Research Article

Ekin
Journal of Crop Breeding and Genetics

10(1):52-58, 2024

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Determination of Resistance to Tomato Yellow Leaf Curl Virus by Molecular Methods in Pink Beef Tomatoes

Mine BULUT* **Sevinç TEKİN** **Elif ÜRÜN KÖKSALAN** **Veysel ARAS**

Alata Horticultural Research Institute, Erdemli, Mersin, TÜRKİYE

* Corresponding author e-mail: minebulut33@gmail.com

Citation:
Bulut M., Tekin S., Köksalan EÜ., Aras V., 2024. Determination of Resistance to Tomato Yellow Leaf Curl Virus by Molecular Methods in Pink Beef Tomatoes. Ekin J. 10(1):52-58.

Received: 20.11.2023 ***Accepted: 18.12.2023*** ***Published Online: 31.01.2024*** ***Printed: 31.01.2024***

ABSTRACT

In both public and private tomato breeding projects, marker assisted selection (MAS) for disease resistance is frequently used. In tomato molecular breeding programs, the development and application of molecular markers have been extensively pursued, particularly for disease resistance to enable the selection of a single resistance gene or a combination of multiple resistance genes. Tomato Yellow Leaf Curl Virus (TYLCV) is one of the most dangerous viruses affecting tomato production and growth worldwide. Using resistant cultivars is the most effective and eco-friendly way to combat TYLCV. In this study, the TYLCV was tested against 155 genotypes of pink beef tomatoes utilizing the MAS (Marker-Assisted Selection) technique. Resistance against TYLCV was determined with the SCAR (P6-25) primer developed in connection with the *Ty-3* gene. 42 pink tomato genotypes were determined to be susceptible (rr), 99 to be heterozygous resistant (Rr), and 8 to be homozygous resistant (RR) to TYLCV as a consequence of MAS testing. Furthermore, no molecular marker was found in any of the six pink beef tomato genotypes. These findings suggested that P6-25 (SCAR) primers could be used successfully in breeding studies to identify disease resistance.

Keywords: Tomato, MAS, TYLCV, resistance breeding

Introduction

The tomato is a member of the nightshade family Solanaceae, which is classified in the following orders: Solanales, suborder Solanineae, division Magnoliophyta, class Magnoliopsida, and subclass Asteridae. It is estimated that the 96 genera and over 2800 species that make up the incredibly diverse and huge Solanaceae family are divided into three sub-families: Solanoideae (which Lycopersicon belongs to), Cestroideae, and Solanineae (Knapp et al., 1992; Knapp et al., 2004). The tomato (*Solanum lycopersicum* L.) is the most necessary after potatoes. Unquestionably, it is the most widely grown vegetable crop worldwide (Bhandari et al., 2017). The crop is grown almost anywhere in the world, despite being a tropical plant (Robertson and Labate, 2007). The tomato is a crop with significant global economic importance (Foolad, 2007). It is estimated that 4.9 million hectares of tomatoes are farmed annually, yielding over 186 million tons of tomatoes (FAO, 2022). Abiotic and biotic stress are the main factors limiting tomato cultivation. Approximately 200 distinct pathogens have been identified for the tomato plant, making it vulnerable to numerous fungus, bacteria, viruses, and microorganisms (Jones et al., 1991). Globally, a number of biotic stress, including as viral infections, are to blame for large losses in tomato output. Whitefly-transmitted geminiviruses (genus: Begomovirus) are among the viral illnesses that significantly limit tomato output in tropical and subtropical areas of the world. Tomato yellow leaf curl disease (TYLCD) and tomato leaf curl disease (ToLCD), which are harmful diseases with a variety of symptoms, are caused by these viruses (Cohen and Lapidot, 2007). One of the most dangerous viruses in the world is the TYLCV. This disease, which is spread by whiteflies, is caused by single-spinning DNA

