The RNA and its nucleotide composition in different parts of healthy and virus X infected potato plants

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INTRODUCTION

Changes in the contents and nucleotide composition of RNA in the course of living organisms development have bean the object of interest of many researchers. Research works concerned both living organism of animals [15] and lower plants — algae [13, 18], as well as higher, vascular plants. In almost all publications it has been stated that younger organisms and organs show in an early developmental stage a higher content of ribonucleic acid than older ones.

Ingle and Hageman [8] found highest contents of RNA and soluble nucleotides in corn sprouts, 5 days after germination. Ledoux and Huart [12] examined the RNA level in germinating barley and proved that highest level of RNA could be observed 4 days after germination, and it subsequently decreases. Roots contained more RNA than coleoptiles. Investigations of Van Parijs [22] show that the initially high quantity of ribonucleic acid present in pea seedlings, was gradually decreasing with the plant development. Similar results were achieved by Olsson and Boutler [14] who analysed *Vicia faba* L.

Tobacco leaves of various age were analysed with respect to RNA contents by Holden [7], Bottger and Wollgiehn [3] and Hageman [6]. They found that the total quantity of nucleic acids is higher in adolescent leaves than in older ones. Similar conclusions were drawn by Kessler who examined leaves of apple trees, Swirski and Tahori [9] as well as Kessler and Engelberg [10]. Sisakian and Odincowa [17] found that not only leaves of various age (of the same plant) differ from each other in regard to the RNA level; similar differences could be also observed in organs of plants of the same variety collected at different stages of development.

Whereas, changes in contents and composition of RNA according to the growth and development of plants were the subject of many publications, changes of RNA level in virus infected plants and their organs have not been commonly investigated. Plant virologists seem to be more concerned with the metabolism of virus nucleic acids and especially with their synthesis. Experiments carried out by Röttger [16] on tobacco leaves of Samsun variety infected with TMV showed that in comparison to healthy leaves RNA contents in infected leaves increase already in a short time after infection. Progressing infection of TMV causes not only an increase of RNA in inoculated leaves but also a slight qualitative displacement in the nucleotide composition of RNA. Bassler and Commoner [2] as well as Altmann and co-workers [1] found a drop of insoluble RNA level in tobacco inoculated with TMV.

Investigations of Ulrychova and Limberek [19, 20] showed that: (1) contents of RNA in stems and leaves of the potatoe variety Apta increased in a 3-weeks period following their infection with leaf-roll virus and subsequently decreased below the characteristic content of healthy plants; (2) in comparison to healthy plants the level of RNA increased in tomato leaves infected with the potato withes'-broom and decreased in stems; (3) leaves and stems of *Nicotiana glauca* plants infected with that virus contained less RNA than healthy ones.

Potato plants cultivated in field conditions were investigated in the present work. Plants used for investigation came from 2 different soil conditions, from loess and calcareous ("rędzina") soil, but were raised in the same climate. The present paper aimed to investigate the contents of ribonucleic acid and its nucleotide composition in organs of healthy potatoes and those inoculated with the potato virus X, in two different developmental stages.

MATERIAL AND METHODS

INVESTIGATED MATERIAL

The tuber material of potatoes of the Epoka variety, superelite class, was cultivated in Polanowice on two kinds of soil. In 1964 in lower, flatter parts Polanowice is covered by loess soils, which change further to the west directly into calcareous (chalk "rędzina") soil, covering higher hilly terrains. Sixty plants in 6-leaves stage grown on calcareous and loess soil were inoculated with potato virus X, and parallel groups were treated as controls. Infection was almost 100%. This was proved by means of the serological method at the flowering stage. Towards the end of the vegetation tubers were harvested separately from under each plant and replanted in the same conditions in the year 1965. One month following germination average samples of virus X — infected and virus free plants were taken from the field for analysis; being examined previously by the serological method.

The plants were characterized by 6 developed leaves, average height 7 cm and average number of haulms 4. A succesive portion of plants was taken to analysis in the flowering stage i. e. about the middle of July. The average height of these plants amounted in the case of loess soil to 54 cm and as regards calcareous soil — to 46 cm. Average number of haulms — 4 and leaves — on the main sprout — 15.

Before collecting, the experimental material it was again examined serologically and optically for the presence of virus X. No symptoms of virus Y (streak), or other viruses were observed.

COLLECTING AND PRELIMINARY TREATMENT OF MATERIAL FOR ANALYSES

Plants for analyses were carefully dug out of the earth, to avoid any damage of the root system, and transported to the laboratory. Hawing been washed in tap water they were divided into individual organs — leaves, stems and roots.

At the flowering stage, leaves were divided into 3 groups: (1) top leaves (from the 9th up); (2) middle leaves (5-8th included); (3) bottom leaves (1st-4th included). Immature tubers constituted at this stage a separate fraction.

The material prepared in such a way and partly triturated was kept at 25°C for at least 48 hrs; then it was liophilized and additionally dried in a vacuum dessicator in presence of P_2O_5. Dry material was pulverized in a mortar and in a homogenizer, put trough a hair sieve, and stored in a refrigerator in tightly closed dark--glass jars.

