საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 10, №4 2016
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 10, no. 4, 2016

Molecular Biology

Report of Recombinant Strains of Potato Virus Y (PVY) in Georgian Potato Seeds

Vladimer Baramidze[*], Joerg Schuberts[*], Nugzar Aleksidze[**], Ekaterine Shubladze[§], Leonid Ushanov[§]

[*] *Julius Kuhn Institute, Quedlinburg, Germany*
[**] *Academy Member, Saint Andrew the First-Called Georgian University of the Patriarchate of Georgia, Tbilisi, Georgia*
[§] *Georgian Agrarian University, Tbilisi, Georgia*

ABSTRACT. This is a report of recombinant variants of Potato Virus Y (PVY) from the potato cultivating regions of Georgia: Akhaltsikhe, Akhalkalaki, Marneuli. It is important to know which PVY recombinant strains are present, and if the verities circulating in the country confer any resistance to them. The most prevalent strains were $PVY^{N}Wi$ - 89%, followed by PVY^{N} -11%. We could not detect any infection with PVY^{NTN}, PVY^{NA-NTN} and PVY^{O}. This finding is congruent with research papers stating that $PVY^{N}Wi$ is found to be most prevalent in Europe. Total PVY infection of potato seeds accounted 63%, which exceeds any certification limits in the world. The highest incidence of PVY was observed for Akhaltsikhe - 78%, followed by Marneuli - 61 % and Akhalkalaki- 50%. According to chi-squared analyses, Akhalkalaki region had a significantly lower number of PVY infection ($P<0.05$) compared to both other regions. This might be attributed to the higher altitude of the growing area. The rate of infection in Georgia is significantly higher than the certification standard of EU (9% with high PVY infection). Furthermore, there was no significant difference ($P>0.05$) of PVY infection between cultivars rated as PVY-resistant and non-resistant, demonstrating the cultivars in the country are not recombinant strain resistant. Widely used varieties Desiree and Spunta formerly were reputed to be well resistant to PVY, but, today these cultivars are counted as sensitive to new recombinant strains such as PVYNTN or $PVY^{N}Wi$.

Key words: PVY, recombinant, multiplex PCR, potato, Georgia

Potato virus Y (PVY) is the type species of the genus *Potyvirus*, family *Potyviridae*, with a single-stranded positive-sense genomic RNA of approximately 9.7 kb [1]. PVY infects important crop plants such as potato, tobacco, tomato, pepper. It is considered as one of the most economically damaging viruses in the world [2].

PVY strains are distinguished based on their hypersensitive resistance response (HR) to particular cultivars of potato and also by symptoms on tobacco plants [3]. Based on symptomatology in tobacco and potato plants and reaction of some resistant cultivars, PVY isolates were divided into different strains. PVY^{c}

induces HR in the potato cv. King Edwards, while PVY^{o} induces HR in cvs. Desiree and Maris [4]. PVY^{z} which is carrying putative *Nz* gene, induces HR in cvs. Maris Bard or Pentland Ivory. The PVY^{N} is not producing HR in the presence of all three known resistance genes, and it is able to overcome PVY resistance in potato [5,4]. PVY^{C}, PVY^{O}, and PVY^{Z} induce mosaic and vein clearing symptoms in tobacco, However, PVY^{N} causes vein necrosis and stunting [6,7,2,4]. Strain PVY^{E} has been shown to produce no HR against any potato resistance gene, similar to PVY^{N}, but inducing only mosaic and vein clearing in tobacco. Just recently by whole genome sequencing two recombinant type strains status $PVY^{n}Wi$ and PVY^{NTN} [8]. PVY^{NTN} causes potato tuber necrotic ringspot disease (PTNRD), while $PVY^{N}Wi$ results in tobacco veinal necrosis and sometimes can induce tuber necrosis symptoms [9-12]. Recently, a novel PVY strain variant NE/11 has been shown in Syria to be recombination between PVY^{N} and PVY^{NTN} [13].

