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Araştırma Makalesi/*Research Article*

Determination of some viruses by serological and molecular techniques in potato production areas in Tokat Province

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Abstract: In this research, it was aimed to determine existence of some viruses, causes problem, by serological and molecular techniques in potato production in Tokat province was carried out by serological and molecular tools. For this purpose, the survey was conducted to collect samples in potato production areas in Başçiftlik, Niksar, and Artova districts of Tokat province. DAS-ELISA test was performed to determine the prevalence of *Potato virus Y* (PVY), *Potato leaf roll virus* (PLRV) and *Alfalfa mosaic virus* (AMV). All samples were also tested by RT-PCR using specific primers to confirm the results DAS-ELISA. The results revealed that 54 % of samples were infected with PVY, PLRV, and AMV. PVY was the most common virus in the survey areas with the ratio of 50 %, followed by PLRV (5.5%) and AMV (1.38%) in all tested samples, respectively. Mixed infections also were detected within collected plants. The mixed infections PVY+PLRV was identified in four samples while each of mixed infection with PLRV+AMV, PVY+AMV and PVY+PLRV was represented with one isolate. RT-PCR amplified the expected PCR products for viruses. The best of our knowledge, AMV was first determined on potato in Tokat province.

Keywords: Tokat, Potato, *Potato virus Y*, *Potato leaf roll virus*, *Alfalfa mosaic virus*

Tokat ili patates üretim alanlarında bazı virüslerin serolojik ve moleküler yöntemlerle belirlenmesi

Öz: Bu araştırmada, Tokat ili patates üretim alanlarında sorun olan viral etmenlerin varlığının serolojik ve moleküler olarak belirlenmesi amaçlanmıştır. Bu amaç kapsamında, Tokat ilinde patates yetiştiriciliğinin en çok yapıldığı Merkez, Niksar, Artova, Başçiftlik ilçelerine surveyler yapılmıştır. Surveyler esnasında toplanan örnekler Patates Y virus (*Potato virus Y*-PVY), Patates yaprak kırılma virüsü (*Potato leaf roll virus*-PLRV) ve Yonca mozaik virüsü (*Alfalfa mosaic virus*-AMV) etmenlerinin varlığını belirlemek için öncelikle serolojik olarak DAS-ELISA ile testlenmiştir. Bütün örnekler DAS-ELISA test sonuçlarını kontrol etmek amacıyla spesifik primerler kullanılarak RT-PCR ile de testlenmiştir. Çalışmalar sonucunda, bölgeden toplanan örneklerin %54'ünde bir veya birden fazla virüs enfeksiyonu tespit edilmiştir. Survey alanlarından toplanan örneklerde en yüksek oran ile %50 PVY tespit edilirken, %5.5 oranla PLRV ve %1.38 oranında AMV enfeksiyonu tespit edilmiştir. Toplanan örneklerde düşük oranlarda karışık enfeksiyonlarda görülmüş olup 4 bitkide PVY+PLRV tespit edilmiş iken, birer bitkide PLRV+AMV, PVY+AMV ve PVY+PLRV karışık enfeksiyonları belirlenmiştir. Çalışma konusu virüslere spesifik primerlerle yapılan RT-PCR çalışmaları sonucunda beklenilen büyüklükte PCR ürünleri elde edilmiştir. Yapılan bu çalışma ile Tokat ilinde patates alanlarında AMV' nin varlığı ilk defa tespit edilmiştir.

Anahtar kelimeler: Tokat, Patates, Patates Y virüsü, Patates yaprak kırılma virüsü, Yonca mozaik virüsü

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important industrial crops which is used for human food. It is the fourth largest cultivated plant in the world with more than 100 countries and has a status of globally traded commodity (He et al. 2012). Turkey is one of the largest potato producer country in Mediterranean region and potato can be cultivated in almost all parts of the country (Yardımcı et al. 2015). Turkey has 4.550.493 tones potato production in 1.359.715 decares (da) in 2018 (FAO 2020). Tokat province has 61.385 tones potato product in a cultivation area of 24.291 da.

