Neth. J. Pl. Path. 86 (1980) 301–309

Mature plant resistance of potato against some virus diseases. III. Mature plant resistance against potato virus Y^N, indicated by decrease in ribosome-content in ageing potato plants under field conditions

J. H. VENEKAMP[1], A. SCHEPERS[2] and C. B. BUS[2]

[1] Centre of Agrobiological Research (CABO), Wageningen
[2] Research Station for Arable Farming and Field Production of Vegetables (PAGV), Lelystad

Accepted 23 october 1979

Abstract

The concurrence of a decrease of the concentration of ribosomes and glycoproteins in leaves of potato plants under field conditions and mature plant resistance against potato virus Y^N was studied. On four of five dates with intervals of one or two weeks plants of randomly chosen plots in the field were inoculated. During plant growth sixth, tenth and fifteenth leaves of main stems were collected at regular intervals. With the aid of cellulose columns and polyethylene glycol-containing solvents the ribosome- en glycoprotein-contents (and in one experiment also the RNA-content) of these leaves were determined. Tubers were harvested from plants, inoculated fourteen days before. Lack of typical virus symptoms on plants grown from these tubers gave information on the presence of mature plant resistance.

It was concluded that in plants which had developed about 20 leaves, there was a high degree of mature plant resistance when ribosome- and glycoprotein-contents in the fifteenth leaf was less than 2 and 4 OD_{260}/ml/g fresh weight respectively. When higher absorbance values are measured, mature plant resistance may or may not occur depending on environmental conditions. The ribosome- and glycoprotein-contents have a remote relation to the mature plant resistance. It is suggested that ribosome- and glycoprotein-contents of the youngest fully expanded leaves give the best indication of mature plant resistance.

Additional keyword: protein synthesis.

Introduction

In a previous paper Venekamp and Beemster (1980a) described a concurrence of the development of mature plant resistance of potato plants against potato virus X (PVX) and the ribosome- and RNA-contents in the leaves. Young leaves have very high contents in the fifteenth leaf coincides with the development of mature plant resistance. rapidly and then remain at a constant level. The decrease of the ribosome- and RNA-contents in the fifteenth leaf coincide with the development of mature plant resistance.

In field experiments Schepers and Reestman (1975) demonstrated mature plant resistance of potato plants against potato virus Y^N (PVY^N). A concurrence of the

increase of this resistance and a rapid decrease of the ribosome-content in certain leaves might be suspected. To obtain information on this, some experiments with PVY^N were performed.

After the experiments described by Venekamp and Beemster (1980a and 1980b) the chromatographic technique was slightly extended by analysis of another fraction. Components of this fraction were identified as glycoproteins. In this paper concentrations of these glycoproteins are given along with those of the ribosomes.

Materials and methods

Plants. Healthy 'Bintje' potato-plants grown from seed potatoes, size 40–45 mm, presprouted in light, were used.

Potato virus Y^N (PVY^N). Pressed sap of potato plants grown from PVY^N-infected tubers, was diluted ten times with water and mixed with carborundum (350 mesh). With a paint spraying gun (Wiersema, 1972) the mixture was applied to the experimental plants, under a pressure of 4–5 bar.

Chromatographic analysis of ribosome, RNA, and of a glycoprotein fraction. A modification of the method of Venekamp and Beemster (1980a) was used. After application of the first column effluent to the second column of 1 g Whatman CF 11 cellulose (height 10 cm and diameter 0.9 cm) successive 50-ml batches of the solvents 3, 4 and 5 (see Table 1 in Venekamp and Beemster, 1980a) passed through the column. Ribosomes were eluted with 50 ml of a solution containing 5% polyethylene glycol, 0.1% ammonium acetate, 4.5% glucose, and 0.01 M tris, pH 7. Finally the percolation of 100 ml 0.01 N NaOH yielded a glycoprotein-containing fraction (see Discussion). The absorbances of the complete effluents were estimated at 260 nm (1 cm pathlength) with a Unicam Spectrophotometer. The concentrations of the ribosomes and glycoproteins were expressed as absorbance at 260 nm per ml and per gram fresh weight. Only in the first experiment RNA was estimated according to the slightly modified method of Venekamp and Beemster (1980a). Fifty ml of solvent 10 (Table 1 in Venekamp and Beemster, 1980a) was used to elute RNA.

