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Marker-assisted selection and pyramiding of *I*1 and *Ph3* genes for multiple disease resistance in tomato through PCR analysis

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Abstract

Fusarium wilt and late blight are the most devastating diseases of tomato that causes significant yield loses all over the world. Genetic host resistance is the most effective way to control these problems. Marker assisted selection (MAS) was carried out to screen 46 genotypes of tomato for the presence of *Fusarium* wilt resistance gene (*I*1) and late blight *resistance gene* (*Ph*3). For this purpose allele specific SSR marker Tom-144 and SCAR marker SCU602 were used that are tightly linked to *I*1 and *Ph*3 genes, respectively. In the present study 17 genotypes showed the presence of *I*1 gene and 13 genotypes showed the presence of *Ph3* gene while five genotypes were found to possess both *I*1 and *Ph*3 genes. Thus we have successfully pyramided *I*1 and *Ph*3 genes into five tomato lines that are accessions 1008, 017878, 017868, 0101 and 1002. These genotypes are highly resistant to both *Fusarium* wilt and late blight of tomato and thus should be released as resistant inbred lines for general cultivation by farmers.

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Introduction

Fusarium wilt is one of the most devastating diseases of tomato all over the world (Abdel- Monaim, 2012). *Fusarium oxysporum f. sp. Lycoperseci* is the causal organism for wilting in tomato. It may causes up to 80% yield reduction. Similarly late blight of tomato caused by *Phytophthora infestans* is also a serious problem of tomato. A number of fungicides have been used to control these diseases (Liggit *et al*., 1997) but the use of fungicides is not suitable because it pollute the atmosphere (Lumsden and Locke, 1989) as well as increase the cost of production (Song and Goodman, 2001).

Several fungal diseases can be present in the same field causing increase in both the environmental impact and financial cost of tomato. So, developing varieties with multiple disease resistance is desirable. Host plant resistance is an effective approach to control fungal diseases of tomato (Singh, 2005).

Marker assisted technology can potentially overcome at least some of the restrictions related with phenotypic selection, major that they are "neutral" in phenotypic responses, that is, they do not have any pleiotropic effect on the phenotype, nor are they influenced in their segregation and inheritance by the growing conditions of the plant. In addition, molecular markers can be detected at any growth stage, strengthening the probability of selecting plants on the basis of expediency to the breeder, in contrast to the season-bound nature of phenotypic selection (Foolad and Panthee, 2012).

Parmar *et al.,* (2013) recommended a set of SSR primer TOM-144 that can be used as a marker for the identification *Fusarium* wilt resistance gene *I*1. Similarly, Truong *et al.,* (2013) developed a sequence characterized amplified region (SCAR) marker SCU602 that is tightly linked to *Ph*3 gene that provide resistance against late blight in tomato.

In the present study SSR Marker TOM144 and SCAR marker SCU602 were used for molecular screening of *Fusarium* wilt resistance gene *I*1 and *Phytophthora infestans* resistance gene *Ph*3 in selected tomato germplasm, in order to pyramid these genes in a single genotype to develop multiple resistance against fungal diseases.

Materials and methods

Plant material used in this study comprised of 46 tomato genotypes. Seeds of 18 genotypes were obtained from the Gene Bank of Plant Genetic Resource Institution (PGRI), National Agriculture Research Centre (NARC) Islamabad while 28 genotypes were obtained from Hazara Agricultural Research Station (HARS) Abbottabad Pakistan. Plant material was grown at HARS, Abbottabad during the year 2014 (Fig. 1).

Mature seeds of these genotypes were grow in randomized complete block design (RCBD) in small pots. After 40 days seedlings were transplanted from pots to the field. Distance between the plants and rows was kept 20 cm, plant protection measures and standard agronomic practices were adopted.

