Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297

jas.ankara.edu.tr

JAS

In Vitro Evaluation of Apricot Cultivars Response to *Pseudomonas syringae* Pathovars: Image Processing as an Alternative Method

Mustafa AKBABA[a*], Kaan HÜRKAN[b], Ahmet Erhan KARAHAN[c]

[a]*Department of Plant Protection, Faculty of Agriculture, Igdır University, Igdır, Türkiye*
[b]*Department of Agricultural Biotechnology, Faculty of Agriculture, Igdır University, Igdır, Türkiye*
[c]*Department of Zootechnic, Faculty of Agriculture, Igdır University, Igdır, Türkiye*

ARTICLE INFO
Research Article
Corresponding Author: Mustafa AKBABA, E-mail: mustafa.akbaba@outlook.com
Received: 12 Dec 2022 / Revised: 13 Feb 2023 / Accepted: 17 Feb 2023 / Online: 19 Sept 2023

Cite this article

AKBABA M, HÜRKAN K, KARAHAN A E (2023). *In Vitro* Evaluation of Apricot Cultivars Response to *Pseudomonas syringae* Pathovars: Image Processing as an Alternative Method. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(3):842-853. DOI: 10.15832/ankutbd.1217921

ABSTRACT

Türkiye, with an apricot (*Prunus armeniaca* L.) production amount of 833,398 tons per year, ranks first in fresh apricot production and dried apricot export in the world. Malatya, Iğdır, and Elazığ constitute the main apricot production centers in Türkiye. Many table and dried apricot cultivars have been grown in Türkiye. Local apricot cultivars such as cv. Şalak (or Aprikoz), cv. Tebereze, cv. Ordubat, cv. Ağcanabat, and cv. Ağerik are widely grown in the Aras Valley, including Iğdır and Kağızman. In this study, DNA barcoding of local cultivars based on the internal transcribed spacer region was performed and their distribution demonstrated in the Aras Valley. The reactions of these apricot cultivars to the causal agents of bacterial canker, which negatively affect the yield and quality of apricot cultivation, were also determined. Alternative methods such as image-processing technology and CHAID analysis have also been successfully used for cultivar reaction tests. It was determined that "Şalak" is economically important and the most common apricot cultivar in the Aras Valley. In addition, the Ağcanabat cultivar was sensitive to the causal agents of disease, while other local apricot cultivars were tolerant to it.

Keywords: Stone fruits, Local cultivars, Bacterial canker, DNA barcoding, CHAID

1. Introduction

Apricot (*Prunus armeniaca* L.) has been cultivated for more than 5000 years across a wide area of the world, including Türkiye, Iran, Turkestan, Afghanistan, Central Asia, and western China (Ercisli 2009; Bakır et al. 2019). Apricots consumed fresh and dried are among the agricultural products with high nutritional value (USDA 2022). With an apricot production of 833,398 tons/year, Türkiye is ranked first in fresh apricot production and dried apricot exports worldwide (FAO 2020). The most economically important apricot-growing area in Türkiye is the Eastern Anatolia region (Akın et al. 2017; Abacı & Asma 2010) where 65-70% of the world's dried apricots are produced in this region (Ercisli 2009). The main apricot production areas in this region are Malatya (389,396 tons/year), Iğdır (42,989 tons/year), and Elazığ (31,179 tons/year) (TÜİK 2021). The Aras Valley, which includes Iğdır and Kağızman, has approximately 6.37% of Türkiye's apricot production (800,000 tons/year), with a production total of 51,033 tons/year (TÜİK 2021). Previous studies have determined the phenological and pomological characteristics of local apricot cultivars (cv. Şalak or Aprikoz, cv. Tebereze, cv. Ordubat, cv. Ağcanabat, and cv. Ağerik) widely grown in the Aras Valley (Özyörük & Güleryüz 1992; Ercisli 2009; Muradoğlu et al. 2011). Referring to the different names of local cultivars or varieties among geographic regions, countries, and continents is a global problem and this issue is particularly important for apricot cultivars in Türkiye. Therefore, to generate a standardized database, it is necessary to resolve the local cultivars of apricots based on DNA barcoding of the universal barcode region internal transcribed spacer (ITS). By doing this it is possible to identify apricot samples by matching them with the generated database that includes known samples as positive controls. DNA barcoding has been successfully applied to some apricot cultivars such as cv. Şalak (Hürkan 2020), Tunusian cultivars (Batnini et al. 2019), and Egyptian cultivars (Sayed et al. 2022).

Due to the small difference in altitude among the apricot orchards, all apricot plantations are exposed to frost in the spring of some years in the Aras Valley, especially in Iğdır (Ercisli 2009). Canker formation on trees is induced by stress factors, such as frost exposure, which makes trees susceptible to infection and injury from frost damage. Pathogen inoculation trials in some studies have consistently shown that increased infection rate and canker length are associated with frost damage (Sobiczewski & Jones 1992; Weaver 1978). *Pseudomonas syringae* van Hall, which has ice nucleation activity, makes some plants susceptible to frost damage at -5 °C (Lindow et al. 1982). Bacterial canker diseases in stone fruits are characterized by the systemic movement of bacteria in all aboveground parts of the plant, especially in the vascular tissues (Lamichhane et al. 2014). It has been reported that disease agents including *P. syringae* pv. *syringae* (*Pss*), *P. syringae* pv. *morsprunorum* race 1 (*Psm* R1), *P. syringae* pv. *morsprunorum* race 2 (*Psm* R2), and *P. syringae* pv. *cerasicola* (*Psc*) are pathogenic in apricot plants and may cause bacterial canker symptoms (Hulin et al. 2020). Disease-causing bacteria infect all aboveground plant organs throughout the season, causing symptoms such as fruit spots, necrosis, dead buds, flower blight, cankers on stems and branches, and bacterial oozing (Kennelly et al. 2007; Hulin et al. 2020). Apricots (Kavak & Çitir 1995; Kotan & Sahin 2002), peaches (Özaktan et al. 2008), cherries (Bülbül & Mirik 2014; Erkek 2017; Akbaba & Özaktan 2021), and citrus (Mirik et al. 2005) have been reported to be causative agents of bacterial canker in Türkiye. The control of diseases caused by *P. syringae* pathogens is almost impossible because of the lack of effective chemical or biocontrol agents, the low number of resistant cultivars, and the endophytic nature of the pathogen (Kennelly et al. 2007). Different pathogenic strains of *P. syringae* have a large genetic variability that is adaptable to different hosts, cultivars, and pedoclimatic conditions, making it difficult to control bacterial canker (Morris et al. 2009). Gilbert et al. (2010) suggested the need for alternative methods for the management of bacterial diseases in orchards, including approaches based on biological control and plant resistance. For this reason, one of the most viable and economical methods for the management of bacterial cancer in apricots is to use resistant cultivars. Only a few apricot cultivars in previous studies worldwide have reported resistance to *P. syringae* pv. *syringae* (Scortichini et al. 1999; Singh et al. 2005; Giovanardi et al. 2018).

