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THE DISTRIBUTION OF TOBACCO MOSAIC VIRUS IN EXCISED TOMATO-ROOTS CULTIVATED IN VITRO[1]

Met een samenvatting: De verdeling van tabaksmozaïekvirus in afgesneden tomatewortels, gekweekt in vitro

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INTRODUCTION

It has been shown by various authors that virus concentrations may vary in different parts of a diseased plant. In some parts, such as in developing leaves, the concentration may be high, in others much lower. No virus has been detected in growing-points of diseased plants belonging to many different species (LIMASSET & CORNUET, 1949). MOREL & MARTIN (1955) succeeded in growing healthy potatoes from excised apical meristems originating from plants infected with virus Y. Later, KASSANIS (1957) obtained the same results with plants infected with potato paracrinkle virus and potato virus S. It may be assumed that these meristems grown in vitro did not contain virus. The application of this method is of special importance for virus-infected plants belonging to species which are propagated vegetatively.

BRANTS (unpublished) obtained virus-free plants of the potato variety 'Munsterse' which is important as a parent of the well-known variety 'Bintje'. The tubers of this variety were diseased to such an extent with the viruses X, Y and S that the plants grown from them were unable to flower and crossings could thus not be performed. The virus-free plants grown from meristem-cultures flowered well and produced many seeds after pollination.

The method of culturing meristems may be applied in combination with a heat-treatment. In this way THOMSON (1956) freed potato varieties from virus Y, and QUAK (1957) cultivated virus-free plants of the carnation varieties 'Pink Sim' and 'Harvest Moon'.

It is still unknown whether in a diseased plant the apical meristem as a whole is virus-free; THUNG (1938) assumed that in the meristem of a tobacco plant infected with virus, some virus-free cells occur. However, WHITE (1943) states that in excised tomato-roots cultivated in a liquid medium and infected with tobacco mosaic virus (TMV), the tip meristems are virus-free. Virus concentration decreases from the base to the tip of the roots (KASSANIS, 1957).

Roots cultivated in this way offer a suitable means for studying the influence of chemical substances added to the nutrient, particularly as the tip meristem of a root is so easily accessible. This is not the case with stem meristems which are covered by primordia and leaves. Moreover, with roots cultivated in a sterile nutrient solution there is no influence of micro-organisms. The advan-

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tages of such root-cultures as test objects are mentioned in more detail by MELCHERS & BERGMANN (1959). A disadvantage is that the roots cultivated in vitro grow under abnormal environmental conditions as compared with the roots of an intact, well-growing plant. Moreover, the roots are no more connected with the stem. Therefore, the results of these experiments may not be unreservedly compared with those obtained in experiments with intact plants.

BERGMANN (1958) found an inhibiting influence on the growth and virus production of isolated tomato roots by thiouracil and cytovirin when added to the nutrient solution. SCHMELZER (1961) states that this method is as yet seldom used to test chemicals for their ability to influence the multiplication of viruses.

The purpose of the present experiments was:

1. to determine whether meristems of excised tomato roots infected with TMV and cultivated in vitro under different conditions contain virus, and
2. to determine the influence of some substances added to the nutrient solution on virus distribution in the roots.

MATERIALS AND METHODS

Sterile young plants of *Lycopersicum esculentum* Mill. cultivar 'Potentaat' were infected with TMV following the method described by GRAAFLAND et al. (1957). After about three weeks the roots were excised and cultivated further in vitro. They were kept in 100 ml Pyrex Erlenmeyer flasks containing 25 ml White's solution. Such root-cultures can be maintained in the laboratory for many years. At 25 °C growth is so vigorous that the roots have to be subcultured every eight to twelve days. This can be done by cutting pieces of two to three cm from the tips of main or lateral roots and transfering them to fresh flasks of sterile White's solution. In this way it is possible to have at one's disposal sufficient test-material during the whole year. At 15 °C, growth is inhibited and sub-culturing is only necessary every month. Growth is also retarded by cultivating the roots in a small volume of White's solution poured on a solidified layer of 0.8% White's agar in an Erlenmeyer flask. From the solution the roots penetrate the agar in which growth is slowed down. Figure 1 shows the difference in morphology between roots grown in solution and those grown in agar. There is no difference in growth-rate between healthy and virus-infected roots when cultivated in White's medium, nor could any anatomical difference be found with regard to the lengths of the region of elongation.