from the geminivirus genus (Laterrot,1995). TYLCV can result in yield losses of up to 100% in tomato disease-affected areas. Early in the 1960s, it began to spread from the Middle East and is currently found over much of Africa, America, and Asia. Turkey was affected by the illness in the early 1980s (Polston and Anderson,1997; Moriones and Navas-Castillo, 2000; Agnihotri et al. 2013). The disease, which was initially found in the Middle East and later spread to many other nations, is now a significant problem restricting the output of tomatoes. There are few methods for controlling TYLCV in tomatoes and they are expensive. The most effective approach to combating nematodes and diseases is to create varieties that are resistant to pests and diseases (Glick et al., 2009; Melomey et al., 2019; Ogunsola and Ogunsina, 2021). In wild species, such as *S. chilense* (*Ty-1, Ty-3, Ty-4*, and *Ty-6*), *S. habrochaites* (*Ty-2*), and *S. peruvianum* (*Ty-5*), many resistance genes against TYLCV have been found. New tomato cultivars have been successfully bred using the genes *Ty-1/Ty-3* and *Ty-2* (Gill et al., 2019; Ji et al., 2007; Ji et al., 2009a; Ji et al., 2009b). The identification, mapping, and transfer of several disease resistance genes and quantitative trait loci (QTLs) in tomatoes have been made easier by the application of MAS approaches and genetic markers. In both public and private tomato breeding projects, marker assisted selection (MAS) for disease resistance is frequently used (Foolad, 2007; Jung et al., 2015). Tomatoes are a major product for both domestic and export in Türkiye, so it is crucial that the information and techniques developed on the topic be applied in Türkiye as well as the rest of the globe to increase tomato competitiveness through the development of new varieties. Therefore, the aim of this research is to determine the resistance against TYLCV of tomato genotypes propagated from a commercially resistant hybrid using molecular methods.

Materials and Methods

The material of this study consists of 155 pink tomato genotypes in the gene pool of Alata Horticultural Research Institute. Seeds were sown in peat-perlite medium at a ratio of 1:1 and DNA was isolated from these seedlings. The plants were employed for DNA analysis when they had three or four true leaves.

DNA isolation was performed by modifying the CTAB method developed by Doyle and Doyle (1990). While Chloroform:Isoamyl alcohol was used in the ratio of 24:1 in the CTAB method developed by Doyle and Doyle (1990), in our study, Chloroform:Octanol was used in the ratio of 24:1. Resistance against TYLCV was determined with the SCAR (P6-25) primer developed in connection with the *Ty-3* gene (Ji et al. 2007). The DNA primers used in the research are given in Table 1. PCR reactions for TYLCV were performed in a total volume of 15 µl; 2 µl master mix, 1 µl each of forward and reverse primers, 1.5 µl DNA and 9.5 µl ddH_2O were added to a total volume of 15 µl.

In the reactions of PCR the first denaturation was started at 94°C for 4 minutes and the cycle was performed 35 times, including denaturation at 94°C for 30 seconds, annealing at 53.7°C for 1 minute and 1 minute at 72°C, and this cycle was performed for 10 minutes at 72°C. The PCR products obtained as a result of the study were conditioned on a 1.5-2% agarose gel and the results were evaluated.

Results

In this study, 155 pink beef tomato genotypes were screened with the SCARP6-25 primer providing resistance against TYLCV. PCR findings (Figure 1) were analyzed genotypically: The homozygous (RR) resistant samples yielded a single 630 bp band, but the heterozygous (Rr) genotype samples showed two bands, one at 630 bp and the other at 320 bp. Lastly, 320 bp was found in a single band in samples with homozygous recessive (rr) genotypes (Table 2). 42 pink tomato genotypes were determined to be susceptible (rr), 99 to be heterozygous resistant (Rr), and 8 to be homozygous resistant (RR) to TYLCV as a consequence of MAS. Furthermore, no molecular marker was found in any of the six tomato genotypes-pink beef.

