



## DEVELOPMENT OF CLUSTER TOMATO VARIETIES RESISTANT/TOLERANT TO TOMATO YELLOW LEAF CURL VIRUS (TYLCV) AND *Fusarium oxysporum* f.sp. *Radicis-lycopersici* (Forl) THROUGH MOLECULAR MARKER-BASED PLANT BREEDING

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**Abstract:** The global spread of viral and fungal diseases has led to a decline in tomato production as farmers are forced to abandon their crops. To combat these diseases, researchers have developed techniques using molecular-assisted selection to identify plant varieties that are resistant to these diseases. This study focused on cultivating cluster tomato varieties that are resistant or tolerant to *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Forl) and *Tomato Yellow Leaf Curl Virus* (TYLCV) using molecular DNA markers. The breeding program involved isolating genomic DNA from 69 cluster tomato varieties and then using PCR with C2-25 and Ty3P6-25 primers to identify which varieties were resistant or tolerant to Forl and TYLCV, respectively. Out of the 66 cluster tomato varieties, 20 were resistant or tolerant (RR) to Forl, 37 were heterozygous resistant or tolerant (Rr), and 9 were susceptible (rr). Among the 3 cluster tomato varieties, some were resistant or tolerant (designated as RR) to TYLCV, while others were heterozygous resistant or tolerant (Rr), and some were susceptible (rr) to the disease. This indicates that DNA molecular markers can reliably determine the presence of resistance or tolerance to Forl and TYLCV in cluster tomatoes. Molecular markers can efficiently screen thousands of tomato plants in a shorter time period, leading to the selection of more high-quality, resistant or tolerant varieties.

**Keywords:** Forl, TYLCV, Resistance, Cluster tomato, Molecular marker, Plant breeding

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### 1. Introduction

Vegetables are an essential part of human nutrition, and one such vegetable that is often underrated is the tomato (*Solanum lycopersicum* L.). Tomatoes are rich in cancer-fighting compounds and are widely traded agricultural products. Their extensive use in the food industry, particularly in canned goods and sauces, has established them as one of the primary tropical vegetables in global agricultural production (Toor et al., 2006; Gull and Nayik, 2020). The tomato is a diploid plant with 24 chromosomes and belongs to the *Solanum* genus and the Solanaceae family of the Solanoideae subfamily (Davies et al., 1981). The *Solanum* genus contains not only cultivated species like *S. lycopersicum*, but also wild species such as *S. pimpinellifolium*, *S. glandulosum*, and *S. cheeseman* (Kil et al., 2016). The tomato, originally from Central and South America, was first cultivated on the coast of Peru (Hedrick, 1919). It is widely cultivated in approximately 144 countries, making it one of the most valuable vegetables (Hassan, 2020). Global tomato production reached 187 million tons in 2020. Türkiye

ranked third with 13.204 million tons, following China and India (FAO, 2022).

There are different varieties of tomatoes that grow as single fruits or clusters. The market value, production quantities, and demand for tomato fruits are determined based on their visual characteristics and quality. These fruits are then presented to the market for consumption and export. Cluster tomatoes generally have a greater market advantage than single harvested tomatoes due to their fresh appearance and visual appeal. Harvesting tomatoes in clusters reduces labor costs and time (Benoumoualem et al., 2004). Tomatoes are an important part of the global economy, but they are susceptible to various diseases caused by pathogens and insects. These diseases can lead to a significant decrease in crop yield, nutrient content, shelf life, and fruit quality, and can even kill the plant. Fungi and viruses are examples of the types of pathogens that can infect tomato plants.

The major viral pathogens that can negatively impact tomato production include: *Tomato Yellow Leaf Curl Virus* (TYLCV), *Tomato Brown Rugose Fruit Virus* (TBRFV)



