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Changes in the quantity of ribosomes in healthy and virus-diseased plants during senescence

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Abstract

The ribosome and virus contents in potato virus X-infected tobacco plants was determined chromatographically. The data were compared with those obtained from measurements of ribosome concentrations in healthy plants of the same age. Bean plants with white clover mosaic virus, and potato and tobacco plants with potato virus X, and tobacco mosaic virus alone and in complex, were similarly tested.

The ribosome content of healthy bean plants was about ten times that of healthy tobacco plants, and of healthy potato plants about 2.5 times that of tobacco plants.

Potato virus X induced a larger increase in ribosome content in tobacco plants than in potato plants. White clover mosaic virus had almost no influence on the ribosome level in bean plants.

During senescence the increase in ribosome and virus contents induced by virus infection declined. In seven-week-old tobacco plants and ten-week-old potato plants the quantity of ribosomes remained constant. Tobacco mosaic virus reduced the increase of ribosome content caused by potato virus X.

Introduction

When isolating potato virus X (PVX) and white clover mosaic virus (WCMV) by chromatography using cellulose as column material and polyethylene glycol-containing solutions, Venekamp et al. (1973) separated virus and ribosomes from the same homogenate. These authors developed a method which now permitted a study on the influence of the virussynthesis on the content of ribosomes in plants under different conditions.

The data in the literature on this influence of virus infection are contradictory. McCarthy et al. (1970) reported an increase in the ribosome content in Mung beans by infection with a strain of tobacco mosaic virus (TMV). Meister (1966), on the contrary, found 10% less ribosomes in southern bean mosaic virus-infected bean leaves than in healthy ones. According to Randles and Coleman (1970) infection of *Nicotiana glutinosa* with lettuce necrotic yellows virus also resulted in a decrease of the ribosome content.

It is known that the protein synthesis depends on the ribosomes. References on changes in the protein content by virus infection are also conflicting. Eskarous and

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Naguib (1964) described an increase in protein content in tobacco by TMV. Reddi (1964) confirmed this. Similar results were obtained by Schumann (1971) with virus-infected grasses and by Solecka et al. (1969) with apple leaves infected by Spy 227 lethal virus. Krzymánska and Hoppe (1969), however, reported a constant level of proteins in potato leaves and tubers infected with PVX and/or potato virus Y. Reid and Matthews (1966) confirmed this unaltered protein level in turnip yellow mosaic virus-infected plants.

Ageing of plants effects their protein and ribosome content also. According to Ecklund and Moore (1969) the protein content of healthy pea plants increased with age. Moreover, the ribosomes are attached to membranes during senescence (Bonner, 1965).

Thus the following questions arise:

1. Do the virus-induced changes in the ribosome contents depend on plant species and virus type?
2. How does the time elapsing between virus inoculation and the analysis influence the change in ribosome content and how is its impact on virus content?
3. What is the effect of plant age at the time of inoculation on the change in ribosome content?
4. What is the influence of plant age on virus multiplication ?
5. What is occurring in case of complex infection by PVX and TMV?

We have now tried to answer these questions by a series of experiments.

Materials and Methods

1. *Virus isolates.* Isolates of three different viruses were used. PVX was provided by Ir A. Rozendaal, Wageningen and WCMV by Dr L. Bos, Wageningen.
2. *Plants.* The experimental plants 'White Burley' tobacco, 'Bataaf' beans (*Phaseolus vulgaris*), and 'Bintje' potatoes, were grown in the greenhouse at a temperature of 20–22 °C. The health of the seed potatoes was certified by the General Netherlands Inspection Service for Field Seeds and Seed Potatoes.
3. *Estimation of ribosome and virus contents.* In all experiments diseased and healthy leaf samples of 100 g fresh weight were analysed for 80 S-ribosomes and – in case of infection – for viruses by the chromatographic procedure no. 7 of Venekamp et al. (1973).
4. *Experiments.* In Experiment 1 seventy 'White Burley' tobacco plants were inoculated with PVX when 21 days old. Another seventy plants of the same age were not inoculated. Samples were taken 8, 12, 16, 19, 21, 26, and 33 days after the inoculation. Similarly bean plants 'Bataaf' half of them inoculated with WCMV at the age of 14 days, were analysed.

