Analele Universităţii din Craiova, seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series) Vol. XLVI 2016

THE INCIDENCE OF POTATO VIRUS Y (NECROTIC STRAINS) IN SEED POTATO GROWN IN SEVERAL ROMANIAN COUNTIES (PRELIMINARY STUDIES)

Bădărău C.L. [1,2] ***, Rakosy-Tican E.*** [3], ***Aurori A.*** [3], ***Chiru S.C.*** [1], ***Olteanu Gh.*** [1], ***Stefan M.*** [1], ***Ghinea A.*** [1]

[1] *National Institute of Research and Development for Potato and Sugar Beet, Brasov*
[2] *Faculty of Food and Tourism, Transilvania University, Braşov, Romania*
[3] *Faculty of Biology, Babeş-Bolyai University, Cluj-Napoca, Romania*

Keywords: potato virus Y, necrotic strains, potato seed

ABSTRACT

Protective measures of culture against Potato Virus Y necrotic strains (PVY^N) infections, diagnosis and control of this pathogen play an important role in potato seed production technology and multiplication. Also, the choice of resistant varieties to the PVY^N infection could be one of the measures recommended for farmers and producers. Surveys during 2 years (2014, 2015), in five main seed potato growing areas of Romania (Braşov, Covasna, Harghita, Cluj, Suceava), for 10 varieties (Christian, Roclas., Riviera, Carrera, Bellarosa, Jelly, Desiree, Red Fantasy, Hermes and Red Lady), revelead significant differences in PVY^N incidence.The tests confirmed the PVY^N presence in all the regions, with high prevalence of this virus especially for the cultivars Hermes and Carrera and very low spread for the cultivars Riviera and Christian.

INTRODUCTION

The present paper provides an updated information on incidence of PVY in certified seed potatoes (several cultivars) produced in Romania, in five main regions where aphid infestations in potato were previously studied [unpublished data].

Potato virus Y (PVY, *potyvirus* genus, family *Potyviridae*) is one of the most important economically virus of *Solanum tuberosum* L. plants, because his frequency and damaging potential. The virus Y occurs worldwide and the plant production with secondary infection can be diminuated with 33-90%, depending on the variety and the virus strains [2]. DeBokx ans Huttinga (1981) state that the PVY infections can reduce yields 10-80% [4]. Rykbost et al. (1999) [12] reported reduction in yield of number 1 tubers of Russet Norkotah by 12-40%. Similary, a reduction in marketable yield of 65% in Russet Norkotah was reported by Hane and Hamm (1999) [7]. Nolte et al. (2004) studyied the effect of tuber borne PVY infection on Russet Bubank, Russet Norkotah and Shepody and reported a yield loss of 0.18 tones/ha for each 1% increase in PVY infection [10].

PVY 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 [6, 8]. The national certification scheme involves sanitation measures such as monitoring aphid populations, filed inspections associated with visual detection, roguing of infected plants and post-harvest testing. Despite all these protection measures, 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 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.

Potato virus Y (PVY) was described for the first time by Smith in 1931 (UK) and for a long time, PVY isolates were classified according to foliar systemic and local symptoms in three main groups PVY^O, PVY^N, PVY^C, depending on the symptoms induced in *Nicotiana tabacum* and *Solanum tuberosum* varieties [13]. In recent years, PVY isolates were found intermediate between PVY^O and PVY^N groups, because they share symptoms, serological and genomic properties with the two groups [5,9,14]. Thus, 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 the necrotic ring staining of tubers (PNRTD)**.** 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). Being very aggressive, these strains can overcome existing resistance to infection with other strains of potato virus Y (PVY^o and PVY^c) [1]. Due to the fact that this patogen affect the resistance of some potato varieties maybe, there are varieties considered resistant unitll now and could passe into the category of sensitive ones, affecting also the production of the potato in our country.

