Prevalence of Some Stone Fruit Viruses in the Almond Orchard of Kahramanmaras Sutcu Imam University

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ABSTRACT: The severity and prevalence of the stone fruit viruses were investigated in the almond orchards at Kahramanmaras Sutcu Imam University located in the Eastern Mediterranean region. A total of 222 samples were individually collected and tested by DAS-ELISA for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Plum pox virus* (PPV), *Apple chlorotic leafspot virus* (ACLSV) and *Apple mosaic virus* (ApMV). A total of 23,4% of ELISA-tested samples was infected at least by single virus. PNRSV (20,2 %) was the most prevalent followed by ACLSV (3,2 %). Surprisingly, the other viruses were present neither individually nor in mixed infections. The almond cultivars with infections were both foreign and local selected ones. These results demonstrated that we are facing to the problem of not having healthy nursery stocks for fruit trees.
Key words: Almond, ACLSV, ApMV, PNRSV, PDV, PPV, ELISA

Kahramanmaraş Sütçü İmam Üniversitesi'ne Ait Badem Bahçesinde Bazı Sert Çekirdekli Virüslerinin Yaygınlıkları

ÖZET: Batı Akdeniz Bölgesi'nde bulunan Kahramanmaraş Sütçü İmam Üniversitesi'nde badem bahçesinde sert çekirdekli meyve türleri virüslerinin yaygınlığı ve şiddeti araştırılmıştır. Toplanan 222 örnek, DAS-ELISA ile *Erik nekrotik halkalı leke virüsü* (PNRSV), *Erik cücelik virüsü* (PDV), *Erik beneklenme virüsü* (PPV), *Elma klorotik yaprak leke virüsü* (ACLSV) ve *Elma mozaik virüsü* (ApMV) için testlenmiştir. Testlenen örneklerin % 23,4'ü en az bir virüsle enfekteli bulunmuştur. En yaygın PNRSV (% 20,2), onu ACLSV (% 3,2) takip etmiştir. Şaşırtıcı şekilde diğer virüslerin tek veya karışık enfeksiyonlarına rastlanmamıştır. Enfekteli badem çeşitleri yabancı ve yerli çeşitler olarak tespit edilmiştir. Alınan sonuçlar, meyve ağaçları için sağlıklı fidanlık materyallerine sahip olmadığımız gerçeğini ortaya koymuştur.
Anahtar kelimeler: Badem, ACLSV, ApMV, PNRSV, PDV, PPV, ELISA

INTRODUCTION

Almond which is a typical species of the countries in Mediterranean Basin has been widely grown in almost all areas of Turkey, except high elevations of Eastern Anatolia (Dokuzoğuz and Gülcan, 1979; Gülcan et al., 1989). Moreover, wild almond types can be very often seen in nature. The main problem of almond cultivations early spring frost and precocity apart from biotic factors especially viral agents. Although various number of studies were carried out about virus and virus-like diseases in stone fruits in Marmara (Sahtiyanci, 1969; Yürektürk, 1984), Central Anatolia (Kurçman, 1973; Elibüyük and Erdiller, 1991 and 1998), the Aegean (Azeri, 1994; Gümüş et al., 2007; Ulubaş Serçe et al., 2009), Eastern Anatolia (Sipahioğlu et al., 1999) and the Mediterranean Regions (Çaglayan-Yildizgördü and Çali, 1994; Baloğlu et al., 1995; Buzkan et al., 2005 and 2006; Koç and Baloğlu, 2006). Elibüyük (2005) reported the natural spread of PPV, particularly the isolate PPV-M by mealy plum aphids (*Hyalopterus pruni*). Pathogenicity test of PPV on some apricot (cvs. Sakit-2, type 11/89) and plum (cvs. Aynali, Papaz, Havran and type 1559) cultivars and types demonstrated no-resistance and/or tolerance to the virus infection (Çağlayan et al., 2004). However almond is the least studied cultivar among the other *Prunus* spp., and the latest study was conducted in very extensive surface area in Turkey. Apart from other stone fruit species, few almond trees in Tekirdağ were found to be infected with PPV (Akbaş et al., 2011).

Kahramanmaras, a city located in the Eastern Mediterranean Region has suitable climatic and geographical conditions for almond growing. However, almond trees are only seen as spread among the other stone fruit varieties. A group of researchers at Horticultural Department of Kahramanmaraş Sutcu Imam University (KSU) has established a fruit research and experimental plot at KSU and arranged two independent almond orchards in 1995 and 1999 to assess the adaptation of various almond varieties to the region under irrigated conditions. This plot was also aimed to use as a nursery to supply propagating materials to growers during plantations, the plant materials were never checked for the presence of infectious disease agents and their symptoms started to be seen within a couple of years. Therefore, it is very important to control the sanitary status of the trees before propagation.

MATERIALS and METHODS

Field survey

Field inspection and collection of samples were carried out in one of the research and experimental almond orchards at KSU in the Eastern Mediterranean.

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The plot was established with the grafted-nursery stocks supplied by Horticultural Departments of Çukurova (Adana) and Harran (Şanlıurfa) Universities and Ataturk Central Horticultural Research Institute, Ministry of Agriculture in 1999. Surveys were done from mid-May onwards. Plants with no-symptoms on the foliage were re-inspected at fruit ripening period. Leaf samples were collected from individual trees and stored at 4°C.