DETERMINATION OF RIBONUCLEIC ACID AS A SUM OF FOUR MONONUCLEOTIDES

An analysis of plant material for the content of ribonucleic acid — with mononucleotides taken into account — was carried out by Fritz and Röttger method [5], in which the technique of column chromatography was applied with the use of Dovex 1×10, 200-400 mesh.

The green liophylized pulver was gradually purified from acid soluble phosphates, pigments and lipids by means of diluted $HClO_4$, ethanol, a mixture of ethanol with ether and finally with pure ether; it was subsequently descicated. 100 mg of this pulver was subjected to alcaline hydrolysis in 1 N KOH in 37°C for 22 hours.

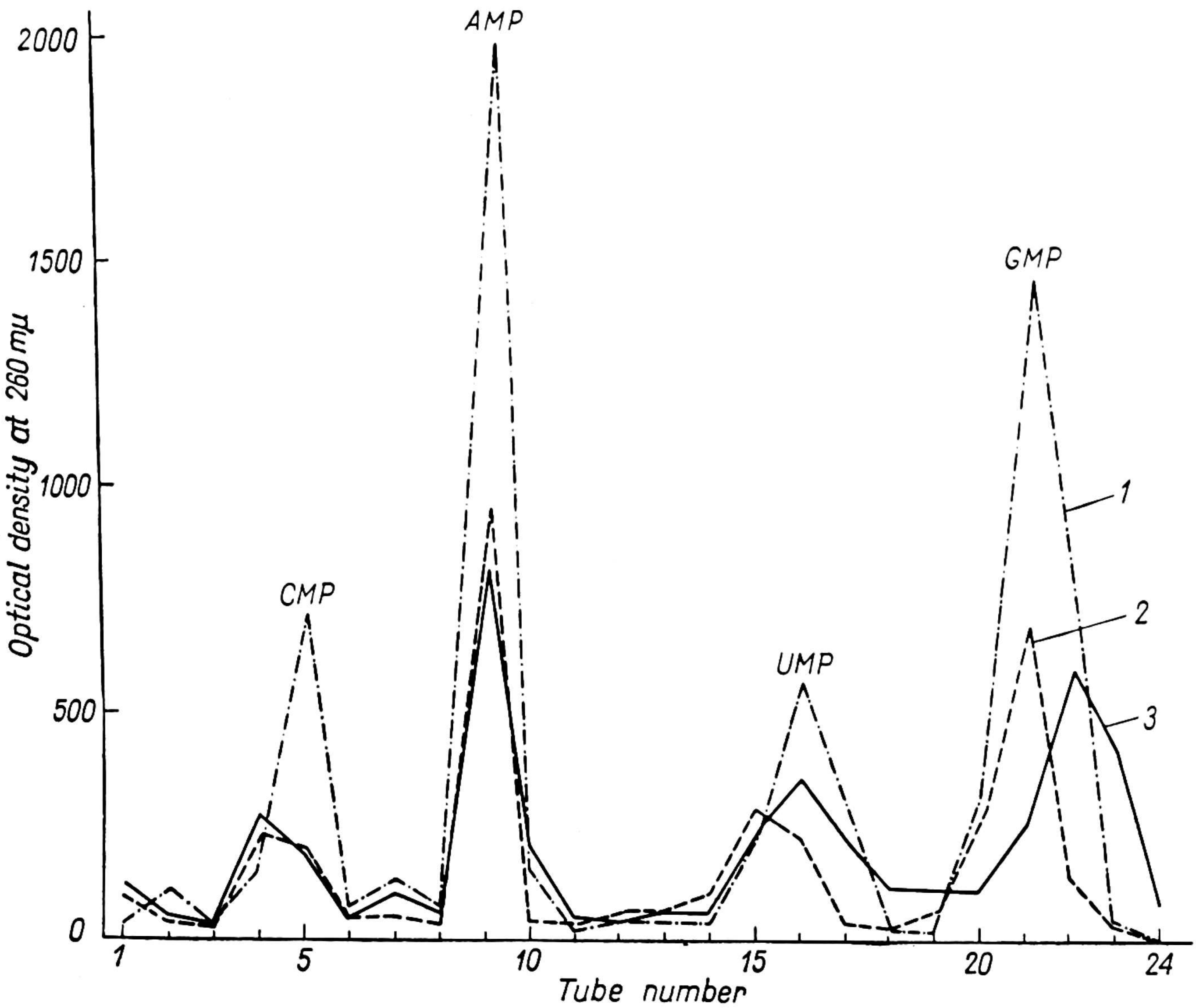

Fig. 1. Example of chromatographic separation of RNA mononucleotides from leaves — *1*, roots — *2*, and stems — *3*, of healthy potato plants, cultivated on calcareous soil at Polanowice. Adolescent stage.

