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Validation of molecular markers for multiple disease resistance in tomato (*Solanum lycopersicum*)

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ABSTRACT

Marker assisted breeding has been successfully used for selecting disease resistance by identifying genetic markers linked to resistance genes/allele. In tomato, availability of molecular markers linked to genes conferring resilience against Tomato leaf curl virus (ToLCV) reaction, late blight, Tomato Mosaic Virus (ToMV) and Tomato gray leaf spot were utilised to develop pyramided MAS derived lines for multiple disease resistance. For this purpose, markers for the tomato leaf curl disease (ToLCD) resistance gene loci *Ty-2, Ty-3* and *Ty-3a,* late blight resistant loci *Ph-3,* ToMV resistant allele $Tm2^2$ and Tomato gray leaf spot resistant gene *Sm* were validated using PCR assay. The assay correctly predicted the genotypes of tomato breeding lines harbouring resistant as well as susceptible alleles at each loci. Duplexing PCR assay combining two SCAR markers (T0302 and P6-25) for detecting simultaneously 3 important resistance gene loci (*Ty-2, Ty-3* and *Ty-3a*) in tomato genotypes and thereby improving the cost efficiency of the PCR assay. Further, we have validated the Tomato leaf curl New Delhi virus (ToLCNDV) infection in tomato leaves by Reverse transcription (RT) PCR with ToLCNDV genome specific *AC4* primers.

Key words: Late blight, Marker-assisted selection (MAS), Tomato, Tomato gray leafspot, Tomato leaf curl virus (ToLCV), Tomato Mosaic Virus (ToMV)

Tomato production is often threatened due to severe losses caused by various pathogens including viruses, bacteria, fungi, and nematodes. Molecular breeding using molecular markers are now being widely used in tomato (Foolad and Sharma 2004). There are more than 40 genes that confer resistance to major classes of tomato pathogens which can be pyramided through MAS, for the introgression of more than one resistance genes into 1 genotype.

In India, tomato leaf curl disease (ToLCD) is one of the most devastating diseases of tomato. Symptoms of ToLCD include stunting, yellowing, leaf curling and flower abortion which results in significant yield loss (Chakraborty *et al.* 2009). Most of tomato cultivars in India are susceptible to ToLCD. However, several wild species of tomato including *Solanum chilense, Solanum habrochaites* and *Solanum peruvianum* (Ji *et al.* 2007a) have been found promising for ToLCD resistance. Currently, 6 resistance genes i. e. *Ty-1*and *Ty-3* on chromosome 6, *Ty-2* on chromosome 11, *Ty-4* on chromosome 3, *ty-5* on chromosome 4 and *Ty-6* on chromosome 10 are available for commercial breeding against ToLCD (Zamir *et al.* 1994, Ji *et al.* 2007b). *Ty-1*and *Ty-3* were demonstrated to be allelic (Verlaan *et al.* 2013). *Ty-2* originated from *S. habrochaites* B6013 (Ji *et al.* 2009a) mapped to a 300 kb interval on chromosome 11 (Yang *et al.* 2014). *Ty-4* (Ji *et al.* 2009b) and *Ty-6* (Hutton and Scott 2013) have also originated from *S. chilense* accessions. Recessive resistance against TYLCV was identified in *S. peruvianum* and designated as *ty-5* (Hutton *et al.* 2012).

The *Ph-3* gene with a coiled-coil nucleotide-binding leucine-rich repeat (NBS–LRR), conferring incomplete resistance against a wide range of *Phytophthora infestans* isolates has been introgressed into cultivated tomatoes from *S. pimpinellifolium* (L3708) (Zhang *et al.* 2014).

Tomato Mosaic Virus (ToMV) disease is another important disease of tomato which causes significant yield losses. Based on molecular studies the $Tm2^2$ allele derived from *Solanum chilense* has proved most efficient in providing resistance against ToMV (Lanfermeijer *et al.* 2003). Tomato gray leaf spot is another devastating disease of tomato caused by *Stemphylium lycopersici.* The *Sm* gene has incomplete dominance and considered as effective source of resistance against 4 species of *Stemphyllium* (Scott and Gardner 2006).

