

BEHAVIOR OF SEVERAL POTATO VARIETIES WITH DIFFERENT STARCH CONTENT TO POTATO TUBER NECROTIC RINGSPOT DISEASE

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Abstract: *The goal of this research was to evaluate the behavior of 10 potato varieties (with different starch content) after mechanical inoculation with a PVY^{NTN} isolate. Excepting the varieties Christian, Riviera and Bellarosa, which were very resistant and resistant to this pathogen, all the other varieties presented 62.5-100% infected plants. After 5 months from harvesting, the frequency of tubers with necrotic symptoms was between 2.2-27.4% for varieties Bellarosa, Jelly, Roclas and 62.6-97.8% for Desiree, Productiv, Red Lady, Carrera and Hermes. The tubers from samples with significantly higher starch content (varieties Riviera and Christian), after harvest and storage, didn't have visible tuber necrotic disease symptoms.*

Key words: *potato virus Y, starch content, varieties, necrotic strain.*

1. Introduction

Potato are available all around the world, however the specific acceptance is different between countries and populations subgroups (e.g. social classes and age groups). Having a high consumption in Romania, the potato has an important position as a basic food. Also, the interest for this vegetable is increasingly for the consumer, nutritional medicine and food processing industry (e.g. potato crisps production, bakery industry). Next to freshly boiled or steamed potatoes, the Romanian consumers request more and more potato products.

The improvement of identification's techniques of pathogen agents, knowing the biochemical composition of this kind of food, especially the components that 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 virus Y (PVY) (*Potyviride*) is one of the most important potato's viruses [2], [4], [6], [14]. High PVY level can cause stand loss, reduced yields, undersized tubers and reduced quality [4], [8]. Over the past 20 years, PVY has become an increasingly serious constraint to seed potato production in the world [2], [7], [11]. Thus, the efforts to control PVY are essential when producing potatoes for market or seed.

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PVY induced symptoms can be diverse and depend on the cultivar and the virus strain. Potato virus Y^{NTN} (PVY^{NTN}) is a very aggressive and virulent strain belonging to the necrotic group of potato virus Y which causes the potato ringspot necrotic disease (PTRND) [3], [7], [13]. Several researchers consider PVY^{NTN} as sub-strain of PVY^N and the identification of non-recombinant PVY^N inducing PTRND revealed that the recombinant structure of the genome is not a necessary prerequisite for PTRND phenotype [7], [12], [15].

The potato tuber ringspot necrotic disease (PTRND) reported in many countries has been identified also in some experimental plots of Bârsa depression-Brasov county. In the last 20 years, on few varieties, sometimes, tubers with superficial necrotic ring 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 (usually, after harvesting).

Potatoes are a good source of valuable proteins and carbohydrates (higher in quality than those of others vegetable source), but regard to the high water content, the nutrient density is low [1], [9], [10]. Available carbohydrates are present both as starch or free sugars, whereas non-available compounds counted among dietary fibers. Concentration of starch varies widely with respect to genotype and environment. Raw potato starch has a high amount of non-digestible units, but gelatinized starch is easily available and the content of resistant starch in potato food is prevailing low. Increased demands on potatoes quality indicate that the starch content will become of greater importance not least concerning the use of potatoes in the processing industry.

The aim of the present work was to compare the starch content of PVY^{NTN} inoculated and infected plants (primary infection) from several *Solanum tuberosum*

L. varieties, which differ in their susceptibility or resistance to this pathogen. Another purpose of this research was to evaluate the behavior of 10 potato varieties to the disease induced by PVY^{NTN} and to estimate the correlation between this behaviour and the starch content of the tubers planted for the experience.

2. Material and methods

Plant material. The positive, negative controls, the material used in experiments (virus free) and the virus source used for inoculation were obtained from the collection of the Institute for Research and Development of Potato and Sugar Beet, Braşov, Romania. *Solanum tuberosum* L. varieties tested in these experiments were the following:

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

The experiments regarding the behavior of these varieties after inoculation were done in green house conditions. From each variety, 8 pots (with 1 eye pieces) were planted in three repetitions. Plants were grown in 18cm pots and were maintained at 18-22°C with 14 hour day length. These plants were maintained under greenhouse conditions for 90 days after planting (DAP) and each pot was allocated to an experimental unit. Before the inoculation and after 45 DAP the presence of PVY was tested by ELISA.

After emergence, plants have been mechanically inoculated, using a PVY^{NTN} isolate (Hermes variety). After the inoculation, disease symptoms were observed and ELISA tests have been made (3 weeks from inoculation). 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 5 months from harvesting).

ELISA test. 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. The analysis was performed following essentially the protocol described by Clark and Adams (1977) [5]. The samples having A_{405} values exceeding the cut-off (two times the average of healthy control samples) were considered virus infected.

