T.C.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE
DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

PLUM POX VIRUS RESISTANCE OF HYBRID F1 APRICOTS PRODUCED BY HYBRIDIZING ZARD AND HACIHALILOĞLU VARIETIES

Prepared
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Supervisor
Assoc. Prof. Dr. Kahraman GÜRCAN

Master's Thesis

August 2022
KAYSERI

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This study was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) with project number 119O846.

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COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in this study was obtained in accordance with academic and ethical rules. I also state that I have fully cited and referenced all materials and results that are not inherent in this study, as these rules and behavior require.

Adil MOHAMED ALI EDRIS

Signature ……

The MSc thesis entitled **plum pox virus resistance of hybrid f1 apricots produced by hybridizing Zard and Hacıhaliloğlu varieties** has been prepared in accordance with Erciyes University graduate school of natural and applied science thesis preparation and writing guide.

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ZARD X HACIHALİLOĞLU MELEZİ F1 KAYISILARNIN ŞARKA HASTALIĞINA DAYANIKLIK DURUMLARININ BELİRLENMESİ.

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Erciyes Üniversitesi, Fen Bilimleri Enstitüsü

Yüksek Lisans Tezi, Ağustos 2022

Danışman: Doç. Prof. Dr. Kahraman GÜRCAN

ÖZET

Kayısı (*Prunus armeniaca L.*), ılıman bölgelerde küresel olarak yetişen bir meyve türüdür. Şarka hastalığına nedenolan plum pox virüsü (PPV), kayısı üretiminin önündeki birincil engel olarak tanımlanmıştır. Türkiye hem kuru hem de taze kayısı üretiminde dünyanın lider üreticisidir. Türkiye'deki önemli kayısı çeşitleri PPV'ye hassastır. Bu çalışmada, PPV'ye hassas kurutmalık "Hacıhaliloğlu ile PPV'ye dayanıklı Zard çeşidi melezlenerek kurutmalık karakterine sahip ve PPV'ye dayanıklı melez kayısıların (F1) elde edilmesi amaçlamıştır. Melezleme 2018 yılında Kayseri'de gerçekleştirilmiştir. 241 adet fidan üretilmiştir. Fidanlar bir yıl fidan poşetinde yetiştirildikten sonra 3x2 m aralıkla deneme parseline dikilmiştir. F1 melez bitkilerin dayanıklılık durumunu belirlemek için dayanıklılığı kodlayan başlıca aday gen lokusu *PPVres* ile ilişkili 5 bp'lik bir delesyondan geliştirilmiş olan ZP002 moleküler markörü kullanılmıştır. F1 bitkilerin 237'u *PPVres* lokusunu heterozigot taşınırken, dört F1 melezin ise *PPVres* için homozigot dayanıklı olduğu bulunmuştur. 3 adet homozigot dayanıklı melez bitkinin tüm genom DNA'sı dizilenmiş, Hacıhaliloğlu ve Zard çeşitlerinin birinci bağlantı grubu üst kolu üzerine yer alan 300 kb uzunluğundaki DNA bölgesi ile eşleştirilmiş, bu bölgede yer alan *PPVres* lokusundaki alelik varyasyon incelenmiştir. ZP002 markörü ve genom dizileme çalışmaları sonucunda Zard çeşidinin *PPVres* için homozigot olduğu belirlenmiştir. Ayrıca F1 popülasyonundaki melez bitkiler PPV-T ırkı ile inoküle edilmiştir. Moleküler testlere ek olarak, ileriki yıllarda elde edilecek biyolojik denemenin sonuçları, melez bitkilerin (F1) şarka hastalığına dayanıklılık durumlarını belirlemede önemli olacaktır.

Anahtar Kelimeler: Melez Kayısı, F1, Erik Pox Virüsü, ZP002 Marker, PPV Dayanıklı.

PLUM POX VIRUS RESISTANCE OF HYBRID F1 APRICOTS PRODUCED BY HYBRIDIZING ZARD AND HACIHALILOĞLU VARIETIES

Adil MOHAMED ALI EDRIS

Erciyes University, Graduate School of Natural and Applied Sciences Master Thesis, August 2022
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ABSTRACT

The apricot (*Prunus armeniaca* L.) is a fruit species that grows globally in temperate regions. Plum pox virus (PPV), which is the cause of Sharka disease, has been found to be the main problem with apricot production. Türkiye is the world's leading producer of both dry and fresh apricots. The main varieties of apricots in Türkiye are susceptible to PPV. In this study, it was aimed to obtain PPV-resistant hybrid apricots (F1) by hybridizing "Hacıhaliloğlu" and the PPV-resistant "Zard" variety. Crossbreeding was conducted in 2018 in Kayseri, Türkiye. A total of 241 seedlings were produced. After the seedlings were grown in a greenhouse for a year, they were planted in the trial parcel at 3 x 2 m intervals. The resistance status of F1 hybrid plants was figured out by using the molecular marker ZP002, which was designed to detect a 5 bp deletion in *PPVres* main candidate gene locus for PPV resistance (*PPVres*). A total of 237 progenies were found to be heterozygous for resistant genes, while four progenies were found to be homozygous for resistant genes. The whole genome DNA of three homozygous resistant progenies were sequenced and compared with a 300 kb long DNA segment of the first linkage group (LG1) of the "Hacıhaliloğlu" and "Zard" varieties. The allelic variation in the locus was detected. The results of the ZP002 marker and genome-wide association studies showed that the "Zard" variety was homozygous for *PPVres*. Furthermore, the progenies in the F1 population were inoculated with an isolate of PPV-T strain. In addition to molecular testing, the results of biological experiments will help us figure out how resistant progenies (F1) are to the Sharka disease.

Keywords: Hybrid Apricots, F1, Plum Pox Virus, ZP002 Marker, PPV Resistance.

CONTENTS

PLUM POX VIRUS RESISTANCE OF HYBRID F1 APRICOTS PRODUCED BY HYBRIDIZING ZARD AND HACIHALILOĞLU VARIETIES

ABBREVIATIONS and SYMBOLS

F1	Family one
bp	Base Pair
DNA	Deoxyribonucleic acid
Mb	Megabyte
nt	Nucleotide
Gb	Gigabyte
RNA	Ribonucleic Acid
QTL	Quantitative Trait locus
µL	Micro liter
°C	Temperature Unit
PCR	Polymerase Chain Reaction
WGS	Whole Genome Sequence
GWA	Genome Wide Association
PPV	Plum Pox Virus
PPVres	Plum Pox Virus Resistance Loci
LG	Linkage Group
MAS	Marker Assisted Selection
SSR	Simple Sequence Repeat
SSLP	Single Sequence Length polymorphisms
HRM	High-Resolution Melting
SNP	Single Nucleotide Polymorphism
ml	Milliliter
EtOH	Ethanol
NaOH	Sodium Hydroxide
nm.	Nanomole
mRNA	Messenger RNA
S	Susceptible
R	Resistance

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INTRODUCTION

Apricot (*Prunus armeniaca. L.*) is a stone fruit tree species primarily found in the Iranian-Caucasian geographical region, Mediterranean countries, Uzbekistan, the United States of America, Pakistan, and China [1], [2].

Turkiye is the world's leading producer of both dried and fresh apricots. The FAO estimates that the global apricot area harvested was ~583k.ha, with a total production volume of ~3.8m. metric tons in 2020. Turkish apricot production was 833k tons, accounting for 22% of global output [3].

Türkiye produces both fresh and dried apricots, widely cultivated in Malatya province, the Aegean and Mediterranean regions. As a result, Turkiye is distinguished by the presence of extremely exciting cultivars of local apricots, which are grown in residential gardens, on roadsides, field borders, abandoned fields and farms for general production. In the province of Malatya, for example, the varieties Hasanbey, ‘'Kabaaşı,'' and ''Hacıhaliloğlu'' are commons [1]. “Hacıhaliloğlu,'' the most common cultivar in Turkiye, accounts for the majority of the country's dried apricot crop production [2].

The *plum pox virus* has been the primary impediment to apricot production. PPV is also known as Sharka disease. PPV is widespread throughout the world, particularly among woody species of trees that produce fruit with a kernel, such as sweet and sour cherries, peaches, apricots, and plums [4]. The PPV is a main member of the genus "Potyvirus," which includes most plant viral species. PPV was reported for the first time in 1917 in peaches in Bulgaria [5]. In 1968, PPV was first reported in Turkiye [1], [6]. PPV strains (PPV-M,-D,-Rec, -EA, -C, -T, -An, -W, -CR, and PPV-CV) have been identified via the biological, serological, and, more recently, molecular analysis of a growing number of isolates from various regions of the globe [7],[8]. Many studies have confirmed that PPV-T", "PPV-M," "PPV-Rec," and "PPV-D" strains are widely spread in Türkiye [4], [9]–[14].

In terms of apricot fruit, the losses caused by Sharka disease are as follows: early maturation, deformation, and reduced crop quality and productivity, which can sometimes result in the complete loss of production during the season [2], [4], [6], [9]. PPV has become a real threat to the production of apricots in the area, especially in Türkiye and Iran, which are known as major producers of dry apricots [4].

Several studies have confirmed that aphids are the primary vectors of the PPV. Due to the disease's characteristics, it is difficult to control through pesticide spraying. Due to the fact that aphids are facilitate virus spread in trees prior to the onset of disease symptoms [4], [9]. As a result, it is important to keep working on the breeding and improvement program, which aims to create new resistant cultivars of high quality that can grow in the area [2], [4], [9]. For example, PPV-resistant cultivars have been made in North America, and a similar project is going on in France, Greece, Spain, Italy, and Austria [2], [5], [9], [15]. Therefore, it is considered to be the most appropriate long-term solution to the Sharka disease problem [2], [5]. This means that the genetic resistance is the most promising future solution to PPV [2]. However, virus resistance genes are rare in local cultivars [5]. In apricot breeding programs in many countries, the American cultivars "Harlayne," "Goldrich," "Stark Early Orange (SEO)," "Harcot," and "Stella" are used as resistance donors [2].

Numerous researchers have proven the nature of resistant variants. Following this method of cross-breeding the susceptible cultivars with the resistant cultivars, the result was apricot seeds that were 100% resistance to Sharka disease [15]. According to Gürcan et al. the inclusion of some new resistance sources expected from Central Asia increases the availability of the source of apricot resistance genes [2], [4]. Furthermore, *PPVres*, a major resistance locus in Linkage Group 1 (LG1), was responsible for 70% of the resistant to PPV [16]. It has been hypothesized that a 5 bp deletion locus which located on chromosome 1 is mostly associated with *PPVres* in prunus species sequences [17]. Moreover, research has demonstrated that *PPVres* alone is insufficient for the generation of resistant varieties [5], [17]. A collection of molecular markers associated with *PPVres* was discovered to help in the early selection of resistant varieties in breeding programs. SSR markers (PGS 1.21, 1.23, and 1.24) have been developed by Soriano et al. [18], and used to screen apricot accessions [2], [18], [19]. Later, a SSLP marker named ZP002 was developed by Decroocq et al. [17] to identify a 5 bp deletion

in the region containing the main candidate resistance gene. Thus, the ZP002- SNP (A/T) was identified by Passaro et al. [20] as being associated with *PPVres*. Additionally, HRM assays for the marker loci PGS 1.221, ZP002, and PGS 1.24 were developed [20]. According to Gürcan et al. [4], the ZP002 marker is more reliable and straightforward to use to determine the *PPVres* resistant allele in apricots [4]. Turkiye is home to a diverse array of apricot cultivars. Recent studies have shown that Turkish apricots have very few sources of resistance to PPV, and other studies have shown that the virus is spreading in some provinces [1], [6],[21],[11],[13],[22]. In terms of Turkish apricot breeding effort, Ulubaş Serçe et al. [1] screened Hacıhaliloğlu, Kabaaşı, Hasanbey, Çöloğlu, Adilcevaz5, Şekerpare, MahmudunEriği, Soğancı and Çataloğlu as common Turkish aprioct varieties. Ulubaş Serçe et al. confirmed that all Turkish genitors have no resistance alleles compared with "Stark Early Orange" (SEO) and "Harcot" varieties. Gürcan et al. [4] attempted to discover sources of resistance to the Plum pox virus in Turkish apricots using biological and molecular new makers assisted selection between 2014 and 2018. It was found that four accessions ('Cebir,' 'Lifos,' 'Karum,' and 'Zard') were resistant to PPV-T and had molecular markers for PPVres. European apricot breeding programs mainly focus on the development of cultivars for fresh consumption, with an emphasis on PPV resistance and early maturity [15], [23]. However, in Turkey, Iran, Pakistan, and Afghanistan, cultivars for dried fruit production are more important. Turkey is the leading producer of dried apricots, supplying almost 70% of the world crop. Late frost in the spring is also a particularly important problem in temperate regions, since occasionally the entire crop is lost.

Among the PPV-resistant accessions, the Western Asian cultivar 'Zard' is late-blooming, frost-tolerant, and has fruit high in soluble solids, and thus has good potential as a parent in dried apricot breeding programs [4]. As a necessary consequence, the inclusion of resistant genes in Turkish apricot crops, which have good qualities, is of extreme importance. This study aimed to examine the inheritance of the new dried resistance apricot Zard variety with the susceptible Turkish variety "Hacıhaliloğlu" and determine the resistance status of apricot progenies (F1).

CHAPTER 1

LITERATURE REVIEW

1.1. APRICOTS-GROWING COMMUNITY

1.1.1. In Classification and Nomenclature

Armeniaca L. is a specie in the *Prunus L. genus.* This genus has three subgenera: Amygdalus, Prunophora, and Cerasus [24], [25]. Amygdalus (L.) Focke and Prunus (L.) Focke were divided into five subgenera by Liu and Shi et al. (2013) based on the inflorescence and endocarp characters of the Rosaceae family [26], [27]. There are about 430 species of Prunus in the world, but most of them found in the northern temperate zones. Many of these fruit crops, like peaches, plums, apricots, and cherries, are important for their economic value. Some species in the Prunus genus stay green all year long and are used as decorations, see (*P. laurocerasus*) as an example [24]. According to different researchers, there are 3 to 12 distinct types of apricots. The species *P. armeniaca* L. accounts for most cultivated apricots, and it is thought that six additional species are most closely related to *P. armeniaca: P. mume, P. dasycarpa,* and *P. brigantina* are Japanese apricot species. China is the origin of *P. sibirica, P. holosericea* Batal., and *P. mandshurica* [28]. In addition, novel interspecific hybrids have been created by artificial cross-pollination in the last few decades. Plumot and aprium are thought to be the result of crossing plums and apricots and then crossing them back to plum (pluots) or apricot (aprium) [29].

Classification of Apricot -*Prunus Armeniaca*

Domain:	Eukaryote
Kingdom:	Plantae
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Dicotyledonae
Order:	Rosales
Family:	Rosaceae
Genus:	Prunus
Species:	*Prunus armeniaca L.*

1.1.2. Botany

The apricot is a diploid (2n = 16), however Bailey and Hough (1975), discovered some tetraploid mutations [30]. A total of 265 and 294 Mbp were determined for the peaches and apricots genomes, respectively, using flow cytometry in 1991. While apricot and peach genomes are almost the same in size, the peach genome was recently sequenced and found to be 227 Mbp in length [31]. Apricots' genome do not get any bigger than other fruit species, like prunes (880 Mbp) or apples (320 Mbp) (750 Mbp) [32], In actuality, apricot is only around twice as large as *Arabidopsis thaliana L. Heynh* (115 Mbp). Grafted apricots can grow well on other Prunus rootstocks, like peaches or plums [33].