The substances of the effluent obtained after passage of 0.01 N NaOH through the column were not characterized by a particular absorption maximum and/or minimum in the ultraviolet absorption spectrum. The positive reaction with the reagent of Elson and Morgan, described by Sandford et al. (1971) indicated that the substances contained hexosamine. From this result and from the fact that the substances eluted through a Sephadex-G75 column with the solvent front, it is assumed that the fraction contained some high-molecular weight glycoproteins. In this paper these are indicated as 'glycoproteins'.

Experiments. The first experiment was conducted in 1976 on a field of the Experimental Farm prof. dr. J. M. van Bemmelenhoeve at Wieringerwerf. This field was divided into 16 plots. The plots consisted of four rows of 50 plants each. Fourteen days before the underlined dates of sampling, given in Table 1, all the leaves of part of the plants of two inner rows of four plots, randomly situated in the field, were inoculated with PVY^N. Two weeks after inoculation 150 tubers were harvested from twenty plants of each of

these plots. On the inoculation dates, and two and six weeks after the last inoculation date a similar number of tubers from the same number of uninoculated plants was collected.

All tubers were planted the next year. PVY^N-infection of the plants grown from these tubers was visually assessed.

On the dates of sampling, given in Table 1, 15 sixth, 15 tenth, and 15 fifteenth leaves

Table 1. Ribosome (R), glycoprotein (GP), and RNA concentrations in potato plants, 'Bintje', in relation to development of mature plant resistance against PVY^N under field conditions (Wieringerwerf). Fourteen days before the underlined dates of sampling part of the plants of each plot was inoculated.

Age of plants in weeks from the day of planting at sampling	Sampled plants inoculated at the age in weeks of	Date of sampling	Leaf-number	Absorbance/ml/g fresh weight			Percentage of infected tubers two weeks after inoculation from	
				R	GP	RNA	inoculated plants	control plants
9		9/6	6	2.63 ±0.21	15.23 ±0.62	7.98 ±0.45		
10		16/6		1.74 ±0.15	6.72 ±0.54	6.73 ±0.22		0
12	10	30/6		1.22 ±0.25	1.94 ±0.09	3.60 ±0.69	96	1
14	12	14/7		0.59 ±0.06	1.08 ±0.07	1.46 ±0.13	97	1
16	14	28/7		0.26 ±0.04	1.94 ±0.09	0.52 ±0.04	14	19
18	16	11/8		0.70 ±0.15	1.54 ±0.19	1.79 ±0.30	25	28
20		25/8		0.75 ±0.07	1.60 ±0.12	1.87 ±0.24		
22		8/9						42
9		9/6	10	6.22 ±0.21	13.45 ±0.59	9.98 ±0.33		
10		16/6		4.25 ±0.41	10.51 ±1.66	7.71 ±0.79		0
12	10	30/6		2.73 ±0.45	2.78 ±0.21	5.56 ±0.96	96	1
14	12	14/7		1.25 ±0.13	1.77 ±0.17	1.29 ±0.15	97	1
16	14	28/7		0.71 ±0.14	2.60 ±0.16	0.51 ±0.04	14	19
18	16	11/8		1.28 ±0.23	2.46 ±0.25	1.70 ±0.20	25	28
20		25/8		1.14 ±0.10	2.20 ±0.24	2.76 ±0.16		
22		8/9		0.45 ±0.04	2.09 ±0.13	0.64 ±0.08		42
14		14/7	15				97	1
15		21/7		2.19 ±0.18	8.25 ±2.05	7.16 ±1.61		
16	14	28/7					14	19
17		4/8		0.92 ±0.15	9.23 ±0.41	0.82 ±0.12		
18	16	11/8					25	28
20		25/8		0.98 ±0.14	2.54 ±0.34	3.06 ±0.63		
22		8/9						42

Tabel 1. Concentraties van ribosomen (R), glycoproteïnen (GP) en RNA in aardappelplanten, 'Bintje', in verband met het optreden van ouderdomsresistentie tegen PVY^N onder veldomstandigheden (Wieringerwerf). Veertien dagen voor de onderstreepte bemonsteringsdata werd een deel van de planten van elk veldje geïnoculeerd.

of main stems were collected from inoculated and uninoculated plants of each plot. The ribosome-, glycoprotein-, and RNA-contents of the leaf samples per plot were estimated.