Discussion

A significant disease that severely reduces tomato yield is TYLCV, a begomovirus belonging to the Geminiviruidae family. Treatment for viral illnesses can be very difficult. Cultivars that are resistant to various diseases and pests during growth are therefore among the most important strategies. Numerous attempts have been attempted to introduce resistance into elite cultivars since host resistance is an economical and environmentally beneficial approach of controlling this virus. Through molecular-assisted selection, TYLCV-resistant genotypes can be generated quickly by screening a large number of plant materials. There have been several reported gene-linked markers for the six TYLCV-resistant genes (*Ty-1* to *Ty-6*) (Ji et al. 2007; Yang et al., 2014; Caro et al., 2015; Jung et al., 2015; Lapidot et al., 2015; Gill et al., 2019). For tomato breeding initiatives to improve MAS, gene-based or functional indicators still need to be established. Several researchers have accepted *Ty-1* and *Ty-3* as the markers that indicate tomato resistance to the TYLCV virus, and these findings have been published

in MAS (Zamir et al. 1994; Agrama and Scott, 2006; Ji et al., 2007). Kim et al., (2020) investigated non-synonymous sequence variations between resistant and susceptible varieties for the *Ty-2* and *Ty-3* genes, and the resulting resistance-associated SNPs and InDels were subsequently used to develop molecular markers for MAS. In their study, Aktaş and Aydın (2022) identified 22 homozygous resistant, 4 heterozygous resistant and 128 susceptible individuals in tomatoes (*S. lycopersicum*) at the F_5-F_8 stage. Using molecular DNA markers, the study assessed the TYLCV resistance of various cherry and cocktail tomato varieties. Additionally, 409 different cherry and cocktail tomato varieties had their TYLCV resistance determined by polymerase chain reaction (PCR) with the Ty3P6-25 primer. Of these, 291 were found to be TYLCV susceptible (rr), 66 to be heterozygous resistant (Rr), and 45 to be homozygous resistant (RR). Furthermore, in seven tomato varieties-cherry and cocktail-no molecular marker was found (Basım et al., 2023). Pınar et al., (2013) found that 24 out of 92 tomato genotypes had both bands, but only 50 had homozygous resistant and susceptible bands following testing of the P6-25 marker for the *Ty-3* resistance gene. Similar results were obtained in our study. In their research, Prasanna et al. (2014) shown that Indian breeding studies can make good use of molecular markers created for the tomato leaf curl virus.

Conclusions

Positive results were found from testing 155 pink tomato genotypes with the SCAR P6-25 marker, which was designed for the tomato leaf curl virus and reported in the literature and it was successfully identified that the pink beef tomato genotype is resistant to the TYLCV disease. The molecular DNA marker that was employed was found to be helpful in identifying pink beef tomato resistance responses to TYLCV and could yield fast, accurate, and repeatable findings. It has been determined that the primers can be used in future breeding experiments due to the availability of this information and the fact that some tomatoes exhibit disease resistance.

