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Purification and Conservation of Infective Sugar Beet Mosaic Virus

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Abstract. Sugar beet mosaic virus (SBMV) was precipitated by polyethyleneglycol (PEG) 6000 from the cell sap of infected sugar beet leaves. After centrifugation and addition of dextrane T 10 the virus was lyophilized. Its infectious activity was demonstrated by mechanical transmission to *Chenopodium quinoa* WILLD. and to sugar beet. Stability of infectious activity of the lyophilized virus was verified.

The aim of our experiments was to find a gentle method for purification and conservation of SBMV which would not disturb the infectivity of the final product. HERBERT (1963) purified the virus of wheat mosaic, tobacco mosaics virus (TMV) and tobacco ring spot virus using precipitation by PEG 2000 which was added to partially purified samples. He found that the samples of rod-like viruses had highest activity if PEG was added in the amount which constituted 3 per cent of final solution. In the case of globular viruses, as TMV, the PEG was dissolved in 0·1 M NaCl. The advantage of this kind of precipitation of plant viruses of this type is that the polymer used is chemically inert and it prevents the inactivation of plant viruses. JERMOLJEV and ALBRECHTOVÁ (1969) purified potato X-virus by using PEG 6000 and the final product was stabilized by the addition of 6% dextrane T 10 before lyophilization. The lyophilized antigen of this virus possessed strong infectious and antigenic properties. A similar method of purification and precipitation was used also for SBMV. We modified the original method for our purposes and for this reason we give a detailed description.

Material and Methods

100 g of frozen sugar beet leaves infected with SBMV were used for preparation of the homogenate. 80 ml of 0·007 M phosphate buffer (pH 7·8) containing 0·01 M veronal, 0·01 M cysteine and 0.007 M EDTA (LINDNER

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et al. 1955, MUNDRY and ROHMER 1958), was added. The cell sap was obtained by pressing the leaves through a silon net and centrifuging for 5 minutes at 9000 × g. 12·5 ml of 40% PEG, dissolved in water, was added to 50 ml of supernatant. 0·73 g of NaCl was added to reach the final concentration 0.2 M. The final concentration of PEG was 10%. At this concentration no precipitation was observed. 30 minutes after mixing the solution was centrifuged for 5 minutes at 11500 × g. The sediment was dissolved in 5 ml of 0·01 M phosphate buffer at pH = 7·5 and centrifuged in a Spinco L ultracentrifuge, with rotor 40, for 60 min at 105000 × g. Before lyophilization, dextrane was added to a final concentration of 6%.

The infectivity of the sample was tested on the indicator plants *Chenopodium quinoa* WILLD. Four leaves of each variant were mechanically infected. Celite was used as abrasive. Ten plants were infected in each variant. The crude cell sap was used as control.

The infectivity of the SBMV sample was measured at first before lyophilization, using the dissolved sample after ultracentrifugation in phosphate buffer. The next test of the activity of the sample was made after lyophilization. The lyophilized sample of SBMV was dissolved in distilled water prior to inoculation to obtain the original volume. Fourteen days after inoculation the cell sap of *Chenopodium album* leaves, showing local and

Table 1

Mechanical infection of *Chenopodium quinoa* WILLD. and sugar beet with samples of SBMV

Sample	Indicator plant	Number of inoculated plants	Number of plants showing infection	Number of local lesions per leaf
Control — crude infectious sap	*Ch. quinoa*	10	8	2—3
SBMV before lyophilization	*Ch. quinoa*	10	10	7—10
SBMV after lyophilization	*Ch. quinoa*	14*	11	2—3
Crude infectious sap from Chenopodium quinoa	*sugar beet*	10	10	systemic infection
SBMV one month after lyophilization	*sugar beet*	10	8	systemic infection

* Two plants died.

Abbreviations used: polyethyleneglycol — PEG, sugar beet mosaic virus — SBMV, tobacco mosaic virus — TMV.

systemic symptoms of SBMV, was extracted and used for mechanical infection of sugar beet (cv. Dobrovická) seedlings. One month after lyophilization we applied the dissolved sample of SBMV by mechanical inoculation of the sugar beet seedlings.

Results and Discussion

The results of our experiments, dealing with the preservation of SBMV with maintenance of infectivity are pooled in Table 1. As seen in the table, the infectious activity of SBMV obtained by precipitation of SBMV from infectious sugar beet cell sap after ultracentrifugation is much higher than the infectivity of the sample was decreased by lyophilization, it remains approximately on the level of the infectivity of the crude cell sap.

The stability of the sample was increased by adding dextrane T-10 before lyophilization. The infectivity is very high and stable, which makes it possible to infect sugar beet seedlings directly, without using *Chenopodium quinoa*. The infectivity is probably maintained for a rather long period after lyophilization, as shown by the mechanical transmission of the infection to sugar beet seedlings one month after lyophilization.

References

HERBERT, T. T.: Precipitation of plant virus by polyethyleneglycol. — Phytophatology **53** : 362, 1963.

JERMOLJEV, E., ALBRECHTOVÁ, L.: Stabilization of purified potato virus X by dextrane T-10 during lyophilization. — Biol. Plant. in press 1969.

LINDNER, R. C., KIRKPATRICK, H. C., WEEKS, T. E.: Some factors affecting the purification of a stone fruit virus. — Phytopathology **45** : 574—576, 1955.

MUNDRY, K. W., ROHMER, I. Über die mechanische Übertragung des Vergilbungsvirus der Rüben. — Phytopath. Z. **31** : 305—318, 1958.

J. CHOD a J. POLÁK, Ústav ochrany rostlin, Praha: **Čištění a konservace viru mozaiky řepy se zachováním infektivity.** — Biol. Plant. **11** : 324—326, 1969.

Virus mozaiky řepy byl vysrážen polyethylenglykolem 6000 ze šťávy z infikovaných listů řepy cukrové. Po následné ultracentrifugaci a po přidání dextranu T 10 byl lyofilizován. Jeho infekční schopnost byla prokázána mechanickými přenosy na *Chenopodium quinoa* WILLD. a řepu cukrovou. Zároveň byla potvrzena stálost infektivity viru v lyofilizovaném stavu.