ACTA BOT. CROAT. VOL. 40, 1—5, 1981.

UDC 58
CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858.8 : 536.5 = 20

THE INFLUENCE OF LOW TEMPERATURE ON THE STABILITY OF KAZAKHSTAN AND COMMON STRAIN OF TOBACCO MOSAIC VIRUS

DAVOR MILIČIĆ and MERCEDES WRISCHER

(Department of Botany, Faculty of Science, University of Zagreb, and Laboratory of Electron Microscopy, Ruđer Bošković Institute, Zagreb)

Received January 4, 1981

Introduction

Goldin (1963) found in Kazakhstan an interesting strain of tobacco mosaic virus (TMV). Brčák (1978) established that this Kazakhstan strain (Ka strain) of TMV was defective and that it infected tobacco plants very slowly or the systemic infection was lacking. Its thermal inactivation point lay between 75 and 80° C. In fresh infective sap virus particles of normal length were found very rarely and very long particles of various length were often present. These data were also confirmed by Miličić et al. (1979) and Miličić and Wrischer (1980) who mostly studied the fine structure of inclusion bodies and the form of virus particles.

In 1971 Kassanis and Bastow investigated the influence of temperature on the state and multiplication of the type strain and three defective strains of TMV. The defective strains were well examined and belonged to the forms PM_2, Ni 118 and the thermophilic strain TC. The studied here Ka strain is similar to the TC strain which at normal temperature also forms very long particles, several times longer than is the normal length of TMV.

Recently we have observed that the length and form of Ka strain particles were very much influenced by the temperature at which infective detached leaves were kept before negative staining.

For comparison, common TMV was also investigated under the same conditions. The results of these experiments are reported in this paper.

Material and Methods

The Ka strain of TMV was examined and described as a natural strain by Goldin (1963). This virus was kindly sent us by Dr. J. Brčák (Prague) and was preserved in dessicated leaves of Samsun tobacco for many years.

In these experiments we also used a strain of TMV which was collected in the surroundings of Zagreb and which reacted to usual test plants like the common strain. The strain was serologically compared with the type strain and was identical with it.

The viruses were cultivated in *Nicotiana tabacum* cv. Samsun during the spring when the temperature in the greenhouse was moderate. The experiments were performed one month after inoculation. Two experiments were made with each virus. In one experiment detached leaves of tobacco cv. Samsun were kept before negative staining for 14 hours at normal temperature of 21° C and in the other for the same time at 10° C.

Electron microscopic investigations were performed by a Siemens Elmiskop I. The preparations of infective sap were negatively stained with potassium phosphotungstate.

Results

Experiments with the Ka strain

Miličić and Wrischer (1980) established that the particles of the Ka strain changed its form when infected living tobacco leaves were exposed to various temperatures for many hours before the staining.

The particles of Ka strain kept at normal temperature of 21° C were often very long (Fig. 1). The length of particles sometimes exceeded 1200 nm, i. e. the length of four particles arranged end to end. However, many particles were shorter, but the particles of normal length were relatively rare.

In addition, many short fragments of particles were present which were obviously at various stages of desintegration. These fragments were often bent and their convex part consisted of protein formations which were about 6 nm thick and were probably built of two helix turns (Fig. 1 c). On the concave part of fragments the turns were not separated and the part formed a compact body.

The fragments in decomposition were sometimes straight and then consisted of small pieces of protein which were stacked near one another (Fig. 1 s). These stacked pieces can derive from stacked discs which are sometimes present in the protein coats of defective strains (Hariharasubramanian and Siegel 1969, Kassanis and Bastow 1971).

Very small protein parts which consisted of one or a small number of turns were placed flat and had the form of a ring with a hole in the centre (Fig. 1f).

When before the negative staining the living leaves were kept in a refrigerator at 10° C for 14 hours, the frequency, at which certain types of particles were present, significantly changed (Fig. 2). It is visible that the number of straight particles is very small and the others are very much altered. The straight particles are not of the normal length of 300 nm. Above that they are swollen and about 21 nm wide. Most of the par-

ticles are in a state of decomposition. Many particle fragments are bent, and groups of protein turns on their convex parts can be distinctly seen. This is not visible on the concave surface because there the turns are joined together.

