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EVALUATION OF VITAMIN C CONTENT IN SAMPLES FROM TEN POTATO CULTIVARS INOCULATED WITH POTATO VIRUS Y *(Necrotic strains)*

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Abstract

Providing basic nutrition to many people, being a staple food, potato tubers with higher levels of bioactive compounds (as vitamin C) could have a positive impact on the people health. This study aimed to evaluate the behaviour of 10 potato varieties with different L ascorbic acid content after inoculation with potato virus Y necrotic strains (PVY^N). Another goal of this research work was to elucidate the biochemical basis responsible for different reaction to infection with potato virus Y among several varieties which differ in their susceptibility or resistance to this pathogen. The potato cultivars evaluated were: Christian, Roclas, Red Lady, Marvis, Castrum, Brasovia, Hermes, Sante, Riviera and Carrera. The vitamin C content was estimated in the flesh matter only, using an enzymatic method. Significant differences in total ascorbic acid content were observed across the varieties before and after virus inoculation, the variety Hermes showing the highest content (746 mg.kg^{-1} DW) in tubers after inoculation. Excepting the cultivars Christian, Riviera and Sante, which were very resistant and resistant to mechanical inoculation, all the other samples tested presented 48.6 - 100% infected plants.

Key words*: total ascorbic acid content, potato, potato virus Y, necrotic strains.*

INTRODUCTION

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) (Bădărău et al., 2015c). Potato is the third most consumed food, after rice and maize (FAO, 2015) and their tubers are recognized as a good source of carbohydrates, vitamin B_1, B_3 and B_6, potassium, phosphorus and magnesium. It has a moderate content of iron, but its high L ascorbic acid levels promote iron absorption. Potato is rich in essential amino acids. It also contains pantothenic acid, folate and riboflavin (Camire et al., 2009). While 50 years ago more than half of the global annual production output was concentrated in Russia, Poland and Germany, now, around 40% comes from China, India and Russia. China and India have seen a dramatic increase, with both countries doubling their production in the last 20 years (FAO, 2015).

Vitamin C is the most abundant vitamin in potato and it is estimated that about 18% of the recommended daily allowance (RDA) of vitamin C in Australia and 21% in the UK are provided by potatoes (Camire et al., 2009; Cahil et al., 2009). Three main biological functions have been identified for L ascorbic acid enzyme cofactor, free radical scavenger and donor/acceptor of electrons al the plasma membrane. Humans have lose ability to synthesize L ascorbic acid and depend on the diet to acquire the necessary amounts required to maintain good health. Deficiency of the vitamin C could cause the disease scurvy characterized by spots on the skin, spongy gums and bleeding from mucous membranes. Is caused by deficient synthesis of collagen in which L ascorbic acid is cofactor (Camire et

al., 2009; Cahil et al., 2009; Schleicher et al., 2009). Although, nowadays, scurvy is considered rare in developed nations, the vitamin C intake of significant part of the population of some of these countries may be below RDA (80 mg per day in European Union EC) (EC, 2008). About 13% of the population in the USA or 1 in 7 young adults in Canada have been reported to be deficient in vitamin C with certain groups such as smokers, pregnant women and people of low socioeconomic status at a higher risk of deficiency is common. L ascorbic acid is particular important because it can reduce the chelating effect that compound phytic acid has on iron, increasing its bioavailability (Camire et al., 2009).

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 potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicum esculentum)* (Kerlan et al., 2009). PVY in potato received (in the last period) a special 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 (Crosslin et al., 2006; Gray et al., 2010; Karasev et al., 2011).

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 the 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 favourable 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, the PVY necrotic strains can overcome existing resistance to infection with other strains of potato virus Y (PVY^{o} and PVY^{c}) (Singh et al., 2008; Boonham et al., 2002a; 2002b).

The goal of this study was to quantify the levels of L ascorbic acid in 10 potato varieties with different L ascorbic acid content before and after inoculation with potato virus Y necrotic strains (PVY^{N}).

Potato with high increase content of vitamin C could have an important impact on human health, especially in populations where potato is the main staple food crop and therefore, would be of interest to consumers, producers and policymakers.