DNA extraction and PCR analysis for the presence of (I1) and (Ph3) genes

Young healthy leaves of each genotype were taken in 1.5 ml Eppendorf tubes and put immediately in liquid nitrogen. Then crushed with squashing needles and 500µl of 2x CTAB buffer (50mM Tris-HCl, pH 8.0, 25mM EDTA, 300mM NaCl and 2% CTAB) was added to it, incubated at 65° C for 1 hour and 500µl Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) was added and left at room temperature for 30 minutes. These tubes were centrifuged at 8000 rpm for 15 minutes. 400µl clear supernatant was transfer to new tubes and equal volumes of 2-propanol was added and incubated at – 20° C for 2 to 3 hours then spin at 8000 rpm for 10 minutes to form DNA pellet. Supernatant was discarded and pellet was washed with 70% ethanol. The tubes were kept invert for few hours to become dried. Then 50µl TE buffer was added to each tube. 5µl DNA sample of each genotype was checked by electrophoresis on 1% agarose gel and stained with ethidium bromide. The concentration of extracted genomic DNA was measured by

Spectrophotometer and was adjusted from 20 to 50 ng/µl by using sterilized distilled water and stored in Eppendorf tubes at 4°C for further use (Ali *et al*., 2016).

Amplification of *I*1 and *Ph*3 genes were carried out using allele specific primers Tom 144 and SCU602, respectively (Table 1). Amplification reactions was carried out in 16 ul reaction volumes containing 1µl genomic DNA (20-50 ng/µl), 0.5µl each of forward and reverse primers (10 µM / µl), 1.2µl of dNTPs (25 mM each) , 0.4 µl of Taq DNA Polymerase (2 units, Enzymomix), 1X Taq Buffer and 1.6µl $MgCl_2$ (2.5 mM).

PCR amplification for TOM-144 was carried out in DNA Thermal Cycler (Applied Bio System) set at an initial denaturation of 5 min at 94°C; 32 cycles of 94° C for 45 sec, 52°C for 45 sec, and 72°C for 45 sec. One additional cycle of 7 min at 72°C was used for final extension. While amplification profile of SCU602 consisted of an initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30s, 55°C for 1min and 72°C for 1min. A final step at 72°C for 10 min was as final extension. Amplified products were resolved by electrophoresis on 2% agarose gel run in 1 X TAE buffer. The amplified products were observed under UV light after staining with ethidium bromide (10 ug/ml). The data was scored for the presence of *I*1 and *Ph*3 genes linked DNA fragments.

Results and discussion

PCR analysis

A molecular survey was conducted for the identification of *I*1 and *Ph*3 genes. Among the 46 selected tomato genotypes 17 showed the presence of *I*1 gene, while the remaining genotypes were lacking this gene. The genotypes that possessed *I*1 gene include 1008, 017868, 1311,017878, 017859, 1173, 017869, 0101, sashaltai, 017890, 017909, 1315, 017872, 017870, 1002, kht-5 and 017887.

Table 1. Detail of primers used in this study.

Primer	Sequence (5' – 3')	Linked gene	Reference
Tom 144	(F) CTGTTTACTTCAAGAAGGCTG	*I*1	Parmar *et al.*, 2013
	(R) ACTTTAACTTTATTATTGCGACG		
SCU602	(F) ACAAACTAAATGGCCAAGTG	*Ph*3	Truong *et al.*, 2013
	(R) ATGATAGCTCTTCTCGGGA		

Table 2. PCR analysis of selected tomato genotypes for the presence of *Fusarium* wilt resistance gene (*I*1) and *Phytophthora infestans* resistance gene (*Ph*3).

S/No	Genotypes	Target gene *I*1	Target gene *Ph*3	S/No	Genotypes	Target gene *I*1	Target gene *Ph*3
1	017863	_	+	24	017872	+	±
2	Coldera	_	_	25	017870	+	±
3	1008	+	+	26	017903	_	+
4	017868	+	+	27	Nepoli	_	±
5	1311	+	±	28	1002	+	+
6	017878	+	+	29	017882	_	±
7	1003	_	_	30	017883	_	_
8	Angina	_	±	31	Naqeeb	_	+
9	9601	_	_	32	Anahi	_	_
10	Roma	_	_	33	017906	_	_
11	1315	_	±	34	Kht-5	+	_
12	017856	_	_	35	1314	_	+
13	017859	+	_	36	0201	_	+
14	1173	+	±	37	Zhezha	_	±
15	017869	+	±	38	017904	_	+
16	0101	+	+	39	Zarnita	_	_
17	017862	_	±	40	Longkeeper	_	_
18	Sashaltai	+	±	41	017887	+	±
19	1004	_	_	42	Bushbeef	_	_
20	017890	+	_	43	Subarctic	_	_
21	017909	+	_	44	Riogrande	_	_
22	1315	+	±	45	017902	_	±
23	017871	_	±	46	1219	_	+

± = heterozygous

Similarly 13 genotypes showed the presence of *Ph*3 gene. These genotypes include accession 017863, 1008, 017868, 017878, 0101, Roma, 017903, 1002, 1314, 0201, 017904, 1219 and Naqeeb.