In Experiment 2 eighteen 70-day-old 'Bintje' potato plants were inoculated with PVX and TMV. Thirty days after inoculation when the plants were 100 days old, three samples each from a group of six plants were used for analysis: a first sample from the four upper leaves, a second from the following four leaves and a third from the next four leaves. When 110 days and 120 days old, respectively, two other groups of six plants were analysed.

Experiment 3 consisted of three parts. In all of them leaf samples of diseased plants and healthy controls were taken 14 days after inoculation.

A. Four groups of ten 'White Burley' tobacco plants were inoculated with PVX at the ages of 28, 35, 42 and 49 days, respectively.
B. Five groups of ten 'Bataaf' bean plants were inoculated with WCMV at the age of 14, 21, 28, 35, and 42 days, respectively.
C. Eight series of six 'Bintje' potato plants were inoculated with PVX at the age of 28, 42, 49, 63, 70, 70, 91, and 91 days, respectively.

In Experiment 4 three series of ten 'White Burley' tobacco plants were simultaneously inoculated with PVX and TMV at the age of 28, 35, and 42 days. These and their healthy controls were also tested 14 days after inoculation.

Results

Effect of plant species and virus type (question 1). Healthy plant species had different levels of ribosome contents, bean plants had about ten times as much as tobacco plants (Table 1), and potato plants an intermediate concentration (Table 2).

According to Table 1 PVX on the average induced a tenfold increase in ribosome concentration in tobacco, but in bean plants the increase caused by WCMV was negligible. In potato plants infected with PVX the increase in the ribosome content was about 2 times (Table 2, see age of plants on the date of inoculation 28 and 42 days).

Influence of time since inoculation (question 2). In tobacco the increased ribosome content again decreased after 21 days after inoculation (Table 1). Diseased and healthy bean plants and healthy tobacco plants had almost constant ribosome levels.

Old diseased potato leaves (Table 3) contained very small amounts of ribosomes. At the beginning of flower production (age 110 days), the ribosome content of top

Table 1. The relative concentrations of ribosomes (Rib.), potato virus X (PVX), and white clover mosaic virus (MCMV) in diseased and healthy plants. The concentrations of ribosomes and viruses are expressed as absorbance at 260 nm wavelength and a pathlenght of 1 cm.

Days after inoculation	Absorbance/ml/g fresh weight					
	tobacco			bean		
	diseased Rib	PVX	healthy Rib	diseased Rib	WCMV	healthy Rib
8	3.21	0.12	0.53	6.75	2.30	5.98
12	8.47	1.18	0.77	9.57	2.10	8.89
16	9.34	2.10	0.49	8.01	2.00	6.79
19	7.15	2.05	0.73	5.01	2.17	6.43
21	8.27	4.86	0.64	7.83	1.27	6.21
26	5.00	0.82	0.69	7.69	1.64	6.77
33	4.48	0.73	0.72	6.51	1.23	5.38

Tabel 1. De relatieve concentraties van ribosomen (Rib), aardappelvirus X (PVX) en witteklavermozaïekvirus (WCMV) in zieke en gezonde planten. De concentraties van de ribosomen en virussen zijn weergegeven als extinctie bij 260 nm golflengte en 1 cm lichtweglengte.

Table 2. The relative concentrations of ribosomes, PVX, and WCMV in diseased and healthy plants.

Plant age at		Absorbance/ml/g fresh weight								
		tobacco			bean			potato		
inocula-tion	sampl-ing	diseased		healthy Rib	diseased		healthy Rib	diseased		healthy Rib
		Rib	PVX		Rib	WCMV		Rib	PVX	
14	28				7.44	3.00	6.42			
21	35				5.73	1.03	6.04			
28	42	8.08	1.24	0.48	7.65[1]	0.18[1]	7.38[1]	4.10	0.68	1.81
35	49	2.33	0.02	0.30	7.05	0.06	7.00			
42	56	1.45	0.04	0.61	6.09	0.18	5.36	2.78	0.59	1.23
49	63	0.62	0.02	0.60				0.99	0.64	0.65
63	91							0.73	0.48	
70	91							0.38	0.50	0.51
70	105							0.50	0.08	
91	105							0.46	0.00	0.44
91	106							0.33	0.00	0.32

[1] Plants started flowering

Tabel 2. De relatieve concentraties van ribosomen, aardappelvirus-X (PVX) en witteklavermozaïekvirus (WCMV) in zieke en gezonde planten.

leaves started to decrease. Ten days later it did not differ appreciably from that in the other leaves.

