



Determination of TYLCV-Resistant Cherry and Cocktail Tomato Cultivars by Molecular Markers

Hüseyin BASIM^{1*}, Osman KANDİL², Mine KARAOĞLAN²

¹Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Antalya, Türkiye

²Istanbul Tarım SAN. and TİC. A.Ş., Antalya, Türkiye

*Sorumlu yazar: hbasim@akdeniz.edu.tr

ARTICLE INFO

Received: 08/09/2023

Accepted: 21/09/2023

Keywords: *Cherry and cocktail tomatoes, Molecular marker, Tomato Yellow Leaf Curl Virus*

DOI: 10.55979/tjse.1357477

ABSTRACT

In this study, resistant cultivars to Tomato yellow leaf curly virus (*TYLCV*) of different cherry and cocktail tomato cultivars were determined using molecular DNA markers. For this purpose, resistance to Tomato Yellow Leaf Curly Virus (*TYLCV*) of a total of 409 different cherry and cocktail tomato cultivars was determined by polymerase chain reaction (PCR) using the primer Ty3P6-25. As a result of the assays, 291 cherry and cocktail tomato cultivars were found to be susceptible (*rr*), 66 cultivars were heterozygous resistant (*Rr*), and 45 cultivars were homozygous resistant (*RR*) to *TYLCV*. In addition, no molecular markers were detected in 7 cherry and cocktail tomato cultivars. It was found that the molecular DNA marker used is useful in determining resistance responses to *TYLCV* in cherry tomato and cocktail tomato and can provide reproducible and reliable results in a short time.

TYLCV'ye Dayanıklı Kiraz ve Kokteyl Domates Çeşitlerinin Moleküler Markörler ile Belirlenmesi

MAKALE BİLGİSİ

Alınış tarihi: 08/09/2023

Kabul tarihi: 21/09/2023

Anahtar Kelimeler: *Domates Sarı Yaprak Kıvrılma Virüsü, Kiraz ve kokteyl domatesleri, Moleküler markör*

DOI: 10.55979/tjse.1357477

ÖZET

Bu çalışmada, farklı kiraz ve kokteyl domates çeşitlerinin Domates sarı yaprak kıvrık virüsüne (*TYLCV*) karşı dayanıklılıkları moleküler DNA markörleri kullanılarak belirlenmiştir. Bu amaçla, Ty3P6-25 primeri kullanılarak polimeraz zincir reaksiyonu (PCR) ile toplam 409 farklı kiraz ve kokteyl domates çeşidinin Tomato Yellow Leaf Curly Virus'a (*TYLCV*) dayanıklılığı belirlenmiştir. Testler sonucunda, 291 kiraz ve kokteyl domates çeşidinin *TYLCV*'ye duyarlı (*rr*), 66 çeşidin heterozigot dayanıklı (*Rr*) ve 45 çeşidin homozigot dayanıklı (*RR*) olduğu tespit edilmiştir. Ayrıca, 7 kiraz ve kokteyl domates çeşidinde herhangi bir moleküler belirteç tespit edilmemiştir. Kullanılan moleküler DNA markörünün kiraz domatesi ve kokteyl domatesinde *TYLCV*'ye karşı direnç yanıtlarını belirlemede yararlı olduğu ve kısa sürede tekrarlanabilir ve güvenilir sonuçlar sağlayabileceği bulunmuştur.

1. Introduction

Vegetables, grown in many parts of the world, are an extremely important source of nutrients needed by living things. Tomato. Tomato is one of the most important vegetables in global agricultural production because it contains various minerals and vitamins, is one of the most produced, consumed, and traded agricultural products in the world, and has many uses in the food industry such as canned food, tomato paste, and ketchup (Tatar & Pirinç, 2017). About 7% of tomatoes, of which 186 822 thousand tons are produced on an area of 5052 thousand hectares around the world, are produced in Turkey, and with this production amount, our country ranks 3rd in tomato production in the world after India and China (FAO, 2021; FAO, 2022). Turkey's vegetable production as of 2021 is approximately 32 million tons. Tomato, which is the most produced vegetable in Turkey, has a share of 41.2% (13.1 million tons) in total vegetable production in 2021 (TUİK, 2022).

