

Detection of Potato Y Virus in Pepper Growing Areas in Isparta Province*

Isparta İli Biber Üretim Alanlarında Patates Y Virüsü'nün Belirlenmesi

Emre YAĞCIOĞLU^{1*}, Handan ÇULAL-KILIÇ²**Abstract**

The aim of the study was to determine the presence of Potato Y Virus (PVY) in pepper-growing areas in Isparta, using mechanical inoculation of indicator plants, Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). A total of 184 pepper plants were collected from pepper fields and all samples were tested for PVY using a specific antiserum in a DAS-ELISA. PVY was detected in 33 samples, amounting to an infection rate of 17.93% of the field samples. The highest PVY infection rate was observed in Senirkent (63.63%), followed by Kuleönü (57.14%). Leaf samples that tested positive for the PVY in the DAS-ELISA test were then used in mechanical inoculation, total RNA isolation, and RT-PCR studies. Following mechanical inoculation of sensitive test plants, typical symptoms of PVY were observed. In RT-PCR studies, primer pairs specific to PVY were used to amplify 801 bp fragments representing the coat protein region of PVY, which were then observed using agarose gel electrophoresis. RT-PCR testing confirmed that all nine DAS-ELISA-positive isolates and all eleven symptomatic plants were infected with PVY. No virus-specific PCR bands were observed in the pepper plants used as negative controls, nor in *Nicotiana tabacum* L. 'White Burley', *N. tabacum* L. 'Samsun NN', *Chenopodium amaranticolor* and *C. quinoa*, all of which remained symptomless in the mechanical inoculation assays. In this study, the presence of PVY was detected for the first time in pepper production areas in the province of Isparta, Turkey. It indicates that sequence analysis of PVY isolates should be carried out in future, that resistance studies should continue and that growers should be kept informed.

Keywords: Pepper, Virus, Potato Y virus, Detection

^{1*}**Sorumlu Yazar/Corresponding Author:** Emre Yağcıoğlu, Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye, E-mail: yagcioglu.emre@outlook.com  ORCID: 0009-0005-4089-6141

²Handan Çulal Kılıç, Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye. E-mail: handankilic@isparta.edu.tr  ORCID: 0000-0003-4020-9442

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Öz

Bu çalışmanın amacı indikatör bitkilerinin mekanik inokulasyonu, Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) ve Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) yöntemleri kullanılarak Isparta'daki biber yetiştirme alanlarında Patates Y Virüsü (PVY) varlığının belirlenmesidir. Tarlalardan toplam 184 biber bitkisi toplanmış ve tüm örnekler DAS-ELISA'da spesifik bir antiserum kullanılarak PVY açısından test edilmiştir. PVY, 33 örnekte tespit edilmiş ve tarla örneklerinin %17,93'ünde enfeksiyon oranı saptanmıştır. En yüksek PVY enfeksiyon oranı Senirkent'te (%63,63) gözlemlenirken, bunu Kuleönü (%57,14) izlemiştir. DAS-ELISA testinde PVY virüsü için pozitif çıkan yaprak örneklerinde elde edilen PVY izolatları, daha sonra mekanik aşılama, toplam RNA izolasyonu ve RT-PCR çalışmalarında kullanılmıştır. Hassas test bitkilerinin mekanik inokülasyonunu takiben PVY'nin tipik belirtileri gözlemlenmiştir. RT-PCR çalışmalarında, PVY için spesifik primer çiftleri kullanılmış ve PVY'nin kılıf protein bölgesine ait 801 bp'lik bölge çoğaltılmış ve agaroz jel elektroforezinde gözlemlenmiştir. RT-PCR testi, DAS-ELISA pozitif dokuz izolatın ve semptomatik on bir bitkinin tümünün PVY ile enfekte olduğunu doğrulamıştır. Negatif kontrol olarak kullanılan biber bitkilerinde, *N. tabacum* L. 'White Burley', *N. tabacum* L. 'Samsun NN', *C. amaranticolor* ve *C. quinoa*'da virüse özgü PCR bantları gözlemlenmedi ve bu bitkilerin tümü mekanik inokülasyon testlerinde semptomsuz kaldı. Bu çalışmada, Türkiye'nin Isparta ilindeki biber üretim alanlarında PVY'nin varlığı ilk kez tespit edilmiştir. Bu durum gelecekte PVY izolatlarının sekans analizinin yapılması, dayanıklılık çalışmalarının sürdürülmesi ve yetiştiricilerin bilgilendirilmeye devam edilmesi gerektiğini göstermektedir.