It was subsequently cooled to 0°C acidified with $HClO_4$, purified from sediment containing DNA and afterwards from the excess of $HClO_4$ by applying 4 N KOH. The supernatant containing hydrolized RNA to mononucleotides was then put onto columns and fractionated; CMP, AMP, UMP and GMP were subsequently washed out by means of formic acid of increasing concentration, then by a mixture of formic acid and ammonium formate. One of the columns free from analyzed material served as control. The material was run averagely for 24 hours. 10 mm fractions were manually collected into test tubes and then kept in a refrigerator. Extinction of every fraction was measured in a Hungarian spectrophotometer "Spektromom 201". Coefficients were calculated for every fraction from the ratio E 280/260 and compared with values characteristic for separate mononucleotides. Subsequently extinction at 260 mμ, of fractions of similar coefficients were summed up and the content of mononucleotides in mg/100 mg dry weight of the purified tissue free from acid-soluble phosphates pigments and lipids was calculated. The total of these mononucleotides corresponded to the RNA content.

Figure 1 shows an example of chromatographic separation of the RNA, from various organs of potatoes harvested in adolescent stage of development.

RESULTS

RNA CONTENTS AND ITS NUCLEOTIDE COMPOSITION IN SEPARATE ORGANS OF POTATO PLANTS, HARVESTED AT TWO STAGES OF DEVELOPMENT

A comparison of average contents of ribonucleic acid as the sum of four mononucleotides in the leaves, stems, and roots of plants harvested from loess and calcareous soil at two stages of development — adolescent and flowering stage —

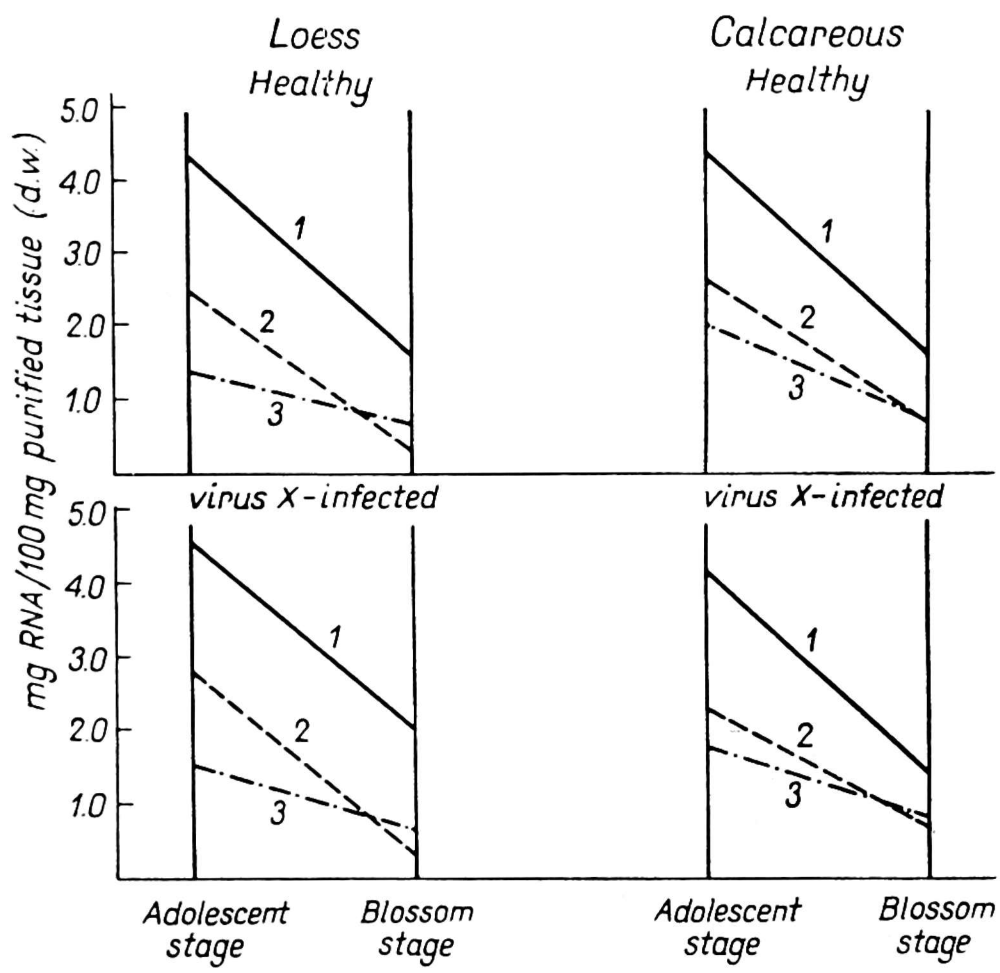

Fig. 2. RNA content in healthy and virus X infected potato plants, in adolescent and blossom stage. *1* — leaves, *2* — stems, *3* — roots.

is shown in Fig. 2. In adolescent plants the RNA content oscillates from 4.2-4.6 mg/100 mg dry weight of purified tissue in the leaves, from 2.3-2.8 in the stems, and from 1.4-2.1 in the roots. With older, flowering plants those quantities amount to: 1.5-2.1, 0.4-0.8 and 0.7-0.8 respectively.

It can be thus clearly seen that the development of plant was accompanied by an evident decrease of RNA level — 2.5-fold in leaves and roots, 6-fold in the loess-soil stems and 3-fold in the calcareous soil stems. In adolescent plants the differences in RNA level between stem and root were clearly visible, especially in loess-soil grown plants. Those differences were obliterated in the flowering stage and in case of material coming from loess soil the RNA contents in stems decreased even slightly below the RNA level in roots.