Moreover, greenhouse cultivation is very common in our country, and tomato production and export can be done at

any time of the year. In 2021, 4.4 million tons of tomatoes were produced under glass in Turkey. When listing tomato cultivation areas in Turkey in 2021 by province, it was found that Antalya ranked first with 19 thousand hectares, Bursa ranked second with 16 thousand hectares and Manisa ranked second with about 13 thousand hectares (TUİK, 2022).

Tomato varieties cultivated in the world differ in their physical properties and bioactive compounds contained in them. The cultivated tomato (*Solanum lycopersicum* L.) is rich in nutrients, economically important and the second most consumed vegetable in the world (Foolad, 2007). Cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) is physically smaller than the cultivated tomato and is the ancestor of the domesticated form of the cultivated tomato (Ranc et al., 2009). Generally, the amounts of components such as tocopherol, folate and potassium, including bioactive phenolic compounds, are higher in cherry tomatoes compared to large tomatoes (Choi et al., 2011). Also, cherry tomatoes contain higher levels of phenolic compounds and nutrients than their processed products.

Therefore, the demand for fresh tomato fruit is increasing, and especially cherry tomato, because of its high nutritional content, its consumption is increasing and it is becoming more and more popular as a fresh salad dish.

Phytopathological and entomological problems arise from failure to properly maintain greenhouse conditions such as cultivation, temperature, humidity, and ventilation that limit tomato production and yields throughout the world and cause economically significant product losses. Despite years of intensive efforts to develop disease-resistant.

tomato breeding programs, numerous viral and fungal plant diseases still threaten tomato production. Major viral diseases that negatively impact tomato production include: *Tomato Yellow Leaf Curl Virus (TYLCV)*, *Tomato Mosaic Virus (ToMV)*, *Tomato Ring Spot Virus (ToRSV)*, *Potato Y Virus (PVY)*, *Tomato Spotted Wilt Virus (TSWV)* (Wani ve et al., 2010), *Tomato Brown Rugose Fruit Virus (TBRFV)* diseases (Salem et al., 2016; Luria et al., 2017) significantly damage tomato production. Tomato yellow leaf curl virus (*TYLCV*) disease is one of the most important virus diseases affecting tomato production worldwide (Czosnek & Laterrot, 1997; Hanssen et al., 2010; Moriones, et al., 2011). *TYLCV*, a member of the whitefly-transmitted genus *Begomoviruses* belonging to the family *Geminiviridae*, has a 2.7-2.8 kb ssDNA genome (Zhe et al., 2021). *TYLCV* was first described by Israel in 1939 (Pico et al., 1996). *TYLCV* is an economically important plant pathogen as it causes yield losses of up to 100% in tomato (Pérfarres et al., 2012; Kil et al., 2016; Thierry et al., 2012). The disease is most common at high temperatures, especially in many tropical and subtropical regions, and shows its most destructive effect at high temperatures (Lapidot et al., 2007). *TYLCV* causes yellowing and upward curling of the plant's upper leaves, flower drop, and stem stunting (Picó et al., 1996; Cohen & Lapidot, 2007). With the onset of breeding studies and the increasing popularity of cherry tomatoes among the population, the demand for cherry tomatoes has increased. This has prompted growers to develop new varieties that are higher yielding as well as more flavorful and disease resistant. Convective tomato breeding studies only allow phenotypic selection for plant susceptibility. However, due to the effects of environmental conditions and specific growing conditions on phenotype, field screening is a time-consuming and complex process (Junker et al., 2015; Moriones et al., 2007). With the development of molecular markers and genetic mapping methods, high-quality and disease-resistant tomato cultivars could be developed in a shorter time. Molecular markers are methods to select parents to be used in breeding programs and screen the desired gene region or gene loci related to disease resistance in the organism (Yorgancılar et al., 2015).

Marker assisted selection (MAS), developed as a modern molecular biology technique and molecular marker, greatly increases the speed and efficiency of developing resistant cultivars in phenotypic selection (Yorgancılar et al., 2015). Simple Sequence Repeat (SSP), Amplified Fragment Length Polymorphic (AFLP), Random Amplified Polymorphic DNA (RAPD), Single Nucleotide Polymorphic (SNP) methods developed based on PCR are molecular techniques to detect resistant gene regions for disease resistant tomato selection (Yang et al., 2014; Hanson et al., 2016; Yorgancılar et al., 2015).