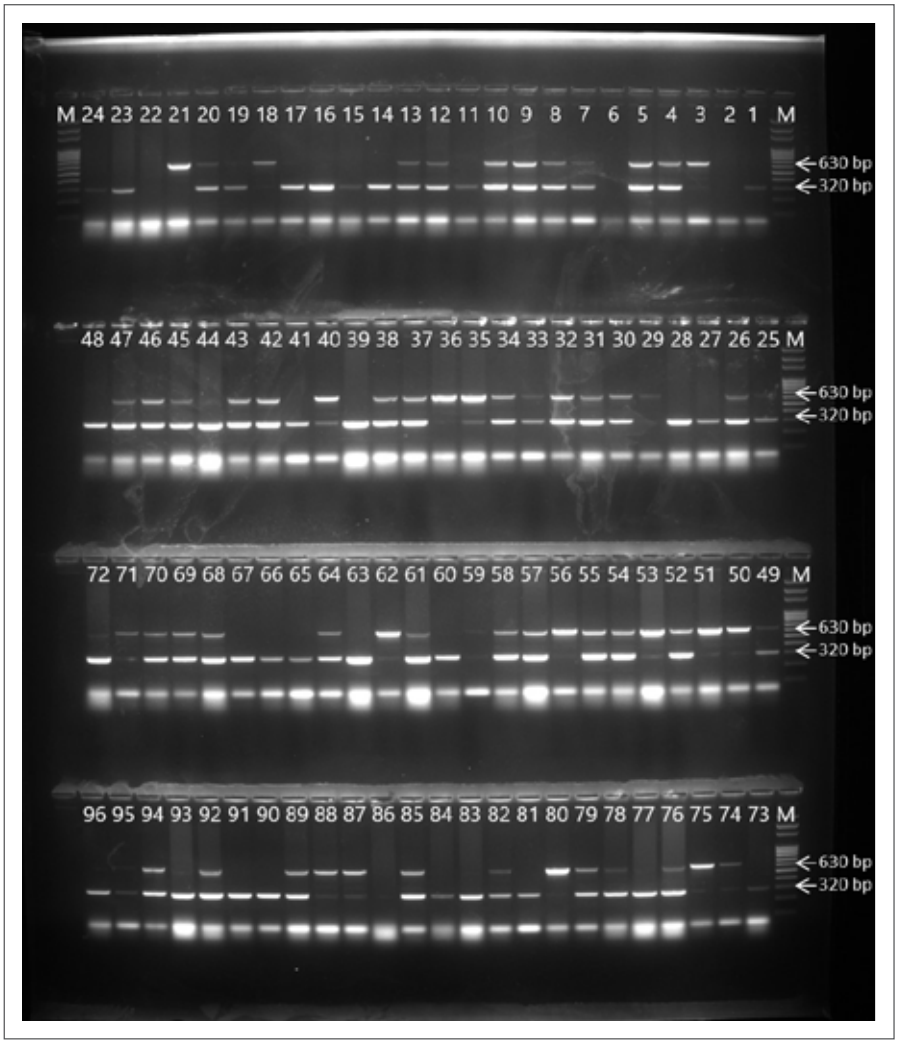

Figure 1. PCR results of tomato genotypes for P6-25. M, Marker 100 bp; Tomato cultivars, 1-96