(Salem et al., 2016; Luria et al., 2017), *Tomato Mosaic Virus (ToMV)*, *Tomato Ring Spot Virus (ToRSV)*, *Potato Y Virus (PYV)*, and *Tomato Spotted Wilt Virus (TSWV)* (Wani et al., 2010). In addition, *Fusarium oxysporum* is known to be one of the most important fungal pathogens affecting tomato plants (Hassan, 2020). Two specific strains of *Fusarium oxysporum* can infect tomatoes. The first one, called *F. oxysporum* f.sp. *radicis-lycopersici* (*Forl*), causes *Fusarium* crown and root rot, while the second one, called *F. oxysporum* f.sp. *lycopersici* (*FoI*), causes vascular wilt (Armstrong and Armstrong, 1981). *Forl* is a pathogenic fungus found in the soil around various plant species worldwide and is known to cause *Fusarium* crown and root rot in tomatoes and other crops, making it an economically significant problem. The pathogen was first discovered in South Florida, USA (Sonoda, 1976) and was later detected in Turkey for the first time in 2004 (Can et al., 2004). Symptoms of crown and root rot caused by *Forl* include yellowing and wilting of plants, as well as severe root rot. These symptoms can worsen under certain conditions, such as low temperatures (10-20 °C), moist soil, saline water, and low pH levels, ultimately resulting in rapid spread and increased impact on the plant (Hassan, 2020).

Given the lack of an effective fungicide to combat *Fusarium* wilt disease in tomato cultivation, as well as the inefficiency of soil solarization, the most viable solution appears to be the use of tomato plants resistant to *Forl* (Szczechura et al., 2013). This resistance is due to a single dominant gene, *Frl*, on the 9th chromosome of the tomato (Fazio et al., 1999; Truong et al., 2011). *Fusarium oxysporum* has become a significant soil-borne pathogen affecting tomato production. It has led to substantial losses in tomato cultivation in various countries, including the United States, Mexico, Israel, and specific regions of Turkey (Geng et al., 2012). *Tomato Yellow Leaf Curl Virus (TYLCV)* is a pathogen belonging to the Begomovirus genus of the Geminiviridae family. It has a single-stranded circular DNA genome of around 2.8 kb. *TYLCV* is responsible for significant losses in the tomato economy (Abhary, 2007; Hull, 2009). Earliest evidence of *TYLCV* was found in Israel during 1939-1940. It was associated with outbreaks of the whitefly (*Bemisia tabaci*) and observed to be a harmful disease agent affecting tomato cultivation in Jordan during the 1960s (Cohen and Nitzany, 1966). It was named *Tomato Yellow Leaf Curl Virus (TYLCV)* (Cohen and Harpaz, 1964). The presence of *Tomato Yellow Leaf Curl Virus (TYLCV)* was first reported in areas where tomatoes were grown in Türkiye (Yılmaz, 1978).

Tomato crops can suffer significant economic losses due to the *TYLCV* disease, resulting in yield losses of up to 100% depending on the stage of infection (Moriones and Navas-Castillo, 2000). *TYLCV* is a disease that poses a significant threat to countries with economically important tomato production, such as China, India, the United States, and Türkiye. The infection caused by this disease has spread to the many regions worldwide, including tropical, subtropical, and temperate regions,

and affects a wide range of hosts, including tomatoes. The severity of the infection in tomato plant populations is directly related to the level of the vector for *Tomato yellow leaf curl virus (TYLCV)* known as *Bemisia tabaci* (Ghanim et al., 1998). To prevent losses caused by the *TYLCV* disease, it is important to take measures to stop the virus from spreading in areas where tomatoes are grown (Czosnek and Laterrot, 1997; Czosnek and Ghanim, 2011). One suggested method is to use tomato plant varieties that are resistant to pests and diseases (Moriones et al., 2007). Genetic studies have revealed that the resistance of tomatoes to *TYLCV* is primarily controlled by multiple genes. The development of resistant tomato varieties relies mainly on Ty-3 (Zamir et al., 1994; Hanson et al., 2000; Ji et al., 2007; Ji et al., 2009; Anbinder et al., 2009).

The use of molecular markers and genetic mapping techniques has sped up the selection of high-quality, disease-resistant tomato varieties in breeding programs. This allows for the screening of thousands of plants in a shorter time. This selection process was first introduced in tomato plants (Tanksley, 1983; Tanksley et al., 1992). Molecular markers are tools that help identify gene loci resistant to certain diseases in an organism. This information aids in selecting parents for breeding programs. Two types of molecular markers have been developed: hybridization-based and PCR (Polymerase Chain Reaction)-based. PCR-based methods include Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), Single Nucleotide Polymorphism (SNP), and Amplified Fragment Length Polymorphism (AFLP). These techniques are used to develop and detect disease-resistant tomato varieties (Yang et al., 2014; Hanson et al., 2016). Sequence Characterized Amplified Region (SCAR) and Cleaved Amplified Polymorphic Sequence (CAPS) are important PCR-based molecular techniques used for the selection of tomatoes resistant to *TYLCV* and *Fusarium* (Nevame et al., 2018).