In addition, Amplified Polymorphic Sequence (CAPS) and Sequence Characterized Amplified Region (SCAR) are important PCR-based molecular methods for identifying *Ty-1/Ty-3* resistant gene regions for *TYLCV*-resistant tomato selection. *TYLCV* resistance in tomato is mostly based on *Ty-3* (Adedze et al., 2018).

The objective of this study is to identify some cherry and cocktail tomato cultivars developed by our breeding program that are resistant to *TYLCV* disease by PCR using *TYLCV*-specific DNA molecular marker.

2. Materials and Methods

Samples taken from the young leaves of a total of 409 cherry and cocktail type tomato cultivars in our breeding program grown in greenhouse were taken into 1 ml eppendorf tubes and their genomic DNAs were isolated by CTAB method (Doyle & Doyle, 1990). The concentration of the obtained genomic DNA samples was measured with the help of spectrophotometer (Thermo ND-1000) (100 ng/ml) and stored at 4 °C for later use.

PCR was performed with gene-specific *Ty3* P6-25 primers (Jensen et al., 2007) to determine the resistance of cherry and cocktail tomato cultivars to *TYLCV*. PCR reactions for *TYLCV* were performed in a total volume of 25 µl; 2.2 µl DNA, 2.5 µl 10X Dream Taq Buffer (containing 20 mM MgCl₂), 4 µl dNTP (each dNTP 2.5 mM), 0.25 µl Taq (5U µl⁻¹ Taq DNA polymerase), 1 µl forward and reverse primers and ddH₂O were added to a total volume of 25 µl.

PCR cycle parameters for *TYLCV*: initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, binding at 53°C for 1 minute, elongation at 72°C for 1 minute, and an additional 10 at 72°C. minutes and 35 cycles in total.

PCR products were transferred to 1.5% agarose gel prepared with 0.5 TAE (Tris-acetate-EDTA) buffer and ethidium bromide (0.5 mg/ml) and run for 120 minutes at 110 volts. PCR results run in gel were visualized and recorded using an ultraviolet (UV) light imaging system (Vilber Lourmat, France).

3. Results ve Discussion

The results of PCR studies with *Ty3P6-25* primers (Figure 1.2.3.4) were examined genotypically: Samples with homozygous (*RR*) resistant genotype produced a single band of 630 bp, while samples with heterozygous (*Rr*) genotype had a band of 630 and 320 bp. had two bands. Finally, samples with homozygous recessive (*rr*) genotype had a single band of 320 bp (Tables 1,2,3,4). As a result of MAS assays, 291 cherry and cocktail tomato cultivars were found to be susceptible (*rr*), 66 cultivars were heterozygous resistant (*Rr*), and 45 cultivars were homozygous resistant (*RR*) to *TYLCSV*. In addition, no molecular markers were detected in 7 cherry and cocktail tomato cultivars.

Resistance to *TYLCSV* has been found in numerous tomato wild species, including *Solanum pimpinellifolium*, *S. peruvianum*, *S. chilense*, *S. habrochaites*, and *S. Cheesmaniae* (Pico et al., 1996). Many loci on tomato genome (i.e. *Ty-1* to *Ty-5*) for *TYLCSV* resistance have been described. The genes conferring resistance to *TYLCSV* contribute to the resistance originating from *S. habrochaites* (Sade et al., 2012; Eybishtz et al., 2009; Eybishtz et al., 2010). Three resistance genes, *Ty-1*, *Ty-3* or *Ty-3a* are primary used for *TYLCD* resistance in many tomato breeding program worldwide.

Evaluation of the results of this study showed that the PCR method using the *Ty3 P6-25* primers (Jensen et al., 2007) used in the determination of *Ty3*, the molecular marker for the presence of Tomato Yellow Leaf Roll

Virus (*TYLCSV*) disease resistance in cherry and cocktail tomato cultivars is very useful and safe. Therefore, the molecular resistance marker (*Ty3*) developed against Tomato Yellow Leaf Roll Virus (*TYLCSV*) should be used in different breeding programs to develop the above *TYLCSV* resistant cultivars. This method provides the opportunity to test more tomato plant material and increase the success rate by reducing the duration of tomato breeding programs.

