none of the examined tomato lines has the dominant allele for *Rx4* resistance gene.

Gene-based CAPS marker for Pto resistance.

A total number of 19 tomato genotypes were subject to CAPS marker analysis. Primer Pto CAPS amplified a 552 bp band from both bacterial speck resistant and susceptible tomato genotypes (Fig. 7a **and** Table 3). The restriction enzyme *RsaI* has not cut the amplicon from the homozygous resistant tomato accessions involving *S. arcanum* 1346, *S. corneliomulleri* 1274 and 1283, *S. pennellii* 1733, 2963 and 1942, *S. huaylasense* 1358, *S. pimpinellifolium* 1342, 1279 and 1332, *S. peruvianum* 1333, *S. habrochaites* 1352 and 1739 and *S. chilense* 56139, but digested the amplicon from the susceptible tomato genotypes into two amplified fragments, 113 and 439 bp (none of the two fragments were obtained in 19 the tested tomato genotypes) (Fig. 7b **and** Table 3). Besides, pto CAPs primer scored three alleles of 113 bp, 439 and 552 bp in the three tomato genotypes, which were heterozygous such as *S. neoricki* 0247, *S. lycopersicon* cv. Super Marmande and *S. lycopersicon* cv. Strain B F1 (Fig. 7b **and** Table 3). In contrast, *S. hirsutum* 24036 and *S. galapagense* 0317 have not shown any bands. None of the tested tomato genotypes carry a recessive allele for the *Pto* gene (Fig. 7 **and** Table 3).

Discussion

Production of tomato is being threatened by multiple diseases e.g., fungi, bacteria, viruses, insects, and nematodes. Marker-assisted selection (MAS) is an indirect screening process; whereas a trait of interest is screened depending on molecular markers, which can be applied in the tomato breeding programs for the selection of resistance alleles of pathogens. In this study, we used seven molecular markers linked with three fungal diseases and two bacterial diseases to select tomato lines carrying resistance loci for MAS programs.

In this work, primer set SCAR I1 gave false-positive results for the presence of the *I-1* locus, responsible for resistance to fusarium wilt disease in the tomato. This marker has not separated resistance and susceptible alleles for the *I-1* gene. In contrast, primer pair SCAR I1 86.1 well separated both dominant and recessive alleles at each locus. The PCR results successfully amplified DNA amplicons for the *I-1* locus from both resistant (314 bp) and susceptible (583 bp) tomato genotypes. As a result, it is expected that the SCAR I1 86.1 marker would be beneficial for MAS to resistance against fungus *F. oxysporum* f. sp. *lycopersici* race 1. These results were in an agreement with Catanzariti and Jones (2010); Takken and Rep (2010) mentioned that fusarium wilt disease threatens tomato production worldwide. Fusarium wilt fungus in tomato is controlled by main genes for resistance introgressed from wild tomato species. The resistance gene *I-1*, introgressed from *S. pimpinellifolium*, refers to resistance against race 1 by recognition of *Avr1*gene (Houterman et al. 2008). The co-dominant SCAR markers used in this study should permit routine marker-assisted selection (MAS) for resistance to wilt fusarium fungus in the tomato breeding programs. This would allow early screening of resistant lines without inoculation steps, waiting for a long period until the appearance of symptoms. Mutlu et al. (2008) mentioned that co-dominant SCAR markers linked to dominant resistance genes against wilt fusarium fungus are more informative and easier in the eggplant breeding programs, compared with other markers.

Our results have not recorded any 19 tested tomato genotypes resistant to verticillium wilt disease. Resistance to *V. dahlia* and *V. albo-atrum* fungi was identified from *S. lycopersicum* line Peru wild and potato plants, respectively (Schaible et al. 1951; Kawchuk et al. 2001). The two resistance loci *Ve1* and *Ve2* have been identified for resistance to verticillium wilt (Diwan et al. 1999). Arens et al. (2010) developed primers as well as SNP markers to amplify either *Ve1* or *Ve2*. Primers specific to *Ve1* and *Ve2* were used to amplify fragments in both susceptible and resistant varieties (homozygous and heterozygous resistance).

