Validation of *Ty-2* Gene in Diverse Tomato(*Solanum lycopersicum* L.) Genotypes for Enhancing Tomato Leaf Curl Virus (ToLCV) Resistance

T. Kanal[1]*, T. Saraswathi[2], L. Pugalendhi[1], N. Manivannan[3], M. Karthikeyan[4] and N. Manikanda Boopathi[5]

[1]Department of Vegetable Science, HC&RI, Tamil Nadu Agricultural University, Coimbatore

[2]Department of Department of Medicinal and Aromatic Crops, HC&RI, TNAU, Coimbatore

[3]Centre of Excellence in Molecular Breeding, CPBG, TNAU, Coimbatore

[4]Department of Plant Pathology,AC & RI (TNAU), Vazhavachanur

[5]Directorate of Research, TNAU, Coimbatore

Corresponding author email: kanal.thorti@gmail.com

Abstract : Tomato (*Solanum lycopersicum* L.) is a globally significant vegetable crop, with India ranking second in production. However, tomato cultivation is prone to several challenges, most notably, the tomato leaf curl virus (ToLCV) disease, causing severe yield losses. Thisstudy was focused on validation of *Ty-2*gene among 14 tomato genotypes. DNA was extracted and PCR amplification using the TES0344 marker associated with the *Ty-2* gene was performed. Gel electrophoresis revealed distinct patterns: genotypes SL159, SL160, SL165, SL167, SL168, and SL169 exhibited a 190 bp band, indicating the presence of the *Ty-2* resistance allele. In contrast, genotypes SL113, SL115, SL124, SL142, SL144, SL147, SL149, and SL154 displayed a 205 bp band, signifying susceptible alleles. Using genotypes with *Ty-2* genotypes in breeding can boost tomato crop resilience against ToLCV, aiding agricultural sustainability and food security.

Keywords: Tomato, Leaf Curl virus, *Ty-2* gene, Molecular marker, Resistance.

Introduction

Tomatoes, scientifically known as *Solanum lycopersicum* L. and classified under the *Solanaceae*family, possess a chromosome count of 2n=24. They hold a prominent status as one of the most vital global vegetable crops. In the realm of tomato production, India claims the second position according to FAOSTAT (2022). The cultivation of tomatoes in India spans approximately 863.98 thousand hectares of agricultural land, resulting in a collective yield of 20.62 million metric tons and an average productivity rate of 23.87 tons per hectare. Among the Indian states engaged in tomato cultivation, Madhya Pradesh emerges as the top producer,

dedicating an area of 121 thousand hectares and yielding an impressive 2.76 million tons as per the report of INDIASTAT(2023). They come in various shapes, sizes, and colors and are a staple ingredient in countless culinary dishes. However, tomato cultivation faces numerous challenges, including the threat of various diseases. The primary challenge faced by tomato growers in India is the presence of the Tomato Leaf Curl Virus (ToLCV) disease, as noted by Moriones & Navas-Castillo (2000). Plants that are susceptible to this disease exhibit indications such as leaf yellowing, curling, and cupping, along with severe growth inhibition and the premature dropping of flowers and fruits as reported by Abhary*et al*.(2007). ToLCV falls under the genus Begomovirus within the Geminiviridae family, and it is transmitted by the whitefly, Bamisia tabaci.Among the six introgressions (*Ty* genes) known to provide resistance against tomato leaf curl disease, *Ty-2*(dominant) and *Ty-3* (partially dominant) hold significant importance due to their distinct gene actions. Prasanna*et al*. (2015) reported that these genes,with their distinct resistance properties, can play a crucial role in developing tomato hybrids with enhanced resistance to tomato leaf curl disease, thereby contributing to improved crop yield and overall agricultural sustainability. At the World Vegetable Centre in Taiwan, tomato lines containing the *Ty-2* resistance gene, along with other established Ty-genes such as *Ty-1, Ty-3,* and *Ty-5,* have been developed. Numerous efforts have been made to delineate the gene structure and create a comprehensive map of the *Ty-2* locus but there is no definitive evidence pointing to a specific gene as the causal factor behind Ty-2-mediated resistance as mentioned by Yamaguchi *et al*. (2018).These tomato lines are extensively employed in breeding initiatives spanning several Asian countries and other parts of the world. Dhaliwal *et al*. (2020) reported that the *Ty-1* and *Ty-2* genes exhibit complete dominance, with *Ty-3* displaying a form of partial dominance and these three genes have been widely utilized in breeding programs aimed at countering both monopartite and bipartite begomoviruses. Hence, the objective of the current study was to contribute to the validation of the *Ty-2* gene across 14 diverse tomato genotypes. This validation process is intended to facilitate and inform future breeding programs, allowing for the strategic incorporation of *Ty-2*-mediated resistance in tomato varieties, ultimately bolstering their resilience against tomato leaf curl disease.

Materials and Methods

Plant Material: Fourteen tomato genotypes were selected for this study, representing diverse genetic backgrounds the details of the genotypes are given in the table1.

