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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

J. H. VENEKAMP[1] and A. B. R. BEEMSTER[2]

[1] Centre for Agrobiological Research (CABO), Wageningen
[2] Research Institute for Plant Protection (IPO), Wageningen

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Abstract

Four or five weeks after planting a group of potato plants 'Bintje' was inoculated with potato virus X (PVX). Other groups were inoculated at intervals of 14 days. Tubers produced by plants inoculated 35 days after planting were all infected. The plants inoculated 49 days or later after planting produced few infected tubers. The latter had developed mature plant resistance against PVX infection.

The ribosome and RNA contents of leaves were measured by application of adsorption chromatography. A rapid decrease in ribosome and RNA contents occurred in plants at the time of rapid increase in the rate of mature plant resistance. The decrease was most distinct in the fifteenth leaf and therefore the contents in this leaf seem to give a good indication of the rapid increase in resistance.

Additional keyword: Protein synthesis.

Introduction

When young potato plants are inoculated with a virus, virus multiplication in the inoculated and also in newly developing leaves and translocation to the developing tubers can be demonstrated. Inoculation of plants which have passed a critical age, results in little or no infection of the tubers. Beemster (1958) described this effect as mature plant resistance.

Limitation of the protein-synthesizing capacity of a plant as a consequence of reduced concentrations of the necessary cell components, such as ribosomes or RNA, may define the beginning of mature plant resistance. In full-grown parts of the plant this capacity is sufficient for the normal physiological functions. In case of virus infections the above-mentioned components may also be considered to be involved in virus synthesis. Leaves of older potato plants may not produce sufficient virus to permit translocation to the tubers. Therefore it may be expected that the rate of mature plant resistance coincides with changes in concentration of protein-synthesizing components. Then, for example, the ribosome concentration in a certain leaf may give an indication of the development of the mature plant resistance. The establishment of a

high level of this resistance by assay of ribosomes may be valuable in the practice of seed potato production.

Several viruses can be isolated and purified chromatographically (Venekamp and Mosch, 1964; Venekamp et al., 1973a). In this technique the presence of magnesium ions in the solvent is required for the adsorption of ribosomes onto the column of cellulose. Subsequent percolation of a solvent without these ions liberates the ribosomes from the column (Venekamp et al, 1973b). These authors gave several criteria on the purity of the obtained ribosomes. The presence of sodium chloride in the solvent has a similar effect on the adsorption of RNA onto cellulose (Venekamp and Chan, unpublished data).

In the present paper the concurrence of the development of mature plant resistance and the decrease in concentrations of ribosomes and RNA was established using chromatographic techniques. The experiments to be described, were limited to potato virus X (PVX) for the following reasons; 1) mainly PVX had been used so far in studies on the application of the above-mentioned chromatography; 2) potato plants show a distinct mature plant resistance against this virus (Beemster, 1958).

Materials and methods

Plants. Potato plants 'Bintje' were grown in the greenhouse at a temperature of 18–20 °C under natural light conditions. Before use each plant was assayed for the presence of PVX and potato virus Y (PVY).

Potato virus X (PVX). The isolate of PVX was the same as used by Venekamp & Taborsky (1973). Purified virus, obtained as described by these authors was used as inoculum after a 1 :100 dilution with water.

Assay methods. PVX was assayed by inoculating expanded leaves of *Gomphrena globosa* (Beemster, 1958). The method of de Bokx (1964) with A6-test plants was used to assay PVY.

Table 1. Composition of the solvents for the chromatographic purification of ribosomes, PVX, and RNA.

Chemical		Solvent									
		1	2	3	4	5	6	7	8	9	10
Dextran	(%)	2	1								
Sucrose	(M)	0.8	0.4								
Polyethylene glycol 6000	(%)			5	5	5	5		5	5	5
Ammonium acetate	(%)			1	1	1	1				
Glucose	(%)			4.5	4.5	4.5	4.5				
Tris pH7	(mM)	20	10	10	10	10	10		10	10	10
Tris pH9	(mM)							50			
Magnesium acetate	(mM)			4	4	4					
Cysteine	(%)	0.2	0.1	0.1	0.1						
Triton X 100	(%)			0.4						0.4	
Sodium chloride	(M)								2	2	

Tabel 1. Samenstelling van de oplossingen voor de chromatografische zuivering van ribosomen, aardappel X-virus en RNA.