RNA CONTENTS IN ORGANS OF PLANTS HARVESTED IN THE ADOLESCENT STAGE

The phenomenon of RNA decrease, accompanying the plant development is presented in Fig. 2. Fig. 3 shows the total RNA contents in potato organs (adolescent

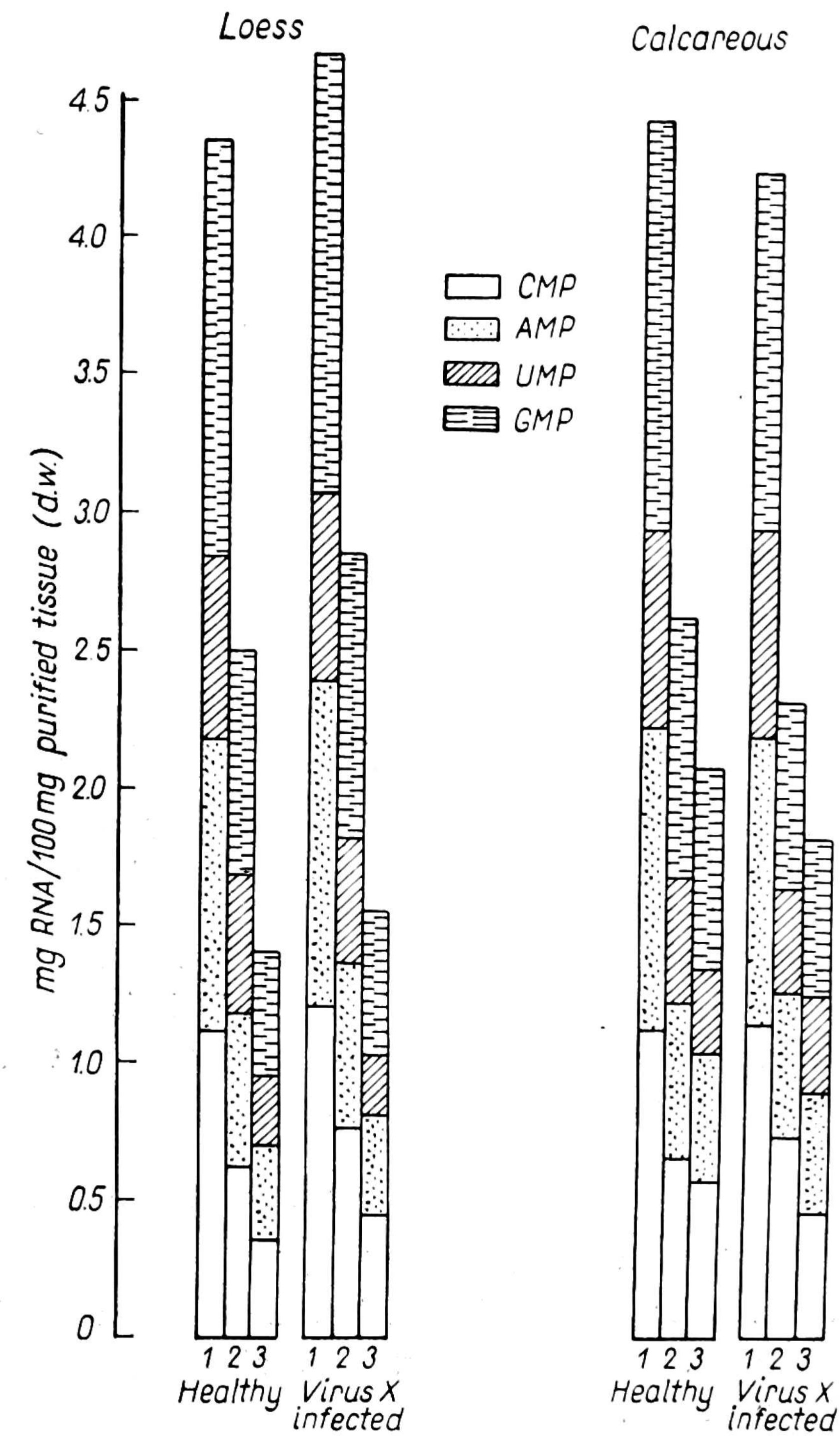

Fig. 3. RNA content as the sum of its mononucleotides in different organs of potato plants, cultivated at Polanowice. Adolescent stage. *1* — leaves, *2* — stems, *3* — roots.

stage) as a sum of four mononucleotides, expressed in mg/100 mg dry weight of purified tissue. It looks clearly that the highest content of ribonucleic acid was found in leaves, much less — in stems, and least of all — in roots. These contents are given in absolute numbers.

