

PRELIMINARY STUDIES REGARDING THE INCIDENCE OF POTATO VIRUS Y IN SEED POTATOES IN ROMANIA (FOR SEVERAL CULTIVARS)

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Abstract

The farmers income is affected strongly by potato virus diseases. Potato Virus Y has become in the last years one of the most damaging pathogen of potato crop. It can cause serious reduced yield, stand loss and decrease of the tuber's quality. The necrotic and recombinant strains PVY play an important role in the spreading of this virus. Elimination of this pathogen from potato supply is essential for seed production and the choice of resistant varieties to the infection with this virus could be one of the measures recommended for farmers and producers. The results of this preliminary study show that varieties resistant to necrotic strains PVY in our study (samples taken from the regions Brașov, Covasna, Harghita, Cluj, Suceava, in 2014) were the following: Riviera, Bellarosa, Jelly, Roclas, Christian.

Key words: potato virus Y, necrotic strains, potato ringspot, necrotic tuber disease

Why are important to know more information about Potato Virus Y? For solving food shortages at the beginning of this millennium, the potato proves to be a product with promising perspectives. Considered by some a common product, cheap food, poor people's food and the plant of poor areas, the potato is actually a product that helps improving the daily diet being rich in carbohydrates, vitamins and minerals. For Romania, the potato is a strategic food, contributing to the national food safety system. Our country is ranked on the third position in Europe in terms of area cultivated with potatoes (after Poland and Germany) (<http://www.insse.ro>).

Virus diseases lead to the reduction of farmer's income from agricultural or national communities. Damages and economic losses are due primarily to the reduction of plant growth leading to reduced production or even its destruction. This is why protective measures of culture against viral infections, diagnosis and control of virus diseases play an important role in potato production technology and multiplication.

Distributed worldwide, *potato virus Y* (PVY, potyvirus genus, family *Potyviridae*) is a major economic disease agent for the crops. This pathogen causes losses in solanaceous crops such as potato (*Solanum tuberosum*), tobacco (*Nicotiana*

tabacum) and tomato (*Lycopersicon esculentum*) (Cojocaru N. 1987; Lacomme L. *et al.*, 2014). PVY in potato received (in the last period) a big attention because this pathogen is one of the most economically important problems in seed potatoes in the world. This virus is responsible for serious decreases yield and quality tubers, but the main problem in seed potato production is the requirement for a strict PVY tolerance limits for certified lot of seed. High levels of PVY are responsible for the rejection of many seed potato lots. Also, a significant reduction of the crop value was noticed and in a certified seed's shortage, too, especially for certain varieties highly susceptible to PVY infection (Lacomme C. *et al.*, 2014). Damages caused in one year (by reducing production by 50%) on a hectare cultivated with potatoes for consumption at an average production of 20 t/ha amount to 8.000 lei/ha (10.000 kg potato x 0,80 lei/kg = 8.000 lei/ha. If it is affected only 50% of the area cultivated with consumption potato in the country, the produced damage is of 8.000.000.000 lei/year (100,000 ha potato x 8.000 lei/year loss/ha = 8.000.000.000 lei/year).

Massive imports of potato in last decades, the continuous "migration" of seed potatoes from one area to another, climate change, inadequate treatments for disease vector control (especially

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aphids), viral pressure, resistance of varieties are just some of the factors that may favor the spread of aggressive strains of the virus Y that recently appeared in the culture. In order to limit the spread of these infections (PVY necrotic) there are useful to conduct research work for:

- ✓ Determining the spectrum of the virus strains in most cultivated varieties in our country (best known genotypes appreciated by farmers, producers and consumers for their production, nutrition and sensory quality);

- ✓ Estimation of geographical areas with epidemiological risk;

- ✓ Identification, promotion and usage of potato lines and varieties with high resistance or tolerance to infection with necrotic strains of the virus Y;

- ✓ Assessment of necrotic PVY strains ecology;

- ✓ Improvement of potato micro-zoning.