Improving the conditions for the control of bacterial canker requires determining the diversity, epidemiology, and distribution of the causal agents of the disease. In addition, the distribution, diversity, and susceptibility of host cultivars are important in the epidemic risk of the disease. The present study aims to generate a DNA barcode database for the local apricot cultivars (cv. Ağcanabat, cv. Ağerik, cv. Aprikoz, cv. Ordubat, and cv. Tebereze) resolve phylogenetic relationships among the cultivars, identify samples with the generated database, and determine the resistance reactions of these cultivars against *P. syringae* pathovars, which are the causal agents of bacterial canker in apricots. Image processing technology and MATLAB software were used to determine the responses of the cultivars as an alternative method, in contrast to previous studies.

2. Material and Methods

2.1. Collection of plant samples

In the field survey, a simple random sampling method was applied to represent the apricot production areas in the Aras Valley (Türkiye) (Bora & Karaca 1970). According to the method; for 1-5 da (1 piece), 5-10 da (2 pieces), 10-50 da (3 pieces), 50-100 da (4 pieces), 100-500 da (5 pieces), and 500-1000 da (6 pieces) were collected as plant samples from apricot orchards. The samples were made by increasing the sampling rate by 1 for every 500 decares after 1000 da area. Leaf samples from the apricot cultivars (Table 1) were collected for DNA Barcoding. "Şalak" is known as "Aprikoz" cultivar in local public in Kağızman.

Table 1- Characteristics of local apricot cultivars in the Aras Valley (Reviewed in Ercisli 2009)

Cultivars	*Fruit weight (g)*	*Fruit shape*	*Skin colour*	*Freedom of pit*	*Type of consumption*	*Flesh firmness*	*TSS (%)*	*Location*
Şalak	63	Cylindiric	Yellow	Free	Table	Soft	14	Iğdır
Ordubat	25	Cylindiric	Orange	Semi-cling	Dried	Medium	18	Iğdır
Tebereze	38	Round	Orange	Free	Dried	Medium	17	Iğdır
Agcanabat	51	Round	Cream	Free	Table	Medium	14	Iğdır
Agerik	45	Round	White	Free	Dried	Soft	14	Iğdır
Şekerpare	22	Round	Cream	Free	Table	Soft	20	Malatya

2.2. DNA extraction and amplification of ITS region for DNA Barcoding

We used 100 mg of fresh leaf tissues from each cultivar as the starting material for DNA extraction, which was performed according to the literature (Aydin et al. 2018). The extracted DNAs were checked for integrity on 1.5% agarose gel electrophoresis, and the concentration was measured using NanoDrop (Maestrogen, Taiwan) spectrophotometer. The DNA extracts were stored at -45 ºC until polymerase chain reaction (PCR). We amplified the partial ITS region using the primers ITS1A (5´-GACGTCGCGAGAAGTCCA-3´), and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) described by Gulyás et al. (2005) and White et al. (1990), respectively. Twenty-five microliter of PCR reaction mix was prepared with a 2X Reaction Buffer (Thermo Scientific, Cat. No. EP0401), 0.1 mM dNTPs, 0.2 µM both primers, 1 U Taq DNA polymerase (Thermo Scientific, Cat. No. EP0401), 1 mM Mg^{+2}, 20 ng DNA and nuclease-free water. Thermal cycling was performed in a SimpliAmp™ instrument (Thermo Scientific, USA) using conditions first denaturation at 95 °C for 1 min and followed by 35 cycles of 95 °C for 30 s denaturation, 54 °C for 30 s annealing, 72 °C for 1 min elongation. PCR was finalised with 72 °C for 10 min final elongation step. All the PCR products were checked for specific amplicons (~800 bp) on 2% agarose gel electrophoresis. After ensuring for specific amplicons, we directly sent the PCR products to Macrogen Inc. (The Netherland) for purification and Sanger sequencing for both directions using the same primers on the PCR.

2.3. Bioinformatics and phylogenetics

The obtained DNA sequences were imported to Geneiour R8 (Dotmatics, Australia) bioinformatics platform and checked for sequencing quality. The primer binding regions and low quality ending parts of the sequences were trimmed, and the sequences for both directions were aligned to generate consensus sequences for each apricot cultivar. The consensus sequences of the positive controls (*P. armeniaca* cv. Ağcanabat, *P. armeniaca* cv. Ağerik, *P. armeniaca* cv. Şalak (Aprikoz), *P. armeniaca* cv. Ordubat, and *P. armeniaca* cv. Tebereze) were submitted to the National Center for Biotechnology Information GenBank and deposited with the accession numbers OP804469, OP804470, OP804471, OP804472, and OP804473, respectively. The ITS sequence of *P. armeniaca* cv. Şalak were obtained from Genbank (MT072696). Then, to identify the 67 apricot samples collected in this study, we compared their sequences with positive controls by aligning all the sequences and constructing a phylogenetics tree using Neighbor-Joining approach with 1,000 bootstrap replicates. *Prunus persica* (DQ006273) was selected as the outgroup.

2.4. Determination of response of cultivars against P. syringae pathovars

Five different local apricot cultivars and cv. Şekerpare in Table 1, commonly cultivated in Türkiye, were used in this study (Ercisli 2009). According to Görmez et al. (2013), cv. Şekerpare is a tolerant cultivar of *P. syringae* pv. *syringae*. *Pseudomonas syringae* strains (*P. syringae* pv. *morsprunorum* R1 strain 25B and *P. syringae* pv. *morsprunorum* R2 strain 732 from Poland and *Pseudomonas syringae* pv. *syringae* strain BAY3 from Türkiye) in bacteriology laboratory stocks were also used as reference pathogenic bacteria.

The methods specified in previous studies by Sobiczewski & Jones (1992) and Li et al. (2015) were optimized and used for the shoot inoculation tests in this study. One-year dormant shoots of apricot cultivars were randomly collected from orchards in and around Iğdır in 2021 (December) and used for cultivar reaction tests. Each shoot was cut into equal lengths of 10 cm. For surface sterilization, the cut shoots were kept in 0.5% sodium hypochlorite for 5 min, rinsed with sterile distilled water, and left to dry on blotting paper for a while.

For shoot inoculation, the strains of pathogenic bacteria were grown in King's B medium (Schaad et al. 2001) for 24-48 h at 24±2 °C. Growing bacterial cultures were suspended in 20 mL of sterile water and prepared for all strains at the same concentration (OD_{600}: 0.1) using a spectrophotometer. Sterile distilled water was used as the negative control. The upper 5 mm of each 10 cm shoot tip was removed with a scalpel and immersed in the bacterial suspension for 5 min. The tips of the shoots immersed in the bacterial suspension were covered with Parafilm. The untreated tips of the shoots were cut (approximately 10 mm) and placed in transparent polyethylene bags containing sterile cotton and sterile distilled water (0.1% sodium hypochlorite dropped) to a depth of 20 mm. To prevent cross-contamination, separate polyethylene bags were used for each treatment. Cultivar × strain treatment was performed with 5 repetitions. The shoots were incubated in closed polyethylene bags at 15 °C for 1 week under a photoperiod of 16 h in light and 8 h in darkness. To show the effect of frost damage on the infection process in the experiment, the shoots were incubated for 1 week at -2 °C. Finally, the 10 mm untreated part of each shoot was discarded, and the shoots were placed in sterile polyethylene bags according to a completely randomized plot experiment design. It was incubated at 15 °C for four weeks under the same light conditions as previously mentioned, maintaining high humidity.