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In this study, different allele specific molecular markers for the *Ty-2, Ty-3, Ty-3a, Ph-3, Tm-2²* and *Sm* resistance loci were validated in available germplasm and F_1 hybrids (Table 1) so that the reproducible markers could be used for Marker-assisted selection (MAS) for gene pyramiding in commercial tomato cultivars. In addition, we have confirmed the ToLCNDV symptoms in the field grown tomato leaf samples ausing one of ToLCNDV gene specific primer *AC4,* using RT PCR.

Table 1 List of representative set of tomato breeding lines for verification of *Ty-2, Ty-3, Ty-3a, Ph-3, Tm2²* and *Sm* alleles

Genotype	Allele R/S
EC904111	R
EC814916	R
EC904112	R
EC814915	R
EC814917	R
Pusa Rohini	S
Pusa Ruby	S
Pusa-120	S
Pusa Sadabahar	S
Pusa Sheetal	S
H-86	S
F_1 (Pusa Ruby × EC814916)	R/S
F_2 (Pusa Ruby × EC814916) populations	R/S
Rohini × EC814916	R/S
H-86 × EC814916	R/S
Pusa-120 × EC814916	R/S
Pusa Rohini × EC814916	R/S
Pusa Sheetal × EC814916	R/S

MATERIALS AND METHODS

The breeding lines and F_1 hybrids used in this study (Table 1) were maintained at the research farm of IARI, New Delhi, India. Young and healthy leaves from each genotype were collected for genomic DNA extraction following the C-TAB method (Murray MG and Thomson 1980). DNA quality and quantity were assessed on a 1% (w/v) agarose gel stained with ethidium bromide (Sigma Aldrich Chemical Pvt. Ltd, Bangalore, India).

Markers used for validation of different genes **(**Table 2**)** were custom synthesized (G- Biosciences, USA). PCR was carried out in 10 µl volumes with 40 ng genomic DNA, 0.5 U *Taq* DNA polymerase (G- Biosciences, USA), 1. 0 µM of each primer, 0.5 µl of 10 mM dNTP mix (G-Biosciences, USA), and 1.0 µl of 10× PCR buffer having 17.5 mM $MgCl_2$ (G- Biosciences, USA). All the primers were amplified using touchdown PCR in an Eppendorf Mastercycler (Germany). For amplification of *Ty* genes the annealing temperatures (T_a) were 53°C for 1 min followed by 72°C for 1 min and a final cycle of 72°C for 5 min for 35 cycles. Amplification conditions used for *Ph-3* genes were, one cycle of 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 57°C for 30 seconds and 72°C for 1 min and final extension at 72°C for 10 min. Amplification conditions for *Tm2²* and *Sm* genes were one cycle of 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 55°C for 30 seconds and 72°C for 1 min and final extension at 72°C for 10 min. Amplified products were resolved on 2.5% agarose gels and observed under UV light (Alpha imager, Cell biosciences, Santa Clara, CA).

Leaf samples for RNA isolation were collected from field, 4 month after transplanting. Total RNA was isolated from the tomato leaves using Spectrum plant total RNA isolation kit (Sigma, USA) following manufacturer instructions. To remove any DNA contamination, RNA samples were subjected to column DNase-I treatment

Table 2 Molecular markers used for validation of *Ty-2 Ty-3 Ty-3a Ph-3 Tm-2²* and *SM* alleles in the genotypes under study and their primers sequences