In Europe, still $PVY^{N}Wi$ is the prevailing strain. This may be caused by its higher aggressiveness as well as the fact that symptoms are hardly recognized on some cultivars during field inspection. Taking into consideration that Georgia is in the group of low yield countries, the yield being only 8-15tons per hectare [14], the aim of our study was to identify the main PVY recombinant strains infecting seed potatoes in Georgia. As far as we know no such survey had been conducted. In order to implement disease management strategies, firstly it is important to know which PVY recombinant strains are present, and if the verities circulating in the country sold confer any resistance to them.

Materials and Methods

Virus Isolate. 122 seed potato tubers were collected, from 8 different cultivars produced in the districts Akhaltsikhe, Akhalkalaki and Marneuli. These tubers were harvested by small hold producers in previous crops to be sold as a seed potatoes in 2013. Three to four eyes from each test tuber were collected, homogenized with PBS (0.01M sodium phosphate buffer, pH 7.2, 0.15 NaCI) buffer and mechanically inoculated to *Nicotiana tabacum* cv. *Samsun* NN (3-4 leaves stage) dusted with carborundum. All plants were grown in insect free greenhouse (20-26°C) at Julius Kuhn Institute Germany.

Molecular Differentiation of PVY isolates. Immunocapture reverse transcription reactions were performed as described by [15]. Briefly, 0.5ml tubes were coated for 4 h at 37°C with PVY-specific IgG (10 µg/ml in 0.1M sodium carbonate buffer, pH 9.5; IgG was kindly provided from Frank Rabenstein). After coating tubes were washed for three times with phosphate-buffer saline (0.01M sodium phosphate buffer, pH 7.2, 0.15NaCI) with 0.05% Tween-20 (PBST). Tobacco plant leafs (200 mg) were homogenized with extraction buffer (1:10/w:v; PBS containing 2% PVP-25 and 2% dried skimmed milk powder). Homogenates were centrifuged at 12000 x g and 50 ml of supernatant were added to the coated tubes and kept overnight 4°C. Afterwards, tubes were washed three times with PBST and once briefly with distilled water. RT reaction was run in 40ml total volume, firstly 32µl of RT reaction premix was added to IgG coated tubes, and they were heated at 72°C for 2 min. RT premix contained 5x concentrated buffer (Promega), dnTps 10mM, 100mM 3'END PVY specific primer. Secondly, to the rest of 8µl of premix was added 0.5µl (200 units/µl) of RT enzyme (Promega) and the mixture was appended to tubes. Reaction proceeded for 60 min at 42°C, final extension at 72°C for 10 minutes. The PCR conditions varied depending on the number of intended viruses to be detected and the number of primers involved. The antisense, sense primers and PCR fragment size are given in (Table 1). For triplex PCR, a final volume of 25µl contained: 3µl of cDNA (approximately 300ng, Nanodrop), strain specific antisense and sense primers for PVY^{NA-NTN}, PVY^{N} and PVY^{O}, final concentration 0.2mM each,10x concentrated reaction buffer (provided by supplier of the Taq DNA polymerase, containing 1.5 mM MgCl2, 0.2mM dNTPs) and 1U of Taq DNA polymerase

Table 1. The primer pairs used for PVY recombinant strains differentiation

Name	sequence [5′-3′]	Specific for strains	PCR Fragment Size (Bp)
PVY 3 end	TCTCCTGATTG	All Strains	-
PVY 3-2558	GGCTCATCTAACAGCAACTGTC	$PVY^{N}Wi$	778
PVY 5-1780	CCGAATGGGACAAGAAAACTTG	$PVY^{N}Wi$	
PVY 3-622	TTGATAAGATGGTTCATTTGTTT	PVY^{NA-NTN}	434
PVY 5-116	TTGATCTTCGTCGTACAAACCG	PVY^{NA-NTN}	
PVY 3-9525	CCACAATGACGAAATCACCCTG	PVY^{NTN}	890
PVY 5 8635	AAGGTAGCATTCAACCAAATCTC	PVY^{NTN}	
PVY 3-2438	GGTTCATCCAGTAGCAATTGCT	PVY^{N}	658
PVY 5-1780	CCGAATGGGACAAGAAAACTTG	PVY^{N}	
PVY 3-2558	GGCTCATCTAACAGCAACTGTC	PVY^{O}	1553
PVY 5-1005	AATTGTACGATGCACGTTCTAGA	PVY^{O}	

