Acta Bot. Croat. *32* (1973) 37—42

THE RELATIVE CONCENTRATION OF RADISH MOSAIC VIRUS IN TURNIP

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Received February 7, 1973

Introduction

On the basis of recent findings (Štefanac and Mamula 1971; Mamula and Miličić 1971; Mamula et al. 1972; Shukla and Schmelzer 1972) it seems that radish mosaic virus (RaMV) often attacks cruciferous plants in Europe. In Yugoslavia the virus causes very common outbreaks in turnip (*Brassica rapa* L. var. *rapa*) in which it exists in the form of a distinct strain (the European strain). According to serological properties and the reaction of certain test plants the European strain differs from the type strain found in California and Japan (Štefanac and Mamula 1971).

Turnip is not only very often infected with RaMV in the fields, but it is also very appropriate for experimental work with the virus. This species grows relatively quickly and apparently does not contain viral inhibitors; it is not difficult to get the virus from turnip in sufficient amounts. This paper presents the results of studies concerning the concentration of RaMV in turnip and seed transmission of the virus.

Material and Methods

Virus isolate and serum. The work was done with the HZ isolate of the European strain of RaMV (Štefanac and Mamula 1971). The virus was transmitted to turnip and other test-plants by mechanical inoculation of leaves dusted with carborundum. A serum against HZ isolate was prepared previously (Štefanac and Mamula 1971). It had a specific titre of 512—1024 with the homologous virus and titre of 8—16 with normal plant proteins.

Inquiry of relative virus concentration. The relative concentration of the virus was estimated serologically in agar-gel by using the serum at dilution of 1 : 60. For these tests, sap extracted from the infected plants was clarified by low-speed centrifugation (3000—4000 rpm/min), then series of twofold dilutions of clarified sap were made in 0.14 M saline and a virus end-point was determined. Appropriate control with healthy plant sap was included in all tests.

Experiments of virus transmission through seed. The seed-transmission was studied on artificially infected plants grown in the glasshouse. The seed was harvested only from plants which, when fructifying, showed obvious symptoms of infection and in which the virus could be detected serologically. After sowing in sterilized soil and after germination, seedlings were transplanted into boxes. All progenies were singly indexed for virus by serology tests and a certain number in addition by inculating sap to *Chenopodium murale* L.

Results

Concentration of the virus in turnip

Several experiments were done with the intention to establish 1) how the concentration of the virus changes in the course of infection, 2) whether the concentration of the virus differs in old and in young leaves and 3) how the virus influences the yield of turnip.

The quantity of the virus in plants was defined by its relative concentration. For these tests young plants were infected in the stage of 6—8 leaves and grown in the glasshouse at an approximate minimum of 7° C and maximum of 28° C.

1. *Change of virus concentration during the infection*

To determine the change of virus concentration during the infection, we first examined the concentration of the virus in plants during the first month of infection. For this purpose a certain number of plants were inoculated and subsequently, at suitable intervals, checked for relative quantity of the virus. Before the appearance of systemic symptoms, the concentration was determined only in inoculated leaves, but later, when after 12 days prominent symptoms in younger leaves developed, all leaves from the pants were collected for assay. Plants were tested in groups of five. From the results recorded in Table 1, it is evident that the virus concentration at the begining of infection rapidly increased and afterwards, i. e. after the fourth and twelfth day, respectively, it did not essentially change.

In the second group of experiments, concentration of the virus was investigated in plants suffering from infection for a longer time. In these tests we did not include the plants (about 20% of inoculated ones) which after a certain time recovered. On every occasion the plants raised at the same conditions were compared. All together we did five measurements. The values recorded in Table 2 indicate that the quantity of the virus in plants was nearly the same in spite of a longer period of infection. In other words, it has been established that the length of infection does not have any significant influence on the virus concentration.

Table 1. Concentration of RaMV in turnip plants during the first month of infection

Duration of infection (in days)	Virus concentration*
2	0
3	0
4	1/4
5	1/16
12	1/16
20	1/16
27	1/16
31	1/32

* The relative concentration measured by means of virus end-point

Table 2. Concentration of the virus in plants infected for short and long periods

Temperature (°C)*		Virus concentration	
min.	max.	short period of infection**	long period of infection***
10	19	1/16	1/16
10	20	1/32	1/32
6	29	1/32	1/64
7	28	1/32	1/32
8	25	1/32	1/32

* The average minimum and maximum temperatures during the week before the experiment. ** Infections approximately a month old. *** Infections more than eight months old.

2. *Virus concentration and age of leaves*

The concentration of the virus in the old (outside) and the young (inside) leaves was determined several times. The plants examined had well-marked symptoms and were infected for a month or more. The virus occurred usually in several times higher concentration in the old leaves than in the young ones (Table 3). The minimal difference in concentration between the old and young leaves was found in samples B and D where the virus concentration in the old

Table 3. Concentration of the virus in old and young leaves

Sample	Temperature (°C)		Virus concentration	
	min.	max.	old leaves	young leaves
A	7	28	1/32	1/2
B	10	23	1/64	1/32
C	7	28	1/32	1/8
D	11	16	1/32	1/16
E	11	16	1/4	0
F	11	16	1/16—1/32	1/4

leaves was one time higher than in the young, and it was the maximal in sample A where the former contained even sixteenfold quantity of the virus present in the later.