This research work could help the spread limiting of this pathogen by identifying and implementing methodological control solutions of PVY necrotic strains in the context of climate change. This concept includes a holistic approach for the development of innovative technologies to streamline control system of PVY necrotic strains. This requires an evaluation (spatial and temporary) of strains spectrum of potato virus Y, the degree of infection with PVY necrotic recombinant strains in some genotypes in accordance with applied pesticide treatments and climate change in Romania. The main benefits, expected in case of this kind of research work, are the following:

- development of innovative technologies for potato crop protection for seed and consumption in Romania against necrotic ring staining of potato tubers (PRNTD) and infection with PVYNTN recombinant strains, in accordance with the principles of sustainable agriculture.

- identification of national and foreign potato lines and varieties with high resistance or tolerance to infection with these necrotic strains for replacing sensitive cultivated varieties, reducing damage, preventing the spread of necrotic ring staining of potato tubers and keeping under control the put break.

Very short information about the classification of PVY strains. Potato virus Y (PVY) was described for the first time by Smith in 1931 (UK). For a long time, PVY isolates were classified according to foliar systemic and local symptoms (*figure 1*) in three main groups PVY^O, PVY^N, PVY^C, depending on the symptoms induced in *Nicotiana tabacum* and *Solanum*

tuberosum varieties (Singh R.P. *et al.*, 2008). While the virus PVY^O is present all over the world, the virus PVY^N was mentioned in Europe, South America, Africa, India (Crosslin J.M. *et al.*, 2006; Karasev A.V. *et al.*, 2011). In recent years, PVY isolates were found apparently intermediate between PVY^O and PVY^N groups, because they share symptoms, serological and genomic properties with the two groups (Galvino-Costa S.B.F *et al.*, 2012; Valkonen J.P., 2011). Thus relatively recent there were identified new subgroups of viruses PVY^N (*table 1*):

- PVY^{N-Wi} and PVY^{N-O} (Valkonen J.P., 2011; Nie X. *et al.*, 2002, Karasev A.V. *et al.*, 2010) that have PVY^N pathotype and PVY^O serotype.

- PVY (NTN) often associated with destructive virus that causes PRNTD on sensitive species. More recently, molecular genotype of PVY^{NTN} was described as follows: NA-PVY (NTN) (Nie X. *et al.*, 2002, Karasev A.V. *et al.*, 2010) and NE-11 PVY (NTN) (Lorenzen J.H. *et al.*, 2006; Boonham *et al.*, 2002; Lorenzen J.H. *et al.*, 2008). PVY in 2000 was considered quarantine plant pathogen in USA and Canada (Crosslin J.M. *et al.*, 2002).

In the last three decades new PVY strains have emerged, some of them (e.g. PVY^{(N)W}) induce barely visible symptoms during the growing season (often being unnoticed during visual inspection) and others (e.g. PVY^{(N)NTN}) produce symptoms on tubers, causing the so-called necrotic ring staining of tubers.

Due to the fact that these viral strains may affect the resistance of some potato varieties compared to other strains of the virus Y (PVY^O and PVY^C) numerous varieties that were considered resistant passed into the category of sensitive ones, which affected the production of the potato in our country. The damage caused by this pathogen agent is both quantitative (significant reduction of production) and qualitative (commercial depreciation of tubers). In case of cultivation of sensitive varieties under favorable conditions, financial losses can be important both for potato consumption (it can become unmarketable) as for seed potatoes (it will be downgraded or rejected from certification). PVY (NTN) strains produce symptoms on tubers, causing the so-called necrotic ring staining of potato tubers (PNRTD). Being very aggressive, these strains can overcome existing resistance to infection with other strains of potato virus Y (PVY^O and PVY^C) (Boonham N. *et al.*, 2002).

Most countries which discovered the presence of necrotic PVY strains have acted to prevent the spread of this pathogen. Initially funds were allocated for research on resistance to infection with this particularly aggressive viral strain, after which there was a drastic revision of

the structure of potato varieties, decision making forums intervening in time to limit expansion of tuber necrotic ring staining induced by PVY necrotic strains that are in a permanent "genetic reviewing-recombination".

