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Identification of *Tm* Genes for *Tomato mosaic virus* (ToMV) Resistance in Tomato Germplasm

B. N. Ashwini and N. Nagaraju
Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : bnashwini00@gmail.com

Abstract

Use of resistant germplasm is an effective, successful and eco-friendly approach to manage *Tomato mosaic virus* (ToMV) in tomato. Identity of ToMV in tomato was confirmed through reverse - transcriptase polymerase chain reaction (RT-PCR), an amplicon size of 300bp was obtained for coat protein gene of ToMV. Totally, 35 entries of tomato were screened for the identification of resistance source through mechanical inoculation under glasshouse condition. As per the severity scale, six were found resistant, 11 moderately resistant, 12 moderately susceptible, six were susceptible and none were immune to ToMV infection. The presence of *Tm* genes in the resistant once were identified using sequence characterized amplified region (SCAR) and allele specific (AS1) markers for *Tm*-1 and *Tm*-2 genes respectively. Both *Tm*-1 and *Tm*-2 genes were identified in hybrid Arka Rakshak and in lines such as LA 0887, TLB 503-3-2 and CK 12. However, *Tm*-2 alone was present in TLB-409 and TLB-504-1-1, similarly *Tm*-1 was detected in *var*. Sankranti which exhibited moderately resistant response. In case of susceptible hybrid Indus 1030, no amplification of *Tm* genes were noticed indicating *Tm*-1 and *Tm*-2 genes have shown to be responsible for resistance to ToMV infection in tomato germplasm.

Keyword : Screening, *Tomato mosaic virus,* resistant *Tm* genes, SCAR and AS markers

Tomato (*Solanum lycopersicum* L.) is the second most important remunerable Solanaceous vegetable after potato. It is often described as 'poor man's orange' and considered to be an important vegetable grown worldwide. Tomato is native to South America and widely cultivated in around 140 countries of the world (Smith, 1994). Tomato has high nutritional value, it also provides a balanced source of ascorbic acid, vitamin 'A' and 'E' which are needed to maintain good health (Terna *et al*., 2017 & Lokesha *et al.,* 2019).

An extensive cultivation of tomato crop in recent years, have increased many fungal, bacterial, viral and nematode diseases with annual losses amounting millions of rupees. Among viruses infecting tomato, *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), *Tomato leaf curl virus* (ToLCV) and *Tobacco mosaic virus* (TMV) are presently known to contribute consistently to yield losses of tomato crop (Massumi *et al*., 2009 & Kavyashri *et al.,* 2018).

Tomato mosaic virus (ToMV) is the type member of the genus *Tobamovirus* belongs to family *Vignaviridae* found worldwide and affects tomatoes and many other wide host range including agricultural crops and weeds (Castello *et al.*, 1995). ToMV infection causes 34.30 per cent and 59.77 per cent reduction in number and weight of tomato fruits, respectively (Ullah *et al*., 2017). Management of viruses is generally difficult since the viruses are systemic and contagious in nature. No effective management strategies have been developed for the control of ToMV associated with tomato.

However, identification of resistant sources has been considered as the most efficient approach against plant viruses. Keeping these aspects in view, an attempt has been made to identify the relative resistant source *i.e., Tm* genes in tomato germplasm against ToMV disease.

The Mysore Journal of Agricultural Sciences

Material and Methods

Maintenance of ToMV Inoculum

Tomato leaf samples showing characteristic symptoms *viz.,* systemic mosaic, downward leaf curling, leaf deformation and stunting symptoms (Plate 1) were collected from tomato plants grown in experimental plot, Main Research Station, Hebbal, Bengaluru. The virus was propagated through sap inoculation using 0.1 M phosphate buffer (Ashfaq *et al.,* 2010) on ToMV susceptible tomato hyb. Indus 1030 and inoculated plants were kept separately under insect proof cage for symptom expression and the same was used as a source of inoculum for further investigations.

Molecular Detection of ToMV through RT-PCR

ToMV maintained on tomato as a source of inoculum was confirmed through RT-PCR. Total RNA isolation was done from 100 mg of leaf sample by TRIzole reagent (Thermofischer, TRIzol user guide). The isolated RNA was converted to cDNA using BIORAD cDNA conversion kit (Catalog no.1708891) and reverse transcriptase polymerase chain reaction (RT-PCR) was performed using primers designed for ToMV coat protein region from sequence data obtained from NCBI (Table 1).

