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SPECTROPHOTOMETRY ANALYSIS OF CARCINOGENIC COMPONENTS OF POTATO VARIETIES WHEN INFECTED BY THE WART CASUATIVE AGENT *Synchytrium endobioticum* (Shilbersky) PERCIVAL

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Summary. The paper proposed a mehod of spectrophotometric analysis for total proteins in various combinations of crossing parent potato forms whith different wart resistance and received after crossing descendants in the scope from 595 - 600 nm. As a result of conducted research after infecting by the wart causative agent of 75 potato samples and spectrophotometric analysis of total proteins, it was found that in resistant potato forms their content(total proteins) was 0.78 – 0.84 mg/ml, in wart - sensitive forms containing pathogenetic proteins – 0.56-0.65 mg/ml. This present spectrophotometric analysis test with a resolution of 1-5 nm in various radiation ranges allows to identify wart compounds in wart formations in the early stages of ontogenesis.

Keywords: potato, infection, wart, pathogenetic proteins, spectroscopy.

Introduction

Spectrometric analysis methods are widely used for research of biological structures, product quality monitoring in pharmacy, ecology, medicine, materials science, scientific research to identify carcinogenic components: carbon complexes -C=O, - C-N-, - C-S-N -, amine nitrogen - (-NH_2), sulfur-containing substances (-S-H-) and other types that can exhibit carcinogenic properties, lead to poisoning of animals and humans.

The wart disease is caused by an intracellular obligate pathogen *Synchytrium endobioticum* (Schilbersky) Percival is the most dangerous potato disease [1].

The nature and degree of harmfulness of the disease depends on the weather - climatic conditions of the zone [2], the applied agricultural technology, the level of implementation of advanced technologies, the variety's resistance, soil fertility, the level of seed production, the system of protective techniques and other factors [3].

Potato wart included into the list of quarantine diseases in 38 countries as per data of European and Mediterranean Plant Protection Organization (EPPO). There were identified 40 pathotypes of casuative agent [3]. The disease spread in 5 regions of Ukraine with a total area of 2337.96 hectares.

The most effective and environmentally friendly technique of combating the wart causative agent is the introduction of wart-resistant potato varieties with a full-sized protein that contains all 8 essential acids and is characterized by high taste qualities [4].

The Ukrainian Academy of Sciences urgently proposed to investigate the possibility of toxic impact of wart affected *Synchytrium endobiotum* (Schilbersky) Percival potato on animals. Such research was started in 1960-1970 at Chernivtsi Medical Institute (now Bukovina State Medical University) by Professor S. P. Zakryvydoroga together with Z. S. Zakryvydoroga [5,6], D. V. Lipsitz [7]. P. I. Lubovsky, V. A. Polatay, M. L. Tarakhovsky, V. L. Fastovsky [6].

It was determined that in animals that were fed wart growths, after the potato defeating by the wart causative agent, the number of hemoglobin and red blood cells decreased (anemia developed) and ESR (erythrocyte sedimentation reaction) accelerated [5]. The degradation sources were noted in the liver lobules. Small – cell infiltration of connective tissue located between the myocardial fibers was detected in the heart, and pronounced peri-vascular edema was detected in the brain. In the cerebral cortex, different degrees of color of nerve cells were noted [6].

Biochemical studies have shown that the most significant decrease in the content of α - globulins in blood serum occurs in rabbits that were fed potato wart (on 0.47%). The content of β – globulins increased on 10-16% [6].

Research by Lipsitz D. B. It was identified that infected potatos by causative agent of wart in tubers, the content of amine nitrogen (-NH_2), sulfur-containing substances (-SH-), nitro compounds (- NO-), connected with carbon atoms, which are products of the metabolism of destructive organisms. They belong to the most dangerous carcinogens that cause malignant tumors of the liver, lungs, kidneys, and digestive tract in 40 animal species [7].

Employees of the UkrSRPQS IPP NAAS continued research on quality and quantitatity analysis of various potato varieties healthy and affected by wart causative agent [8,9]. Biochemical methods for wart identifying in various potato varieties have also been developed.