In this research, we indicated new tomato genotypes have a dominant allele of *Ph3* i.e., *S. hirsutum* 24036, *S. galapagense* 0317, *S. pennellii* 1733, *S. pimpinellifolium* 1342, *S. peruvianum* 1333 and *S. chilense* 56139. Besides, two resistant tomato wild types were heterozygous involving *S. neoricki* 0247 and

S. arcanum 1346. The latter genotypes may be introgressed from lines containing the dominant allele. Resistance sources to late blight disease in tomato are supplied by *Ph3* gene produced from *S. chilense* (Miranda et al. 2010; Elsayed et al. 2011), *S. hirsutum* (Elafifi et al. 2019), *S. pennellii* (Li et al. 2011), *S. pimpinellifolium* (lrzhansky and Cohen 2006; Zhang et al. 2014), *S. arcanum* (Akhtar et al. 2016) and *S. habrochaites* LYC4 (Finkers et al. 2007). Our results showed that a co-dominant SCAR marker was effective in differentiation between the homozygous and heterozygous of *Ph3* allele. This marker gave results matched to results observed by Hittalmani et al. (2000) and Jung et al. (2015) who used the SCAR marker for screening of resistance gene *Ph3* that will be a powerful tool in tomato breeding programs. Besides, molecular markers can reduce the breeding period. It is clear that the SCAR marker applied in this work would be beneficial for screening tomato lines made by crossing plants that are resistant to late blight. Consequently using gene-based markers, such as strenuous crossing and offspring testing to genotype the *Ph3* gene could be averted.

In the current investigation, we discovered that the indel marker discriminated tomato genotypes carrying *Cf-9* from *Cf-4*. Genotyping with the Indel marker showed that all tested tomato lines carry the *Cf-9* allele, except *S. habrochaites* 1739. In addition, indel marker amplified products not only from the *Cf-9* gene but also from its homologs. Interestingly, eight tomato accessions carry both the *Cf-9* and the *Cf-4* resistance loci including *S. neoricki* 0247, *S. arcanum* 1346, *S. corneliomulleri* 1274 and 1283, *S. huaylasense* 1358, *S. peruvianum* 1333, *S. lycopersicon* cv. Strain B F1 and *S. pennellii* 1942. These lines will be useful in the tomato breeding programs of resistance against leaf mold disease. Similar data were obtained by Kruijt et al. (2005) mentioned that the resistance gene *Cf-9* was found in two wild tomato types viz., *S. habrochaites* and *S. pimpinellifolium*, while its close homolog, the *Cf-4* resistance allele was indicated in six tomato accessions e.g., *S. chilense*, *S. peruvianum*, *S. habrochaites*, *S. parviflorum*, *S. lycopersicum* and *S. chmielewskii*. Kim et al. (2017) distinguished between *Cf-9* and *Cf-4* alleles using SNP and InDel markers that will be beneficial for MAS of tomato varieties resistant to leaf mold. Durable resistance to the leaf mold disease caused by fungi *C. fulvum* has been the main purpose for breeders (Stevens and Rick 1988; Rivas and Thomas 2005). Introgressions of *Cf* genes inside *S. lycopersicum* supplied with genetic resources resistant to leaf mold (Thomas et al. 1997; Kruijt et al. 2005). The *Cf-9* resistance gene was highly homologous with the *Cf-4* gene with 95.5% and 91% at the DNA and amino acid levels, respectively (Parniske et al. 1997; Parniske and Jones 1999).

In our study, all tested tomato lines recorded susceptible to bacterial spot disease, using pcc12 Indel marker, except *Solanum hirsutum* 24036 and *S. galapagense* 0317 have not shown any products. Similar studies were made by Yuqing et al. (2018) mentioned that no commercial tomato cultivars are resistant to bacterial diseases. Pei et al. (2012) found that resistance genes to bacterial spot disease from wild tomato species and incorporating them into tomato cultivars are important for disease resistance. The resistant accession *S. pimpinellifolium* PI128216 that carries the *Rx4* gene on chromosome 11 referring to hypersensitivity response (HR) and field resistance to *Xanthomonas campestris* pv. *vesicatoria* strain T3 (Robbins et al. 2009).

For *Pto* locus, PCR products of DNA from 19 tomato genotypes and subsequent digestion by *RsaI* were performed using the CAPS marker. After restriction with *RsaI*, 14 wild tomato types have resistance gene *Pto* such as *S. corneliomulleri* 1274 and 1283, *S. peruvianum* 1333 and *S. chilense* 56139 (Hörger 2011), *S. arcanum* 1346, *S. pennellii* 1733, 2963 and 1942, *S. huaylasense* 1358, *S. pimpinellifolium* 1342, 1279 and 1332 (Orsi et al. 2012), *S. habrochaites* 1352 and 1739 (Thapa et al. 2015). Furthermore, three tomato lines were heterozygous e.g., *S. neoricki* 0247, *S. lycopersicon* cv. Super Marmande and *S. lycopersicon* cv. Strain B F1. These lines were introgressed from tomato germplasms carrying the dominant allele of *Pto*. These findings were synchronized with results previously obtained by Yang and Francis (2005) identified the *Pto* gene responsible for resistance to bacterial speck by a co-dominant CAPS marker, which is more exhausting and less easy compared with the SCAR marker. Orsi et al. (2012) determined tomato cultivars resistant to *Pseudomonas syringae* pv. *tomato* by a semi-dominant allele of *S. pimpinellifolium* that was introgressed into *S. lycopersicum* in the past century. Pedley and Martin (2003) mentioned that the *Pto* dominant allele was widely applied to bacterial speck resistance in tomato. Because the *Pto* gene is semi-dominant, symptoms of infection with *P. syringae* pv. *tomato* were obtained in hybrids, which have one copy of the *Pto* gene (Pedley and Martin 2003). Completely resistant lines avert any damage caused by the pathogen, so decreasing agrochemical operations. Besides, seed production companies can benefit from molecular markers linked to the dominant allele (*Pto*) to generate tomato cultivars resistant to *P. syringae* pv. *tomato* for breeding programs depending on marker-aided selection (MAS) (Collard and Mackill 2008).