Table 1

Primer used for amplification of ToMV coat protein (CP) region

Primer name	Primer sequence	Amplification size (bp)
ToMV CPDF	CAGAGTCCGACAACAGCTG	300
ToMV CPDR	GGGTCAGCCCATACAGATG	

Initial denaturation was done at 95 °C for 5 min. followed by 35 cycles consisting of denaturation at 94 °C for 1min., annealing at 53 °C for 30s, extension at 72 °C for 1 min. and final extension for 10 min. at 72 °C. Amplification was confirmed by agarose gel electrophoresis (Sambrook and Russel, 2001).

Tomato Germplasm for ToMV Screening

Thirty five germplasm / lines / varieties of tomato procured from different sources were screened for resistance to ToMV in glasshouse in UAS, GKVK, Bengaluru. Seeds were sown in pro-trays contained coco-peat with nutrient mixture and maintained upto two leaf stage. Then the seedlings were transplanted to polythene covers containing sterilized soil for inoculation studies.

Mechanical Inoculation of ToMV

The tomato plants at two leaf stage were inoculated mechanically with standard sap extract as described

Mosaic Downward curling Leaf deformation

Plate 1 : Tomato leaves showing different types of ToMV symptoms upon mechanical inoculation

by Ashfaq *et al*. (2010). After inoculation, plants were sprayed with water to remove superfluous inoculum. Tomato hyb. Indus 1030 was used as a susceptible check throughout the studies. Thirty days after inoculation, disease severity was scored with the scale given by Imran *et al*., 2012 (Table 2)

TABLE 2

Disease severity scale for ToMV (0-10) (Imran *et al*., 2012)

Score	Type of symptoms
0	No symptoms
2	Vein clearing
4	50 % of leaves showing mosaic symptoms
6	100 % of leaves showing mosaic symptoms
8	50 % of leaves showing severe mosaic and malformation
10	100 % of leaves showing severe mosaic and malformation

and the per cent disease index (PDI) was calculated using formula given by Wheeler (1969).

Based on the PDI values obtained, the genotypes were classified into five categories *viz.*, Immune (I) where PDI=0; resistant (R) where PDI=1-25 per cent; moderately resistant (MR) where PDI = 26-50 per cent; moderately susceptible (MS) where PDI=51-75 per cent and susceptible (S) where PDI=76-100 per cent (Havey, 1996).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

Identification of Resistance Genes for ToMV by SCAR and Allele-Specific Markers

For genotyping of *Tm*-1 and *Tm*-2 loci the following (SCAR and allele-specific) markers were used (Table 2). The DNA was extracted from germplasm / lines / varieties showing resistant reaction *i.e*., visually virus-free tomato plants after 30 days of virus inoculation using CTAB protocol (Edwards *et al*., 1991). Primers were synthesized from Sigma Aldrich and PCR amplification for both the markers were performed in 25 µl reaction mixture consisted of TAKARA PCR Mastermix 12.5 µl, 2 µl (20pmol / µl) of each primer, 100 ng genomic DNA as template and volume was made to 25 µl by adding double distilled water. PCR condition for the reaction consisted of initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30s, annealing at 55 °C and 58 °C for SCAR and AS markers, respectively for 30s, 72 °C for 1 min and a ûnal extension at 72 °C for 10 min was executed. The amplification was confirmed by agarose gel electrophoresis (Sambrook and Russel, 2001).

RESULTS AND DISCUSSION

Molecular Confirmation of ToMV Infecting Tomato

The successful amplification of CP region of ToMV using RT-PCR was obtained with an expected size of ~300 bp in infected tomato samples but not in healthy samples of tomato (Plate 2). The ToMV culture was maintained through sap inoculation of confirmed infected samples and

TABLE 3

SCAR and allele-specific markers for genotyping *Tm*-1 and *Tm*-2 loci

Marker name	Resistant allele	Primer name	Primer sequence	Source
SC110	*Tm*-1	TM1SCF1	ACAACGCGAGGCCAAATCCCATCA	Pasev *et al*., 2016
		TM1SCR1	ACAACGCGAGTAGGTTTAGGGTG	
AS1	*Tm*-2	TM2SF1	CAGTGATCCGAGTGAGCAAA	
		TM2SR1	TTCCGATAAACTGATTCCGC	

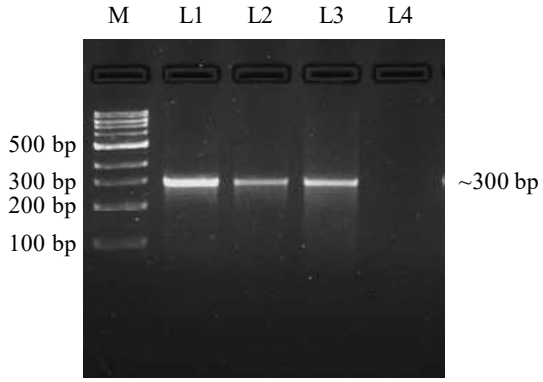

Plate 2 : Agarose gel picture showing amplification of CP gene of ToMV in infected tomato samples

Lane M : 100bp ladder (Thermoscientific)
Lane 1 : Positive control
Lane 2 and 3 : ToMV infected sample
Lane 4 : Non template control

the same is used as ToMV inocula for screening the germplasm / lines / Varieties. Arinaitwe *et al*. (2018) showed the presence of ToMV by detection of PCR fragment of expected size (450bp) showing 99.0 per cent identity with ToMV accessions AJ132845, FN985165 from China.