As a result of the analysis of the qualitative composition of proteins by isoelectrofocusing them in a polyacrylamide gel in a pH gradient of 3.5 – 10.0, the protein spectrum is represented by 48 protein components in unaffected potato varieties, and 53 in affected ones. Redox enzymes activity analysis in different potato varieties an original potato parent forms combinations. Amino acid composition analysis in potato wart causative agent affected showed the increase in the content of cystin, methionine (sulfur-containing), lysine (has two amino groups), valine and histidine [10].

Together with scientists of department of computer systems and networks of Chernivtsi National University after Yuriy Fedkovych we started the researches for developing test_methods and using computer equipment for identifying and express-analysis for cancerogenic components in bioactive medium and materials [11]. These studies are still ongoing.

The work purpose was to identify potentially dangerous compounds in potato wart formation by the spectrometric technique.

Materials and methods

Identification of wart on various potato varieties was carried out according to the author's technique by UkrSRPQ IPP NAAS and the Department of Computer Systems and networks of Chernivtsi National University named. Yu. Fedkovich on the basis of the laboratory of quarantine diseases and pests during 2020-2022. Parent potato forms and hybrids received after crossing (75 pieces) were infected by wart causative agent zoospores [12]. Spectrophotometric analysis of total proteins of potato varieties with wart resistance contrast and obtained hybrids after crossing was performed using the Bradford M. method. 0.5% solution Coomassie blue G-250 was used as a dye for proteins [13].

A sample of vegetable material (5g) of potato sprouts was ground in the cold with 0.01g of Tris-glycine buffer + 0.1% EDTA + 0.5% ascorbic acid in 4C0 (1:5). The homogenate was centrifuged for 15 minutes at 12 thousand revolutions per wave, was purified through a column with cefadex G-25. For determining the protein content they were stained with a 0.5% solution of Commassie blue G-250 and at a wavelength of 595 Nm and 600 Nm on a Lomo-46 spectrophotometer (Russia), the light transmission value P (in %) was determined by the formula: P= Ro - PK, where P is the final result of the sample as a percentage of light transmission, or a unit of optical density, Ro is the percentage of light transmission of the prototype, PK is the percentage of light transmission of the lysing buffer. The extinction value was determined: E = -lg P. The protein concentration (in mcg/ml) was determined by the Formula C= E/K, where C is the protein concentration; E is the extinction value, and K is the light transmission ratio [14]. Statistical data processing was carried out according to Maslov Yu. I. [15].

Results and discussion

As a result of spectrophotometric analysis at a wavelength of 595 nm, it was revealed that in resistant potato forms after infection with the cancer pathogen, the amount of total protein was 0.78 – 0.84 mg/ml, in wart – sensitive forms – 0.58-0.65 mg/ml (table).

In the crossing option *resistant x resistant* (Gitte x 81 459c19) the protein content was 0.84 – 0.78 mg/ml (hybrids 2 - 19).

When crossing the original parent forms sensitive x resistant (Poliska rozheva x Ghitte), resistant x sensitive (81.459 C19 x Poliska rozheva) the protein content, containing pathogenic iron proteins with carcinogenic components in sensitive parent forms and in hybrids obtained after crossing was 0.58 – 0.65 mg/ml. In wart-resistant initial forms and received hybrids, 0.78 – 0.84 mg/ml (hybrids 20-58).

Table

Content of total proteins of the original parent forms of potatoes and hybrids received after crossing during infecting by the wart causative agent (in mg/ml)

