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DETECTION OF PELARGONIUM LEAF CURL VIRUS IN YUGOSLAVIA

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An isometric virus was isolated from an unknown cultivar of ***Pelargonium zonale*** with yellow/green rounded and stellate spots and uneven growth of the leaves. On the basis of reactions of diagnostic test plants, stability in crude sap, serological and immunoelectrophoretic tests the virus was identified as an isolate of pelargonium leaf curl virus (PLCV). It belongs to strain 2 of PLCV and to the serological cluster comprising most PLCV isolates. This is the first report of PLCV and the second report of a tombusvirus in Yugoslavia.

Introduction

Except of some viruses which occur also in other crops, five specific or true pelargonium viruses have been isolated from different pelargonium cultivars up to now (Stone 1980). None of them causes severe damage to pelargoniums and they are mostly symptomless during the warmer period of the year. However, virus mixtures are quite common in pelargoniums (Stone and Hollings 1973, Stone 1974, Paludan 1976, Stone 1980) and combinations of viruses may cause growth reduction and loss of flower guality.

A few years ago we isolated and described a specific pelargonium virus, i. e. pelargonium line pattern virus (PelLPV) (Pleše and Štefanac 1980, Štefanac et al. 1982) which had been previously reported only from Great Britain (Stone and Hollings 1977, Stone 1980).

Recently we have detected another true pelargonium virus in Yugoslavia i.e. pelargonium leaf curl virus (PLCV), so far recorded in several European countries, the Mediterranean area and in the United States (cf. Martelli 1981). The present paper deals with the Yugoslav isolate (P-718) of PLCV which seems to belong to the cluster of common and prevalent PLCV isolates (Hollings 1962, Hollings and Stone 1965, 1975).

Material and Methods

P-718 was isolated from an unknown cultivar of *Pelargonium zonale* of a private collection. During winter and early spring the infected plant showed yellow small rounded and stellate, in places necrotic, spots and uneven growth of the leaves (Fig. 1 A). The virus was isolated without difficulty by grinding the symptoms bearing leaf tissue in 0.06 M phosphate buffer pH 7.6 and by inoculation to *Chenopodium quinoa*. A few chlorotic local lesions, which soon became necrotic, appeared on the rubbed leaves a week later. *C. quinoa* was used in further investigations as assay and as propagation species.

For the purpose of comparative studies and identification of our isolate P-718, an isolate of PLCV, i. e. PLCV-456 of Hollings and Stone (1965, 1975), and the spinach strain of tomato bushy stunt virus (TBSV) (Štefanac 1978) were included in these investigations. The isolate PLCV-456 was used for comparative symptomatological, serological and immunoelectrophoretic analyses, and the spinach strain for comparative serological tests only. In most serological test and immunoelectrophoresis the isolates P-718 and PLCV-456 were employed in purified preparations and the spinach strain of TBSV in crude sap.

The isolates were partially purified by n-butanol method previously used for PLCV and several other stable isometric pelargonium viruses (Hollings 1962, Stone and Hollings 1973, Hollings and Stone 1975, Pleše and Štefanac 1980). The inoculated leaves of *C. quinoa* (on average 150 g) covered with innumerable local lesions were used as a source of the virus for purification. Purified preparations consisted usually of c. 50 mg of virus/ml. To avoid a possible damage of virus particels during electron microscopic analysis, the preparations were fixed in 1% formaldehyde prior to negative staining with potassium phosphotungstate (2%, pH 6.8). Until use, suspensions of purified isolates were stored at 258 K (—15°C) by adding 20% (1:5 v/v) of glycerol as preservative.

The serum against our isolate P-718 was prepared by 6 intravenous injections (each of 1 ml) over a period of 15 days and it was collected within 2 weeks after the final injection. Homologous titre of the serum in agar gel was 1/512 against the virus and 1/4 to normal plant components The serum was stored with an equal volume of glycerol at 275 K (2°C). Serological tests were done with the serum against isolate P-718 and the serum against spinach strain of TBSV (homologous titre 1/1024). For the tests the sera and the purified antigens were diluted to suitable equivalent concentrations established in preliminary serological tests. Serological experiments were performed in agar gel by double diffusion method.