On some straight fragments, it was possible to observe that protein parts looked like stacked discs and were obviously separated from one another (Fig. 2 s). A straight particle marked with b in Fig. 2 is interesting because its upper end has begun to decay but the lower part is still preserved.

In the material kept in a refrigerator many small fragments were placed flat and therefore had the form of a ring (Fig. 2 f).

Experiments with common TMV

The investigation of the particles of common TMV was first performed on leaves kept a the temperature of 21° C. It was established that most of the particles were 300 nm long (Fig. 3). The fragments were relatively rare and small. In one area we found 70 normal particles of 300 nm and 20 small fragments which together had the length of about 7 normal particles. The virus protein decomposition (Fig. 3) was low.

In the leaves which were kept for 14 hours at the temperature of 10° C before negative staining, also a large number of well built normal particles (Fig. 4) was found. Above that some fragments of virus particles were found, but their number was approximately equal to the number of fragments in the material kept at normal temperature.

A large quantity of decomposed virus protein was present among virus particles (Fig. 4). It consisted of fragments which were in decomposition and of ring shaped small fragments placed flat (Fig. 4 f). Consequently, the treatment with temperature of 10° C also alters the common TMV, but a only small part of protein is decomposed. The grade of decomposition is considerably smaller than in Ka strain (Fig. 2) where the complete virus protein is involved in this process.

Discussion

As follows from the experiments described, Ka strain is very sensitive to the change of temperature. A difference of only 11 centigrades can entirely change the form of virus particles (Fig. 1 and 2). On the contrary, the particles of common TMV are relatively stable so that a large number of particles preserve the normal form in changed conditions.

Electron micrographs of Ka particles kept at the normal temperature of 21° C showed a considerable number of very elongated particles (Fig. 1). Brčák (1978) drew our attention to this fact. The presence of these very elongated particles is an important characteristic of defective Ka strain. With regard to this property Ka strain agrees with the thermophilic TC strain which was studied by Kassanis and Bastow (1971) at temperatures of 20° and 35° C.

Lebeurier and Wurtz (1968) have found on the basis of spectrophotometric investigations that the particles of TC strain do not contain RNA at normal temperature. Kassanis and Bastow (1971) also confirmed by means of a spectrophotometer that the relation E 260/E 280 indicated the absence of RNA in TC strain at 20° C. It would be necessary to establish the relation E 260/E 280 for purified preparations of Ka strain in order to find whether the particles of Ka strain contain RNA or not.

It is known that purified preparations of defective TMV strains kept at 21° C can have many broken particles and that they nevertheless can contain RNA. This was established by Kassanis and Bastow (1971) during the investigation of the strain Ni 118. They found that purified preparations of the strain Ni 118 had broken particles but the E 260/E 280 showed that the particles contained RNA.

It must be pointed out that TC strain is not a HNO_2 mutant as e. g. the strain Ni 118 is. The TC strain was obtained from common TMV by selection at high temperature. This thermophilic strain was first named LB and was able to multiply very actively at 36° C (Lebeurier and Hirth 1966). From this thermophilic LB strain, three strains were isolated, one of which was the strain TC (Lebeurier and Wurtz 1968).

The second virus investigated during these experiments was common TMV. It was established that this virus was also sensitive to low temperature, under the influence of which some particles became desintegrated (Fig. 4).

It is possible that the following event is also connected with the lack of resistance of common TMV to low temperature. Some years ago we cultivated tobacco plants infected with common TMV in greenhouse. In the tissues of these plants many hexagonal prisms built of virus particles were present (cf. Warmke and Edwardson 1966, Miličić 1968). Later the plants were transferred to a cold room and kept there for many days. Probably under the influence of low temperature the hexagonal prisms disappeared from the tissues. This was an unpleasant experience because the hexagonal prisms were to serve for demostration purposes.

Summary

The Kazakhstan strain (Ka strain) of tobacco mosaic virus is defective and particles of normal length of 300 nm are rarely found in preparations. However, particles over 1,000 nm long and many fragments shorter than 300 nm are very frequent. Such particles appeared in preparations made by negative staining method from living leaves kept at 21° C (Fig. 1).

As the Ka strain is sensitive to low temperatures, the form of the particles in preparations made from leaves kept for 14 hours at low temperature differs very much from those kept at normal temperature. The preparations from the material kept at 10° C mostly contained particles at various stages of decomposition (Fig. 2).