At the end of the incubation period, the necrotic areas on the shoots were evaluated for the severity of the bacterial canker. First, the uppermost layer of the bark was stripped from the top to reveal symptoms. The shoots were then cut from the bottom to approximately 8 cm long and digitally photographed to detect the necrotic areas. According to the classical method used by Sobiczewski & Jones (1992), the length of the necrosis was measured using a ruler, and the percentage of disease severity was determined by proportioning it to the entire length of the shoot. As an alternative to this evaluation method, a new method based on image processing using MATLAB was used in this study. This new method allows for the detection of the entire necrotic area compared with the widely used subjective evaluation method based on the measurement of necrosis length specified in previous studies.

In the detection of disease by image processing method, the (red, green, and blue) values of the diseased parts of the apricot shoot were examined at the first stage, and the black-and-white (binary) image obtained by following the instructions given in Supplemantary data 1 and the processing steps specified in Figure 1 was used in calculating the area of the apricot area and the diseased area in the apricot. Perimeter and area measurements are meaningful only for binary images, for this purpose the percentage of diseased area was calculated using the "bwarea" command (Pratt 1991) in MATLAB.

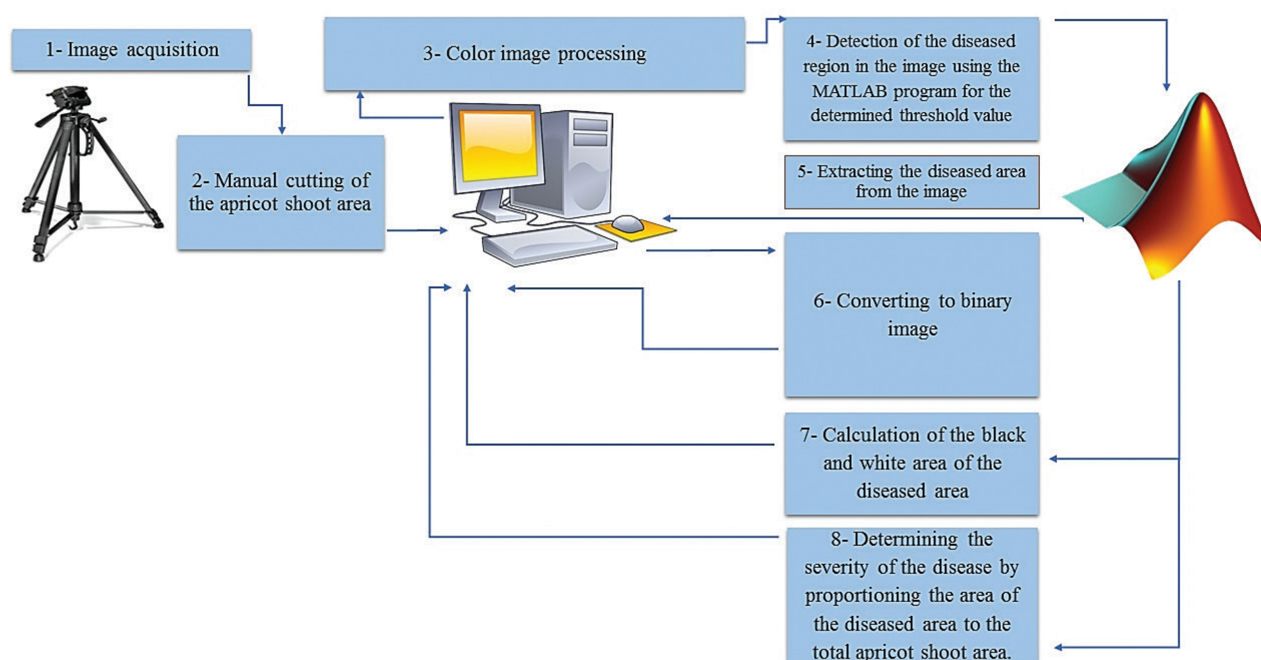

Figure 1- Image processing steps using MATLAB

2.5. Statistical analysis

In the evaluation of the cultivar x treatment reaction was used CHAID analysis which is a non-parametric analysis, it is a statistical method that is independent of the preconditions and assumptions required by parametric tests and does not require any transformation in the data. In the chaid analysis, the parent and child node ratio is taken as 10:5 and tree depth 3.

3. Results

3.1. Collecting of apricot samples

Based on the results published by Özyörük and Güleryüz (1992), local cultivars cultivated in the Aras Valley were determined, as shown in Figure 2. Leaf samples of local apricot cultivars were collected from 71 points representing the apricot production areas in the Aras Valley (Supplementary data 2).

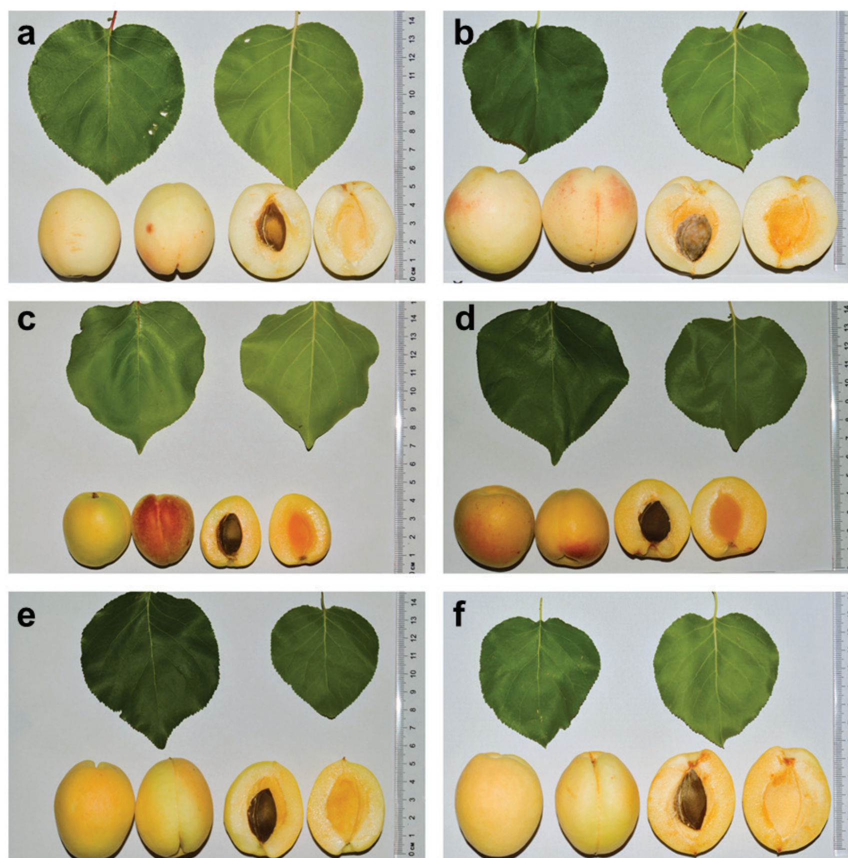

Figure 2- Local cultivars cultivated in the Aras Valley; Ağerik (a), Ağcanabat (b), Ordubat (c), Tebereze (d), Şalak (e), Aprikoz (f)

3.2. DNA barcoding of the apricot samples

We successfully extracted DNA from the samples with the concentrations ranging from 18.39-84.42 ng μL^{-1}, the A260/A230 ratio was 1.30-1.69, and the A260/A280 ratio was 1.83-2.30. The nanodrop results confirmed that the extracted DNAs were ready for PCR reaction. Agarose gel electrophoresis validated ITS barcode region were amplified successfully for all the samples (data not shown). Nucleotide sequences for ITS region were obtained with sequencing quality scores ranging from 80.6-96.7. After trimming and aligning the sequences, the aligned length was 716 bp.