Immunoelectrophoresis was done at 275 K (2°C) for a period of 4 h with a LKB-Gelman 6800A-1 microelectrophoresis apparatus. Slides were coated with 0.9% Ionagar no. 1 (Oxoid) dissolved in 0.03 M phosphate buffers pH 7.6 and pH 7.86 in the repeated test. The same phosphate buffers were used throughout as conducting liquids. Antigen wells, 1 mm diameter, were filled with suitably diluted (1/4) purified preparations. A constant voltage supply of 140 V gave c. 5 V/cm across the agar bed. After electrophoresis the serum against P-718 (diluted 1/8) was placed in the channels and slides were incubated for 24 h at c. 297 K (24°C) in a humid chamber. Mobilities were calculated from the average movement from the centre of the origin well (see Hollings and Stone 1975).

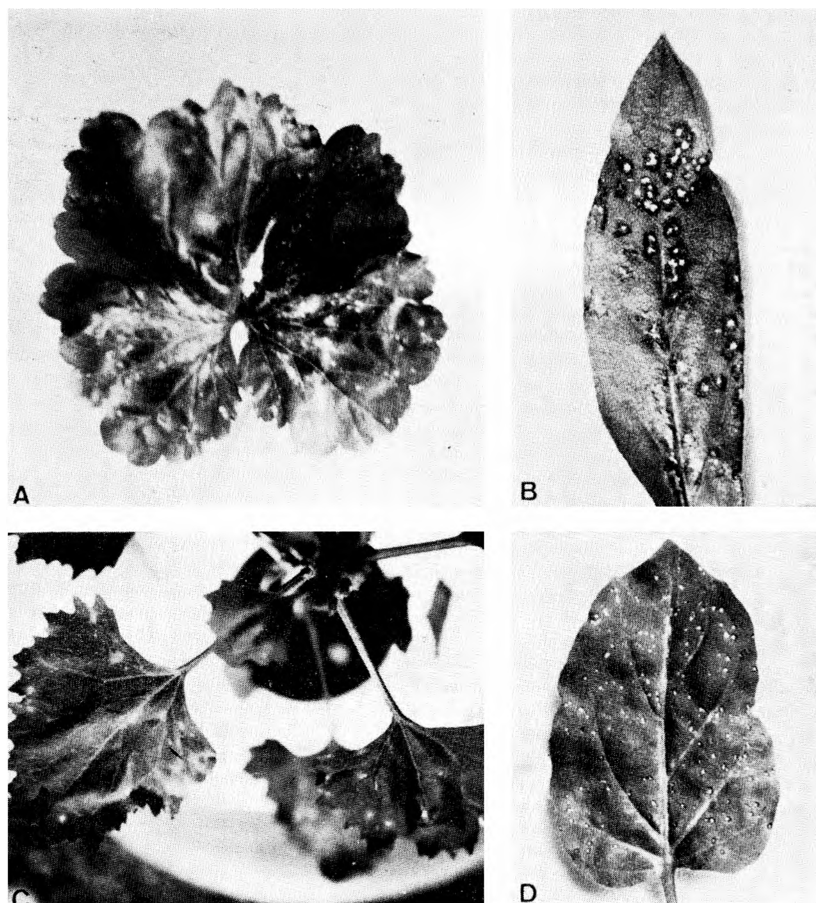

Fig. 1. **A** Symptoms in *Pelargonium zonale* from which P-718 was isolated. **B** Local necrotic dots with red-rimmed rings in inoculated leaf of *Gomphrena globosa*. **C** Systemic symptoms with chlorotic and necrotic flecks and dots and leaf buckling in *Chenopodium amaranticolor*. **D** Small local necrotic dots in *Nicotiana glutinosa*.