Resistance Gene	Marker	Resistant allele (bp)	Susceptible allele (bp)	R. E	Primer sequence (5′-3′)	Reference
Ty-2	T0302	900	791	-	F.P: TGGCTCATCCTGAAGCTGATAGCGC R.P: AGTGTACATCCTTGCCATTGACT	Yang *et al.*(2014)
Ty-2	TG36	520	450		F.P: AACCACCACAAGAAAGATCCC R.P: TCCTGAAATGGAAGATTGCC	Schmitz *et al.*(2002)
Ty-3	P6-25	450	320	-	F.P: GGTAGTGGAAATGATGCTGCTC R.P:GCTCTGCCTATTGTCCCATATATAACC	Jensen *et al.*(2007)
Ty-3	TY3-SCAR1	519	269	-	F.P: GCTCAGCATCACCTGAGACA R.P: TGCAGGAACAGAATGATAGAAAA	Dong *et al.*(2016)
Ph-3	TG328	260 + 240	500	*Bst*NI	F.P: GGTGATCTGCTTATAGACTTG GG R.P: AAGGTCTAAAGAAG GCTGGTGC	Robbins *et al.*(2010)
Sm	CT-55	200 + 140	330+200+140	*Dde*I	F.P: CATCTGGTGAGGCGGTGAAGTA R.P: TCCGCCCAAACAAAACAGTAATA	Ji *et al.*(2009)
Tm-2²	NCTm-019	270 + 600	870	*Hae*III	F.P: AATTTGGGCATACTGACATC R.P: GTTGCACACATT□GGTTGTAG	Panthee *et al.*(2013)

(Sigma, USA). 1 µg of RNA was taken for cDNA synthesis (Applied biosystem cDNA synthesis kit). Concentration of cDNA was checked using Nanodrop spectrophotometer (Thermo Scientific, USA). RT PCR was done to check the *AC4* transcripts in the cDNA samples. *AC4* primers (F CTAGAACGTCTCCGTCTTTGTCGATGT, R GGGTCTCCGCATATCCATGTTCTCA) specific to the ToLCNDV viral genome were procured (Sigma Aldrich Chemical Pvt. Ltd, Bangalore, India). The reaction was set up with single strand cDNA (template) 2 µL (100 ng), Buffer (10×) 5 µL, dNTP (10mM) 1.5 µL, *AC4* Forward primer 1 µL (20 pmol), *AC4* Reverse primer 1 µL (20 pmol), Taq polymerase 1 µL (3U), final reaction volume of 50 µL. The amplification was performed in a Thermal cycler (Eppendorf, Germany) at temperatures (T_a), 60°C for 30 seconds followed by 72°C for 30 seconds. Final extension at 72°C for 10 min. Amplified products were visualized on gel.

RESULTS AND DISCUSSION

Validation of markers for Ty-2, Ty-3 and Ty-3a genes*:* The markers for ToLCD resistance were identified from wild species like *S. chilense* (*Ty-1, Ty-3, Ty-4 and Ty-6*), *S. habrochaites* (*Ty-2*) and from *S. peruvianum* (*ty-5*) (Ji *et al.* 2009b, Hutton *et al.* 2012, Hutton and Scott 2013, Verlaan *et al.* 2013, Yang *et al.* 2014). Availability of reproducible and reliable markers can greatly aid in molecular breeding for important agronomic traits like resistance to various diseases. The resistant donors, susceptible lines and F_1 population (Table 1) derived from donor lines (*S. chilense* and *S. habrochaites*) were screened for the introgression of *Ty-2* and *Ty-3* genes. For genotyping of *Ty-2* gene, we have used (TG36) a CAPS marker identified by Schmitz *et al.* (2002). It is clear from Fig. 1a that marker (TG36) (Schmitz *et al.* 2002) is capable to discriminate resistant donor line, susceptible var. (Pusa Ruby) and F_1 hybrids by amplifying 520 bp and 450 bp fragments. In case of heterozygotes both fragments were amplified (Fig.1a).