The aim of this study is to develop tomato varieties that are resistant or tolerant to *TYLCV* and *Forl* diseases using molecular marker-assisted selection techniques developed with the advancement of modern biotechnology in our breeding program.

## 2. Materials and Methods

In this study, samples from the young leaves of 69 cluster tomato varieties grown in a greenhouse were placed into sterile 1.5 mL Eppendorf tubes. Genomic DNA was then extracted from the samples using the CTAB method (Doyle and Doyle, 1990). The concentration of the genomic DNA samples was measured using a Thermo ND-1000 spectrophotometer, which showed a concentration of 100 ng/mL. Finally, the samples were stored at +4°C for further use.

To determine the resistance or tolerance of cluster tomato varieties (69) to *TYLCV* and *Forl*, PCR (Polymerase Chain Reaction) was performed using gene-specific primers *C2-25* and *Ty3P6-25*, respectively (Table

1). The PCR reactions for *Forl* and *TYLCV* consisted of 1.2 µL DNA (100 ng/µL), 1.25 µL 10X Dream *Taq* Buffer (containing 20 mM MgCl<sub>2</sub>), 1 µL dNTP (2.5 mM), 0.25 µL *Taq* polymerase (5U), 0.25 µL forward and reverse primers (10 mM). The final volume was adjusted to 12 µL with ddH<sub>2</sub>O.

For the *Forl* test, the PCR cycling parameters were as follows: initial denaturation at 94 °C for 1 minute, followed by denaturation at 94 °C for 25 seconds, annealing at 55 °C for 35 seconds, extension at 72 °C for 1 minute and 30 seconds, for a total of 35 cycles. This was followed by a final extension at 72 °C for an additional 5 minutes.

Similarly, for the *TYLCV* test, the PCR cycling parameters were as follows: initial denaturation at 94 °C for 3 minutes, denaturation at 94 °C for 30 seconds, annealing

at 53 °C for 1 minute, extension at 72 °C for 1 minute for a total of 35 cycles. This was followed by a final extension at 72 °C for an additional 10 minutes.

PCR products for *Forl* were digested using *XapI* enzyme. For each sample, a reaction was set up with 10.5 µL of PCR product, 0.66 µL of 10X buffer, 0.66 µL of *XapI* enzyme (500 U), and the final volume was adjusted to 20 µL with ddH<sub>2</sub>O. The samples were incubated overnight at 34 °C. The PCR products (*Ty3P6-259*) and cleavage products (*C2-25*) were loaded onto a 1.5% agarose gel prepared in 0.5X TAE (Tris-acetate-EDTA) buffer containing ethidium bromide (0.5 mg/mL). The gel was run at 100 volts for 150 minutes. The PCR and cleavage results were visualized using an ultraviolet (UV) light imaging system (Vilber Lourmat, France) and recorded for further analysis.