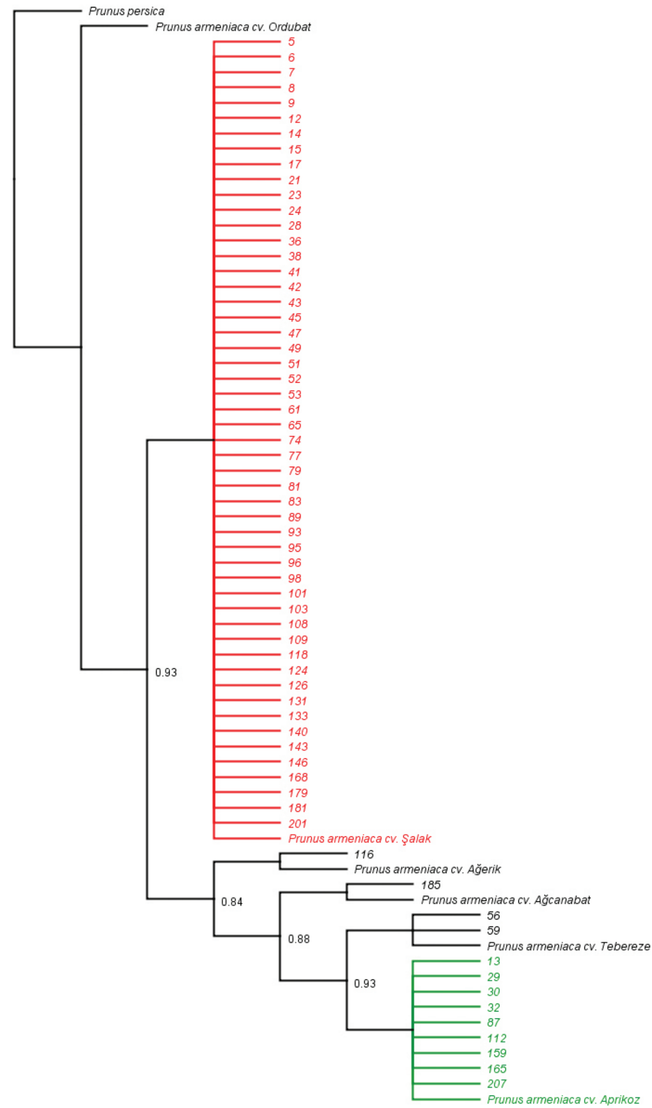

Figure 3- Phylogram reconstructed with internal transcribed spacer sequence of 71 apricot *(Prunus armeniaca L.)* samples. The Aprikoz cultivar groups were coloured as green, the Şalak cultivar group were coloured as red. Bootstrap support values were placed on the nodes

The phylogram reconstructed with ITS sequences identified the sampled by grouping the samples with closely related positive controls (Figure 3). The outgroup was placed as the natural outgroup on the tree. The branchings were highly supported by bootstrap values (85 to 93%). According to the tree, most of the (52 samples) samples matched with *P. armeniaca* cv. Şalak, 9 samples were matched with *P. armeniaca* cv. Aprikoz, two samples (56 and 59) matched with *P. armeniaca* cv. Tebereze, the sample 185 matched with *P. armeniaca* cv. Ağcanabat, and the sample 116 matched with *P. armeniaca* cv. Ağerik. There was no matching with *P. armeniaca* cv. Ordubat. According to the DNA barcoding results in these 65 locations, 80% cv. Şalak, 14% cv. Şalak (Aprikoz), and 3% cv. Tebereze cultivars were identified, and the remaining 3% belonged to the Ağcanabat, Ordubat, and Ağerik cultivars (Figure 4).

