



Exploring allelic status of selected disease resistance genes in a set of tomato genotypes using gene-linked molecular markers

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ABSTRACT

The production of the world-wide important vegetable crop tomato is threatened by different diseases and pests. Naturally, breeding approaches targeting the development of disease and pest resistant tomato genotypes is of pivotal importance. With the availability of molecular markers, efficient selection of resistant genotypes in environment-independent manner has become feasible. In this study, we report the identification of resistance alleles for 4 important tomato diseases (i.e., tomato leaf curl, tomato root knot, tomato late blight and tomato mosaic) in 20 tomato genotypes using molecular markers. The *Ty3* resistance allele (for leaf curl disease) was found in 5 genotypes, the *Mi1.2* resistance allele (for root knot disease) was found in 2 genotypes and the *Ph3* resistance allele (for late blight disease) was found in 9 genotypes. The tetra-primer amplicon refractory mutation system (tetra-primer ARMS) assay for the *Tm2* resistance gene (for tomato mosaic disease) revealed the resistance allele in 1 genotype.

Keywords: Molecular markers, tomato late blight, tomato leaf curl, tomato mosaic, tomato root knot

Tomato (*Solanum lycopersicum* L., $2n = 2x = 24$) is a globally extensively grown and highly consumed (fresh as well as in processed forms) vegetable crop. Tomato production is threatened by prevalence of several diseases and pests. Among the different diseases and pests, incidence of tomato leaf curl disease has been reported to cause even a complete crop-loss (Reddy *et al.*, 2011). In a similar manner, heavy occurrence of late blight disease has been documented to cause complete crop-loss in a short span of time (Lima *et al.*, 2009). The root knot disease has been reported to be a major problem, particularly in protected cultivation of tomato and has been reported to cause ~25 to 100 % yield loss (Seid *et al.*, 2015). In addition, the occurrence of tomato mosaic disease has been documented as a serious threat to tomato production (Bhandari, 2018). In order to protect the crop from a massive load of diseases and pests, the farmers mainly depend on application of chemical pesticides, which have become notorious from the point of view of environmental and health related issues associated with them. Genetic resistance to diseases and pests has naturally gained pivotal importance in tomato breeding. However, proper screening of disease resistance has remained a challenge to the breeder, as occurrence of diseases depends on several environmental factors (Peries, 1971), which are tough to create through epiphytotic conditions. Development of DNA-based reproducible molecular markers linked to disease resistance alleles has provided

a viable alternative in this regard, where resistance alleles can be identified in environment-independent manner. Several molecular markers have been and are being developed in tomato (Foolad, 2007; Foolad and Panthee, 2012), which has paved the way for marker assisted selection (MAS) in molecular breeding programmes of tomato.

Among the different viral diseases of tomato, leaf curl disease, transmitted by white fly has remained as a serious threat to tomato production, owing to the destructive capability of the virus. Till date, 6 resistance/tolerance genes (*Ty1* to *Ty6*) have been reported in wild relatives of tomato, where *Ty1*, *Ty3*, *Ty4* and *Ty6* have been reported in *Solanum chilense*, *Ty2* in *Solanum habrochaites* and *ty5* in *Solanum peruvianum* (reviewed in Dhaliwal *et al.*, 2019). Among them, *Ty1* and *Ty3* genes present on chromosome 6 have been documented to be allelic (Verlaan *et al.*, 2013) and *Ty3* has been shown to provide high level of resistance (Prasanna *et al.*, 2015). The co-dominant sequence characterized amplified region (SCAR) marker P6-25 for detection of the *Ty3* resistance allele has been developed (Ji *et al.*, 2007) and widely used in breeding programmes. In case of the root knot disease caused by the nematode *Meloidogynespp.*, 9 resistance genes (*Mi1* to *Mi9*) have been reported (reviewed in El-Sappah *et al.*, 2019). The *Mi1* locus present on chromosome 6 has been documented to contain 2 open reading frames (ORFs) along with a pseudo gene (*Mi1.1*, *Mi1.2* and *Mi1.3*),