Estimation of the ribosome, PVX and π-RNA concentrations. For each analysis 10 g fresh leaves were homogenized in 50 ml of solvent 1 (Table 1). The homogenate was applied on the first column of 5 g ground filter paper Whatman No. 1 (height 10 cm, diameter 2 cm), previously washed with 50 ml of solvent 2 (Table 1). The homogenate was extracted by percolation of solvent 2 until the volume of the eluate (= effluent 1) was 250 ml. Polyethylene glycol 6000, ammonium acetate and magnesium acetate were added to effluent 1 to final concentrations of 5%, 1% and 4 mM, respectively. This mixture passed a second column of 1 g Whatman CF11 cellulose (height 10 cm and diameter 0.9 cm), previously washed with 10 ml of solvent 3 (Table 1). The eluate (= effluent 2) was applied to a third column as described below. The second column was washed successively with 50 ml amounts of the solvents 3, 4 and 5 (Table 1). Then the column was placed on the LKB fraction collector and a gradient elution from solvent 6 to 7 (Table 1) was applied. To form the gradient a lower vessel in direct connection with the column contained 150 ml of solvent 6 and an upper vessel in connection with the first vessel contained 500 ml of solvent 7. Fractions of the eluate were recorded at 254 nm by a LKB Uvicord I absorption meter. This resulted in a chromatogram with a peak of ribosomes; when diseased plant material was used, this peak was followed by a peak of PVX. Fractions of each peak were combined. The concentrations of ribosomes and PVX in the obtained suspensions were measured by means of a Beckman DB-G spectrophotometer with a Sargent recorder and expressed as absorbances at 260 nm wavelength (pathlength 1 cm) per gram fresh weight.

Sodium chloride was added to effluent 2 to a final concentration of 2 M. This mixture passed a third column similar to the second column, in size and material, previously washed with 50 ml of solvent 8 (Table 1). After successive washings with 50 ml of each of the solvents 9 and 8 (Table 1) the third column was placed on the LKB fraction collector and 100 ml of solvent 10 (Table 1) passed the column with simultaneous recording of the effluent with the aid of the LKB Uvicord I absorption meter at 254 nm. This resulted in a chromatogram with one peak. The substance of this peak appeared to be an RNA fraction (molecular weight < 60000) and is referred as RNA. The absorbance of the combined peak fractions was measured in the same way as that of the ribosomes or of PVX.

Experiments. In experiment 1 48 plants were used of which a group of 24 plants remained uninoculated. On each of the dates, given in Table 2, all the leaves of three plants of the second group of 24 plants were mechanically inoculated with PVX. Fourteen days after each inoculation mixed leaf samples of 10 g fresh weight from the three plants were analysed according to the method given above. On the sampling dates the tubers were collected and kept at 5 °C for three months. The young plants from these tubers were bioassayed.

Two similar groups of plants were used in experiment 2. On the dates given in Table 3 the available fifth, tenth, fifteenth and twentieth leaves of six plants of the second group were inoculated with PVX. Fourteen days after each inoculation the fifth, tenth, fifteenth and twentieth leaves (counted from the base of the plant) and higher top-leaves, if present, of both groups were harvested. The contents of ribosomes, RNA, PVX in 10 g fresh sample weight and the percentages of infected tubers were estimated as described above.

Experiment 3 was similar to experiment 2. In this experiment, however, no group of healthy plants was included. The dates of inoculation and sampling are given in Table 4.

Results

Before use for the experiments the plants appeared to be free of PVX or PVY according to the assay method, given in 'Materials and methods'. In Figure 1 the chromatograms of the samples of the first experimental plants, both healthy and diseased (40 days after planting), are given as an example of the results. All other samples in this study yielded similar chromatograms. Ribosomes eluted first from the column which in case diseased material was used, were followed by PVX.

Fig. 1. Fractionation of ribosomes (R) and PVX from potato plants 'Bintje' on cellulose columns by use of polyethylene glycol-containing solvents.

