Advances in Bioresearch
Adv. Biores., Vol 16 (1) January 2025: 237-240

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.1.237240

Advances in Bioresearch

ORIGINAL ARTICLE

Determination of *Apple mosaic ilarvirus* (ApMV) disease in pome fruitin Azerbaijan,using DAS-ELISA-test.

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ABSTRACT
Apple mosaic virus (ApMV), which is one of the widespread viral diseases of fruit plants, is one of the most dangerous diseases to the roses (RosaL. Spp.), blackberry (Rubus fruticosus L.), raspberry (Rubus idaeus L.), black raspberry (Rubus occidentalis L.), red current (Ribes rubrum L.), apple and crab apple trees (Malus pumila L., Malus silvestris L.),apricot, cherry, almond, plum and peach (Prunus spp.), mountain ash (Sorbus aucuparia L.), hazelnut (Corylus avellana L.),silver birch (Betula pendula L.), chestnuts (Aesculus hippocastanum L., AesculusxcarneaL., Aesculusflava L., Aesculusparviflora L.) and hop (Humulus lupulus L.) in Azerbaijan and in the world.ApMV shows typical symptoms on its hosts, such as yellowish-white coloration on leaves, mosaic ring spots and vein banding. This research work was carried out in 2021-2022 in order to determine Apple mosaic ilarvirus (ApMV) disease in the apple fruit growing areas of Guba and Ganja region of our republic.A total of 27 apple and pear trees suspected to be infected with ApMV were sampled and 7 apple (25.9%), 3 pear (11,1%) were found to be infected as a result of DAS-ELISA tests. At the same time, in contrast to Apple chlorotic leaf spot trichovirus, the maximum limit of the optical density of the infected samples was 2.69 and the minimum was 2.02. Four samples without external symptoms were infected with Apple mosaic ilarvirus. All symptomatic plants were infected with the virus. It should be noted that the plants with Apple mosaic ilarvirus symptoms described in the literature were not often seen in apple plantations.
Keywords: *Apple mosaic ilarvirus, pome fruit, apple, pear, DAS-ELISA test*

Received 24.10.2024 Revised 01.11.2024 Accepted 11.12.2024

How to cite this article:
E.A.Abdullayev, E.M.Khankishiyeva A. Determination of *Apple mosaic ilarvirus* (ApMV) disease in pome fruitin Azerbaijan,using DAS-ELISA-test. Adv. Biores. Vol 16 [1] January 2025. 237-240

INTRODUCTION

Fruit growing is an important component of the agrarian economy of Azerbaijan. In the current period, when the world population is growing rapidly and global climate change is intensifying, the state's need for an effectively functioning agricultural sector is felt more prominently. An efficient agricultural sector means that the economy can achieve higher production of agricultural products using less resources. In this sense, the low or high productivity in agriculture of different countries with the same natural and economic conditions in international practice shows how effective the agricultural policy of those countries is.There are many abiotic and biotic factors that cause product quality indicators as well as economic losses in the apple plants in Azerbaijan.The pathogen was first named Apple mosaic virus (ApMV) based on its symptoms observed on apple plants and was studied in different years by White (1928), Bradford and Joley (1933) and Christoff (1934) [2,5]. During the conducted studies, it was found that the Apple mosaic virus infects 65 plant species from 19 families [15]. According to researchers, the natural vector of this virus is unknown [13]. In general, the virus is transmitted mechanically by plant sap, use of virus-infected inoculum material, root grafting between plants and vegetative propagation materials. The pathogen produces typical symptoms such as yellowish-white discoloration of leaves, mosaic ring spots and vein streaking in its hosts.