Dry matter and starch content. Dry matter (thermoventilated oven at 105°C), starch content (enzymatic kit method, Starch EnzyPlus from Loewe, Germany) were determined on healthy tubers before planting them in the pots. Starch content was analyzed by two methods: by using specific gravity based on the weight of the potatoes weight in water. The underwater weight was converted to starch percentage according to the EC Regulation No 1949/95. We choose a representative sample of tubers per plot. The other method was the Megazyme total starch assay kit (Loewe, Germany, ICC Standard method No. 168). The sample for these analysis were choose from each 2 tubers (2 tubers/sample). The chemical and physic-chemical tests were made in three repetitions.

Statistical analysis. Each set of comparable assay was conducted at the same time and with the same bulk sample. Data were analyzed by ANOVA and Duncan's Multiple Range Test and scored as significant if $P < 0.05$ (IBM SPSS Statistics software).

3. Results and Discussion

After mechanical inoculation, about all of tested plants presented mosaic symptoms on leaves, associated with crinkling top leaves (Red Lady, Desire, Jelly and Hermes) or with necroses and streak on leaves, veins, petioles and stems followed by wilting of leaves (Carrera, Roclas and Productiv). Our results were similar with the former investigations of Le Romancer and Kerlan [13] that Y^{NTN} is more virulent than Y^N strains. In most of the plants the virus began to multiply in the inoculated leaves four to five 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 vigorously in the potato cv. Carrera and Red Lady similar phenomena observed to the extremely susceptible variety Hermes, the percentage of infected plants being maximal in these situations (table 1). As expected, the virus did not multiply in the highly resistant cv. Riviera and Christian. Excepting these two varieties and Bellarosa 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 identified on the tubers from all the other varieties, excepting the cv. Riviera, and Christian. Regarding the other varieties, the appearance and evolution of symptoms on tubers is going on immediately after harvesting.

Table 1

*Frequency of PVY infected plants (after mechanical inoculation) and of tubers with potato tuber ringspot necrotic disease (PTRND) symptoms**

| Variety | % plants infected with PVY after inoculation** | % tubers with necrotic symptoms*** | |
|-----------|--|------------------------------------|-----------------------------|
| | | At harvest | After 5 months from harvest |
| Riviera | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 |
| Christian | 16.67 ± 9.433 | 0.00 ± 0.000 | 0.00 ± 0.000 |
| Bellarosa | 16.67 ± 9.433 | 2.20 ± 1.905 | 2.20 ± 3.810 |
| Roclas | 50.00 ± 0.000 | 3.30 ± 0.000 | 13.60 ± 7.967 |
| Jelly | 58.33 ± 7.121 | 4.50 ± 0.000 | 27.48 ± 4.446 |
| Desiree | 79.17 ± 14.434 | 8.17 ± 3.176 | 62.60 ± 11.588 |
| Red Lady | 87.5 ± 0.000 | 18.27 ± 7.159 | 88.41 ± 1.576 |
| Carrera | 100.00 ± 0.000 | 53.10 ± 0.000 | 93.45 ± 4.076 |
| Productiv | 87.5 ± 0.000 | 12.30 ± 9.168 | 89.03 ± 2.656 |
| Hermes | 100.00 ± 0.000 | 59.120 ± 5.427 | 97.77 ± 2.113 |

* Data are mean values (3 repetitions, 8 pots for each repetition) ± standard deviation

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

*** Tuber symptoms characterized by raised or sunken necrotic lesions, were scored at harvest and after 5 months storage at 4-8°C

Table 2

Dry matter and starch content of the biological material used for estimate the behaviour of inoculated plants with potato virus Y^{NTN} (PVY^{NTN})*

| Variety | Dry matter (% FW) | Starch (g/100g FW) ±SD** |
|-----------|-------------------|--------------------------|
| Riviera | 23.2 ± 0.095 | 21.128 ± 0.829 (a)*** |
| Christian | 24.1 ± 0.103 | 19.790 ± 0.115 (b) |
| Bellarosa | 23.8 ± 0.057 | 17.867 ± 0.023 (c) |
| Roclas | 20.8 ± 0.058 | 16.777 ± 0.656 (d) |
| Jelly | 23.6 ± 0.061 | 16.734 ± 0.180 (de) |
| Desiree | 22.6 ± 0.049 | 16.525 ± 0.318 (de) |
| Red Lady | 23.4 ± 0.121 | 16.484 ± 0.356 (de) |
| Carrera | 21.8 ± 0.062 | 15.967 ± 0.077 (ef) |
| Productiv | 22.6 ± 0.114 | 16.109 ± 0.541 (def) |
| Hermes | 22.2 ± 0.028 | 15.783 ± 0.104 (f) |

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

** Mean values for 3 repetitions ± standard deviation. The starch content (mg/100g fresh matter) was evaluated using the enzymatic kit Starch EnzyPlus and the protocol recommended by Loewe (ICC Standard method No. 168, Germany).