Apricot is a small tree growing to a height of 8–12 m, with a trunk diameter of up to 40 cm and a dense, spreading canopy. Apricot's leaves are oval in form, averaging 5–9 cm long by 4–8 cm wide, and are serrated on the margins and base. The flowers in apricot are 2–4.5 cm in diameter and have five white to pinkish petals; the bloom singly or in pairs in early spring, prior to the leaves emerging. The apricot's fruit is about 1.5–2.5 cm in diameter (larger in some modern cultivars), yellow to orange in color, and often reddish on the side that faces the sun. The fruit has a distinct flavor and changes to sweet or tart. The single seed in the apricot is encased in a tough, stony shell, frequently referred to as a "stone," [34]. As with all other *Prunus* species, *armeniaca* L. develops a single ovary containing two ovules, of which one devolves immediately after anthesis; and in the large majority of cases, only one seed is produced [35]. To initiate blooming, apricot trees require a particular level of cooling, measured as the number of units (hours) below 7.2°C. [36]. The chilling requirements for flowering vary according to apricot variety, ranging from 300 to 1,200 CU [39]. The heat requirement following chilling is extremely low, which results in apricot trees blooming early in most locations. As a result of its early bloom habit, the apricot is susceptible to frost injury, and thus its production area is constrained by the threat of spring frost [38]. The apricot fruits take between three and six months to develop, and their flesh may range from sweet to sour [39].

The apricot fruit ranges in weight from 30 to 120 grams and has a high sugar content of 8,700 to 14,200 mg/100g fresh fruit and a low acidity [40]. Due to its high concentration of important microelements for human health, the apricot is one of the most promising foods, due to its high fiber content (Table 1.1), vitamin A content (Table 1.2), and potassium level (Table 1.2) [41].

Table 1.1. Apricot fruit content in raw and dried form per 100g.

Fruit content	Raw fruits	Dry fruits
Water (%)	86.35	30.89
Carbohydrates (%)	11.12	62.64
Total sugars (%)	9.24	53.44
Crude fibers (%)	2	7.3
Protein (%)	1.4	3.39
Ash (%)	0.75	2.57
Fat (%)	0.4	0.51
Energy (Kcal)	48	241

source: USDA Nutrient Database, 2012

Table 1.2. Apricot fruit Vitamins and microelements content per 100g.

Fruit content	raw fruits	dry fruits
Vitamin A (IU)	1926 IU	3604 IU
Vitamin, B1 (mg)	0.03	0.015
Riboflavin, B2 (mg)	0.04	0.074
Niacin (mg)	0.6	2.59
Vitamin C (mg)	10	1
Calcium (mg)	13	55
Phosphorus (mg)	23	71
Iron (mg)	0.39	2.66
Sodium (mg)	1	10
Potassium (mg)	259	1162
Magnesium (mg)	10	32

source: USDA Nutrient Database, 2012

1.2. ECONOMIC SIGNIFICANCE OF APRICOT

The apricot production is distinguished by high demand; as a result, the apricot is one of the few temperate tree fruits that is unaffected by production surplus [42]. The apricot fruits, both raw and processed, frequently command premium prices [38]. This popularity is a result of the fruits' attractiveness, which is defined by their flavor, ease of preparation, and versatility of use [42]. Fruits could use fresh, dried, or canned; those that could made into jams, juices, wines, alcohol, or puree for infant food; and apricots were preserved in ancient China by salting or smoking. The apricot kernel oil is used to create soap in the cosmetic business. Traditional Chinese medicine uses the kernels to cure asthma and coughing [43], and in Japan, it is believed that *P. mume* fruits possess antibacterial and fungicidal properties [44]. Because of their strong flavor and bitterness, the apricot kernels of some species are edible and employed in the food business. However, kernels of some other varieties contain amygdalin, which in the human body degrades to cyanide and causes significant toxicity [45]. Additionally, trees could be grown for their ornamental value [46], [47]. Apricots are perceived differently in distinct cultures. For example, in the United States, the majority of the apricot fruits production are not eaten fresh, but rather as dried or canned [48]. The apricot fruit is one of the most important fruits commercially in Türkiye. Apart from fresh consumption, it is used throughout the summer to make marmalade, jam, and jelly, as well as canned in slices or processed as fruit juice [49].

1.2.1. Worldwide production

Apricots are commercially produced in more than 70 countries listed by the FAO [3], (Table 1.3.). According to the FAO, the world's apricot area harvested was ~583 k. ha and that produced a total of ~3.8m metric tons of fresh and dried apricots. Fruit production and yield production are declining globally, while harvested area is increasing (Figure 1.1.) [3]. The apricot is a temperate zone fruit that grows well in mountainous areas with a hot, dry summer and a consistent, frigid winter. Nonetheless, there are certain types that have minimal cold needs and can be cultivated in subtropical temperatures [50]. The Mediterranean area accounts for 95 % of the fresh apricot market, which is mostly imported and consumed by the European population [24].

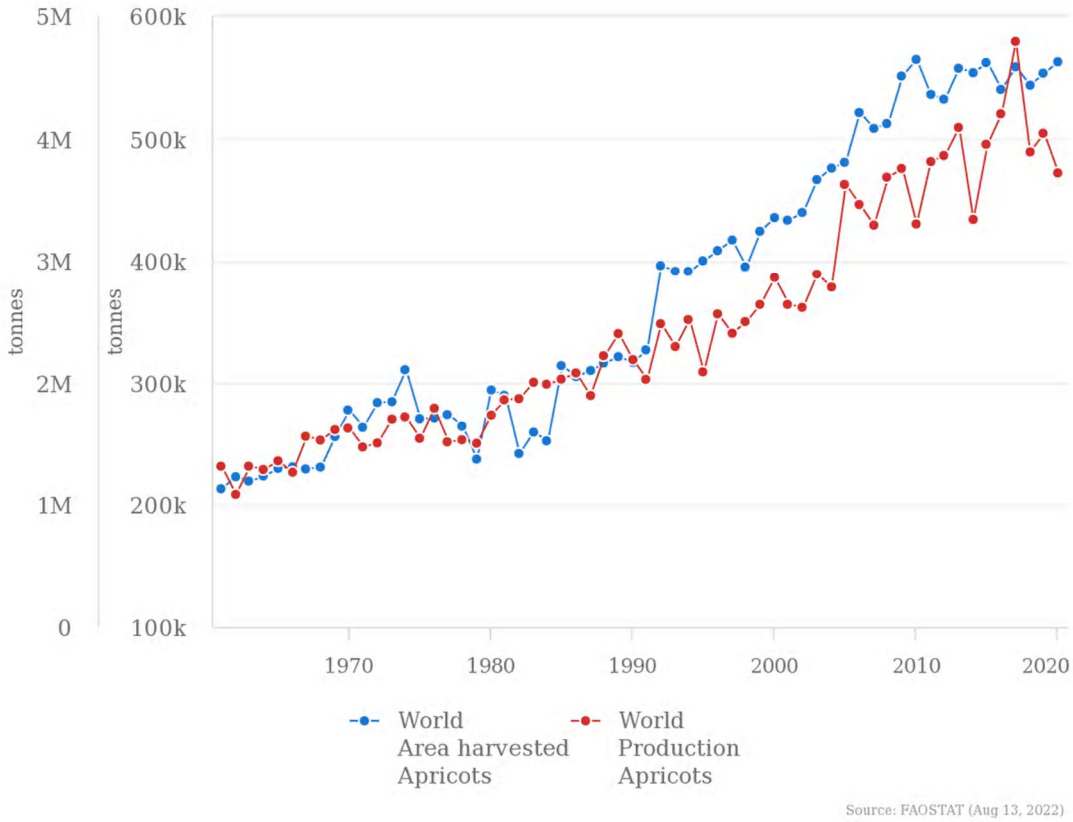

Figure 1.1. Apricot production timeseries in the world [3].

Table 1.3. FAO: Apricot production (t),Harvested Area (ha), Yield (t/ha) [3].

Country	Production (t). %	Area (Ha). %	Yield (t/ha)
World	3791702 (100)	583302 (100)	6.5
Türkiye	833398 (22)	132748 (22.76)	6.3
Uzbekistan	529109 (14)	44262(7.59)	12.0
Iran	334408 (8.8)	58515 (10.03)	5.7
Algeria	187273 (4.9)	29719 (5.09)	6.3
Italy	173380 (4.6)	17810 (3.05)	9.7
Afghanistan	131788 (3.5)	17481 (3.0)	7.5
Spain	128700 (3.4)	19780 (3.39)	6.5
Greece	125640 (3.3)	12240 (2.1)	10.3
Pakistan	97045 (2.6)	17062 (2.93)	5.7
Morocco	93008 (2.5)	10594 (1.82)	8.8
France	85830 (2.3)	12190 (2.09)	7.0
Egypt	84135 (2.2)	5085 (0.87)	16.5
Russian	72800 (1.9)	11923 (2.04)	6.1
China	71728 (1.9)	21057 (3.61)	3.4
Japan	71100 (1.9)	14100 (2.42)	5.0
Ukraine	69480 (1.8)	7300 (1.25)	9.5
Armenia	53191 (1.4)	10551 (1.81)	5.0
China, mainland	53121 (1.4)	17603 (3.02)	3.0
Tunisia	38000 (1.0)	7336 (1.26)	5.2
Syrian	35014 (0.9)	13984 (2.4)	2.5
Turkmenistan	34667 (0.9)	2631 (0.45)	13.2
Iraq	34609 (0.9)	6523 (1.12)	5.3
Lebanon	34110 (0.9)	3810 (0.65)	9.0
Tajikistan	31920 (0.8)	11625 (1.99)	2.7
Serbia	30494 (0.8)	5985 (1.03)	5.1
USA	30299 (0.8)	3586 (0.61)	8.4
Azerbaijan	28977 (0.8)	3366 (0.58)	8.6
Jordan	28632 (0.8)	1977 (0.34)	14.5
Argentina	27105 (0.7)	2355 (0.4)	11.5
Kyrgyzstan	26967 (0.7)	8221 (1.41)	3.3
Romania	26770 (0.7)	2030 (0.4)	13.2
Libya	25990 (0.7)	4648 (0.8)	5.6
Kazakhstan	23092(0.6)	4345 (0.7)	5.3
South Africa	21520 (0.6)	2512 (0.4)	8.6
Taiwan	18607 (05)	3454 (0.6)	5.4
India	14233 (0.4)	5042 (0.9)	2.8
Hungary	10620 (0.3)	5940 (1.02)	1.8
Bulgaria	9520 (0.3)	1840 (0.32)	5.2

Table 1.3. continued

Country	Production (t). %	Area (ha). %	Yield (t/ha)
Switzerland	8080 (0.2)	741 (0.13)	10.9
Australia	6986 (0.2)	6700 (1.15)	1.0
Chile	5728 (0.2)	609 (0.1)	9.4
Moldova	5487 (0.1)	3599 (0.62)	1.5
Albania	5143 (0.1)	346 (0.06)	14.9
Israel	5000 (0.1)	565 (0.1)	8.8
North Macedonia	3434 (0.1)	358 (0.06)	9.6
Portugal	3240 (0.1)	520 (0.09)	6.2
Poland	2900 (0.1)	900 (0.15)	3.2
New Zealand	2725 (0.1)	376 (0.06)	7.2
Georgia	1900 (0.1)	1859 (0.32)	1.0
Nepal	1782 (0.0)	289 (0.05)	6.2
Austria	1580 (0.0)	830 (0.14)	1.9
Yemen	1445 (0.0)	645 (0.11)	2.2
Madagascar	1442 (0.0)	172 (0.03)	8.4
Mexico	1094 (0.0)	207 (0.04)	5.3
Palestine	1074 (0.0)	423 (0.07)	2.5
Cameroon	933 (0.0)	181 (0.03)	5.2
Cyprus	870 (0.0)	180 (0.03)	4.8
Canada	808 (0.0)	101 (0.02)	8.0
Croatia	800 (0.0)	290 (0.05)	2.8
Bosnia and Herzegovina	753 (0.0)	519 (0.09)	1.5
Czechia	590 (0.0)	1170 (0.02)	0.5
Peru	484 (0.0)	96 (0.02)	5.0
Slovenia	470 (0.0)	80 (0.01)	5.9
Ecuador	356 (0.0)	72 (0.01)	4.9
Slovakia	140 (0.0)	200 (0.03)	0.7
Kenya	76 (0.0)	16 (0)	4.8
Zimbabwe	46 (0.0)	23 (0)	2.0
Bhutan	36 (0.0)	5 (0)	7.2

1.2.2. Apricot production in Türkiye

Apricot is a significant species in Turkiye's fruit production. While apricots are not indigenous to Turkiye, they are grown in most Anatolia's regions (Figure 1.2.). Until the work of Vavilov (1934), the apricot was thought to has originated in Armenia. However, subsequent research indicates that the apricot's native range includes Central Asia, China, and Siberia [51]. Turkiye produced 834 thousand tons of fresh and dried apricots. Turkish apricot production represents 22% of global production for the year 2020 (Table 1.3.). Each year, Turkiye exports dried apricots to approximately 90 countries (Russia, the United States of America, England, and Germany, among others), and apricot cultivation generates a sizable amount of revenue. While dried apricot production is greater than fresh apricot production, Turkiye is growing as a fresh apricot exporter in recent years. Apricot production and exports are a significant source of income for farmers. Turkiye's most important apricot producing provinces are as follows: Malatya, Erzincan, Icel (Mut), the valley of Aras (Igdir-Kagizman), Sivas, Elazig, Kayseri, Nigde, Nevsehir, Hatay, and Kahramanmaras [49]. As a result, Turkiye has increased apricot cultivation annually, as illustrated in Figure 1.3.

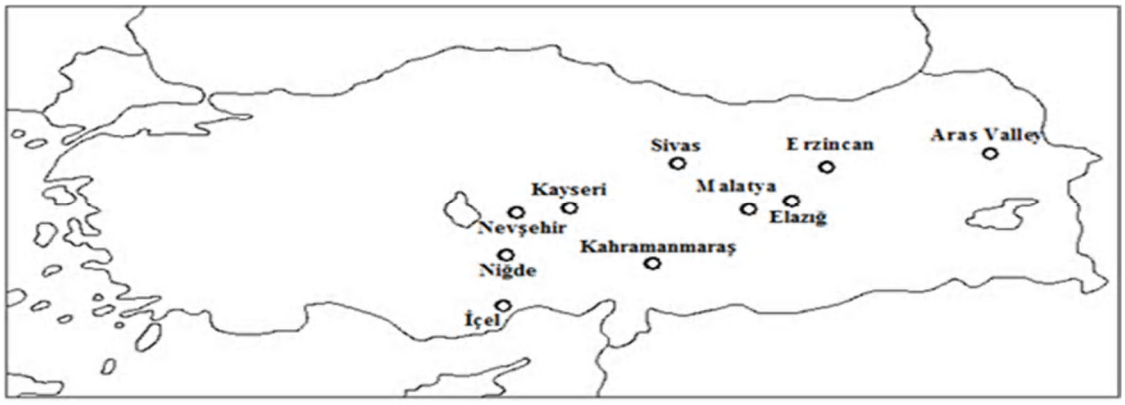

Figure 1.2. The Primary Apricot Production Regions of Türkiye [49].