To establish the presence or absence of TMV in the different parts of the roots, the latter were removed from the culture medium and divided into small sections under a binocular. These root-sections were homogenized on a rough glass-slide with a thin glass stick. The presence of virus in the sap was determined in two different ways: a. by inoculating leaves of *Nicotiana glutinosa* and b. by means of electron-microscopical photographs.

The first method has the disadvantage that the presence of still non-infectious virus-material cannot be detected. It is possible that such material is present in the youngest parts of the roots, becoming infectious only when these parts have matured. In applying the first method, two halves of one leaf of *Nicotiana glutinosa* were inoculated with sap of two different root-fragments respectively. In general the sap of one root-fragment was tested on two halves of different *Nicotiana glutinosa* leaves. The main-root was divided into sections

over a distance of 5 to 12 mm, commencing at the tip. The length of the sections was 0.5 mm, in the neighbourhood of the root tip varying from 0.25 to 0.5 mm. In many cases the lateral roots were also tested.

To test a root-fragment electron-microscopically the unpurified sap was put on a holder and photographed after shadow-casting with gold. The enlargment was 21,000 to 30,000 ×. This method was only used in a few cases.

The anatomy of the roots was also studied. Root tips of 1 to 2 cm in length were fixed in the fixative of Bouin, Carnoy or Craf. After 24 hours fixation the root tips were upgraded through alcohol and benzene and embedded in paraffin of m.p. 58 °C. Microtome sections, 4 to 15 μ thick, were deparaffinized in xylene and graded down via aethanol and water and finally stained in a solution of ferrous-haematoxylin. Other sections were left unstained.

Anatomy

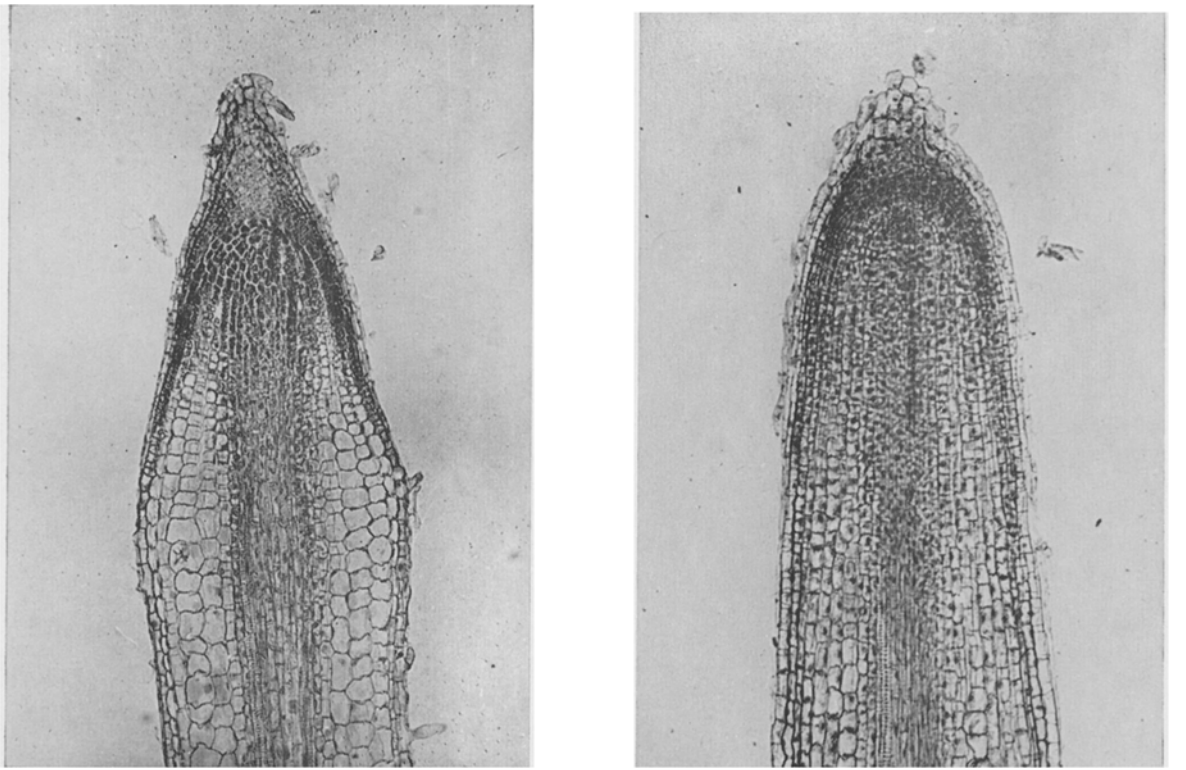
The growing point of a root consists of a small group of cells. The peripheral cells form the periblem, the innermost cells producing the plerome. Rootlets arise in the outer cell layer of the plerome at loci directly outside the protoxylem and usually at points about 20 mm from the tip. The first evidence of rootlet formation consists in the radial enlargment of two to several pericyclic cells adjacent to a protoxylem point. The cells of the periblem and the plerome occurring in the neighbourhood of the growing point continue dividing and retain their meristematic character over some distance from the tip. Together with the growing point they constitute the region of dividing of the root, comprising small actively dividing cells in which no vacuoles have been formed yet. Directly behind of this region is a zone known as the region of elongation, in which the newly formed cells are increasing in size. This region is relatively short and merges imperceptibly into what may be termed the zone of differentiation, in which many of the cells may continue to enlarge and some still divide, but the majority begins to develop the special characteristics of the several tissues which constitute the primary regions of the root axis (Hayward, 1938).