where *Mil.2* has been documented to be most useful for resistance to nematode and also for resistance to potato aphid (Milligan *et al.*, 1998; Rossi *et al.*, 1998). Though different molecular markers for the detection of the *Mil.2* resistance allele have been developed (Devran and Elekçioflu, 2004; Bendezu, 2004; El Mehrach *et al.*, 2005), the Mi23 SCAR marker (Garcia *et al.*, 2007) has been found to be most useful (Bhavana *et al.*, 2019). Among the different resistance genes for tomato late blight disease caused by *Phytophthora infestans*, the *Ph3* gene from *S. pimpinellifolium* present on chromosome 9 has been documented to provide incomplete but broad resistance against different isolates of *P. infestans* (Black *et al.*, 1996; Zhang *et al.*, 2013). In a recent past, a gene-based SCAR marker (Ph3-SCAR) for detection of the *Ph3* resistance allele has been developed (Jung *et al.*, 2015) as a robust molecular diagnostic tool. The *Tm2* and *Tm2²* resistance alleles on chromosome 9 from *S. peruvianum* has been documented to provide resistance against tomato mosaic virus race 0, 1 and virus races 0,1 and 2, respectively (Lanfermeijer *et al.*, 2003; Lanfermeijer *et al.*, 2005). Depending upon the sequence variation existing between the susceptible (*tm2*) and resistance (*Tm2* or *Tm2²*) alleles, a robust tetra-primer amplicon refractory mutation system (T-ARMS) assay has been developed for the detection of *Tm2* (or *Tm2²*) resistance allele in tomato genotypes (Arens *et al.*, 2010).

In this study, we used the P6-25 SCAR, Mi23 SCAR, Ph3-SCAR and *Tm2* T-ARMS markers discussed above for detection of *Ty3*, *Mil.2*, *Ph3* and *Tm2* resistance alleles, respectively, in a collection of 20 tomato genotypes. We observed the presence of *Ty3* resistance allele in 5 genotypes, *Mil.2* resistance allele in 2 genotypes, *Ph3* resistance allele in 9 genotypes and *Tm2* resistance allele in 1 genotype. The dendrogram prepared on the basis of presence and absence of these resistance alleles grouped the genotypes in 4 clusters indicating the target recipient and donor (for single or double resistance alleles) parents for future molecular breeding programme to develop multiple disease resistant lines in tomato.

MATERIALS AND METHODS

Plant materials

Detail of the tomato genotypes used in this study is presented in table 1.

Genomic DNA extraction and polymerase chain reaction (PCR)

Genomic DNA of the 20 tomato genotypes was isolated from the young leaves through a rapid DNA isolation protocol (Kumar *et al.*, 2017). From the isolated genomic DNA, 2 μ l was used for a 12 μ l PCR volume

containing 1X reaction buffer [10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ and 0.1 % (v/v) Triton X-100], 0.1 mM dNTPs, 5 pmole of each forward and reverse primers (Table 2) and 1U of *Taq* DNA polymerase (Xcelris). PCR was programmed as initial denaturation for 4 min at 94 °C, 40 cycles of denaturation at 94 °C for 30 s, annealing at appropriate temperature (Table 1) for 30 s and extension at 72 °C for 30 s followed by final extension at 72 °C for 7 min and hold at 4 °C for 2 min. The amplicons generated were visualized and imaged in gel documentation system (Genei, Bangalore) after electrophoresis in 1.5 (for P6-25 SCAR, Mi23 SCAR and *Tm2* tetra-primer ARMS assays) or 2.5 (for Ph3-SCAR assay) % (w/v) agarose gel containing ethidium bromide.

Construction of dendrogram

The amplicon profile generated by all the markers were converted to 1/0 matrix on the basis of the presence/absence of a specific band. This matrix was used to generate Dendrogram in Newick format using the web tool D-UPGMA (<https://usuaris.tinet.cat/debb/UPGMA/>). The phylogenetic tree was visualized using the web tool TreeDyn (http://www.phylogeny.fr/one_task.cgi?task_type=treedyn).

RESULTS AND DISCUSSION

Genomic DNA isolated from the 20 tomato genotypes were subjected to polymerase chain reaction (PCR) to amplify resistance or susceptible alleles of the *Ty3*, *Mil.2*, *Ph3* and *Tm2* disease resistance genes. The co-dominant P6-25 SCAR marker for the *Ty3* gene has been reported to produce resistance allele-specific ~450 bp amplicon and susceptible allele-specific ~320 bp amplicon. Among the 20 tomato genotypes of the present study, 5 genotypes (BRDT-1, Kashi Aman, Kashi Chayan, VRTOLCV-16 and VRTOLCV-32) were found to carry the resistance allele at the *Ty3* locus (Fig. 1A). In case of the *Mil.2* gene, the Mi23 SCAR marker-derived amplicons of ~380 bp and ~430 bp have been reported to be resistance and susceptible allele-specific, respectively. In the present study, only 2 genotypes (IIHR-2614 and H-88-78-1) were found to possess the resistance allele at *Mil.2* locus (Fig. 1B). The gene-based co-dominant Ph3-SCAR marker has been developed to generate resistance and susceptible allele-specific ~176 bp and ~154 bp amplicons, respectively (Jung *et al.*, 2015). In this study, we identified the resistance allele at the *Ph3* locus in 9 genotypes (Superbug SPS, Aka Vikash, IIHR-2612, Kashi Chayan, VRTOLCV-16, VRTOLCV-32, H-88-78-1, Sel 18 and Sun Cherry) out of 20 (Fig. 1C). The tetra-primer ARMS assay for the *Tm2* gene has been designed to generate ~509 bp common amplicon in all genotypes along with either resistance allele-