Potato is exposed to infection of many viruses reduce yield and tuber quality. Potato is infected by at least 40 viruses and 2 viroids (Jeffries et al. 2005). *Potato virus Y* (Potyvirus, Potyviridae, PVY), *Potato virus X* (Alphaflexiviridae, Potexvirus, PVX), *Potato virus S* (Betaflexiviridae, Carlavirus, PVS), *Potato virus A* (Potyviridae, Potyvirus, PVA), *Potato virus M* (Betaflexiviridae, Carlavirus, PVM) and *Potato leaf roll virus* (Leutoviridae, Polerovirus, PLRV) are important due to their distribution and incidence (Salazar 1996, Hameed et al. 2014).

Some viruses as such PVY, PLRV, PVS, PVA and AMV have been reported in potato fields in Turkey (Çıtır 1982, Yılmaz et al. 1990; Gümüş ve Erkan 1998, Bostan and Açıkgöz 2000, Bostan ve Haliloğlu 2004, Arlı-Sökmen et al. 2005, Sertkaya and Sertkaya 2005; Guner and Yorganci 2006, Sertkaya and Calıskan 2009, Sertkaya et al. 2009, Sertkaya 2013). There is little information about viruses infecting potato in Tokat province. This study was conducted to occurrence and incidence of PVY, PLRV and AMV in potato-growing areas in Tokat.

2. Material and method

2.1 Survey

The comprehensive survey was conducted to collect leaf samples from plants showing foliage symptoms indicative of virus infection in potato fields in Niksar, Basçiftlik, Pazar and Artova district of Tokat. Total 288 potato samples were collected by random sampling from potato growing areas (Table 2) (Bora ve Karaca 1970).

2.2 Serological tests

All samples were tested by DAS-ELISA (Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay) (Clark and Adams 1977) using ELISA kit (BIOREBA) according to manufacturer's instructions against to PVY, PLRV, and AMV. Purified polyclonal antibodies of spesific viruses were diluted 1000 times in carbonate buffer (pH 9.6), and each well of ELISA plates was coated with the antibodies (100 µl/well) and incubated for 4 h at 37°C. The leaf samples were grinded (1/10 w/v) in extraction buffer, following three washes with washing buffer. The plate was coated with diluted extracted plant samples (100 µl/well) and incubated for 24 h at 4°C. After five times washes with washing buffer, 100 µl of conjugated antibody diluted in conjugate buffer (pH 7.4). Alkaline phosphate conjugated polyclonal virus-specific antibody was added in each well and incubated for 5 h at 37°C. After washing, the plates were incubated with fresh p-nitrophenyl phosphate substrate buffer (1 mg/ml p-nitrophenyl phosphate tablets in substrate buffer) for 60-90 minutes at room temperature in the dark. Absorbance values were measured at 405 nm by an ELISA reader. Test was assessed as positive when the average absorbance value of tested sample was greater than two times of healthy (uninfected) control (Abou-Jawdah et al. 2000).

2.3 Molecular tests

Total Nucleic Acid (TNA) was extracted from positive samples detected in the result of DAS-ELISA. TNA isolation was made according to protocol described by Astruc et al. (1996). Complementary DNA (cDNA) synthesis was performed using extracted TNA with hexamer primers. The cDNA synthesis was carried out in a total reaction mixture of 20 µl including 2.5 µl RNA, 1.0 µl hexamer primers, 0.2 µl of 25 mM dNTPs, 0.5 µl RNase inhibitor, 2.0 µl of 10x RT buffer, 1.0 µl reverse transcriptase enzyme (Thermo Scientific, USA) and sterile ultra-pure water. The reaction mixture was incubated at 25°C for 5 min and 42°C for 60 min, followed by incubation at 85°C for 5 min. cDNA was used as template for reverse transcriptase-polymerase chain reaction (RT-PCR). PCR was carried out in a 25 µl mixture containing 2.5 µl of cDNA, 0.2 µl of 25mM dNTPs, 2 µl of 25 mM $MgCl_2$, 5 µL of 5X green reaction buffer and 0.5 µl of 10 µM of each specific primers, 0.25 µl of 5 units μl^{-1} *Taq* DNA polymerase (Promega, USA), and sterile ultra-pure water. Primer sets are listed in Table 1. PCR products were electrophoresed in 1% agarose gel including ethidium bromide.

