SOME RESEARCHES CONCERNING THE RESISTANCE MECHANISM DETERMINATION OF POTATO TO WART PRODUCED BY *Synchytrium endobioticum* THROUGH BIOCHEMICAL ANALYSES

Gheorghe SAGHIN[1], Dumitru BODEA[1], Ioan Cătălin ENEA[1], Daniela MURARIU[2]

e-mail: g.saghin@yahoo.com

Abstract

The paper presents results concerning the resistance mechanism determination of potato to wart, caused by *Synchytrium endobioticum*, through biochemical analysis, in 10 potato resistant and susceptible genotypes to above pathogen, relating to: dry matter and moisture contents, ash, total nitrogen, total crude protein and starch contents , titratable acidity, catalase and polifenoloxidaze activities, ascorbic acid and total free amino acids contents. Following the analysis carried out on resistant and susceptible potato genotypes to the pathogen was found that indicators refering to dry matter and moisture content, ash, ascorbic acid, titratable acidity and starch contents no guarantee expression of the resistance degree or susceptibility level to pathogens. It is interesting the analyzes of total nitrogen and crude protein contents from tubers, catalase and polifenoloxidaze activities and total free amino acids contents. Thus we can say, after the first analyzes conducted in this direction, the potato genotypes resistant to pathogens, contain over 0.9g nitrogen/100g tissue tuber crude protein over 5.6% total free amino acids content, over 0.40% from d.s., catalase units less than 110 and below 1.70 micromoles of ascorbic acid oxidized by enzyme in 1 gram of tuber tissue for one minute. It is necessary to continue this type of research on a much larger number of resistant and sensitive potato genotypes and take into account other analyzes to those mentioned in this paper, regarding to quality of the protein content, the essential amino acids content, alkaloids, amides, the study of albuminoidal substances compozition etc., which would prevent the cellular system development of the fungus in potato tubers.

Key words*:* wart, *Synchytrium endobioticum*, biochemical analyses, resistance, susceptibility.

One of the main diseases that cause great damages to potato crop is wart (potato cancer) caused by *Synchytrium endobioticum*, which in favorable years for fungus development can reach till 50-100%. The rapid spread of this disease, especially in the first decades of the nineteenth century in Europe, has made this disease to be considered very dangerous and declared as phytosanitary quarantine disease due to large yield losses with economic implications.

In the ssortment of potato genotypes can find varieties with different reaction to infection with the pathogen, from the highly sensitive, light sensitive, medium sensitive to resistant and very resistant and even immune. There are also some varieties names limit which sometimes they behave as poorly sensitive and sometimes as resistant (Zadina, J., 1988). The opinions regarding the definition of potato resistance to wart are not unanimous. It starts from the two sides separate assessment of potato resistance to wart – necrotic reaction of defense and reaction of tumor formation. To this, occurs the training zoosporangium resistance factor that determines the character of the disease quarantine. The susceptible varieties maintain the infection in soil, can transmit the infection and may be a source of aggressive races developement (Potočec, J., 1991). We mention that the only measure to combat the potato wart, it is genetic way, through cultivation of potato varieties resistant to this pathogen, in the private and asociative sectors.

Referring to hereditary transmission of potato varieties resistance to this pathogen they have a very different attitude, it is assigned some genes of which have a dominant character (Potoček, J., 1991 Mc Donnell, MB, 1980).

Regarding the mechanism of potato resistance to pathogens, from biochemical point of view, it observed that the resistance must be depend of certain substances contained in the chemical composition of tubers, which contribut to prevent or encourage the development of the fungus in the host plant, the two groups genotypes

[1] Agricultural Research and Development Station of Suceva
[2] Suceava Genebank

(resistant and susceptible). In this context, for the first time this kind of research was initiated in our country.

MATERIAL AND METHOD

The research concerning the resistance testing to wart, of Romanian potato genotypes and import material, which undergoing breeding process is carried out at the Centre for Agricultural Research Pojorâta subordinated to the Agricultural Research and Development Station of Suceava, under field conditions by natural infection and in laboratory conditions by artificial infection. The center has a field, strongly infested with akinetosporanges of fungus *Synchytrium endobioticum,* used exclusively for this activity. The research referring to the mechanism of resistance of potato genotypes to the pathogen from biochemical point of view, aims to eliminate a huge amount of work concerning the testing under field conditions by natural infection. This method is faster and more precise, when infection in field conditions occurs but it is not vizible. Research of this type were made in two consecutive years (2010-2011). Following the analysis and observations in the field conditions, in years 2010 and 2011 it was selected 10 sensitive potato genotypes and 10 resistant potato genotypes to pathogens, and were made biochemical analysis relating to: dry matter and moisture content, ash, total nitrogen respectively crude protein, starch, titratable acidity, catalase activity, polifenoloxidaze activity, ascorbic acid and total free amino acids contents.

