Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Plant Pathology

ISSN 1819-1541
DOI: 10.3923/ajppaj.2017.185.190

Research Article
Survey of Viral Infection of Apple in Shida Kartli Region of Georgia

Iveta Megrelishvili, Zurab Khidesheli, Zviad Bobokashvili and Nino Chikovani

Scientific Research Center of Agriculture, Marshal Gelovani Ave., 0159 Tbilisi, Georgia

Abstract

Background and Objective: Pome fruits are infected by many viruses, it is important to know the way of transmittance and causing agents of disease. To produce virus free propagating material it is necessary to assess the main viruses affecting pome fruits. The research aimed to record the incidence of apple viral infection in commercial fruit tree nursery in Shida Kartli region of Georgia. **Materials and Methods:** Nine hundred and nine symptomatic and asymptomatic samples were analyzed by means of Double Antibody Sandwich-Enzyme linked Immunosorbent Assay (DAS-ELISA) using commercial kits from BIOREBA AG (Switzerland). **Results:** According to DAS-ELISA results, 213 from 909 tested samples reacted positively to at least one virus with total incidence of 23.43%. Apple stem grooving virus (10.67%), apple stem pitting virus (8.47%) were found to be frequently presented in infected apple cultivars. Apple chlorotic leaf spot virus has revealed minor spreading -3.3%, but apple mosaic virus infection was not found. **Conclusion:** Among all tested viruses on apple cultivars, apple stem grooving virus was recorded the highest infection rate and apple chlorotic leaf spot virus was characterized by the lowest distribution.

Key words: Apple viruses, cultivars, commercial apple trees, DAS-ELISA, pome fruits

Citation: Iveta Megrelishvili, Zurab Khidesheli, Zviad Bobokashvili and Nino Chikovani, 2017. Survey of viral infection of apple in Shida Kartli region of Georgia. Asian J. Plant Pathol., 11: 185-190.

Corresponding Author: Iveta Megrelishvili, Scientific Research Center of Agriculture, Marshal Gelovani Ave., 0159 Tbilisi, Georgia Tel: (+995) 577387222

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Apple is the principal pome fruit crop for Georgia. Understanding of the apple pathogen is economically important. Among viruses affecting trees, apple stem grooving virus (ASGV), apple stem pitting virus (ASPV), apple chlorotic leaf spot virus (ACLSV), apple mosaic virus (ApMV) are widespread and can cause substantial economic losses[1-3].

The ASPV is transmitted by grafting and is spread through infected propagating material. It is unknown whether the vectors are able to transmit this virus[4]. Many trees diseases are associated with ASPV such as pear vein yellows, pear necrotic spots, pear stony pit, graft incompatibilities such as top-working disease[5]. It is also known that ASPV was the most prevalent virus in Tunisia-(46%) and in Iran (5.12%)[6,7]. The ACLSV is one of the graft-transmissible pathogens worldwide among fruit trees, which induces symptoms of deformation, reduced the size, chlorotic leaf spots and ring pattern mosaic on leaves of the susceptible cultivars of apple and pear[8]. In the case of the mix of two latent viruses ACLSV and ASPV infection up to 30% reduction in yield has been reported[9]. The relevant information about virus distribution within individual plants, its concentration and detectability according to season, resistance or tolerance of hosts and interactions with other viruses are important[10].

ApMV is the most important virus, which can cause severe damage to plants populations[11]. The other three viruses: ACLSV, ASPV and ASGV are latent[12]. Virus-free apple production protocol using heat treatment and shoot tip culture have been developed in Korea[13]. Some researchers think that virus elimination using cryotherapy produced virus-free shoots for seven of nine cultivars and is a promising technique for developing a virus-free apple collection[14].

Shida Kartli, Georgia is the major production region of apple, where 60-65% of fresh trees in Georgia is produced. The leading cultivars grown in the country are 'Golden delicious', 'Kekhura', ''Winter banana'', 'Champagne Rennete', 'Red delicious' and 'Idared'. The cultivars 'Canadian Rennete' and 'Georgian Sinapi' are of regional importance. In the new orchards various strains of 'Gala', 'Fuji', 'Jonagold' and 'Granny Smith' are widely planted[15].