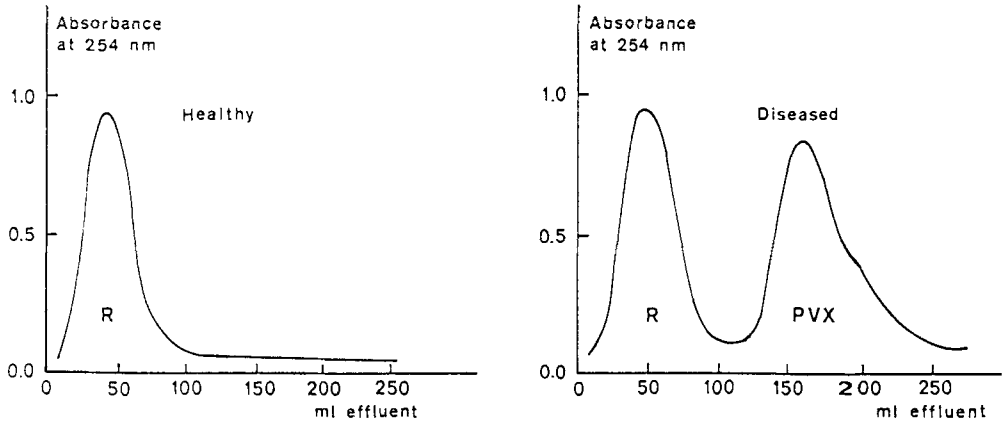

Fig. 1. Fractionering van ribosomen (R) en PVX uit aardappelplanten 'Bintje' over cellulose kolommen met behulp van oplossingen die polyethyleenglycol bevatten.

The results of the analyses of experiment 1 are summarized in Table 2. Plants inoculated at 47 days produced 4% infected tubers two weeks after inoculation. This indicates that mature plant resistance which gradually had developed, as can be seen from the infection percentages, had reached a high level and may have started when the plants were about 47 days old. In the week before the 47th day the ribosome content decreased from 4.9 to 1.5. In the same week the RNA content dropped from 20.5 to 7.8. In this comment, but also in those on Tables 3 and 4, the rate of mature plant resistance (as percentage of infected tubers) on a certain day in the series of inoculation dates (for example day 47, Table 2) corresponds with the ribosome and RNA contents on the same day in the series of sampling dates (also day 47).

Table 3 gives the results of experiment 2. The difference in percentages of infection resulting from plants inoculated 50 and 64 days after planting (85% and 12%, respectively) suggests that the plants inoculated when 64 days old, had developed a degree of mature plant resistance to PVX at this time. When fifteenth leaves were

Table 2. The relative concentrations of ribosomes (R), RNA, and PVX in healthy and diseased potato plants 'Bintje' of different ages. Concentrations measured at 260 nm and 1 cm lightpath and expressed as absorbance per ml and per g fresh weight. Group 1 = healthy plants; group 2 = plants inoculated with PVX.

Group	Age of the plants in days on the date of		Absorbance/ml/g fresh weight of mixed leaf sample			Percentage of infected tubers
	inoculation	sampling	R	RNA	PVX	
1		40	3.7	15.2		
		47	1.2	9.1		
		54	0.3	6.5		
		61	0.8	6.1		
		68	0.6	3.5		
		75	0.4	2.6		
		82	0.9	2.7		
		89	0.5	0.6		
2	26	40	4.9	20.5	3.4	100
	33	47	1.5	7.8	0.4	93
	40	54	0.4	5.4	0.5	62
	47	61	0.5	4.8	0.8	4
	54	68	0.8	2.7	0.2	2
	61	75	0.6	1.8	0.4	3
	68	82	0.6	1.0	0.4	7
	75	89	0.7	2.0	0.1	4

Tabel 2. De relatieve concentraties van ribosomen (R), RNA en PVX in gezonde en zieke aardappelplanten 'Bintje' van verschillende leeftijden. Concentraties gemeten bij 260 nm en lichtweg van 1 cm, weergegeven als extinctie per ml en per g vers gewicht. Groep 1 = gezonde planten; groep 2 = planten geïnoculeerd met PVX.

sampled on the 64 th day the ribosome and RNA contents were 4.2 and 8.2. During the fourteen days before these contents dropped from 10.8 to 4.2 and from 24.4 to 8.2, respectively. Ageing of the leaves decreased the contents only to 2.0 and to 7.1, respectively, on the last day of sampling.