Table 1

The classification of necrotic strains of Potato Virus Y (PVY) (after Singh *et al.*, 2008)

Name of necrotic strain	Simiral cod	Description
PVY ^N not recombinant	PVY ^{EU-N} , PVY ^{NA-N} , NA-PVY ^N , PVY ^R , PVY ^{TVN}	Symphoms of tabacco leav, There are'nt isolates that could cause Potato Necrotic Ringspot Tubers Disease
PVY ^{NTN}	EU-PVY ^{NTN} , Eu-PVY ^{NTN} , PVY ^{EU-NTN} , PVY ^{NN} , PVY ^{NA-NTN} , NA-PVY ^{NTN}	Symphoms of tabacco leav, they could cause Potato Necrotic Ringspot Tubers Disease
PVY ^{N-Wi}	PVY ^{N-Wilga} , PVY ^{N-W} , PVYN-Wi-P, PVY ^{N:O}	Strains resulted by recombination, phenotipic express as like the PVY ^N strains but serologic have a reaction similary with PVY ^O

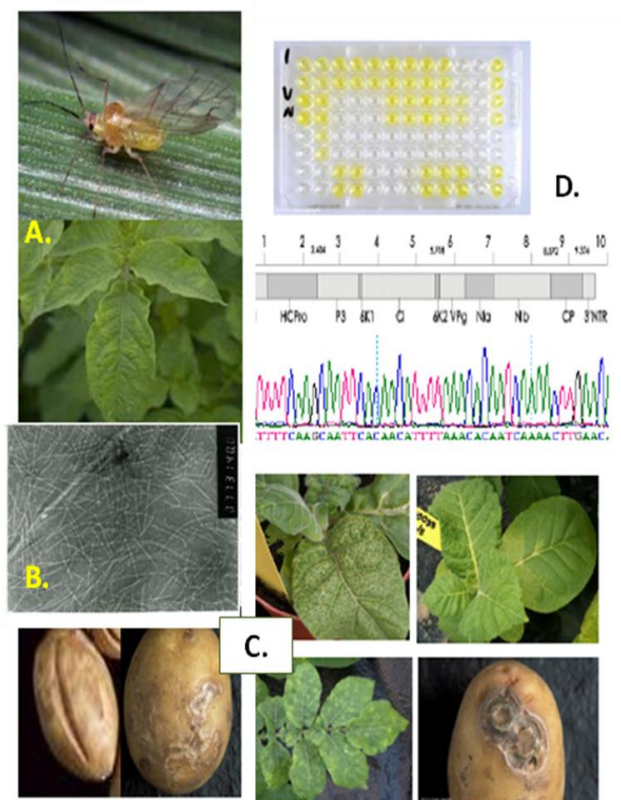


Figure 1 A. *Myzus persicae* - one of the PVY vectors (aphicides are not efficient in controlling PVY spread) (Lancomme C. *et al.*, 2014). B. Potato virus Y (virus suspension) (Cojocaru N. *et al.*, 2009). C. Symptoms induced to test plants after PVY mechanical inoculation: leaf of *Nicotiana tabacum*, tubers of susceptible variety. D. Molecular characterization (partial genome sequencing) (Lancomme C. *et al.*, 2014).

In our country, the disease has been reported sporadically in some susceptible potato varieties, beginning in the fall of 1988, in some experimental plots, further more plant protection inspectorates in the country have reported the presence of this disease in the state and private producers (Cojocaru N. *et al.*, 1995). The study aimed to remind some of the most important problems caused by the spread of necrotic strains and to present several preliminary results

regarding the level of infection with necrotic strains PVY of some varieties cultivated in different geographical regions of Romania in 2014.

MATERIALS AND METHODS

The potato samples were taken from the following geographical regions of our country

(different potato seed producers and farmers): Brasov, Covasna, Harghita, Cluj and Suceava.

The varieties tested in this research work were:

- Christian, Roclas, Productiv (Romanian varieties);
- Bellarosa, Jelly, Red Fantasy, Carrera, Riviera, Hermes, Desiree (foreign varieties).