Conclusions

The gene-based markers (SCAR, CAPS, SNP, and InDel) used in this work should permit routine marker-assisted selection (MAS) for resistance against fungal and bacterial pathogens in tomato. In this study, we identified new tomato lines resistant to multiple diseases, and their pyramiding into domesticated tomato will take a short time compared with the classical breeding ways, which require inoculation steps and waiting for a long period till the appearance of symptoms. In addition, the classical breeding ways produce only heterozygous lines, while gene-based markers identify non-segregating homozygous resistant tomato genotypes.

Declarations

Local and national regulations

All Studies complies with local and national regulations.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this work already exist in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by National Research Centre (NRC).

Authors' contributions

Dr. Heba A. Mahfouze carried out SCAR, CAPS, SNP and InDel markers and Prof. Dr. Sherin A. Mahfouze performed writing of the manuscript.

Acknowledgements

The authors are thankful to the National Research Centre (NRC) to fund this study.

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Figures

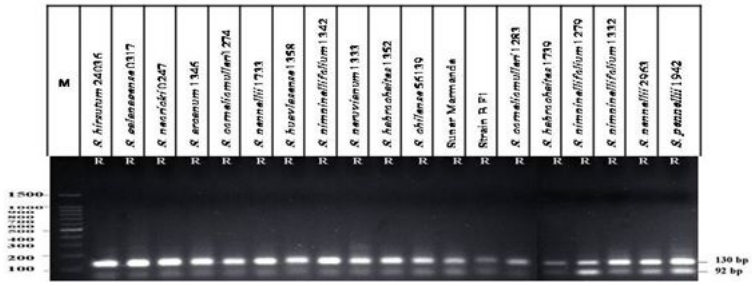

Figure 1

PCR fragments represent primer pairSCAR I1 amplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M= 100 bp DNA ladder; R= homozygous resistant genotypes.

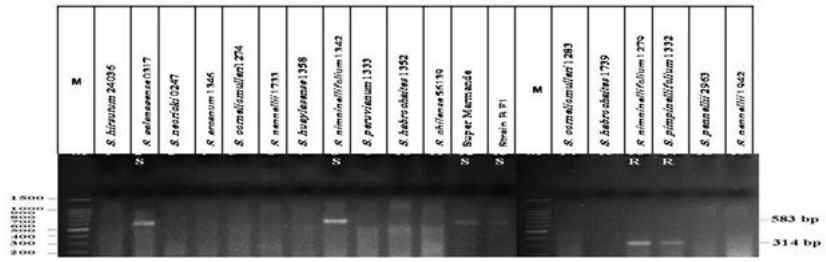

Figure 2

PCR fragments represent primer set SCAR I1 86.1 amplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder; R= homozygous resistant genotypes; S= susceptible genotypes.

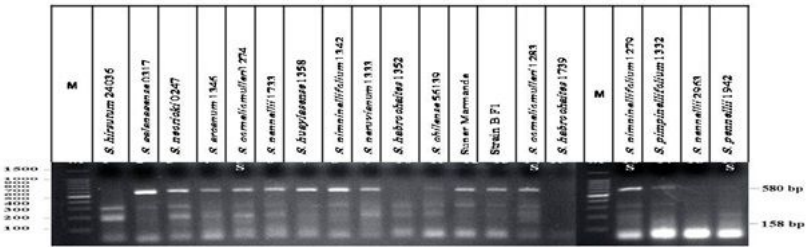

Figure 3

PCR fragments represent primer pair Ve SNPamplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder; S= susceptible genotypes.