The PVX content in the tobacco plants (Table 1) had a maximum value 21 days after inoculation. Then the content decreased along with that of the ribosomes.

The WCMV content in bean already reached a maximum 8 days after the inoculation. The content decreased about three weeks after the inoculation.

In old potato plants (100 days) the top leaves contained still more PVX than the other leaves (Table 3) but at the age of 110 days this difference had disappeared. TMV could be detected in the oldest leaves only.

Effect of plant age at the moment of inoculation (question 3). According to Table 2 the increase of the ribosome content of virus-infected plants diminished with increasing age of the plants at the moment of inoculation, at least with potato and tobacco. In rather young potato plants, the increase in ribosome content induced by infection remained at a lower level than in young tobacco plants. The increase in potato plants declined when inoculated at ages of about six weeks. In contrast to tobacco and bean plants the ribosome content in healthy potato plants decreased as the plants grew older.

The influence of plant age on virus production (question 4). This is demonstrated by Table 2. The bean plants of 28 and 35 days old of this table are of comparable age as those of Table 1. The virus and ribosome contents of diseased and healthy plants of

Table 3. The relative concentration of ribosomes, PVX and TMV in potato leaves 'Bintje'. Plants inoculated with the viruses when 70 days old.

Plant age in days	Leaf position	Absorbance/ml/g fresh weight		
		Rib	PVX	TMV
100	top	1.27	0.43	–
	middle	0.36	0.13	–
	basis	0.12	0.05	0.19
110	top	0.43	0.17	–
	middle	0.21	0.23	0.10
	basis	0.20	0.04	0.28
120[1]	top	0.19	0.06	–
	middle	0.09	0.15	–
	basis	0.11	0.04	0.03

[1]Plants started flowering

Tabel 3. De relatieve concentraties van ribosomen (Rib), aardappelvirus-X (PVX) en tabaksmozaïekvirus (TMV) in aardappelbladeren 'Bintje'. Planten van 70 dagen oud werden met de virussen geïnoculeerd.

both experiments correspond also. Maturation seemed to reduce the capacity of virus production. This was obvious when the plants started to flower.

Effect of complex infection (question 5). The presence of PVX+TMV in tobacco plants induced a small increase in ribosome content (Table 4). Moreover, the PVX concentration was very low. When this concentration decreased, the amount of TMV increased. The same tendency occurred in the potato leaves of Table 3.

Table 4. The relative concentrations of ribosomes, PVX and TMV in tobacco plants, inoculated with a mixture of the viruses, and in healthy controls.

Plant age at		Absorbance/ml/g fresh weight			
inoculation	sampling	diseased			healthy Rib
		Rib	PVX	TMV	
28	42	1.45	0.19	2.91	0.57
35	49	2.12	0.07	3.90	0.78
42	56	2.42	0.08	6.28	0.77

Tabel 4. De relatieve concentraties van ribosomen (Rib), aardappelvirus-X (PVX) en tabaksmozaïekvirus (TMV) in tabaksplanten, geïnoculeerd met een mengsel van de virussen, en in gezonde tabaksplanten.

Discussion

The ribosomes isolated here had a sedimentation constant of 80 S. This was confirmed by criteria described by Venekamp et al. (1973).

In general PVX induced a considerable increase in ribosome content in tobacco. This may result in an enhanced protein synthesis. In healthy bean plants the ribosome content was higher than in healthy tobacco plants and virus infection did not increase it in bean plants as much as in tobacco plants. The ribosome level of healthy bean plants was obviously already at maximum and a further rise seemed not possible. PVX infection induced an increase in ribosome content in tobacco plants as to the same maximum as in bean plants.

Older tobacco plants (age of about 35 days) seemed to have a much lower capacity to synthesize ribosomes. Plants of 49 days old were even not able to increase the ribosome concentration after inoculation.

The growth rate of the virus-diseased bean plants retarded in comparison to that of the healthy bean plants. Virus production claimed part of the protein-synthesizing activity, thus decreasing normal processes. This is indicated by the constant level of ribosome content. In tobacco plants PVX did not retard the growth rate. Likely, the ribosome concentration, indicated by the absorbance /ml/g fresh weight of approximately 0.50, was still sufficient for the normal plant functions.