**Table 1.** Molecular markers and primers used

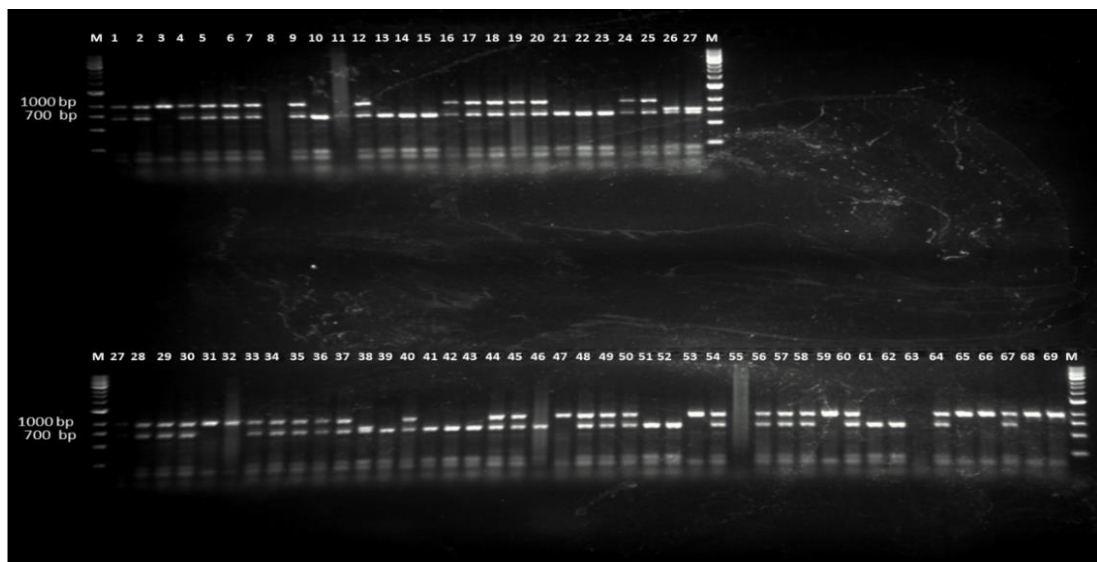
Gene	Marker	Primer Sequence 5'.....3'	Reference
<i>Ty</i>	<i>Ty3 P6-25</i>	F:GGTAGTGGAAATGATGCTGCTC R:GCTCTGCCTATTGTCCCATATATAACC	Jensen et al, 2007
<i>frl</i>	<i>C2-25</i>	F:ATG GGC GCT GCA TGT TTC GTG R:ACACCTTTG TTGAAAGCCATC CC	Staniaszek et al, 2014

### 3. Results

Based on the results of PCR tests using the *C2-25* and *Ty3P6-25* primers to determine the genotype of 69 cluster tomato varieties, it was found that 20 varieties were homozygous resistant/tolerant (*RR*) to *Forl*, 37 varieties were heterozygous resistant/tolerant (*Rr*), and 9 varieties were susceptible (*rr*) to the disease. Similarly, for *TYLCV*, 3 varieties were homozygous resistant/tolerant (*RR*), 27 were heterozygous resistant/tolerant (*Rr*), and 39 were susceptible (*rr*). No varieties were found to be simultaneously resistant or tolerant to both diseases. It is worth noting that some samples did not show any band formation. The genotypic analysis was provided in Table 2.

Analysis was conducted to determine the genotypes

using *C2-25* primers for *F. oxysporum* subsp. *radicis-lycopersici*. Samples with the homozygous resistance (*RR*) genotype showed a 700 bp band, while samples with the heterozygous resistance (*Rr*) genotype displayed bands at 700 and 1000 bp (Figure 1). Similarly, samples with the recessive genotype (*rr*) exhibited a single band at 1000 bp. Additionally, genotypic analysis using *Ty3P6-25* primers for Tomato yellow leaf curl virus (*TYLCV*) revealed that samples with the homozygous resistance (*RR*) genotype produced a single band at 630 bp. Meanwhile, samples with the heterozygous resistance (*Rr*) genotype showed bands at 630 and 320 bp, and samples with the recessive genotype (*rr*) displayed a single band at 320 bp (Figure 2).