In experiment 3 (Table 4) the plants inoculated 59 days after planting, yielded only 9% infected tubers as compared to 96% for plants inoculated 45 days after planting. The ribosome and RNA contents of the fifth and tenth leaves were almost constant during the preceding period. However, for leaf number 15 of the 59-day-old plants they were 4.0 and 5.7, respectively, and already much lower than those of plants of 46 days old (8.8 and 18.7, respectively). Mature plant resistance appeared to be almost complete when the plants were about 60 days old.

In Table 5 the differences in the ribosome and RNA contents at about the date that the rapid raise of mature plant resistance started, are compared with those of fourteen days earlier for the analysed leaves. It can be noted that large differences were found in the fifteenth leaf. The contents of this leaf seem therefore to give a good indication for the rapid increase in the rate of the mature plant resistance. This rapid increase appeared to occur when the ribosomal content of the fifteenth leaf corresponded with

Table 3. The relative concentrations of ribosomes (R), RNA, and PVX in leaves of diseased and healthy potato plants 'Bintje' of different ages. For explanation see Table 2. * Leaf was not yet present.

Age of the plants in days on the date of		Leaf number	Absorbance/ml/g fresh weight					Percentage of infected tubers
			healthy		diseased			
inoculation of the leaves	sampling of the leaves		R	RNA	R	RNA	PVX	
36	50	5	2.9	1.2	3.9	6.8	3.3	100
50	64		1.8	0.8	2.1	4.1	4.6	85
55	69		1.8	1.0	2.0	2.2	2.4	35
64	78		1.5	0.9	1.6	2.0	2.4	12
36	50	10	3.6	4.1	8.8	13.0	10.6	100
50	64		3.0	2.9	3.6	7.1	3.8	85
55	69		2.9	1.9	2.5	4.8	1.7	35
64	78		2.5	1.9	1.9	4.3	1.5	12
*	50	15	10.1	15.9	10.8	24.4	11.6	100
50	64		4.0	4.4	4.2	8.2	0.8	85
55	69		3.4	1.8	3.5	7.2	0.5	35
64	78		3.3	1.7	3.2	7.2	0.4	12
71	83		1.1	3.5	2.0	7.1	0.4	12
*	50	20	14.2	25.5	17.3	32.0	5.8	100
*	64		4.0	5.7	3.8	17.8	0.5	85
55	69		3.8	3.3	4.0	11.5	0.1	35
*	78		3.0	3.2	2.5	10.0	0.0	12
71	83		0.9	2.0	1.6	7.4	0.3	12
*	64	top	13.5	11.3				85
*	69		4.3	8.1	6.2	9.5	0.1	35
*	78		3.2	7.7	6.0	8.2	0.0	12
*	83		1.2	1.2	2.5	3.9	0.6	12

*Tabel 3. De relatieve concentraties van ribosomen (R), RNA en PVX in bladeren van zieke en gezonde aardappelplanten 'Bintje' van verschillende leeftijden. Voor verklaring zie Tabel 2. * Blad was nog niet aanwezig.*

an absorbance per ml and per gram fresh weight of less than 4.5. Then the absorbance of the RNA fraction from this leaf seemed to be less than 8.5.

Contrary to the high ribosome and RNA contents of the young leaves the PVX concentrations in general had a tendency to be higher in the older leaves than in the younger ones. In several cases fifteenth and/or twentieth and/or higher top leaves were not yet present on the inoculation dates (see note 1 in Tables 3 and 4). In the still growing plant the virus translocation to the young leaves took a certain time and therefore virus synthesis started later than in inoculated leaves. This is not the only reason for the lower PVX content in the younger leaves. Probably ageing of the leaves may be another cause of reduced ability to synthesize virus.