MATERIALS AND METHODS

Biological material

Ten potato varieties were chosen for this study. The biological material included:

- commercial cultivars such as Riviera, Carrera, Red Lady, Hermes and Sante which represented about 40% of the production area in Romania in 2015 (Bădărău et al., 2015a).
- Christian and Roclas, Romanian varieties very appreciated in Romania for their nutritional quality (data not show)
- Sarmis, Marvis, Castrum, Brasovia (new Romanian varieties).

The health tubers were obtained from the Breeding Department, National Institute of Research and Development for Potato and Sugar Beet Brasov (NIRDPSB Bv). From each variety, 6 pots (with 1 eye pieces) in three repetitions were planted. Plants were grown in 18 cm pots in green house conditions. After emergence, plants have been mechanical inoculated, using a PVY^{N} source (secondary infection Record variety).

After the inoculation, disease symptoms were observed and ELISA tests have been made, in

the aim to confirm the infection. At harvesting, we select 2 tubers from each pot and there were tested 3 samples (4 tubers/samples) for each variety. The other tubers were keep and the percentage of tubers with necrotic symptoms was estimated at harvesting time and later (after 3 months storage at 4-8°C).

Detection of PVY^N infections

The analysis was performed following the protocol Clark and Adams (1977). The absorbance values were estimated at 405 nm (A_{405}) using a Tecan SunRise reader (software Magellan). In the first stage, the material was tested for Potato virus Y (polyclonal antibodies) and only the PVY infected material was used, for identify the samples infected with necrotic strains (PVY^N). This biological material was retested using monoclonal antibodies (mAb) or polyclonal (PCA). The plates 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 alkalin phosphatase (AP) linked to anti–PVY-NOC mAb (Bioreba, Switzerland, specific for the strains PVY^N).

Sample preparation

For the healthy material, composite samples were prepared by pooling tubers. Tubers were peeled with a potato peeler, the flesh of each tuber quartered from stem to bud and one of the quarters sliced. Flesh tissues were dried, ground to a fine powder (using a coffee grinder), stored to -20°C until analysis.

Vitamin C analysis

Dry matter (at 105°C), vitamin C (a spectrophotometric method, L ascorbic acid test kit, Megazyme, Bioreba) were determined on healthy tubers before planting them in the pots. A representative sample of tubers per plot was used. The sample for these analysis was used from each 2 tubers (2 tubers/sample). The characteristics determination was made in 3 repetitions (Bădărău et al., 2015b).

Statistical interpretation

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

RESULTS AND DISCUSSIONS

After the inoculation, about half of plants presented mosaic symptoms on leaves (Carrera, Red Lady and Hermes) or with necrosis on leaves, veins, petioles and stems followed by wilting of leaves (Marvis, Castrum). The first foliar symptoms from primary infections on the leaves have been observed on Hermes, Carrera and Red Lady varieties and later on cv. Castrum and Marvis.

Table 1. Percent of PVY^N infected material and of tubers with necrotic symptoms

Variety	% PVY^N infected plants after inoculation**	% PVY^N infected tubers (with necrotic symptoms)***	
		At harvest	After 3 months from harvest
Riviera	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Christian	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Sante	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Roclas	44.44 ± 10.000	0.00 ± 0.000	8.20 ± 6.000
Brasovia	66.66 ± 0.000	0.00 ± 0.000	8.20 ± 6.000
Marvis	83.33 ± 0.000	5.152± 1.533	34.700 ± 15.000
Castrum	83.33 ± 0.000	7.180± 1.203	34.700 ±15.000
Red Lady	100.00 ± 0.000	15.667 ± 2.887	69.200 ± 10.000
Carrera	100.00 ± 0.000	30.244 ± 15.248	87.633 ± 2.300
Hermes	100.00 ± 0.000	48.267 ± 12.648	98.267 ± 1.700

* Data represents the mean values (3 repetitions, 6 pots for each repetition) ± standard deviation

** ELISA test made after 4 weeks after inoculation (for identify PVY^N infected plants)

*** Tuber symptoms characterized by raised or sunken necrotic lesions, were scored at harvest and after 3 months storage at 4-8°C

In several plants, the virus began to multiply in the leaves six days after PVY^N inoculation. As we observed in other paper (Bădărău et al., 2015b), simultaneously, the virus spread to the stem, followed by the upper, green parts of the plants. In this way, the virus multiplied vigorously in the potato variety Carrera and Red Lady similar phenomena observed to the extremely susceptible variety Hermes, the percentage of infected plants being maximal in these situations. As waited, the virus did not multiply in the cultivars Riviera, Sante and Christian. Excepting these three cultivars, which were very resistant and resistant to mechanical inoculation, all the other varieties presented 44.4 - 100% infected plants.