For establishing a new pome fruit orchard virus-free propagated material was always used because infected plants have a major risk of transmitting infection by grafting. Virus-free apple trees are generally more vigorous and productive than virus infected trees[16]. Viral infections of all of the trees are common pathogens in commercial cultivars and are economically critical[17].

This study was undertaken to survey the presence of the main viruses (ASGV, ASPV, ApMV and ACLSV) affecting pome fruits (apple) in commercial fruit tree nursery of Shida Kartli region and to verify their distribution by using serological methods.

MATERIALS AND METHODS

A survey was carried out in the pome fruit growing areas in Eastern Georgia (Fig. 1) Nine hundred and nine (909) symptomatic and asymptomatic samples were collected during spring, summer and early autumn in 2015 and 2016

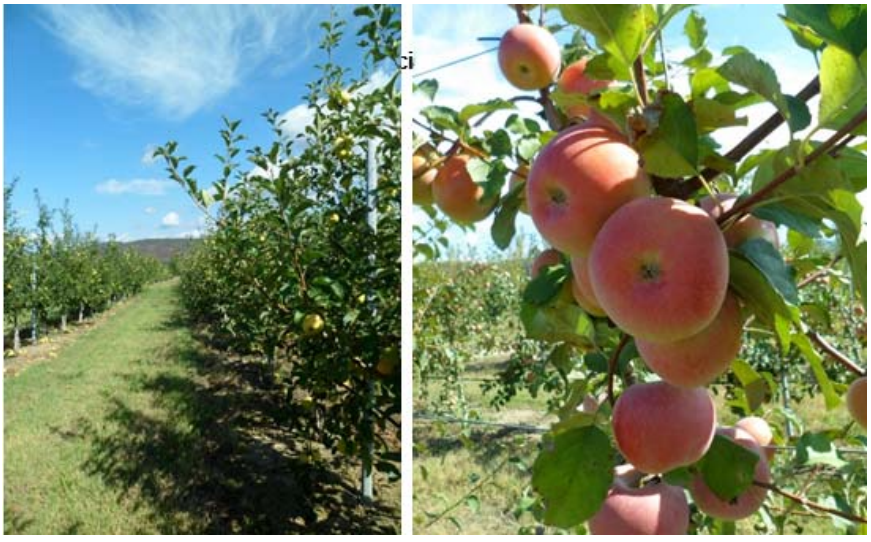

Fig. 1: Survey of 7 Year Old Commercial Fruit Tree Nursery Located in Shida Kartli Region of Georgia Where Samples Were Collected in 2015-2016

from commercial fruit tree nursery of Shida Kartli. During the same season adult leaves were collected from 168 trees of 'Kekhura', 212 trees of ''Achabetura'', 157 trees of 'Georgian Sinapi', 183-''Winter banana'', 189-'Canadian Rennete'. The studied varieties has various origin: ''Winter banana'' is American variety, 'Canadian Rennete' is from Europe and other-'Kekhura', 'Achabetura' is the local varieties. All varieties was grafted on MM106 rootstock and planted in 2008. Eight leaflets in the 4 directions at the height of 2 m above the ground from each sample tree were collected. In 2015 and 2016, a total of 909 trees of apple cultivars grown in commercial fruit tree nursery of Shida Kartli were analyzed by DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay) as reported by Clark and Adams[18] for ASGV, ACLSV, ASPV and ApMV. The DAS-ELISA test was performed by using extracts from young leaves of the collected samples and the serological commercial kits provided by company BIOREBA AG (Switzerland) according to the manufacturer's instructions: Purified polyclonal antibodies of respective virus were diluted 1000 times in coating buffer (pH 9.6) and were coated to each well of microtiter plates (200 µL/well) for 24 h at 4°C. Following three washes with PBS-Tween, the plates were coated with diluted leaf sap (200 µL/well) for 24 h at 4°C. Leaves were crushed [(w/v) 1:5] in extraction buffer (pH 8.2) containing 2% polyvinylpyrrolidone (PVP MW 24,000), 0.02% NaN_3 and 0.05% Tween 20. After four washes with PBS-Tween, 200 µL of conjugate antibody (alkaline phosphate conjugated polyclonal virus-specific antibody) was coated in each well for 5 h at 30°C. Next after final washing, the plates were incubated with fresh pNPP (para-nitrophenyl phosphate) substrate buffer [adding p-nitrophenyl phosphate tablets (1 mg mL^{-1}) in substrate buffer] for 3 min at 25°C preferably in dark. Absorbance was determined at 405/450 nm on ELX800 Microplate Reader (Bio-Tek Instruments, Winooski, VT) and the sample was considered positive if its optical density was 3 times higher than the negative control.