The second experiment was conducted at the same Experimental Farm in 1977. The field was divided into twenty plots of five rows, each of 60 plants. Fourteen days before the underlined dates of sampling, given in Table 2, part of the plants of three rows of four plots, randomly situated in the field, was inoculated with PVY^N. The ribosome- and glycoprotein-contents of each sample of 15 leaves were estimated. In this experiment samples of 150 tubers of each plot were also taken two weeks after inoculation. Uninoculated plants were sampled on the inoculation dates and two weeks after the last inoculation date of 1th August.

The third experiment was conducted in 1977, also in four replications, on the Experimental Farm of the General Netherlands Inspection Service for Field Seeds and Seed Potatoes, 'Friesland', at Sint Anna Parochie. This field was divided in 24 plots. Each plot consisted of four rows of 40 plants, of which part of two inner rows was inoculated. Leaf and tuber sampling was done as in the former experiments. Table 3 gives the scheme of inoculations and sampling.

Results

Table 1 gives the results of the first experiment. Young leaves show high ribosome-, glycoprotein-, and RNA-contents. In the investigated samples of the sixth and tenth leaves these concentrations decreased during the experimental period to a minimum at 14 to 16 weeks. Then a small increase in the concentrations occurred in both the sixth and tenth leaves. In the tenth leaves the concentrations reached a lower level than the above-mentioned minimum at the age of 22 weeks.

The tubers of 14-weeks-old plants, inoculated at the age of 12 weeks, showed an infection percentage of 97. The plants inoculated when 14 weeks old, showed two weeks later an infection percentage of the tubers of only 14. The infection percentages of the tubers from untreated plants, however, increased considerably when the plants were older than 12 weeks. From the 16th week on the infection percentages of the treated and untreated plots were at about the same level, which means that from that date on artificial inoculation had no additional effect on PVY^N infection.

Table 2 gives the data of the second experiment. The absorbances corresponding to the ribosome- and glycoprotein-contents of the sixth leaves were already 1.10 ± 0.03 and 2.33 ± 0.04 respectively when the plants were ten weeks old. At that time the contents of both components in the tenth leaves were still very high. They decreased to levels comparable to those in the sixth leaves within the next four weeks. The decrease in the contents in the fifteenth leaves was even later.

The tubers derived from plants which were inoculated at 16 weeks, showed an infection percentage of 39. The tubers from control plants had a rather low infection percentage throughout the growing period. It increased to 12 percent by the 18th week. At the age of 16 weeks, the effect of the inoculation was still very clear but decreased percentages of infected tubers indicated some mature plant resistance.

Table 3 shows a slower decrease in the ribosome-, and glycoprotein-content of the plants of the third experiment than those of the second experiment. The absorbance corresponding to the ribosome fraction in the tenth leaves of the 11-weeks-old plants

Table 2. Ribosome (R) and glycoprotein (GP) concentrations in potato plants, 'Bintje', in relation to development of mature plant resistance against PVY^N under field conditions (Wieringerwerf). Fourteen days before the underlined dates of sampling part of the plants of each plot was inoculated.

Age of plants in weeks from the day of planting at sampling	Sampled plants inoculated at the age in weeks of	Date of sampling	Leaf number	Absorbance/ml/g fresh weight		Percentage of infected tubers two weeks after inoculation from	
				R	GP	inoculated plants	control plants
10		20/6	6	1.10 ±0.03	2.33 ±0.04		2
12	10	4/7		0.22 ±0.05	1.33 ±0.16	66	3
10		20/6	10	5.40 ±0.55	6.88 ±0.71		2
12	10	4/7		3.45 ±0.12	4.10 ±0.13	66	3
14	12	18/7		1.61 ±0.07	2.58 ±0.14	94	4
14	12	18/7	15	2.50 ±0.14	3.53 ±0.18	94	4
16	14	1/8		1.15 ±0.03	1.50 ±0.07	78	10
18	16	15/8				39	12