Table 4. The relative concentrations of ribosomes (R), RNA and PVX in leaves of diseased potato plants 'Bintje' of different ages. For explanation see Table 2. * Leaf was not yet present.

Age of the plants in days on the date of		Leaf number	Absorbance/ml/g fresh weight			Percentage of infected tubers
			R	RNA	PVX	
inoculation of the leaves	sampling of the leaves					
32	46	5	1.2	1.4	3.0	100
45	59		1.3	1.1	3.2	96
59	73		1.1	0.5	4.5	9
73	87		0.0	0.2	2.6	0
32	46	10	2.0	3.7	2.8	100
45	59		2.3	2.1	2.4	96
59	73		2.2	0.7	2.4	9
73	87		0.0	0.2	1.9	0
*	46	15	8.8	18.7	0.8	100
45	59		4.0	5.7	0.7	96
59	73		3.8	5.4	0.4	9
73	87		0.0	0.4	1.0	0
*	46	20	9.5	19.5	1.0	100
*	59		9.6	17.3	1.3	96
59	73		4.3	5.5	1.2	9
73	87		0.1	0.8	0.7	0

*Tabel 4. De relatieve concentraties van ribosomen (R), RNA en PVX in bladeren van zieke aardappelplanten 'Bintje' van verschillende leeftijden. Voor verklaring zie Tabel 2. * Blad was nog niet aanwezig.*

Table 5. The differences in concentrations of ribosomes and RNA in leaves of the diseased plants on the date of the beginning of the mature plant resistance and those on the date fourteen days before the beginning of the mature plant resistance. The figures were derived from those of Table 3 and 4. In experiment 2 the mature plant resistance started when the plants were 64 days old and in experiment 3 the mature plant resistance started when the plants were 60 days old. Because of missing of 60-day samples in experiment 3 the results of the 59-day-old plants were presented.

Leaf number	Difference in absorbance/ml/g fresh weight			
	experiment 2		experiment 3	
	ribosomes	RNA	ribosomes	RNA
5	1.8	2.7	–0.1	0.3
10	5.2	5.9	–0.3	1.6
15	6.6	16.2	4.8	13.0
20	13.5	14.2	–0.1	2.2

Tabel 5. De verschillen in ribosoom- en RNA-concentraties in bladeren van de zieke planten op de datum van de intrede van de ouderdomsresistentie en die op de datum veertien dagen voor het beginnen van de ouderdomsresistentie. De cijfers werden van die van de Tabellen 3 en 4 afgeleid. In proef 2 begon de ouderdomsresistentie toen de planten 64 dagen oud waren en in proef 3 begon de ouderdomsresistentie toen de planten 60 dagen oud waren. Vanwege het ontbreken van de monsters van 60 dagen in proef 3 werden de gehalten van de 59 dagen oude planten in beschouwing genomen.

Discussion

In previous studies Venekamp and Taborsky (1973) used samples of 100 g fresh plant material to estimate ribosome contents with the aid of the chromatographic technique using columns of cellulose and polyethylene glycol-containing solvents. In the present study the method was slightly modified and adjusted to the analysis of 10 g fresh plant material. Moreover the complete separation of the ribosomes and PVX was a consequence of a gradual decrease of the polyethylene glycol, ammonium acetate and glucose contents of the eluting solvent with a simultaneous increase of the pH from 7 to 9. Venekamp and Chan (unpublished data) isolated and purified an RNA fraction from effluent after removal of ribosomes and PVX. The homogenous substance had a characteristic ultraviolet absorption spectrum with a maximum at 258 nm and a minimum at 230 nm. The ratio of these absorptions was 2.60. Application of gel filtration on Sephadex G 75 revealed that the moleculair weight of this nucleic acid was smaller than 60000. Venekamp and Taborsky (1973) reported that PVX induced a considerable increase in ribosome content of young tobacco and potato plants. In the first experiment the young potato plants (Table 2), inoculated 26 and 33 days after planting, respectively, also demonstrated this effect. A similar effect on the RNA concentrations was found only in plants sampled on day 40. When plants were 54 days or older the difference was not present anymore. At the same age mature plant resistance had developed sufficiently to be of practical importance.