Agricultural Research Centre of Pojorâta is situated in a very favorable area for this pathogen development: altitude 740 m, the average annual precipitation sum on 850 mm, the average precipitation sum during vegetation period (April-September) 500 mm, average annual temperature of 6℃ average temperature during vegetation period of 14.4℃ and sandy loam alluvial soil.

RESULTS AND DISCUSSIONS

a) The dry matter, moisture, ash , ascorbic acid and starch contents and titratable acidity

If we analyze the data referring to dry matter and moisture contents at resistant potato genotypes to this pathogen, it appears that values of d.m. ranged between 19.0% - 24.39% and 75.61 - 81.00% for moisture content and at sensitive potato genotypes to this pathogen the values ranged between 20.39% - 24.10% and 75.90% - 79, 61%. Analyzing the individual values but mostly average samples of 20 resistant and sensitive variants to *Synchytrium endobioticum* we found values on 22.21% d.m. and 22.47% d.m.; 77.78% moisture content and 77.53% moisture content. It is found that the difference between the values of resistant potato genotypes to pathogens and those sensitive is small and insignificant on 0.26% d.m. and 0.25% moisture content (table 1). Concerning the ash content the analyzes reveal higher values in some resistant genotypes in comparison with susceptible genotypes, but while some resistant genotypes showed lower values in comparison with susceptible genotypes. Values of studied resistant genotypes were ranged between 1.89% and 4.71% ash content and at sensitive potato genotypes to pathogen between 2.58% and 4.23% ash content, the difference between the mean values of the resistant and sensitive potato genotypes (3.25% and 3.21%) is small and insignificant on 0.04% (*table 1*). In this context we can say that these three analyzed indicators not guarantee expression of resistance or susceptibility levels to potato wart. Furthermore it is known that the soil type influenced the zoospores mobility, being able to produce the infection very early, at high humidity of the soil (80% of field capacity), but in the case of the moisture content from the tubers and susceptibility of the potato genotypes there is no any correlation.

The obtained results from the determination of titratable acidity, expressed as grams of oxalic acid in 100 g biological tissue from potato tubers, reveal that individual values in the resistant potato inbred line to pathogen are higher than those susceptible. Thus, the variation amplitude of resistant potato genotypes to pathogens ranged between the 0.36 g oxalic acid/100 g tuber and 0.63 g oxalic acid/100 g tuber and in sensitive inbred lines, ranged between the 0.27 g of oxalic acid and 0.48 g oxalic acid (table 1). If we compare the average values of resistant and susceptible inbred lines it found that the resistant inbred lines contain 0.11g oxalic acid/100g tuber more than in the sensitive inbred lines, low and insignificant value that does not confirm that the resistance or susceptibility of potato genotypes to pathogen is influenced by the amount of oxalic acid contained in the potato tuber.

Ascorbic acid content of the examined samples were between 5.84 and 8.36 mg/100 g tuber tissue in the resistant potato genotypes to pathogen and 5.40 to 7.10 mg/100 g tuber in the sensitive genotypes. Even if the content of ascorbic acid is higher in the resistant potato genotypes on 0.686 mg/100g tuber in comparison with the average of the sensitive potato genotypes, we can not say that this indicator represent the genotypes clasification garantee as resistant or sensitive, taking into consideration that the individual values are very close between some inbred lines of the two analyzed groups genotypes (*table 1*).