Tabel 2. Concentraties van ribosomen (R) en glycoproteïnen (GP) in aardappelplanten, 'Bintje', in verband met het optreden van ouderdomsresistentie tegen PVY^N onder veldomstandigheden (Wieringerwerf). Veertien dagen voor de onderstreepte bemonsteringsdata werd een deel van de planten van elk veldje geïnoculeerd.

was 1.73 ± 0.05. The absorbance of the glycoprotein fraction from these leaves was 2.30 ± 0.10. These values of the fifteenth leaves of the 11-weeks-old plants were 3.29 ± 0.04 and 4.18 ± 0.09 respectively. Fourteen days later 41 percent of the tubers were infected which means that some mature plant resistance had developed. The natural infection at that time was only 8%.

In all the experiments the standard errors of the absorbance data from samples of young leaves were much greater than from samples of older leaves.

Discussion

Venekamp and Taborsky (1973) found that inoculation of potato plants, more than 10 weeks old, with PVX or tobacco mosaic virus had no influence on the ribosome-content of these plants. It is assumed that this also holds for inoculation of potato plants with PVY^N. In the first two experiments of the present paper the plants were also ten weeks old or older at the time of inoculation. Therefore it was not necessary to sample the leaves from inoculated and control plants separately. As a consequence the contents, given in the tables, are averages derived from analyses of similar leaves from plants of different plots.

To avoid effects of the margin of the plots, rows of sampled plants were distributed throughout the field among plants which were not included in the experiment.

Table 3. Ribosome (R) and glycoprotein (GP) concentrations in potato plants, 'Bintje', in relation to development of mature plant resistance against PVY^N under field conditions (St. Anna Parochie).

Age of plants in weeks from the day of planting at sampling	Sampled plants inoculated at the age in weeks of	Date of sampling	Leaf number	Absorbance/ml/g fresh weight		Percentage of infected tubers two weeks after inoculation from	
				R	GP	inoculated plants	control plants
6		1/7	6	0.59 ±0.13	1.01 ±0.12		
7		8/7		0.28 ±0.04	0.57 ±0.09		
6		1/7	10	4.54 ±0.06	5.67 ±0.34		0
7		8/7		3.14 ±0.08	5.17 ±0.13		2
8	6	15/7		2.79 ±0.12	4.63 ±0.08	80	0
9	7	22/7		2.39 ±0.13	3.60 ±0.14	96	0
10	8	29/7				84	4
11	9	5/8		1.73 ±0.05	2.30 ±0.10	78	3
8	6	15/7	15	5.00 ±0.06	5.66 ±0.22	80	0
9	7	22/7		4.32 ±0.14	5.26 ±0.16	96	0
10	8	29/7				84	4
11	9	5/8		3.29 ±0.04	4.18 ±0.09	78	3
13	11	19/8				41	8

Tabel 3. Concentraties van ribosomen (R) en glycoproteïnen (GP) in aardappelplanten, 'Bintje', in verband met het optreden van ouderdomsresistentie tegen PVY^N onder veldomstandigheden (St. Anna Parochie).

In the first experiment the highest standard errors of the data were found, when the plants were 10 or 12 weeks old. Standard errors of data corresponding to low contents of the analysed components from leaves which were developed during a very dry period, were much smaller. Rainfall after the date when the contents were minimal, very likely induced renewed growth of the plants and a small increase in the concentrations of ribosomes, glycoproteins, and RNA.

Venekamp and Beemster (1980a) established that there was high mature plant resistance of potato plants against PVX under greenhouse conditions when the absorbances corresponding to the ribosome- and RNA-contents in the fifteenth leaves decreased to below 4.5 and 8.5, respectively. It was concluded that mature plant resistance has a remote relation to these contents. In the first experiment, described here, hardly any fifteenth leaves were available as a consequence of a drought during the experimental period. The estimated absorbances corresponding to the above-mentioned contents of the fifteenth leaves from the 15-weeks-old plants had already a value of 2.19 ± 0.18 and 7.16 ± 1.61. However, there were insufficient data from the fifteenth leaves to establish the concurrence of the rate of mature plant resistance, with the decrease in ribosome- and RNA-contents.