Experiments and results

The distribution of virus was determined in a great number of roots cultivated in White's solution. In table 1 the results are given for a root which may be considered as representative. In the main root as well as in the lateral roots, a zone occurred behind the root tip in which no virus could be demonstrated with the *Nicotiana glutinosa* test. Usually the length of this virus-free part was 2 to 3 mm, though sometimes it was as small as 0.5 mm. In other cases even a length of 11 mm was free of virus.

In our experiments the length of the root cap was about 0.075 mm, that of the region of dividing was about 0.360 mm. The boundary between the region of dividing and the region of elongation occurred at about 0.4 mm from the tip.

The length of the virus-free part at the tip depended on the virus concentration in the root as a whole. When the virus concentration was low, the first three mm or more from the tip appeared to be virus-free (table 1, root no. 1); in cases of high concentration only the first 0.5 mm from the tip was not in-



A B

FIG. 1. Tips of excised tomato roots cultivated in vitro, × 100.
Toppen van afgesneden tomatewortels, gekweekt in vitro, 100 ×.

A: in White's solution / *in White-oplossing*

B: in White's agar / *in White-agar*

TABLE 1. The number of TMV local lesions on half-leaves of *Nicotiana glutinosa* after inoculation with sap from sections of tomato roots. Length of each section 0.5 mm.
Het aantal lokale lesies, veroorzaakt door TMV op bladhelften van Nicotiana glutinosa *na inoculatie met perssap van stukjes tomatewortel. Lengte van elk stukje 0,5 mm.*

Distance in mm from the base of the section to the root tip *Afstand van de basis van het stukje tot de worteltop in mm*	Number of local lesions on 2 half-leaves inoculated with the same sample *Het aantal lokale lesies op 2 bladhelften geïnoculeerd met sap van hetzelfde wortelstukje*	
	Root grown in White's solution Root No. 1 *Wortel no. 1*	Root grown in White's agar Root No. 2 *Wortel no. 2*
0.5	0 0	0 0
1.0	0 0	2 3
1.5	0 0	0 4
2.0	0 0	3 2
2.5	0 0	4 2
3.0	0 0	6 4
3.5	1 1	4 8
4.0	1 0	4 12
4.5	3 2	6 9
5.0	4 4	3 14

fected with virus. The virus concentration of a fragment increased with its distance from the tip. Virus distribution in the lateral roots was similar to that in the main root. More than 50 roots have been tested. Always the apical meristem appeared to be virus-free.