NUCLEOTIDE COMPOSITION OF RNA IN THE ORGANS OF PLANTS HARVESTED IN THE ADOLESCENT STAGE

The nucleotide composition of RNA was considered adopting the molar percentage as the basis. The sum of CMP, AMP, UMP and GMP moles was treated as 100. As it can be seen from Fig. 4, in organs of adolescent plants no essential differences in this respect could be observed. In all cases guanylic acid constitued the biggest part i. e. up to 34.3 molar percentage, then cytydylic acid, adenylic acid and urydylic acid came in succesion. The percentage of the last one oscillated within the range

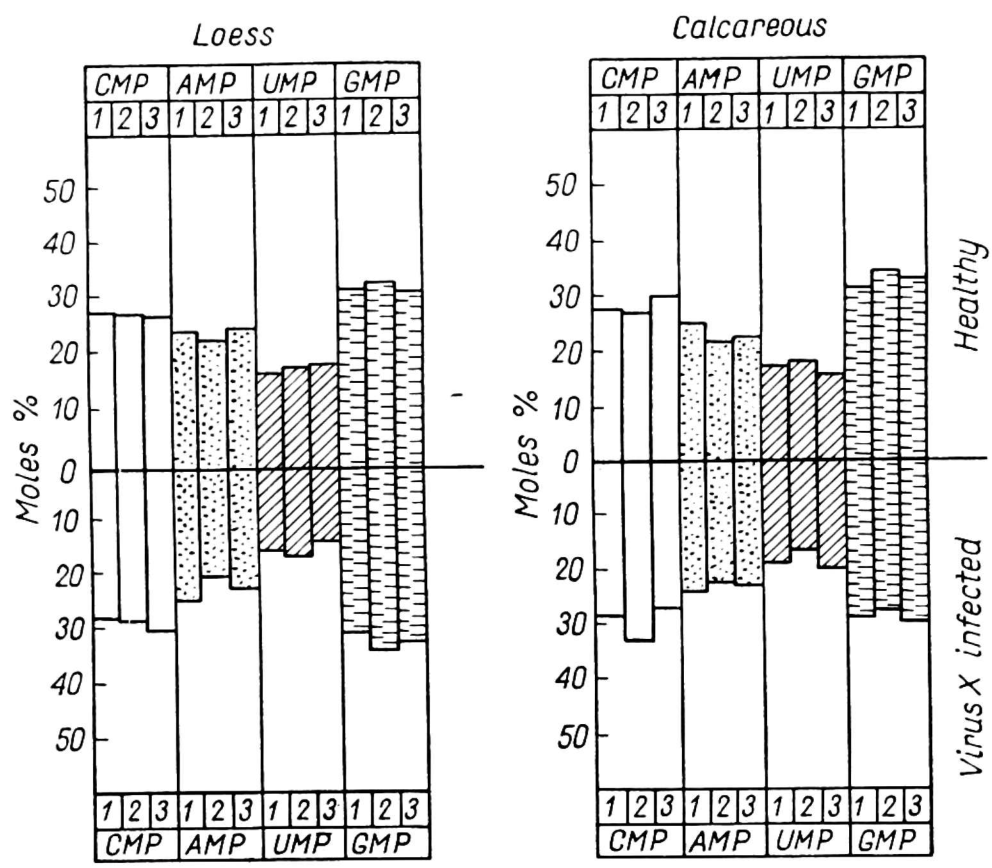

Fig. 4. RNA composition in different organs of potato plants. Adolescent stage. *1* — leaves, *2* — stems, *3* — roots.

of 18.0 both in leaves, stems and roots. Thus, the ribonucleic acid of adolescent plants was rich in GMP and CMP. This is in accordance with results of Biełozierski (1959) and Röttger [16] who found that RNA of vascular plants such as tobacco, poppy, beans or wheat belongs to the G—C type.

RNA CONTENTS IN PLANTS HARVESTED IN THE BLOSSOM STAGE

Similar but more detailed analyses were carried out at the blossom stage of plants. Leaves were divided into 3 groups according to their age; besides stems and roots, immature tubers were also examined for RNA contents. Results are shown in Fig. 5.