(Promega, GoTaq). For dupliplex PCR we have used primers pair's specific for $PVY^{N\text{-}Wilga}$ and PVY^{NTN} strains. The thermo cycling conditions for both dupliplex and tripliplex PCR were the same: 96°C 3min; 96°C 30s, 62°C 1 min 15s, 72°C 2 min, 32 cycles, final extension at 72°C for 10 min. The expected bands were analyzed on a 1% agarose gel in TAE buffer, including ethidium bromide (EthBr) and viewed under UV illumi-nation using a digital imaging system (INTAS UV system P93DW). Statistical analysis about prevalence of PVY and its distribution in potato producing regions of Georgia were tested by the chi square (χ^2) tests for independence. One-way ANOVA test was used to find the relation between PVY and plant resistance**.**

Results

Molecular differentiation of PVY isolates. In initial experiments to differentiate PVY recombinant variants in the country, we identified $PVY^{N}Wi$ as predominant strain group, constituting 56% of total PVY population. Nevertheless of significantly low number, PVY^{N} strain is present in all potato producing regions of Georgia and constitutes 7% of total PVY infection (Table 2, Fig. 1-representative agarose gel lane 2.3). Highest incidence rate of $PVY^{N}Wi$ was observed in Akhaltsikhe region 68%, followed with Marneuli 59% and Akhalkalaki 42%. These regions differed in the distribution patterns of the PVY^{N} infection rate. The highest load of infection was in Akhaltsikhe 10%, followed Akhalkalaki 8%, and Marneuli 2 %.

In our experiments we had 6 cases with mixed infection from $PVY^{N}Wi$ and PVY^{N} strain. We couldn't detect any infection with PVY^{NA-NTN}, PVY^{O}, and PVY^{NA}. The results obtained indicate that PVY infection is a limiting factor for potato production in Georgia. Our records revealed 63% PVY infection from tested 122 farm saved seed potatoes (Table 1). Comparison of incidence of PVY infection in different potato producing regions resulted in highest rate from Akhltsikhe - 78%, followed with Marneuli - 61% and Akhalkalaki- 50%. A chi-squared test of PVY infection distribution into regions, has resulted in a significant difference ($P<0.05$). The comparison of infection rate between regions revealed that PVY level in Akhalkalaki significantly differs from both Marneuli and Akhaltsikhe ($P<0.5$). On the other hand there is no significant difference between infection rate of Marneuli and Akhaltsikhe ($P>0.05$).

According to the European Cultivated potato database, among the varieties that are spread in Georgia only Spunta and Desiree carry medium resistance to PVY infection (Table 1). With statistical analyses (ANOVA), there is not a significant difference in an infection rate of PVY between resistant and non-resistant cultivars (df=1; Value=45.8 P= 0.826).

Table 2. Differentiation of PVY recombinants in different cultivars and regions of R. Georgia

			% tubers Infected with different strains of PVY			
Region	**Cultivar**	**Tested Tubers (No)**	**Resistance to PVY ***	$PVY^{N-Wilga}$	PVY^{N}	**% TOTAL PVY**
Akhalkalaki	Spunta	18	Medium	67	11	78
	Marabella	7	NO	57	14	71
	Mariana	3	NO	33	0	33
	Milva	12	NO	0	0	0
TOTAL		**40**		**42.5**	**7.5**	**50**
Akhaltsikhe	Redish	7	NO	57	14	71
	Pikaso	8	NO	50	25	75
	Desire	6	Medium To High	17	17	33
	Unknwon	20	NO	95	0	95
TOTAL		**41**		**68**	**10**	78
Marneuli	Jely	10	NO	40	10	50
	Unknown	31	Unknown	65	0	65
TOTAL		**41**		**59**	2	**61**
TOTAL for Georgia		**122**		**56**	**7**	**63**

* European cultivated potato database (www.europotato.org.)