Anahtar Kelimeler: Biber, Virüs, Patates Y virüsü, Teşhis

1. Introduction

Vegetables are a rich food source containing high levels of protein, carbohydrates, vitamins, minerals and antioxidants (Abak et al., 2010; Sadeghi et al., 2015). Parts of vegetables such as fruits, flowers, leaves, stems, roots and seeds can be used as food sources and can be consumed fresh or processed (Erkan et al., 2013). The anti-cancer properties of vegetables and their effects on reducing cardiovascular diseases have been proven many times (Sadeghi et al., 2015). It is therefore of great importance for the health of a society to ensure that vegetables are grown to high quality standards (Salehzadeh et al., 2020).

Pepper is a vegetable species of the Solanaceae family with the scientific name *Capsicum annuum* L. (Bayram et al., 2019; Vural et al., 2000). *Capsicum* spp. are widely distributed crops of economic and nutritional importance throughout the world, including both wild and cultivated species, some of which are grown in regions with warm climates (Demirel et al., 2012). The fruits produced by these plants are commonly known as pepper, chili or red chili peppers and show a high variability in color and morphology (Sáez et al., 2024). The current classification of the genus *Capsicum* includes 38 wild and 5 cultivated species (Barboza et al., 2019).

Situated in transitional zone between the Central Anatolia and Mediterranean regions, Isparta province plays a crucial role in vegetable cultivation. In this region, an annual yield of 138.437 tons is obtained from 42.558 hectares of vegetable production area, of which 4.500 tons consist of pepper (processing, bell, long, Charleston types) (TUIK, 2021).

Viral pathogens such as pepper mild mottle virus (PMMoV), pepper veinal mottle virus (PVMV), pepper ringspot virus (PRSV), potato y virus (PVY), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), tomato spotted wilt virus (TSWV) cause significant damage in peppers (Arogundade et al., 2012; Buzkan et al., 2006; Fidan and Barut, 2019; Ozaslan et al., 2006; Ryu et al., 2009; Waweru et al., 2019).

Potato Y virus (PVY) was first described in 1931 as a potato virus transmitted by aphids. The virus, which belongs to the genus *Potyvirus* of the family Potyviridae, has a wide host range and causes significant economic losses in crops such as potato, pepper, tomato and tobacco. PVY has been renamed as Potyvirus yituberosi (ICTV, 2024). PVY, one of the most damaging plant pathogens in the world, has also caused damage to crops such as petunia in Europe, eggplant in India and golden strawberries in Hawaii (Kerlan, 2008).

The virus causes symptoms such as stunting, vein clearing, mosaic, dark green vein banding, necrosis, rugose and deformation of fruits. It is reported to cause 10-100% crop loss depending on host susceptibility, virus strain, infection time and environmental conditions (Quenouille et al., 2013). PVY can be mechanically transmitted by plant sap and at least 50 aphid species, mainly *Myzus persicae* (Karavina et al., 2021).

The management of viral diseases is highly challenging and requires the integration of multiple disciplines to achieve effective outcomes. Furthermore, the development program necessitates a comprehensive understanding of local, environmental, regional host-pathogen-vector interactions. Given these complexities, accurate identification of the causal agents is of primary importance in preventing viral disease. To ensure reliable detection, the application of multiple detection methods is strongly recommended. Therefore, this study aims to identify the presence of PVY in pepper production areas in Isparta using biological, serological and molecular methods.

2. Materials and Methods

This study was conducted in Isparta's pepper production areas between 2022 and 2023. During the surveys, a total of 184 pepper leaf samples were collected, which were suspected of being infected with the virus and exhibited symptoms including, but not limited to, mosaic symptoms, deformation, chlorosis, necrotic local lesions, vein banding, stunting, deformity and discoloration in fruits (Arli-Sokmen et al., 2005). The samples were then placed in nylon bags and stored in -18°C. In addition to DAS-ELISA (Double antibody sandwich-enzyme linked immunosorbent assay), symptomatologic studies with indicator plants and RT-PCR (Reverse transcription polymerase chain reaction) studies, which are more sensitive and specific than ELISA method, were carried out for the diagnosis of the virus in pepper samples stored in deep freezer.