The study aimed to provide an updated information on incidence of PVY in certified seed potatoes (several cultivars) produced in Romania, in five geographical regions (where aphid infestations in potato were previously studied), in 2014 and 2015.

MATERIAL AND METHOD

Biological material The potato samples were taked from different potato seed producers and farmers, from the following geographical regions of our country: Braşov, Covasna, Harghita, Cluj and Suceava (table 1).

The varieties tested in this research work were:

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

Before planting and after emergence, the biological material was PVY virus free.

Detection of PVY infections

The analysis was performed following the protocol Clark and Adams (1977) [3] and for testing the tubers (taken in 2014 and 2015) we used sap from tubers and from their sprouts. Rinsed microplates filled with substrate solution (p-nitro-phenyl-phosphate) were incubated one hour and the absorbance values were estimated at 405 nm (A_{405}) using a Tecan SunRise 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. 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, Switzerland).

Table 1

Number of PVY and PVY^N infected samples (material collected in 2014 and 2015)

Region	Number samples PVY infected	Number samples PVY^N infected	Number total samples tested
Year 2014			
Brasov	138	86	600
Covasna	260	119	1150
Harghita	212	69	478
Cluj	145	93	320
Suceava	128	44	200
TOTAL	883	411	2748
Year 2015			
Brasov	338	159	920
Covasna	225	154	500
Harghita	178	70	1080
Cluj	207	97	650
Suceava	149	60	250
TOTAL	1097	540	3400

Biotyping and serotyping. Infection was confirmed by mechanical inoculation of the positive samples in *Nicotiana tabacum* (cv. Samsun) under insect proof greenhouse post inoculation. Symptoms on tobacco plantlets were observed daily and recorded at 20-22 days post inoculum. Tobacco samples were ELISA tested to indentify PVY^N using an anti-PVY serum (Bioreba, Switzerland), and anti-PVY(N) specific monoclonal antibodies (Bioreba, Switzerland). The methodology used for preliminary typing the PVY isolates is presented in table 2.

Table 2

Differentiation methodology for typing PVY isolates

Symptoms *Nicotiana tabacum* (cv.Samsun)	Patotip	Serotip	*Group of strains/ Subgroup*	Products PCR [1]*	PINTC tubers*
Mosaic (M)	O	N specific -	O	-	-
		O/C specific +		-	-
Leaf necroses (N)	N	N specific +	N/N	No	-
			N/NTN	No	-
		O/C specific +	N/W	Yes	Yes
			N/W	No	No

*data not shown in this study

Statistical interpretation

Analysis of variance (ANOVA) and Duncan's multiple range test were used to analyze the data.

RESULTS AND DISCUSSIONS

For this study, 3,400 potato samples were tested in 2014 and respectively 2,748 samples in 2015 (table 1). The samples were collected from differents sites of 5 Romanian counties (9 producers from Brasov, 11 farmers from Covasna, 5 from Harghita, 6 from Cluj and 5 from Suceava).

Concerning the **%** PVY infection of the material tested, the lowest value was obtained in Harghita in 2014 (16.5%) and the highest in 2015 in Suceava (64.0%) (figure 2A). On the other hand, percent of infection rates in Harghita in 2015 was 2.6 times higher than in 2014. PVY Infection percentage was higher in Cluj and Suceava in 2015 (figure 2A). On the whole, in both years, there were significant differences depending on the site and area of sampling (figure 1).

There were interesting the closed values obtained for total % PVY infection of the biological material collected in 2014 (32.3%) and 2015 (32.1%) (figure 2A). Very closed values were obtained too, for the total % PVY^N infection calculated from total samples PVY infected and from total samples tested in 2014 (49.2%, 15.9%) and 2015 (46.4%, 15.0%) (figure 2B&C).