Although cherry and cocktail tomato cultivars resistant to *TYLCSV* have been determined in this study by using molecular marker, pathogenicity tests are essential for a safer *TYLCSV* resistance. Therefore, testing different genotypes of the pathogen against plants that have been identified as resistant may provide a more accurate and stable resistance against genotypes of the pathogen from different geographic areas. For this purpose, in the next step of this study, cherry and cocktail tomato cultivars determined to be resistant *TYLCSV* by molecular marker will be tested *in vivo* against different genotypes of Tomato Yellow Leaf Curl Virus (*TYLCSV*).

The use of molecular markers and pathogenicity tests together in determining the resistance reactions against diseases is of great importance in terms of the reliability and sustainability of the resistance obtained. In such studies, it is essential to have different strains or genotypes of pathogens for pathogenicity tests.

Table 1. Genotypic characteristics of cherry and cocktail type tomato cultivars (1-96) analyzed by PCR

Cultivar No	<i>Ty3P6</i>	Cultivar No	<i>Ty3P6</i>	Cultivar No	<i>Ty3P6</i>
1	<i>rr</i>	33	<i>rr</i>	65	<i>Rr</i>
2	-	34	<i>rr</i>	66	<i>rr</i>
3	<i>rr</i>	35	<i>rr</i>	67	<i>rr</i>
4	<i>rr</i>	36	<i>rr</i>	68	<i>rr</i>
5	<i>rr</i>	37	<i>rr</i>	69	<i>rr</i>
6	<i>rr</i>	38	<i>rr</i>	70	<i>rr</i>
7	<i>rr</i>	39	<i>Rr</i>	71	<i>rr</i>
8	<i>rr</i>	40	<i>rr</i>	72	<i>rr</i>
9	<i>rr</i>	41	<i>rr</i>	73	<i>rr</i>
10	<i>rr</i>	42	<i>rr</i>	74	<i>rr</i>
11	<i>rr</i>	43	<i>rr</i>	75	<i>rr</i>
12	<i>rr</i>	44	<i>rr</i>	76	<i>rr</i>
13	<i>rr</i>	45	<i>rr</i>	77	<i>rr</i>
14	<i>rr</i>	46	<i>rr</i>	78	<i>rr</i>
15	<i>rr</i>	47	<i>rr</i>	79	<i>rr</i>
16	<i>rr</i>	48	<i>rr</i>	80	<i>rr</i>
17	-	49	<i>rr</i>	81	<i>rr</i>
18	<i>rr</i>	50	<i>rr</i>	82	<i>rr</i>
19	<i>rr</i>	51	<i>rr</i>	83	<i>rr</i>
20	<i>rr</i>	52	<i>rr</i>	84	<i>rr</i>
21	<i>rr</i>	53	<i>rr</i>	85	<i>rr</i>
22	<i>rr</i>	54	<i>rr</i>	86	<i>rr</i>
23	<i>rr</i>	55	<i>rr</i>	87	<i>rr</i>
24	<i>rr</i>	56	<i>rr</i>	88	<i>rr</i>
25	<i>rr</i>	57	<i>rr</i>	89	<i>rr</i>
26	<i>Rr</i>	58	<i>rr</i>	90	<i>rr</i>
27	<i>RR</i>	59	<i>RR</i>	91	<i>rr</i>
28	<i>RR</i>	60	<i>RR</i>	92	<i>rr</i>
29	<i>rr</i>	61	<i>rr</i>	93	<i>rr</i>
30	<i>rr</i>	62	<i>rr</i>	94	<i>rr</i>
31	<i>rr</i>	63	<i>rr</i>	95	<i>rr</i>
32	<i>rr</i>	64	<i>rr</i>	96	<i>rr</i>