For comparison leaves infected with common TMV were treated in the same manner. Many normal particles of 300 nm were found in the preparations from leaves kept at 21° C (Fig. 3). However, in the preparations from the material kept at 10° C, many particles were decomposed. Nevertheless, the largest number of particles had preserved the normal form and length of 300 nm (Fig. 4).

References

Brčák, J., 1980: Defective character of the Kazakhstan strain of tobacco mosaic virus. Plant Virology, Proc. 8th Conf. Czechoslov. Plant Virologists, pp. 187—194.

Goldin, M. I., 1963: Virusnye vključenija v rastitelnoj kletke i priroda virusov. Izdat. AN SSSR, Moskva.

Hariharasubramanian, V., A. Siegel, 1969: Characterization of a new defective strain of TMV. Virology *37*, 203—208.

Fig. 1. Kazakhstan strain of tobacco mosaic virus at the temperature of 21° C. Negative staining with potassium phosphotungstate. In the middle a very long particle. Curved fragment (c), stacked fragment (s), flat fragment (f). Bars for figures 1 to 4 represent 200 nm.

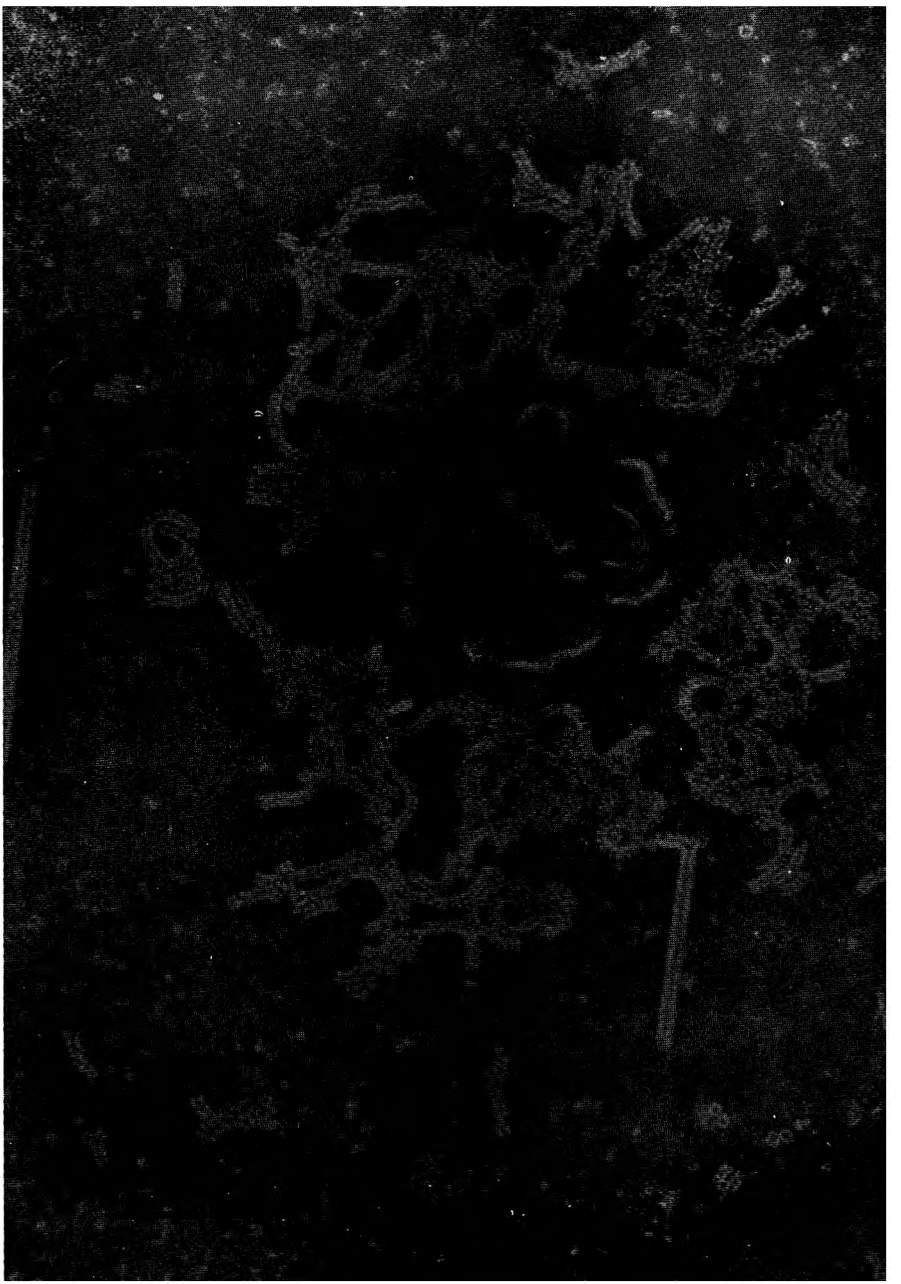

Fig. 2. Kazakhstan strain at the temperature of 10° C. Particles are mostly decomposed. Long swelled particle (l), straight particle (b). Other signs as in Fig. 1.

Fig. 3. Common strain of tobacco mosaic virus at the temperature of 21° C. The particles are mostly 300 nm long. Short fragments are relatively infrequent.

Fig. 4. Common strain at the temperature of 10° C. Short fragments (k) and flat ring-shaped fragments (f) are visible. The particles have mostly preserved the length of 300 nm.

Kassanis, B., C. Bastow, 1971: The relative concentration of infective intact virus and RNA of four strains of tobacco mosaic virus as influenced by temperature. J. gen. Virol. *11,* 157—170.

Lebeurier, G., L. Hirth, 1966: Effect of elevated temperature on the development of two strains of tobacco mosaic virus. Virology *29,* 385—395.

Lebeurier, G., M. Wurtz, 1968: Propriétés d'un clone isolé a partir d'une souche thermophile du virus de le mosaïque du tabac. Compte rendu Acad. Sci. Paris, Sér. natur. *267,* 871.