Discussion

The identification of PVY recombinant strains in Georgia appears to be the first report. Our results show that most prevalent strains are PVY^{N}Wi 56% and $PVY^{N.}$ 7%. We could not detect any infection with PVY^{NA-NTN}, $PVY^{O,}$ and PVY^{NA}. This finding is congruent with research papers about PVY recombinant strains and their distribution in other parts of the world [16]. PVY^{N}Wi is found to be more infectious in potatoes infecting larger number of potato cultivars, than the other recombinant variants [17,18]. It has spread in Poland representing more than 90% of PVY infections in some growing areas [19]. Similar situations were also observed in Spain [20], in France [21], in Russia, in Finland and in Germany. The reason for such wide spread can be associated with very mild symptoms [17]. Usually they are totally symptomless and undetectable by visual inspections in the fields. In most cases, such infections are lately detected by laboratory tests during post-harvest controls. The abundant presence of these two strains in Georgia can be explained by the absence of laboratory control system. To combat drastic spread of these recombinant strains it requires implementation of specific control measures against these PVY variants. A prerequisite is set up an IC-RT-PCR methodology, which could be applied in seed certification and breeding programs.

This report along with similar studies [14] confirms the presence of PVY in Georgia. The infection rate of PVY for Georgia accounted 63% in farm produced seed potatoes. The results are extremely starling and it needs urgent action from the government. The rate of infection from Georgia is significantly higher than the certification standard of EU (9% with high PVY infection). Comparative analyses revealed that there is a significant difference between infection rate of Akhalkalaki with that of Akhaltsikhe and Marneuli. The only explanation for significantly low number of PVY in Akhalkalaki could be associated with geographic locations of growing areas. Akhalkalaki is 1700m above sea level when Akhaltsikhe is 1000m, and Marneuli at 420m. The higher location of the Akhalkalaki provides not affordable climatic conditions for vector populations. In cooler climates most aphids of potato crops

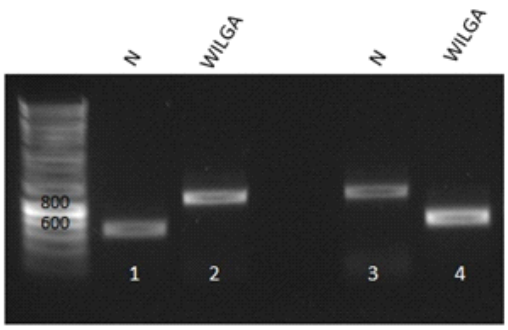

Fig. 1. Representative agarose gel of amplification with PCR: Lanes 1-3- PVY^{N} expected band size 658BP, Lanes 2-4, $PVY^{N\text{-}Wilga}$ expected bands of 758bp

Table 3. Chi-squared test: Regions versus PVY and regional differences

		df	Value	P Value
Regions vs PVY		2	6.649	0.036
Akhalkalaki vs	Akhaltsikhe	1	7.569	0.0059
Akhaltsikhe vs	Marneuli	1	0.0488	0.8252
Akhalkalaki vs	Marneuli	1	6.454	0.0111

overwinter either as eggs an primary host (holocyclus) or as viviparae in protected sites on secondary hosts (anholocyclus). Primary hosts are usually fruit trees, secondary hosts, mainly biannual or perennial weed species. This allows aphid numbers to increase before potato plants are available for colonization and provide a source of migrants to crops [22]. In milder, climates parthenogenesis (asexual) reproduction is around the year on weeds possible, this availability of green plants provides an immediate aphid and virus source when potatoes are planted [23,24]. The vector populations from potato growing areas of Georgia had never been investigated. Thus making any inference about vector population behaviors is not scientifically correct.