The 16-weeks-old plants, inoculated two weeks before, yielded tubers with an infection percentage of 14. Considering a period of 14 days from the inoculation of the leaves till the occurrence of a demonstrable amount of virus in the tubers, these plants showed a distinct mature plant resistance when they were 14 weeks old. At that time the estimated absorbances for sixth and tenth leaves had almost minimum values. These were less than 1.5 for ribosomes and less than 2.0 for glycoproteins and RNA.

Due to natural infection the percentages of infected tubers from control plants increased with the age of the plants. The percentage of infected tubers from plants, inoculated when 14 weeks old, was 14 in spite of the mature plant resistance. Two weeks later the inoculated plants yielded 25% infected tubers. then the control plants yielded 28% infected tubers and on September 8 even 42%. These data may indicate that the multiplication and translocation of virus brought into the plants by aphid infection in an earlier susceptible stage of growth, was still going on, even after mature plant resistance against new (artificial) inoculations had developed.

In the second experiment the plants had developed a certain degree of mature plant resistance when they were 16 weeks old. In relation to the period of 14 days, mentioned above, the plants which were 18 weeks old, yielded tubers with a decreased infection percentage. It is assumed that a decrease in the values of the absorbances continued when the plants were older than 14 weeks. The values pertaining to the fifteenth leaves of the 16-weeks-old plants were 1.15 ± 0.03 and 1.50 ± 0.07 respectively. These values were less than the above-mentioned critical value of 4.5 for the fifteenth leaves.

The high value of 8.25 for glycoproteins, found in the first experiment at the time a high rate of mature plant resistance was reached, may have been due to the regrowth of leaves following the previously mentioned drought.

According to Table 3 the plants of the third experiment did not develop a high rate of mature plant resistance during the experimental period. The planting date of this experiment was delayed six weeks because of unsuitable soil conditions, so that the plants were inoculated and sampled at a younger stage than those of the other experiments. Yet the ribosome- and glycoprotein-contents in the tenth leaves were already very close to the low values of experiment 1 in which experiment the plants showed a high rate of mature plant resistance. In spite of the fact that the rate of mature plant resistance of the 11-weeks-old plants of experiment 3 was very similar to that of the 16-weeks-old plants of the second experiment (about 40% tuber infection 2 weeks after inoculation), the corresponding absorbance values for ribosomes and glycoproteins in the fifteenth leaves were still higher in the last experiment than in those of experiment 2.

Besides a difference in the geographic situation between experiments 2 and 3, carried out in the same year, the growth conditions were also different since the planting of experiment 3 was delayed 6 weeks. As a consequence of this delay the plants of experiment 3 were exposed to a longer day-length and higher temperatures during the beginning period of their development and the growth period of this experiment was shorter than that of experiment 2.

Venekamp and Beemster (1980b) stated that environmental factors affect the starting time of the concurrence of the decrease in the contents of protein-synthesizing components and the increase of mature plant resistance. Therefore the estimation of the degree of mature plant resistance of a seed potato crop, based only on the ribosome- and glycoprotein-contents, cannot be relied upon, unless additional information concerning the growing conditions is known. From the present experiments it may be

concluded that absorbance values at 260 nm, of 2 for ribosome- and of 4 for glycoprotein-content in the fifteenth leaves, may indicate that a certain rate of mature plant resistance had been reached in crops which had developed about twenty leaves per main stem. Higher absorbance values did not necessarily mean the absence of mature plant resistance.

In the establishments of a concurrence of decrease in ribosome- and RNA-contents and development of mature plant resistance Venekamp and Beemster (1980b) suggested that absorbance values measured in the youngest fully expanded leaves give better indications than those of older leaves. The present experiments under field conditions strengthen this suggestion.

Acknowledgments

The authors are greatly indebted to dr D. H. Wieringa-Brants (Phytopathologisch Laboratorium Willie Commelin Scholten, Baarn, the Netherlands) for valuable suggestions in the text and to dr R. F. Bozarth (Dept. of Life Sciences, Indiana State University, Terre Haute, Indiana, USA) for helpful criticism of the manuscript and correcting the English text.