Screening for Resistance Against *Tomato mosaic virus*

Out of 35 germplasm / lines / varieties screened for resistance against ToMV, none of them showed immune reaction whereas, six germ plasm / lines / varieties *viz.*, TLB-409, CK 12, LA 0887, TLB-504-1-1, TLB-503-3-2 and Arka Rakshak with PDI 1-25 per cent were categorized as resistant and 11 germplasm/ lines / varieties (TLB-514-2-1, TLB-511-2-1, TLB-504-2-1, TLB-514-3-1, TLB- 409-1-2, TLB-503-2-1, TLB-509-1-1, TLB-416-1-4, LA 3914, LA 3924 and Sankranthi) as moderately resistant. 12 germplasm / lines / varieties *viz*., TLB-407-1-4, TLB-414-1-2, TLB-511-1-1, TLB-514-2-7, Nandi, Arka Meghali, Arka Sourabh, KTR, Vybhav, Prabhat, TY-12 and TY-11 and six germplasm / lines / varieties (TLB-503-1-1, TLB-601-1, Arka Alok, Arka Vikas, Arka Avinash and Indus 1030) with PDI between 51-75 per cent and 76-100 per cent exhibited moderately susceptible and susceptible reaction respectively (Table 4 and Table 5).

TABLE 4

Phenotypic reaction of tomato germplasm / lines / varieties to *Tomato mosaic virus* infection under glasshouse conditions

Germplasm / lines / varieties	Disease severity (%)	Disease score	Disease reaction
TLB-414-1-2	57.78	3	MS
TLB-514-2-1	46.67	2	MR
TLB-503-1-1	75.56	4	S
TLB-407-1-4	66.67	3	MS
TLB-511-2-1	48.89	2	MR
TLB-601-1	82.22	4	S
TLB-504-2-1	35.56	2	MR
TLB-514-3-1	46.67	2	MR
TLB- 409-1-2	31.11	2	MR
TLB-503-3-2	2.22	1	R
TLB-503-2-1	31.11	2	MR
TLB-509-1-1	46.67	2	MR
TLB-504-1-1	17.78	1	R
TLB-511-1-1	66.67	3	MS
TLB-514-2-7	64.45	3	MS
TLB-416-1-4	40.00	2	MR
TLB-409	11.11	1	R
CK 12	15.56	1	R
TY-11	55.56	3	MS
TY-12	51.12	3	MS
LA 0887	15.56	1	R
LA 3924	33.33	2	MR
LA 3914	33.33	2	MR
Nandi	57.78	3	MS
Sankranthi	26.67	2	MR
Prabhat	60.00	3	MS
Vybhav	52.32	3	MS
KTR	57.78	3	MS
Arka Rakshak	20.00	1	R
Arka Meghali	57.78	3	MS
Arka Sourab	73.33	3	MS
Arka Alok	80.00	4	S
Arka Avinash	75.56	4	S
Arka Vikas	80.00	4	S
Indus 1030	82.22	4	S

R - Resistant, MR - Moderately Resistant, MS - Moderately Susceptible, S - Susceptible

TABLE 5

Grouping of tomato germplasm / lines / varieties based on phenotypic reaction to ToMV disease under glasshouse conditions

PDI (%)	Disease reaction	Germplasm/ lines/ varieties
0	Immune	-
1-25	Resistant	TLB-409, CK 12, LA 0887, TLB-504-1-1, TLB-503-3-2, Arka Rakshak
26-50	Moderately resistant	TLB-514-2-1, TLB-511-2-1, TLB-504-2-1, TLB-514-3-1, TLB- 409-1-2, TLB-503-2-1, TLB-509-1-1, TLB-416-1-4, LA 3914, LA 3924, Sankranthi
51-75	Moderately susceptible	TLB-407-1-4, TLB-414-1-2, TLB-511-1-1, TLB-514-2-7, Nandi, Arka Meghali, Arka Sourabh, KTR, Vybhav, Prabhat, TY-12. TY-11
76-100	Susceptible	TLB-503-1-1, TLB-601-1, Arka Alok, Arka Vikas, Arka Avinash, Indus 1030

The findings of screening experiment are in line with reports of Ullah *et al*. (2017) who screened twenty different genotypes against ToMV, among them one genotype (017902) was highly resistant, one resistant (017883), four moderately susceptible and 15 were found susceptible.