Analysis f variance showed no significant difference of PVY infection between resistant and non resistant varieties ($P>0.05$). European varieties which are widely used in Georgia are sold as varieties with moderate resistant against PVY. This information provided is not based on true evidence. Because, these varieties Desiree and Spunta formerly were reputed to be well resistant to PVY, but, today these cultivars are counted as sensitive to new isolates such as PVY^{NTN} or $PVY^{N}W$ [25].

In conclusion, present study demonstrated that $PVY^{N}Wi$ and PVY^{N} are currently the predominant forms infecting seed potatoes in Georgia. We could not detect any other $PVY^{NA/NTN,}$ $PVY^{O,}$ PVY^{NTN} in the potato producing regions. Our study revealed that 63% percent of farm produced seed potatoes are infected with PVY infection and there is a significantly low number of infection in Akhalkalaki region. Most cultivars, which are sold as resistant to PVY, are not resistant to the recombinant variants widely dispersed in the regions. This study is a scientific proof and clearly reflects the reasons of potato production problems in the country.

Acknowledgement. The authors acknowledge financial support from DAAD program. We are grateful to Martina Nielitz for her constant support, to Edgar Schilephake, Bärbel Apel and Uta Brunngräber.

მოლეკულური ბიოლოგია

ქართული კარტოფილის თესლზე კარტოფილის Y ვირუსის რეკომბინანტული შტამები

ვ. ბარამიძე*, ი. შუბერტი*, ნ. ალექსიძე, ე. შუბლაძე§, ლ. უშანოვი§**

** იულიუს კუნ-ის სახ. ინსტიტუტი, ქვედლინბურგი, გერმანია*
*** აკადემიის წევრი, საქართველოს საპატრიარქოს წმიდა ანდრია პირველწოდებულის სახელობის ქართული უნივერსიტეტი, თბილისი, საქართველო*
§საქართველოს აგრარული უნივერსიტეტი, თბილისი, საქართველო

ნაშრომში წარმოდგენილია საქართველოში ჩატარებული პირველი კვლევა კარტოფილის Y ვირუსის რეკომბინანტული ვარიანტების შესახებ, რომლებიც კულტივირებულია საქართველოს სხვადასხვა რეგიონებში არსებული კარტოფილიდან: ახალციხე, ახალქალაქი და მარნეული. ყველაზე მეტად გავრცელებული შტამები იყო: $PVY^{N}Wi$ - 89% და PVY^{N} -11%. უნდა ითქვას, რომ არ გამოვლინდა არანაირი ინფექცია PVY^{NTN}, PVY^{NA-NTN} და PVY^{O}-ის შემთხვევაში. კარტოფილის თესლების ტოტალური ინფექცია, რომელიც გამოწვეული იყო კარტოფილის Y ვირუსით, მოიცავდა 63%-ს, რომელიც სცდება მსოფლიოში მიღებულ სასერტიფიკაციო ზღვარს. კარტოფილის Y ვირუსის ყველაზე მაღალი ინფიცირების დონე დაფიქსირდა ახალციხეში - 78%, შემდეგ - მარნეულში - 61%, ხოლო ახალქალაქში - 50%. Chi-squared ანალიზის მიხედვით ახალქალაქის რეგიონში აღინიშნებოდა კარტოფილის Y ვირუსით გამოწვეული ინფექციის მნიშვნელოვნად დაბალი მაჩვენებელი ($P<0,05$) დანარჩენ სხვა ორ რეგიონთან შედარებით, რაც შეიძლება ახალქალაქის ზღვის დონიდან სიმაღლით იყოს განპირობებული. გარდა ამისა, არ აღინიშნებოდა არსებითი განსხვავება კარტოფილის Y ვირუსით დაინფიცირებულ ($P>0,05$) კარტოფილის სხვადასხვა ჯიშებს შორის, კარტოფილის Y ვირუსის მიმართ არსებულ რეზისტენტულ ჯიშებს არ გააჩნიათ გავრცელებული რეკომბინანტული შტამების მიმართ მდგრადობა. ყოველივე საჭიროებს ქვეყანაში პრევენციული და საკონტროლო მექანიზმების მოქმედების შემუშავებას და ამოქმედებას.