The analysis was performed following the protocol Clark and Adams (1977) (Clark M.F. and Adam A.N., 1977) and for testing the tubers (taken in 2014) we used sap from tubers and from their sprouts (Bădărău C.L. *et al.*, 2014). Rinsed microplates filled with substrate solution (p-nitrophenyl-phosphate) were incubated one hour and the absorbance values were estimated at 405 nm (A_{405}) using a TecanSunRise reader (software Magellan). The samples that have A_{405} values exceeding the cut-off (two times the healthy control samples average) were considered PVY infected (Bădărău C.L. *et al.*, 2014). The material was tested for 6 viruses (Potato virus Y, Potato

Leaf roll Virus, Potato virus M, Potato virus X, Potato virus S and Potato virus A) and we keep only the PVY infected material, for identify the samples infected with necrotic strains. This biological material was retested using monoclonal antibodies (mAb) or polyclonal (PCA). The microplates were coating with anti PVY-NOC mAb (Bioreba, Switzerland, antibodies that could recognize all the PVY strains excepting the PVY^O) and the virus was detected using alcalin phosphatase (AP) linked to anti -PVY-NOC mAb (Bioreba, Switzerland, specific for the strains Y^N) or linked to anti-PVY-NOC mAb (Bioreba).

RESULTS AND DISCUSSIONS

The results of the tests made to samples taken from 5 geographical areas of Romania (know like potato seed producers) are presented in brief in the figures 2 and 3.

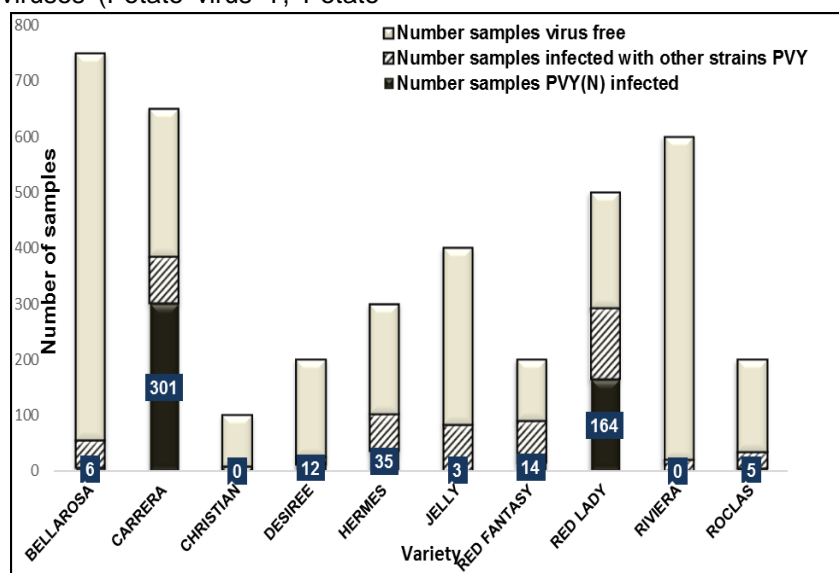


Figure 2 Results regarding the number of infected samples with PVY (necrotic strains) to the material taken in 2014 (several varieties more cultivated by seed potato producers)

As show in these 2 figures, in case of samples taken in 2014, the highest infection level with necrotic strains of PVY was noticed to varieties Carrera and Red Lady, Hermes and Red Fantasy.

The data presented synthetic in figure 2 and 3 will be used in the future for identify favorable and risk areas and improving potato microzoning (based on spatial and temporary assessment of potato virus Y necrotic strains spectrum, the degree of infection with PVY necrotic strains correlated with climate change in Romania). So the source of the samples tested in this study was several geographical areas, different counties (9 producers from Brasov, 11 farmers from Covasna, 5 from Harghita, 6 from Cluj and 5 from Suceava). Regarding the total number of samples tested, 920

samples were taken from Brasov, 1080 from Covasna, 650 from Harghita, 500 from Cluj and 250 from Suceava.

These results are only preliminary, because we have to continue the experiments minimum 3 years. Because of this reason, this paper didn't purpose to make a statistical analysis. Also, the main objective was to estimate some varieties more resistant to PVY (necrotic strains) cultivated in several counties of Romania. These genotypes very resistant in our study (in the conditions of the studied counties in 2014) were the following: Riviera, Bellarosa, Jelly, Roclas, Christian (figure 2).

Regarding the most favorable regions for seed potato producing (in case of varieties taken in this study) we cannot give more results until this

moment because it is necessary to repeat the experiments in the future. However, some preliminary data are presented in figure 2 and 4. In the context of intensify the measures to prevent and control virus Y, the contribution of this paper to the current state of research will result in estimation of PVY spectrum strains spread to

some genotypes grown in our country in order to assess the degree of infection with necrotic strains of PVY to several national and foreign varieties more cultivated in different geographical areas of the country and to identify some potato varieties with high resistance or tolerance to infection with viruses PVY (necrotic strains).