№ items	Potato varieties and hybrids	Resistance degree	Number of defeated tubers	Protein content (mg/ml)	
				At 595nm	At 600 nm
1	*2*	*3*	*4*	*5*	*6*
1.	Ghitte(♀) (resistant)	1	0	0,84±0,66	0,85±0,66
2.	П94 27–11	1	0	0,81±0,33	0,82±0,33
3.	П94 27–12	1	0	0,78±0,66	0,78±0,66
4.	П94 27–21	1	0	0,80±0,66	0,81±0,66
5.	П94 27–22	1	0	0,79±0,33	0,79±0,33
6.	П94 27–26	1	0	0,80±0,66	0,80±0,66
7.	П94 27–39	1	0	0,84±0,66	0,83±0,66
8.	П94 27–46	1	0	0,82±0,66	0,82±0,66
9.	П94 27–49	1	0	0,79±0,33	0,78±0,33
10.	П94 27–52	1	0	0,78±0,66	0,78±0,66
11.	П94 27-53	1	0	0,79±0,66	0,79±0,66
12.	П94 27-54	1	0	0,81±0,33	0,82±0,33
13.	П94 27-55	1	0	0,84±0,66	0,83±0,66
14.	П94 27-56	1	0	0,82±0,66	0,82±0,66
15.	П94 27-57	2	0	0,83±0,66	0,83±0,66
16.	П 94 27-58	1	0	0,81±0,66	0,81±0,66
17.	П 94 27-59	1	0	0,84±0,66	0,84±0,66
18	П 94 27-60	1	0	0,79±0,66	0,79±0,66
19.	81 459c19 (♂) (resistant)	1	0	0,78±0,33	0,78±0,33
20.	Poliska rozheva (♀) (sensitive)	3	3	0,58±0,66	0,56±0,33
21.	П95 49/3	1	0	0,83±0,66	0,83±0,66
22.	П95 49/5	3	3	0,58±0,33	0,56±0,33
23.	П95 49/21	2	0	0,78±0,33	0,78±0,33
24.	П95 49/25	1	0	0,84±0,66	0,84±0,66
25.	П95 49/28	1	0	0,83±0,66	0,83±0,66
26.	П95 49/35	3	3	0,58±0,33	0,57±0,33
27.	П95 49/48	3	3	0,59±0,33	0,56±0,33
28.	П95 49/53	2	0	0,74±0,66	0,74±0,66
29.	П95 49/54	3	3	0,58±0,66	0,58±0,66
30.	П95 49/55	1	0	0,80±0,33	0,81±0,33
1	*2*	*3*	*4*	*5*	*6*
31.	П95 49/56	3	3	0,59±0,66	0,60±0,66
32.	П95 49/57	1	0	0,64±0,66	0,64±0,66
33.	П95 49/58	1	0	0,78±0,33	0,78±0,33

34.	П95 49/59	3	3	0,59±0,66	0,60±0,66
35.	П95 49/60	1	0	0,84±0,33	0,84±0,33
36.	П95 49/61	3	3	0,65±0,33	0,65±0,33
37.	П95 49/62	1	0	0,78±0,33	0,78±0,33
38.	П95 49/63	3	3	0,65±0,33	0,65±0,33
39.	81 459c19 (♂) (resistant)	1	0	0,82±0,66	0,82±0,66
40.	Ghitte(♀) (resistant)	1	0	0,84±0,66	0,83±0,66
41.	П95 9–1	3	3	0,65±0,66	0,65±0,66
42.	П95 9–6	2	0	0,58±0,66	0,58±0,66
43.	П95 9–7	2	0	0,63±0,33	0,63±0,33
44.	П95 9–8	3	3	0,60±0,33	0,61±0,33
45.	П95 9–10	3	3	0,62±0,66	0,62±0,66
46.	П95 9–12	3	3	0,63±0,66	0,64±0,66
47.	П95 9–22	3	3	0,61±0,33	0,62±0,33
48.	П95 9–23	3	3	0,59±0,66	0,59±0,66
49.	П95 9-24	1	0	0,84±0,33	0,84±0,33
50.	П95 9-25	1	0	0,83±0,66	0,83±0,66
51.	П95 9-26	1	0	0,84±0,66	0,84±0,66
52.	П95 9-27	1	0	0,83±0,33	0,83±0,33
53.	П95 9-28	1	0	0,80±0,33	0,80±0,33
54.	П95 9-29	3	3	0,65±0,66	0,65±0,66
55.	П95 9-30	3	3	0,63±0,66	0,63±0,66
56.	П95 9-31	1	0	0,80±0,66	0,80±0,66
57.	П95 9-32	2	0	0,78±0,33	0,78±0,33
58.	Poliska rozheva (♂) (sensitive)	3	3	0,58±0,66	0,56±0,33
59.	Poliska rozheva (♀) (sensitive)	3	5	0,58±0,66	0,56±0,33
60.	П95 11/16	3	3	0,62±0,33	0,62±0,33
61.	П95 11/22	3	3	0,65±0,66	0,64±0,66
62.	П95 11/26	3	3	0,64±0,66	0,62±0,66
63.	П95 11/28	3	3	0,61±0,66	0,61±0,66
64.	П95 11/30	3	3	0,59±0,33	0,59±0,33
65.	П95 11/37	3	3	0,58±0,66	0,58±0,66
66.	П95 11/43	3	3	0,60±0,66	0,60±0,66
67.	П95 11/46	3	3	0,58±0,66	0,58±0,66
68.	П95 11/47	3	3	0,58±0,33	0,58±0,33
69.	П95 11/57	1	0	0,78±0,33	0,78±0,33
70.	П95 11/68	3	3	0,59±0,66	0,59±0,66
71	П95 11/70	3	3	0,60±0,66	0,60±0,66
72.	П95 11/75	3	3	0,58±0,33	0,58±0,33
73.	П95 11/76	3	3	0,60±0,66	0,61±0,66
74	П95 11/77	3	3	0,63±0,66	0,63±0,66
75.	Teteriv (♂) (sensitive)	2	0	0,68±0,33	0,68±0,33
S.P.D ≤ 0,6				0,01	0,02

