OPTIMUM CONDITIONS FOR THE STORAGE OF POTATO VIRUS S

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Summary. – The effect of storage conditions on the serological activity of two strains of potato virus S (PVS), Andean and the ordinary, was studied by ELISA. Virus purificates, infected leaves and their homogenates, stored in lyophilized, frozen and dissolved form at various temperatures were tested. Virus purificates were most stable in lyophilized form, their activity decreased after 9 months only by 20 – 30%. Also non-purified virus was most stable as a lyophilized leaf homogenate, its activity decreased after 12 months by 30 %. When lyophilized leaves were stored, the virus activity dropped after 12 months by 45 %. Both the Andean and the ordinary strain of PVS behaved similarly during storage under the conditions tested.

Key words: potato virus S; storage conditions; ELISA

For checking the health of plants, enzymological tests are usually employed with a requirement of a positive control (e.g. virus-infected plant) for their evaluation. The aim of our work was to find optimum conditions for the storage of PVS in plant tissues and in the purified form.

The Andean strain of PVS (PVS-ASS isolate) was obtained from Dr. G. Rose, Agricultural Scientific Service, Scotland, and several isolates of the ordinary strain of PVS were obtained from the Institute of Potato Research and Breeding, Havlíčkuv Brod, Czech Republic. Both PVS strains were maintained in *Lycopersicon esculentum cv.* Nevskij. The leaves of infected plants were harvested 3 weeks after inoculation and ground in a mortar with the extraction buffer (0.02 mol/l phosphate pH 7.4, 0.8% NaCl, 0.05% Tween 20, 2% polyvinylpyrrolidone, 0.2% bovine serum albumin (BSA) and 0.05% NaN_3) at the ratios of 1:5 and 1:20. A negative control was prepared from healthy leaves by the same method. One ml portions of prepared antigen were stored at +4 °C, -20 °C, -70 °C and in a lyophilized form at -20 °C. The virus purification was carried out according to Čeřovská and Filigarová (1995). The serological activity of PVS was determined by the double antibody sandwich ELISA according to Clark and Adams (1977). We used rabbit IgG antibodies prepared from an antiserum by precipitation with caprylic acid (Steinbuch and Audran, 1969) and conjugated to alkaline phosphatase (Avrameas, 1969) for the assay. The values given in the tables are means from 3 experiments performed in duplicates. In short-term experiments we followed the activity of PVS in homogenate (sap) stored at +4 °C in the course of several weeks. In long-term experiments we tested the virus activity in lyophilized samples stored at -20 °C and samples stored frozen at -70 °C after 1, 3, 6, 9 and 12 months.

The lyophilization of leaf homogenate in the extraction buffer was the best method for a long-term storage of both strains of PVS. The results with the Andean strain are shown in Table 1. The serological activity of the virus decreased after 12 months in average only by about 30% at the 1:5 dilution. In samples stored in the form of lyophilized leaves, the activity dropped after the same time by 40 – 50%. In leaves stored at -20 °C, the virus lost about 60% of its activity after 9 months, and after 12 months it was quite inactive. There was no difference in the serological activity in leaves stored at -20 °C and -70 °C (data not shown). The activity of purified PVS (both strains) was preserved best by lyophilization (Table 2). In average, the activity of preparations stored under these conditions did not decrease after 4 weeks at all. After 9 months, the activity of lyophilized purificates dropped by about 40 % independently on the addition of stabilizing proteins (ovalbumin, BSA). In purificates stored at -20 °C or -70 °C, the virus lost approximately 70% of its activity already after 3 months, and after 6 months it was serologically quite inactive (data not shown). Virus purificates stored at +4 °C were fully inactive already after 4 weeks.

Table 1. The serological activity of PVS during storage of infected leaves and leaf homogenate

Time of storage (months)	Serological activity (%)			
	Lyophilized homogenate		Leaves	
			Lyophilized -20 °C	
	1:5	1:20		
0	100	100	100	100
1	100	80	100	60
3	100	60	100	52
6	95	65	90	45
9	80	55	75	37
12	70	45	55	0

The Andean strain of PVS was used.

Table 2. Effect of storage conditions on the serological activity of purified PVS

PVS strain	Purificate						Lyophilized purificate			
	+4 °C		-20 °C		-70 °C					
	A^0	A^1	A^1	A^3	A^1	A^3	A^0	A^1	A^3	A^9
Ordinary	2.5	0	1.4	0.48	0.8	0.45	2.1	2.1	1.8	1.6
Andean	2.5	0	1.5	0.60	1.1	0.80	2.0	2.0	1.9	1.7

A^0, A^1, A^3 and A^9 represent A_{405} values of samples assayed after 0, 1, and 9 months of storage.

Table 3. The serological activity of PVS in homogenate of infected leaves stored at +4 °C

Time of storage (weeks)	Serological activity (%)	
	Dilution of homogenate	
	1:5	1:20
0	100[a]	100[a]
1	80	100
3	30	52
4	0	12

[a]100% corresponds to A_{405} of 2.5.

Table 3 illustrates the loss of the serological activity of virus in a leaf homogenate during a short-term storage at +4 °C. Samples were diluted in the extraction buffer. The sample diluted to 1:5 was inactivated in 4 weeks. For comparison, the activity of virus in a lyophilized homogenate dissolved in the extraction buffer was inactivated at +4 °C

Table 4. The serological activity of PVS in lyophilized leaf homogenate dissolved in the extraction buffer and stored at +4 °C

Time of storage (weeks)	Serological activity (%)	
	Dilution of homogenate	
	1:5	1:20
0	100[a]	100[a]
1	50	60
2	30	40
3	0	0

[a]100% corresponds to A_{405} of 2.5.

faster (Table 4). Already after 3 weeks it was impossible to prove the presence of virus by ELISA in both virus dilutions.

In all the experiments presented here we did not observe any substantial differences between the two PVS strains tested.

In our opinion the serological activity of PVS could be reduced during storage in our experiments by some proteases which attack the virus coat protein and destroy it. From this reason the structure of the virus epitopes may be damaged and such virus (tissue homogenate) reacts neither with polyclonal nor monoclonal antibodies directed to different epitopes or polypeptide subunits on PVS particle (Čeřovská *et al.*, 1995).

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