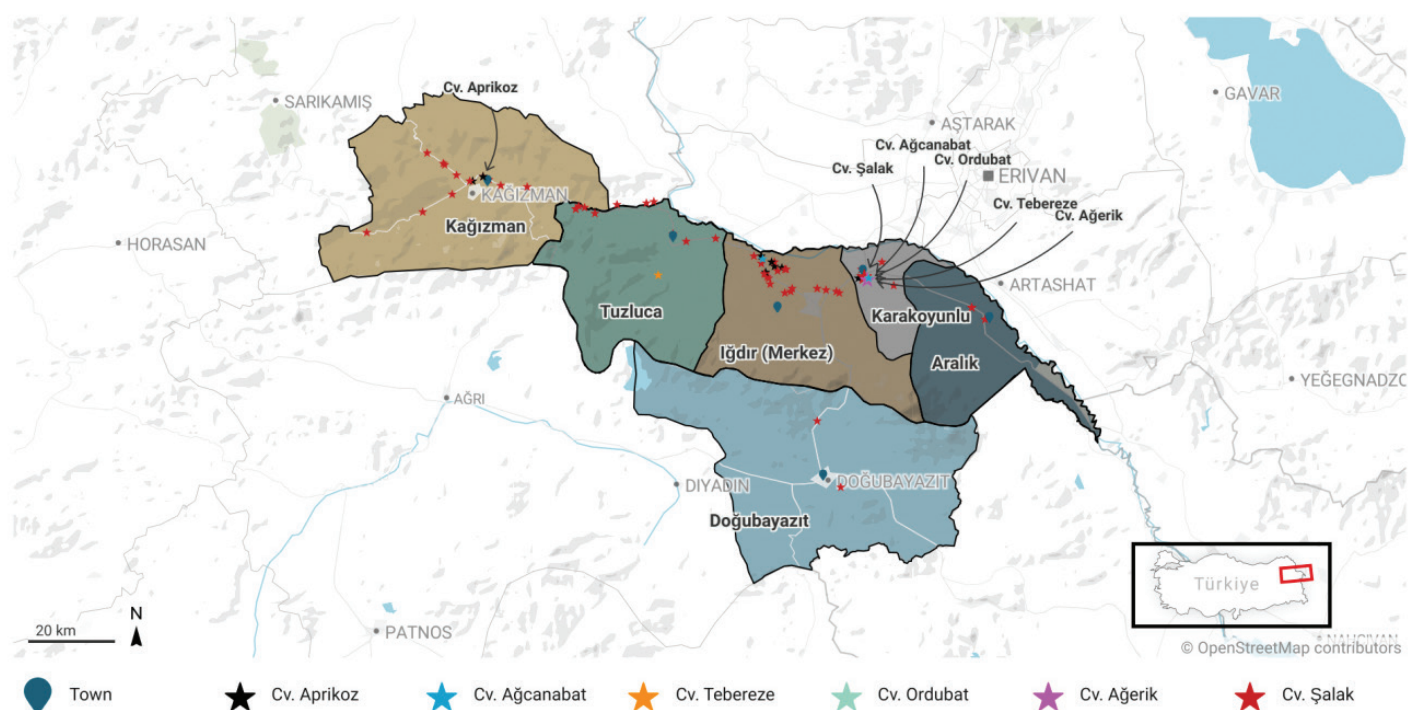

Figure 4- Distribution map of apricot cultivars in Aras Valley based on GPS data

3.3. An alternative method to detect the reactions of apricot cultivars to pathogens

In this study, results were obtained regarding the reactions of six different apricot cultivars against three different *P. syringae* pathovars, which are causal agents of bacterial canker in apricot (Giovanardi et al. 2018). An alternative new method based on image processing technology instead of the classical method has been attempted to obtain results (Figure 1). Data on disease severity obtained using MATLAB were statistically compared with the results obtained using the classical method (Figure 5). A correlation of 0.7587 was found between the disease severity data determined using these two methods.

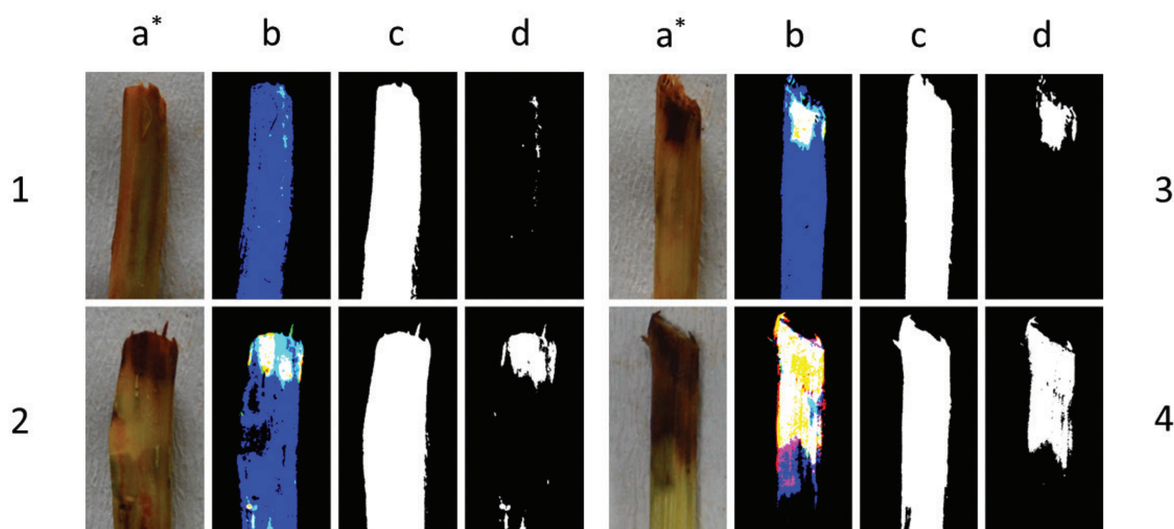

Figure 5- Stages of image processing (IP) at cultivar reactions to bacterial pathogens; Apricot cultivar (cv. Ağcanabat*); treatments (NC[1], strain BAY3[2], strain 25B[3], strain 7324); Image processing (Original[a], RGBcolour[b], RGBblack_white[c], RGBdiseased_area[d])

In the classical method, different researchers can detect the disease severity at different rates. However, the image-processing method provides the same results as an objective tool for researchers who use the same command and threshold values. Therefore, in the next step of the study, the data obtained from the image processing method using MATLAB was used to determine the average disease severity (%) values showing the response of the cultivars to the pathogens.

Table 2- Reactions of cultivars with *P. syringae* pathovars (%)

Cultivars	*NC*	*Strain BAY3 (Pss)*	*Strain 25B (Psm R1)*	*Strain 732 (Psm R2)*
Ağcanabat	0.75	9.13	1.69	15.72
Şalak	0.08	0.80	1.24	2.22
Tebereze	0.00	1.68	0.45	0.85
Ordubat	0.01	3.73	1.29	1.32
Ağerik	0.10	5.52	3.43	2.73
Şekerpare	0.39	1.74	2.82	4.33

The results were evaluated with reference to cv. Şekerpare, which has been reported to be tolerant to *Pss* in previous studies. According to the results of the CHAID analysis shown in Figure 6, disease severity was determined at an average rate of 7.032% in 120 apricot shoots (node-0). Pathogens (treatments) affected the disease severity more than the cultivar factor; therefore, the first node (node-0) was attached to the node of the treatments. Disease severity was divided into three groups based on the pathogens. In the negative control group, an average of 0.033% disease severity was determined (node-1). Strains BAY3 and 732 were included in the same group in terms of their effect on disease severity and caused 11.075% of disease on average (node-2). The disease severity rate caused by strain 25B was determined to be 5.943% and was included in a different group from the others (node-3). Strains BAY3 (*Pss*) and 732 (*Psm* R2) were in the same statistical group according to the disease severity values created by pathogen application on the cultivars. The Ağcanabat cultivar was in the sensitive group with a disease severity rate of 17.810% (node-4) for these two pathogens, whereas other cultivars were in the same statistical group with the more tolerant cv. Şekerpare with an average disease severity of 9.728% (node-5). According to the results, the Ağcanabat cultivar was more sensitive than the cv. Şekerpare (Table 2). The reaction differences between cultivars other than the Ağcanabat cultivar against these two pathogens were statistically insignificant. There was no statistical difference between the cultivars with respect to strain 25B (*Psm* R1). All cultivars for this strain were in the same statistical group as that of the tolerant reference cultivar. When the image processing method was used to evaluate the results, an average of 0.033% disease severity values were measured in the negative control. According to expert opinions, the error of the method is a tolerable level for plant samples (Figure 5, Table 2, node-1). The coefficient of determination (R^2) for the model was 0.599.