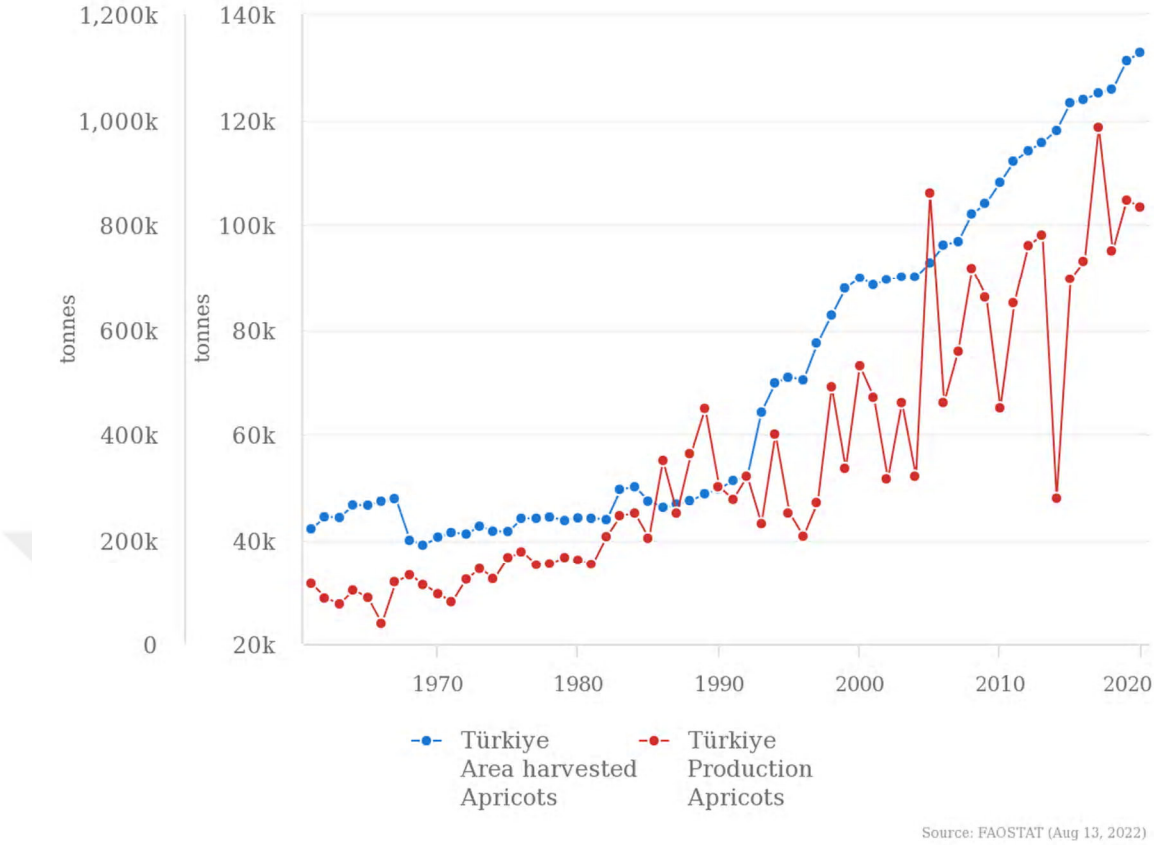

Figure 1.3. Timeseries of Apricot Production in Türkiye-2020 [3].

1.3.THE IMPORTANT DISEASES OF APRICOTS

Several serious fungal, bacterial, nematode, and viral diseases are common to apricot fruits and should be of concern to all growers. Symptoms of several common diseases and their control measures are discussed.

1.3.1. Fungal Diseases

1.3.1.1. Monilinia Laxa

One of the most damaging apricot diseases is brown rot, caused by *Moniliana laxa Ehr*. This disease has the potential to destroy an entire annual crop during the blooming stage and can kill shoots up to 30 centimeters away from the initial infection point [52], [53]. Fungicides are typically utilized in Türkiye to treat brown rot. The downsides of chemical control include excessive energy consumption, soil and environmental damage, and pesticide residues on fruits. There has been a recent rise in interest in ecologically friendly sustainable agriculture techniques [53], [54].

1.3.1.2. *Rhizopus Stolonifer*

Rhizopus is a fungus that can rot ripe apricots, peaches, and plums. however other species may also be included. *Monilinia and Rhizopus* rot could grow because of the conditions. a lot less brown rot and soft rot happened on damaged nectarine, apricot, and peach fruits, and the size of the damage was much smaller [52], [55].

1.3.2. Bacterial Diseases

1.3.2.1. *Xanthomonas Campestris PV.*

Several bacterial pathogens are negative for stone fruit. Bacterial spot, which is caused by Xanthomonas campestris, is a fundamental problem all over the country. Even though trees rarely die, they can die back if nodes and lenticels on fruiting arms get infected and cause diseases. In some seasons, a lot of the fruit may also be damaged. Bacterial spot-on vegetative tissue thrives in warm, wet conditions, like most bacterial diseases. It can cause fruit loss wherever stone fruit is grown [56].

1.3.2.2. *Pseudomonas syringae PV.*

P. syringae pv. persicae, which causes bacterial decline. This pathogen is the cause of nectarine and peach trees starting to fail and dying. It can also cause Japanese plum trees to die back, but this usually does not do much damage to trees. P. syringae, on the other hand, is what Phytophthora (Klebahn) Klebahn causes spots on apricots, peaches, and nectarines that look very similar to those caused by Pseudomonas syringae pv. syringae. It has been linked to stone fruits with rotten collars [56], [57].

1.3.2.3. *Cytospora Spp*

Cytospora spp. have been reported as pathogenic to Prunus spp. In Türkiye a survey was conducted. A total of 6327 apricot trees in 125 plantations were inspected. According to survey findings, 90% of plantations contained trees that were contaminated in some way, and 36% of trees evaluated displayed disease signs. Additionally, Cytospora-induced rapid mortality of apricot trees has been seen on a rare basis [58].

1.3.3. Viral Disease

1.3.3.1. *Plum Pox Virus* (PPV)

PPV the agent that causes Sharka disease, is a member of the genus *Potyvirus*. This virus's natural host range is Prunus spp (Peaches, Plums, and Apricots) [2]. Many countries in Europe, Asia, and North and South America have a high prevalence of PPV despite the fact that the infection rates levels differ wildly from one country to another [59]. PPV is obstinately transmitted by many aphid species, grafting, and propagation of infected plants, but seed transmission has not been reported. PPV has been classified into 10 strains/groups based on molecular characteristics (PPV-M, -D, -Rec, -EA, -C, -T, -An, -W, -CR, and PPV-CV [7],[8]. Despite the availability of numerous screening methods for the responsive and/or specific detection of PPV, the virus's uneven distribution within infected woody hosts and its low viral load outside of the active growth period make detection difficult. In the absence of resistant varieties to PPV, a combination of prophylactic measures, such as tree eradication, vector control, and virus-free propagation material are generally used to control the virus in areas where it has not yet become endemic [60].

The "PPV-T" strain was found to be the most prevalent despite numerous studies confirming the presence of the "PPV-M," "PPV-Rec," and "PPV-D" strains in Turkiye. Several Turkish provinces were identified as carrying the "PPV-T" strain, including Ankara, Istanbul, Kayseri, and Konya, and PPV is causing massive economic losses in the farming production in these provinces [4],[21],[11],[13],[22].

PPV Virus particles are small, measuring 750 nm in length and 15 nm in diameter. The PPV virus has a single-stranded RNA genome that is shaped like a pinwheel and is found in the cytoplasm of infected cells [61]. Numerous strains of PPV are being studied to glean as much information as possible about the virus, and serval RNAs are cloned and sequenced to obtain the complete or partial nucleotide sequence. The PPV genome structure was extensively studied, and its detection and analysis are now possible [62], [63]. While PPV has long been classified as a potyvirus, a related virus was discovered in 1994 and named Asian prunus latent potyvirus. DNA primers and their cross-reaction with other tests can be used to determine the difference between Asian prunus latent potyvirus and PPV [64].

PPV Classification.

Domain: Virus

Group: "Positive sense ssRNA viruses"

Group: "RNA viruses"

Family: Potyviridae

Genus: Potyvirus

Species: Plum pox virus

1.3.3.1.1. Hosts species for PPV

The primary woody host of the PPV virus is the prunus family of fruit trees. This category holds apricots, peaches, and plums. Almonds can also function as a virus host, but only in a few instances. Although almond is naturally PPV-infected in Chile, the survey conducted at a different location discovered that almond is not naturally PPV-infected. The cherry plant is injected with the D and M strain of PPV virus for the experiment. After the experiment is completed, it is seen that the PPV virus stays locally and does not spread to another tree. However, in the case of the P strain, the PPV virus spread across multiple cherry blossom and sweet cherry varieties. In general, stone fruits such as plum, peach, nectarine, apricots, , sweet and sour cherries, and ornamental prunus species serve as hosts for PPV [59], [65].

1.3.3.1.2.PPV Symptoms

Sharka disease symptoms can be seen by analyzing leaves, fruits, blossoms, and seeds (figures 1:4,5 and 6) [2], [6], [43]. After infection, leaves turn yellow with bands on the surface; similar symptoms are seen on fruits as well. Due to the virus's uneven distribution on the surface, some areas become yellowish with bands. The newly infected tree may be devoid of symptoms for three or more years. In some cases, the tree may be symptomless; in this case, laboratory testing may aid in the detection of PPV. As a result, many guideline programs place a premium on laboratory testing over visual examination.[66].

1.3.3.1.3. Leaves

Sharka disease has many symptoms that may appear on the leaves (Figure 1.4). Small green or yellow rings may grow on leaves. The young leaves may not show signs in the initial stages of spring. Only a few tree branches produce leaves without PPV signs. Young trees show more symptoms than older ones. Plum tree symptoms are more obvious than peach and apricot tree symptoms [10].

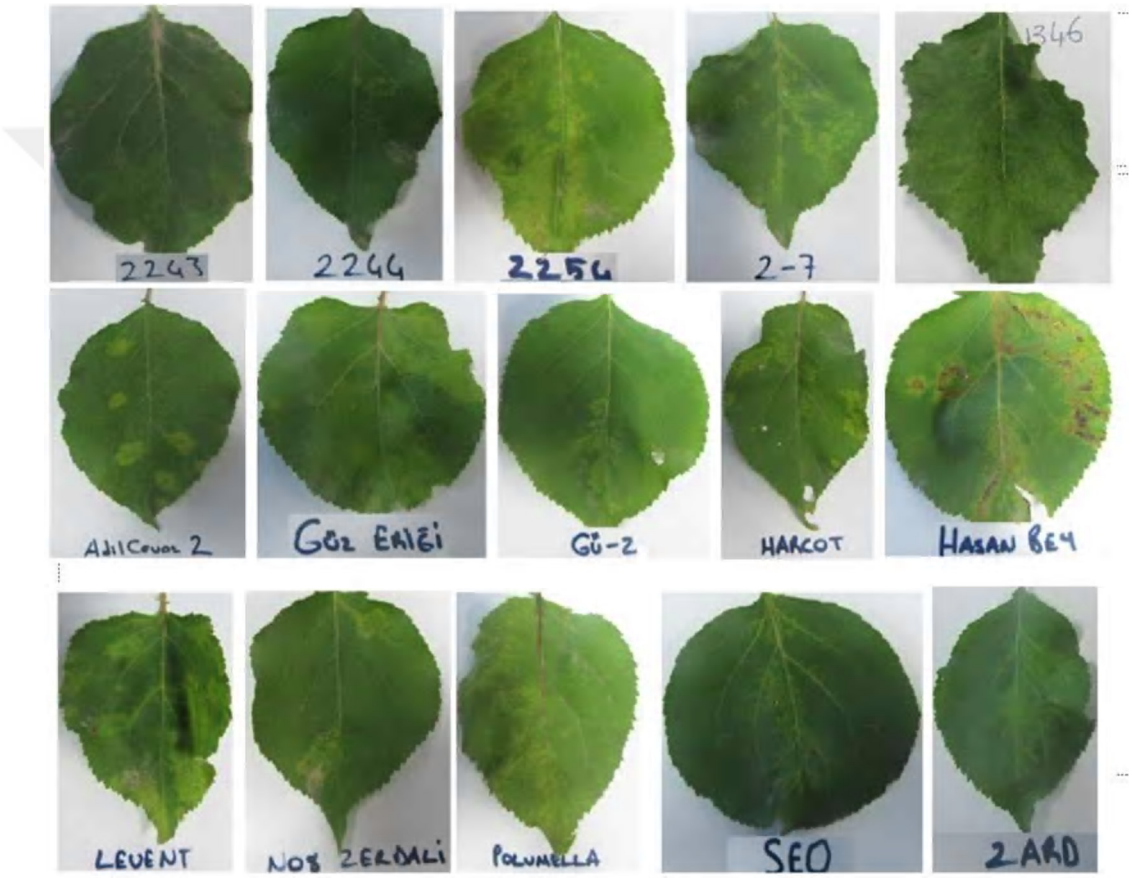

Figure 1.4. Sharka Disease Symptoms on Infected Apricots Varieties' Leaf [6]

1.3.3.1.4. Fruit

In the case of fruit, in the initial stages of PPV infection, the fruits show no symptoms, but after a few years, the surfaces of the fruits have a dark yellow ring. As PPV develop, all fruits exhibit infection signs, making it simple to identify (Figure 1.5 and 1.6). As the virus spreads, the fruit loses sweetness, flavor, and prematurely falls off [4], [6], [67].

Figure 1.5. Sharka Disease symptoms on apricot fruits and seeds [2].

Figure 1.6. Sharka Disease Symptoms on Apricot Stone [43].

1.3.1.1.5. PPV Distribution

The first reported to PPV was in 1917 in peach trees in Bulgaria [5]. Since then, PPV is spreading in several countries that produce stone fruits (Figure 1.7.) [43]. Thousands of trees infected with Sharka disease have been removed across Canada and USA, and the breeding program is underway in Europe and Türkiye [2], [5], [9], [15]. Moreover, the Sharka disease was reported to has reached Japan last decade [68], [69]. Early, In 1968, PPV was first reported in Türkiye [1], [6]. According to recent studies, Türkiye is the primary source diversity of the strains PPV-D , PPV-M and -REC (Figures 1.8, 9, 10 and 12) [11], [13], [14], [70] . Furthermore, there are also two PPV isolate groups found only in Türkiye, the PPV-T and the PPV-MIs (Figure 1.8 and 11) [6], [12], [71]. Moreover, recombination research revealed PPV-MIs isolates to be the donors of the PPV-M segment of PPV-Rec, for that called PPV-M Istanbul strain [11]. However, no strain was discovered in Malatya, which is considered the primary producer province of Turkish apricots [1], and this may be an opportunity to hurry in an apricot breeding program in Türkiye.