Table 1. The used primers in this study
Çizelge1. *Çalışmada kullanılan primerler*

Primer	Sequence (5' to 3')	Amplified Region	Annealing ºC	Product size (bp)	References
PLRV F	CGCGCTAACAGAGTTCAGCC	Coat protein	62	336	Singh and Singh, 1998
PLRV R	GCAATGGGGGTCCAACTCAT				
PVY sense	ACGTCCAAAATGAGAATGCC	Coat protein	58	480	Singh et al. 1995
PVY antisense	TGGTGTTCGTGATGTGACCT				
AMV F	CCATCATGAGTTCTTCACAAAAG	Coat protein	58	351	Xu and Nie 2006
AMV R	TCGTCACGTCATCAGTGAGAC				

3. Results and Discussion

During surveys, totally 288 samples were collected from the potato field located in Tokat province. The symptoms including mosaic patterns on leaves, malformations, veinal necrosis, necrosis and stunting on plants were observed in surveyed area (Figure 1 and 2). Similarly, these symptoms were reported by previous studies (Brunt 2001, Stevenson et al. 2001, Guner and Yorgancı 2006, Yardımcı et al. 2015)

Figure 1. Mosaic and stunting symptoms (a), and rolling in the top leaves and coloring on potato plants (b)
Şekil 1. *Mozaik ve bodurlaşma belirtileri (a), ve üst yapraklarda kıvrılma ve patates bitkilerinde sararma*

Figure 2. AMV symptoms on potato plants
Şekil 2. *Patates bitkilerinde AMV belirtileri*

All 288 potato leaf samples were tested by DAS-ELISA against to PVY, PLRV and AMV. The incidences of PVY, PLRV and AMV viruses infecting potato are given in Table 2. According to the results of DAS-ELISA, 54% of samples were infected with PVY, PLRV and AMV. PVY was the most common viruses in the survey area with the ratio of 50%. They are followed by PLRV and AMV 5.5% and 1.38% in all tested samples, respectively. Mixed infections also were detected on tested plants. Four mixed infections with PVY+PLRV were determined. One sample was found to be with PLRV+AMV in Başçiftlik samples while mixed infections with PVY+PLRV and PVY+AMV were determined in one sample taken from Center. Among the surveyed districts, the highest incidences of the viruses were detected in Tokat Center with the ratio of 61%.

Tablo 2: Results of DAS-ELISA
Çizelge 2.*DAS-ELISA sonuçları*

Region	Total	Infected samples with		
		PVY	PLRV	AMV
Center	116	71	1	1
Niksar	63	14	3	2
Başçiftlik	65	37	9	-
Artova	44	21	3	1
Total	288	143	16	4
Rate		50%	5.5%	1.38%

RT-PCR tests with primers specific to PVY, PLRV and AMV produced the expected bands about 480 bp, 336 bp, 351 bp respectively (Figure 3).

In this research, PVY (50%) was determined as the most widespread virus in potato-planting area in Tokat province. Similarly, Çıtır et al. (1999) reported that PVY, PLRV, and PVX were presented in seedling in potato-production area from Kazova and Niksar districts of Tokat province, and PVX was prevalent surveyed area. Kutluk Yılmaz et al. (2003) examined serologically mixed infection of PVY with PLRV, PVX, and PVS in leaf samples of potato and found 2.98% and 10% rates of PVY infection in tubers with PVY and PVS, respectively. Guner and Yorganci (2006) found PVY, PVA, PVX, PVS and PLRV viruses as single or mixed infections in both potato leaves and tubers by

using DAS-ELISA method in Nigde and Nevsehir provinces. Bostan and Haliloglu (2004) determined PVY (16.8%) PLRV (13.28%), PVX (6.9%), and PVS (6.4%), in seed tubers in potato growing provinces. In potato plants and tubers were collected from potato fields in Hatay province of Turkey in 2013-2014, the most common viral infection was determined as PVY (49.5%), following by PLRV (5.4%) and AMV infections (5.4%). PVY and PLRV were found as mixed infection with the rate of 1.6% (Çarpar and Sertkaya 2016).

