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BRIEF COMMUNICATION

Optimum conditions for the storage of potato virus Y^{NTN} strain

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Abstract

The effect of storage conditions on the serological activity of two isolates of potato virus Y^{NTN} strain (PVY^{NTN}) was studied by ELISA. Purified virus, intact and homogenized infected leaves stored freeze-dried and frozen at various temperatures were tested. Purified virus was the most stable at +4 °C and non-purified virus was best preserved as a freeze-dried leaf homogenate at -20 °C. Their serological activity did not change after three months of storage.

Additional key words: ELISA, *Solanum tuberosum*.

Potato virus Y (PVY) is widely distributed in the potato-growing areas of all the world. It infects mostly plants from the family *Solanaceae* causing various symptoms depending on the virus strain isolate. PVY isolates inducing necrosis on tubers belong to the PVY^{N} strain according to their reactions on *Nicotiana tabacum* (Beczner *et al.* 1984). However, these isolates, initially called PVY^{NTN} (Le Romancer and Kerlan 1991), are distinguished from other PVY^{N} isolates by their behaviour on several herbaceous non-cultivated plants and also on some potato cultivars (Beczner *et al.* 1984, LeRomancer and Kerlan 1991).

Potato tuber necrotic ringspot disease has been first mentioned in Hungary (Beczner *et al.* 1984) and then in Germany (Weidemann 1985). Isolates of this strain are not serologically distinct from standard PVY^{N} isolates (Le Romancer and Kerlan 1991, Le Romancer 1993, Le Romancer *et al.* 1994). The aim of our work was to find optimum conditions for the storage of PVY^{NTN} in plant tissues and in the purified form.

We used two isolates of PVY^{NTN}: isolate H kindly provided by Dr. C. Kerlan, INRA, Rennes, France, and isolate Lebanon provided by Dr. P. Dědič, Potato Research Institute, Havlíčkův Brod, Czech Republic. The isolates were maintained in *Nicotiana tabacum* cv. Samsun. The leaves of infected plants were harvested 2 weeks after inoculation and ground in a mortar with the extraction buffer (0.02 mol dm^{-3} Na-K phosphate, pH 7.4, 0.8 % NaCl, 0.05 % Tween 20, 2 % polyvinylpyrrolidone, M_r 6000, 0.2 % bovine serum albumin, BSA, and 0.05 % NaN_3) at the ratio of 1:10. A negative control was prepared from healthy leaves by the same method. One cm^3 portions of prepared antigen were stored at -20 °C, -70 °C and in freeze-dried form at -20 °C. The virus purification was carried out according to Čeřovská *et al.* (1997). The serological activity of PVY^{NTN} was determined by the double antibody sandwich ELISA according to Clark and Adams (1977). We used rabbit IgG antibodies against PVY^{NTN} 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 long-term experiments we tested the virus activity in freeze-dried samples and samples stored frozen at -20 °C and -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 PVY^{NTN}. The results with the isolate Lebanon (Table 1) shown that the activity of homogenate stored under these conditions did not decrease after three months. After six months of storage the activity of lyophilised homogenates dropped by about 30 %. The serological activity of the virus decreased after 9 months in average by about 60 % at the 1:10 dilution. For comparison the serological activity of the virus in leaves

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stored at -20 and -70 °C did not change after one month but was almost fully inactivated after 6 months of storage. During the first month of storage the serological activity of purified PVY^{NTN} (Table 2) did not changed regardless procedures tested. From the Table 2 was evident, that the purificate was best preserved at + 4 °C.

In all the experiments presented here we did not observe any substantial difference between the two PVY^{NTN} isolates tested.

Table 1. The serological activity of PVY^{NTN} isolate Lebanon after storage of infected leaves and leaf homogenate. 100 % corresponds to A_{405} of 3.5.

Storage period [months]	Serological activity [%] freeze-dried homogenate	leaves frieze-dried	-20 °C
0	100	100	100
1	100	100	100
3	100	80	67
6	67	20	6
9	35	8	0
12	0	0	0

Table 2. Effect of storage conditions on the serological activity of purified PVY^{NTN} isolate Lebanon. 100 % corresponds to A_{405} of 3.5.

Storage period [months]	Serological activity [%] purified virus +4 °C	-20 °C	-70 °C	freeze-dried purificate
0	100	100	100	100
1	100	100	100	100
3	100	86	80	77
6	53	35	17	14
9	19	7	0	0
12	0	0	0	0

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