The ultimate objective of this researches on pome virus infection was the control of these diseases in the tree nursery of Shida Kartli region.

Assessment of disease occurrence: Survey and sampling of pome fruits in Shida Kartli region, Georgia was carried out by the following method[19]. Disease incidence (percentage of diseased fruits) and disease severity (percentage of area affected on the fruit on average) was then obtained using the following equation[20]:

$$\text{Diseases incidence (DI)} = \frac{X}{N} \times 100$$

where, X is a number of infected fruits and N is a total number of fruit samples:

$$\text{Disease severity (DS)} = \frac{\Sigma(a+b)}{N} + \frac{100}{Z}$$

where, Σ(a+b) is the sum of symptomatic fruits and their corresponding score scale, N is total number of fruit samples and Z is highest score scale. The highest disease rating is five.

RESULTS

The study was focused on determination of the spreading of different viruses affecting five types of apple cultivars: 'Kekhura', 'Achabetura', 'Georgian Sinapi', 'Winter banana', 'Canadian Rennete' in commercial fruit tree nursery of Shida Kartli in 2015 and 2016. Apple varieties were tested for 4 types of viruses: Apple stem grooving virus (ASGV), apple stem pitting virus (ASPV), apple mosaic virus (ApMV) and apple chlorotic leaf spot virus (ACLSV). During the field survey, the following symptoms were observed in different species of trees: Weak development of trees, stem grooving and pitting, chlorotic, tatter leaves, riddled leaves. In many cases, it was impossible to observe field symptoms associated with viruses.

Two hundred and thirteen out of 909 tested samples reacted positively to at least one virus and showed an infection rate 23.43%. Among all studied apple varieties, the highest infection rate was reported on ASGV (10.67%), followed by ASPV (8.47%), the distribution of ACLSV (3.3%) was minor, but ApMV did not show any positive reaction with the antibodies of ACLSV, ApMV, ASPV, ASGV (Table 1). The survey of commercial fruit tree nursery in Shida Kartli region showed that most of the different apple cultivars were carrying several viral infections, as evidence is presented in Table 2. It has been discovered that ASGV was presented only in three types of cultivars: 'Kekhura' (42.85%), 'Achabetura' (3.77%), 'Winter banana' (9.28%), but as the Table 1 presented ASGV showed the highest intensity of infection development as compared to others. Among these 4 viruses ACLSV was

Table 1: Viral infected result of DAS-ELISA test in commercial fruit tree nursery in the Eastern region of Georgia

	Number of samples	
Virus varieties	Infected	Total infected rate (%)
Apple stem grooving virus (ASGV)	97	10.67
Apple stem pitting virus (ASPV)	77	8.47
Apple chlorotic leaf spot virus (ACLSV)	30	3.30
Apple mosaic virus (ApMV)	-	-

Table 2: Assessment of each viral infection in 2015 and 2016 using DAS-ELISA test according to apple varieties in commercial fruit tree nursery located in the eastern region of Georgia

	Virus varieties			
Species	ApMV	ACLSV	ASPV	ASGV
Infected rate (%)				
Kekhura	-	6.54	5.35	42.85
Achabetura	-	2.35	17.92	3.77
Georgian sinap	-	3.18	-	-
Winter banana	-	2.18	8.74	9.28
Canadian renette	-	2.64	7.4	-