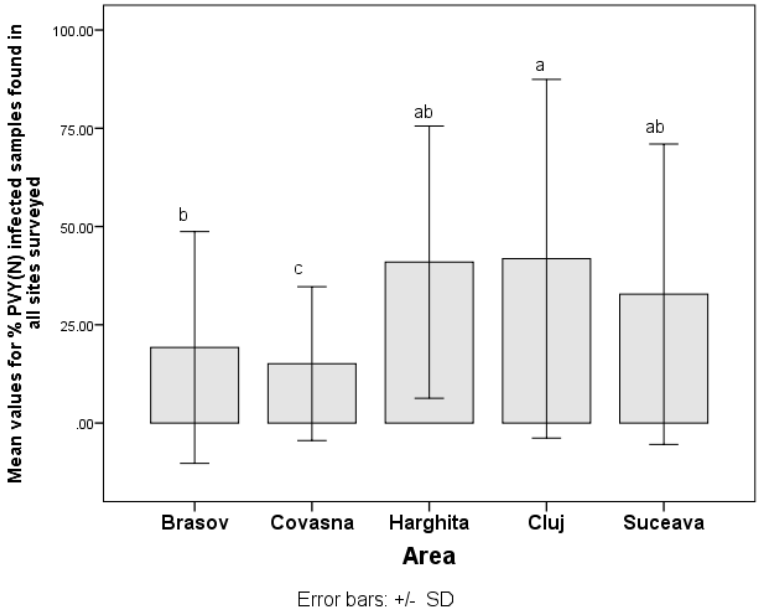

Figure 1. Incidence of PVY^N (preliminary results). Data represent mean values of the % PVY^N infection in all the sites surveyed (material collected in 2014 and 2015). Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test.

Regarding the PVY^N infection level of the material tested in both years, the highest infection level with necrotic strains of PVY was noticed in case of cultivars Carrera, Hermes and Red Lady (figure 3).

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).

These results are only preliminary, because we have to continue the experiments. Untill this moment, as results of our study, in the conditions of the studied counties in 2014 and 2015, the genotypes with low PVY^N infection level were the following: Riviera, Bellarosa, Jelly, and Christian (figure 3).