Repeatedly a decrease in the virus concentration was found in sections at a distance of about 10 mm from the tip, followed by an increase again at about 20 mm from the tip. The presence of virus-free meristematic cells in the pericycle from which rootlets may arise could be an explanation for the decrease in virus content. However, primordia of rootlets did not arise at less than 20 mm from the tip and, moreover, it became evident that at places where a primordium of a lateral was present or where a rootlet had just developed, no decrease in virus concentration occurred. Thus the decrease in virus content in fragments at a distance of 10 mm from the tip could not be ascribed to the presence of meristematic tissue.

Electron-microscopical research confirmed that in the sap of the apical fragments no TMV-particles occurred. In sap obtained from sections lying at increasing distances from the tip an increasing amount of particles appeared to be present. A high virus concentration was found in the fragments at 18 to 20 mm from the tip. This result agreed with that of the *Nicotiana glutinosa* test.

It is possible that the rapidly growing root tip cannot be reached by virus, if virus transportation from the older parts of the roots towards the apex occurs too slowly. If so, inhibition of the growth would allow virus material to penetrate the extreme tip. Roots grown in White's solution over solidified agar met with mechanical resistance in attempting to penetrate the agar and their growth was retarded by about 30%. However, the first 0.5 mm from the tip of the root remained virus-free. Root number 2 (table 1) may be considered as representative of about 20 tested roots grown in agar. It may be concluded that even after growth-retardation, infectious virus material could not be detected in the apical meristem, though the virus-free part at the tip of roots grown in agar was smaller than that of roots grown in nutrient solution.

In a virus-diseased tobacco plant a rather sharp boundary can be found between virus-free meristematic tissue and the developing primordia and young leaves (A. F. SCHIPPERS-LAMMERTSE, unpublished). By analogy it was supposed that in the root also a distinct anatomical boundary could be found between the virus-free and the virus-containing parts. However, in a root the boundary between the meristematic zone and the region of elongation does not coincide with the borderline of the virus-free portion. This may be due to the transformation of embryonic cells into mature cells, which occurs gradually.

THE INFLUENCE OF CHEMICAL SUBSTANCES ON VIRUS DISTRIBUTION

According to CROWLY & HANSON (1961), TMV is able to penetrate the tips of tomato roots under influence of both versene and ethylene-diamine-tetra-acetic acid (EDTA). Their experiments were carried out with root fragments of 2 mm length. A more detailed examination of the portion within 2 mm from the tip was not performed. In order to test their conclusions we examined the influence of EDTA on virus distribution in tomato roots cultured in vitro. EDTA added to a White's solution at a concentration of 6×10^{-4} M advanced the penetration of TMV into the root tips to 0.5 mm from the tip. In all cases the first 0.5 mm from the tip proved to be virus-free. The data obtained for a root representative of these experiments are given in table 2. The opinion of CROWLY & HANSON that TMV can infect the meristematic cells of tomato roots was therefore not confirmed in our experiments. However, the virus had always penetrated further into the tips than in the control roots and, moreover, the virus concentration in the fragments lying 3 mm or more from the tip was somewhat higher than in the controls. These results agreed with those obtained when testing roots grown in agar without EDTA (table 1, root No. 2).

TABLE 2. The number of TMV-local lesions on half-leaves of *Nicotiana glutinosa* after inoculation with sap from sections of tomato roots grown in White's solution, treated with EDTA. Length of each section 0.5 mm.
Het aantal lokale lesies, veroorzaakt door TMV op bladhelften van Nicotiana glutinosa *na inoculatie met perssap van stukjes van tomatewortels, gekweekt in White's oplossing, behandeld met EDTA. Lengte van elk stukje 0,5 mm.*

Distance in mm of the base of the section to the root tip *Afstand van de basis van het stukje tot de worteltop in mm*	Number of local lesions on 2 half-leaves inoculated with the same sample *Het aantal lokale lesies op 2 bladhelften geïnoculeerd met sap van hetzelfde wortelstukje*	
0.5	0	0
1.0	2	2
1.5	7	1
2.0	6	5
2.5	6	8
3.0	8	12
3.5	15	11
4.0	22	17
4.5	20	32
5.0	21	34

A more detailed examination of the first half mm from the tip of roots treated with EDTA was performed by cutting fragments of 0.2 mm length with a freezing-microtome. The sap of each section was then tested on *Nicotiana*

glutinosa leaves. In three roots, a piece of about 0.6 mm from the tip appeared to be virus-free. As it is still unknown how EDTA influences the metabolism of cells it is difficult to explain why cells at the tip of the root remain virus-free in the face of a treatment which tends to increase virus concentrations.