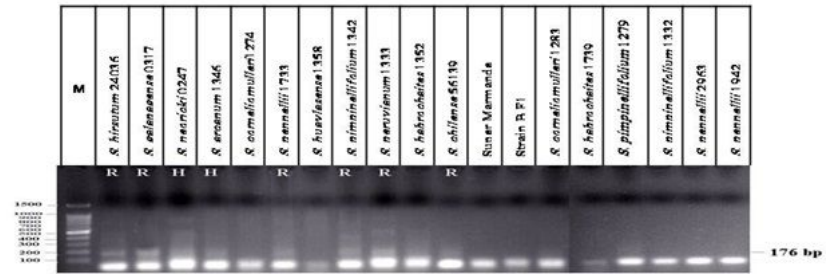

Figure 4

PCR fragments represent primer pair SCAR Ph3amplified from 19 tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder; R= homozygous resistant genotypes; H= heterozygote resistant genotypes.

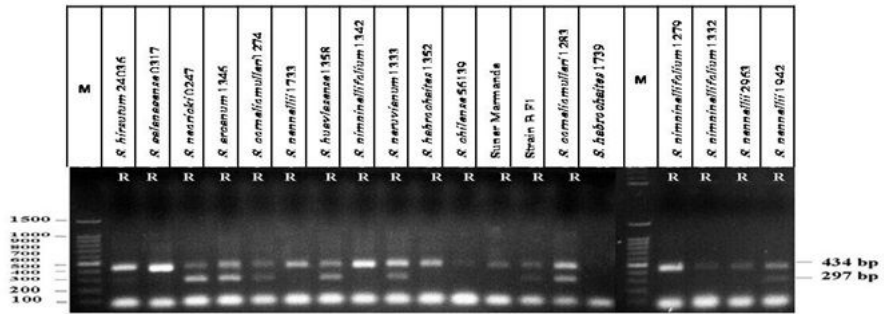

Figure 5

PCR fragments represent primer pair InDel2_Cf-9/Cf-4amplified from 19 tested tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder, R= homozygous resistant genotypes

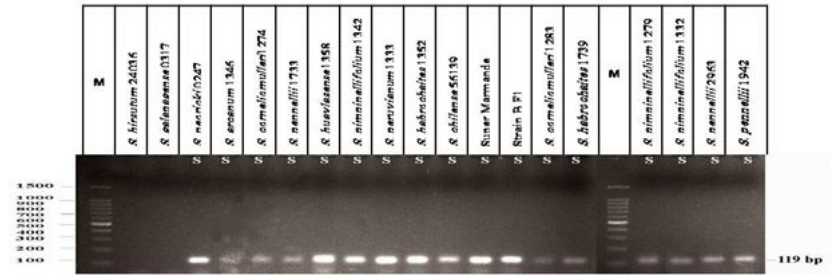

Figure 6

PCR fragments represent primer pair pcc12 Indel Rx4marker amplified from 19 tested tomato genotypes, resolved in 1% agarose gel. Lane M: 100 bp DNA ladder; S= susceptible genotypes.

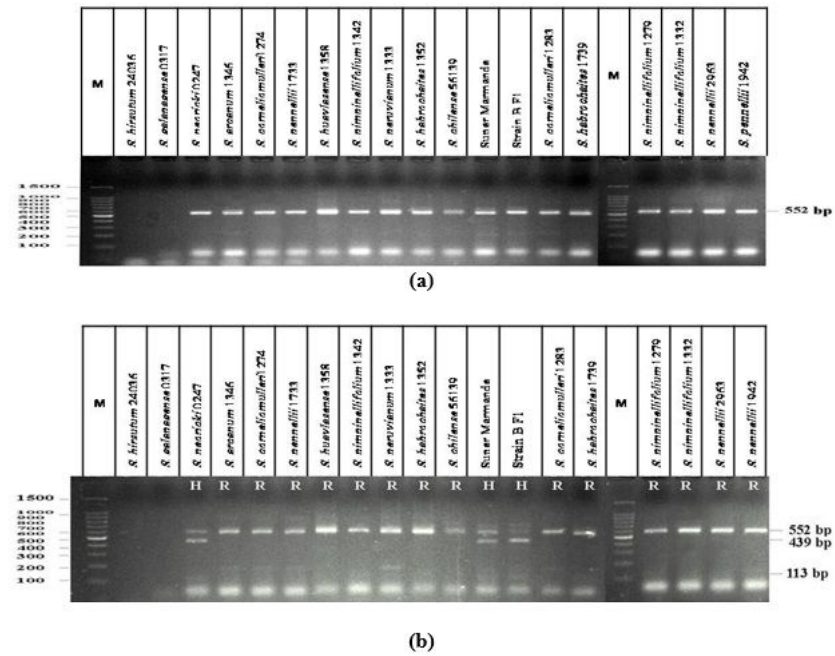

Figure 7

(a) PCR profiles of Pto amplified by CAPS marker from 19 tomato genotypes.(b) RsaI digestion of PCR products amplified by CAPS marker. Lane M= 100 bp DNA ladder; R= homozygous resistant genotypes; H= heterozygote resistant genotypes.