Table 1. Details of the Plant Material and their source.

S.No	Genotypes	Source
1	SL 113	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
2	SL 115	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
3	SL 124	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
4	SL 142	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
5	SL 144	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
6	SL 147	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
7	SL 149	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
8	SL 154	Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu
9	SL 165	AVRDC, Taiwan
10	SL 167	AVRDC, Taiwan
11	SL 159	AVRDC, Taiwan
12	SL 160	AVRDC, Taiwan
13	SL 168	AVRDC, Taiwan
14	SL 169	AVRDC, Taiwan

DNA Extraction

DNA extraction was carried out in the leaf tissue of tomato genotypes using CTAB method suggested by Bernatzky&Tanksley (1986), with little modifications. 250 mg of leaf sample was weighed and cut it into small pieces using sterile scissors.The leaf bits were macerated by adding 500 µl of CTAB buffer using a pestle and mortar, which was then transferred to 2 ml microfuge tubes to make up the volume to 1 ml.The tubes were incubated at 65 ºC for 30 minutes in a water bath with occasional inversion for thorough mixing.Equal volume of chloroform: Isoamyl alcohol mixture (24:1 v/v) was added to the tubes, and they were mixed well.The tubes were then centrifuged at 12,000 rpm for 10 minutes in a refrigerated (4º C) centrifuge.A new sterile 1.5 ml microfuge tube was taken to transfer the clear supernatant, to

which an equal volume of ice-cold isopropanol and 200 µl of Sodium acetate were added.These tubes were incubated overnight at -20º C to allow the DNA to precipitate.The tubes were centrifuged at 10,000 rpm for 10 minutes the following day, and the supernatant was discarded.200 µl of 70% ethanol was added to the precipitate, and the tubes were centrifuged at 10,000 rpm for 10 minutes, and then the supernatant was discarded.The DNA pellets were then air-dried and dissolved in 50 µl of TE Buffer.

PCR Amplification

PCR was performed using TES0344, a SSR marker associated with the *Ty-2* gene, which is one of the commonly employed markers for marker-assisted selection at AVRDC - The World Vegetable Center. Forward sequence of marker is5'-GCCTTTTCCCACTTATATTCCTCTC-3' and Reverse sequence is 5'-ACACATACGACGTTCCGTCA-3'. The expected amplicon size is 190 and 205 for resistance and susceptibility respectively (Yang *et al*.,2012). Amplification was carried out using Eppendorf Master Thermal Cycler. The amplification profile was initial denaturation at 95°C for 10 min, followed by 35 cycles of 94°C for 45 sec, annealing temperature of 59.4°C for 1min, 72°C for 2 min and final extension of 72°C for 10 min as per Chen *et al*.(2015). The PCR products were size-fractiones on 2% agarose gel with ethidium bromide in 1xTBE buffer. After electrophoresis, the gel was visualized under ultraviolet light.

Results and Discussion

The marker will produce a resistant band at 190 base pairs (bp), while susceptible band at 205 (bp). Such marker-based assessments are instrumental in precisely identifying and selecting tomato genotypes with the desired resistance traits for future breeding efforts (Chen *et al*., 2015). The gel electrophoresis results (Figure 1) displayed a distinct pattern among the 14 tomato genotypes tested for the presence of the *Ty-2* gene.Occurrence of band at 190bp indicated the presence of resistance allele in the lines SL159, SL160, SL 165, SL167, SL168 and SL169 which are sourced from AVRDC, Taiwan.These genotypes may possess a crucial genetic advantage The study involved the verification of 14 breeding lines using the PCR-based marker TES0344, to identify the presence of the *Ty-2* gene, a critical determinant of resistance to tomato leaf curl

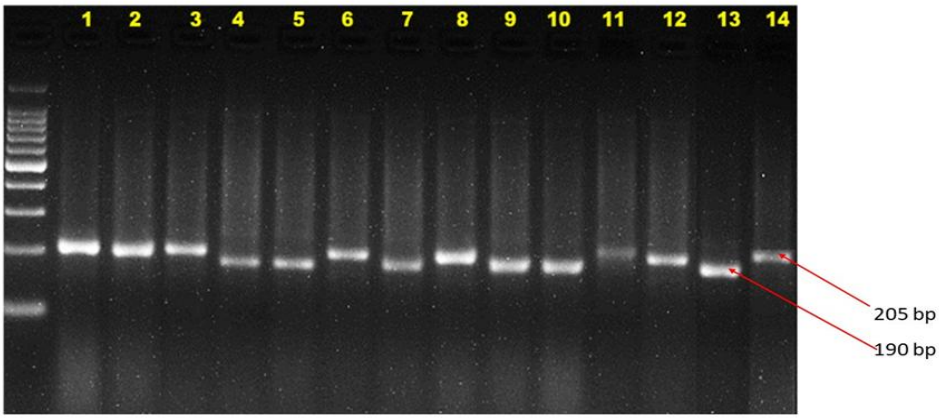

Figure 1. PCR profile amplified by the *Ty-2* primer- TES0344; 1= SL 113, 2=SL 115, 3= SL 124, 4= SL 165,5=SL 167, 6=SL 142, 7= SL 159, 8= SL 144, 9= SL 160, 10= SL 168, 11=SL 147, 12= SL 149, 13= SL 169,14= SL 154

able candidates for breeding programs aimed at enhancing ToLCV resistance in the cultivars as reported bySadashiva*et al.* (2017).