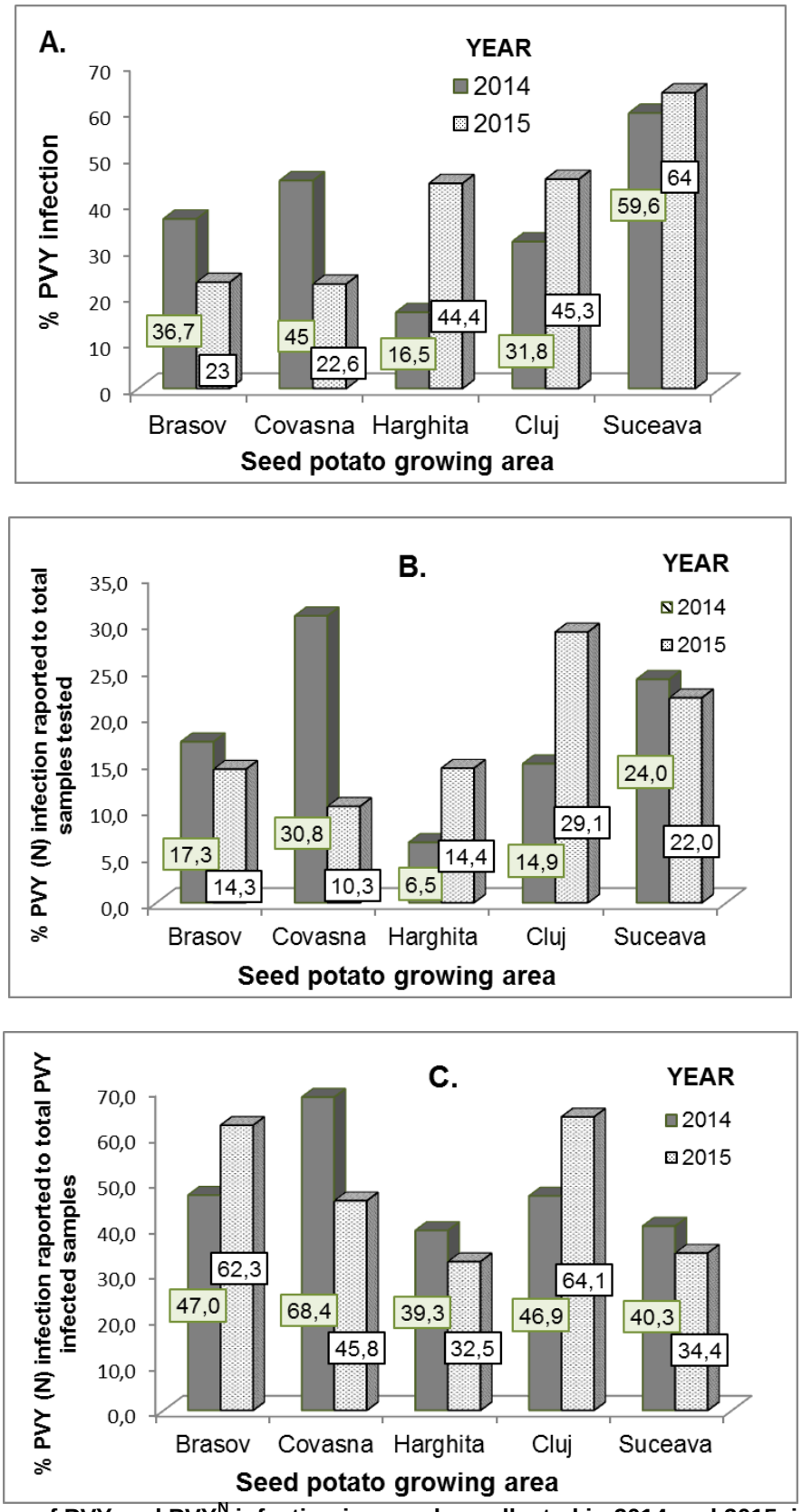

Figure 2. Percentage of PVY and PVY^N infection in samples collected in 2014 and 2015, in five seed potato growing areas in Romania A. % PVY infection; B. % PVY^N infection (samples PVY necrotic infected reported to total material tested); C. % PVY^N infection calculated from PVY infected material (samples PVY^N infected reported to total PVY infected samples).