REFFERNCE:

1. *Hull R.* (2009) Comparative Plant Virology. 2nd ed, Elsevier 376 pp.
2. *Kerlan C.* (2006) Potato virus Y. AAB descriptions of plant viruses. No 414.
3. *de Bokx J. A., Huttinga H.* (1981) Potato virus Y. Descriptions of plant viruses, No. 242. Common Mycol. Inst/Assoc Appl Biol. England.
4. *Singh R. P., Valkonen J.P., Gray S.M., Boonham N., Jones R.A., Kerlan C., Schubert J.* (2008) Arch. Virol. **153** (1): 1–1.
5. *Jones RAC* (1990) Ann. Appl. Biol. 117:93–105.
6. *Ellis P., Stace-Smith R., Billiers G.* (1997) Plant Dis. 81: 481-484.
7. *Fanigliulo A., Comes S.* (2005) Arch. Virol. **150** (4): 709-720.
8. *Karasev A.V., Gray S.M.* (2013) Annu. Rev. Phytopathol. 51: 571–586.
9. *Beczner L., Hovarth H., Romanyi I., Forester H.* (1984) Potato Res. **27** (4): 339-353.
10. *Ramirez-Rodriguez V.R., Frias –Trevino G., Avina-Padilla K., Martiney Soriano J.P.* (2009) J. Virology. **6**: 47-48.
11. *McDonald J.G., Singh R.P.* (1996) American Potato J. **73** (7): 309-315.
12. *Kogovsek P., Gow L., Pompe-Novak M., Gruden, K., Foster G,D., Boonham N., Ravnikar, M.* (2008) J. Virol. Methods. 149: 1-11.
13. *Lorenzen J.H., Meacham T., Berger P.H., Shiel P.J., Crosslin J.M., Hamm P.B., Kopp H.* (2006) Arch. Virol. 151: 1055-1077.
14. CIP report 2010.
15. *Schubert J., Fomitecheva V., Sztangert-Wisniewska J.* (2007) J. Virol. Methods. 140: 66-74.
16. *Kerlan C., Mouray B.* (2008) Potatoe virus Y. Ency. Virology, Academic Press, Oxford, pp.287-296.
17. *Chrzanowska M.* (1991) Potato Res. **34** (2): 179-182.
18. *Chrzanowska M.* (1994) Phytopathol. Pol. 8: 15-20.
19. *Chrzanowska M., Doroszewska T.* (1997) Phytopathol. Pol 3: 63-71.
20. *Blanco-Urgoiti B., Tribodet M., Leclere S., Ponz F., Perez de San Roman C., Legorburu F.J., Kerlan. C.* (1998) Eu. J. Plant. Path 104: 811-819.
21. *Kerlan C., Chauvin A., Tribodet L. D.* (1999) Abstracts of the 14th Triennial Conference of the European Association for Potato Research. Sorrento: 700 p.
22. *Radcliffe E. B., Ragsdale D. W.* (2002) J. Potato Res. 79: 353-386.
23. *Daiber C.C., Schöll S.E.* (1959) J. Entom. Soc. South. Africa. 22: 495-520.
24. *Radcliffe E. B.* (1982) Annual Review of Entomology. 27: 173-204.
25. *Haverkort A.J., Boris V.* (2007) Potatoes production and innovative technologies. p. 345.

Received July, 2016