Dong *et al*. (2016) designed gene based SCAR marker *Ty-3* SCAR1 that could be effectively used for the selection of *Ty-3* locus **(***ty-3/Ty-3* loci.). In our genotypes marker *Ty-3* SCAR1 (Fig. 1b) was found to detect the *Ty-3* introgressions on the basis of an amplicon of 519 bp and *ty-3* susceptible allele with 269 bp amplicon. It was reported that the sequences for *Ty-3* alleles were different for lines derived from *S. chilense* LA2779 and LA1932 (Maxwell *et al.* 2007) and the 2 different introgressions were designated as *Ty-3* from LA2779 and *Ty-3a* from LA1932. Jensen *et al* (2007) designed a set of PCR primers, viz. P6-25F2/P6-25R5 which could discriminate *Ty3* and *Ty3a* introgressions and a newly discovered introgression from *S. chilense* LA1969. These primers gave 450-bp fragments in lines derived from either the *S. Chilense* LA2779, while a 320-bp *ty-3* fragment was amplified from susceptible breeding lines. In addition, a 630-bp *Ty-3a* allele was found in lines derived from *S. Chilense* LA1932. Respective heterozygous alleles were detected in hybrids.

We have used marker P6-25 which specifically distinguished between *ty-3*, *Ty-3* and *Ty-3a* alleles on the basis of fragment sizes of 320 bp, 450 bp and 630 bp, respectively and, hence, it was found to be an efficient marker for selection of introgression from S. *chilense* LA2779 or *S. Chilense* LA1932 lines (Fig.1c). Since *Ty-1* allele was originally introgressed from *S. Chilense* LA1969 and both the loci *Ty-1* and *Ty-3* are known to be allelic, therefore, this may be a reason for occurrence of *Ty-3a* specific band in this line. For genotyping of *Ty-2* gene, we have also used (T0302) a tightly linked SCAR marker identified by Yang *et al.* (2014). Marker (T0302) (Yang *et al.* 2014) is capable to discriminate resistant donor line, susceptible var. (Pusa Ruby) and (F_1 hybrids) by amplifying 900 bp and 800 bp fragments in a duplex assay with P6-25 (Jensen *et al.* 2007) (Fig.1c). In case of heterozygote both fragments were amplified (Fig.1c).

Testing of markers for Ph-3, Tm2^2 and Sm genes: For *Ph-3* gene a CAPS marker TG328 developed by Robbins