Table 1

The dry matter,moisture, ash , ascorbic acid and starch contents and titratable acidity

Genotype	Dry matter %	Moisture content %	Ash content %	Ascorbic acid content (mg/100g tuber)	Starch content (g/100g tubercul)	Titratable acidity (g oxalic acid/100 g tuber)
R	23,96	76,04	4,71	7,93	15,68	0,58
R	21,20	78,80	3,22	8,24	14,90	0,36
R	22,41	77,59	4,65	6,56	15,30	0,41
R	19,00	81,00	4,26	6,81	16,15	0,50
R	24,39	75,61	2,92	7,15	14,78	0,36
R	23,59	76,41	3,37	8,36	15,55	0,43
R	23,07	76,93	4,05	6,16	16,12	0,37
R	20,36	79,64	1,89	5,84	15,80	0,63
R	22,38	77,62	3,64	7,44	16,40	0,41
R	21,80	78,20	3,47	6,30	15,25	0,56
AVERAGE	**22,26**	**77,78**	**3,25**	**7,10**	**15,59**	**0,46**
S	21,70	78,30	3,30	6,80	17,02	0,34
S	21,07	78,93	2,60	7,04	16,20	0,29
S	23,83	76,17	2,97	6,40	16,38	0,48
S	21,83	78,17	4,23	6,16	14,24	0,29
S	23,99	76,01	2,58	5,83	15,72	0,39
S	22,85	77,15	2,71	6,95	16,44	0,27
S	21,92	78,08	3,38	5,40	16,52	0,31
S	20,39	79,61	3,87	5,56	17,16	0,34
S	24,10	75,90	3,30	7,10	14,86	0,39
S	23,06	76,94	3,23	6,08	16,30	0,43
AVERAGE	**22,47**	**77,53**	**3,22**	**6,33**	**16,10**	**0,35**

R= resistant genotype to pathogen;
S = sensitive genotype to pathogen.

Table 2

The total nitrogen and crude protein contents, catalase and polifenoloxidaze activities

Genotype	Total nitrogen content (g N/100g tuber)	Crude protein content (% from d.m.)	Catalase activity (catalase units)	Polifenoloxidaze activity (µmoli oxidized ascorbic acid/1g tub./minute)	Total free aminoacids content (% from d.m.)
R	1,10	6,87	1102,03	1,67	0,41
R	0,84	5,25	1163,26	1,52	0,42
R	0,89	5,56	1163,26	1,52	0,40
R	0,89	5,56	1124,48	1,69	0,50
R	1,23	7,68	1102,03	1,52	0,39
R	0,95	5,93	1040,81	1,57	0,56
R	1,17	7,31	1102,03	1,52	0,43
R	0,90	5,63	1134,48	1,75	0,39
R	1,37	8,56	1185,70	1,52	0,58
R	1,17	7,31	1163,26	1,52	0,40
Average	**1,05**	**6,57**	**1128,13**	**1,58**	**0,47**
S	0,26	1,63	1346,93	2,53	0,21
S	0,42	2,66	1408,15	2,02	0,17
S	0,33	2,10	1346,93	2,53	0,19
S	0,58	3,63	1224,48	2,53	0,20
S	0,60	3,75	1469,38	2,02	0,16
S	0,36	2,25	1408,15	2,02	0,22
S	0,57	3,56	1285,70	2,53	0,15
S	0,56	3,50	1224,48	2,02	0,16
S	0,40	2,53	1346,93	2,53	0,22
S	0,30	1,88	1408,15	2,02	0,22
Average	**0,44**	**2,75**	**1346,92**	**2,27**	**0,19**

R = resistant potato genotyp to pathogen
S = sensitive potato genotype to pathogen;
Catalase units = the enzyme quantity which decompose in one minute one micromole of hydrogen peroxide (0,034 mg H_2O_2/minute/1g tuber).

The values of starch content ranged from 14.78g to 16.40 g/100 g tuber tissue in the potato resistant genotypes to pathogen and between 14.24g and 17.02g/100 g tuber tissue in the potato susceptible genotypes (*table 1*). The values differences between the two groups of genotypes are small and insignificant, we stated that neither the starch content can not classify in resistant or susceptible potato genotypes to the pathogen.

b) Total nitrogen and crude protein contents, catalase and polifenoloxidaze activities

Analyzing data on total nitrogen and crude protein contents we notice the significant differences between resistant and susceptible genotypes to pathogen (*table 2*). Thus, the total nitrogen content expressed in grams of nitrogen per 100 g tuber tissue (%) and crude protein (% of dry matter), in the resistant inbred lines to pathogen those two indicators, varied between 0.50g N_t/100g tuber and 1.37g N_t/100g and 3.12% crude proteine and 8.56% crude proteine. In the sensitive inbred lines to pathogen the variation amplitude of total nitrogen content and crude protein is between 0.26g N_t/100 g tuber and 0.70 g/100g tuber and 1.87% c.p. and 4.37% c.p. It can make a first statement that the resistance level to wart must depend to some extent on the percentage of total nitrogen and crude protein content in potato tubers.