2.1. DAS-ELISA Procedure

DAS-ELISA method was applied to samples exhibiting virus symptoms obtained during the surveys. DAS-ELISA test for the detection of PVY was performed according to the instructions of the supplier of the test kits (Bioreba, AG, Switzerland).

2.2. Mechanical inoculation method

The test plants were inoculated mechanically at the 3-4 leaf stage using the samples that gave positive results when tested by ELISA. Leaves from PVY-infected plants were prepared in 0.01 M phosphate buffer (pH 7.2) (containing 0.01% 2-mercaptoethanol) at a 1:1 (W/V) ratio and crushed in a sterile porcelain mortar. Carborundum powder, used as an abrasive, was poured on the leaves of the test plants and the prepared inoculum source was thoroughly applied to the leaves using cheesecloth. The leaves of the inoculated plants were then washed with tap water (Romero et al., 2001).

Test plants were placed in growth chambers at 18-26°C for symptom development. Plants were observed daily, and symptoms were scored according to literature (Abdel-Shafi et al., 2017; d'Aquino et al., 1995; Faurez et al., 2012; Moodley et al., 2019; Mostafaei et al., 2008).

2.3. Total RNA extraction

Total RNA extraction from leaf samples was performed using the Ribospin™ Total RNA Extraction Kit. Total RNA extraction steps were performed according to the instructions for use of the Ribospin™ Total RNA Extraction Kit. One-step RT-PCR studies were performed on the RNAs obtained from the extraction studies.

2.4. RT-PCR studies

A one-step RT-PCR method was used for the molecular identification of PVY in pepper plants. For this purpose, total RNAs obtained from serologically and biologically detected PVY-infected samples and negative samples were used.

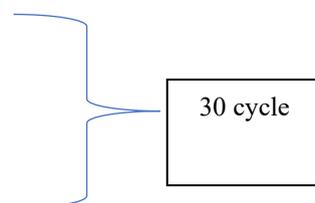
In the RT-PCR reaction, 1 µl of total RNA was added to 10 µl master mix buffer, 1 µl forward primer, 1.5 µl reverse primer and 6.5 µl ultrapure water to make a volume of 20 µl (GeneAll, One-step RT-PCR master mix kit). The samples were then loaded into the PCR instrument (Techne-TC-5000)

The primer pair (cpf- 5' TCAAGGATCCGCAAATGACACAATTGATGCAGG 3'; cpr- 5' AGAGAGAATTCATCACATGTTCTTGACTCC 3') used was that of Shalaby et al. (2002), which amplifies an 801 bp fragment specific for the coat protein gene region.

The binding temperature (55°C) was calculated in the Thermacycler programme, considering the melting temperature of the primer to be synthesised.

The temperature values and times used for PVY are as follows:

- 50°C 30 min
- 94°C 2 min
- 94°C 30 sec
- 55°C 30 sec
- 72°C 1 min
- 72°C 3 min
- +4°C ∞



2.5. Agarose Gel Studies

RT-PCR products were run for 1 hour at 100 V using 1% agarose gel and TBE (Tris, boric acid, EDTA) buffer solution with DNA markers of known size (100 bp DNA ladder, 1 KB DNA ladder). They were then photographed under UV transilluminator light.

3. Results and Discussion

Symptoms such as mosaic, vein banding, deformation, chlorosis, plant stunting, fruit deformation and discoloration were observed in pepper production areas during surveys of pepper growing fields in Isparta, Türkiye (Figure 1).