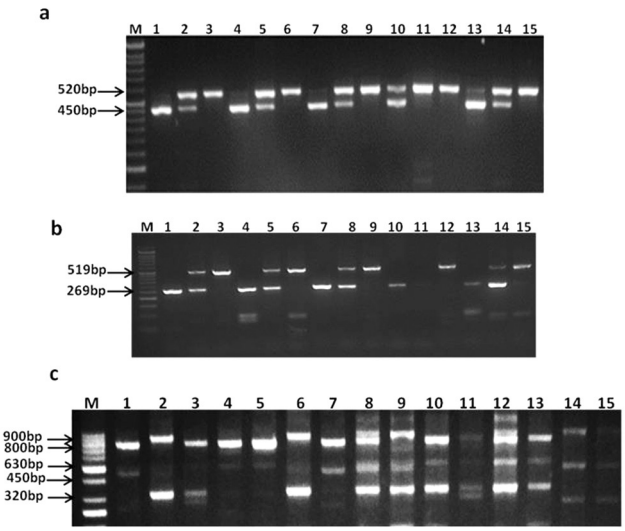

Fig 1. Verification of markers for *Ty-2, Ty-3* and *Ty-3a* genes. 1a. Gel photograph of PCR amplicon of 520 bp for resistant *Ty-2* allele and 450 bp for susceptible *ty-2* allele. M: 50 bp ladder, Lane: 1 (Susceptible var. Pusa Ruby), lane 2-14 (2-Pusa Ruby × EC814916; 3-EC814916; 4-Pusa-120; 5- Pusa-120 × EC814916; 6- EC814916; 7- Pusa Rohini; 8- Pusa Rohini × EC814916; 9-- EC814916; 10- Pusa Sadabahar × EC814916; 11- EC814915; 12- EC814917; 13-Pusa Sheetal ;14- Pusa Sheetal × EC814916;) , lane 15 (Donor line EC814916). 1b. amplification of 519 bp for *Ty-3* allele and 269 bp for *ty-3* allele. M: 50 bp ladder, Lane: 1 (Susceptible var. H-86), lane 2-14 (1-H-86; 2-H-86 × EC814916; 3-EC814916; 4-Pusa-120; 5- Pusa-120 x EC814916; 6- EC814916; 7- Pusa Rohini; 8- Pusa Rohini × EC814916; 9-EC814916; 10- Pusa Sadabahar; 11- No amplification; 12- EC814917; 13-Pusa Sheetal ;14- Pusa Sheetal × EC814916;15- EC904112). 1c. PCR product of 320 bp for *ty-3* allele, 450 bp for *Ty-3* allele, 630 bp for *Ty-3a* allele, 800bp for *ty-2* allele and 900bp for *Ty-2* allele. M: 50 bp ladder. Lane: 1-Pusa Ruby; 2- EC814916, lane 3-15 F_2 populations of Pusa Ruby × EC814916.

et al. (2010) was used. This marker produced an amplicon of approximately 260/240 bp in the resistant line, whereas susceptible lines amplified a longer fragment of about 500 bp (Fig 2a). To validate $Tm2^2$ allele for ToMV resistance, NCTm-019 marker developed from $Tm2^2$ locus by Panthee *et al.* (2013) based on restriction site differences. The resistant line gave a fragment of 270+600 bp and in heterozygotes 3 fragments were produced (270+600 bp and 870 bp) upon restriction of amplified product with Hae III (Fig 2b). For confirmation of *Sm* gene linked to tomato gray leaf spot resistance in the germplasm lines (Table 1) recessive CAPS marker CT-55 developed by Ji *et al.* (2009) was used. The resistant line generated a fragment of 200 bp and 140 bp, while an additional fragment of 330 bp amplified in susceptible line along with 200 bp and 140 bp fragment (Fig 2c).

Screening for Tomato leaf curl New Delhi virus (ToLCNDV): ToLCNDV is the most predominant begomovirus in northern India. The prenent study was underfatceh to know the ToLCNDV status in the tomato lines (*Ty-2* and *Ty-3* donor lines, susceptible var. and F_1 hybrids) (Table 1) grown in the IARI field. ToLCNDV genome specific *AC4* primers were used to detect viral titre by RT PCR in the cDNA samples of above mentioned lines. An amplicon of 189 bp specific for *AC4* transcript was detected which indicates the presence of virus in all the samples tested under study (Fig.3). The presence of virus also in donor lines indicates that *Ty* genes provide immunity but not resistance.

Multiplexing reduces the workload and costs for marker-assisted selection (Maxwell *et al.* 2007). Therefore, in the present investigation 6 markers, viz. TG36, T0302, SCAR1, P-625, *Ph-3,* $Tm2^2$, and *Sm* were tested on genotypes