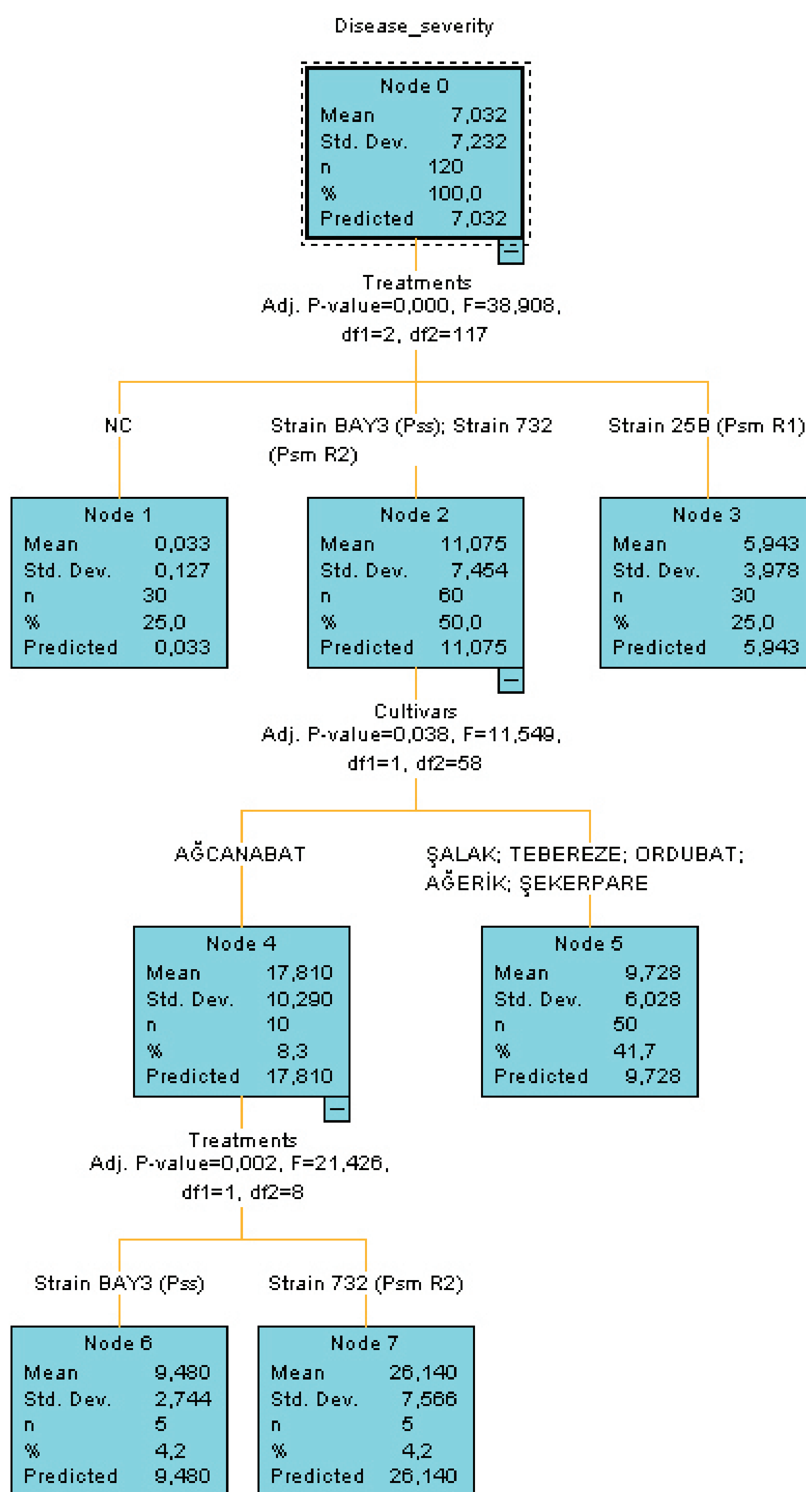

Figure 6- CHAID diagram: Effect of cultivar and pathogen treatments on disease severity

4. Discussion

Many table [Hasanbey, Şalak (Aprikoz), Şekerpare, Şam, Turfanda İzmir, Tokaloğlu, Alyanak, Ethembey, Karacabey, Mahmudun Eriği, Adilcevaz 5, İri Bitirgen, Precoce de Tyrinthe, Precoce de Colomer, Canino, Luizet, Roxana, Ninfa, Aurora ect.] and dried apricot cultivars (Hacıhaliloğlu, Kabaaşı, Soğancı, Çataloğlu) are grown in Türkiye, placing the country first in world apricot production (Paydaş Kargı et al. 2015). The Aras Valley, which includes Iğdır and Kağızman, accounts for approximately 6.37% of this production (TÜİK 2021). Economically important apricot cultivars such as Şalak (Aprikoz), Tebereze, Ordubat, Ağcanabat, and Ağerik are widely grown in the Aras Valley, and their phenological and pomological characteristics have been determined in previous studies (Özyörük & Güleryüz 1992; Ercisli 2009; Muradoğlu et al. 2011). Therefore, it is crucial to identify the apricot cultivars for breeding and commercial use. Bourguiba et al. (2010) reported sequence-tagged microsatellites (SSR) and amplified fragment length polymorphism (AFLP) markers could be used to identify apricot cultivars and discover synonyms and homonyms. Genotyping studies were limited to AFLP, SSR, and random-amplified fragment length polymorphism (RAPD) techniques for Turkish apricot genotyping studies. The next level genotyping method based on DNA sequencing was also applied to identify and discriminate apricot cultivars from closely related apricot types. In a study performed by Hürkan (2020), the DNA sequences of barcoding regions ITS, LEAFY, matK, rbcL, and ycf1 for *P. armeniaca* cv. Şalak were obtained and tested for their discrimination performance. The results of the study showed that ITS and LEAFY DNA sequences successfully discriminated the Şalak cultivar from other cultivars. Previous studies have mentioned that “Aprikoz” and “Şalak” are the same cultivars (Asma 2000, Alim & Kaya 2005; Asma & Ozturk 2005). However, these cultivars differed according to the DNA sequencing results based on the ITS gene region. In the present study’s DNA sequencing based analysis, the cultivars distributed as Şalak (80%), Aprikoz (14%), and Tebereze (3%) in the Aras Valley. The remaining 3% included the Ağcanabat, Ağerik, and Ordubat cultivars. The results confirmed the previous studies identification of apricot cultivars distributed in the Aras Valley. Alim & Kaya (2005) reported Şalak (Aprikoz) was the most cultivated cultivar with 85%, and Tebereze, Ağerik and Ordubat cultivars was 15%. *P. syringae* pathovars have high genetic diversity, making bacterial canker difficult to control (Morris et al. 2009). One of the most important methods for controlling the causal agents of bacterial canker is the use of resistant apricot cultivars (Donmez et al. 2010). However, only a few apricot cultivars have been reported to be resistant to *Pss* until now (Scortichini et al. 1999; Singh et al. 2005). In a study conducted on apricot cultivars in Türkiye, Hacıhaliloğlu was reported to be very sensitive to *Pss*, while the Roxana, Hasanbey, and Şekerpare cultivars were tolerant to *Pss* (Görmez et al. 2013). Apricot cultivars that show resistance or sensitivity to the causal agents of bacterial canker in Türkiye and the world have been determined by many similar studies (Donmez et al. 2010; Bibi et al. 2022; Cetinkaya et al. 2022). However, the local cultivars in the Aras Valley were not included in these studies. Therefore, in this study, the reactions of apricot cultivars in the Aras Valley against *P. syringae* pathovars caused bacterial canker on apricot were detected for the first time using the image processing method.

The determination coefficients of the disease severity values obtained by image processing and classical methods were calculated as R^2=0.5756. The most important reason for not obtaining a high level of determination coefficient is that the region of the disease is in the form of a line in some cases, whereas in some cases, it is spread over the entire shoot (Figure 5). In the classical method, disease severity is determined by measuring the length of necrosis and dividing it by the shoot length. For this reason, even if the disease symptom was a thin line, the entire shoot area with symptoms was considered to be diseased. However, in the image-processing method, disease severity is only obtained by the ratio of the specified necrotized area to the area of the shoot, which eliminates this disadvantage (Figure 5). In this study, an image processing method was developed using the MATLAB package program to obtain faster and more objective results than the classical method (Sobiczewski & Jones 1992) in the evaluation of disease severity. In a similar study, a general, rapid and reliable approach was used for lesion measurement using image recognition and an artificial neural network model, and it was suggested that it produced more objective results than visual scoring (Li et al. 2015).