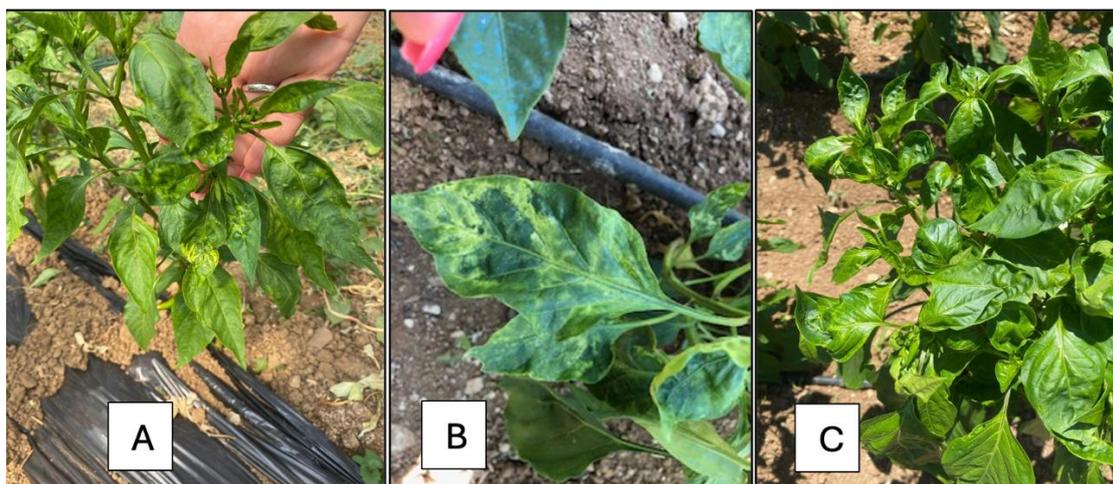


Figure 1. Symptoms observed on pepper leaves (A: Deformations, vein banding and vein wrinkling; B: Mosaic symptoms and deformations; C: Spoon shaped deformation)

3.1. DAS-ELISA Results

It was found that 33 out of 184 pepper samples collected were found infected with PVY and the infection rate was 17.93%. The highest PVY infection rate was observed in Senirkent (63.63%), followed by Kuleönü (57.14%). In other regions, the infection rates were as follows: Küçükgökçeli 27.77%, Aksu 17.07%, İslamköy 15%, Büyükgökçeli 14.28%, Isparta-Merkez 11.11%, Atabey 10%, Keçiborlu 6.25%. The samples collected in Gönen and Büyükkabaca tested negative for PVY. The detailed DAS-ELISA results are given in Table 1.

Table 1. The presence of PVY by DAS-ELISA in samples collected from pepper production areas in the province of Isparta

Sampled locations	Number of collected samples	Number of infected samples	Detection of PVY% (%)
Isparta-Merkez	18	2	11.11
Kuleönü	7	4	57.14
Büyükkabaca	6	0	0
Atabey	20	2	10
Aksu	41	7	17.07
Gönen	13	0	0
Keçiborlu	16	1	6.25
İslamköy	20	3	15
Küçükgökçeli	18	5	27.77
Büyükgökçeli	14	2	14.28
Senirkent	11	7	63.63
Toplam	184	33	17.93

ELISA plates were measured at a wavelength of 405 nm using an ELISA reader (Versamax). Samples with absorbance values at least twice the mean absorbance value of the negative control and greater were considered as positive. Observationally, yellow coloration was observed in the wells where these samples were found. (Çulal-Kılıç, 2019).

3.2. Results of mechanical inoculation studies

When test plants were inoculated mechanically, symptoms appeared 5-14 days after inoculation. These symptoms were scored and photographed. The symptoms observed in the test plants as a result of mechanical inoculation were given in Table 2 and some of these symptoms were illustrated in Figure 2.

Table 2. Symptoms caused by PVY on test plants

Test plants	Symptoms observed after post-inoculation (day)	Symptoms
<i>Chenopodium amaranticolor</i>	*	*
<i>Chenopodium quinoa</i>	*	*
<i>Capsicum annuum</i> L.	10	Def, Y
<i>Nicotiana glutinosa</i>	12	Def, M
<i>Nicotiana benthamiana</i>	12-14	Def, VR.
<i>Nicotiana rustica</i>	5-13	NLL, Def
<i>Nicotiana tabacum</i> L. "White Burley"	*	*
<i>Nicotiana tabacum</i> L. "Xanthii"	12	Si, M
<i>Nicotiana tabacum</i> L. "Samsun NN"	*	*

*No Symptoms, Def: Deformation Y: Yellowing M: Mosaic VR: Vascular retraction NLL: Necrotic local lesion Si: Systemic infection



Figure 2. Symptoms caused by PVY on test plants (A: Symptoms of PVY on pepper leaves after inoculation; B: Symptoms of *N. benthamiana* after mechanical inoculation. 3: control plant 4: inoculated plant; C: Symptoms on leaves after inoculation. 8: control plant; 5,6,7: inoculated leaves)

3.3. Total RNA Extraction

Leaves from 9 positive and 2 negative samples and leaves from 11 test plants were used in the extraction studies. In addition, leaves from symptomless pepper plants were used as a negative control.