Concerning the catalase activity (amount of enzyme that breaks down in one minute, one micromole of hydrogen peroxide - 0.034 mg H_2O_2/min/1g tuber tissue, there is a big difference in the values obtained from analyzes conducted between resistant and sensitive genotypes to wart produced by *Synchytrium endobioticum*. Catalase activity in the resistant potato genotypes to pathogen is much lower than in sensitive potato genotypes. Thus, in the resistant potato genotypes, catalase activity was between 1040.81 c.u. and 1185.70 c.u. and in the susceptible potato genotypes between 1224.48 c.u. and 1469.38 c.u. (Table 2). If we compare individual differences and averages between resistant and susceptible potato genotypes, it noticed a significantly higher value in the sensitive potato genotypes of 218.79 c.u. In this context, it can make a quarrel first assertion that the higher catalase activity is less than 1200 c.u., the potato genotypes show a higher degree of resistance to the pathogen.

Polifenoloxidaze activity (micromolar oxidized ascorbic acid by the enzyme in a gram of tuber tissue - in one minute), the resistant potato genotypes, have values between 1.52 micromolar oxidized ascorbic acid and 1.75 mmol/1 g.tuber/1 minute and mean value on genotypes of 1.58 µmoli oxidazed ascorbic acid/1 g.tuber/minute. In the sensitive potato genotypes to pathogen, thepolifenoloxidaze activity has higher values in comparison with resistant potato genotypes, between 2.02 and 2.53 µmole oxidized ascorbic acid/1g tuber/1 minute, with an average of 2.27 µmoles ascorbic acid/1 g tuber/minute, with the finding that all individual values of the resistant potato genotypes are significantly lower in comparison with values of susceptible potato genotypes (table2).

Because the difference between the average values of the two analyzed genotypes groups is significantly being a value of 0.69 µmoli ascorbic acid/1g tuber/minute, it can be said, as in the case of catalase activity, when the values of polifenoloxidaze activity are below 2.00 µmoli oxidezed ascorbic acid/1g tuber/1minute, those potato varieties are resistant to wart, which highlights the importance of the polifenoloxidaze activity to the assessment of resistance or sensitivity of potato genotypes to the pathogen.

The total free amino acid content was higher for resistant potato genotypes, variation amplitude has values between 0.39 and 0.62% of d.m. presenting mean value of 0.47% and the variation amplitude in the susceptible potato genotypes varied between 0.15 and 0.22% of d.m. with an average value of 0.19%. The difference between the average values of the two analyzed genotypes groups is significant (0.26%), So we can say that the potato genotypes which have a total free amino acid content more than 0.40% of d.m. are resistant to pathogen.

CONCLUSIONS

We believe that the method for determining the resistance of potato to wart, produced by fungus *Synchytrium endobioticum*, through biochemical analysis, is faster and more accurate (where, under field conditions, infection with the pathogen occurs later and is not visible until summer or autumn), which will greatly ease the work of testing of potato genotypes to pathogen by eliminating a large volume of works which can be occur under field conditions or in laboratory, by artificial infection.

As follow of performed biochemical analyzes on resistant and susceptible potato genotypes to pathogen was found that indicators relating to: dry matter and moisture contents, ash, ascorbic acid and starch contents and titratable acidity no guarantee expression of the resistance or susceptibility degrees of potato genotypes to wart.

From the first studies referring to correlation between resistance of potato genotypes to wart

and some biochemical elements contained in potato tubers, it show interest the analyzes referring to total nitrogen and crude protein contents, catalase and polifenoloxidaze activities.

Following the results of such research we can say that resistant potato genotypes to pathogens (*Synchytrium endobioticum*), contain over 0.9 g total nitrogen/100 g tissue tuber, over 5.6% crude protein, less than 1100 catalase units, below 1.70 µmoli oxidized ascorbic acid by enzime in one gram tuber tissue during one minute and over 0.40% total free amino acids contents.

Being the first results of this type, for determining the resistance of potato genotypes to pathogen, it is necessary to continue these kind of research through the mechanism determination of potato resistance to *Synchytrium endobioticum*, through biochemical analysis, in a much larger number of resistant and susceptible potato genotypes to pathogenic and taking into account and other biochemical analyzes, relating to protein quality, essential amino acids content, alkaloids, amides, study of albuminoidal substances composition etc. These studies will prevent or encourage the development of the fungus in cellular system of potato after infection producing.

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