In this study, CHAID analysis, which allows for easier interpretation of the data, was used as a statistical method to evaluate the reactions of local cultivars to *P. syringae* pathovars. In most scientific studies, the findings obtained cannot provide the assumptions of the ANOVA test (normality, homogeneity of variances, etc.). In this situation, applying one of the transformation methods (angle, log, ln, etc.) to the data, the dataset was transformed to be suitable for a normal distribution, an ANOVA test was applied to these new data, and the results obtained were evaluated. The CHAID analysis, which has become increasingly popular in recent years, was used as an alternative to the classical method in this study. The CHAID analysis is a decision tree method in which the relationship between the dependent and independent variables in the dataset and the relationships among the independent variables are examined (Akın et al. 2017). CHAID is a nonparametric analysis, is independent of the preconditions and assumptions required by parametric tests, and does not require any transformation of the data (Koc et al. 2019).

5. Conclusion

According to our results of DNA Barcoding based on ITS, “Şalak” is the most common apricot cultivar in Aras Valley. Alternative methods such as image-processing technology and CHAID analysis have also been successfully used for cultivar reaction tests in this study. It was also determined that the Ağcanabat cultivar, which is a local apricot cultivar in the Aras Valley, is sensitive to strains BAY3 (*Pss*) and 732 (*Psm* R2), whereas the other local cultivars were found to be tolerant. All cultivars were in the tolerant group of strain 25 B (*Psm* R1), and the Ağcanabat apricot cultivar was highly sensitive to strain 732 (*Psm* R2).

Data availability: Data are available on request due to privacy or other restrictions.

Authorship Contributions: Concept: M.A., K.H., A.E.K., Design: M.A., K.H., A.E.K., Data Collection or Processing: M.A., K.H., A.E.K., Analysis or Interpretation: M.A., K.H., A.E.K., Literature Search: M.A., K.H., A.E.K., Writing: M.A., K.H., A.E.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: We are grateful to Dr. Monika Kaluzna (Poland) and Prof. Dr. Hatice Özaktan (Türkiye) for providing the reference strains that were used in this study. Furthermore, we also thank Sezer Şiktaş and Assoc. Prof. Nihal Acarsoy Bilgin who helped us with the survey of apricot orchards. The study was funded by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) - contract no: 120O825.

References

Abacı Z T & Asma B M (2010). Bazı kayısı çeşitlerinin farklı ekolojik alanlardaki biyolojik özelliklerinin analizi. *Research Journal of Biological Sciences* 3(1):165-168.

Akbaba M & Özaktan H (2021). Evaluation of bacteriophages in the biocontrol of *Pseudomonas syringae* pv. *syringae* isolated from cankers on sweet cherry (Prunus avium L.) in Turkey. *Egyptian Journal of Biological Pest Control* 31(1):1-11. doi.org/10.1186/s41938-021-00385-7

Akın M, Eyduran E & Reed B M (2017). Use of RSM and CHAID data mining algorithm for predicting mineral nutrition of hazelnut. *Plant Cell, Tissue and Organ Culture* 128(2):303-316. doi.org/10.1007/s11240-016-1110-6

Alim M & Kaya G (2005). Iğdır'da kayısı tarımı ve başlıca sorunları. *Doğu Coğrafya Dergisi* 10(14): 47-65.

Asma B M (2000). Kayısı Yetiştiriciliği. Evin Ofset.

Asma B M & Ozturk K (2005). Analysis of morphological, pomological and yield characteristics of some apricot germplasm in Turkey. *Genetic Resources and Crop Evolution* 52: 305-313. doi.org/10.1007/s10722-003-1384-5

Aydin A, Ince A G, Uygur Gocer E & Karaca M (2018). Single cotton seed DNA extraction without the use of enzymes and liquid nitrogen. *Fresenius Environmental Bulletin* 27(10): 6722-6726.

Bakır M, Dumanoğlu H, Erdoğan, V, Ernim C & Macit T (2019). Characterization of wild apricot (Prunus armeniaca L.) genotypes selected from Cappadocia Region (Nevşehir-Turkey) by SSR markers. *Journal of Agricultural Sciences* 25(4): 498-507. doi.org/10.15832/ankutbd.457850

Batnini M A, Bourguiba H, Trifi-Farah N & Krichen L (2019). Molecular diversity and phylogeny of Tunisian *Prunus* armeniaca L. by evaluating three candidate barcodes of the chloroplast genome. *Scientia Horticulturae* 245: 99-106. doi.org/10.1016/j.scienta.2018.09.071

Bibi S, Inam-ul-Haq M, Riaz A & Malik S I (2022). Identification of Host Resistance against bacterial canker disease on apricot, plum and peach grown in Punjab and KPK. *International Journal of Plant Pathology* 11(1): 43-48. doi.org/10.33687/phytopath.011.01.4130

Bora T & Karaca İ (1970). Kültür bitkilerinde hastalığın ve zararın ölçülmesi, *Ege Üniversitesi Ziraat Fakültesi Yardımcı Ders Kitabı* 167: 43.

Bourguiba H, Krichen L, Audergon J M & et al. (2010). Impact of mapped SSR markers on the genetic diversity of apricot (Prunus armeniaca L.) in Tunisia. *Plant Molecular Biology Reporter* 28: 578-587. doi.org/10.1007/s11105-010-0189-x

Bülbül M & Mirik M (2014). Tekirdağ’da kiraz ağaçlarında bakteriyel kanser etmenlerinin yaygınlığı, izolasyonu ve tanımlanması üzerinde araştırmalar. *Journal of Turkish Phytopathology*, 43(1-3): 15-24.

Cetinkaya Yildiz R, Karatas A, Horuz S & et al. (2022). Determination of susceptibility of citrus and stone fruit cultivars against Pseudomonas syringae pv. syringae in Turkey. *Gesunde Pflanzen* 74: 639-646. doi.org/10.1007/s10343-022-00638-x

Donmez MF, Karlidag H & Esitken A (2010). Identification of resistance to bacterial canker (Pseudomonas syringae pv. syringae) disease on apricot genotypes grown in Turkey. *European Journal of Plant Pathology* 126: 241-247. doi.org/10.1007/s10658-009-9536-x

Ercisli S (2009). Apricot culture in Turkey. *Scientific Research and Essays* 4(8): 715-719.

Erkek E (2017). Determination of Epiphytic and Pathogenic Bacterial Agents in Cherry Trees in Amasya Province. Master Thesis.

FAO (2020). Production of Apricots: top 10 producers. Accessed 13 October 2022 https://www.fao.org/faostat/en/#data/QCL/visualize

Gilbert V, Planchon V, Legros F, Maraite H & Bultreys A (2010). Pathogenicity and aggressiveness in populations of *Pseudomonas syringae* from Belgian fruit orchards. *European Journal of Plant Pathology* 126: 263-277. doi.org/10.1007/s10658-009-9538-8

Giovanardi D, Ferrante P, Scortichini M & Stefani E (2018). Characterisation of *Pseudomonas* syringae isolates from apricot orchards in north-eastern Italy. *European Journal of Plant Pathology* 151(4): 901-917. doi.org/10.1007/s10658-018-1424-9

Görmez A, Sahin F, Gulluce M & Aslan, I (2013). Identification and characterization of Pseudomonas syringae isolated from apricot trees in the Erzurum province of Turkey and evaluation of cultivar reaction. *Journal of Plant Pathology* 95(3): 525-532.