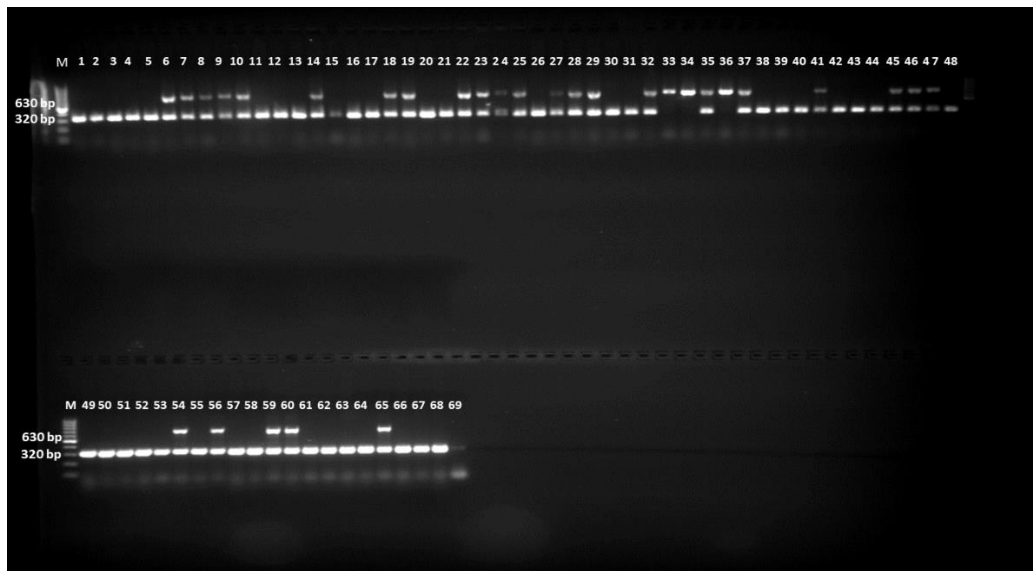


**Figure 1.** PCR of Cluster Tomato Varieties with *C2-25* primer (M = Marker, 1 kb; 1-69, Cluster Tomato Varieties).

**Table 2.** Molecular marker-based analyses of cluster tomato varieties (1-69) analyzed by PCR

Test No	Ty3P6-25	C2-25	Test No	Ty3P6-25	C2-25
1	<i>rr</i>	<i>Rr</i>	36	<i>RR</i>	<i>Rr</i>
2	<i>rr</i>	<i>Rr</i>	37	<i>Rr</i>	<i>Rr</i>
3	<i>rr</i>	<i>rr</i>	38	<i>rr</i>	<i>Rr</i>
4	<i>rr</i>	<i>Rr</i>	39	<i>rr</i>	<i>RR</i>
5	<i>rr</i>	<i>Rr</i>	40	<i>rr</i>	<i>RR</i>
6	<i>Rr</i>	<i>Rr</i>	41	<i>Rr</i>	<i>Rr</i>
7	<i>Rr</i>	<i>Rr</i>	42	<i>rr</i>	<i>RR</i>
8	<i>Rr</i>	-	43	<i>rr</i>	<i>RR</i>
9	<i>Rr</i>	<i>Rr</i>	44	<i>rr</i>	<i>RR</i>
10	<i>Rr</i>	<i>RR</i>	45	<i>Rr</i>	<i>Rr</i>
11	<i>rr</i>	<i>RR</i>	46	<i>Rr</i>	<i>Rr</i>
12	<i>rr</i>	<i>Rr</i>	47	<i>Rr</i>	<i>RR</i>
13	<i>rr</i>	<i>RR</i>	48	<i>rr</i>	<i>rr</i>
14	<i>Rr</i>	<i>RR</i>	49	<i>rr</i>	<i>Rr</i>
15	<i>rr</i>	<i>RR</i>	50	<i>rr</i>	<i>Rr</i>
16	<i>rr</i>	<i>Rr</i>	51	<i>rr</i>	<i>Rr</i>
17	<i>rr</i>	<i>Rr</i>	52	<i>rr</i>	<i>RR</i>
18	<i>Rr</i>	<i>Rr</i>	53	<i>rr</i>	<i>RR</i>
19	<i>Rr</i>	<i>Rr</i>	54	<i>Rr</i>	<i>rr</i>
20	<i>rr</i>	<i>Rr</i>	55	<i>rr</i>	<i>Rr</i>
21	<i>rr</i>	<i>RR</i>	56	<i>Rr</i>	-
22	<i>Rr</i>	<i>RR</i>	57	<i>rr</i>	<i>Rr</i>
23	<i>Rr</i>	<i>RR</i>	58	<i>rr</i>	<i>Rr</i>
24	<i>Rr</i>	<i>Rr</i>	59	<i>Rr</i>	<i>Rr</i>
25	<i>Rr</i>	<i>Rr</i>	60	<i>Rr</i>	<i>rr</i>
26	<i>rr</i>	<i>RR</i>	61	<i>rr</i>	<i>Rr</i>
27	<i>Rr</i>	<i>RR</i>	62	<i>rr</i>	<i>RR</i>
28	<i>Rr</i>	<i>Rr</i>	63	<i>rr</i>	<i>RR</i>
29	<i>Rr</i>	<i>Rr</i>	64	<i>rr</i>	-
30	<i>rr</i>	<i>Rr</i>	65	<i>Rr</i>	<i>Rr</i>
31	<i>rr</i>	<i>Rr</i>	66	<i>rr</i>	<i>rr</i>
32	<i>Rr</i>	<i>rr</i>	67	<i>rr</i>	<i>rr</i>
33	<i>RR</i>	<i>rr</i>	68	<i>rr</i>	<i>Rr</i>
34	<i>RR</i>	<i>Rr</i>	69	<i>rr</i>	<i>rr</i>
35	<i>Rr</i>	<i>Rr</i>			