3.4. Results of RT-PCR studies

In RT-PCR studies, 9 isolates that were positive by DAS-ELISA and 11 test plants that showed symptoms following inoculation of test plants gave bands at the expected level and were found to be infected with PVY. No PVY-specific PCR bands were obtained from the pepper plants used as negative controls, nor from the mechanically inoculated *N. tabacum* L. 'White Burley', *N. tabacum* L. 'Samsun NN', *C. amaranticolor* and *C. quinoa* all of which remained symptomless following inoculation. The RT-PCR results of the test plants and field samples were shown in Figure 3.

Symptomatic studies based on observation alone are not sufficient for the diagnosis of viruses. Investigations should be supported by biological, serological and molecular studies. Measures can be taken to control this viral disease once the factor has been identified.

This study was carried out to investigate PVY in pepper growing areas of Isparta province. Biological, serological and molecular methods were used to identify the virus. All these methods were mutually supportive.

Symptoms such as mosaic, necrosis, chlorosis, deformation, vein banding symptoms, stunting, and small fruit formation were observed on leaves during the surveys in pepper production areas in Isparta province and its districts. These symptoms were consistent with those reported in previous studies (Choi et al., 2005; Deligöz et al., 2023; Fanigliulo et al., 2005; Ibaba and Gubba, 2011).