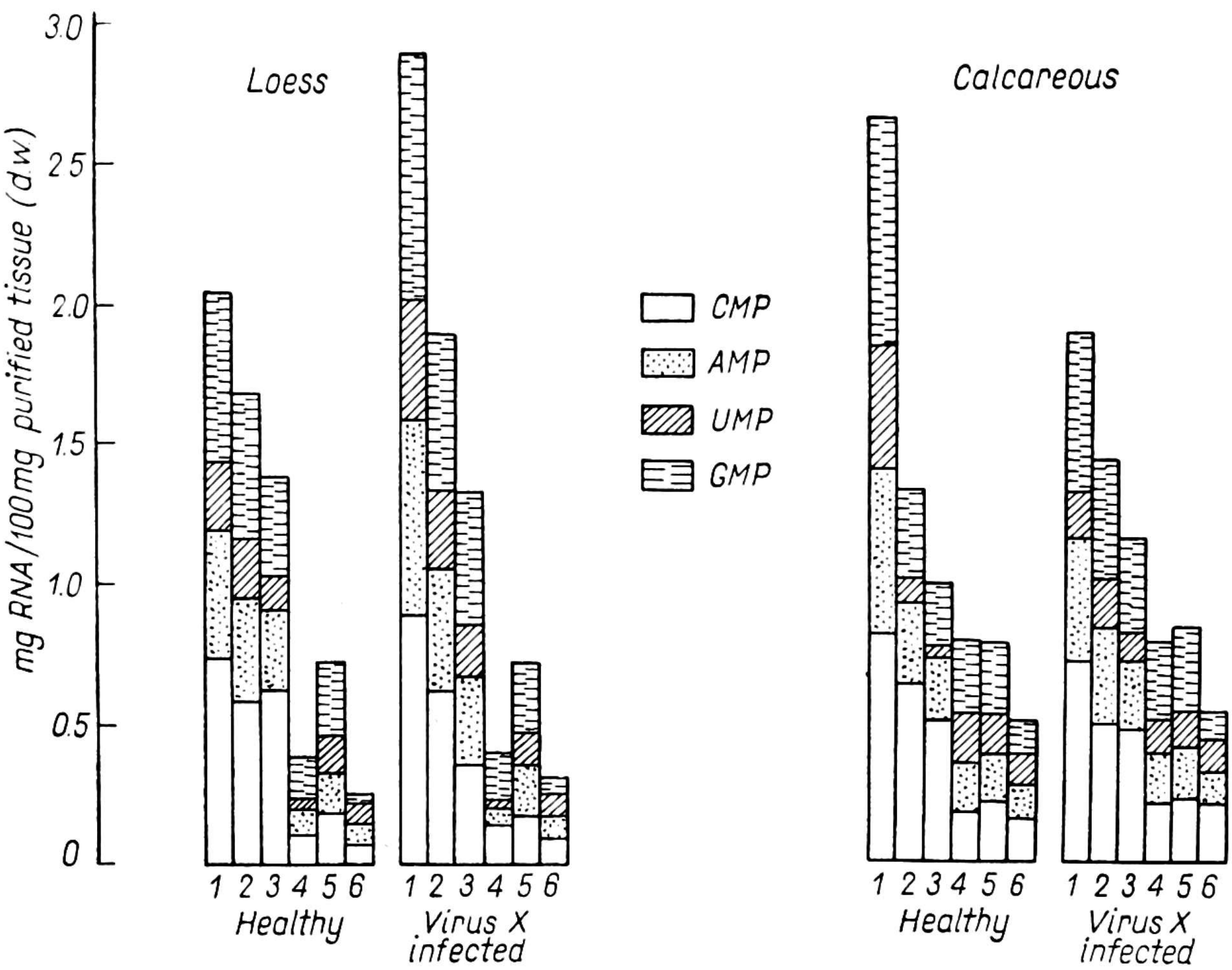

Fig. 5. RNA content as the sum of its mononucleotides in different organs of potato plants, cultivated at Polanowice. Blossom stage. *1* — top leaves, *2* — middle leaves, *3* — bottom leaves, *4* — stems, *5* — roots, *6* — tubers.

In accordance with the data given by authors quoted in the introduction, highest content of RNA was found in top leaves, the youngest ones, smaller quantities in middle leaves, and the lowest ones in the bottom leaves — the old ones. In the remaining organs the RNA level was found to be — in comparison to leaves — much lower. An insignificant quantity of that compound was discovered in stems of potatoes raised in loess soil and also in tubers. Similarly as was the case with young plants, the RNA contents in the roots and stems raised in calcareous soil were similar. In plants raised on loess soil those RNA contents differed. In tubers the RNA level, although generally low, was in the loess-soil cultivated plants lower than in those grown on calcareous soil.

NUCLEOTIDE COMPOSITION OF RNA IN THE ORGANS OF PLANTS HARVESTED AT THE BLOSSOM STAGE

The nucleotide composition of ribonucleic acid of individual organs is illustrated in Fig. 6. In all parts of flowering plants, except of tubers, coming from loess soil, the G—C system dominates in RNA. This means that the ratio G+C/A+U, or the so called "coefficient of specificity" [4] is higher than 1. Generally speaking an especially high quantity of cytydylic acid was discovered in leaves. Oldest leaves had the highest quantity of cytydylic acid and this was accompanied by a low contents of urydylic acid, which was in those organs even below 10 molar percentage. Nucleotides of the G—C type were prevailing especially in the leaves of