Ertunç et al. [4] carried out molecular characterization and sequence analysis of apple mosaic virus isolates according to the envelope protein gene region. The researchers collected samples infected with the virus from apple and hazelnut orchards. The collected samples were tested by mechanical inoculation, DAS-ELISA method and RT-PCR methods. Thus, they discovered the presence of the pathogen. Finally, 7 apple and 9 hazelnut isolates from different regions of Turkey were sequenced. Ertunç et al. [4] compared leaf samples collected from apple orchards in Ukraine in 2008-2009 with samples collected from different apple and hazelnut orchards in Turkey. According to the information provided by the researchers, the main sequences of Ukrainian and Turkish isolates are similar to each other. During research, it was found that Turkish isolates are divided into 9 different genotypes, but Ukrainian isolates are similar to each other. Korkhmaz [12] collected a total of 481 infected apple samples from commercial apple orchards in the are an empirical social research method in order to determine the prevalence intensity of ApMV in Van and Malatya provinces of Eastern Anatolia Region. They tested Dot-blot hybridization and RT-PCR techniques. As a result, they determined the ApMV contamination rate to be 0.8%. During the conducted studies, they cloned the shell protein of ApMV-G and ApMV-M isolates and determined their nucleotide sequence. Grimova et al. [5] conducted a study to reduce the spread of *Apple mosaic virus* in large areas around the world and its negative effects on the production of many fruit crops. During the study, taxonomic classification of the virus, genome structure, symptoms, intensity of spread, diagnosis of the virus and methods of combating them were compiled. The main aim of the study was to detect ApMV in modern apple orchards in Konya and Niğde provinces and to collect isolates, as well as to study the molecular characteristics of some ApMV isolates obtained from Konya and Niğde provinces. For these purposes, leaf samples were taken from apple trees suspected to be infected with the virus, examined by serological (DAS-ELISA) and molecular (PCR) methods, and molecular characterization of the obtained isolates was carried out. In 2019, a research study was conducted to detect Apple mosaic virus (ApMV), in modern apple orchards in Konya and Nigde regions of Turkey, and the molecular characteristics of ApMV isolates were studied. The collected samples were first tested by serological (DAS-ELISA) and then by molecular (Polymerase Chain Reaction, PCR) methods to detect whether they were infected with the virus or not. During the investigation, samples were taken from 130 apple trees suspected to be infected with ApMV, and as a result of DAS-ELISA test, 28 samples from Konya-Kütören region, 1 from Niğde-Zengen region and 3 from NiğdeÇukurkuyu region were found to be infected with ApMV [1] In general, the obtained data on the infection of apple mosaic ilarvirus virus of umbel fruit plants do not characterize the general level of spread of viruses in the plantations of Azerbaijan, because the plant sample for the DAS ELISA-test method was initially made up of externally damaged plants with some addition of asymptomatic samples to the sample. The study is aimed at the initial detection of viral pathogens in the country in order to assess phytosanitary risks and develop schemes to protect nurseries from mass replication of virus-infected material.

MATERIAL AND METHODS

Plant material. Visual diagnosis of plants with disease symptoms (viruses) was carried out in the collection gardens of pome fruit plants (apple, pear). Depending on the presence of symptoms, the general condition of the plants, leaves, fruits, and shoots is evaluated depending on the disease and the examination period. Each tree was inspected in small plantations, nurseries and farms, up to 20% of plants selected from 1 ha to 3 ha in fruit plantations, and up to 10% of plants in commercial plantations larger than 3 ha were visually inspected. The optimal time for sampling and DAS ELISA testing of viruses of fruit plants is considered to be May - early June (flowering period, shoot growth and active development phase).Samples were stored at -20^{0}C until used for serological testing. The “Double Antibody Sandwich-ELISA” (DAS-ELISA) method was applied to all collected samples to detect the presence of ApMV viruses (Clark and Adams 1977) [3]. ELISA kits containing polyclonal antiserum obtained from Bioreba were used in the DAS-ELISA test. DAS-ELISA test was performed for each virus in accordance with the recommendations of the manufacturer.The results were evaluated according to the absorbance values at 405 nm wavelength in the ELISA reader (ELx800 Universal Microplate Reader). Samples giving values twice or more than the value given by the negative control were evaluated as positive.