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

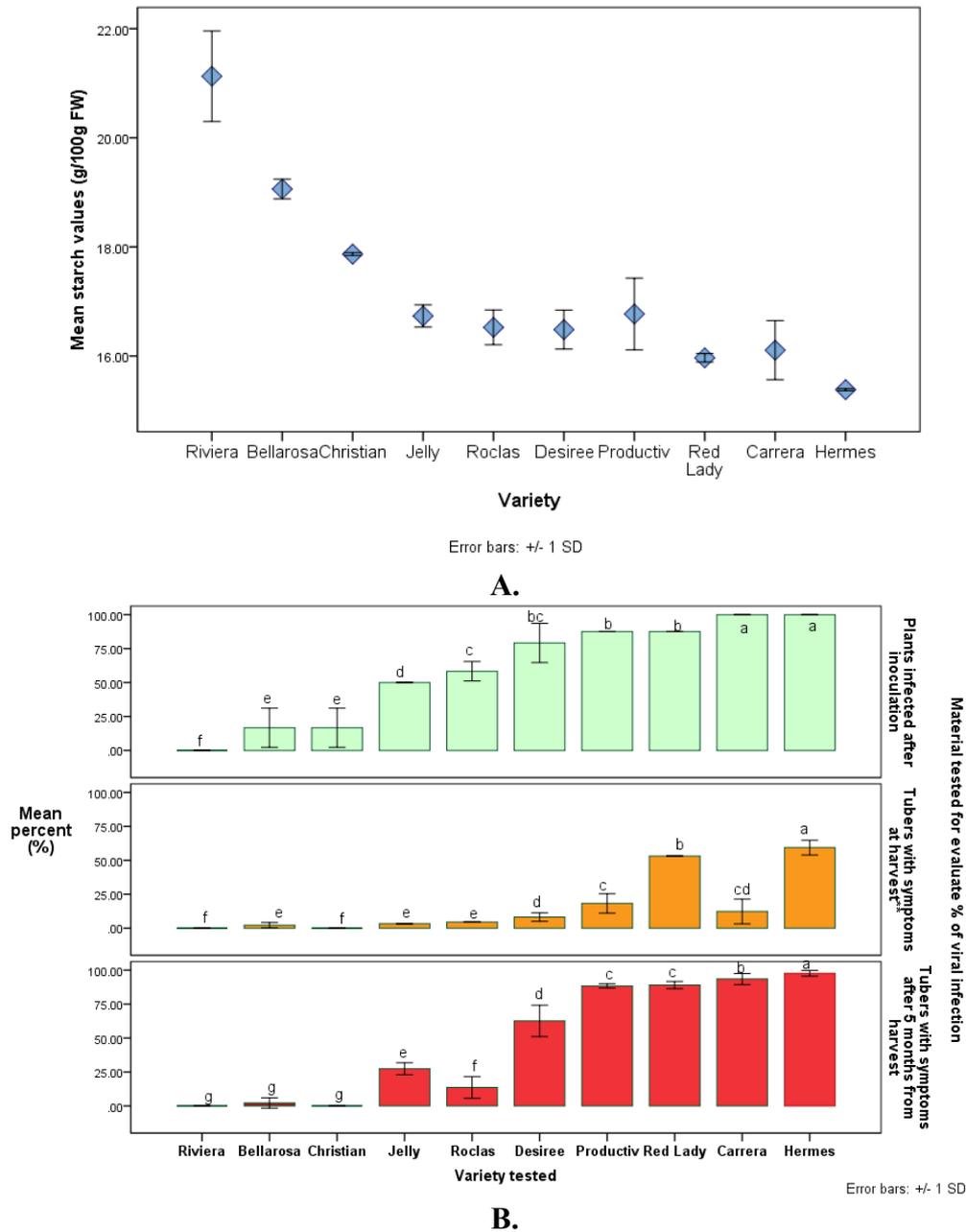


Fig. 1. Starch content (mean values, 3 repetitions, starch content evaluated by gravimetric method in % fresh weight) for the material planted in greenhouse (A). The potatoes cultivars behavior to the Potato Tuber Ringspot Necrotic Disease (PTRND) induced by mechanic inoculation with an isolate PVY^{NTN} from Hermes secondary infected (B). Data = means (n=3). Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05).