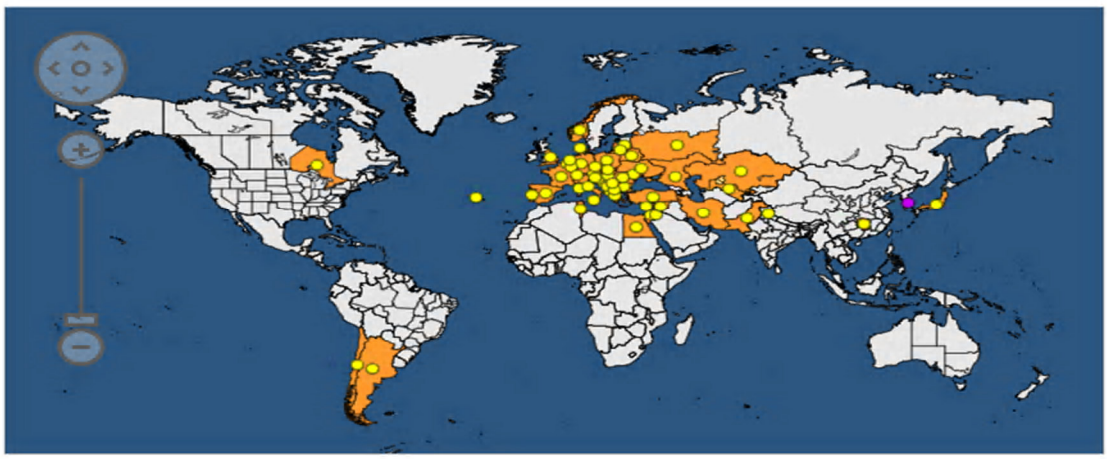

Figure 1.7. Plum pox virus (PPV000) Distribution [43]

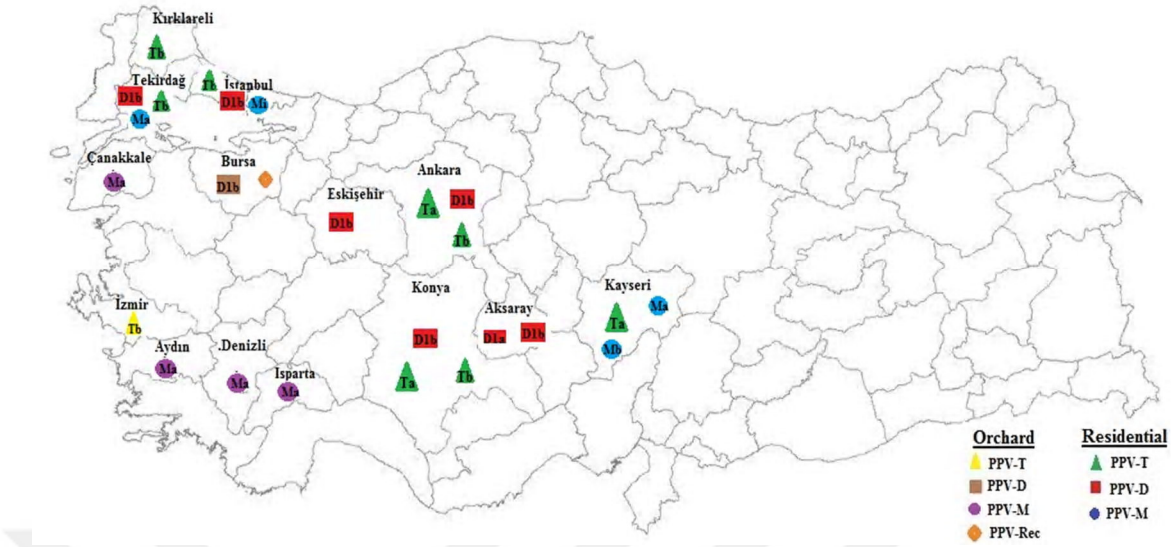

Figure 1.8. Location of PPV Strains isolated in Türkiye [6].

Figure 1.9. Locations of PPV- D Strain isolates in Türkiye [14].

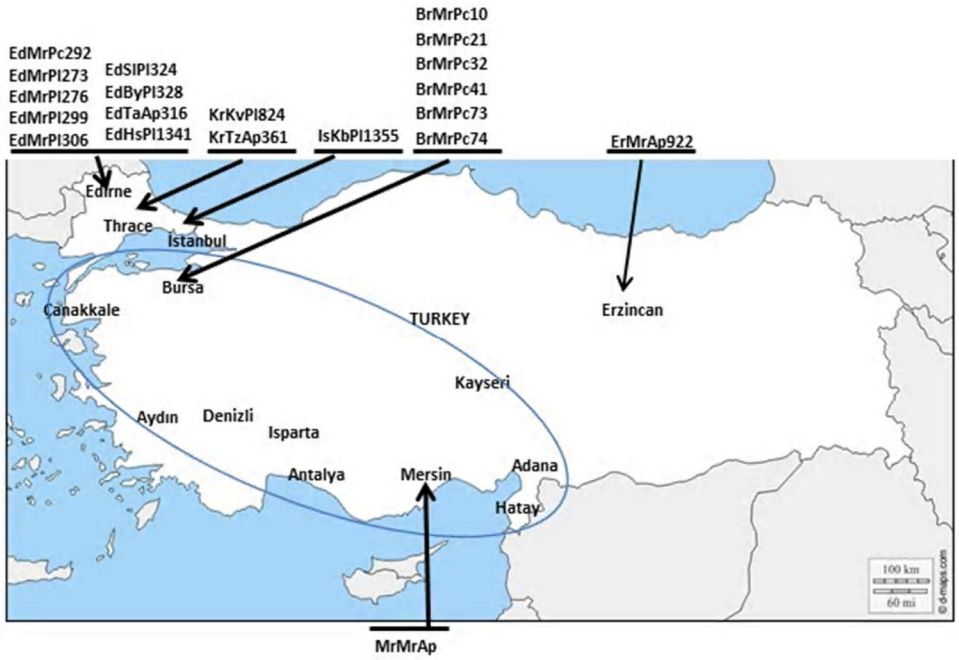

Figure 1.10. Locations of PPV- M isolates in some Turkish Regions [72].

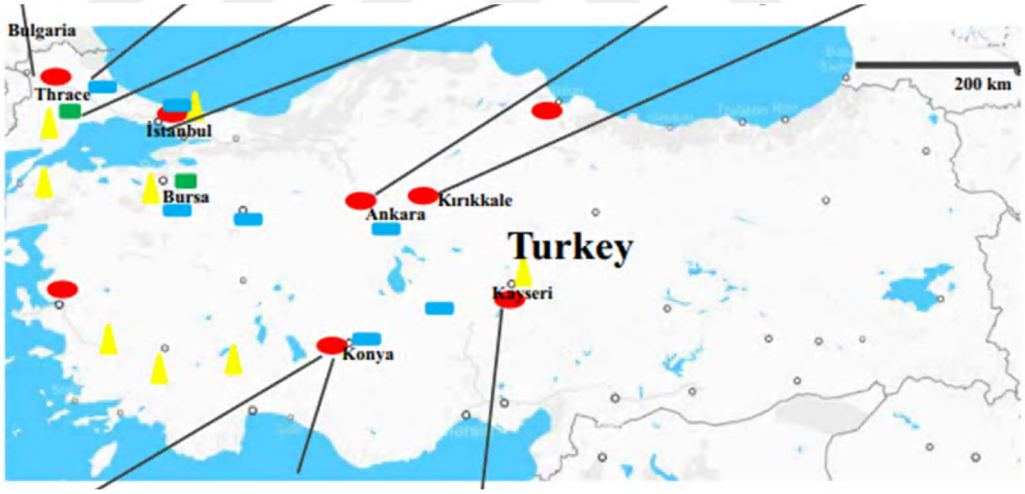

Figure 1.11. Locations of PPV- T Strain isolates in some Turkish Regions [12].

Figure 1.12. Locations of PPV- REC Strain isolates in some Turkish Regions [13]

1.3.3.2. PPV Assessment

Numerous approaches, including biological assessment, ELISA, PCR and WGS, are frequently employed to identify PPV. Natural infection studies and resistant cultivations to PPV was assessed by planted the cultivars to be tested next to infected trees [6], [73]. Additionally, studies of artificial infection were continued, using aphids and budding is a viable method of transmitting PPV. Moreover, It has been reported that GF-305 peach rootstocks rapidly develop symptoms of disease [18], [74]. Wherefore, two methods for determining PPV resistance have been developed using the GF-305 rootstock. The first method involves grafting the desired apricot cultivar or types onto the GF-305 rootstock, which can be grown from seed or a clone, and apricot buds are expected to appear. The disease is then transmitted by grafting PPV-infected eyes onto sprouted apricots [18]. In the second method, the GF-305 mother is infected first and then vaccinated with the apricot eye. The researchers compared the two methods and concluded that the second was more effective [74].

The ELISA method is commonly used to determine the molecular presence of viruses. With the discovery of polyclonal antibody 5B-IVIA and monoclonal antibodies for a variety of PPV strains, ELISA became the standard method for identifying all PPV strains [6], [67], [75]–[77].

By amplifying the variable N-terminal section of the viral protein (CP) and the conserved noncoding region, PCR was utilized to identify the virus generally or strain-specifically [77]–[80].

Sequence analysis is widely considered as the most accurate approach for viral identification. As with other plant viruses, PPV was investigated primarily by sequencing of the viral CP gene [77], [81].

1.4. APRICOT BREEDING STUDIES

Since the 1950s, breeding efforts for PPV have been ongoing in the United States and Europe. Many researchers mentioned that Kegler et al 1998. reviewed 280 studies on Oriental resistance in the Prunus family over a 50-year period in their review article [18], [82]. Martinez-Gomez et al. [83] summarized previous researches on apricot resistance and listed the resistance status of different types and varieties to Sharka. Thus, several apricot cultivars resistant to PPV have been identified in Europe through resistance breeding and molecular studies. The apricot cultivars include "Goldrich", "Harlayne", "Stark Early Orange, SEO", "Stella", and "Harcot" are frequently used in breeding studies as resistant parents [16], [84]–[86]. Thus, resistance is attempted to economically significant apricot varieties. Many producer countries that breed apricots include Spain, Greece, France, Italy, and the Czech Republic are still working to produce resistance local cultivars. The varieties "Lito" and "Pandora" were developed in Greece, "Avilara" in France, and "Leronda" in the Czech Republic and more that 11 resistance cultivars were developed though crossing with "Goldrich" [18], [47], [84], [87], [88].

Elibuyuk and Erdiller [89] conducted a study in Türkiye on seven apricot cultivars and concluded that they were susceptible to Sharka disease. In addition, Caglayan et al. [90] determined the susceptibility of apricot cultivars and seven plum cultivars to Sharka disease using the Sakt-2 and 11/89 types. However, Ulubaş Serce et al.[1] screened a subset of the previous hybrid populations with two markers associated to the SEO/Harlayne resistance locus. Ulubaş Serce discovered that 62 hybrid plants carried resistant markers. However, due to the absence of phenotypic testing, the resistance status of these hybrid plants could not be determined. Flowing that, Gurcan et al. [2], [4] examined over 200 apricot cultivars found in Turkish germplasm, identified five resistant apricot cultivars, and concluded that the remaining cultivars are susceptible to

Sharka disease. Two of the Gurcan et al. apricots (Kaniş and Fracasso) are tolerant or lack the North American resistance, while Lifos Karum , and Cebir are confirmed to be both resistant Turkish apricots. Moreover, another study developed hybrid plants by crossing significant dried apricot cultivars from Türkiye with disease-resistant cultivars from North America [86].

Researchers have concentrated on deciphering the disease's epidemiology, deciphering the function of viral genes, developing virus-resistant varieties, and deciphering the genetic mechanism of resistance. Numerous segregation and molecular mapping studies conducted to identify the origins and molecular genetics of apricots resistant to Sharka disease have revealed that they are controlled by a single gene. Despite this, a few researchers observed that by combining two genes, and even some said by combining three genes [91]–[94]. Many studies mentioned that Linkage Group 1 (LG1) and linkage Group 5 (LG5) show a minor quantitative part (LG5). Numerous loci (quantitative trait loci) have been found. Minor loci of endurance LG3 genes are a subset of minor endurance genes [17], [95], [96] and LG5 [95], [97].

Marandel et al. [94] conducted a QTL meta-analysis on six previously published studies and identified four regions on LG1 and LG3. Although the genetic mechanism of resistance and the number of loci controlling resistance are debatable, researchers agree that the major locus LG1 in the apricot genome's upper region is effective at conferring resistance. A bacterial artificial chromosome (BAC) library has been made available for the purpose of identifying the LG1 locus [98]. SSR markers were developed using DNA fragments sequenced and generated using LG1 from the BAC library [16], [98]. Two SSR markers ("ssrPACITA5" and "ssrPACITA17") have been identified for use in marker assisted selection studies (MAS) [95]. Moreover, subsequent research has revealed that a large locus called *PPVres* located upstream of LG1 accounts for 70% of resistance [99], [100]. At the *PPVres* locus, molecular markers have been identified for use in MAS studies. Soriano et al. (2012) developed three simple sequence repeat (SSR) markers (PGS 1.21, 1.23, and 1.24) on the *PPVres* locus [18]. Then, in their study, Zuriaga et al. [101] narrowed the *PPVres* locus to a 196-kb region [101]. Decroocq et al. [17] identified a 5 bp deletion in the condensed 196kb region and hypothesized that it was responsible for resistance. Furthermore, Decroocq created the ZP002 single sequence length polymorphism marker to detect 5 bp deletions [17]. Passaro et al. [20]

subsequently developed high resolution melting (HRM) analyses for the PGS 1.21, ZP002, and PGS 1.24 markers. Therefore, Gürcan et al. [4] used all the above-mentioned markers to screen Turkish apricots, and a correlation between markers and resistance loci has been established. There have been reports of discrepancies between phenotypic and genotypic testing for PPV resistance. Rubio et al. (2014) genotyped 80 apricot cultivars and types using the *PPVres* locus marker (PGS1.21, PGS1.23, and PGS1.24) and discovered that the perishable cultivar 'Alba' and the (1002)8-6 genotype also contained the *PPVres* locus markers [17]. Similarly, Decroocq et al. (2014) concluded that markers associated with the *PPVres* locus are insufficiently reliable for selecting resistant apricots. While primers have been developed, the major gene responsible for PPV resistance has yet to be identified. Additional research is required in this area, as well as the discovery of reliable markers [102].