3. *The effect of the virus on the yield*

The influence of the virus concentration on the yield of turnip was examined by serological testing of tuberous root during the period of seed maturation. The results show (Table 4) that a certain relation existed between the quantity of the virus present and the root weight. On the average, the presence of the virus could be proved serologically only in the roots which weighed between 30 and 90 g. In samples which exceeded 90 g the virus could not be detected serologically in spite of the fact that their leaves showed signs of infection. It is evident that the virus concentration in the second case was too low to be detected serologically.

In addition we noticed that the infected plants yielded less seed than the healthy ones.

These results are in agreement with our observations in the field that RaMV can remarkably reduce the yield of turnip.

Table 4. Weight of the tuberous root and concentration of the virus

Root weight (g)	31	34	38	41	46	48	64	70	80	86	87	88	93	98	110	127	132	177
Virus concentration	1/4	1/4	1/4	0	0	1/8	0	1/4	1/2	1/4	0	1/2	0	0	0	0	0	0

Experiments on seed transmission

All together we examined 150 young plants of turnip (*B. rapa* L. var. *rapa*), 45 of *Capsella bursa pastoris* (L.) Med., 50 of *Sinapis alba* L. and 30 of *S. arvensis* L. the parents of which were infected with RaMV. No symptoms of infection were noticed in any of the progenies. To detect the possible presence of the virus in these plants, we tested them serologically against RaMV. None of them reacted with the serum. In addition, 50 young turnips, derived from seed of infected plants, were also tested by inoculating sap to *Chenopodium murale* but the results were also negative.

Discussion

There are no literature data concerning the concentration of RaMV in plants. Since turnip represents an important source of RaMV for experimental work, we have investigated the virus concentration in this species. The results obtained show that the concentration of RaMV at the beginning of infection increases relatively fast and later it keeps more or less unchanged, even in plants which have been infected for a very long time. In other words, the virus concentration does not decrease by prolonged infections. These results agree with the data obtained for cowpea mosaic virus (Agrawal 1964), which also belongs to comoviruses (Harrison et al. 1971), and drew our attention because with most other viruses the concentration decreases after reaching a maximum, in some cases even rather quickly.

Moreover we found that RaMV is regularly present in the older leaves in concentration which considerably exceeds that in the younger ones. In this respect the behaviour of RaMV is similar to many other plant viruses. A high concentration was established also in the tuberous roots which remained weakly developed under the influence of the virus. As this virus reduces the yield of tuberous root very much it is also of a certain economic importance.

Neither previous workers (Cambell and Colt 1967) nor we could establish the transmission of RaMV through seed. However, on the basis of work done so far it cannot be stated that RaMV is not carried by seed at all, since seed-transmission was established in some other comoviruses (Freitag 1956; Shepherd 1964). It is possible that RaMV is carried by seed of some other hosts or in very low percentage by seed of plants investigated.

Summary

The concentration of radish mosaic virus (RaMV) was investigated in leaves and in tuberous roots of turnir (*Brassica rapa* L. var. *rapa*). During the first 12 days of infection the virus multiplied in leaves relatively fast and the concentration soon reached a maximum; afterwards the concentration did not diminish, but remained at the same level for a very long time. Besides, the virus concentration was higher in old than in young leaves. The influence of RaMV on the yield of turnip was very great so that the tuberous roots of infected plants were reduced in size.

The transmission of the virus through seed could not be detected in the following cruciferous species: *Brassica rapa* L. var. *rapa*, *Capsella bursa pastoris* (L.) Med., *Sinapis alba* L. and *S. arvensis* L.

We thank Mrs. A. Škof, Department of Botany, Faculty of Natural Sciences and Mathematics, Zagreb, for her technical assistance.

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SADRŽAJ

RELATIVNA KONCENTRACIJA VIRUSA MOZAIKA ROTKVE U POSTRNOJ REPI

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U ovom radu istražili smo kretanje koncentracije virusa mozaika rotkve (VMR) u postrnoj repi (*Brassica rapa* L. var. *rapa*). Rezultati istraživanja pokazuju da koncentracija virusa u početku zaraze naglo raste, a kad postigne maksimum bitno se ne mijenja niti nakon vrlo dugog trajanja zaraze. Biljke koje rastu u jednakim uvjetima sadrže otprilike jednaku količinu virusa, bez obzira da li su zaražene samo mjesec dana ili čak osam mjeseci. Osim toga utvrdili smo da se virus redovno nalazi u mnogo većoj koncentraciji u starijim (vanjskim) nego u mlađim (unutarnjim) listovima rozete te da znatno smanjuje prinos repe.

Nismo mogli ustanoviti da se VMR prenosi sjemenom. Ukupno smo ispitali 150 primjeraka vrste *B. rapa* L. var. *rapa*, 45 primjeraka vrste *Capsella bursa pastoris* (L.) Med., 50 primjeraka vrste *Sinapis alba* L. i 30 primjeraka vrste *S. arvensis* L., koji su bili uzgojeni iz sjemena sakupljenog sa zaraženih biljaka, ali nisu sadržavali virus.

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