Gulyás G, Sramkó G, Molnár V A, Rudnóy S, Illyés Z, Balázs T & Bratek Z (2005). Nuclear ribosomal DNA ITS paralogs as evidence of recent interspecific hybridization in the genus Ophrys (Orchidaceae). *Acta Biologica Cracoviensia Series Botanica* 47(2):61-67.

Hulin M T, Jackson R W, Harrison R J & Mansfield J W (2020). Cherry picking by pseudomonads: after a century of research on canker, genomics provides insights into the evolution of pathogenicity towards stone fruits. *Plant Pathology* 69(6): 962-978. doi.org/10.1111/ppa.13189

Hürkan K (2020). Analysis of various DNA barcodes on the turkish protected designation of origin apricot "Iğdır Kayısısı" (*Prunus armeniaca* cv. Şalak). *Turkish Journal of Agriculture - Food Science and Technology* 8(9): 1982-1987. doi.org/10.24925/turjaf.v8i9.1982-1987.3594

Kavak H & Çitir A (1995). Malatya ili merkez ilçede kayisilarda görülen hastaliklarin tanilari ve yayginlik oranlari üzerine araştırmalar. *VII. Türkiye Fitopatoloji Bildirileri* 531-534.

Kennelly M M, Cazorla F M, de Vicente A, Ramos C & Sundin G W (2007). Pseudomonas syringae diseases of fruit trees: progress toward understanding and control. *Plant Disease* 91(1): 4-17. doi.org/10.1094/PD-91-0004

Koc A, Karahan A E & Bingül M (2019). Determination of relationship between land surface temperature and different land use by chaid analysis. *Applied Ecology and Environmental Research* 17(3): 6051-6067. doi.org/10.15666/aeer/1703_60516067

Kotan R & Sahin F (2002). First record of bacterial canker caused by *Pseudomonas syringae* pv. *syringae* on apricot trees in Turkey. *Plant Pathology* 51: 798. doi.org/10.1046/j.1365-3059.2002.00768.x

Lamichhane J R, Varvaro L, Parisi L, Audergon J M & Morris C E (2014). Disease and frost damage of woody plants caused by *Pseudomonas syringae*: seeing the forest for the trees. *Advances in Agronomy* 126: 235-295. doi.org/10.1016/B978-0-12-800132-5.00004-3

Li B, Hulin M T, Brain P, Mansfield J W, Jackson R W & Harrison R J (2015). Rapid, automated detection of stem canker symptoms in woody perennials using artificial neural network analysis. *Plant Methods* 11(1): 57. doi.org/10.1186/s13007-015-0100-8

Lindow S E, Arny D C & Upper C D (1982). Bacterial ice nucleation: a factor in frost injury to plants. *Plant Physiology* 70(4): 1084-1089. doi.org/10.1104/pp.70.4.1084

Mirik M, Baloglu S, Aysan Y, Cetinkaya-Yildiz R, Kusek M & Sahin F (2005). First outbreak and occurrence of citrus blast disease, caused by Pseudomonas syringae pv. syringae, on orange and mandarin trees in Türkiye. *Plant Pathology* 54(2): 238. doi.org/10.1111/j.1365-3059.2005.01134.x

Morris C E, Bardin M, Kinkel L L, Moury B, Nicot P C & Sands D C (2009). Expanding the paradigms of plant pathogen life history and evolution of parasitic fitness beyond agricultural boundaries. *PLoS Pathogens* 5(12): e1000693. doi.org/10.1371/journal.ppat.1000693

Muradoğlu F, Pehluvan M, Gündoğdu M & Kaya T (2011). Iğdır yöresinde yetiştirilen bazı kayısı (Prunus armeniaca L.) genotiplerin fizikokimyasal özellikleri ile mineral içerikleri. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 1(1): 17-22. dergipark.org.tr/en/pub/jist/issue/7924/104203

Özaktan H, Akkopru A, Bozkurt A & Erdal M (2008). Information on peach bacterial canker in Aegean Region of Turkey. In Proceedings of STF Meeting on "Determination of the incidence of the different pathovars of Pseudomonas syringae in stone fruits"COST Action 873. *Bacterial diseases of stone fruits and nuts* 8.

Özyörük C & Güleryüz M (1992). Iğdir ovasinda yetişen kayisi çeşitleri üzerinde pomolojik biyolojik ve fenolojik araştırmalar. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi* 23(1): 16-28.

Paydaş Kargı S, Kafkas E & Dikkaya Y R (2015). Düşük soğuklamalı bazı kayısı çeşitlerinin KKTC ekolojik koşullarına adaptasyonları (TAGEP PROJESİ: TAE-P2000014). Çukurova Üniversitesi Ziraat Fakültesi - KKTC Tarım ve Doğal Kaynaklar Bakanlığı (in turkish)

Pratt WK (1991). *Digital Image Processing*. New York, John Wiley & Sons 634.

Sayed H A, Mostafa S, Haggag I M & Hassan N A (2022). DNA barcoding of prunus species collection conserved in the national gene bank of Egypt. *Molecular Biotechnology* 65: 410-418. doi.org/10.1007/s12033-022-00530-z

Schaad N W, Jones J B & Chun W (2001). Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society (APS Press) St Paul USA p.373.

Scortichini M, Pellegrino S & Berra L (1999). Susceptibility of apricot germplasm to natural infection by Pseudomonas syringae pv. *syringae* in Piedmont. *Rivista di Frutticoltura e di Ortofloricoltura* 61: 83-86

Singh H V, Mir M S & Wani M A (2005). Effect of host genotypes and age of plant on resistance to gummosis disease in apricot of Kargil. *Annals of Plant Protection Sciences* 13: 152-155.

Sobiczewski P & Jones A L (1992). Effect of exposure to freezing temperatures on necrosis in sweet cherry shoots inoculated with Pseudomonas syringae pv. *syringae* or *P s* morsprunorum. *Plant Disease* 76: 447-451. doi.org/10.1094/pd-76-0447

TÜİK (2021). Bitkisel Üretim İstatistikleri (Kayısı)

United States Department of Agriculture (USDA) (2022). Apricots, raw.

Weaver D J (1978). Interaction of Pseudomonas syringae and freezing in bacterial canker on excised peach twigs. *Phytopathology* 68: 1460-1463. doi.org/10.1094/phyto-68-1460

White T J, Bruns T, Lee S J W T & Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In PCR protocols: a guide to methods and applications* 18(1): 315-322. doi.org/10.1016/b978-0-12-372180-8.50042-1

Supplementary File Link: http://glns.co/eolo4

Copyright © 2023 The Author(s). This is an open-access article published by Faculty of Agriculture, Ankara University under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium or format, provided the original work is properly cited.