1.4.1. The Origin of PPV Resistance in Turkish Apricots

Türkiye ranks number one in the world in terms of apricot production and export of dried apricots. Hacıhaliloğlu (60-65%) and Kabaaş (30-35%) are two of the most commonly grown apricot cultivars in Malatya, which is widely regarded as the world's most important apricot production center[86]. In terms of Turkish apricot breeding effort, Ulubaş Serçe et al. [1] screened Hacıhaliloğlu, Kabaaşı, Hasanbey, Çöloğlu, Adilcevaz5, Şekerpare, MahmudunEriği, Soğancı and Çataloğlu as common Turkish aprioct varieties. Ulubaş Serçe et al. confirmed that all Turkish genitors have no resistance alleles compared with "Stark Early Orange" (SEO) and "Harcot" varieties. Gürcan et al. [4] attempted to discover sources of resistance to the Plum pox virus in Turkish apricots using biological and molecular new makers assisted selection between 2014 and 2018. It was found that four accessions ('Cebir,' 'Lifos,' 'Karum,' and 'Zard') were resistant to PPV-T and had molecular markers for PPVres (Table 1.4.). European apricot breeding programs mainly focus on the development of cultivars for fresh consumption, with an emphasis on PPV resistance and early maturity [15], [23]. Thus, the American cultivars "Harlayne," "Goldrich," "Stark Early Orange (SEO)," "Harcot," and "Stella" are used as resistance donors [2]. On the other hand, in countries like Türkiye, Iran, Pakistan, and Afghanistan, dried fruit cultivars are more crucial. In terms of quantity, Türkiye supplies over 70% of the global supply of dried apricots, making it the world leader in production. There is a significant issue with late spring frost in

temperate zones, since it can wipe out an entire crop on rare occasions. The Western Asian cultivar 'Zard' has great promise as a parent in dried apricot breeding projects since it is late-blooming, frost-tolerant, and produces fruit with an elevated level of soluble solids [4].

Table 1.4. Turkish and other Apricot variety associated with PPVres molecular marker

Accession	Origin	PPV inoculation test	SSRs			SSLP	HRM Assay		
			PGS1.21	PGS1.23	PGS1.24	ZP002	PGS1.2 1 SNP	PGS1.2 4 SNP	ZP002 SNP
Harlayne	Canada	Resistant	194/240	161/155	119/121	127/132	+	+	+
Lifos	Turkey	Resistant	212/240	161/173	119/123	127/132	+	+	+
SEO	USA	Resistant	194/240	161/155	119/121	127/132	+	+	+
Yılbat	Turkey	Susceptible	200/240	161/159	119/123	127/132	+	+	+
Zard	Central Asia	Resistant	194/240	161/163	119/119	127/132	+	+	+
Cebir	Turkey	Resistant	210/212	161/163	119/123	127/132	+	+	+
Aurora	Greece	Resistant	194/194	159/163	119/121	127/132	–	+	+
Karum	Turkey	Resistant	212/214	173/175	123/157	127/132	–	–	+
Kaniş	Turkey	Resistant	216/220	155/155	121/147	132/132	–	–	–
Fracasso	Italy	Resistant	194/200	157/159	121/149	132/132	–	–	–
Geç Abligoz	Turkey	Susceptible	214/216	153/155	147/147	132/132	–	–	–
Hacıhaliloğlu	Turkey	Susceptible	214/220	153/177	123/123	132/132	–	–	–
Harcot	Canada	Susceptible	194/208	157/159	121/125	132/132	–	–	–
Hungary Best	Hungary	Susceptible	194/210	153/155	121/149	132/132	–	–	–
Markuleşti	Turkey	Susceptible	194/210	157/159	121/123	132/132	–	–	–
Ninfa	Italy	Susceptible	194/210	153/159	121/149	132/132	–	–	–
Ordubat B.	Turkey	Susceptible	214/216	171/173	123/123	132/132	–	–	–
Şekerpare B.	Turkey	Susceptible	194/210	153/159	121/149	132/132	–	–	–
Mektep	Turkey	Susceptible	194/194	155/155	121/121	132/132	–	–	–
Mektep 8	Turkey	Susceptible	214/216	153/153	147/158	132/132	–	–	–

PPVres lilnked aleles are higligted as red.

1.4.2. Molecular Markers

Molecular markers are DNA fragments connected to and associated with a particular gene segment or genomic area. Because they are based on DNA, molecular markers are also known as DNA markers. The discovery of polymorphic DNA regions is the foundation of molecular markers. The term polymorphism refers to the numerous amino acid variations present in the DNA sequence. These markers may be codominant or dominant. Because molecular markers are genetic markers based on nucleic acids, plant breeders employ them for functional genomics. It can even identify morphologically identical cultural variants. In addition to QTL analysis, genetic mapping, cultivar identification, detecting genetic connections, seed purity analysis, characterization of gene resources, and understanding the structure of genetic resources, molecular markers are utilized in a range of other applications. Some features of molecular markers are needed, like an important level of polymorphism and the ability to distinguish genotypes; the ability to tell heterozygous organisms from homozygous dominant organisms; a cheap application cost; and the ability to repeat the test [103]–[105].

1.4.1.1. Molecular Marker Types

Molecular markers are classified into two broad categories based on their method of production: hybridization-based markers and PCR-based markers. RFLP is a hybridization-based marker. There are four types of PCR-based markers. These include SSRs, SNPs, SSLP), and RAPDs. Aside from these markers, DNA sequencing has revealed SRAP (Sequence Related Amplified Polymorphism), SCAR (Sequence Characterized Amplified Regions), and CAPS (Cleaved Amplified Polymorphism) markers [105]–[108].

1.4.1.2. Mutation Types

There are mutations that involve single base changes (point mutations), transitions, or crossovers (transversions). Replace Adenine (A) with Guanine (G) or Cytosine (C) with Thymine (T) in the transition model results in a same-sex change; this is referred to as a transition mutation from purine to purine or from pyrimidine to pyrimidine. This transformation takes four distinct forms: Transition mutations are A G or C T changes. Cross-transfers are referred to as transversion mutations. In this case, a purine undergoes conversion to a pyrimidine, or a pyrimidine undergoes conversion to a purine. There are eight possible variants of this: A C; AT; GC or GT. It refers to the transformation of a purine into one of two pyrimidines or a pyrimidine into one of two purines. In genetics, insertion is the process of adding one or more base pairs to a DNA sequence. Base substitutions can take the form of a base being substituted for another base (base substitution) or a base being deleted or added (indel mutations). Genetic variation is caused by chromosome mutations. These mutations were used as markers to pinpoint the gene's location on a particular chromosome [105], [109], [110].

1.5. LITERATURE REVIEW

Hurtado et al. [111] used AFLP, RAPD, RFLP, and SSR markers to detect genetic linkage maps for two apricot varieties. A total of 81 progenies (F1) by crossing "Goldrich" with "Valenciano" were evaluated. Hurtado et al. found that the SSRs are strongly associated with Sharka resistance. As such, the study's findings suggest that screening at least six plants per genotype test is necessary. The study was the first report on genetic linkage maps for apricots. These maps are very important for geneticists and breeders because they show where important genes are located.

Karayiannis, et al. [112] determined Sharka resistance in a total of 1178 apricot progeny. In addition, the inheritance of PPV resistance was investigated for the first time in a BC1 population of 95 apricot hybrids over four vegetative periods. Karayiannis has used GF-305 and double-antibody sandwich enzyme-linked immunosorbent assay tests to assess each hybrid's response to PPV-M. Karayiannis's findings indicate that PPV resistance is controlled by a single dominant gene locus. The variety that carries this gene may exhibit disease symptoms on its leaves at first, but it will be controlled by this gene. But the susceptible variety is not. Although PPV resistance in apricots is monogenic, the mechanisms of resistance are distinct in Stella, implying that different resistance loci exist among Stella variety.

Marandel et al. [113] revealed the presence of at least two additional loci on chromosome one and a third on chromosome three in the apricot genome. While most PPV-resistant varieties have the resistance alleles, a substantial number of PPV-susceptible varieties have the same resistant alleles. Moreover, the influence of the PPV strain used for phenotyping was also demonstrated. Consequently, the presence of additional components or genes implicated in the process of Sharka resistance in apricots may account for these surprising results. Given that the success of MAS is based on the stability of the underlying model, it is anticipated that it will be less successful in the presence of digenic or higher-order determinism, especially if not all QTLs have been identified and targeted by MAS markers. The study found that the existing collection of PGS markers is insufficient for MAS and that marker-assisted breeding utilizing the *PPVres* locus alone is insufficient for choosing PPV-resistant apricot cultivars without ambiguity. For MAS of PPV resistance to work in apricot, the additional locus or loci that may make *PPVres* work better must be found and targeted with molecular markers.

Zhivondov and Milusheva [114] released a selection of hybrids based on visual assessment under natural infection settings and serological analysis. Four hybrids (21-3 and 21-47), and (21-55 and 21-67) were developed by mating "Pasific" with "Serdika," "Pasific" with "Stanley" prospectively. Milusheva used a mix of biological indexing and field tests to determine the status of resistance to PPV. During the biological indexing, the hybrids were intentionally injected with viral isolates representing the PPV-M, PPV-D, and PPV-Rec strains. Until then, biological indexing data indicated that hybrid 21-47

had no PPV symptoms, and ELISA findings were negative. Three more hybrids were infected with PPV isolates. Simultaneously, the examined hybrids were propagated and planted without the use of chemical aphid repellents under natural PPV infection pressure. After five years of development, hybrid 21-47 remained symptom-free and was determined to be negative for PPV using an antibody directed, but the virus was discovered in 7.7, 23.5, and 42.1% of the examined plants in hybrids 21-3, 21-55, and 21-67, respectively. Based on the summarized results, it was feasible to determine at that time in the investigation that the hybrid 21–47 had a significant level of PPV resistance. The outcomes of the field trial matched those of the artificial inoculation. Milusheva suggested conducting more research utilizing biological indexing using other PPV isolates to corroborate the findings acquired regarding hybrid 21-47, in addition to continuing field observation.

Zuriaga et al. [101] produced a map of the *PPVres* locus in the PPV-resistant variety 'Goldrich.' Using the Illumina-HiSeq2000 technology, the complete genomes of seven apricot variants (four susceptible and three resistant variants) were sequenced along with three other apricot species. Zuriaga categorized putative resistance genes. As per the study findings, the candidates for PPV resistance in apricot include a cluster of meprin and TRAF-C homology domain (MATHd)-containing proteins. Interestingly, Zuriaga revealed that MATHd proteins control potyvirus in resistant apricots. Even though the roles of MATHd-like proteins are unclear, evidence shows that they may serve as a connection between certain protein substrates and ubiquitin ligase complexes, hence clarifying the involvement of TRAF-like proteins in PPV resistance. The results of the study may help with the use of new ways to improve resistance breeding, such as using MAS to test resistance transgenes.

Ulubaş Serçe et al. [1] obtained crossbreeding between ‘SEO’ and 'Harcot' as resistant varieties with Turkish apricot varieties including 'Hacıhaliloğlu', 'Kabaaşı', and 'Hasanbey. A total of 189 progenies were screened with PGS1.21 and PGS2.23. Further, future virus strains may be able to overcome current resistance. The conclusion of the study was that it is essential to create new methods for screening new indigenous germplasm for PPV resistance. New local apricot cultivars are still being made, and the same offspring are still being judged by new markers.

Gürcan et al. [2] evaluated a total of 278 apricot accessions. Three SSR loci (PGS1.21-

240, PGS1.23-161, and PGS1.24-119) were linked to the 'SEO' and 'Harlayne' resistant varieties. These loci were rare in Turkish accessions (1.7%), but they were common in Pakistani accessions (41.7%). Kahraman demonstrated that the SSR profile data may be utilized to build identification charts for variety resistant status. The findings emphasized the need for biological assessment to evaluate PPV resistance in natural or controlled situations. At present, seven varieties of Turkish apricot have been examined in the field and determined to be susceptible. On the other hand, Turkish varieties 'M2243' and 'M2244' were found to have heterozygous resistance alleles at two loci (PGS1.21 and PGS1.24) and a homozygous resistance allele at PGS1.23.

Rubio et al. [115] conducted the PPV sensitive-resilient Gene Expression Analysis. The apricot genotypes of "Rojo Pasión" (PPV-resistant) and Z506-7" (PPV susceptible) were detected by ELISA and RT-PCR. Moreover, Rubio sequenced RNA for these varieties by using the Illumina HiSeq2000 to obtain the sequences of cDNA libraries. Rubio found a total of 283.057 variants in the "Rojo Pasion" variety and a total of 293,565 variants in the "Z506-7" variety. Rubio reported that 5,266 of the 277,792 SNPs detected in "Rojo Pasion" were INDEL (deletion) sites, while 5,939 of the 287,062 SNPs detected in "Z506-7" were INDEL sites. In addition, Rubio et al. found 124 SNPs at the *PPVres* locus that were specific for the resistant genotype "Rojo Pasión" (196 kb region).

Mariette et al. [19] employed a genome-wide association (GWA) technique in apricot (*Prunus armeniaca*) germplasm with significant genetic diversity, next-generation sequence-based genotyping, and a high-quality peach genome reference sequence to detect SNPs. The GWA analysis verified previously known QTL intervals for PPV resistance, discovered further possible resistance loci, and narrowed each down to a small group of candidate genes for future investigation. Mariette et al. showed that an association genetics technique can be used to find candidate genes for QTL in apricots. They also suggested that this method could be used to find candidate genes for other marked trait intervals in the germplasm.

Passaro et al. [20] utilized HRM to define resistant and vulnerable *PPVres* alleles. A total of 51 apricot cultivars were evaluated for PPV resistance. A coupling dye to detect the change in fluorescence intensity caused by DNA denaturation was used. Prior studies have demonstrated that SSR markers are inadequately straightforward and

dependable. Passaro therefore created two new resistance markers (PGS1.21 SNP and PGS1.24 SNP). As a replacement for the ZP002 DEL (ZP002 SNP) marker, Passaro created a deletion-associated marker based on the presence of the A/T polymorphism in the first intron of the ppb22225m gene. These markers were obtained from the compilation of the BAC libraries of the "Lito" variety. These three HRM markers were verified in an F1 population of 'Lito' (Resistant) x 'BO81604311' (Susceptible), together with 73 samples previously genotyped with SSR markers for PPV resistance. Passaro et al. found that just six of the 42 individuals assessed contained the resistance allele. Consequently, they underlined the significance of advancing endurance research by improving HRM tests for specific *PPVres* gene markers. Passaro et al. produced a quick and cheap way to find out if an apricot is resistant to PPV.

Gürcan et al. [4] used a total of 227 apricot accessions and 48 F1 progeny. Gürcan et al., used 10 markers, including PGS1.21, PGS1.23, and PGS1.24 [18], ZP002 marker [17], [20], and two CAPS markers (SNP 8156.254 and SNP 8.157.485) [17]. In addition, the LightCycler® Nano Instrument-Real-time PCR system was used to perform HRM analyses (Roche, Switzerland). Gürcan reported that while eight of ten markers used in the study produced bands, two CAPS markers did not. Therefore, HRM analysis revealed resistance to PPV-T in four genotypes (Algebra, Lifos, Karum, and Zard). The results show that "Poodle" and "Fracasso" do not have any resistance alleles. Also, Gürcan concluded that the ZP002 marker was more accurate and made it easier to choose the resistance allele at the *PPVres* locus.

CHAPTER 2

MATERIALS AND METHODS

2.1. Study design

This study aimed to obtain PPV-resistant hybrid apricots (F1) by hybridizing "Hacıhaliloğlu" and the PPV-resistant "Zard" variety, thus, to reveal resistance patter of Zard accession. For this purpose, after crossing both accessions, F1 population was screened with ZP002 primer to determine F1 carrying Zard type resistance. Later, the PPVres loci covering 300 kb region of tree F1 accessions together with Zard were further investigated by high throughput DNA sequencing and subsequent bioinformatic analysis. The experiment design was diagrammed at Figure 2.1.

