

Behaviour Of Some Potato Varieties With Different L Ascorbic Acid Content To Potato Aucuba Mosaic Virus

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ABSTRACT

The research target was to evaluate the L ascorbic acid content of 10 potato samples (varieties with different resistance to potato aucuba mosaic virus: Armonia, Sevastia, Christian, Temerar, Gared, Marvis, Castrum, Bellaroasa, Carrera, Hermes) and the behaviour of these samples to potato aucuba mosaic virus (PAMV) infection. The L ascorbic acid content was significantly higher to the varieties resistant to PAMV (cv. Armonia and Sevastia). In this case, after 3 months from harvest, the stored tubers didn't have visible tuber necrotic ringspot disease symptoms.

Keywords – Potato Aucuba Mosaic Virus, symptoms, L ascorbic acid, varieties

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I. INTRODUCTION

L ascorbic acid (vitamin C) is the main vitamin in potatoes. Its nutritional contribution is about 40% of daily recommended intake [13]. The content of vitamin C in freshly tubers is in range 10-30mg/100g fresh matter [9,12], whereas according to Brown (2005) [5] the average content 20mg/100g can represent up to 13% of total antioxidant capacity of tuber.

The improvement of identification's techniques of pathogen agents, knowing the biochemical composition of this kind of food, especially the components which could affect its sanitary status, is required for improving the quality of potato. Among the known pathogen agents infecting potato, the viruses produce serious damages to potato quality.

Potato Aucuba Mosaic Virus (PAMV) is transmitted mechanically and by *Myzus persicae* in a nonpersistent manner [7, 8]; aphid transmission requires a helping potyvirus such as PVA or PVY [11]. Nucleotide sequence analysis of the coat protein gene of PAMV indicated that amino acids residues 14 and 16 from the N-terminus have the DAG sequence [11]. This sequence, also found in the coat of potyviruses, is required for aphid transmissibility [11].

Symptoms of PAMV differ depending on virus strains and potato cultivar. Several virus isolates cause yellow leaf flecking, whereas other cause deformation and stunting. Some cultivars develop necrosis in the tubers or sunken patches on the tuber surface; such symptoms occur when tubers are stored at high (20-21°C) temperature. The Potato Aucuba Mosaic virus symptoms resemble those of Potato Mop-Top virus; both viruses cause necrosis on the tuber surface, but those of PAMV are less clearly patterned than the necrotic rings caused by PMTV [11].

These are variable but leaf symptoms include occasional bright yellow spots to more extensive flecking, blotching and mottling, necrotic spots between the veins, top necrosis and stunting. Tuber symptoms of external and internal necrosis and also net necrosis occur during storage at high temperature (20-24°C), but only in some cultivars. Surface symptoms may resemble those caused by Potato mop-top virus (PMTV).

Aphids (*Myzus persicae*) in a non-persistent manner from plants which are co-infected with Potato virus A or Potato virus Y. Also mechanically including by plant-to-plant contact [10] is another transmission modality.

This pathogen reported in European countries has been identified also in experimental plots of Bârsa depression- Braşov county. In the last

10 years on few varieties sporadically tubers with superficial necrotic spots or bows occurred. Firstly these are fairly protruding and light brown, then later becoming fairly deep and brown-black and the skin could be cracked (normal after harvesting).

The research work target was to estimate the L ascorbic acid content of several potato (*Solanum tuberosum* L.) tubers cultivars (varieties with different susceptibility or resistance to PAMV). Another purpose of this research was to evaluate the behaviour of 10 potato varieties to the disease induced by PAMV and to estimate the correlation between this behaviour and the vitamin C content of the tubers planted for the experience.

Until now, the quality of potato has been judged practically only from the aspect of cooking quality and taste, while the content of biologically important substances as L ascorbic acid has been overlooked. Increased demands on quality indicate that the vitamin C content will become of greater importance not least concerning the use of potatoes in the processing industry.

II. MATERIAL AND METHODS

2.1. Potato material

The positive and negative controls were used from the virus collection of the National Institute of Research and Development for Potato and Sugar Beet Brasov. Varieties tested:

- Christian, Sevastia, Gared, Armonia, Marvic, Castrum, Temerar (roumanian cv.)
- Carrera, Hermes, Bellarosa (foreign cv.)

Our researches regarding the behaviour of these varieties were done in green house conditions. From each variety, 8 pots (with 1 eye pieces) were planted. Plants were grown in 18 cm pots and were maintained at 18-22°C with 14 hour day length. After emergence, plants have been mechanically inoculated, using an PAMV infected material (Carrera variety). After the inoculation, disease symptoms were observed and ELISA tests have been made. The infection of this material was confirmed by using antiserum from Bioreba (Switzerland). The percentage of tubers with necrotic symptoms was estimated at harvesting time and later (after 3 months).