The ribosome contents of the diseased leaves in experiment 2 (Table 3) equalled those of the healthy leaves when the plants were 64 days old. Here again the ribosomes had the same tendency of decrease as the percentages of infected tubers. However, in this experiment the infected leaves showed an increase of RNA concentrations for a longer period.

The ribosome and RNA contents of the plants in experiment 1 are averages of the analyses of all leaves together. However, separate analysis of young leaves of 50-day-old plants, e.g. leaf number 15 (Table 3), resulted in a ribosome value of 10.8 which is considerably higher than any of the values found in experiment 1. Two weeks later this value decreased to 4.2 (a difference of 6.6). During the following two weeks the value decreased very slowly to 3.2 (a difference of only 1.0). The absorbance of the ribosomes of the 40-day-old plants of experiment 1 (Table 2) was 4.9. This value was 1.5, giving a difference of 3.4, when the plants had developed a high rate of mature plant resistance (47 days after planting). A similar reasoning holds for RNA. The difference for RNA in the fifteenth leaves of 50-and 64-day-old plants was 24.4–8.2 = 16.2 (Table 3) and that in the whole plants 20.5–7.8 = 12.7 (Table 2). The decrease in both ribosome and RNA concentration, when analyzing a separate leaf, appeared to be much greater than when a total sample of all the leaves of the plants was analysed. Therefore the contents of the two components in a certain leaf will be a better indication of the (rapid) increase in the rate of the resistance.

To a certain extent the ribosome content in the fifteenth leaf on the date of a high level of mature plant resistance differed very much from the ribosome content in the fifteenth leaf fourteen days earlier (see Table 5). The same holds for the RNA contents. This leaf may therefore be a valuable indicator for the establishment of a high level of mature plant resistance under the conditions of the present experiments. Another leaf may be a better indicator when the plants are grown under other circumstances. To

select that leaf, the influence of the temparature, for example, on the described phenomena should be investigated.

Mature plant resistance may be postulated to result from limitation of the protein synthesizing capacity of the plant, evidenced by decrease of ribosome and RNA contents. Both contents were found to decrease in concurrence with development of mature plant resistance. However, the data do not exclude the possibility of the drop in ribosome and RNA contents setting in long before mature plant resistance reaches a level of practical importance. Therefore, the causal relationship may be only remote.

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Samenvatting

Ouderdomsresistentie van aardappelplanten tegen enkele virusziekten. I. Ouderdomsresistentie tegen aardappel X-virus, samengaand met afname van ribosoom- en RNA-concentraties in verouderende planten

Van een groep aardappelplanten 'Bintje' werd een eerste groepje ongeveer 4 à 5 weken na het poten met PVX geïnoculeerd. Veertien dagen later werd een volgend groepje van deze planten met PVX besmet. Deze inoculaties werden met tussenpozen van 14 dagen enige keren voortgezet. Planten die bij de inoculatie ongeveer 35 dagen oud waren, vormden volledig met virus besmette knollen. Het besmettingspercentage van knollen, van later geïnoculeerde planten, was gering. Deze planten vertoonden ouderdomsresistentie tegen X-virusinfectie. De ribosoom- en RNA-gehalten, zowel van gehele planten als van verschillende bladeren van deze planten werden met behulp van chromatografische analyse gemeten. In deze techniek werden cellulosekolommen en oplossingen die polyethyleenglycol bevatten, toegepast. De bepalingen toonden aan dat een snelle toename van de ouderdomsresistentie samengaat met een snelle afname in ribosoom- en RNA-gehalte bij ouder wordende planten. Bij het vijftiende blad was deze gelijktijdigheid het duidelijkste. Onder de gegeven proefomstandigheden zouden de gehalten in dit blad een goede aanwijzing kunnen geven voor het moment van snelle toename van ouderdomsresistentie tegen PVX.

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Addresses

Centrum voor Agrobiologisch Onderzoek (CABO), Bornsesteeg 65, Postbus 14, 6700 AA Wageningen, the Netherlands.

Instituut voor Plantenziektenkundig Onderzoek (IPO), Binnenhaven 12, Postbus 42, 6700 AA Wageningen, the Netherlands.