RR: Homozygous Resistant; *Rr*: Heterozygous; *rr*: Sensitive, -: Not detected

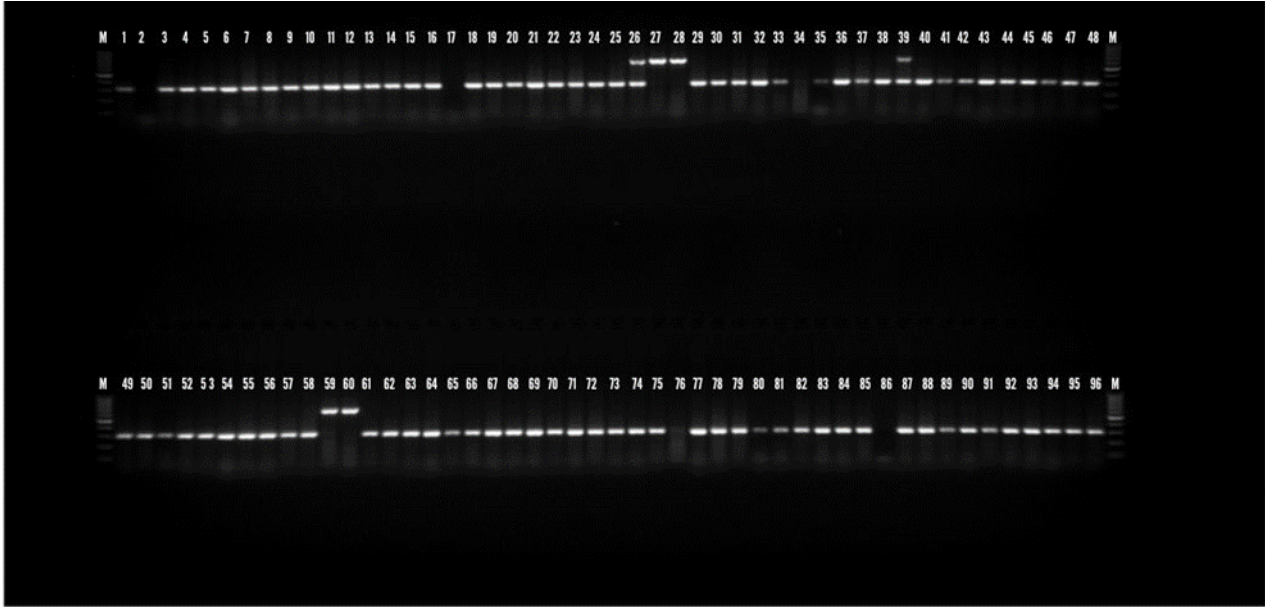


Figure 1. PCR results of cherry and cocktail type tomato varieties for *Ty3 P6-25*. M, Marker 100 bp; Tomato cultivars, 1-96

Table 2. Genotypic characteristics of cherry and cocktail type tomato varieties (101-196) analyzed by PCR

Cultivar No	<i>Ty3P6</i>	Cultivar No	<i>Ty3P6</i>	Cultivar No	<i>Ty3P6</i>
101	rr	133	Rr	165	rr
102	rr	134	Rr	166	rr
103	rr	135	Rr	167	Rr
104	rr	136	Rr	168	Rr
105	rr	137	Rr	169	Rr
106	rr	138	Rr	170	RR
107	rr	139	rr	171	RR
108	rr	140	rr	172	RR
109	rr	141	Rr	173	rr
110	rr	142	Rr	174	rr
111	rr	143	rr	175	-
112	rr	144	rr	176	Rr
113	rr	145	rr	177	Rr
114	rr	146	rr	178	RR
115	rr	147	rr	179	RR
116	rr	148	rr	180	rr
117	rr	149	rr	181	rr
118	rr	150	rr	182	rr
119	rr	151	rr	183	rr
120	rr	152	rr	184	rr
121	rr	153	rr	185	Rr
122	rr	154	rr	186	Rr
123	rr	155	rr	187	rr
124	rr	156	rr	188	RR
125	RR	157	rr	189	RR
126	Rr	158	rr	190	Rr
127	RR	159	rr	191	RR
128	RR	160	rr	192	RR
129	Rr	161	-	193	RR
130	RR	162	RR	194	Rr
131	rr	163	RR	195	Rr
132	rr	164	rr	196	rr