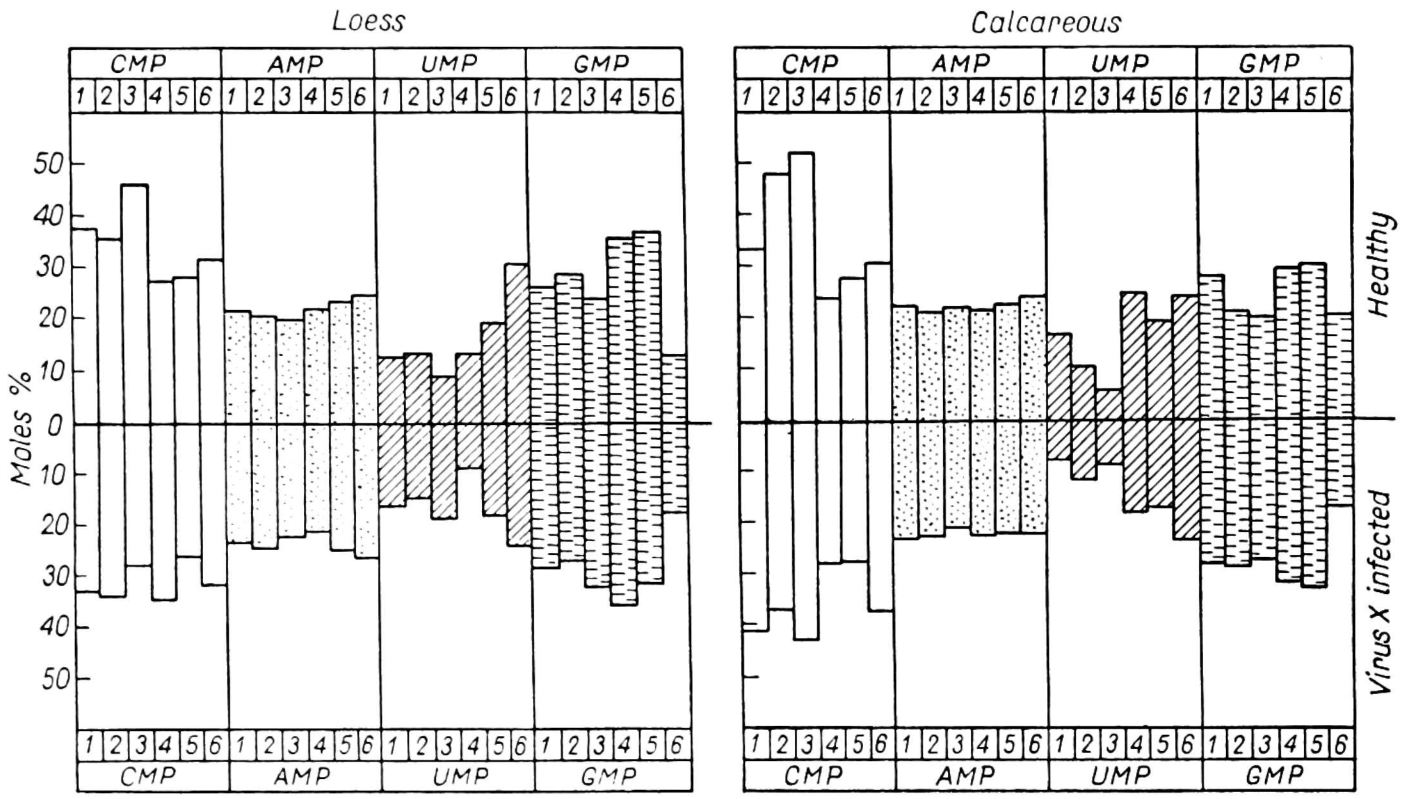

Fig. 6. RNA composition in different organs of potato plants. Blossom stage. *1* — top leaves, *2* — middle leaves, *3* — bottom leaves, *4* — stems, *5* — roots, *6* — tubers.

plants cultivated on calcareous soil and this is indicated by a high coefficient of specificity. In the RNA of stems and roots GMP constitutes the highest molar percentage of the nucleotide composition. In comparison to the leaves the molar percentage of UMP increases here too. In RNA of immature tubers the GMP contents is comparatively low, whereas the UMP in the RNA of those organs constitutes a comparatively high molar percentage. These displacements cause a drop of the tuber coefficient of specificity even below 1.

CONTENTS AND NUCLEOTIDE COMPOSITION OF RNA IN HEALTHY PLANTS IN COMPARISON TO PLANTS INFECTED WITH POTATO VIRUS X

Some differences in the contents and nucleotide composition of ribonucleic acid appeared between the healthy plants and those infected with potato virus X. It ought to be noted that the investigated potatoes raised in the field were in a state of virus degeneration and showed symptoms of the disease in form of a delicate mosaic. In all cases their serological reaction with diagnostic antiserum was positive.

Analyses showed that differences in the contents of total RNA between healthy and diseased plants were most evident in the top leaves at the blossoms stage. The appearance of those differences differed with the soil on which plants had been grown (Fig. 5). Loess soil offered optimal conditions for the development of potatoes and it was here that youngest virus-infected leaves contained more RNA than healthy ones. In calcareous soil a reverse situation could be observed. Here, the virus X influenced to a high degree the inhibition of RNA synthesis in the top leaves. A similar phenomenon was observed in leaves, stems and even roots of plants harvested at the adolescent stage, but at this stage the changes were much less clearly marked. Leaves from lower positions on the stem and the remaining organs of flowering plants, both infected and healthy ones, differed slightly or showed no divergencies in the RNA level.

It was important to find whether a long-lasting virus infection had or had not caused any displacements in the nucleotide composition of RNA. According to Knight [11] the potato virus X has following quantities of individual nucleotides: CMP — 23.8 molar percentage; AMP — 32.2; UMP — 22.2 and GMP — 21.8. Since RNA PVX contains most of the adenylic acid, the question was to find out whether in potatoes infected with that virus any displacements occurred which could tend to increase the AMP content. It was also important to show whether the ratio G+C/A+U which in normal plant RNA is higher than 1, will be shifted to the direction characteristic of virus X, that is 0.82.