Serological tests

In the course of field survey, the total number of 222 samples was individually collected and tested by DAS-ELISA for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Plum pox virus* (PPV), *Apple chlorotic leafspot virus* (ACLSV) and *Apple mosaic virus* (ApMV) (Clark and Adams, 1977). Leaf tissue (1:10 w:v) was extracted in 0.1 M Phosphate Buffer Saline-Tween-Polyvinylpyrolidone (PBST-PVP), pH 7.4, containing 2% PVP, 0.05% Tween-20, 0.15 M NaCl, 4 mM KCl and 0.02% NaN_3. Antibodies for the viruses were commercially provided by the Loewe Biochemica GmbH, (Germany) and the reaction was detected colorimetrically at 405 nm using an ELISA reader (Titertek Multiskan SIRIO apparatus). Two wells were used per sample. The test was considered positive when the mean absorbance value was greater than twice that of the healthy controls (Singh and Singh, 1995).

Mechanical inoculation

A 10% aliquot of randomly selected samples was used as inoculum source to check for the presence of mechanically transmissible (PNRSV, PDV, PPV, ACLSV, ApMV) viruses. Young leaves were ground in 0.1 M phosphate buffer, pH 7.2, containing 2.5% nicotine and inoculated to *Nicotiana benthamiana* Domin and *N. occidentalis* Wheleer. Inoculated hosts were grown in a greenhouse at 22-24°C and inspected for symptom expression. Symptomatic plants were tested by ELISA. Negative controls were prepared by inoculating healthy plants with only inoculation buffer.

RESULTS

Symptoms observed in the orchards

The almond trees were homogenous in terms of tree age (around 5-6 years old) and planting time; however, some almond trees showed leaf symptoms 3-4 years after the planting. No symptoms were observed on trees at the beginning of the vegetation between May and June. The symptoms (chlorotic spot and ringspot) appeared in mid-july which turned into necrosis. The recently developed leaves showed yellowing (Figure 1) and severe vein clearing (Figure 2). Some trees had stunted growth (Figure 2). Visual inspections were repeated during fruit-set and ripening period and there was no symptom on fruits.

Serological test and mechanical inoculation

According to ELISA results, single infections of PNRSV and ACLSV were found in 52 samples (23.4%) of the total tested samples. PNRSV was the most prevalent, being detected in 20.2% followed by ACLSV infection around 3.2% of the samples. PDV, ApMV and PPV were not present in the almond orchard (Table 1). Virus infections were detected on some foreign cultivars (PNRSV on Laurenne, Ferragnes, Masbovera, Picantily; ACLSV on Cristomorto, Laurenne, Yaltsinki, Nikitski) and local selected clones (PNRSV on 300/1; ACLSV on A15/1 and 48/2).

Although the level of virus infections was very low and PPV, the most important one in stone fruit viruses, was not present in the samples, although it was recently reported by Buzkan et al., (2006) in other stone fruit orchards nearby.

No virus was recovered by sap transmission from any of the samples tested.

DISCUSSION

The present study showed that the sanitary status of the research experimental almond orchard which has been established in 1999 in collaboration with the Agricultural Research Directory (TAGEM). The orchard consisted of 28 varieties and grafted nursery plantings (10 trees per variety) were obtained from different sources (Çukurova University-Adana, Harran University-Şanlıurfa and Ataturk Central Horticultural Research Institute-Yalova).

Although few viruses were detected in the trees, it is generally satisfactory considering that no sanitary selection and certification programs have been carried out. PNRSV was the most common virus also found in the peach and apricot plots nearby almond orchard (Buzkan et al., 2005 and 2006) and the results support the idea that the viruses should have been introduced into the plot due to the implantation of the infected materials. It is a great problem to use a material of which sanitary status is unknown in order to establish fruit orchards. Since the aim of this plot to introduce new varieties to the area and to evaluate their adaptation to the regional conditions by fruit quality and quantity, it would be hard to have reliable data from the trees carrying virus infections which can affect these aspects. Moreover, this may cause a further spread of the viruses if any material is used for propagation purpose.

Table 1. The detected viruses by DAS-ELISA at the almond research and experimental orchards at KSU.

No of samples			Infection				
Tested	Infected	%	PNRSV	PDV	ACLSV	ApMV	PPV
222	52	23,4	45	0	7	0	0

A **B**

Figure 1. Chlorotic spots and rings that turned necrotic areas **(A),** yellowing **(B).**

A **B**

Figure 2. Severe vein clearing symptoms (A) and stunting (B).

All efforts should be made to produce healthy nursery materials that will certainly avoid the introduction of new pathogens. It is advisable and reinforce the certification schemes that mandate using and trading with only certified propagating materials for fruit trees. Freedom from viruses and other pathogens in deciduous planting stocks is important because nearly all plants for plantings are produced by vegetative propagation. If present, disease agents will be readily perpetuated, albeit unwittingly, in the progeny. Moreover, once diseased plants are established in commercial orchards they are not amenable to any curative or therapeutic control measures. In most instances, the most effective disease control option is removal of infected plant or plants. Further, several disease agents are spread secondarily by natural vector species, i.e., aphids, mealy bugs, mites, leafhoppers, and nematodes, or even, in some cases, by pollen, and infected stocks can serve as sources of inoculum. First and foremost, the principal method proven most efficient in controlling virus and virus-like diseases in perennial crop plants involves the application of pathogen exclusion protocols in advance of wholesale plant propagations. These protocols are often performed in the framework of clean stock/certification programs.

Certification schemes worldwide share a common objective: to identify healthy sources for propagation through the application of time-tested indexing procedures as well as more recently developed molecular assays. Even so, the actual procedures and protocols can vary widely depending on the specific pathogens being targeted, the endemic disease agents in a production region, the availability of techniques and financial resources, and the expectations of industries served. The first step is the establishment of foundation or nuclear source plants: plants, testing free from all known harmful viruses and professionally identified for true-to-type phenotype.

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