After 3 months from harvesting, the frequency of tubers with symptoms was between 8.2 - 34.7% for varieties Roclas, Brasovia, Marvis, Castrum and for Red Lady, Carrera, Hermes cultivars this percentage was higher (69.2-98.2%) (Table 1).

The vitamin C percentages (% from dry matter) of tubers planting in the pots were very different. As shown in table 2, these values were significantly low to the varieties resistent and very resistent to the inoculation like cv. Riviera, Christian and Sante compared with the sensible cultivars Hermes, Carrera and Red Lady.

The simple correlation coefficient Pearson revealed significantly higher values regarding the vitamin C content (as compared to the percent of infected material for the most resistents potato varieties) both in healthy tubers (before virus inoculation) and in tubers harvested from PVY^N inoculated plants (table 3). As shown in figure 1(A, B), there is a correlation between the vitamin C content of tubers planted in the pots and the behaviour of inoculated material. So, the variants which started in vegetation with low percentage of vitamin C were resistent to the PVY^N inoculation (Riviera, Christian, Sante, Roclas, Brasovia). Concerning these cultivars, the percentage of tubers with necrotic symptoms visible fast after harvesting and after 3 months from the harvest was 0.0%.

The variants which started in vegetation with high content of vitamin C were sensible to the virus inoculation (Hermes, Red Lady and Carrera) (Figure 1 A,B). In our study, the total vitamin C content in the flesh tissues were investigated in 10 varieties of potato grown under uniform greenhouse cultural conditions. Values reported for this compound contents were, in general, lower to those found in the literature (Hamouz et al., 2007; Mazurczyk, 2001; Kolbe et al., 1997; Han et al., 2004) maybe because of the cultural conditions, especially the soil composition. A previous study also found higher ascorbic acid levels in potatoes grown in basic soil (Burgos et al., 2009).

Significant differences were seen between varieties vitamin C content and for behaviour to PVY^N inoculation (necrotic symptoms on the tubers at harvest and on the material stored 3 months at 4-8^{o}C).

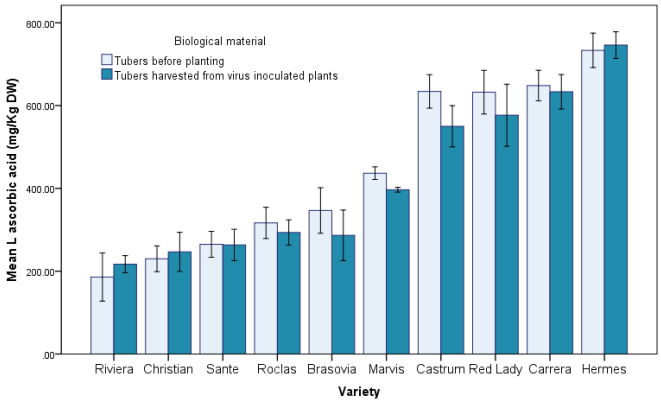
Table 2. Dry matter and vitamin C content of the biological material