The evaluation of foliar symptoms of the plants was observed in addition with the evaluation of the photosynthetic pigments.

2.2. Quality characteristics

Dry matter (thermoventilated oven at 105°C), vitamin C (enzymatic kit method, L ascorbic acid EnzyPlus from Loewe, Germany) were determined on healthy tubers before planting them in the pots. For vitamin C analysis we used a

Spectronic Genesys 5 spectrophotometer (Milton Roy Company, USA). We choosed a representative sample of tubers per plot. The sample for these analysis were choosed from each 2 tubers (2 tubers/sample). The quality characteristics determination was made in four repetitions.

2.3. Material testing by DAS ELISA

A press with smooth roles was used for preparation leaf samples. For the tuber testing, the sap was extracted, diluted and dispensed directly into the plate using the extractor Microlab 500B/C (Hamilton). We tested sprouting tubers after natural break of dormancy, when the sprouts were 2-3mm long. Microplates-NUNC microplates were coated with antibodies for overnight incubation in the refrigerator. The analysis was performed following essentially the protocol described by Clark and Adams (1977). We used 100 µl from each reactives solutions in each well of the plate [1-3, 6]. All experiments were repeated four times. Rinsed microplates were filled with substrate solution (p-nitrophenylphosphate) incubated 60 minute and the absorbance values were estimated at 405 nm (A_{405}) on TecanSunRise reader (software Magellan). The samples having A_{405} values exceeding the cut-off (two times the average of healthy control samples) were considered virus infected.

2.4. Statistical analysis

Each set of comparable assay was conducted at the same time and with the same bulk sample. Analysis of variance (ANOVA) and Duncan's multiple range test were used to analyze the data. In the aim to illustrate the precision of the mean we use confidence interval (CI).

III. RESULTS AND DISCUSSION

After mechanical inoculation, about all of tested plants presented mosaic symptoms on leaves, associated with crinkling top leaves (Bellarosa, Sevastia, Marvis, Gared and Christian) or with necroses and necroses on leaves, veins, petioles and stems followed by wilting of leaves (Temerar, Castrum, Carrera and Hermes).

In most of the plants the virus began to multiply in the inoculated leaves five to six days after inoculation, at the time when the first local lesions appeared. We evaluated the foliar symptoms from primary infections in a greenhouse conditions.

The virus then spread to the stem, followed by the upper, green parts of the plants and the roots at the same time. The virus multiplied hardly in the potato cv. Hermes and Carrera similar phenomenon observed to the extremely susceptible variety

Temerar, the percentage of infected plants being maximal in these situations (table 1).

Table 1. The frequency of tubers with potato aucuba mosaic virus (PAMV) symptoms

Variety	% plants infected after inoculation*	% tubers with symptoms**	
		At harvest	After 3months
Armonia	0 (0/8)***	0	0,0
Sevastia	50 (4/8)	0	0,0
Christian	50 (4/8)	3,3	6.6
Carrera	100 (8/8)	4	8,0
Gared	62,5 (6/8)	4,5	9,0
Marvis	87,5 (7/8)	4	12,0
Bellarosa	62,5 (6/8)	0	22,2
Temerar	100 (8/8)	10	60,0
Castrum	87,5 (6/8)	53,1	87,5
Hermes	100 (8/8)	62,5	100

* ELISA test - 3 weeks after inoculation

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

*** In the brackets, the first number represents nr of plants found infected after inoculation, the following one represents the total number of plants inoculated for every variety.

Table 2. The biochemical analysis results (tubers*)

Variety	Dry matter (% f.w.)	Acid L ascorbic (mg/100g fresh weight) ±SD**
Armonia	21.2	21.341± 0.077 a***
Sevastia	20.1	14.540±0.111 g
Christian	19.8	14.746 ±0.077 f
Carrera	20.6	14.322 ±0.075 h
Gared	19.6	17.148 ±0.148 c
Marvis	22.4	15.854 ±0.100 d
Bellarosa	20.8	14.180 ±0.137 h
Temerar	21.8	15.263 ±0.140 e
Castrum	22.6	19.321 ±0.279 b
Hermes	22.2	13.928 ±0.115 i

* These characteristics were made to the tubers before planting them in the pots. Tissue was taken from tubers stored at 6-8°C six weeks after harvest. Half of every tuber was tested and the other one was planted in the pot.

** Mean values for 4 repetitions ± standard deviation. The L ascorbic acid content (mg/100g fresh matter) was determined using the enzymatic kit Lascorbic acid EnzyPlus and the protocol recommended by Loewe (Germany).

*** Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test.

Abbreviation: f.w. = fresh weight; S.D.=standard deviation.