It is not certain that the healthy potato plants were really virus free. However, contaminants were not detected by the isolation procedure used. A contaminant might have been responsible for the absorbance/ml/g fresh weight of more than 0.50. PVX did not induce an increase in ribosome content in potato plants older than two months.

TMV induced a decrease in ribosome content in tobacco plants; Venekamp et al. (1973) could not isolate ribosomes from these infected plants. In the present paper the authors already suggested that the TMV was produced at the expense of the ribosome concentration. In the case of simultaneous occurrence of TMV and PVX in tobacco plants, PVX increased and TMV decreased the ribosome concentration. This resulted in a final increase smaller than the increase by PVX alone (Table 4). Moreover, the growth rate retardation was smaller when the tobacco plants contained TMV as well as PVX than when infected with PVX alone.

At the beginning of flowering, the upper leaves behaved like old mature leaves in spite of the fact that these top leaves were not fullgrown (Table 3). The ribosome level was obviously too low for normal growth. In all cases of such a low level the PVX content was almost nil. Then TMV could be detected. This also shows the opposite effect of these viruses.

The virus-synthesizing capacity decreased with age of the plant. Sometimes this decrease started after attaining a maximum virus level (Table 1, see tobacco). In potato plants of more than 100 days old (Table 2) no PVX could be detected. These plants obviously had mature resistance.

Samenvatting

Veranderingen in het ribosoomgehalte van gezonde en viruszieke planten gedurende veroudering

Het gehalte aan ribosomen en virus van tabaksplanten van verschillende leeftijden, geïnoculeerd met aardappelvirus-X werd chromatografisch bepaald en vergeleken met op overeenkomstige wijze bepaalde ribosoomgehalten van even oude gezonde planten. Dezelfde analysen werden uitgevoerd met boneplanten, geïnoculeerd met witte-klavermozaïekvirus, en met aardappelplanten en tabaksplanten, geïnoculeerd met aardappelvirus-X en tabaksmozaïekvirus zowel afzonderlijk als in complex.

Het ribosoomgehalte van gezonde boneplanten bleek meer dan tien keer zo hoog als dat van gezonde tabaksplanten (Tabel 1) en van aardappelplanten ongeveer 2,5 × zo hoog als dat van gezonde tabaksplanten (Tabel 2).

Aardappelvirus-X had een verhoging van het ribosoomgehalte bij tabak tot gevolg (Tabel 1). Bij aardappel was deze verhoging geringer (Tabel 2). Witte-klavermozaiekvirus verhoogde het ribosoomgehalte bij bonen nauwelijks (Tabel 1).

Bij veroudering nam de verhoging door virusinfectie sterk af. Bij tabak van zeven weken oud was geen verhoging merkbaar. Bij aardappel trad de verhoging niet meer op, wanneer de planten tien weken oud waren (Tabel 2).

Tabaksmozaïekvirus verminderde de verhoging van het ribosoomgehalte dat door aardappelvirus-X werd veroorzaakt (Tabel 4).

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Book review

L.Chiarappa (Ed.): Crop loss assessment methods. FAO Manual on the evaluation and prevention of losses by pests, diseases and weeds. Published by arrangement with the Food and Agriculture Organization of the United Nations by the Commonwealth Agricultural Bureaux, Farnham Royal, Slough SL2 3BN, England, 1971. Price £ 5.

This FAO manual is loose-leaf to allow insertion of supplements and amended pages, obtainable by subscription.

The manual has four sections: Introduction, Field experiments and Sample surveys, Methods, and Miscellanea. In 'Methods' techniques and apparatus, as well as worked out examples of techniques of measuring disease with 84 host-pathogen combinations are described. 'Miscellanea' contains useful conversion tables and diagrams to determine growth stages of important crops.

The list of contributors is impressive. The book will help those in charge of crop loss assessment programmes, especially in areas where this type of work is being newly developed. The manual will be a stimulus towards more work in crop loss assessment, for which there certainly is a need. In future supplements extensions are desirable not only in the technical sections, but also with regard to principles and backgrounds of crop loss assessment.

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