Meanwhile band at 205bp was noticed in SL113, SL 115, SL 124, SL 142, SL 144,SL 147, SL 149 and SL 154 lines with indicate the presence of susceptible alleles in the loci.This emphasizes the significant genetic diversity observed among different tomato genotypes. It also underscores the importance of developing strategic breeding approaches that aim to introduce resistance genes into susceptible varieties. Tabein*et al.* (2017) also stated that, such efforts ofdeveloping strategic breeding approaches are essential for enhancing the resilience of a broader range of tomato cultivars against tomato leaf curl disease and other similar challenges.

Conclusion

In this comprehensive study, our objective was to validate the presence of the *Ty-2* resistance gene in 14 diverse tomato genotypes. Our ultimate goal is to provide valuable insights for enhancing future breeding programs that focus on resistance to tomato leaf curl disease. Among these genotypes, six sourced from AVRDC were found to contain resistant alleles, while eight genotypes from TNAU were found to have susceptible alleles.In future research, the integration of the genotypes with*Ty-2* gene into breeding programs holds great promise for

strengthening tomato crops against this widespread threat. This development has the potential to significantly improve agricultural sustainability and food security

References

Abhary, M., Patil, B. L., &Fauquet, C. M. (2007). Molecular biodiversity, taxonomy, and nomenclature of tomato yellow leaf curl-like viruses. In *Tomato yellow leaf curl virus disease: management, molecular biology, breeding for resistance* (pp. 85-118). Dordrecht: Springer Netherlands.

Bernatzky, R., &Tanksley, S. D. (1986). Majority of random cDNA clones correspond to single loci in the tomato genome. *Molecular and General Genetics*, *203*: 8-14.

Chen, H., Lin, C., Yoshida, M., Hanson, P., &Schafleitner, R. (2015). Multiplex PCR for detection of tomato yellow leaf curl disease and root-knot nematode resistance genes in tomato (*Solanum lycopersicum* L.). *International Journal of Plant Breeding and Genetics*,*9*(2): 44-56.

Dhaliwal, M. S., Jindal, S. K., Sharma, A., &Prasanna, H. C. (2020). Tomato yellow leaf curl virus disease of tomato and its management through resistance breeding: A review. *The Journal of horticultural science and biotechnology*,*95*(4): 425-444.

FAOSTAT. (2022).**http://www.fao.org/faostat/en/#home**

INDIASTAT.(2022). Season-wise area, production and productivity of tomato in India (1987–1988, 1991-1992 to 2022–2023 – 1st advance estimates). Retrieved from https://www.indiastat.com/table/agriculture/area-production-productivity-tomato-india-1987-198/14911

Moriones, E., &Navas-Castillo, J. (2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus research*,*71*(1-2): 123-134.

Prasanna, H. C., Kashyap, S. P., Krishna, R., Sinha, D. P., Reddy, S., &Malathi, V. G. (2015). Marker assisted selection of *Ty-2* and *Ty-3* carrying tomato lines and their implications in breeding tomato leaf curl disease resistant hybrids. *Euphytica*, 204: 407-418.

Sadashiva, A.T., Hanson, P., Reddy, M.K., Ravishankar, K.V., Prasad, M., Prasanna, H.C., Reddy, K.M., Singh, T.H., Saritha, R.K., Hussain, Z. &Mythili, J.B. (2017). Breeding tomato (*Solanum lycopersicum* L.) for resistance to biotic and abiotic stresses. *Journal of Horticultural Science & Biotechnology 12*(2): 91-105.

Tabein, S., Behjatnia, S. A. A., Laviano, L., Pecchioni, N., Accotto, G. P., Noris, E., &Miozzi, L. (2017). Pyramiding *Ty-1*/*Ty-3* and *Ty-2* in tomato hybrids dramatically inhibits symptom expression and accumulation of tomato yellow leaf curl disease inducing viruses. *Archives of Phytopathology and Plant Protection50*(5-6): 213-227.

Yamaguchi, H., Ohnishi, J., Saito, A., Ohyama, A., Nunome, T., Miyatake, K., & Fukuoka, H. (2018). An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the *Ty-2*Begomovirus resistance locus of tomato. *Theoretical and Applied Genetics, 131*: 1345-1362.

Yang M., Zhao T., Yu W., & Zhao L. (2012). New SSR marker linked to *Ty-2* resistance to tomato yellow leaf curl virus. *Jiangsu Journal of Agricultural Sciences*,*28*(5): 1109–1113.