In the present study five genotypes were found to possess both *I*1 and *Ph*3 genes.

Thus we have successfully pyramided *I1* and *Ph3* genes into five tomato lines that are accessions 1008, 017878, 017868, 0101 and 1002. These genotypes are highly resistant to both Fusarium wilt and late blight of tomato and thus should be released as resistant inbred lines for general cultivation by farmers.

Fig. 1. Pyramided tomato lines grown at Hazara Agricultural Research Station (HARS), Abbottabad, Khyber Phakhtunkhwa Pakistan.

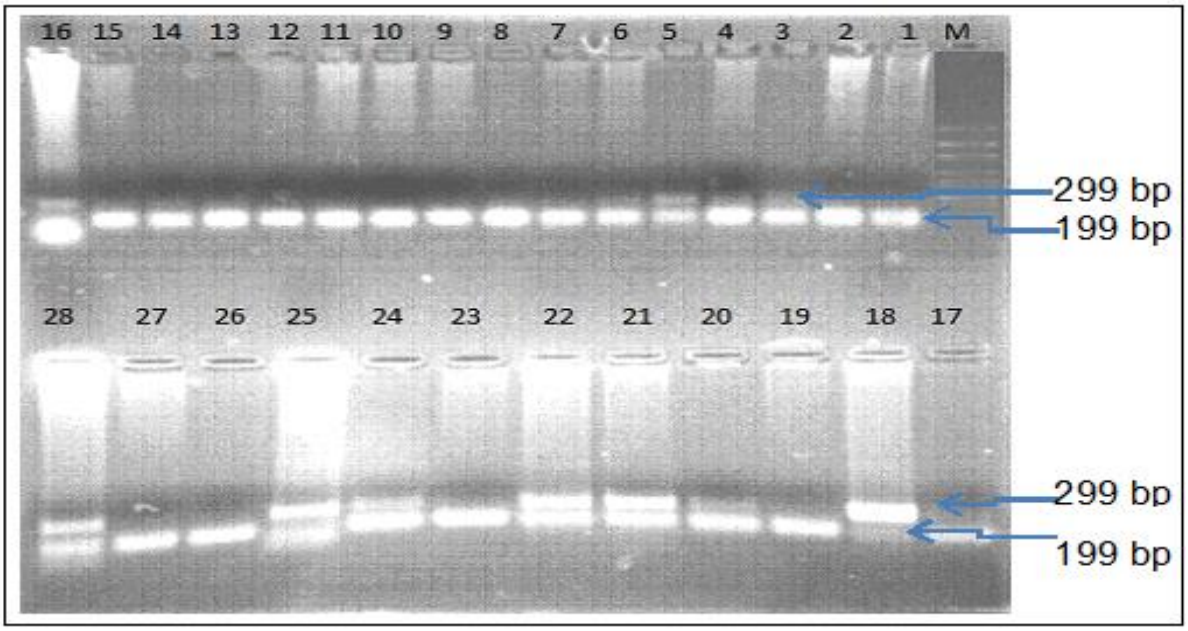

Fig. 2. PCR analysis of tomato genotypes for the presence of *I*1 gene using SSR primer Tom 144. Genotypes having both 299 bp and 199 bp fragments are resistant to race 1 of *Fusarium oxysporum* f. sp. *lycoperseci*.

The data was scored using "+" sign for presence and "–" sign for absence of gene (Table 2 and Fig. 2, 3).