After 5 months, the frequency of tubers with symptoms was between $2.2\% \pm 3.810 - 27.366\% \pm 4.446$ for varieties Bellarosa, Jelly, Roclas and for Desiree, Productiv, Red Lady, Carrera, Hermes varieties these percentage values were between $62.600\% \pm 11.58 - 97.767\% \pm 2.113\%$ (table 1).

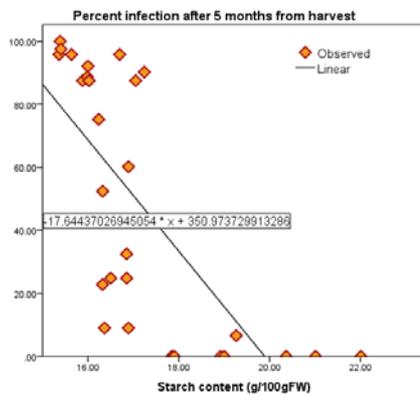


Fig. 2. The tubers starch content level's influence on the percent of tubers with PTNRD symptoms after 5 months from harvest ($R^2=0.524$).

The starch percentages (% from fresh matter) of tubers planting in the pots were very different. As shown in table 2 and in figure 1, these values were significantly high to the varieties resistant and very resistant to the inoculation like cv. Riviera, Christian and Bellarosa compared with the sensible cultivars Hermes, Carrera, Red Lady and Productiv. On our opinion, there

are strong correlations between the starch content of tubers planted in the pots and the behaviour of inoculated material to Potato Tuber Ringspot Necrotic Disease. Also, the variants which started in vegetation with high percentage of starch were resistant to the inoculation (Riviera, Christian and Bellarosa) (fig.1). Concerning these cultivars, the percentage of tubers with PTNRD symptoms visible immediately after harvesting and after 5 months from the harvest was significantly lower ($0.0\% - 2.2 \pm 3.810\%$) comparatively with the other varieties.

Regarding the correlation between the starch content and the PVY percentage infection of inoculated material (% of plants after inoculation, % of tubers with PTNRD symptoms after 5 months from harvest), the simple correlation coefficient Pearson had significantly values. As was checked using simple correlation coefficient Pearson, there were significant differences between starch content values recorded in the experimental variants. However, the regression curve (fig. 2) shows an insignificantly relationship between the starch content and the % of tubers with PTNRD symptoms, the regression coefficient having a very low value ($R^2=0.524$).

Table 3

The correlation between tubers starch content and percentage of different biological infection (% tubers with PTNRD symptoms after 5 months from harvest, % PVY infected plants after inoculation)

| | | Starch content* (% FW, different varieties) |
|--|---------------------------------|---|
| Percent of tubers with necrotic symptoms after 5 months from harvest (%) | Correlation coefficient Pearson | -0.853** |
| | Significance threshold | 0.005 |
| | N | 30 |
| Percent of infected plants after PVY ^{NTN} inoculation (%) | Correlation coefficient Pearson | -0.566** |
| | Significance threshold | 0.001 |
| | N | 30 |

* Starch content evaluated by gravimetric method (% fresh weight). Analysis were made before planting the tubers (N=10 varieties x 3 repetitions).

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



Fig. 3. Symptoms of potato tuber ringspot necrotic disease (PTNRD) on two Romanian varieties after 5 months from the harvest of the inoculated material: Roclas (A); Productiv (B).

The behaviour of cultivars Red Lady, Carrera and Hermes (which started in vegetation with lower starch content) was different. So, after 5 months from harvesting the inoculated plants, the percents of tubers with Potato Tuber Ringspot Necrotic Disease symptoms were the highest ($89.033\% \pm 2.656$, $93.447\% \pm 4.076$ and $97.776\% \pm 2.116$) (fig. 1). Symptoms of potato tuber ringspot necrotic disease on the Romanian varieties after 5 months from the harvest of the inoculated material are visible in figure 3.

4. Conclusions

These preliminary studies showed that the variety and the starch content (% fresh weight) of tubers used for these experiments influenced the behavior of the material after the inoculation with PVY^{NTN} (source: secondary infected plants from Hermes variety).

At harvesting, Potato Tuber Ringspot Necrotic Disease symptoms could be identified on the tubers from all the other varieties, excepting the cultivars Riviera and Christian.

After 5 months from harvesting the inoculated material, the frequency of

tubers with symptoms was between $2.20\% \pm 3.810$ and $27.36\% \pm 4.446$ for varieties Bellarosa, Jelly, Roclas and for Desiree, Productiv, Red Lady, Carrera and Hermes varieties the values of this percentage was between $62.60\% \pm 11.58$ and $97.77\% \pm 2.113$. The samples with significantly starch content (Riviera and Christian) were resistant to PVY^{NTN} inoculation. These tubers stored 5 months didn't have visible tuber necrotic ringspot disease symptoms.

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