*RR*= homozygous resistant/tolerant, *Rr*= heterozygous resistant/tolerant, *rr*= susceptible, -= not detected.



**Figure 2.** PCR for clustert varieties with Ty3P6-25 primer (Marker = Marker 100 bp; 1-69, Cluster Tomato Varieties).

#### 4. Discussion

The tomato (*Solanum lycopersicum* L.) is a popular and nutritious component of the human diet. It contains various vitamins and phenolic compounds that are believed to be effective against many types of cancer. Furthermore, it is one of the most extensively produced and traded agricultural commodities worldwide. However, the widespread occurrence of fungal and viral diseases has forced many farmers to give up on tomato farming altogether. In response to these challenges, modern breeding techniques have been developed to combat these diseases. Traditional breeding methods have primarily focused on selecting plants based only on their physical traits rather than their genetic sensitivity to disease. However, this approach is complicated by the influence of environmental factors and specific farming practices on plant traits, making field screening a complex and time-consuming process (Hanssen et al., 2010; Junker et al., 2015). The most effective way to manage diseases is by selecting resistant varieties. Not only is this method affordable and straightforward, but it's also environmentally safe (Hanssen et al., 2010).

Molecular-assisted selection can accelerate the process of selecting resistant plants. However, not all molecular markers known are suitable for tomato breeding programs. Therefore, further research is necessary to identify and develop allele-specific molecular markers that can improve the use of the molecular-assisted selection method in tomato breeding programs (Foolad and Panthee, 2012).

Marker-Assisted Selection (MAS), has significantly increased the speed and effectiveness of developing more resistant varieties through phenotypic selection (Grube et al., 2000).

"The spread of *TYLCV* presents a significant threat to tomato yield and production. Producers are looking for solutions, and studies suggest that using virus-resistant plant species offers advantages. Modern MAS techniques can greatly facilitate the selection and cultivation process of resistant varieties, reducing the time and effort required (Grube et al., 2000).

Studies have shown that domesticated tomatoes are susceptible to the *Tomato Yellow Leaf Curl Virus (TYLCV)*. However, certain wild tomato species such as *S. arcanum*, *S. cheesmaniae*, *S. chilense*, *S. galapagense*, *S. chmielewskii*, *S. corneliomulleri*, *S. habrochaites*, *S. neorickii*, *S. peruvianum*, *S. pimpinellifolium*, and *S. pennellii* exhibit symptoms when infected with the virus. This has sparked further investigation into the resistance of these species. Previous studies have identified different gene regions that provide resistance to *TYLCV* (*Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5*, *Ty-6*), with *Ty-1*, *Ty-2*, *Ty-3*, and *Ty-4* being dominant, and *Ty-5* being recessive (Zamir et al., 1994; Hanson et al., 2000; Ji et al., 2007; Ji et al., 2009; Anbinder et al., 2009). Both the *Ty-1* and *Ty-2* genes have shown high resistance to the *Tomato Yellow Leaf Curl Virus (TYLCV)* and have been widely utilized by breeders. In later studies, it was discovered that *Ty-2* and *Ty-3* genes

have no impact on begomoviruses, and their resistance has been overcome by specific strains of *TYLCV* (Ji et al., 2007). The *Ty-2* and *Ty-3* genes may not be as effective as the *Ty-3* and *Ty-3a* genes in providing resistance against *TYLCV*. Resistance to *TYLCV* is dependent on the *Ty-3* and *Ty-3a* genes (Ji et al., 2007; Hanson et al., 2016; Nevame et al., 2018). The *Ty-3* gene, which has two allelic genes obtained from the *S. chilense*, were named *Ty-3* and *Ty-3a*. *Ty-3* was derived from *LA2779* and *Ty-3a* from *LA1932*. Co-dominant markers were developed for both genes, and it was found that the *P6-25* marker yielded results for both *Ty-3* and *Ty-3a*. While the *Ty-3* gene provides broader resistance, the *Ty-3a* gene is preferred due to its association with fewer undesirable traits, while still retaining the resistance gene (Ji et al., 2007).