RESULTS AND DISCUSSION

Azerbaijan has a significant place in terms of genetic resources of fruit plants (especially apples). The scientific research carried out in our republic once again proves that the cultivation of pome fruit plant varieties with high genetic characteristics, suitable for production areas, different according to ripening periods, resistant to various diseases (fungus (*Venturia inaequalis* Wint.), virus, etc. .) will provide high

added value to the country's economy, benefit the environment and human health [6,7,8,9,10,11]. The presence of *Apple mosaic ilarvirus* (in 25.9% of the selected plants) was determined by the DAS ELISA-test method in apple and pear plantations in Azerbaijan. Of the 27 selected samples, 7 apple trees (25.9%) were infected with apple mosaic virus (the OD of the samples was greater than the OD of the negative control, not less than 2) (Table 1). At the same time, the maximum limit of the optical density of infected samples was 2.69, and the minimum was 2.02. Visual diagnosis made it possible to detect 3 plants with chlorotic mosaic symptoms on the leaves. Four samples without external symptoms were also infected with *Apple mosaic ilarvirus*. All symptomatic plants were infected with the virus. It should be noted that the plants with Apple mosaic virus symptoms described in the literature are not often seen in apple plantations. *Apple mosaic ilarvirus* pathogen was detected by DAS ELISA-test method in 3 pear trees in the collection plantations of the teaching farm of Azerbaijan State Agrarian University. The obtained data on the infection of apple and pear plants with *Apple mosaic ilarvirus* viruses do not characterize the general prevalence of viruses in the plantations of Azerbaijan, because the plant sample for the DAS ELISA-test method initially consisted mainly of externally affected plants with a small addition of asymptomatic samples. The research is aimed at the initial detection of viral pathogens in the country in order to assess phytosanitary risks and develop schemes to protect phytosanitary farms from mass replication of virus-infected material.

Table 1. Results of determination of *Apple mosaic ilarvirus* pathogen in apple and pear trees by ELISA-test method

№	Selected area (region)	Presence of symptoms	Result			
			ApMV			
			Optical density		($A_0 \geq A_к$+ 100 %) = 0,286	Presence of the virus
			(Aк)	(Ao)		
1	Guba (old plants)	no	0,143	0,207	1,45	-
2		no	0,143	0,21	1,47	-
3		no	0,143	0,289	2,02	+
4		no	0,143	0,255	1,78	-
5		no	0,143	0,25	1,75	-
6		no	0,143	0,234	1,64	-
7		no	0,143	0,265	1,85	-
8		no	0,143	0,242	1,69	-
9		yes	0,143	0,385	2,69	+
10		no	0,143	0,244	1,71	-
11		no	0,143	0,176	1,23	-
12		no	0,143	0,167	1,17	-
13		no	0,143	0,23	1,61	-
14		no	0,143	0,182	1,27	-
15		no	0,143	0,157	1,10	-
16		no	0,143	0,184	1,29	-
17		no	0,143	0,209	1,46	-
18	Plantations of Azerbaijan State Agrarian University	no	0,143	0,161	1,13	-
19		no	0,143	0,212	1,48	-
20		no	0,143	0,297	2,08	+
21		no	0,143	0,294	2,06	+
22		no	0,143	0,251	1,76	-
23		yes	0,143	0,349	2,44	+
24		no	0,143	0,285	1,99	-
25		yes	0,143	0,303	2,12	+
26		no	0,143	0,292	2,04	+
27		no	0,143	0,278	1,94	-
28		yes	0,336	2,51	2,09	+
29		yes	0,342	2,33	2,51	+
30		yes	0,261	2,46	2,42	+

CONCLUSION

The first step in determining the solution to the problem of transition to the production of certified planting material for fruit crops is the assessment of the presence and spread of viruses and pathogens in the country. The preparation of the list of the most common pathogens in garden crops and systemic pathogens in fruit plants of Azerbaijan will allow us to start developing regulatory documents for the certification of our entrepreneurial nursery plantations and to create our own database of virus-free mother plants for import/export of planting material and inclusion in the country's nursery system.

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