Marker assisted detection of disease resistance alleles in tomato

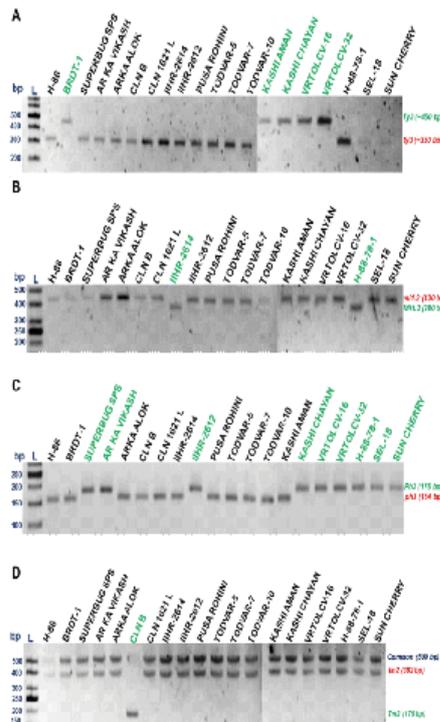


Fig. 1. Detection of resistance alleles through molecular markers.
 A. Inverse image of 1.5 % agarose gel showing presence of *Ty3* resistance allele specific amplicon (~450 bp).
 B. Inverse image of 1.5 % agarose gel showing presence of *Mi1.2* resistance allele specific amplicon (~380bp).
 C. Inverse image of 2.5 % agarose gel showing presence of *Ph3* resistance allele specific amplicon (~176 bp).
 D. Inverse image of 1.5 % agarose gel showing presence of *Tm2* resistance allele specific amplicons (~509 bp + ~179 bp). L = 100 bp DNA ladder in A; 50 bp DNA ladder in B, C and D.

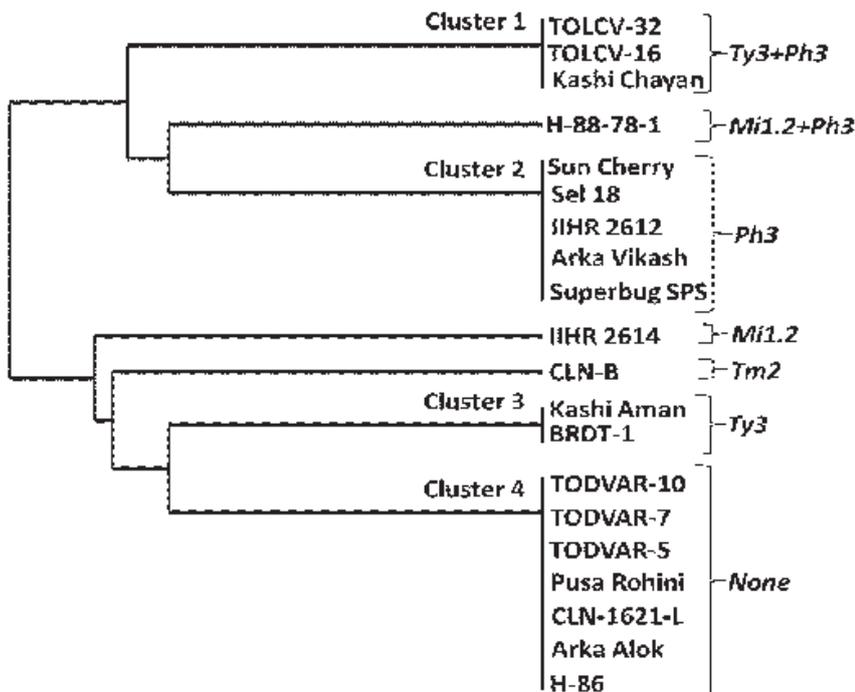


Fig. 2: Dendrogram of 20 tomato genotypes generated on the basis of presence and absence of resistance alleles at the *Ty3*, *Mi1.2*, *Ph3* and *Tm2* loci.