This study was an experimental study.

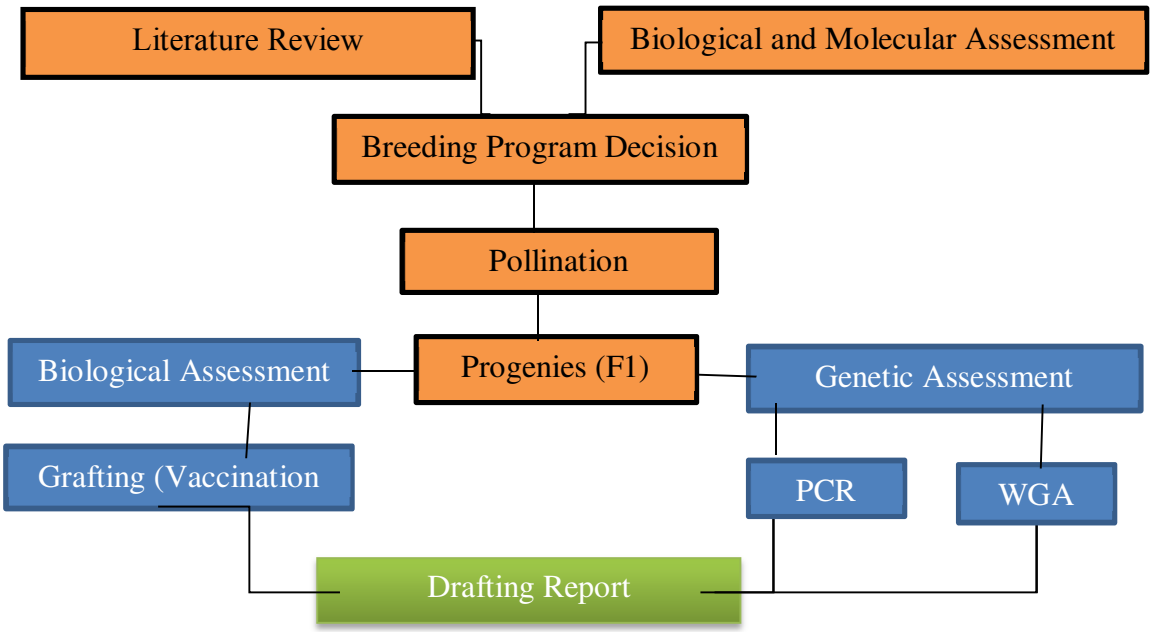

Figure 2.1 The Study Experimental Design.

2.2. Study Setting

The study was conducted at Erciyes University, in Kayseri 2018-2022.

2.3. Plant

2.3.1. Hacıhaliloğlu Variety

Hacıhaliloğlu variety was used in this study as paternal. The most widely planted apricot varieties in Türkiye, including Hacıhaliloğlu are susceptible to PPV[1], [2], [4] . Hacıhaliloğlu accounts for the vast majority of Türkiye's dry apricot crop production. Hacıhaliloğlu produces medium-sized fruits that are oval, yellow in color. The matter ratio ranges between 24 and 30% in fruits [49].

2.3.2. Zard Variety

Zard variety was used in this study as maternal. It is a Western Asian apricot variety that is resistant to PPV [4], [16]. Due to the lateness of the flowering time of this variety, it is more tolerant of frost than other varieties. Zard fruits are high in nutritional value because they have between 22 and 24 percent dry matter. The fruits of the Zard variety are soft when ripe and have a greenish-yellow color [116].

2.4. Hybridization program

Hybridization programs were conducted between the susceptible variety "Hacıhaliloğlu" as the paternal and the resistant Western Asian variant "Zard" as the maternal in 2018.

2.5. Genotyping Assessment

2.5.1 PCR Based Genotyping

2.5.1.1.DNA Extraction

DNA was extracted from the leaves of 241 seedlings by the Cetyltrimethylammonium Bromide (CTAB) method [2]. As flown below:

2. 0.5 g of young leaf sample and powdered with liquid nitrogen were placed in 2 ml tubes.
3. 0.7 ml of CTAB buffer solution (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 2% PVP, mercaptoethanol, 0.1% Na2S2O5) was added to the tubes and mixed.

4. The samples, which were kept in a water bath for 60 minutes, were mixed by inverting every 15 minutes. After the water bath process, the samples were kept at room temperature for 5-10 minutes to reduce the temperature.
5. 0.7 ml of chloroform: isoamyl alcohol (24: 1) was added. It was kept at room conditions for 15 minutes by mixing every 3 minutes.
6. It was centrifuged at 13,000 rpm for 15 minutes to allow the leaf residues to settle to the bottom.
7. After centrifugation, the upper phase was transferred to epandorf tubes with the help of a pipette and isopropanol kept at -20 ^{o}C was added. At this stage, the tubes were slightly shaken to allow the DNA to precipitate.
8. g) After the samples were kept at -20 ^{o}C for 1 night, the tubes were centrifuged at 10,000 rpm for 10 minutes, DNA was precipitated and the isopropanol in the tube was emptied.
9. 0.5 ml 76% ethanol washing buffer solution containing 10 mM ammonium acetate was added to the tubes and kept at +4 ^{o}C for 1-2 hours.
10. The washed DNA was dried and dissolved in pure water. To check the isolated DNA quality, firstly DNA was run on a 1% agarose gel and its concentration was checked.

2.5.1.2.PCR Amplification

The extracted DNA was amplified using ZP002 as a single sequence length polymorphism (SSLP) marker. The primer sequences were (Forward 5'-AACATTTTCTGATTCAATGCCA-3' and Reverse 5'-TGTATCCAGCTTCAAAGTC-3'). PCR with a total volume of 18 was prepared by combining 10.5 of PCR water, 2 of 10X Taq Buffer, 2 of 25 mM MgCl, 2 of 2 mM dNTP, 0.5 U of Taq polymerase, 0.5 M of Forward primer, 0.5 M of Reverse primer, and Thermal Cycler. It is a function of the Cycler gadget. The PCR program was 3 minutes at 95 °C, 45 seconds at 95 °C, 34 cycles of amplification (45 seconds at 60 °C, 1 minute at 72 °C), and 5 minutes at 72 °C for final elongation.

2.5.1.3. Agarose Gel Electrophoresis

The PCR products were visualized using the gel-electrophoresis method and ultraviolet

light. DNA samples were loaded into wells of an agarose gel containing a concentration of 3%. The electrophoresis was carried out using a 1 x TBE buffer solution (89 mM Tris-Cl, 89 mM boric acid, 20 mM EDTA). The electrophoresis device was set at 90 volts. After 180 minutes, the running time was over, and an image on a gel was taken and looked at under ultraviolet light.

2.5.2. Bioinformatic Analysis of PPVres loci sequence

The Zard and Hacıhaliloğlu varieties' genomes were available in the lab, and the three samples that showed a homozygous band in PCR based genotyping were sequenced using Illumina technology. The data on Peach chromosome one was retrieved from the National Center for Biotechnology Information (NCBI). The website is https://www.ncbi.nlm.nih.gov/by accession number (CM031363.1). A total of 300 k from the first linkage group of Chr. one was selected. The sequences of SSR markers (PGS1.21 and PGS1.24), SSLP markers (ZP002[2]) and SNP makers (ZP002-SNP, PGS1.21-SNP, SNP8156254, and SNP8157485) were all included in this region [17], [18], [20]. Besides, Then, the region was used as a reference and mapped with the whole genome of the Hacıhaliloğlu variety by using the Genesius Prime program. A high-quality consensus was produced. The Hacıhaliloğlu variety consensus was used as a point of reference to be mapped with Zard and (ZxHH-70, ZxHH-98, and ZxHH-146) samples to detect the *PPVres* loci that have been associated with resistant varieties in many studies.

2.6. Biological Assessment

In 2021, after transferring the progenies seedlings to the apricot garden at Erciyes University, a T-shaped budding was performed on 241 samples in double to ensure grafting success. The cuttings from nearby trees were used because their leaves appear to carry PPV. The infected buds were directly grafted onto the upper portion of the tested progenies seedlings. A space of 15 cm was left between each grafted bud on the seedling. The grafted seedlings were spaced 2m apart in rows and 3m apart in the field. In the second half of May and June 2022, visual controls of leaf symptoms (vascular clearing, yellow rings, spots and stains, leaf distortion and deformation) were observed weekly for shoots grown from grafted buds on tested apricot seedlings. As described by [4], [16], symptoms are scored as 0 (no symptoms), 1 (very mild symptoms), 2 (moderate symptoms on several leaves), and 3 (severe symptoms on the entire plant).

CHAPTER 3

RESULT

3.1. Hybridization of "Hacıhaliloğlu" and "Zard"

In 2018, traditional hybridization was carried out between the susceptible variety "Hacıhaliloğlu" as the paternal and the resistant Western Asian variant "Zard" as the maternal. In 2019, through the hybridization process, 241 seeds were harvested. The dormancy was broken, and 241 hybrid seedlings (F1) were obtained.

3.2. Molecular Assessment

3.2.1. PCR Based Genotyping

The ZP0022 marker was employed. The locus of this marker may mostly be found in resistance varieties. The genotype shows two bands of 112 bp, which are heterozygous, and one band of 107 bp, which is homozygous. A total of 237 progenies were heterozygous, while only four progenies were homozygous. The experiment was done again to confirm that result, and the same results were found (Figure 3.1). The results showed double bands are heterozygous and single bands are homozygous, as seen in sample number 11.

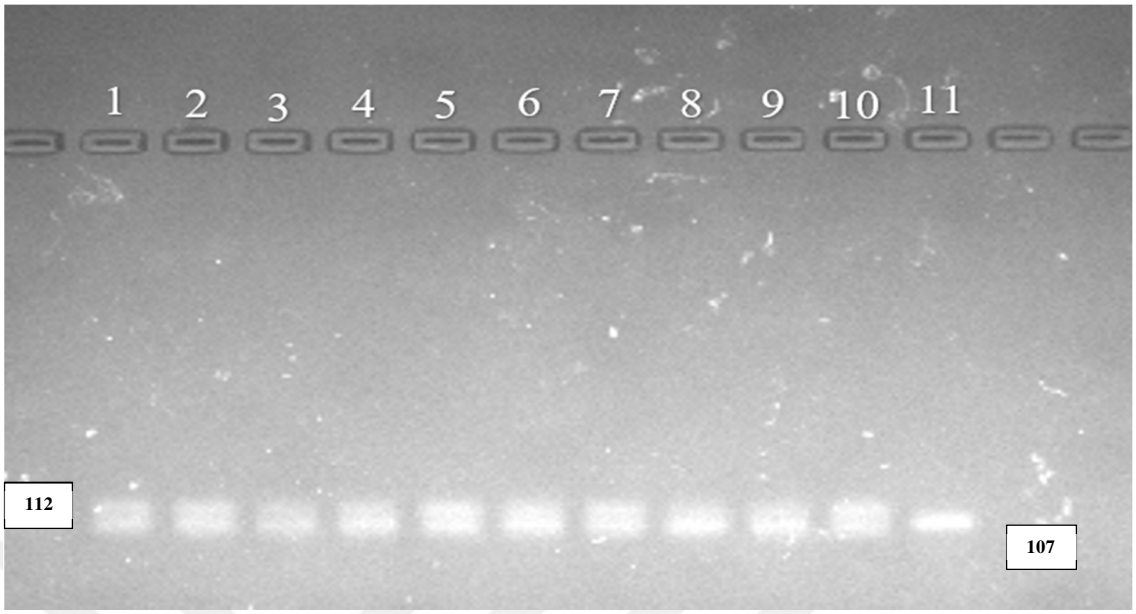

Figure 3.1. PCR-based genotypes employed the ZP0022 marker. In the figure, only 10 samples including homozygote loci were depicted.

3.2.2. Sequencing Based Genotyping

3.2.2.1 Detection of Variants

By using sequence mapping for three progenies that showed one band in PCR detection, the progenies (ZxHH-70, ZxHH-98, and ZxHH-146) and their maternal Zard are mapped with the high reference consensus of the "Hacıhaliloğlu variety. According to the "Hacıhaliloğlu reference, which is used as paternal in this study, the association mapping showed a total of 29904 variants in Zard, 21495 variants in ZxHH-146, 20685 variants in ZxHH-98 and 21173 variants in ZxHH-70 respectively (Table 3.2 and Figure 3.2). Besides that, to detect the variants between the Zard and the three previous progenies, the consensus of the Zard was employed as a reference in Geneious prime program. A total of 20948 variant in ZxHH-70, 20856 in ZxHH-98, and 20595 variants in ZxHH-146 (Table 3.2. and figure 3.3). The type of polymorphism variants in (Table 3.2.) was compared in (Figure 3.4.).

Table 3.1. The variant on 300 k. pb of Zard and three progenies according to the "Hacıhaliloğlu.

Polymorphism Type	Zard	ZxHH -70	ZxHH-98	ZxHH-146
SNP	16307	12864	12534	13769
SNP (transversion)	5911	3331	3235	3220
SNP (transition)	5112	2975	2902	2851
Substitution	1518	1152	1229	902
Deletion	515	351	313	342
Insertion	318	293	297	233
Deletion (tandem repeat)	125	109	88	100
Insertion (tandem repeat)	98	98	87	78
Grand Total	**29.904**	**21.173**	**20.685**	**21.495**

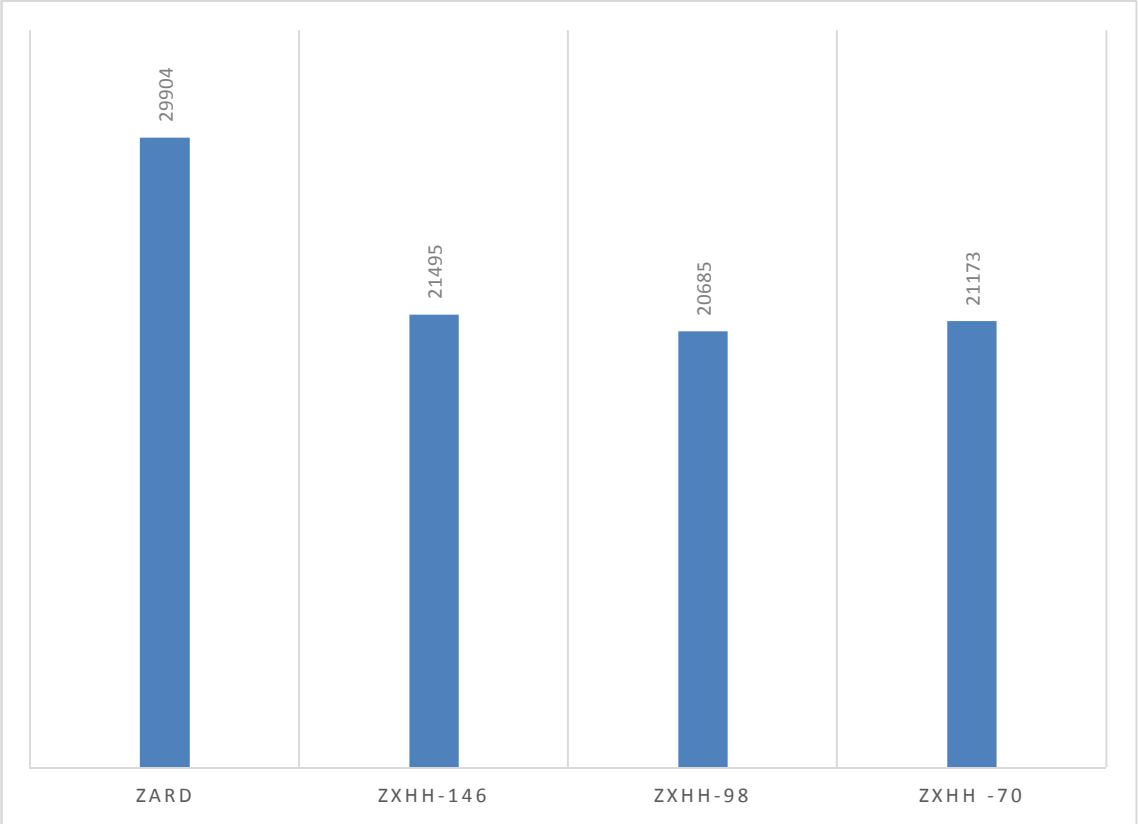

Figure 3.2. Total of Polymorphism variants in Zard and three progenies according to the "Hacıhaliloğlu reference.