In the first, adolescent stage, when infected plants were averagely about 7 cm high and 6 developing leaves, changes in nucleotide composition of RNA in their organs were — in comparison to healthy plants — comparatively insignificant and irregular (Fig. 4). An increasing tendency was observed in CMP, exept RNA of roots from calcareous soil, and a slightly decreasing tendency was noted in GMP, especially on calcareous soil. In UMP and AMP the oscillations were not significant and no regularity in this respect could be observed.

In virus X infected plants harvested at the blossom stage the changes found in the nucleotide composition of RNA (Fig. 6) were as follows: the AMP contents in the RNA of leaves was slightly higher. This increase did not, however, exceed 2.7 molar percentage. A distinct decrease of cytydylic acid level was marked here, too. What is striking here is that only youngest virus infected leaves from calcareous soil, i. e. those in which a decrease of RNA was observed, contained more CMP that healthy ones. In stems an increase of cytydylic acid by about 5 molar percentage was noted; this does not point to any accumulation of virus in the conductive tissues.

UMP in the RNA of virus X infected leaves slightly increased and in RNA of remaining organs decreased; this is especially evident in stems. The molar percentage of GMP in RNA of all parts of plants cultivated on calcareous soil, exept tubers and youngest leaves, tends to increase. In the loess soil material, the increase of GMP occurs only in the RNA of top and bottom leaves and in tubers.

Despite apparent irregularities in the changes of nucleotide composition of RNA of virus infected plants in relation to healthy ones, a certain tendency of shifting of the RNA coefficient of specificity towards the ratio characteristic of RNA-PVX is observed. This is shown in Table 1. At the adolescent stage the disease brought an insignificant decrease of the RNA coefficient of specificity in leaves of plants grown on both knids of soil and in roots from calcareous soil. In stems this coefficient slightly increases or remains unchanged.

The tendency of the coefficient of specificity to decrease at the flowering stage of plants is shown in leaves i. e. organs most active in the biosynthetic process and in the roots coming from loess soil. Quite a contrary phenomenon can be observed in stems, tubers and roots as well as in youngest top leaves cultivated on calca-

Table 1

C+C/A+U

Organs	Loess		Calcareous	
	healthy	PVX-infected	healthy	PVX-infected
Adolescent stage				
Leaves	1.50	1.40	1.41	1.32
Stems	1.49	1.65	1.56	1.53
Roots	1.35	1.70	1.64	1.30
Blossom stage				
Top leaves	1.85	1.55	1.57	2.22
Middle leaves	1.85	1.55	2.19	1.87
Bottom leaves	2.41	1.47	2.64	1.34
Stems	1.78	2.35	1.15	1.47
Roots	1.50	1.35	1.38	1.53
Tubers	0.80	0.96	1.05	1.30

reous soil. In the latter case this fact can be associated, as it has been already mentioned, with inhibited synthesis of RNA in those leaves as compared to healthy ones.

CONCLUSIONS

In the course of investigations of the contents of the total ribonucleic acid in potatoes of the variety Epoka grown in Polanowice on loess and calcareous soil, following statements have been made:

1. Plants harvested in the adolescent stage contained more RNA than plants in the blossom stage.

2. In both stages of development biggest concentration of RNA was observed in leaves a much smaller amount of this compound was found in stems and roots, and least of all in immature tubers.

3. All organs of adolescent plants have a more or less analogous nucleotide composition; in the organs of flowering plants certain differences in that respect have appeared.

4. In RNA of potato plants nucleotides of G—C type are predominant.

5. RNA in leaves of plants derived from calcareous soil is richer in CMP and GMP, as compared to loess, and this is evidenced by a higher coefficient of specificity.

6. In loess soil, the potato virus X influenced the increase of RNA synthesis in top leaves in the blossom stage, whereas in calcareous soil the RNA synthesis in those organs was inhibited as the result of infection.

7. In leaves of virus infected plants a displacement of the coefficient of specificity in the direction of ratio characteristic of RNA-PVX was noted.

SUMMARY

Organs of healthy and virus X infected potatoes of the Epoka variety harvested at two stages of development — adolescent and blossoming, were examined for the contents of RNA mononucleotides. Analyses were made by means of Fritz and Röttger method of column chromatography. Potatoes were grown in fields situated in one locality and within the range of the same climate, but on two different soils — loess and calcareous soil.

The results of investigations showed the presence of considerably higher quantity of RNA in the adolescent plants, as compared with the blossoming plants regardless of their state of health. Individual organs contained different quantities of RNA. The highest contents were found in leaves, the lowest in tubers. The RNA found in potatoes belonged to the G—C type what coincides with the data given in literature. The nucleotide RNA composition of the adolescent plant organs was approximately the same, and in the organs of flowering potatoes it slightly differed.

Virus infection shifted the coefficient of RNA specificity in the direction of ratio characteristic of PVX; this was distinctly marked in the leaves. The infection also caused a decrease of RNA synthesis in top leaves of potatoes grown on calcareous soil and harvested at blossom stage, and a decrease of that synthesis in parallel organs of blossoming potatoes grown on loess soil.

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