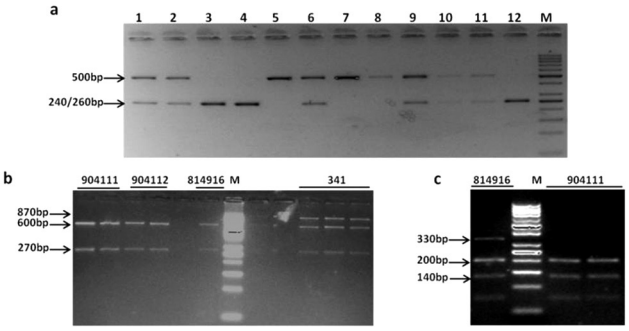

Fig 2. Validation of markers for *Ph-3,* $Tm2^2$ and *Sm* genes. 2a. Gel picture showing PCR amplicon of 260/240 bp for resistant *Ph-3* allele and 500 bp for susceptible *Ph-3* allele. M: 50 bp ladder, Lane: 1,2- Pusa Ruby (Susceptible line) 3,4 (EC814916 resistant lines for *Ph-3*) lane 1,2,5,6,7,8,9,10,11 (Resistant lines for *Ph-3*). 2b. PCR amplicon of 270+600 bp in the resistant line for ToMV and 270+600 +870 bp in the heterozygous line for ToMV. M: 50 bp ladder, (904111, 904112, EC814916 resistant lines for $Tm2^2$; 341 (F_1) for $Tm2^2$. 2c. PCR amplicon of 200 bp +140 bp for *Sm* resistant allele and 330bp for susceptible *Sm* allele. M: 50 bp ladder (904111 homozygous resistant lines for *Sm;* EC814916 homozygous lines for *Sm*).

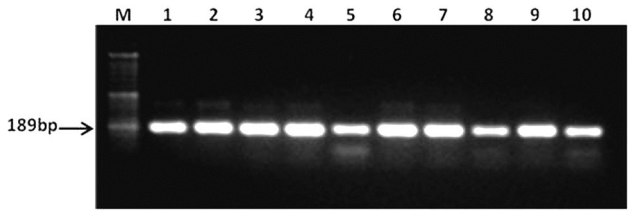

Fig 3. RT PCR analysis for confirmation of *AC4* specific transcript. Gel picture showing amplification of 189 bp *AC4* specific amplicon in the tested lines; 1-7 F_2 populations of Pusa Ruby × EC814916; 8,9- EC814916, 10- EC814917.

harbouring various resistance alleles for ToLCV, late blight, ToMV and grey leaf spot using uniplex and multiplex assay. The current study also demonstrated the feasibility of combining TG0302 and P6-25 markers for a duplex assay for simultaneously detecting three important resistance gene loci, viz. *Ty-2, Ty-3* and *Ty-3*a for breeding tropical tomato. The present study validated 4 highly efficient markers for identification of ToLCV, late blight, ToMV and grey leaf spot resistant loci and these will enable breeders to exploit these markers for pyramiding of resistant loci in the pursuit of stable and broad spectrum resistance to multiple diseases.

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REFERENCES

Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H, Chen L, Lapidot M and Levin I. 2009. Molecular dissection of Tomato leaf curl virus resistance in tomato line TY172 derived from *Solanum peruvianum. Theoretical and Applied Genetics* **119**(3): 519–30.

Chakraborty S. 2009. Tomato leaf curl viruses from India, pp. 339–47. (Eds) Mahy B and Regenmortel M V. *Desk encyclopedia of plant and fungal virology.* Academic press.

Dong P, Siddique M I, Zhao, M and Kang B C. 2016. Gene-based markers for the Tomato yellow leaf curl virus resistance gene Ty-3. *Plant breeding and biotechnology* **4**(1): 79–86.

Foolad M R and Sharma A. 2004. Molecular markers as selection tools in tomato breeding. International Symposium on Tomato Diseases **695**: 225–40.

Hutton S F and Scott J W. 2013. Fine-mapping and cloning of *Ty-1* and *Ty-3* and mapping of a new TYLCV resistance locus Ty-6. Tomato Breeders Round Table. University of Florida.

Hutton S F, Scott J W and Schuster D J. 2012. Recessive resistance to Tomato yellow leaf curl virus from the tomato cultivar Tyking is located in the same region as Ty-5 on chromosome 4. *Horticultural Science* **47**(3): 324–7.

Jensen K S, Van Betteray B, Smeets J, Yuanfu J, Scott J W, Mejia L, Havey M J and Maxwell D P. 2007. Co-dominant SCAR Marker for Detection of the Ty-3 and Ty-3a alleles at 25cM of chromosome 6 of Tomato. *Tomato Genetics Cooperative* **57**: 6–25.