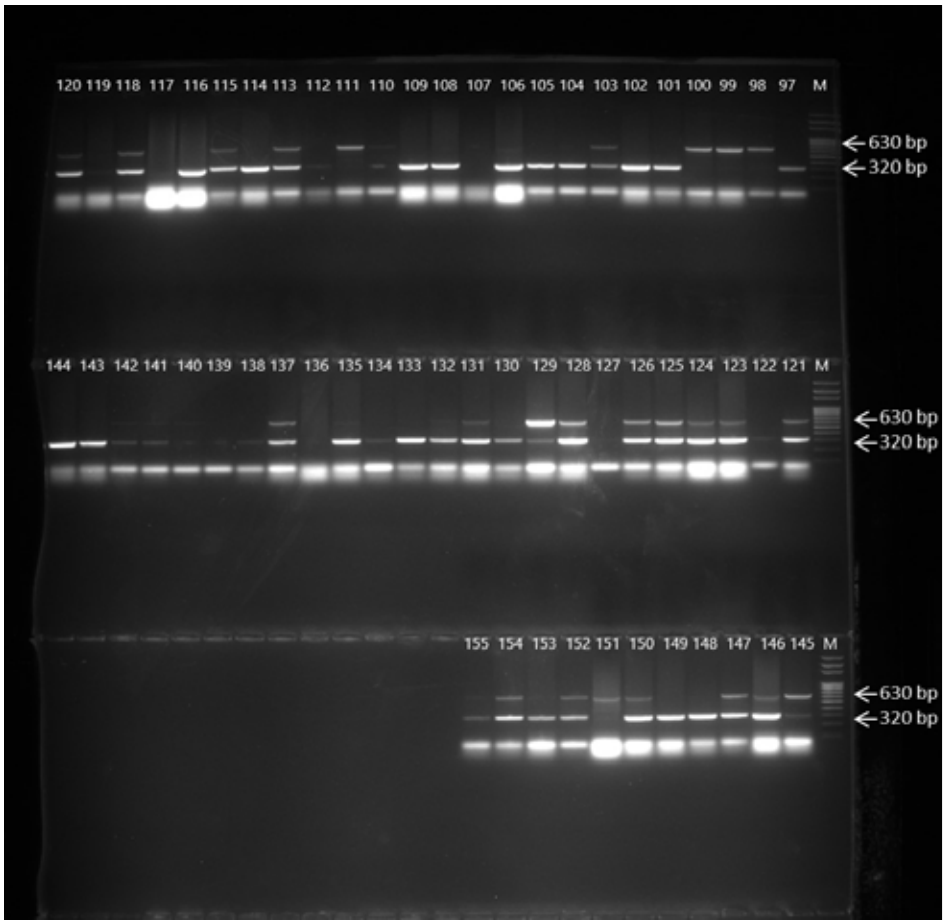

Figure 1 continued, 97-155

Table 1. Used Primer Names and Sequences.

Gene	Primer Name	Primer Sequences	Amplified Product (bp)	
			R***	S*
Ty-3	P6-25-F2 P6-25-R5	5' GGTAGTGGAAATGATGCTGCTC-3' 5' GCTCTGCCTATTGTCCCATATATAACC-3'	450(*Ty3*) 630(*Ty3a*)	320

Table 2. Genotypic characteristics of tomato genotypes (1-155) analyzed by PCR.

Genotype No	P6-25	Genotype No	P6-25	Genotype No	P6-25	Genotype No	P6-25
1	rr	40	Rr	111	RR	118	Rr
2	-	41	rr	112	rr	119	rr
3	Rr	42	Rr	113	Rr	120	Rr
4	Rr	43	Rr	114	Rr	121	Rr
5	Rr	44	Rr	115	Rr	122	rr
6	-	45	Rr	116	rr	123	Rr
7	Rr	46	Rr	65	rr	124	Rr
8	Rr	47	Rr	66	rr	125	Rr
9	Rr	48	rr	67	rr	126	Rr
10	Rr	49	Rr	68	Rr	127	-
11	rr	50	Rr	69	Rr	128	Rr
12	Rr	51	Rr	70	Rr	129	Rr
13	Rr	52	Rr	71	Rr	130	rr
14	rr	53	Rr	72	Rr	131	Rr
15	rr	54	Rr	73	rr	132	Rr
16	rr	55	Rr	74	Rr	133	Rr
17	Rr	56	Rr	75	Rr	134	rr
18	Rr	57	Rr	76	Rr	135	Rr
19	Rr	58	Rr	77	rr	136	-
20	Rr	59	Rr	78	Rr	137	Rr
21	RR	60	rr	79	Rr	138	rr
22	-	61	Rr	80	Rr	139	rr
23	rr	62	Rr	81	rr	140	Rr
24	rr	63	rr	82	Rr	141	Rr
25	Rr	64	Rr	83	rr	142	Rr
26	Rr	97	Rr	84	rr	143	rr
27	rr	98	RR	85	Rr	144	rr
28	rr	99	RR	86	rr	145	Rr
29	Rr	100	RR	87	Rr	146	Rr
30	Rr	101	rr	88	Rr	147	Rr
31	Rr	102	rr	89	Rr	148	rr
32	Rr	103	Rr	90	rr	149	rr
33	Rr	104	Rr	91	rr	150	Rr
34	Rr	105	Rr	92	Rr	151	RR
35	Rr	106	Rr	93	Rr	152	Rr
36	RR	107	RR	94	Rr	153	rr
37	Rr	108	rr	95	Rr	154	Rr
38	Rr	109	rr	96	Rr	155	Rr
39	rr	110	Rr	117	-		