Besides those chemicals which decrease the virus-free zone at a root tip, other chemicals which increase the virus-free part are also of interest, particularly auxins.

LIMASSET & CORNUET (1949, 1950), experimenting with TMV-infected tobacco plants, supposed that the virus is unable to penetrate the tip meristem as a result of the high auxin concentration. In a few preliminary experiments we tested the influence of indole-butyric acid (IBA). This substance added to White's solution to a final concentration of 0.7 mg/l did not cause a change in virus distribution in the roots. A higher concentration proved to be phytotoxic. It is possible that other growth substances may have an influence on virus concentration and distribution in a tomato root but they have not been tested.

According to MELCHERS & BERGMANN (1959) who experimented with thiouracil, the virus production of the roots was not decreased when this chemical was added in a concentration of 1.5 to 3.5 $\times$ 10^{-6} g/ml. Thiouracil at a higher concentration than 10^{-6} g/ml solution inhibited the growth of both infected and healthy tomato roots. At a concentration of 10^{-5} g/ml growth was entirely inhibited. Besides an inhibition of root growth thiouracil induced an abundant production of root hairs.

From our experiments it became evident that thiouracil in a concentration of 1.5 $\times$ 10^{-6} g/ml solution increased the virus-free zone in the roots to five mm, reckoned from the tip. At this concentration growth was not inhibited. A number of other substances were also tested for their ability to change the virus distribution in a tomato root. For each chemical the highest concentration was determined at which no phytotoxic effect occurred (table 3).

TABLE 3. The influence of various chemicals on virus distribution in tomato roots cultivated in vitro.
De invloed van enkele stoffen op de virusverdeling in tomatewortels, gekweekt in vitro.

Substance *Stof*	Maximum conc. in p.p.m. not phytotoxic *Maximum niet-fytotoxische conc. in d.p.m.*	Length of virus-free zone in mm from the top *Lengte der virusvrije zone in mm vanaf de top*	Number of roots tested *Aantal getoetste wortels*
1. 6-amino-benzimidazole.HCl	0.01	0.5–3	6
2. 5-amino-benzimidazole.HCl	5.0	1–3	6
3. 2-propyl-6-chloro-desazapurine	1.0	0.5–3	6
4. 2-propyl-6-chloro-3-desazapurine	2.0	4.5–5	10
5. 1-desaza-adanine	1.0	>5	8
6. 2-methyl-3-desaza-adenine	1.0	0.5–5	3
7. control	–	0.5–3	9

The substances Nos 1, 2 and 3 did not change the virus distribution in the root. No. 4 exercised a distinct influence: in none of the roots tested could virus be demonstrated in fragments within 4,5 mm from the tip. Neither the growth rate nor the morphology of the roots was influenced by this substance. No. 5

increased the virus-free zone to a greater extent: the first five mm from the top were virus-free in all cases. No. 6 produced varying results: in one root virus occurred already beyond 0.5 mm from the tip. In all roots this substance exercised a remarkable morphological influence. Normally the first rootlets appear at about three to five cm behind the root tip. However, under influence of this substance rootlets developed at 1.5 to 10 mm from the top, and in one case the vegetative point was thickened.

The substances 4, 5 and 6 which influence the virus distribution are purine or adenine derivatives. 1-Desaza-adenine proved to be the most active in increasing the virus-free zone.

According to MATTHEWS (1952), free adenine is probably necessary for the protein synthesis of viruses. For this reason it is possible that adenine derivatives exercise a great influence on virus multiplication and distribution. How far these substances influence the metabolism of the cells in an excised or intact root is unknown. Further experiments will be carried out with some of these derivatives.

DISCUSSION

The method of half-leaves used here to compare virus concentrations in the different root sections makes an exact conclusion on virus quantities impossible, as the number of *Nicotiana glutinosa* leaves used was only small. However, the results of the experiments confirmed by those of electron-microscopical studies, establish that a virus-free part, or at least a part with non-infectious virus material, exists. The virus-free tip zone may vary in length. This variation is probably due to differences in growth rate of the roots and in virus concentrations in the rest of the root. In a root cultivated in agar in which growth was inhibited, the virus was present closer to the tip than in a root growing vigorously in a solution.