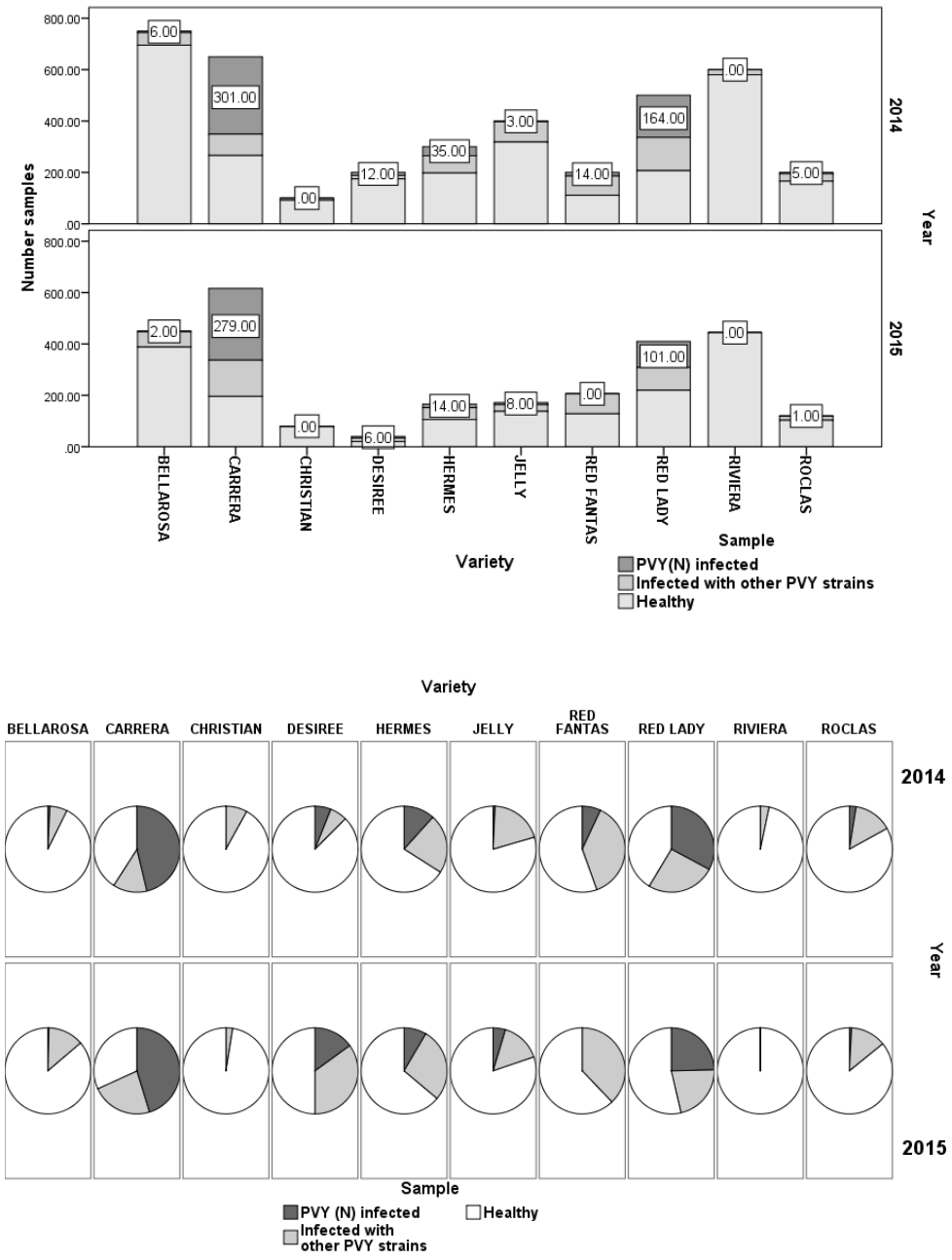

Figure 3. Distribution of healthy, PVY and PVY^{N} infected samples, for the cultivars tested - material collected in 2014 and 2015.

Preliminary typing from a first set of samples collected in 2014

140 samples were keep from the material collected in the post-harvest testing plots in September - October 2014 and were ELISA tested with anti PVY serum and serotype-monoclonal antibodies. Symptoms observed on potatoes from each sample collected were especially severe (severe mosaic and crinkling), or rare, such as leaf necrosis.14 samples were virus negative in all tests. 104 of the 126 positive samples (82.5%) reacted positively only with anti PVY^{N} antibodies (with high ELISA signals for most of them). 12 of the 126 samples (9.5%) reacted positively only with anti-PVY^{O-C} antibodies. Ten samples (7.9%) reacted positively with both anti PVY^{O-C} (high ELISA signals) and anti-PVY^{N} (low ELISA signals) antibodies (table 4). These samples were analyzed in detail. The infection virus from these samples was successfully transferred onto tobacco (*Nicotiana tabaccum* cv. Samsun) and was biologically analysed. Results are summarized in table 3.

Table 3

Analysis of 126 selected samples in 2014: serotyping and biotyping on *Nicotiana tabacum* cv. Samsun

Variety	Number isolates	Serotype	Pathotype*	Preliminary typing
Hermes	28	N	N**	PVY^N
	2	O	N	
Carrera	60	N	N**	PVY^N
	2	O	N	
	7	O+N	N	PVY^N
Red Lady	23	N	N	PVY^N
	3	O	N	
	2	O+N	N	PVY^N
Red Fantasy	2	N	N	PVY^N
	1	O	N	
	1	O+N	N	PVY^N
Desiree	1	N	N	PVY^N
	1	O	O	PVY^O
Total	126	N=104 O=12 O+N=10	N=125 O=1	

* necrotic symptoms on tobacco leaves inoculated

**Symptoms of the pathology caused usually by PVY mosaic, crinkling, necrotic leaves patterns, stunting were forms with increased virulence.