Samenvatting

Ouderdomsresistentie van aardappelplanten tegen virusziekten. III. Ouderdomsresistentie tegen aaardappel Y^N-virus aangegeven door de afname in ribosoomconcentraties in verouderende aardappelplanten onder veldomstandigheden

In veldproeven werd het verband tussen de concentraties van ribosomen en glycoproteïnen in bladeren van aardappelplanten en de ouderdomsresistentie tegen aardappel Y^N-virus (PVY^N) bestudeerd. Op vier of vijf data met tussenpozen van een of twee weken werden planten in het veld met PVY^N geïnoculeerd. Gedurende de groeiperiode van de planten werden zesde, tiende en vijftiende bladeren op vastgestelde tijden verzameld. Chromatografische analyse met behulp van cellulosekolommen en solvents die polyethyleenglycol bevatten, leidde tot de bepalingen van de ribosoom- en glycoproteïnegehalten (en in één proef ook het RNA-gehalte) in de verzamelde bladeren. De oogst van de knollen vond plaats 14 dagen na inoculatie. Het niet tot ontwikkeling komen van de typische virussymptomen bij planten, die uit deze knollen waren gegroeid, was een aanwijzing voor het optreden van de ouderdomsresistentie.

Geconcludeerd wordt dat waarschijnlijk enige mate van ouderdomsresistentie tegen PVY^N in een pootaardappelgewas aanwezig is, als het ribosoom- en glycoproteïnegehalte in het vijftiende blad van hoofdstengels met ongeveer twintig bladeren, leidt tot corresponderende extinctiewaarden bij 260 nm per ml en per gram vers gewicht van respectievelijk minder dan 2 en 4. Wanneer hogere extinctiewaarden worden gevonden kan ouderdomsresistentie wel of niet in belangrijke mate voorkomen, afhankelijk van de omstandigheden waaronder het gewas is gegroeid. Gesuggereerd wordt de jongste volledig ontwikkelde bladeren te gebruiken voor het aantonen van een gelijktijdig optreden van de afname in de ribosoom- en glycoproteïnegehalten en een praktisch waarneembare mate van ouderdomsresistentie.

References

Beemster, A. B. R., 1961. Een vergelijking tussen het transport van X-virus en twee verschillende stammen van Y-virus in aardappelplanten (with a summary). T.Pl.-ziekten 67: 278–279.

Sandford, P. A., Nafziger, A. J. & Jeanes, A., 1971. Reaction of sodium hypochlorite with amines and amides; a new method for quantitating polysacharides containing hexosamines. Analyt. Biochem. 44: 111–121.

Schepers, A. & Reestman, A. J., 1975. Mature plant resistance against PVY^N in relation to some morphological characteristics of the potato plant. Abstracts of Conference Papers, 6th triennial Conf. E.A.P.R., Wageningen, p. 100–102.

Venekamp, J. H. & Beemster, A. B. R., 1980a. Mature plant resistance of potato against some virus diseases. I. Concurrence of development of mature plant resistance against potato virus X, and decrease of ribosome and RNA content. Neth. J. Pl. Path. 85: 47–56.

Venekamp, J. H. & Beemster, A. B. R., 1980b. Mature plant resistance of potato against some virus diseases. II. Mature plant resistance and the influence of temperature on the ribosome and RNA content in leaves. Neth. J. Pl. Path. 85: 57–62.

Venekamp,, J. H. & Taborsky, V., 1973. Changes in the quantity of ribosomes in healthy and virus-diseased plants during senescence. Neth. J. Pl. Path. 79: 62–69.

Wiersema, H. T., 1972. Breeding for resistance. In: J. A. de Bokx (Ed.): Viruses of potatoes and seed potato production. Pudoc, Wageningen, p. 174–187.

Addresses

J. H. Venekamp: Centrum voor Agrobiologisch Onderzoek (CABO), Postbus 14, 6700 AA Wageningen, the Netherlands.

A. Schepers and C. B. Bus: Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond (PAGV), Postbus 430, 8200 AK Lelystad, the Netherlands.