Table 3.2. The variant on 300 k. pb of (ZxHH-70, ZxHH-98, and ZxHH-146) progenies according to the "Zard.

Variant	ZXHH-70	ZXHH-98	ZXHH-146
SNP	15422	14754	15184
SNP (transition)	2392	2497	2329
SNP (transversion)	1876	2108	1776
Substitution	778	966	803
Deletion	242	243	265
Insertion	163	208	149
Deletion (tandem repeat)	43	45	56
Insertion (tandem repeat)	32	35	33
Grand Total	**20.948**	**20.856**	**20.595**

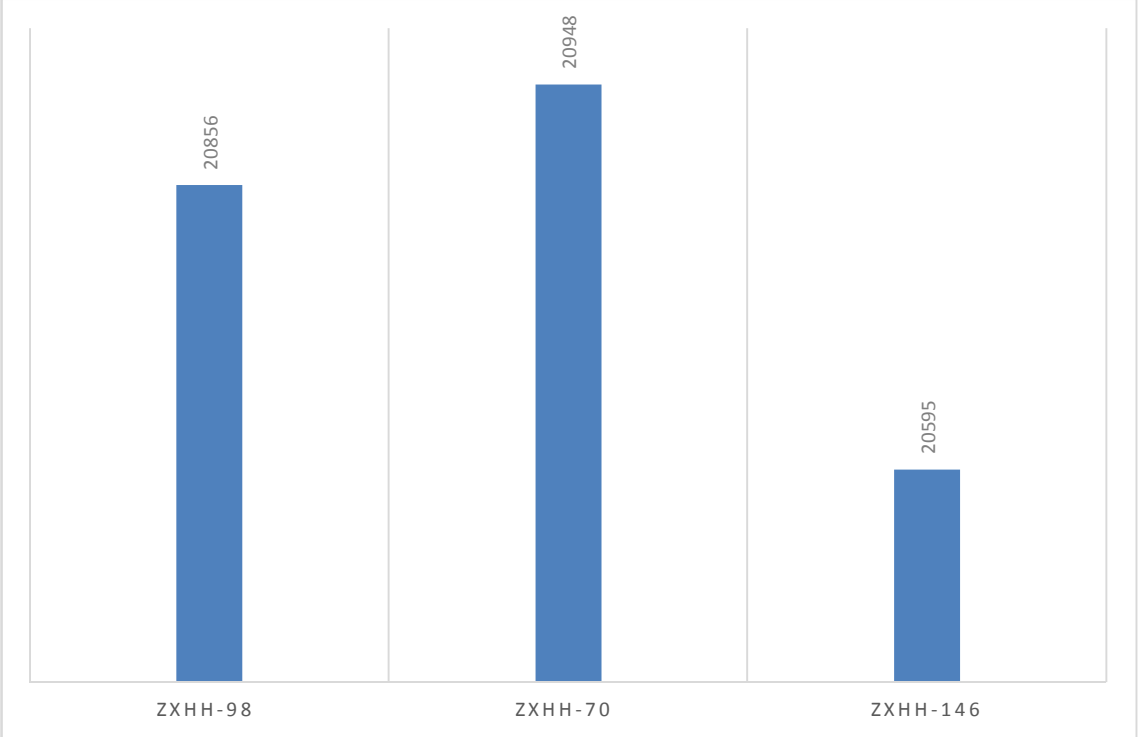

Figure 3.3. Total of variants in (ZxHH-70, ZxHH-98, and ZxHH-146) progenies according to the "Zard consensus.

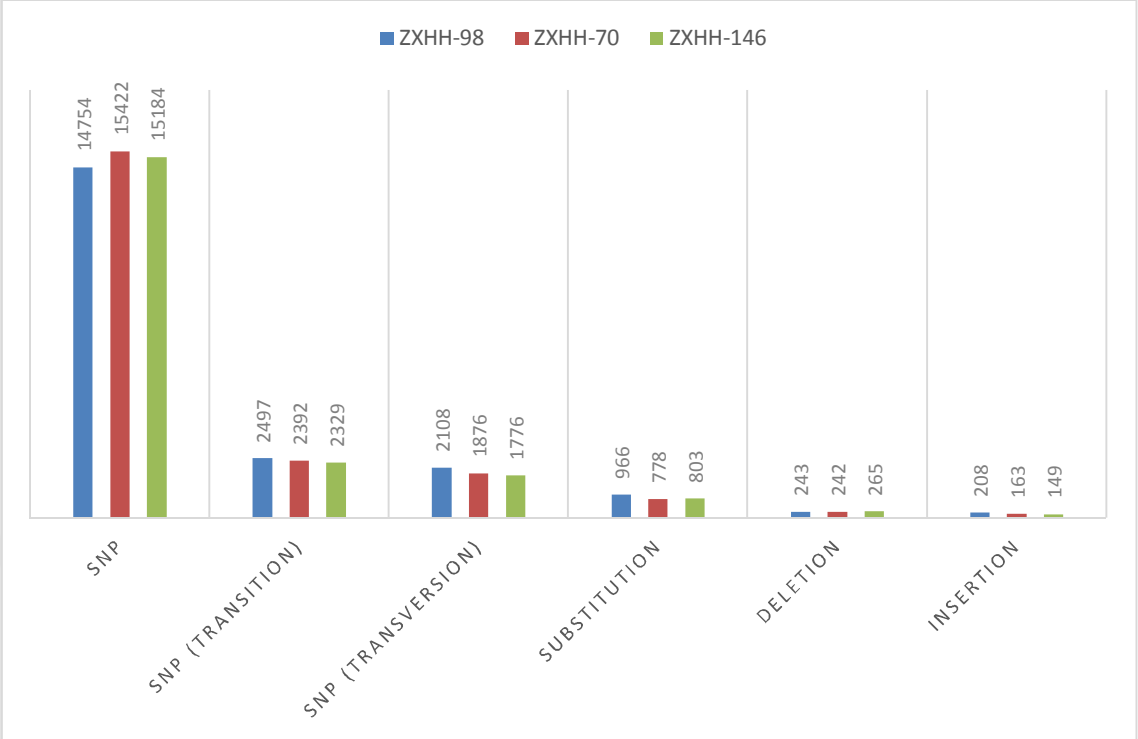

Figure 3.4. comparison of variant types in (ZxHH-70, ZxHH-98, and ZxHH-146) progenies according to the "Zard consensus.

3.2.2.2. Detection of Specific Marker Regions Associated with PPVres.

A Wide Genome Association (WGA) was obtained by using the BioEdit program version 7.2 and Geneious Prime. A total of 300 k pb consensuses and contigs of the paternal "Hacıhaliloğlu, the maternal "Zard", and the three progenies were aligned. The region of markers was determined according to the marker size between the forward and reverse primer. Some reverse's markers were not found in the consensus sequences but determined according to size based on reference studies (Table 3.2.). The mechanism of ZP002 marker sequence was figured by "Hacıhaliloğlu, variety consensus due to five alleles abstention (Figure 3.5, 6,7 and 8).

Table 3.2 Marker Sequences Associated with *PPVres* Genes in Apricot

Marker name	Marker type	size	Forward primer	Reverse primer	Reference
ZP002[2]	SSLP	107-112	TGGCATTGAATCAGAAAATGTT	TGTATCCTCCAGCTTCAAAGTC	[17]
PGS1.21-SNP	SNP	A/G	ACCCGGTGAAAGAAAAGTGA	CTTGACATGTAGAAGCGATCCA	[20]
ZP002_SNP	SNP	A/T	GCCAGTTTCCTGTAGCAAAACCAC	TAATGCAGCTTGGAAATAAGAAT	[20]

1. Figure 3.5. Hacıhaliloğlu variety consensus-locus of ZP002[2]- SSLP marker.

TGTATCCTCCAGCTTCAAAGTCCTCCGATTCATATCTCTCCAGTGAATGTTTGGACATCAATG
AAAACGACTGTATTTTGAACGTGTAATGAGTTGGCATTGAATCAGAAAATGTT

2. Figure 3.6. Hacıhaliloğlu variesty consensus-locus of PGS-1.21- SNP marker

ACCCGGTGAAAGAAAAGTGAAATATCTAGCAAAACCACCCAACTAAAATATCACAGTAAGA
AGGCAATCTGACCAATAAGCATGCGAATTCCAATTCTTGACATGTAGAAGCGATCCA

3. Figure 3.7. Hacıhaliloğlu variesty consensus-locus of ZP002-SNP marker

GCCAGTTTCCTGTAGCAAAACCTCAATTGAGAGTACTCTAATGCAGCTTGGAAATAAGAAT

3.4.2.3. High-throughput Estimation of Allele Frequencies in PPVres Markers

After determining the marker locus on the Geneious program, the resistance alleles associated with these markers were detected and estimated. The genotype was determined according to the coverage and the frequency of associated alleles.

1. ZP002 SSLP marker

The resistance allele of the ZP002 marker was associated with the five deletions of nucleotides in the resistance varieties (Figure 3.9). The genotype of this marker locus was figured out in (Table 3.3).

Table 3.3 Allele Frequencies Associated with ZP002 Marker (*PPVres*).

Population	Variant	Coverage	Frequency	Genotype
Hacıhaliloğlu	TTTGG/ TTTGG	Ref	Ref	Ref
Zard	(-/-)	222	100%	Homozygote
ZxHH-146	(-/-)	114	100%	Homozygote
ZxHH-98	(-/-)	29	100%	Homozygote
ZxHH -70	(-/-)	75	100%	Homozygote

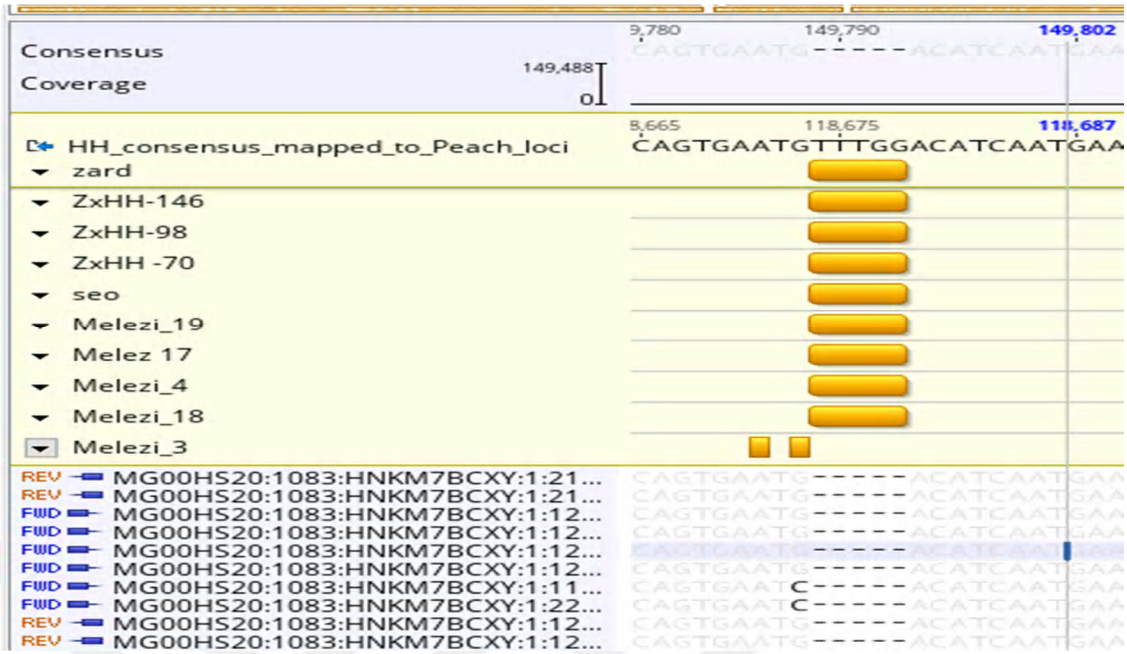

Figure 3.9. allele locus in ZP002 Marker Associated with *PPVres*.

2. PGS-1.21-SNP marker.

The resistance allele of the PGS-1.21 SNP marker was associated with the frequency of the adenine (A) allele in the susceptable variety and the guanine (G) in the same locus as the resistance variety. The result in this locus showed that (G) was absent in the parents' "Hacıhaliloğlu, and Zard varieties and also absent in ZxHH-146) progeny, but this SNP mutation was found in ZxHH-70 and ZxHH-98 (Figure 3.10). The genotype of this marker locus was figured out in (Table 3.4).

Table 3.4 Allele Frequencies in PGS-1.21-SNP (A/G) Marker Associated with (*PPVres*).