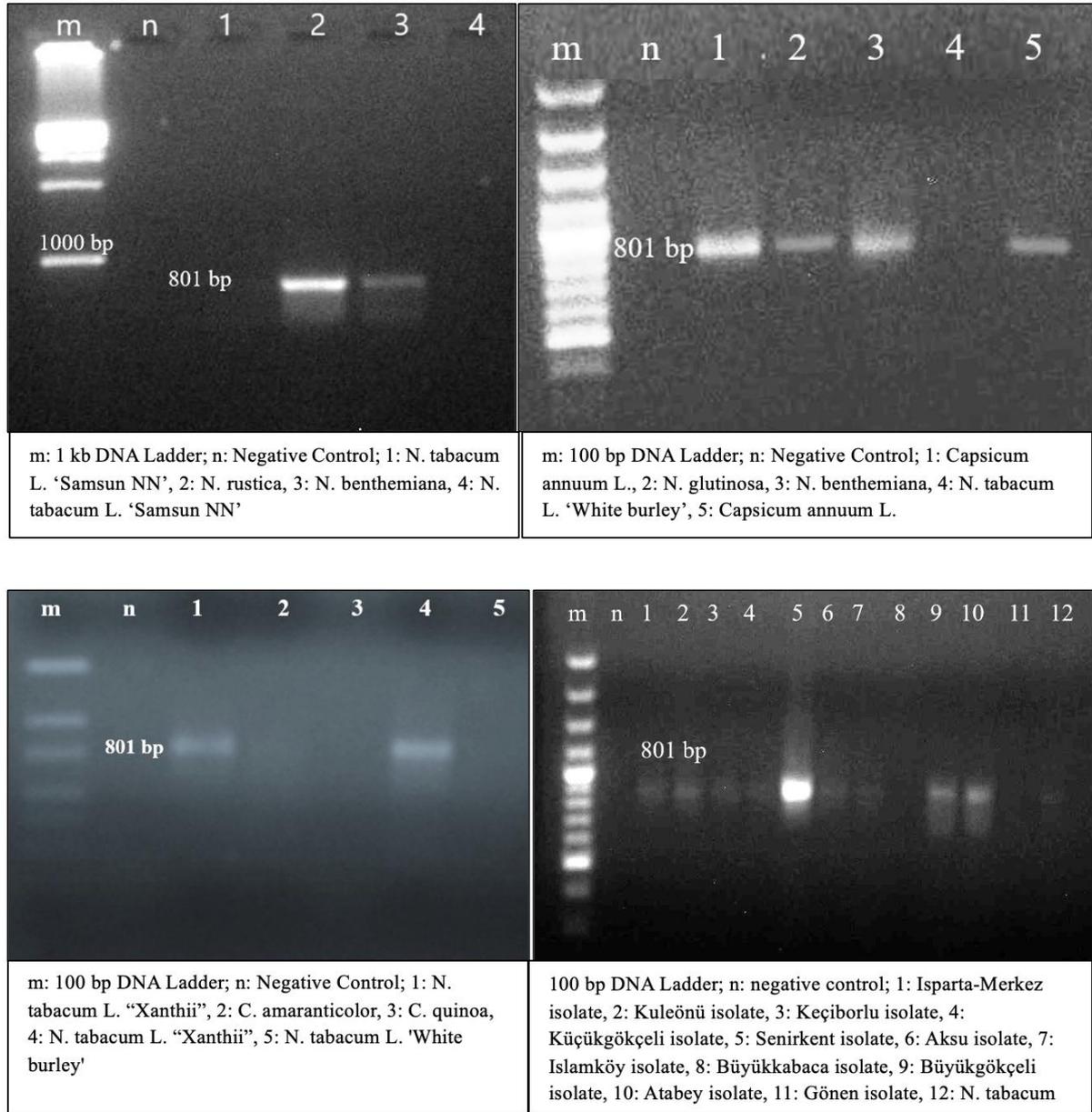


Figure 3. RT-PCR Results from Test Plants and Field Samples

The study used DAS-ELISA to diagnose the virus. This method is widely used because of its advantages, such as the ability to test many samples at the same time and its reliability. Many other researchers have also used DAS-ELISA to diagnose PVY (Arli-Sokmen et al., 2005; Buzkan et al., 2006; Deligöz and Sokmen, 2020; Echer and Costa, 2002; El-Borollosy, 2015; Milošević et al., 2018).

The highest PVY infection ratio in the region was observed in Senirkent (63.63%), followed by Kuleönü (57.14%). In other regions, the infection ratios were as follows: Küçükgökçeli 27.77%, Aksu 17.07%, İslamköy 15%, Büyükgökçeli 14.28%, Isparta-Merkez 11.11%, Atabey 10%, Keçiborlu 6.25%. The samples from Gönen and Büyükkabaca had no evidence of PVY infection. The samples identified as infected with PVY by DAS-ELISA were used for biological and molecular studies.

PVY is a virus that can be easily transported by mechanical inoculation. Previous researchers have also reported that the virus can be easily transferred to test plants by mechanical inoculation (Abdel-Shafi et al., 2017; d'Aquino et al., 1995; Mostafaei et al., 2008; Romero et al., 2001; Saydam et al., 2017). El-Banna et al. (2015) used *Capsicum annuum* L., *Datura stramonium* L., *Solanum tuberosum* L., *N. tabacum* L. White Burley and *Lycopersicon esculentum* L. as test plants. Özdağ and Sertkaya (2017) used *C. annuum*, *L. esculantum*, *N. benthamiana*, *N. glutinosa* and

Phaseolus vulgaris as test plants. In a Korean study on PVY in chilli, *C. amaranticolor*, *C. quinoa*, *N. benthamiana*, *N. tabacum* cv. 'bright yellow', *N. tabacum* cv. Samsun, *Physalis floridana* and *Petunia* spp. were preferred for mechanical inoculation (Ryu et al., 2009). Romero et al. (2001) preferred *N. tabacum* cv. Xanthii nc, *N. benthamiana*, *Chenopodium amaranticolor*, *Capsicum annuum* cv. Yolo Wonder and *Solanum tuberosum* plants; d'Aquino et al. (1995) used a large number of test plants in their host range studies, such as *N. tabacum* L. White Burley, *N. tabacum* cv. Samsun NN, *N. tabacum* cv. Xanthi, *N. glutinosa*, *N. rustica*, *N. debneyi*, *Datura stramonium*, *D. metel*, *C. amaranticolor*, *C. album* and *C. quinoa* (d'Aquino et al., 1995; Romero et al., 2001).

In this study, *C. amaranticolor*, *C. quinoa*, *Capsicum annuum* L., *N. glutinosa*, *N. benthamiana*, *N. rustica*, *N. tabacum* L. 'White Burley', *N. tabacum* L. "Xanthii" and *N. tabacum* L. Samsun NN plants were used to determine the local and systemic hosts of the virus. No symptoms were observed on *C. amaranticolor* and *C. quinoa* plants in the mechanical inoculation studies. Similar results on these plants were reported by d'Aquino et al. (1995) and Mostafaei et al. (2007). In addition, Romero et al. (2001), Choi et al. (2005) and El-Borollosy (2015) stated that *C. amaranticolor* and *C. quinoa* are local lesion hosts of PVY.