Specific markers known as *Ty3P6-25* markers were created to identify the *Ty3* gene region. PCR analysis using *Ty3P6-25* revealed that samples with a homozygous (*RR*) resistance genotype produced a single band of 630 bp. On the other hand, samples with a heterozygous (*Rr*) resistance genotype showed two bands at 630 bp and 320 bp. Additionally, samples with a homozygous recessive (*rr*) genotype exhibited a single band of 320 bp (Jensen et al., 2007). Tomato varieties that are resistant or tolerant against the *Tomato Yellow Leaf Curl Virus (TYLCV)* and have both homozygous and heterozygous traits were developed using the *Ty-3* gene-specific *Ty3P6-25* primer. Currently, there is no effective fungicide to combat *Fusarium wilt (Forl)* disease in tomato cultivation. Though soil solarization is inadequate, the use of *Forl*-resistant tomato plants has been considered the most viable method to combat the disease (Szczuchura et al., 2013).

The first mapping study concerning *Forl* in tomatoes identified a *Forl*-resistant gene region in *Solanum peruvianum*, a wild species of tomato, known as *Forl* (Laterrot and Moretti, 1991; Fazio et al., 1999). The gene (*frl*) provides genetic resistance to tomato and is controlled by a single dominant gene on the 9th chromosome (Laterrot and Moretti, 1991).

A study was conducted to identify markers associated with *Frl* and explore the connection between the *Forl*-resistant *Frl* locus in tomato and the *Tm-2* locus, which confers resistance to multiple strains of *Tobacco mosaic virus (TMV)*. A cross was made between the 'Motelle' breeding line and 'IRB-301-31', and fifteen to sixty seedlings from the F3 generation were tested for resistance to *Fusarium* and the *TMV* "0" strain. The results showed a strong linkage between *Frl* and *Tm-2* (Vakalounakis et al., 1997).

The gene-specific marker *C2-25* for the *frl* gene region was created by Staniaszek et al. (2014). In PCR analysis using this marker, samples with homozygous resistant (*RR*) genotype displayed a 700 bp band, while samples with heterozygous resistant (*Rr*) genotype showed two bands at 700 and 1000 bp. Susceptible varieties (*rr*) produced a single 1000 bp band. This study developed homozygous and heterozygous resistant or tolerant cluster tomato varieties among the selected cluster

tomato varieties using the *C2-25* marker specific to the *Frl* gene region.

## 5. Conclusion

Molecular markers have been developed against two economically important diseases caused by *Forl* and *TYLCV*. These molecular markers can be used successfully to create resistant/tolerant cluster tomato varieties against these diseases. The use of molecular markers in plant breeding programs has been proven to be fast, easy, and advantageous in numerous studies. However, it is important to note that genotypes developed through molecular marker-assisted selection may produce different results in real-world conditions due to the complex nature of pathogens, the emergence of new variants, and variability in virulence. A genotype that is resistant in one region may exhibit susceptibility in another region. Therefore, it is necessary to confirm the reliability of the marker and the true resistance of genotypes to the disease after inoculation with region-specific pathogens.

After completing this study, we will conduct further research to confirm the resistance or tolerance of cluster tomato varieties obtained by conducting pathogenicity tests using pathogens from different geographical regions. Additionally, we will separately measure the susceptibility of *Forl* and *TYLCV* resistant or tolerant varieties to different biotic markers. This will enable us to develop resistance to different biotic agents within the same cluster tomato variety.

This method will enable testing a larger variety of materials and reduce the duration of breeding programs, ultimately increasing the success rate. By using modern breeding methods, individuals with desired traits can be hybridized among heterozygous individuals selected from the two disease resistances. This will result in cluster tomato varieties that are resistant or tolerant to both diseases simultaneously.

## Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.B.	O.K.
C	70	30
D	70	30
S	100	
DCP	60	40
DAI	70	30
L	100	
W	100	
CR	50	50
SR	50	50
PM	30	70
FA		100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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