KASSANIS (1957) did not find a virus-free tip in his experiments. His results can probably be explained by the fact that he tested combinations of several tips each of 10 mm length. However, the roots vary individually and the virus-free parts can differ in length. It is possible, therefore, that some of the combined fragments contained virus within 10 mm from the tip.

SHEFFIELD (1942) tested the combined sap of 20 isolated tip meristems of tomato plants infected with TMV or aucuba-mosaic virus for the presence of virus. However, if some virus-containing tissue was removed together with the meristems, the sap therefrom may have contained virus. Her conclusion that the tip meristem of a virus-infected plant contains virus is therefore doubtful.

LIMASSET & CORNUET (1949, 1950) stated that only in 50% of the meristems which they examined virus was detectable. They are convinced that in isolating meristem parts of 100 μ length, even virus contamination may occur from infected tissue adjacent to the excised parts. CROWLEY & HANSON (1961) combined two mm fragments of different roots, so it is possible that in their experiments too the first 0.5 mm did not contain virus. However, this could not be demonstrated with the method used.

The fact that it was not possible to correlate the anatomical border line of the virus-free part with the limit between the meristematic zone and the region of elongation points rather to a biochemical than an anatomical barrier. The

action of EDTA on virus distribution in a root results in a decrease of the virus-free zone. It is possible that EDTA, which is able to bind heavy metal ions, exercises an inhibiting action on the synthesis of normal protein. In that way building materials would be available for protein synthesis by the virus. The increase of the virus-free zone in roots treated with 2-propyl-6-chloro-3-desazapurine, 1-desaza-adenine and 2-methyl-3-desaza-adenine cannot yet be explained.

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Summary

The purpose of the experiments was to determine the distribution of tobacco mosaic virus in excised tomato roots cultivated in vitro. To demonstrate the presence of TMV in the different root parts, the roots were divided into fragments of 0.5 mm in length. Sap was obtained by rubbing each such section on a rough glass slide with the aid of a thin glass stick. The sap was then tested for the presence of virus on *Nicotiana glutinosa* leaves. In a few cases the sap was tested electron-microscopically.

It was found that in the roots a virus-free region occurred just behind the tip. The length of this zone varied in different roots and was considerably reduced when the growth of the roots was inhibited by cultivating them in a nutrient solution poured on a layer of agar.

Behind the virus-free tip zone the virus concentration increased, followed by a decrease at a distance of 10 mm and again by an increase at a distance of 20 mm from the tip. It could not be proved that development of side roots was responsible for this decrease. It was also not possible to correlate the virus-free zone with a particular anatomic region since the border of the meristematic tissue in a root is not clearly defined. The fact that a virus-free tip zone is always found, makes it probable that the virus is not able to enter the apical growing point.

According to Crowley & Hanson (1961), TMV is able to penetrate the tips of tomato roots under influence of ethylene-diamine-tetra-acetic acid (EDTA). However, they experimented with root sections of 2 mm length. In our experiments the first 0.5 mm of the tip remained virus-free when EDTA was added to the solution.

Besides EDTA, the influence of other substances on virus distribution in a tomato root was also tested. Addition of thiouracil in a concentration of 1.5 p.p.m. was followed by an increase of the virus-free tip zone up to 5 mm; 2-propyl-6-chloro-3-desazapurine at a concentration of two p.p.m. caused a virus-free zone of about 4.5 mm. The same effect was obtained with 1 p.p.m. 1-desaza-adenine. 2-Methyl-3-desaza-adenine exercised a morphological influence besides increasing the virus-free zone. Whereas normally the first rootlets occur 3 to 5 cm behind the root tip, after adding 2-methyl-3-desaza-ade-

nine to the solution, the first rootlets appeared at 1.5 to 10 mm from the tip.

The influence of those purine and adenine derivatives able to increase the virus-free zone will be tested more extensively.