PVY has been reported to cause mosaic, mottling and vein banding symptoms on *N. tabacum* cv. Xanthii and is widely used as a vector (Abdel-Shafi et al., 2017; d'Aquino et al., 1995; Faurez et al., 2012; Moodley et al., 2019; Mostafaei et al., 2008). It has also been reported that PVY causes symptoms such as mosaic, leaf deformation, vein retraction and stunting of the plants in *N. glutinosa*, *N. benthamiana* and *N. rustica* tobacco varieties. In fact, in this study, symptoms such as deformation of new leaves, mosaic, leaf blade shrinkage were observed in *N. glutinosa* plants 12 days after inoculation. In *N. benthamiana*, symptoms such as stunting of the plants, downward curling of the leaves and deformation were observed. These symptoms are similar to those observed by researchers working with PVY (El-Borollosy, 2015; Fakhrabad et al., 2012; Gürsoy, 2011; Romero et al., 2001).

In this study, no symptoms were observed in *N. tabacum* L. Samsun NN and *N. tabacum* L. White Burley plants inoculated with PVY. However, Choi et al. (2005), Mostafaei et al. (2007) and Fakhrabad et al. (2012) observed symptoms such as mosaic, deformation and vein banding in plants. These differences may be due to reasons such as virus strain, plant species and cultivar, and environmental conditions.

As given in the literature, symptoms of vein retraction, mosaic and leaf deformation appeared 10 days after mechanical inoculation of *Capsicum annuum* L. (Abdel-Shafi et al., 2017; Deligöz and Sokmen, 2020; El Banna et al., 2015). The fact that the symptoms caused by mechanical inoculation of virus-susceptible test plants from positive samples in DAS-ELISA studies were consistent with the results obtained in previous studies, supports that the virus is PVY (Abdel-Shafi et al., 2017; d'Aquino et al., 1995; Faurez et al., 2012; Moodley et al., 2019; Mostafaei et al., 2008).

Nine isolates that tested positive in DAS-ELISA, along with mechanically inoculated test plants, were subjected to molecular methods. RT-PCR reactions were performed using primer pair specifically amplifying a 801 bp fragment of the PVY coat protein gene. The analyses revealed that nine DAS-ELISA-positive plant samples, as well as the nine symptomatic test plants, produced bands of the expected size, confirming infection with PVY. In contrast no PCR band was observed in symptomless *N. tabacum* L. "Samsun NN", *N. tabacum* L. "White burley", *C. amaranticolor* and *C. quinoa* plants and in negative controls inoculated with pepper isolates samples. Similarly, some researchers (Shalaby et al., 2002; El Banna et al., 2015; Elmahdy and El Salam, 2016) also used unique primers to amplify part of the sheath protein gene region of PVY and obtained results similar to ours (Shalaby vd., 2002; El Banna vd., 2015; Elmahdy ve El Salam, 2016). It can therefore be said that the results of our study are consistent with the literature.

4. Conclusions

This study confirmed the presence of PVY in pepper production areas of Isparta province for the first time, using DAS-ELISA and RT-PCR. Both methods provided consistent results. Mechanical inoculation also confirmed the observation of the typical symptoms on the test plants. The detection of PVY in the region is highly significant for pepper cultivation, as it emphasises the importance of continuous monitoring and the implementation of effective management strategies. Future research should focus on the molecular characterisation and sequence analysis of PVY isolates, the evaluation of resistant cultivars and the development of integrated control measures. It is also critical to raise awareness among growers to minimise yield losses and prevent the further spread of the virus.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Yağcıoğlu, E., Çulal Kılıç, H.; Design: Yağcıoğlu, E., Çulal Kılıç; Data Collection or Processing: Yağcıoğlu, E., Çulal Kılıç, H.; X.; Statistical Analyses: Yağcıoğlu, E., Çulal-Kılıç, H.; Literature Search: Yağcıoğlu, E., Çulal Kılıç, H.; Writing, Review and Editing: Yağcıoğlu, E., Çulal Kılıç, H.

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