RR: Homozygous Resistant; Rr: Heterozygous; rr: Sensitive, -: Not detected

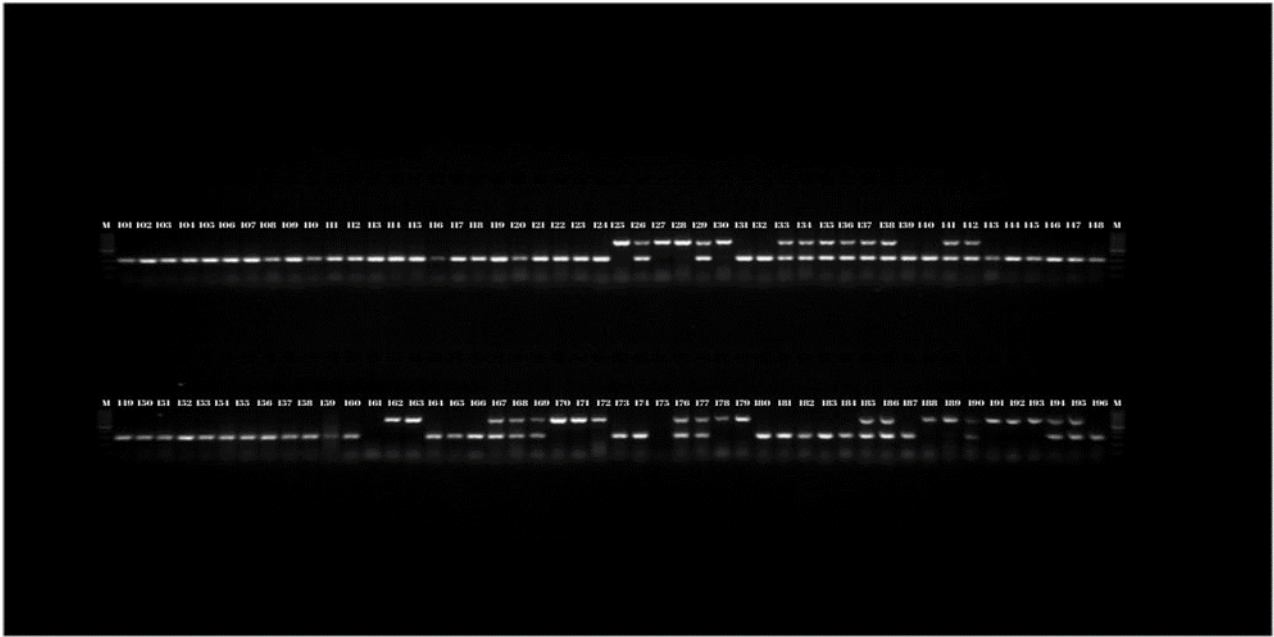


Figure 2. PCR results of cherry and cocktail type tomato cultivars for *Ty3 P6-25*. M, Marker 100 bp; Tomato cultivars, 101-196.

Table 3. Genotypic characteristics of cherry and cocktail type tomato varieties (201-296) analyzed by PCR

Cultivar No	<i>Ty3P6</i>	Cultivar No	<i>Ty3P6</i>	Cultivar No	<i>Ty3P6</i>
201	Rr	233	rr	265	rr
202	Rr	234	-	266	Rr
203	rr	235	Rr	267	Rr
204	rr	236	Rr	268	Rr
205	rr	237	rr	269	Rr
206	RR	238	rr	270	Rr
207	RR	239	Rr	271	RR
208	rr	240	RR	272	Rr
209	rr	241	RR	273	RR
210	Rr	242	rr	274	Rr
211	RR	243	rr	275	Rr
212	rr	244	Rr	276	Rr
213	RR	245	Rr	277	Rr
214	rr	246	RR	278	Rr
215	rr	247	RR	279	Rr
216	rr	248	rr	280	Rr
217	-	249	rr	281	Rr
218	rr	250	rr	282	Rr
219	rr	251	rr	283	RR
220	rr	252	rr	284	rr
221	Rr	253	RR	285	Rr
222	Rr	254	rr	286	rr
223	Rr	255	rr	287	rr
224	rr	256	RR	288	Rr
225	rr	257	RR	289	Rr
226	rr	258	Rr	290	rr
227	rr	259	rr	291	-
228	rr	260	Rr	292	rr
229	rr	261	Rr	293	rr
230	rr	262	rr	294	rr
231	rr	263	rr	295	rr
232	rr	264	rr	296	rr