ApMV: Apple mosaic virus, ACLSV: Apple chlorotic leaf spot virus, ASPV: Apple stem pitting virus, ASGV: Apple stem grooving virus, ApMV: Apple mosaic virus

Table 3: Evaluation of total viral infection in 2015-2016 using DAS-ELISA test according to apple varieties in commercial fruit tree nursery located in the East of Georgia

		Number of samples		Total infected
Species	Origin	Tested	Infected	rate (%)
Kekhura	Georgia	168	92	54.76
Achabetura	Georgia	212	51	24.05
Georgian sinap	Georgia	157	5	3.18
Winter banana	USA	183	46	25.13
Canadian reinette	Europe	189	19	10.05

found positively for all researched apple cultivars, but ASPV was presented in all studied apple cultivars except 'Georgian Sinapi'. The results showed that viral diseases were characterized by different spreading that depends on apple cultivars. According to the DAS-ELISA results in 3 viral infections: ASGV, ACLSV and ASPV were of common occurrence in apple cultivars 'Kekhura', 'Achabetura' and 'Winter banana'. The ACLSV and ASPV were main infecting agents in Canadian rennet and only ACLSV was presented in Georgian sinap. Mixed infection was not found (Table 2).

As the results showed that highest total infected rate was found in apple cultivars 'Kekhura' (54.76%), followed by 'Winter banana' and 'Achabetura' (25.13 and 24.05%, respectively), 'Georgian sinapi' was the least infected cultivar (3.18%) (Table 3).

DISCUSSION

The study has confirmed that the most important and devastating virus (ApMV) was not detected in the collected samples. The ASGV was characterized by relatively high distribution (10.67%) in three types of apple cultivars (''Kekhura'', '''Achabetura''' and '''Winter banana'''). The virus ASPV showed less spreading evidence (8.47%) than ASGV, but it was revealed in four kinds of apple varieties ('Kekhura', 'Achabetura', 'Winter banana' and 'Canadian Rennet'). The total infected rate of ACLSV (3.3%) was low as compared to others, but it was revealed in all studied apple cultivars.

The apple production has thousand years history in Georgia. Today it is distributed in all regions of Georgia mostly in Shida Kartli. The areas of orchards are estimated as 10000-11000 ha[21], average production of apple cultivars varies between 41500 (2008)-87400 (2014) MT/annum[22].

Viral diseases are considered a serious problem in Georgia. The presence of the three latent viruses (ACLSV, ASGV and ASPV) symptoms on apple varieties in Georgia increases the distribution risk through grafting and top working. Investigation of apple viral infections using precise methods has not been conducted in Georgia. Assessment of viral infections was performed only on grapevine varieties[23]. Quantitative detection of four pome fruit viruses was studied in many countries[24-26].

Therefore, in order to promote the establishment of effective monitoring system, it is necessary to control the introduction and dissemination of all these pathogens. All viruses must be detected in apple tree leaf tissue by using laboratory techniques. The large-scale survey allowed us to detect heavy virus and phytoplasma infections, especially in mother plant blocks that are considered the main source of propagating material distribution and can therefore, contribute to the rapid and wide dissemination of these agents[27,28]. This study is based on the selection of virus-free mother trees for establishing healthy orchards, ensuring high-quality crops and for the management of apple virus spreading. Based on our results of DAS-ELISA immune-assay, distribution of selected viruses among apple varieties was different. The DAS-ELISA assay from leaves represents the suitable tool for a large-scale disease diagnosis allowing early detection of virus infection including symptom-free samples. The study represents the first large-scale survey conducted in Shida Kartli region of Georgia. The results showed a possible relationship between viral infections and apple varieties, various distributions of viral infections among apple cultivars depend on several transmission pathways, vectors, plant immunity, insect presence etc. The degree of impact depends on the pathogen varieties in combination with apple cultivars.

All viruses of apple mainly spread through vegetative propagation, grafting and top working, ACLSV, ASGV, ASPV and ApMV do not have known insect vectors and are not transmitted through seeds[29]. Thus, viral dissemination results among apple cultivars might be related to their transmission pathways. These viruses are transmitted when a virus-infected scion bud is grafted on a healthy rootstock or an already virus-infected rootstock.