Population	Variant	Coverage	Frequency	Genotype
Hacıhaliloğlu	G	Ref	Ref	Ref
Zard	G	240	100%	Homozygote
ZxHH-146	G	105	100%	Homozygote
ZxHH-98	A/G	19/10	65.5%/34.5%	Heterozygote
ZxHH -70	A/G	37/38	49.3%/50.7%	Heterozygote

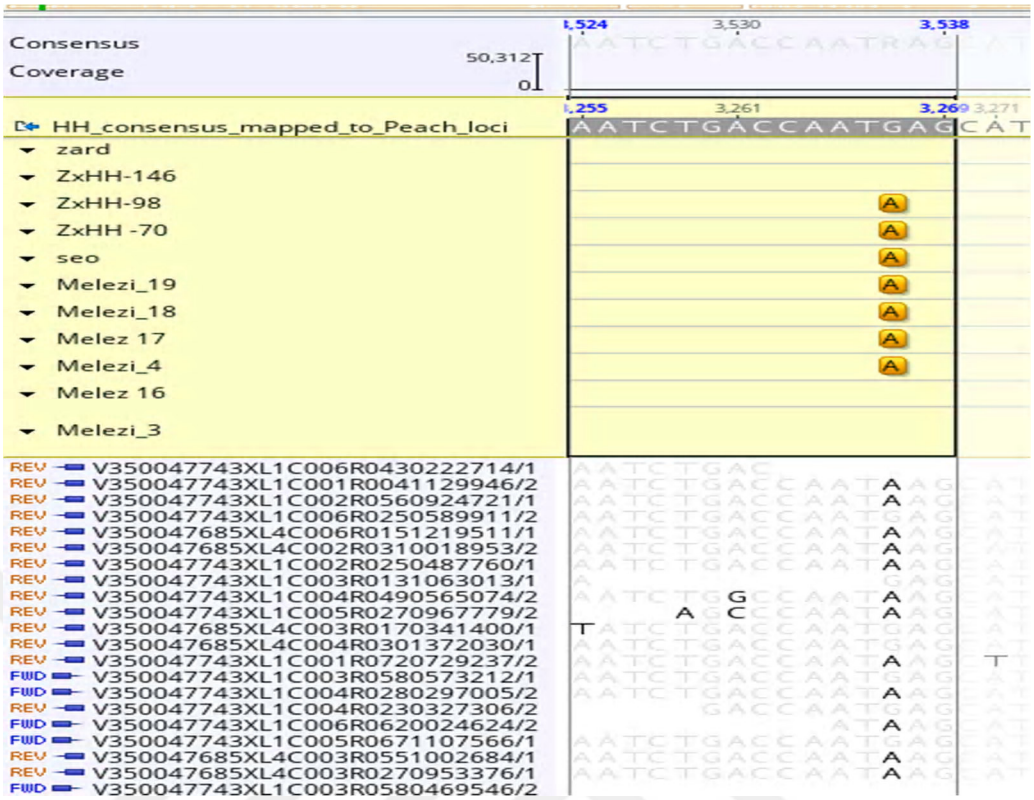

Figure 3.10 allele locus in PGS-1.21-SNP (A/G) Associated with *PPVres*.

3. ZP002-SNP marker.

The resistance allele of the ZP002-SNP marker was associated with the frequency of the thaimine (T) allele in the susceptable variety and the adanine (A) in the same locus as the resistance variety. The result in this locus showed the (T) in the "Hacıhaliloğlu variety and the (A) in "Zard," "ZxHH-146, "ZxHH-98, and ZxHH-70 respectively (Figure 3.11). The genotype of this marker locus was figured out in (Table 3.5).

Table 3.5. Allele Frequencies in ZP002-SNP (A/T) Marker Associated with (*PPVres*).

Population	Variant	Coverage	Frequency	Genotype
Hacıhaliloğlu	T	Ref	Ref	Ref
Zard	A	204	100%	Homozygote
ZxHH-146	A	120	100%	Homozygote
ZxHH-98	A	22	100%	Homozygote
ZxHH -70	A	66	100%	Homozygote

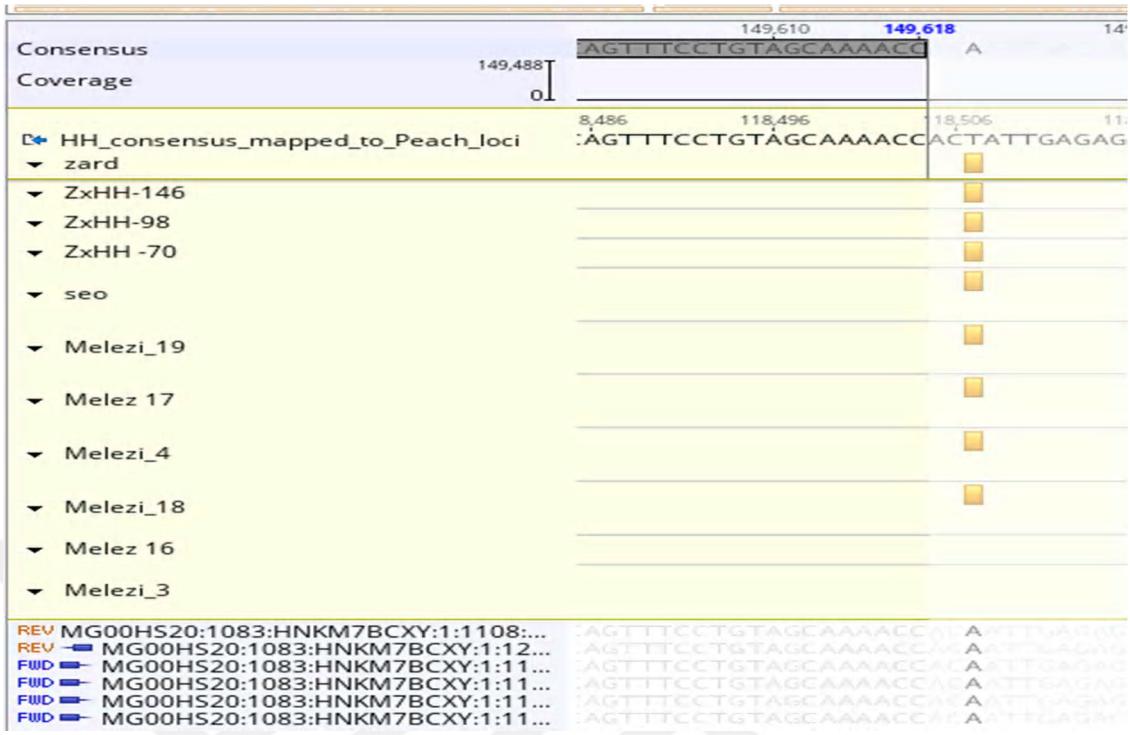

Figure 3.11 allele locus in ZP002-SNP (A/T) Associated with *PPVres*.

The results of the four markers used in this study to determmine the genotype based sequince in "Hacıhaliloğlu, Zard varieties and ZxHH-146, ZxHH-98, and ZxHH-70 were summarized in (Table 3.7).

Table 3.7. Genotype detection of *PPVres*-specific loci Markers by WGA.

Accession	ZP002(pb)	PGS1.21-SNP	ZP002-SNP
Hacıhaliloğlu	112	G	T
Zard	107	G	A
ZxHH-146	107	G	A
ZxHH-98	107	A/G	A
ZxHH-70	107	A/G	A

3.4. Biological Assessment

For the biological assessment, the grafting was implemented in 2021. In the second half of May and June 2022, visual controls of leaf symptoms (vascular clearing, yellow rings, spots and stains, leaf distortion and deformation) were observed weekly for shoots grown from grafted buds on tested apricot seedlings (Figure 3.12.). From 2022 to 2026, on a continuous basis.

Figure 3.12. Biological assessment for hybrid apricot Zard X Hacıhaliloğlu. in May 2022.

CHAPTER 4

DISCUSSION AND CONCLUSION

In brief, in this study, initially PPV-resistant hybrid apricots (F1) by hybridizing "Hacıhaliloğlu" and the PPV-resistant "Zard" variety were obtained. F1 population was screened with ZP002 primer and all the F1 accessions were found heterozygote for resistance according to ZP002 loci showing that Zard variety is homozygote for the PPVres loci. Interestingly four F1 seedlings pose ZP002 loci as homozygote. DNA sequence of three of these ZP002 homozygote F1 and Zard were obtained by high throughput sequencing and 300 kb PPVres region were aligned. Homozygote resistance manner for PPVres region of these 3 F1 seedlings were confirmed by high throughput sequence analysis.

The most apricot cultivar in Turkey is ' Hacıhaliloğlu,' which accounts for the majority of the country's dry apricot crop production [116]. On the other hand, the Western Asian apricot variety ‘Zard’ has a later flowering time and is more frost tolerant than other varieties. Zard fruits contain between 22 and 24 percent dry matter, indicating that they have a high nutritional value. The fruits of this variety are small and delicate, turning a greenish-yellow color when ripe [1], [6]. As part of our efforts in apricot breeding program, to enhance the number of resistant gene donors in dry-resistant apricot varieties, we employed the "Zard" variety for the first time. In this study, we determined the *PPVres* loci in hybrid F1 of "Zard x Hacıhaliloğlu" using SSLP markers (ZP002). During the genotyping evaluation, the PCR-based genotype showed that 237 progenies were found to be heterozygous, while only four progenies were found to be homozygous (Figure3.1). In addition, by employing sequence mapping in 300 k of the genome of a susceptible source—the "Hacıhaliloğlu" variety—and a resistant source—the "Zard" variety—for three progenies that exhibited one band in PCR detection, all three progenies (ZxHH-70, ZxHH-98, and ZxHH-146) as well as their maternal "Zard" have been mapped using the high reference consensus for the "Hacıhaliloğlu" variety.

Table 3.1 and Figure 3.2 show that the association mapping found a total of 29904 variants in the "Zard" allele, 21495 variants in the "ZxHH-146" allele, 20685 variants in the "ZxHH-98" allele, and 21173 variants in the "ZxHH-70" allele. In addition to this, the consensus of the Zard was used as a reference in the Geneious Prime Program in order to identify the differences that existed between the "Zard" and the three progenies. There are a total of 20948 variations in the ZxHH-70 offspring, 20856 in the ZxHH-98 offspring, and 20595 in the ZxHH-146 offspring (Table 3.2 and figure 3.3 and 4). The results of the detecting variations indicated that there were significant differences between the "Zard" variety and the "Hacıhaliloğlu" variety (Figure 3.2). Furthermore, there was a high level of genetic diversity between the "Zard" variety and its offspring (Table 3.2 and Figure 3.4). Thus, it may be necessary to look at them with a large number of genomes to find the susceptible alleles that are shared between them from the "Hacıhalıloğlu" variety. Thus, in this study, we focused on markers that were previously confirmed to be associated with *PPVres* (Table 3.3 and Figure 3. 5, 6, and7). The high-throughput for ZP002 (TTTGG), PGS2.21-SNP (A/G), and ZP002-SNP (A/T) markers' regions was determined by using markers sequences in our associated region genomes.

The genotype of each accession was determined according to the frequency and coverage of the alleles in the marker. The resistance allele of the ZP002 marker was associated with the five deletions of nucleotides in the resistance varieties (Figure 3.9). The results showed that Zard and the three offspring were homozygote by frequency ranged between 89 to 100%. In comparable results, Decroocq et al. (2014) found that, in the mapping population that includes "Vestar" as a susceptible parent, a high number of progenies showed a homozygous banding pattern due to the presence of a ZP002 deleted allele in the susceptible parent [17]. There was a correlation between the susceptibility allele of the PGS-1.21 SNP marker and the frequency of the adenine (A) and the resistance allele of the guanine (G) at the same locus. The "Hacıhaliloğlu" and "Zard" parents did not have the SNP mutation at this locus, nor did the "ZxHH-146" offspring, but the "ZxHH-70" and "ZxHH-98" offspring did (Figure 3.10). The results in marker allele may be not related to the *PPVres* due to absent of (G) in our parents (Table 3.4). The allele may be related to unknown pathogen. A similar result was also obtained for PGS1.21_SNP, since homozygotes for A and G alleles show a Tm difference of about 0.4 °C. The melting curve profile, on the other hand, makes it easier to spot the wider peak in the heterozygote genotype A/G [20]. However, the frequency of the

thiamine (T) in the susceptible variety and the adenine (A) in the same locus in the resistant variety was associated with the resistance allele of the ZP002-SNP marker. The result at this locus showed the (T) in the "Hacıhaliloğlu" variety and the (A) in the "Zard" variety. This means that the "Hacıhaliloğlu" variety is susceptible and the "Zard" variety is resistant. The (T) allele was found in the "Hacıhaliloğlu" variety, while the (A) allele was found in the "Zard," "ZxHH-146," "ZxHH-98," and "ZxHH-70" varieties (Figure 3.11). The results in this marker as like ZP002 marker and showed homozygote in the Zard and its offspring (Table 3.5). In contrast, Passaro et.al (2017), reported that the ZP002_SNP marker does not provide the expected results since the resistant genotype (homozygote for the A allele) shows a higher Tm compared to the susceptible one (homozygote for the T allele) allele[20]. Our result demonstrates that the genitor Zard is homozygous on the main candidate PPV resistant genes. In similar, resistance to PPV was also investigated in 365 F1 hybrids by crossing the resistant cultivar "Stella" with the susceptible cultivar "Bebecou". The resistance/susceptibility segregation ratio was 1:1, showing that "Stella" is homozygous for the resistance trait [112]. Thus, our results showed that 100% of apricot progenies F1 have a resistant allele (Fig 3.1). It may be due to used resistant source ‘Zard’ as maternal in this breeding program, and this confirm the Gurcan et al. results that showed ‘Zard’ has resistance alleles [2], [4]. However, As seen in PCR based genotyping results, a total of 237 progenies successfully carried insertion of 5nt from the variety "Hacıhaliloğlu." Instead of that, four progenies have not carried the 5nt insertions, but they are carried the same alleles from their maternal ‘Zard’ (Fig 3.1 and 4). It may be due to self-pollination, if just one gene is responsible for PPV resistance such it confirmed by Zuriaga et al. [101], and Dunez et al. [63]. In contrast, many researchers observed that the resistance in apricot controlled by combining two genes, and even some said by combining three genes [91]–[94]. Due to that, whole genome sequence analysis (WGS) is required to these four homozygous progenies and comparing with their genitors. At first biological assessment, all progenies have no Sharka symptoms (Figure 3.12). In similar, Zhivondov and Milusheva [114] used natural infection and ELISA in PPV-D and M to assess four hybrids’ apricots (21.3, 21.47, 21.55, and 2167) produced by crossing between ‘Pasific’ with ‘Stanley’ and ‘Serdika’ prospectively. Zhivondov and Milusheva have been reported no symptoms and negative in ELISA results in the first years. The flowing years, instead of ’21.47’ the others three progenies have demonstrated

symptoms and positive results in ELISA. Due to that, the symptoms may not appear in our progenies in the first year of biological assessment. In principle, our findings supported prior report that "Zard" is a promising accession for PPV resistance breeding programs [4], [117].

The limitation of this study includes that the evaluation of resistance in hybrid F1 was carried out by genetic and biological methods, therefore, additional biological assessment the field planning and WGS are required to confirm the resistance in the F1 population.

In conclusion, the hybridization between the Turkish 'Hacıhaliloğlu' variety and the Western Asian 'Zard' variety was successful. A total of 237 seedling progeny were determined to be heterozygous resistant, while four were found to be homozygous resistant. Developing new, high-quality apricot cultivars that are resistant to PPV is essential, so the breeding and development effort must be maintained. Using the "Zard" variety as a resistant gene donor was the first step in increasing the number of donors in apricot-resistant cultivars. Hybridization is a promising method for introducing the PPV resistance gene into the Turkish apricot population. A breeding effort in the future will expand the sources of resistance to Sharka disease by integrating more and diverse resistance genes into vulnerable varieties. In addition, the results show that the accession Zard is homozygous for the major potential PPV resistance genes.

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RESUME

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2. Mohamed Ali Edris, A., & GÜRCAN, K. (2022). *PLUM POX VIRUS RESISTANCE OF HYBRID F1 APRICOTS PRODUCED BY CROSSİNG "ZARD" AND "HACIHALILOĞLU" VARIETIES*.