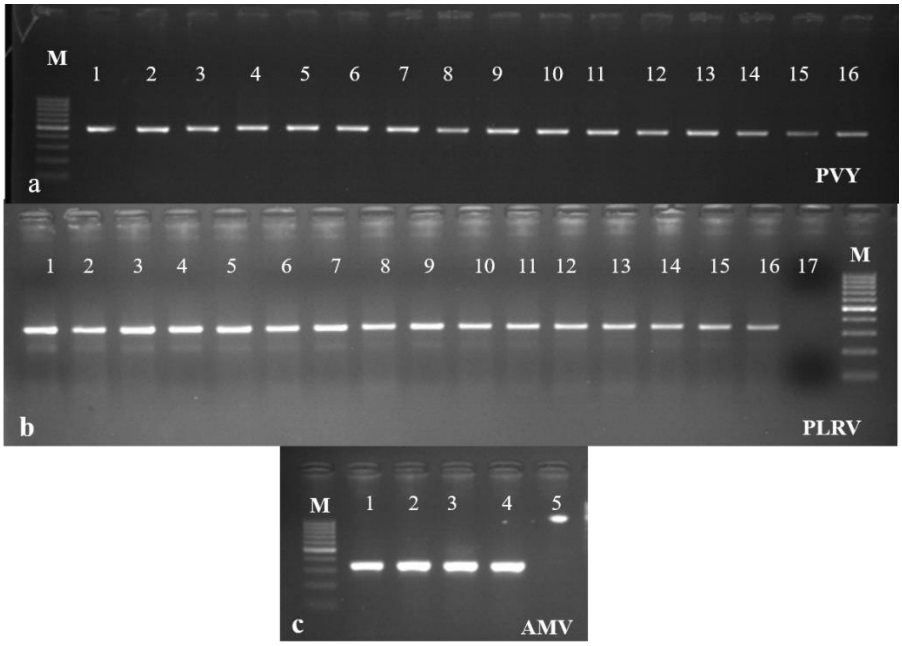

Figure 3: RT-PCR tests results of primers specific to PVY, PLRV and AMV. **a**) PVY, M 100bp ladder (Thermo), 1-16: PVY infected samples (partly). **b**) PLRV, M 100 bp ladder (Thermo), 1-16: PLRV infected samples, 17: Negative control, **c**) AMV, M 100 bp ladder (Thermo), 1-4: AMV infected samples and 5: Negative control.
Şekil 3. *PVY, PLRV ve AMV primerlerine ait RT-PCR sonuçları. a) PVY, M 100bp ladder (Thermo), 1-16: PVY enfekteli örnekler (kısmen).* ***b****) PLRV, M 100 bp ladder (Thermo), 1-16: PLRV enfekteli örnekler, 17: Negatif kontrol,* ***c****) AMV, M 100 bp ladder (Thermo), 1-4: AMV enfekteli örnekler ve 5: Negatif kontrol.*

PVY and AMV transmitted mechanically, in a nonpersistent manner by aphids and by contact with diseased plants in nature, through tubers (Hooker 1986, Burrows and Zitter, 2005). PLRV is transmitted by the aphid *Myzus persicae* in a persistent manner. Vector aphids plays a major role in the spread of common viruses like PVY, PLRV, PVA AMV. Therefore, management with aphids is very important to control virus infection. The incidence and wide distribution of PVY in potato plants were most likely related to the large abundance of vector pests and the inoculation of seedling tuber which infected virus.

AMV infects more than 600 plant varieties and transmitted mechanically, seed, weed seeds and aphids with non-persistent manner (Bol 2003). In previous study, Ozdemir et al. (2011) have found AMV in potato areas located in Balıkesir province. During the potato production in 2014 and 2015 years, 15.3% rates of AMV in *Physalis angulata* infection and on potato plant showing virus symptom %5.4 and %4.6 AMV infection were detected in Hatay province by Sertkaya et al. (2017). No information about prevalence of AMV was obtained by any previous study in Tokat province. AMV infection, thus, was determined firstly in this study for this region with the rate of 1.38%.

PVY, PLRV, PVX, PVA, PVS and PVM are affecting potato crops singly or in mixed. Because of infection by these important potato viruses, yield reduction is usually higher than 50% in most susceptible cultivars (Salazar 2003). Also, mixed infection was detected low rates with PVY+PLRV, PLRV+AMV, PVY+AMV and

PVY+PLRV. Mixed infections of PVY and PLRV caused severe yield losses in point of reduced tuber size and inferior crop quality than that of single infection of either PVY or PLRV in China (Wang et al. 2011).

In this study shows that PVY is prevalent in potato fields in Tokat province while PLRV and AMV are rarely. For management with viruses, producer must use free seeds in the production and eliminate the vector insects from potato fields. Further studies are needed for prevalence of other potato infecting virus in the region.

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