Table 1: Details of the tomato genotypes used in this study

Sl. No.	Genotype	Source	Sl. No.	Genotype	Source
1	H-86	IIVR, Varanasi	11	TODVAR-5	AICRP/2017
2	BRDT-1	BAU, Sabour	12	TODVAR-7	AICRP/2017
3	Superbug SPS	IIVR, Varanasi	13	TODVAR-10	AICRP/2017
4	Arka Vikash	IIHR, Bengaluru	14	KashiAman	IIVR, Varanasi
5	ArkaAlok	IIHR, Bengaluru	15	KashiChayan	IIVR, Varanasi
6	CLN-B	BCKV, WB	16	VRTOLCV-16	IIVR, Varanasi
7	CLN-1621-L	IIVR, Varanasi	17	VRTOLCV-32	IIVR, Varanasi
8	IIHR-2614	IIHR, Bengaluru	18	H-88-78-1	IIVR, Varanasi
9	IIHR-2612	IIHR, Bengaluru	19	Sel 18	IIVR, Varanasi
10	PusaRohini	IIVR, Varanasi	20	Sun Cherry	IIVR, Varanasi

Table 2: Details of molecular markers used in this study

Marker	Forward primer (5' - 3')	Reverse primer (5' - 3')	Annealing Temperature
P6-25 SCAR	GGTAGTGGAAATGATGCTGCTC	GCTCTGCCTATTGTCCCATATATAACC	53 °C
Mi23 SCAR	TGGAAAAATGTTGAATTTCTTTTG	GCATACTATATGGCTTGTTACCC	56 °C
Ph3- SCAR	CTACTCGTGCAAGAAGGTAC	TCCACATCACCTGCCAGTTG	55 °C
<i>Tm2</i> T-Out: ARMS In:	CGGTCTGGGGAAAACAACCTCT CAAATTGGACTGACGGAACAGAAAGTT	Out: CTAGCGGTATACCTCCACATCTCC In: GCAGGTTGTCCTCCAAATTTCCATC	55 °C

specific ~179 bp amplicon or susceptible allele-specific ~382 bp amplicon (Arens *et al.*, 2010). In our study, we identified the resistance allele at the *Tm2* locus to be present only in 1 genotype, *i.e.*, CLN-B (Fig. 1D).

The *Ty1* and *Ty3* gene(s) in tomato have been cloned and found to be allelic (Verlaan *et al.*, 2013) and documented to be effective in providing resistance to leaf curl disease. Naturally, the P6-25 SCAR marker, tightly linked to this gene has been well-explored in molecular breeding programmes and marker assisted selection (Prasanna *et al.*, 2015; Neha *et al.*, 2016; Tabein *et al.*, 2017). In a similar manner, molecular markers developed for cloned and characterized resistance genes *Ph3*, *Mil.2* and *Tm2* have been explored in breeding programmes to pyramid multiple resistance genes in suitable cultivars (Kumar *et al.*, 2019; reviewed in Oladokun and Mugisa 2019). Though most of the individual resistance genes have been documented to provide race-specific vertical resistance, marker assisted pyramiding of similar and/or diverse resistance genes is supposed to provide durable disease resistance in tomato. The gene-based and/or very tightly linked nature of these molecular markers is expected to have high diagnostic value in marker assisted selection. On the basis of the presence or absence of resistance allele-specific amplicons, the 20 genotypes of the present study were clustered. The genotypes were found to be distributed

in 4 major clusters (Fig. 2), where cluster 4 contained 7 genotypes (H-86, Arka Alok, CLN-1621-L, Pusa Rohini, TODVAR-5, TODVAR-7 and TODVAR-10). These lines were found not to contain resistance allele at any of the 4 aforementioned loci. The 3rd cluster contained 2 genotypes, *i.e.*, BRDT-1 and KashiAman. Both these genotypes contained resistance allele at the *Ty3* locus only. The 2nd cluster was composed of 5 genotypes (Superbug SPS, Arka Vikash, IIHR-2612, Sel 18 and Sun Cherry). All these lines were found to possess resistance allele at the *Ph3* locus only. The 1st cluster contained 3 genotypes (KashiChayan, VRTOLCV-16 and VRTOLCV-32). These genotypes were found to contain resistance allele at both the *Ty3* and *Ph3* loci. The genotypes H-88-78-1 (containing resistance alleles at *Mil.2* and *Ph3* loci), IIHR-2614 (containing resistance allele at *Mil.2* locus only) and CLN-B (containing resistance allele at *Tm2* locus, only) were found to be unique, on the basis of their resistance allele profile.

Molecular markers have become indispensable tools in resistance breeding programmes. Owing to their diagnostic values, robust molecular markers facilitate the selection of desirable disease resistant genotypes in environment-independent manner. In the present study, we explored 4 co-dominant markers to identify the resistance alleles at *Ty3*, *Mil.2*, *Ph3* and *Tm2* loci in 20 tomato genotypes. Along with the different resistance

donors (13 genotypes), we identified 7 genotypes lacking all the resistance alleles at the aforementioned loci. These lines can be improved through introgression of single or multiple resistance alleles from potential donors identified in this study. The co-dominant nature of the molecular markers used in this study will facilitate the early-generation identification of fixed loci in segregating populations. Breeding programme in this direction has already been undertaken by us.

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