Development of resistant germplasm is the most effective and environment friendly approach for the management of tomato diseases. In breeding programs which intend to introgressed novel traits into breeding lines, such as pathogen resistance, bioassays are necessary to assess the inheritance of the introgressed trait in the breeding lines. Additionally, breeding programs often plan at introducing genes for resistance to two or more different diseases concurrently (Stevens and Rick, 1986). Exposing plants in the breeding programs to infection by numerous pathogens may be troublesome, and sometimes may lead to loss of important breeding resources. Hence, the development of reliable molecular markers would be useful for prompt screening of progeny lines for the presence of resistance genes.

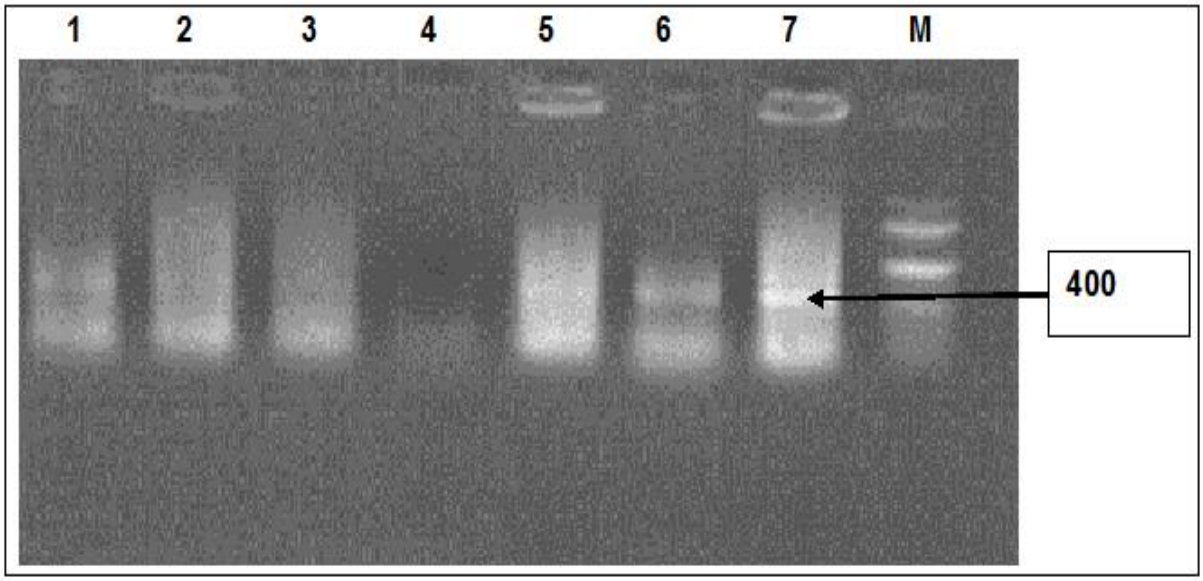

Fig. 3. PCR analysis of tomato genotypes for the presence of *Ph*3 *gene* using SCAR primer SCU602. Genotypes having 400 bp fragments are resistant to *Phytophthora infestans* race 2.

Keeping in view the importance of molecular markers in tomato breeding programs, Parmar *et al.,* (2013), recommended a set of SSR marker TOM-144 for the identification of *Fusarium wilt* resistant genotypes among the tomato germplasm. It has different allele size that is 199 + 299 base pair that can be amplified in resistant genotypes only in contrast to susceptible one with 199 base pair allele only. TOM 144 is linked to *I*1 gene that confer resistance against race 1 of *Fusarium oxysporum f.sp. Lycoperseci* causing wilt disease in tomato.

Similarly, Truong *et al.,* (2013), developed a sequence characterized amplified region (SCAR) marker SCU602 that is tightly linked to *Ph*3 gene that provide resistance against late blight in tomato was also used in the present study.

The successful amplification of these markers was useful in pyramiding *I*1 and *Ph*3 genes in five accessions that are highly resistant to both *Fusarium* wilt and late blight of tomato.

Conclusion

In the present study 17 genotypes out of 46 showed the presence of *Fusarium wilt* resistance gene (*I*1) and 13 genotypes showed the presence of *Phytophthora infestans* resistance gene (*Ph*3). Five genotypes that are accessions 1008, 017878, 107868, 0101 and 1002 were found to possess both *I1* and *Ph3* genes. Thus we recommend these five genotypes for general cultivation by farmers.

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