As expected, the virus did not multiply in the highly resistant cv. Armonia and Sevastia. Excepting cv. Armonia, Christian and Sevastia which were very resistant and middle resistant to mechanical inoculation, all the other varieties presented 62.5-100% infected plants.

At harvesting, symptoms could be indentify on the tubers from all the other varieties, excepting the cv. Carrera, Temerar and Castrum. Concerning the other varieties, the appearance and evolution of symptoms on tubers is going on imediatly after harvesting.

After 3 months, the frequency of tubers with symptoms was between 6.6-22.2% for varieties Christian, Gared, Marvis, Carrera, Bellarosa and for Temerar, Castrum, Hermes varieties this percentage was between 60-100% (table 1). The vitamin C content (mg/100g fresh weight) of tubers planting in the pots were very different.

As shown in table 2, these values were significantly high to the varieties resistant and very resistant to the inoculation like cv. Armonia, Sevastia, Christian and compared with the sensible cultivars Hermes, Temerar and Carrera.

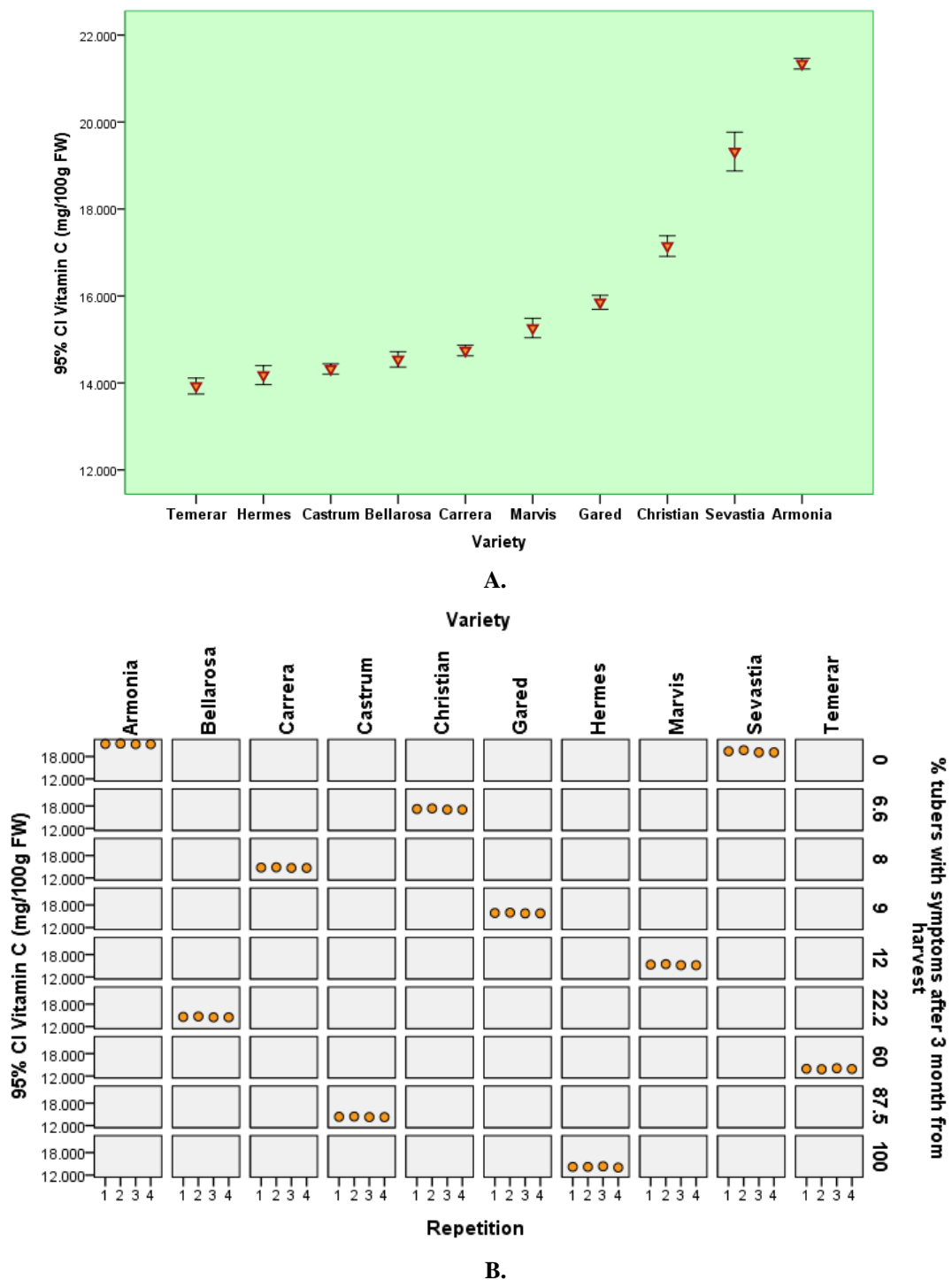


Fig. 1. Vitamin C content (mg/100g fresh weight) for the material planted in pots function on the variety (A) and on the behaviour to the Potato Aucuba Mosaiv Virus (PAMV) induced by mechanic inoculation (B). 95% CI= 95% confidence interval of the difference; (Error bars show 95% CI of mean).