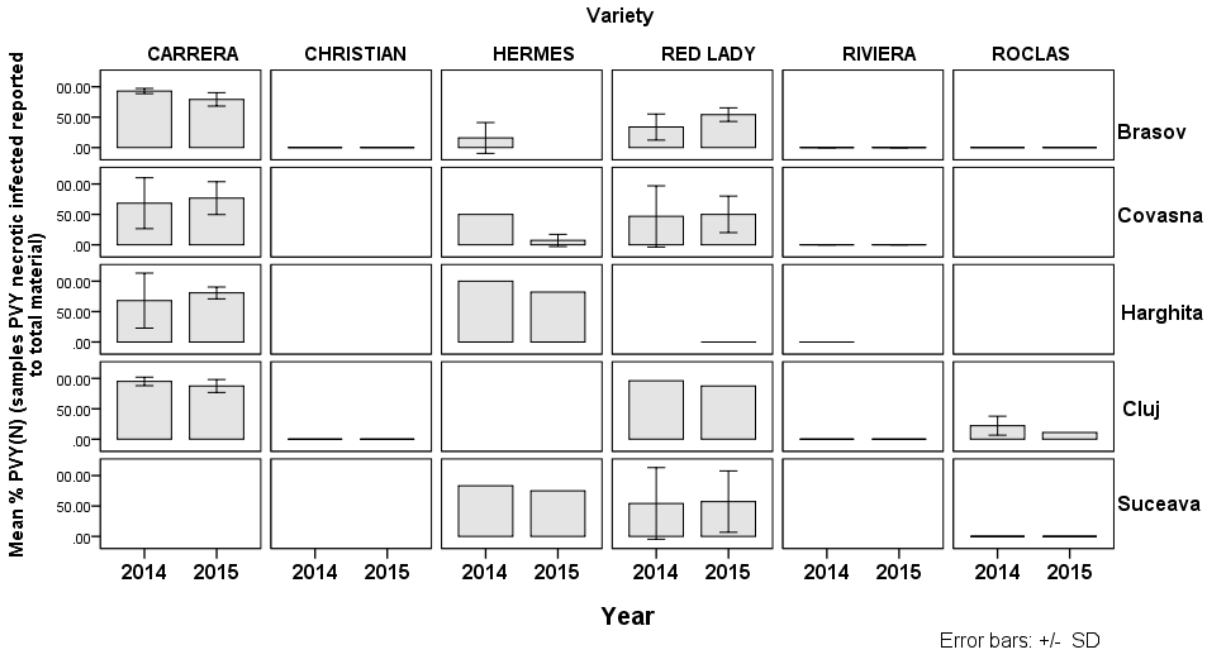

Figure 4. Preliminary results – incidence of PVY^N for the varieties with the lowest and highest level of infection in the studied counties in 2014 and 2015.

Excepting an isolate from cv. Desiree, the inoculation onto tobacco of all the other samples generated PVY^N symptoms, typical for most of them, e.g. a first step of vein clearing rapidly followed by necrotic patterns among which necrosis of the main veins,

associated with bending of some leaves, significant size reduction of most leaves and stunting of the whole plant. Despite the PVY^N like symptoms induced in all the plants, only for several of them there were recorded positive serological PVY^N reactions (table 3).

Regarding the most favorable regions for seed potato producing (in case of varieties tested in this study) we cannot give more results until this moment because it is necessary to repeat the experiments in the future. However, preliminary data regarding the cultivars with the lowest and higher PVY^N infection level (potato seed grown in different sites from 5 Romanian couties) are presented in figure 4.