RR: Homozygous Resistant; Rr: Heterozygous; rr: Sensitive, -: Not detected

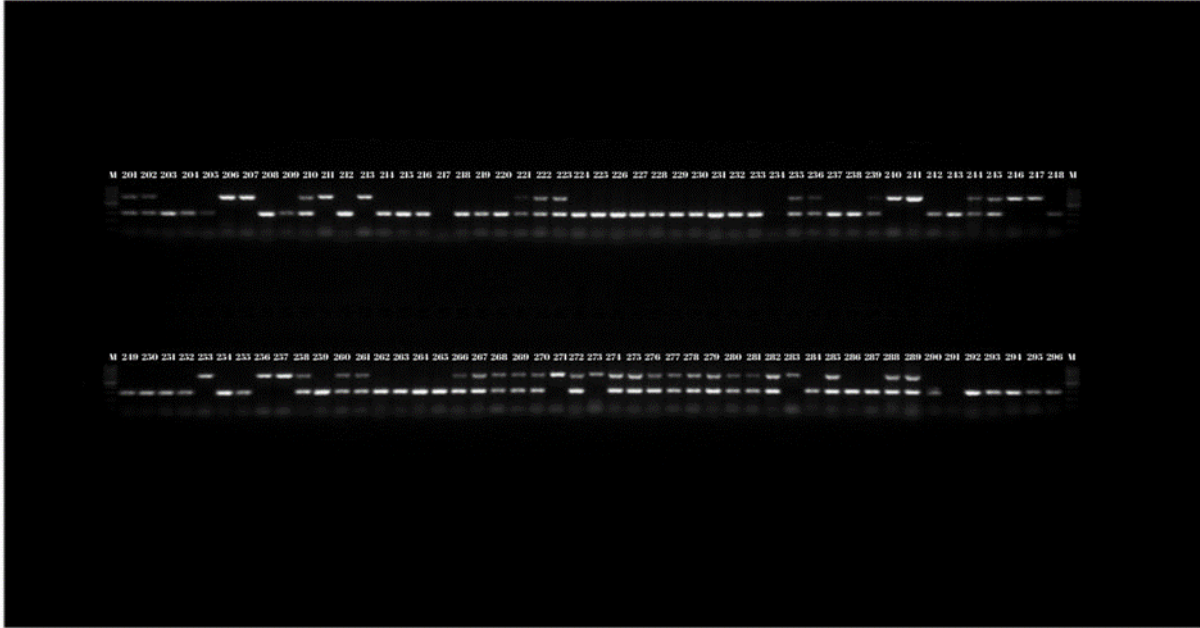


Figure 3. PCR results of cherry and cocktail type tomato cultivars for *Ty3* P6-25. M, Marker 100 bp; Tomato cultivars, 201-296.