Miličić, D., 1968: Zelleinschlüsse von Holmes, ribgrass virus, eines Verwandten des Tabakmosaikvirus. Naturwissenschaften 55, 90—91.

Miličić, D., M. Wrischer, 1980: Further investigations of the defective Kazakhstan strain of tobacco mosaic virus. Acta Bot. Croat. *39,* 1—7.

Miličić, D., M. Wrischer, J. Brčák, N. Juretić, 1979: Intracellular changes induced by the defective Kazakhstan strain of tobacco mosaic virus. Acta Bot. Croat. *38,* 1—7.

Warmke, H. E., J. R. Edwardson, 1966: Electron microscopy of crystalline inclusions of tobacco mosaic virus in leaf tissue. Virology *30,* 45—57.

SAŽETAK

UTJECAJ NISKE TEMPERATURE NA STABILNOST KAZAHSKOG SOJA I OBIČNOG SOJA VIRUSA MOZAIKA DUHANA

Davor Miličić i Mercedes Wrischer

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu i Laboratorij za elektronsku mikroskopiju Instituta »Ruđer Bošković« u Zagrebu)

Kazahski soj (Ka soj) virusa mozaika duhana je defektan. Čestice normalne dužine od 300 nm su rijetke. Međutim, čestice duže od 1000 nm i mnogi fragmenti kraći od 300 nm razmjerno su česti. Takve se čestice obično nalaze u preparatima priređenim metodom uranjanja iz živih listova koji su stajali na temperaturi od 21° C (sl. 1).

Budući da je Ka soj osjetljiv na niže temperature, oblik čestica razlikuje se u preparatima priređenim pri niskoj temperaturi. Tako su preparati iz materijala držanog na temperaturi od 10° C sadržavali većinom čestice u raznim fazama raspadanja (sl. 2).

Radi usporedbe izvršeni su jednaki pokusi sa živim listovima inficiranim običnim sojem virusa mozaika duhana. U preparatima priređenim pri 21° C nađen je velik broj čestica normalne dužine od 300 nm (sl. 3). Međutim u preparatima materijala na temperaturi od 10° C mnoge su čestice bile razgrađene, ali je velik broj čestica ipak zadržao normalan oblik i dužinu (sl. 4).

Prof. dr. Davor Miličić
Botanički zavod
Prirodoslovno-matematičkog fakulteta
Maruličev trg 20/II
YU-41000 Zagreb (Jugoslavija)

Dr. Mercedes Wrischer
Institut Ruđer Bošković
Bijenička cesta 54
YU-41000 Zagreb (Jugoslavija)