	Before inoculation*		After virus inoculation, at harvest**	
Variety	Dry matter (% FW)	Vitamin C content (mg/kg DW) ±SD**	Dry matter (% FW)	Vitamin C content (mg/kg DW) ±SD**
Riviera	24.2±0.050	186.000±58.2068 (f)***	22.2±0.054	217.000±20.663 (e)***
Christian	25.1± 0.130	230.000±31.225(fg)	23.1± 0.132	246.6667±47.258(e)
Sante	24.8±0.070	265.000±82.004 (ef)	24.8±0.057	263.333±37.8593(e)
Roclas	21.8±0.080	316.667±37.859 (de)	20.8±0.0248	293.333± 30.55 (e)
Brasovia	24.6±0.010	346.667±55.075 (d)	22.6±0.016	286.6667± 61.101 (e)
Marvis	23.6±0.049	436.667±15.275 (c)	21.6±0.097	396.6667±5.773 (d)
Castrum	24.4±0.121	634.167±40.324 (b)	22.4±0.141	550.000±500 (c)
Red Lady	23.8±0.020	632.333±52.595(b)	20.8±0.402	576.667±75.055 (bc)
Carrera	23.6±0.140	648.333±36.855 (b)	21.6±0.121	633.333±41.633 (b)
Hermes	24.2±0.080	733.333±43.633(a)	21.2±0.088	746.000±31.187 (a)

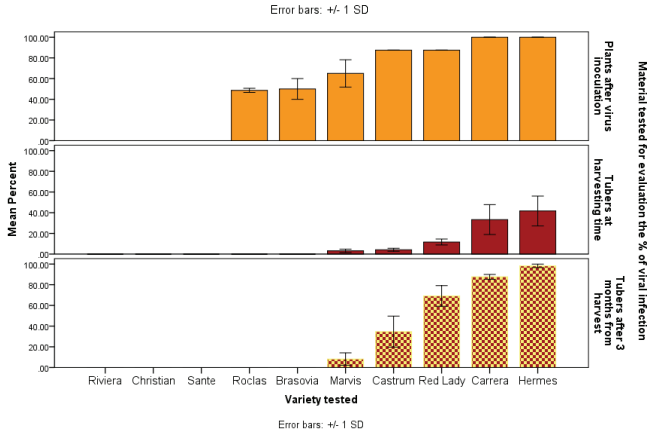
* These analysis were made using tubers before planting them in the pots. Tissue was taken from tubers stored at 6-8^{o}C. Half of every tuber was tested and the other one was planted in the pot.

** For testing vitamin C in tubers harvested from the inoculated plants (6x3 pots for each variety), 2 tubers from each pot were selected and there were tested 3 samples (4 tubers/samples) for each variety.

*** Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test. Abbreviation: FW= fresh weight; DW = dry weight; SD=standard deviation.



A.



B.

Figure 1. Vitamin C content for the material tested before planting (healthy tubers) and at harvesting time (from plants inoculated with PVY^N) (A). The potato cultivars behavior to diseases induced by mechanic inoculation with an isolate PVY^N from Record secondary infected (B).

Table 3. The correlation between content of vitamin C (in tubers, before and after PVY^N inoculation) and the percentage of the infected material at harvest and after 3 months (for 10 varieties tested)

Variables	Statistical indicators	Percent of PVY^N infected tubers (for the 10 varieties tested)	
		at harvest	after 3 months
Vitamin C content (mg/Kg DW) Healthy tubers **before** planting	Correlation coefficient Pearson	**0.742****	**0.886****
	Significance threshold	0.000	0.000
	N	90	90
Vitamin C content (mg/Kg DW) Tubers **harvested** from inoculated plants	Correlation coefficient Pearson	**0.808****	**0.945****
	Significance threshold	0.000	0.000
	N	90	90

** Correlation is significant for $p<0.01$.
N =90 (3 samples x 10 varieties x 3 repetitions)

CONCLUSIONS

The variety and the vitamin C content (% dry weight) of tubers used for the experiment influenced the behaviour of the material after the inoculation with potato virus Y (Y^N strain-variety Record).

The samples with significantly lower vitamin C content (cv. Carrera, Sante and Christian) were resistant to PVY^N inoculation. Thus, after 3

months from harvest, the stored tubers didn't have visible tuber necrotic disease symptoms.

Results of this study show a significant difference between the total vitamin C content of the healthy and PVY^N infected tubers from the varieties tested (cultivars with different behaviour to inoculation with necrotic strain of PVY). However, it must be considered that the results presented in this paper arise from working with only a few of biological material and upper greenhouse growing conditions. Also, extended field trials would be made to confirm our research results.

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