RR: Homozygous Resistant, Rr: Heterozygous, rr:Susceptible, -;Non detected

References

Aktaş H, Aydın G, (2022). Determination of the response of wild and cultivated tomato genotypes to some disease and pests by molecular markers, HortiS (2022) 39(1):15-21, https://doi.org/10.16882/HortiS.1069414

Agnihotri MK, Gothwal RK, Moranıya N, Singh R, Verma S, (2013). breeding for resistance to tomato yellow leaf curl virus (TYLCV)/ tomato leaf curl virus (ToLCV), Agriculture for Sustainable Development, 1(1), 113-117.

Agrama HA, Scott JW, (2006). Quantitative trait loci for tomato yellow leaf curl virus and tomato mottle virus resistance in tomato. Journal of the American Society for Horticultural Science, 131:267-272.

Bhandari HR, Srivastava K, Eswar Reddy G, (2017). Genetic variability, heritability and genetic advance for yield traits in tomato (*Solanum lycopersicum* L.). International Journal of Current Microbiology and Applied Sciences.; 6(7):4131-4138. doi:10.20546/ijcmas.2017.607.428

Basım H, Kandil O, Karaoğlan M, (2023). Determination of TYLCV-resistant cherry and cocktail tomato cultivars by molecular markers, Turkish Journal of Science and Engineering, 5(2): 89-96, doi: 10.55979/tjse.1357477

Caro M, Verlaan MG, Julián O, Finkers R, Wolters A-MA., Hutton SF, Scott JW, Kormelink R, Visser RGF, Diez MJ, Perez-de-Castro A, Bai Y, (2015). Assessing the genetic variation of *Ty-1* and *Ty-3* alleles conferring resistance to tomato yellow leaf curl virus in a broad tomato germplasm. Molecular Breeding, 35(6). doi:10.1007/s11032-015-0329-y

Cohen S, Lapidot M, (2007). Appearance and expansion of TYLCV: A historical point of view. In: Czosnek H (ed) Tomato yellow leaf curl virus disease: management, molecular biology, breeding for resistance. Springer, The Netherlands, pp 3-12. doi:10.1007/978-1-4020-4769-5

Doyle JJ, Doyle JL, (1990). Isolation of plant DNA from fresh tissue, Focus, 12(1):13-15.

FAO, (2022). Food and agriculture organization, agricultural statistical database. https://www.fao.org/faostat/en/#data (accessed on 16 Januar 2024).

Foolad, MR, (2007). Genome mapping and molecular breeding of tomato. International Journal of Plant Genomics, 2007, 1–52. doi:10.1155/2007/64358

Gill U, Scott JW, Shekasteband R, Ogundiwin E, Schuit C, Francis DM, Sim SC, Smith H, Hutton SF, (2019). *Ty-6*, a major begomovirus resistance gene on chromosome 10, is effective against tomato yellow leaf curl virus and tomato mottle virus. Theoretical and Applied Genetics, 132, 1543-1554, doi: 10.1007/s00122-019-03298-0

Glick E, Levy Y, Gafni Y, (2009). The viral etiology of tomato yellow leaf curl disease - a Review. Plant Protect. Sci., 45:81–97.

Ji Y, Schuster DJ, Scott JW, (2007). *Ty-3*, A begomovirus resistance locus near the tomato yellow leaf curl virus resistance locus *Ty-1* on chromosome 6 of tomato, Mol. Breed., 20:271-284. doi:10.1007/S11032-007-9089-7

Ji Y, Scott JW, Schuster DJ, (2009a). Toward fine mapping of the tomato yellow leaf curl virus resistance gene *Ty-2* on chromosome 11 of tomato. HortScience, 44:614-618, https://doi.org/10.21273/HORTSCI.44.3.614

Ji Y, Scott JW, Schuster DJ, Maxwell DP, (2009b). 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, 281-288, https://doi.org/10.21273/JASHS.134.2.281

Jones JB, Jones JP, Stall RE, Zitter TA, (1991). Compendium of tomato diseases. American Phytopathological Society Press, St.Paul.