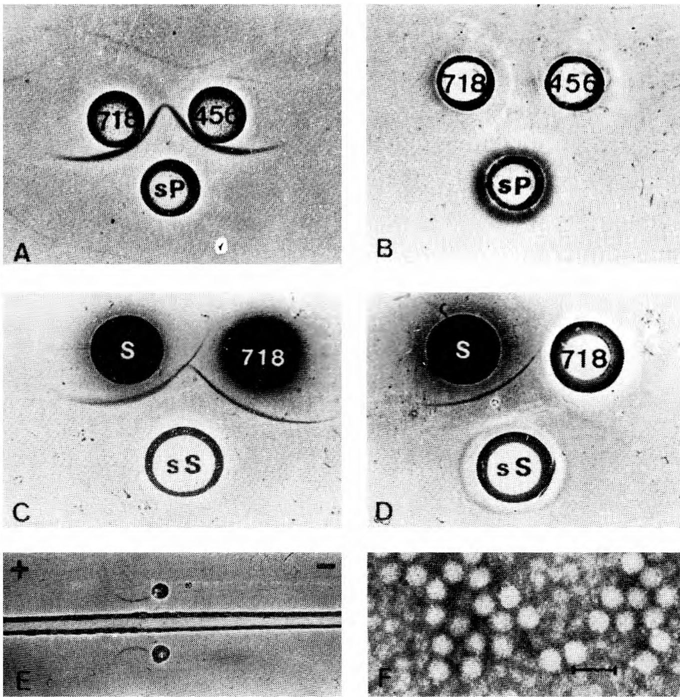

Fig. 2. Immunodiffusion tests of (**A, B, D**) partially purified isolates P-718 and PLCV-456, (**C**) P-718 in crude sap and of (**C, D**) spinach strain (S) in crude sap with the serum against isolate P-718 (sP) and the serum against spinach strain (sS). In **B** the sP was absorbed with PLCV-456 and in **D** the sS with P-718. **E** Gel immunoelectrophoresis showing very slight displacement of P-718 and PLCV-456 from origin wells towards the anode. **F** Electron micrograph of partially purified preparation of P-718 in sodium phosphotungstate. Many particles are disrupted. Bar represents 50 nm.

Results

Reaction of test plants

The investigated isolate P-718 and the isolate PLCV-456 were inoculated to the main diagnostic species for PLCV in order to compare the symptoms produced.

After 2—3 days both isolates provoked in *Chenopodium quinoa* numerous chlorotic local lesions which soon became necrotic. *C. amaranticolor* reacted with local necrotic dots, but systemic infection with chlorotic and necrotic flecks and dots and leaf buckling (Fig. 1 C) ordinarily occurred only at lower temperatures. The leaves which developed later on the top of plants often did not contain detectable virus. In *Gomphrena globosa* pale local necrotic dots, enlarging to red-rimmed rings (Fig. 1 B), appeared in about 5 days. Systemic infection with chlorotic and reddisch coloured flecks and leaf deformations occurred occasionally. Both isolates also produced chlorotic and necrotic lesions in inoculated leaves of *Nicotiana clevelandii,* followed by systemic chlorotic and necrotic spots and rings, leaf deformations and dwarfing. In *N. glutinosa* the isolates provoked small local necrotic dots (Fig. 1 D). Inoculated leaves of *Spinacia oleracea* showed single whitish necrotic lesions. Systemic infection was latent; it developed slowly and failed at high summer temperatures.

Consequently, the investigated isolate P-718 provoked the same characteristic reactions in diagnostic test plants as the isolate PLCV-456 did, which suggests that it also represents an isolate of PLCV.

Stability in sap

The dilution end point of the isolate P-718 was not determined, but it should be very high in view of the high concentrations of purified preparations from inoculated leaves of *C. quinoa* (see Material and methods). Curde sap of *C. quinoa* lost its infectivity after 10 min at 358—363 K (85—90°C) and after 6 weeks at c. 297 K (24°C). These values coincide with those obtained for PLCV by other authors (Hollings 1962).

Electron microscopy

Electron microscopic analysis revealed that purified preparations contain abundant isometric virus particles (28—30 nm) but most of them were disrupted (Fig. 2 F) despite prior fixation with formaldehyde. It is very likely that potassium phosphotungstate was not a suitable stain for our virus preparations (see Martelli et al. 1971, Martelli 1981). However, we did not have the possibility to try other contrasting media.