It is important to continue investigations and analyses in order to know the real phytosanitary status with regards to transmissible infections through the propagating material, which may support the success of any certification program for pome fruits in Georgia. Molecular analysis (qPCR) is in progress in order to further precise all doubtful results from DAS-ELISA method.

CONCLUSION

The survey of apple viral infection in Shida Kartli region was conducted during 2 years. According to DAS-ELISA result, 213 from 909 tested samples reacted positively to at least one virus with a total incidence of 23.43%. Apple stem grooving virus (10.67%) followed by apple stem pitting virus (8.47%) were widely spread among all researched apple varieties, distribution of apple chlorotic leaf spot virus (3.3%) was low and apple mosaic virus infection was not found. This is the first report on the presence of viruses infecting pome fruits in Georgia. According to the results, virus-free samples were labeled for further selection as a propagating material. Careful selection of clean propagated materials is a warranty of reduction of the impact of viruses in apple; this study supports the success of any certification program for producing virus-free pome fruits in Georgia.

SIGNIFICANCE STATEMENTS

This study discovers viral infections according to apple varieties in Shida Kartli region of Georgia. It is very important to identify healthy mother apple trees for nurseries in order to avoid yield losses from viruses and reduced risk of viral infection spread. This study can be beneficial for the production of virus-free apple propagating materials in Georgia.

This study supports the success of certification programs for pome fruits in Georgia. Understanding of phytosanitary status of existing apple viral infections helps local farmers to monitor commercial apple orchards for a presence of viral diseases.

ACKNOWLEDGMENTS

Authors acknowledge a gratitude to the LEPL Scientific-Research Center of Agriculture (village Jighaura, Mtskheta Municipality, 0146, Georgia) and the Ministry of Agriculture of Georgia for the financial and technical support for accomplishing this Project No. 03.04.04.

REFERENCES

1. Dar, N.A., 2013. Apple stem grooving virus-A review. Int. J. Modern Plant Anim. Sci., 1: 28-42.
2. Katwal, V.S., A. Handa, P.D. Thakur and M. Tomar, 2016. Prevalence and serological detection of apple viruses in Himachal Pradesh. Plant Pathol. J., 15: 40-48.
3. Nickel, O. and T.V.M. Fajardo, 2014. Detection of viruses in apples and pears by real time RT-PCR using 5'-hydrolysis probes. J. Plant Pathol., 96: 207-213.
4. Eastwell, K.C. and W.E. Howell, 2014. Apple Stem Grooving. In: Compendium of Apple and Pear Diseases and Pests, Sutton, T.B., H.S. Aldwinckle, A.M. Agnello and J.F. Walgenbach (Eds.)., APS Press, USA., pp: 96–97.
5. Eastwell, K.C. and W.E. Howell, 2014. Apple Stem Grooving. In: Compendium of Apple and Pear Diseases and Pests, Sutton, T.B., H.S. Aldwinckle, A.M. Agnello and J.F. Walgenbach (Eds.)., APS Press, USA., pp: 96–97.
6. Abtahi, F., M. Shams-Bakhsh, N. Safaie, C.R. Autonell and C. Ratti, 2017. Occurrence, distribution and molecular characterization of apple stem pitting virus in Iran. J. Agric. Sci. Technol., 19: 217-230.
7. Mahfoudhi, N., M. El Air, R. Moujahed, W. Salleh and K. Djelouah, 2013. Occurrence and distribution of pome fruit viruses in Tunisia. Phytopathol. Mediterr., 52: 136-140.
8. Eastwell, K.C. and W.E. Howell, 2014. Chlorotic Leaf Spot (Apple Topworking Disease). In: Compendium of Apple and Pear Diseases and Pests, Sutton, T.B., H.S. Aldwinckle, A.M. Agnello and J.F. Walgenbach (Eds.)., APS Press, USA., pp: 95-96.
9. Cieniewicz, E. and M. Fuchs, 2016. Apple Chlorotic Leaf Spot Virus. New York State IPM Program, New York, pp: 1-2.
10. Van Den Bosch, F., G. Akudibilah, S. Seal and M. Jeger, 2006. Host resistance and the evolutionary response of plant viruses. J. Applied Ecol., 43: 506-516.
11. Grimova, L., L. Winkowska, M. Konrady and P. Rysanek, 2016. Apple mosaic virus. Phytopathol. Mediterr., 55: 1-19.
12. Eastwell, K.C., 2014. Apple Mosaic. In: Compendium of Apple and Pear Diseases and Pests, Sutton, T.B., H.S. Aldwinckle, A.M. Agnello and J.F. Walgenbach (Eds.)., APS Press, USA., pp: 97-98.
13. Lee, G., J.H. Kim, H.R. Kim, I.S. Shin and K.H. Cho *et al*., 2013. Production system of virus-free apple plants using heat treatment and shoot tip culture. Res. Plant Dis., 19: 288-293.
14. Romadanova, N.V., S.A. Mishustina, D. Gritsenko, M.Y. Omasheva, N.N. Galiakparov, B.M. Reed and S.V. Kushnarenko, 2016. Cryotherapy as a method for reducing the virus infection of Apples (*Malus* sp.). Cryo Lett., 37: 1-9.
15. Bobokashvili, Z., K. Dzeria and V. Kvaliashvili, 2014. The apple breeding of georgia: Past, present and perspectives. Acta Hortic., 1032: 197-201.