It is a strong correlation between the vitamin C content of tubers planted in the pots and the behaviour of inoculated material to Potato Aucuba Mosaic Virus (as seeing in table 3).Symptoms ranged from mild (yellow markings on leaflets and no distortion or stunting of the plant or

of the leaflet) on plants from cvs. Gared, and Hermes to severe (stunting and mopping of most of stems) on plants of cvs. Carrera and Temerar.

So, the variants wich started in vegetation with high percentage of vitamin C were resistant to the inoculation (Armonia, Sevastia, Christian).

Concerning these cultivars, the percentage of tubers with PAMV symptoms visible immediately after harvesting and after 3 months from the harvest was significantly lower (0.0%-3.3%) comparatively with the other varieties. Symptoms of potato tuber infection on roumanian varieties visible after 3 months from the harvest of the inoculated material

are visible in the figure 2. The behaviour of cultivars Hermes, Temerar and Carrera (which started in vegetation with lower vitamin C content) was different. So, after 3 months from harvesting the inoculated plants, the percents of tubers with PAMV symptoms were the highest (60-100%) (figure 1A&B).

Table 3. The correlation between the level of vitamin C, the variety and the behaviour of samples to PAMV

	Vitamin C (mg/100g FW)	Variety	% of symptoms after 3 months from the harvest
Vitamin C (mg/100g FW)	1	-0.861**	-0.614**
Variety	0.861**	1	0.889**
% of symptoms after 3 months from the harvest	-0.614**	-0.614**	1

** Correlation is significant for $p < 0.01$.

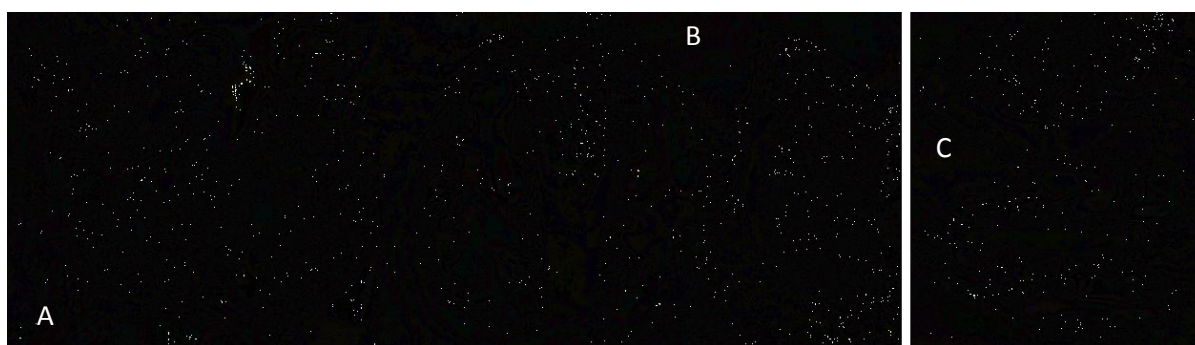


Fig. 2. Symptoms of potato Aucuba mosaic virus (PAMV) on roumanian varieties after 3 months from the harvest of the inoculated material (A-Hermes, B-Gared and C-Temerar).

IV. CONCLUSION

The variety and the vitamin C content (mg/100g fresh material) of tubers used for the experiment influenced the behaviour of the material after the inoculation with potato Aucuba nosaic virus.

Excepting the cultivars Armonia, Sevastia and Christian, wich were very resistant and resistant to mechanical inoculation, all the other varieties presented 62.5-100% infected plants. The first symptoms on the leaves have been observed on Hermes, Temerar and Carrera varieties and later on cv. Castrum, Marvis, Gared, Bellarosa At harvesting, Potato Tuber Ring Necrotic Disease symptoms could be indentified on the tubers from all the other varieties, excepting the cv. Armonia, Christian and Bellarosa. Concerning the other varieties, the appearance and evolution of symptoms on tubers is going on imediatly after harvesting.

The samples with significantly higher vitamin C content (cv. Armonia and Sevastia) were resistant to PAMV inoculation. In this case, after 3

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

The research work for estimate the influences of vitamin C content on the behaviour of several potato inoculated with PAMV awaits further investigation, because the efectiveness of vitamin C in plants is often influenced by the presence of other compunds (bioflavonoides, flavone rutin and flavonol quercetin beeing the main effective ones).

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