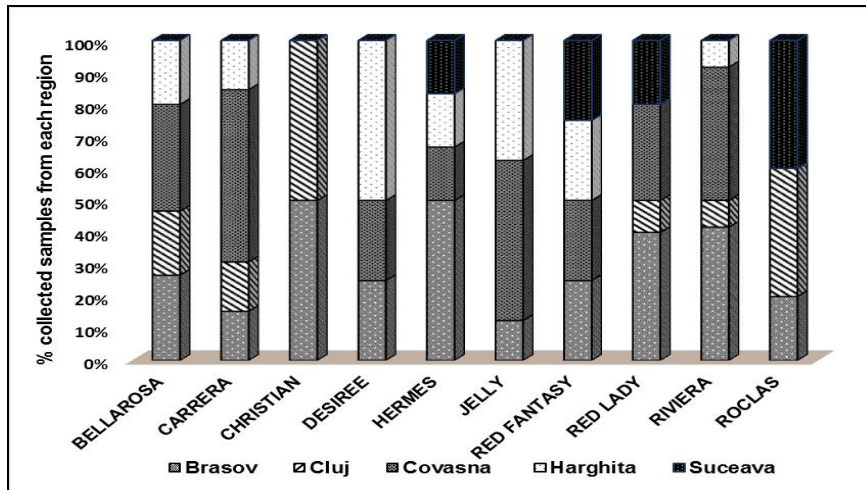


Figure 3 Source of samples tested (the region from that the samples were taken in 2014)

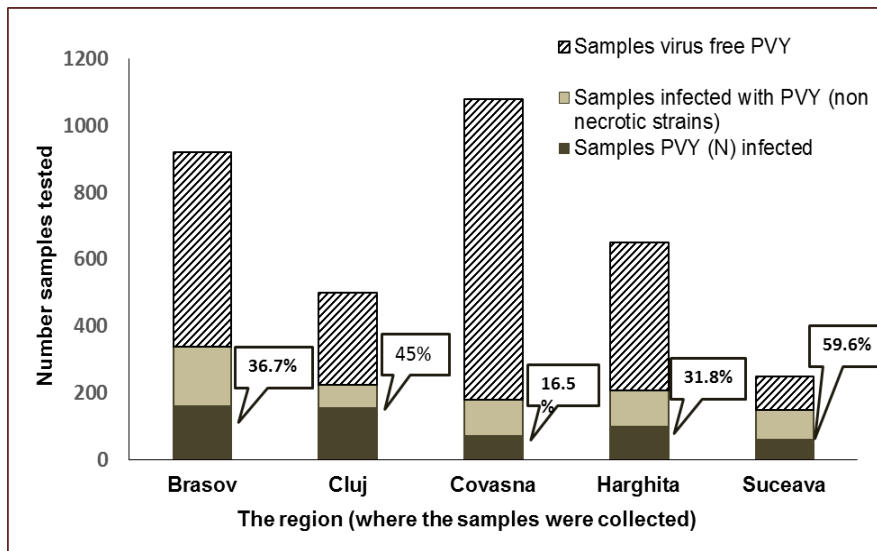


Figure 4 The number of samples infected with PVY and PVY (N) necrotic strains in function of the region (county) from that the material was taken in 2014

CONCLUSIONS

In our country, although it is known that financial damage brought by necrotic strains of PVY are major in case of growing susceptible varieties under favorable conditions both for consumption potatoes (it can become unmarketable) and for seed potato (it will be downgraded or rejected from certification), to date there has not been conducted a comprehensive study on a spatial expansion of the spectrum of these viral strains, study that will contribute to the

development of the control of emerging necrotic strains of potato virus Y.

In this preliminary study, between the varieties tested in 2014 (samples taken from the following counties: Cluj, Suceava, Brasov, Harghita and Covasna) the lowest level of infection with necrotic strains PVY had the following genotypes: Riviera, Bellarosa, Jelly, Christian and Roclas.

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