In the context of intensify the measures to prevent and control the potato 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).

Acknowledgements This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, PN-II-PT-PCCA-2013-4-0452, project number 178/2014 and partially funded by the Romanian grant – MoniCult, project PN-II-PT-PCCA-2013-4-1629.

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 and 2015 (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.

BIBLIOGRAPHY

1. **Boonham, N., Walsh, K., Hims, M., Preston,, S., North, J., Barker, I.,** 2002- *Biological and sequence comparisons of Potato virus Y isolates associated with potato tuber necrotic ringspot disease*. Plant Pathology 51: 117-126.
2. **Cojocaru, N.,** 1987- *Mozaicul Y (Virusul Y al cartofului – Potato virus Y).* In: Protecţia cartofului: boli, dăunători, buruieni, Editura Ceres Bucureşti, p.63 – 67.
3. **Clark, M.F. and Adams, A.N**. , 1997- *Characterization of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant virus*. Journal of General Virology 34: 475-483
4. **De Bokx, J.A. and Huttinga, H.,** 1981- *Potato virus Y.* Descriptions of Plant Viruses No.242. Commonwealth Mycological Institute/ Association of Applied Biologists, Kew, England.
5. **Galvino-Costa, S.B.F., Figueira, A., Camargos, V.V., Geraldino, P.W., Hu, X., Nikolaeva, O.V., Kerlan, C., Karasev, A.,** 2012- *A novel type of Potato virus Y recombinant genome, determined for the genetic strain* PVY^E*.* In: Plant Pathology (2012), 61, p. 388-398

6. **Gray, S., De Boer, S., Lorenzen, J., Karasev, A., Whitworth, J., Nolte, P., Singh, R., Boucher, A., Xu**., **H.** 2010- *Potato virus Y. An evolving concern for potato crops in the United States and Canada.* Plant Disease 94 :1384-1397
7. **Hane, D.C., and Hamm, P.B.,** 1999 - *Effects of seed borne potato virus Y inection in two potato cultivars expressing mild disease symptoms*. Plant Diseases 83: 43-45.
8. **Lacomme C., Kim Davie, Ross Holmes, Jon Pickup, 2014 -** *PVY^{N} Prevalence in Potato Crops: Impact of Strain Competition and Ability to Overcome Plant Resistance Mechanisms*. SASA Edinburgh, UK. The 19 Triennial EAPR Conference, 6-12.07.2014, Brussels, Belgia
9. **McDonald, J.G., and Singh R.P.**, 1996 - *Host range, symptomology and serology of isolates of potato virus Y (PVY) that shared properties with both the PVY^{N} and PVY° strain groups.* American Potato Journal 73: 309-315
10. **Nolte P., Whitwoeth, J.L., Thornton, M.K, McIntosh., C.S.,** 2004 - *Efects of sed borne Potato virus Y on performance of Russet Burbank, Russet Norkotah and Shepody potato*. Plant Diseases 88: 248-252.
11. **Rigotti, S., Gugerli, P.,** 2007 - Rapid identification of potato virus Y strains by one-step triplex RT-PCR. Journal of Virological Methods, 140: 90–94
12. **Rykbost, K. A., Hane, D.C., Hamm, P.B., Voss, R., Kirby ,D.,**1999 - *Effects of seed – borne potato virus Y on Russet Norkotah performance*. American Journal of Potato Research 76: 91-96.
13. **Singh, R.P., Valkonen, J.P., Gray, S.M., Boonharn, N., Jones, R.A., Kerlan, C. , Schubert, J..** 2008 - *Discussion paper: The naming of Potato virusY strains infecting potato.* In: Archives of Virology 153: 1-13
14. **Valkonen, J.P**., 2011 - *Family Potyviridae.* In: *Virus taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses,* A. King, M. Adams, E. Carstens, and E. Lefkowitz, (ed.), Oxford: Elsevier,p. 1069-108