Note: 1, 2, 3 – defeating degree of potato samples by the wart causative agent.

As a result of crossing the original parental forms (Poliska rozheva x Teteriv), sensitive x sensitive in the original forms and the resulting hybrids, the content of total proteins (pathogen-dependent proteins with carcinogenic components) was in the range of 0.56 - 0.65 mg/ml (hybrids 60 - 74) .

Determination of the total protein content by spectrophotometry in 75 potato samples infected with the wart pathogen at a wavelength of 600 nm was slightly changed.

In the variant of crossing the initial parent forms of potatoes *resistant x resistant* (Ghitte x 81. 459c19) protein content was 0.78-0.85 mg/ml (hybrids 2 - 19); in crossbreeding combinations *sensitive x resistant* (Poliska rozheva x Ghitte), *resistant x sensitive* (81.459 C19 x Poliska rozheva) protein content was 0.56-0.85 mg/ml; *sensitive x sensitive (*Poliska rozheva x Teteriv) – 0.56-0.61 mg/ml. Thus, these spectrophotometric analysis tests with a resolution of 1-5 nm in different radiation ranges allow us to determine the content of total proteins, including pathogen-dependent proteins with carcinogenic components in warts in the early stages of ontogenesis.

Conclusions

The researches results for spectrophotometric analysis for identifying potentially dangerous combinations in potato during potato wart causative agent *Synchytrium endobioticum {Schilb.) Perc.*, allows to give principally for foundamentally important conclusions:

- It was found that in resistant potato forms, the content of total and pathogenic proteins along the wavelength of 595 nm was 0.78 – 0.84 mg/ml, in wart – sensitive forms – 0.58-0.65 mg/ml.
- At a wavelength of 600 nm, the content of total proteins in resistant potato forms was in the range of 0.78 – 0.85 mg/ml; in sensitive forms, the content of total proteins was lower, since only pathogenic proteins were detected. Their content was in the scope of 0.56 - 0.68 mg/ml.
- Thus, these spectrophotometric analysis tests with a resolution of 1-5 nm in different radiation ranges allow us to determine the content of total proteins, including pathogen-dependent proteins with carcinogenic components in warts in the early stages of ontogenesis.
- Spectrophotometric analysis can be relatively easily adapted for the study of complex nanodisperse biointegrated systems.

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Section B: *Principles and proceedings of increasing and quantification of genetic variability*