DNA isolation was done from six resistant (TLB-409, CK 12, LA 0887, TLB-504-1-1, TLB-503-3-2, Arka Rakshak) and from one moderately resistant (Sankranti) germplasm / lines / varieties. However, Indus 1030 was used as susceptible control. Amplification for both SC110 and AS1 markers at an expected size of 1100 bp and 310 bp, respectively, was observed in Arka Rakshak, LA 0887, TLB 503-3-2 and CK 12 showing the presence of both *Tm*-1 and *Tm*-2 loci substantiated the reason for resistance against ToMV. But, in TLB-409 and TLB-504-1-1 amplification was seen only to AS1 marker (*Tm*-2) indicating that *Tm*-2 was sufficient to impart resistance against ToMV. In case of Sankranthi which was moderately resistant to ToMV presence of *Tm*-1 was observed but not *Tm*-2. Susceptibility of Indus 1030 to ToMV was proved by the absence of both *Tm*-1 and *Tm*-2 loci since no amplification was seen against SC110 and AS1 markers respectively (Plate 3 and Plate 4).

The results are in agreement with that of Pasev *et al*. (2016) who reported that *Tm*-1 confers resistance to pathotype 0 and 2, but not to pathotype 1 of ToMV. They found that pheno

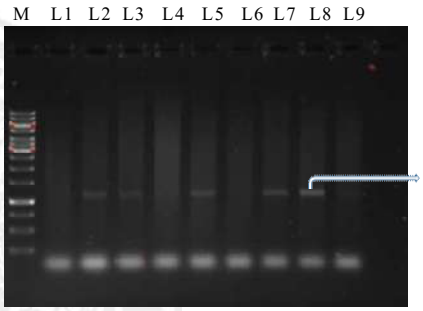

Plate 3 : Agarose gel electrophoresis of SCAR marker (SC110) of *Tm*-1 locus

Lane M: 1000bp ladder
Lane 1: Non template control
Lane 2: Arka Rakshak
Lane 3: CK 12
Lane 4: TLB-409
Lane 5: LA 0887
Lane 6: TLB 504-1-1
Lane 7: TLB 503-3-2
Lane 8: Sankranthi
Lane 9: Indus 1030

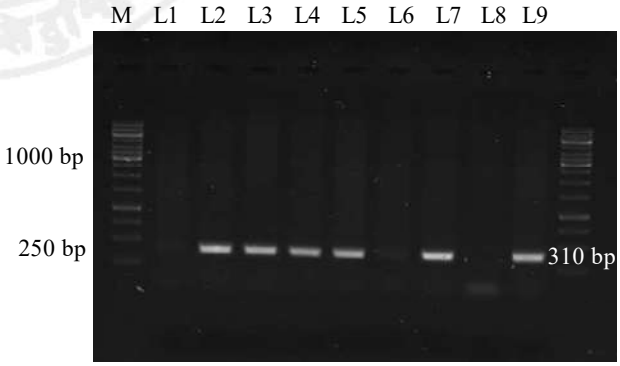

Plate 4 : Agarose gel electrophoresis of allele specific marker (AS1) of *Tm*-2

Lane M : 1000bp ladder
Lane 1 : Non template control
Lane 2 : Arka Rakshak
Lane 3 : CK 12
Lane 4 : TLB 503-3-2
Lane 5 : LA 0887
Lane 6 : Sankranthi
Lane 7 : TLB 504-1-1
Lane 8 : Indus 1030
Lane 9 : TLB 409

The Mysore Journal of Agricultural Sciences

typic expression of *Tm*-1 would have been masked in infection tests if the genotype possesses resistant allele of *Tm*-2 locus. Genotypes possessing the resistant allele of the both loci (*Tm*-1 and *Tm*-2) were resistant to all three pathotypes.

The resistant source identified against ToMV in tomato can be used as a resistant source in various crosses to transfer resistant trait in susceptible varieties having good agronomic characters. The determination of genetic basis of their resistance *i.e.,* presence of natural resistant genes *Tm*-1 and *Tm*-2 genes could be explored in resistance breeding programmes. The molecular domains of *Tm*-1 and *Tm*-2 genes can be discovered to identify the domain which impart resistance and its signals against ToMV by interfering virus synthesis and systemic movement.

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