SAMENVATTING

Het doel van dit onderzoek was, de virus-verdeling na te gaan in afgesneden, in vitro gekweekte tomatewortels, besmet met tabaksmozaïekvirus (TMV). Het was de bedoeling niet alleen wortels te toetsen die gekweekt werden in een White-voedingsoplossing, maar ook te onderzoeken, welke invloed verschillende aan de vloeistof toegevoegde chemische stoffen op de virusverdeling uitoefenen.

Verschillende onderzoekers (WHITE, 1943; MELCHERS & BERGMANN, 1959) hebben reeds vastgesteld, dat groeipunten van tomatewortels, geweekt in White-vloeistof, vrij van TMV zijn. Anderen zijn van mening, dat het TMV wel het groeipunt kan bereiken (KASSANIS, 1957), zij het dan na toevoegen van een bepaalde stof zoals ethyleen-diamine-tetra-azijnzuur aan het medium (CROWLEY & HANSON, 1961).

Om de aanwezigheid van TMV in de verschillende worteldelen na te gaan, werden de wortels in fragmenten met een lengte, variërend van 0,25 tot 0,5 mm, verdeeld. Deze stukjes werden elk afzonderlijk met een glasspateltje op een ruw gemaakt objectglas fijngewreven. Door uitwrijven van het aldus verkregen sap op *Nicotiana glutinosa*-bladeren werd met toepassing van de bladhelftenmethode de aanwezigheid van TMV in het perssap nagegaan.

Steeds bleek in de wortel een zone voor te komen, gelegen achter de uiterste top, waarin geen virus kon worden aangetoond. Enkele elektronen-microscopische foto's bevestigden dit resultaat. Dit virusvrije gedeelte wisselde bij de verschillende wortels in lengte, maar was meestal 2 tot 3 mm lang.

De snelle groei van de wortels deed vermoeden, dat het in de wortel aanwezige virus niet snel genoeg vermenigvuldigd of getransporteerd wordt om de snel groeiende top bij te houden. Het lukte nu de groei te vertragen door de wortels te kweken in een laagje vloeistof op een agarlaag, waarbij de wortels in de agar binnendrongen. Bij deze wortels was de top stomper en eveneens virusvrij, hoewel slechts over een korte afstand. Op enige afstand van de worteltop nam de virusconcentratie eerst toe, dan volgde een daling en vervolgens weer een stijging. De gedachte, dat op die plaatsen waar zijwortels optreden de virusconcentratie zou dalen door de aanwezigheid van meristematische cellen, kon niet bevestigd worden. Het meristeem in een wortel is niet scherp te begrenzen. In het virusvrije gedeelte liggen naast sterk gedifferentieerde elementen ook nog meristematische cellen. Het bleek onmogelijk, de grens van de virusvrije zone te correleren met een anatomische grens.

Daar ook bij een geringe groeisnelheid toch het apicale deel van de top virusvrij blijft, lijkt het waarschijnlijk, dat het groeipunt van de wortel een barrière vormt die het virus belet in de uiterste top binnen te dringen. Volgens CROWLEY & HANSON (1961) kan het TMV onder invloed van EDTA in de toppen van tomatewortels doordringen. Zij combineerden echter wortelfragmenten van 2 mm lengte, die zij tezamen toetsten. In onze experimenten was ondanks de toevoeging van EDTA de eerste 0,5 mm van de top virusvrij.

Naast EDTA werd ook de invloed van andere stoffen op de virusverdeling

nagegaan. Thiouracil bleek in een concentratie van 1,5 d.p.m. de virusvrije zone van de wortels te vergroten tot gemiddeld 5 mm, van de top af gerekend. 2-Propyl-6-chloro-3-desaza-adenine gaf in een concentratie van 2 d.p.m. een virusvrije topzone van 4,5 mm. Eenzelfde effect werd verkregen met 1 d.p.m. 1-desaza-adenine. Behalve dat 2-methyl-3-desaza-adenine de virusvrije zone vergrootte, had het ook morfologische veranderingen ten gevolge.

Terwijl normaal de eerste zijwortels gemiddeld 30 tot 50 mm achter de worteltop optreden, ontstonden deze onder invloed van 2-methyl-3-desaza-adenine reeds op 1,5 tot 10 mm.

De purine- en adenine-derivaten, die in staat zijn de virusvrije zone te vergroten, zullen nog nader op hun werking onderzocht worden.

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