Ji Y, Schuster, D J and Scott J W. 2007. Ty-3, a begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus Ty-1 on chromosome 6 of tomato. *Molecular Breeding* **20**(3): 271–84.

Ji Y, Scott J W and Schuster D J. 2009 . Toward fine mapping of the tomato yellow leaf curl virus resistance gene Ty-2 on chromosome 11 of tomato. *Horticultural Science* **44**(3): 614–8.

Ji Y, Scott J W, Hanson P, Graham E and Maxwell D P. 2007. Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting begomoviruses, pp. 343–62. *Tomato Yellow Leaf Curl Virus Disease.* Springer, Dordrecht.

Ji Y, Scott J W, Schuster D J and Maxwell D P. 2009. Molecular mapping of Ty-4, a new Tomato yellow leaf curl virus resistance locus on chromosome 3 of tomato. *Journal of the American Society for Horticultural Science* **134**(2): 281–8.

Ji Y, Scott J W and Maxwell D P. 2009. CAPS marker linked to the tomato gray leafspot (*Stemphyllium* sp.) resistance gene Sm. *Report of Tomato Genetics Cooperative* **59***:* 29–31

Lanfermeijer F C, Dijkhuis J, Sturre M J, de Haan P and Hille J. 2003. Cloning and characterization of the durable tomato mosaic virus resistance gene Tm-2^2 from *Lycopersicon esculentum*. *Plant molecular biology* **52**(5): 1039–51.

Maxwell D P, Martin C T, Garcia B E, Salus M S, Jensen K S, Havey M J and Mejia L. 2007. Markers for tomato chromosomes. www. plantpath. wisc. edu/Geminivirus Resistant Tomatoes.

Murray M G and Thompson W F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic acids research* **8**(19): 4321–6.

Panthee D R, Brown A F, Yousef G G, Ibrahem R and Anderson C. 2013. Novel molecular marker associated with Tm2a gene conferring resistance to tomato mosaic virus in tomato. *Plant Breeding* **132**(4): 413–6.

Robbins M D, Masud M A, Panthee D R, Gardner R G, Francis D M and Stevens M R 2010. Marker-assisted selection for coupling phase resistance to tomato spotted wilt virus and *Phytophthora infestans* (late blight) in tomato. *Horticultural Science* **45**(10): 1424–8.

Schmitz G, Tillmann E, Carriero F, Fiore C, Cellini F and Theres K. 2002. The tomato Blind gene encodes a MYB transcription factor that controls the formation of lateral meristems. *Proceedings of the National Academy of Sciences* **99**(2): 1064–9.

Scott J W and Gardner R G. 2006. Breeding for resistance to fungal pathogens, pp. 421–56. *Genetic Improvement of Solanaceous Crops.* CRC Press, Taylor & Francis Group.

Verlaan M G, Hutton S F, Ibrahem R M, Kormelink R, Visser R G, Scott J W, Edwards J D and Bai Y. 2013. The tomato yellow leaf curl virus resistance genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA–dependent RNA polymerases. *PLoS genetics* **9**(3): p. e1003399.

Yang X, Caro M, Hutton S F, Scott J W, Guo Y, Wang X, Rashid M H, Szinay D, de Jong H, Visser R G and Bai Y. 2014. Fine mapping of the tomato yellow leaf curl virus resistance gene Ty-2 on chromosome 11 of tomato. *Molecular Breeding* **34**(2): 749–60.

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, Van-Oss H and Kedar N. 1994. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene Ty-1. *Theoretical and Applied Genetics* **88**(2): 141–6.

Zhang C, Liu L, Wang X, Vossen J, Li G, Li T, Zheng Z, Gao J, Guo Y, Visser R G and Li J. 2014. The Ph-3 gene from *Solanum pimpinellifolium* encodes CC-NBS-LRR protein conferring resistance to *Phytophthora infestans*. *Theoretical and applied genetics* **127**(6): 1353–64.