16. Door, A.C. and U. Grote, 2009. The role of certification in the Brazilian fruit sector. Rev. Econ. Contemp., 13: 539-571.
17. Stankiene, J., I. Mazeikiene, D. Gelvonauskiene, J.B. Siksnianiene and C. Bobinas, 2012. Virological assessment of stock planting material of apple and raspberry cultivars. Agriculture, 99: 93-98.
18. Clark, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.
19. Cooke, B.M., 2006. Disease Assessment and Yield Loss. In: The Epidemiology of Plant Diseases, Cooke, B.M., D.G. Jones and B. Kaye (Eds.). 2nd Edn., Springer, Netherlands, ISBN-10: 1-4020-4580-8, pp: 43-80.
20. Kranz, J., 1988. Measuring Plant Disease. In: Experimental Techniques in plant Disease Epidemiology, Kranz, J. and J. Rotems (Eds.). Springler, Berlin, pp: 35-50.
21. Geo Stat., 2004. Orchards. In Main Results of the First National Agricultural Census of Georgia. Agricultural Census, National Statistics of Georgia, pp: 127-128.
22. Geo Stat., 2014. Production of permanent crops, 2014-2016. In Agriculture, National Statistics Office of Georgia, pp: 1.
23. Megrelishvili, I., Z. Khidesheli, L. Ujmajuridze and N. Chiqovani, 2016. The study of viral diseases in Georgian vine grafted nurseries. Int. J. Dev. Res., 6: 8299-8302.
24. Winkowska, L., L. Grimova and P. Rysanek, 2016. Quantitative detection of four pome fruit viruses in apple trees throughout the year. Phytopathol. Mediterr., 55: 207-224.
25. Zhao, L., C.H. Feng, B.Q. Li, X.A. Hao, H. Liu, Y.F. Wu and Q.C. Wang, 2014. Rapid detection of apple stem Grooving virus by reverse transcription loop-mediated isothermal amplification. J. Plant Pathol., 96: 407-409.
26. Ferretti, L., B. Corsi, L. Luongo, C.D. Cortivo and A. Belisario, 2017. A survey of cherry leaf roll virus in intensively managed grafted English (Persian) walnut trees in Italy. J. Plant Pathol., 99: 423-427.
27. Fuchs, M., 2016. Virus transmission and grafting practices. N. Y. Fruit Quart., 24: 25-27.
28. Mowat, W.P. and S. Dawson, 1987. Detection and identification of plant viruses by ELISA using crude sap extracts and unfractionated antisera. J. Virol. Methods, 15: 233-247.
29. Pedrazzoli, F., M. Filippi, M. Deromedi, P. Bragagna and I. Battocletti *et al*., 2008. Apple proliferation transmission by grafting in different periods of the year. Acta Hortic., 781: 489-494.