Jung, J., Kim, H. J., Lee, J. M., Oh, C. S., Lee, H.-J., and Yeam, I, (2015). Gene-based molecular marker system for multiple disease resistances in tomato against Tomato yellow leaf curl virus, late blight, and verticillium wilt. Euphytica, 205(2), 599-613. doi:10.1007/s10681-015-1442-z

Kim M, Park Y, Lee J, Sim SC, (2020). Development of molecular markers for *Ty-2* and *Ty-3* selection in tomato breeding. Scientia Horticulturae, 265, 109230. doi:10.1016/j.scienta.2020.109230

Knapp S, Hawkes JG, Lester RN, Nee M, Estrada-Ramos N, (1992). Solanaceae III: Taxonomy, chemistry, evolution. Kew Bulletin, 47(4), 785. doi:10.2307/4110730

Knapp S, Bohs L, Nee M, Spooner DM, (2004). Solanaceae - A model for linking genomics with biodiversity. Comparative and Functional Genomics, 5(3), 285-291. doi:10.1002/cfg.393

Lapidot M, Karniel U, Gelbart D, Fogel D, Evenor D, Kutsher Y, Makhbash Z, Nahon S, Shlomo H, Chen L, Reuveni M, Levin I, (2015). A novel route controlling begomovirus resistance by the messenger RNA surveillance factor pelota. PLOS

Genetics, 11(10), e1005538. doi:10.1371/journal.pgen.1005538

Laterrot H, (1995). Breeding network to create tomato varieties resistant to tomato yellow leaf crul virus (TYLCV), Fruits, 50:439–444.

Melomey LD, Danquah A, Offei SK, Ofori K, Danquah E and Osei M, (2019). Review on tomato (*Solanum lycopersicum*, L.) improvement programmes in Ghana. Recent advances in tomato breeding and production, 49.

Moriones E, Navas-Castillo J, (2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Research, 71:123-34, https://doi.org/10.1016/S0168-1702(00)00193-3

Ogunsola OA, Ogunsina GA, (2021). Tomato production and associated stress: A case of african climate single cell biology, Vol.10 Iss.4 No:1000003.

Pınar H, Atilla A, Keleş D, Mutlu N, Denli N, Mustafa Ü, (2013). Determination of *Fusarium oxysporum* f. sp. *lycopersici* resistance in tomato lines with the help of molecular markers. Derim, 30(1):15-23.

Prasanna HC, Sinha DP, Rai GK, Krishna R, Kashyap SP, Singh NK, Singh M, Malathi V G, (2014). Pyramiding *Ty-2* and *Ty-3* genes for resistance to monopartite and bipartite tomato leaf curl viruses of India. Plant Pathology, 64(2): 256-264. doi:10.1111/ppa.12267

Polston JE, Anderson PK, (1997). The Emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere, Plant Dis., 81, 1358-1369, doi: 10.1094/PDIS.1997.81.12.1358

Robertson LD, Labate JA, (2007). Genetic resources of tomato (*Lycopersicum esculentum* Mill) and wild relatives. In: Razdan MK, Mattoo AK, editors. Genetic Improvement of Solanaceous Crops. Vol. 2. Tomato. New Hampshire: Science Publishers. pp. 25-75

Yang X, Caro M, Hutton SF, Scott JW, Guo Y, Wang X, Rashid MH, Szinay D, de Jong H, Visser RG, Bai Y, Du Y, (2014). Fine mapping of the tomato yellow leaf curl virus resistance gene *Ty-2* on chromosome 11 of tomato. Molecular Breeding. doi:10.1007/s11032-014-0072-9

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleben H, Van-Oss H, Kedar N, Rabinowitch HD, Czosnek H, (1994). Mapping and introgression of tomato yellow leaf curl virus tolerance gene, *ty-1*, theor. Appl. Genet., 88:141-146.