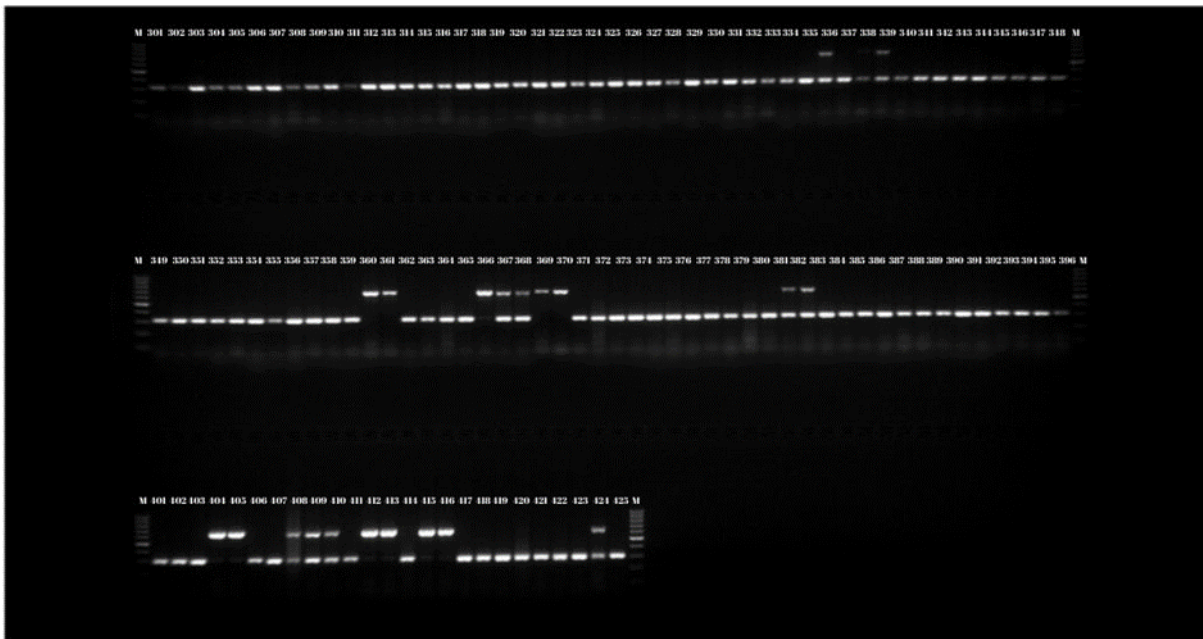


Figure 4. PCR results of cherry and cocktail type tomato cultivars for *Ty3* P6-25. M, Marker 100 bp; Tomato cultivars, 301-396 and 401-425.

Table 4. Genotypic characteristics of cherry and cocktail type tomato varieties (301-396 and 401-425) analyzed by PCR

Cultivar No	Ty3P6	Cultivar No	Ty3P6	Cultivar No	Ty3P6
301	rr	342	rr	383	Rr
302	rr	343	rr	384	rr
303	rr	344	rr	385	rr
304	rr	345	rr	386	rr
305	rr	346	rr	387	rr
306	rr	347	rr	388	rr
307	rr	348	rr	389	rr
308	rr	349	rr	390	rr
309	rr	350	rr	391	rr
310	rr	351	rr	392	rr
311	rr	352	rr	393	rr
312	rr	353	rr	394	rr
313	rr	354	rr	395	rr
314	rr	355	rr	396	rr
315	rr	356	rr	401	rr
316	rr	357	rr	402	rr
317	rr	358	rr	403	rr
318	rr	359	rr	404	RR
319	rr	360	RR	405	RR
320	rr	361	RR	406	rr
321	rr	362	rr	407	rr
322	rr	363	rr	408	Rr
323	rr	364	rr	409	Rr
324	rr	365	rr	410	Rr
325	rr	366	RR	411	Rr
326	rr	367	Rr	412	RR
327	rr	368	Rr	413	RR
328	rr	369	RR	414	rr
329	rr	370	RR	415	RR
330	rr	371	rr	416	RR
331	rr	372	rr	417	rr
332	rr	373	rr	418	rr
333	rr	374	rr	419	rr
334	rr	375	rr	420	rr
335	rr	376	rr	421	rr
336	Rr	377	rr	422	rr
337	rr	378	rr	423	rr
338	rr	379	rr	424	Rr
339	Rr	380	rr	425	rr
340	rr	381	rr		
341	rr	382	Rr		

RR: Homozygous Resistant; Rr: Heterozygous; rr: Sensitive, -: Not detected

4. Conclusion

In this study, cherry and cocktail tomato cultivars resistant to Tomato Yellow Leaf Curl Virus (TYLCV) disease was successfully determined. The resistance of the resistant cherry and cocktail tomato cultivars identified in this study will be verified by pathological testing using different genotypes of TYLCV.

Concept: H.B. (100%), Design: H.B. (100%), Supervision: O.K. (100%), Data collection and/or processing: O.K. (50%) and M.K. (50%), Data analysis and/or interpretation: H.B. (100%), Literature search: H.B. (50%), O.K. (25%), and M.K. (25%), Writing: H.B. (100%), Critical review: H.B. (100%). Submission and revision H.B. (100%). All authors reviewed and approved final version of the manuscript.

Acknowledgments

This work was supported by Istanbul Tarım AŞ.

Conflict of Interest

The authors declared that there is no conflict of interest.

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