Serological tests

The first tests in the investigation of serological identity of the isolate P-718 showed that this isolate and the isolate PLCV-456 were serologically closely related, i. e. the isolate PLCV-456 reacted with the serum against P-718 up to the homologous titre. However, the heterologous titre of the serum to spinach strain of TBSV was with both pelargonium isolates two steps lower (1/256) from its homologous titre.

Further serological comparisons among both isolates and the spinach strain were also done by using sera to P-718 and to spinach strain. In double diffusion tests the isolates P-718 and PLCV-456 formed precipitin

lines which always joined without spur formation (Fig. 2 A). On the other hand, the tests performed with the spinach strain always showed very clear spur formations with both isolates (Fig. 2 C). In absorption tests the serum to P-718 absorbed with antigen PLCV-456 did not additionally react with homologous antigen (Fig. 2 B). On the contrary, the serum to spinach strain formed — after absorption with either of two heterologous isolates — a clear precipitin line with homologous antigen (Fig. 2 D).

According to serological tests performed, the isolate P-718 appeared serologically identical to isolate PLCV-456 of PLCV. Both isolates were serologically related but not identical with the spinach strain of TBSV.

Immunoelectrophoresis

The isolates P-718 and PLCV-456 were also compared in immunoelectrophoresis tests. In 0.03 M phosphate buffers pH 7.6 and pH 7.86, both isolates migrated very slowly towards the anode as a single moving component (Fig. 2 E). The electrophoretic movement was calculated to be 0.8 mm/h for both pH values. No indication of differences in electrophoretic mobility of the isolates could be observed.

Discussion

PLCV is the best known specific pelargonium virus which occurs quite frequently in pelargoniums. Because several times we have isolated only PelLPV from pelargoniums with PLCV-like symptoms (Pleše and Štefanac 1980), we wanted to know whether PLCV is also present in pelargoniums in our country.

Reactions of some diagnostic herbaceous species have allready indicated that isolate P-718 represents an isolate of PLCV (cf. Hollings 1962, Hoollings and Stone 1965). The symptoms in *Nicotiana clevelandii* and *N. glutinosa* suggest that the isolates P-718 and PLCV-456 belong to the strain 2 of PLCV (Hollings 1962, Hollings and Stone 1965). According to earlier literature data (Hollings 1962, Hollings and Stone 1965, Fischer and Lockhart 1977, Štefanac 1978) PLCV and many other tombusviruses infect *Spinacia oleracea* locally. However, we have established that both isolates P-718 and PLCV-456 occasionally provoke latent systemic infection in *S. oleracea*, which may have been overlooked.

Following the results of serological tests performed both pelargonium isolates seem to be closely related or identical each other and different from although related to spinach strain of TBSV. The serological investigations have demonstrated that our isolate P-718 belongs to the group comprising most PLCV isolates (Hollings and Stone 1975).

Immunoelectrophoresis tests displayed no differences in the electrophoretic run of P-718 and PLCV-456, but in our tests both isolates migrated slowly towards the anode and not towards the cathode as cited for PLCV-456 earlier (Hollings and Stone 1975). However, as known, electrophoretic movement of viruses is sometimes influenced by certain effects which change their charge (Paul and Querfurth 1979).

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SAŽETAK

NALAZ VIRUSA KOVRČANJA LISTA PELARGONIJE U JUGOSLAVIJI

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Iz nepoznatog kultivara vrste *Pelargonium zonale* sa žutozelenim okruglim i zvjezdastim pjegicama i neravnim lisnim plojkama izoliran je izometričan virus. Na osnovi reakcije dijagnostičkih pokusnih biljaka, stabilnosti u soku, seroloških i imunoelektroforetskih pokusa istraživani je virus identificiran kao izolat virusa kovrčanja lista pelargonije (VKLP; pelargonium leaf curl virus). Izolat pripada soju 2 VKLP i serološkoj skupini VKLP koja uključuje većinu izolata toga virusa. To je prvi nalaz VKLP i drugi nalaz jednog tombusvirusa u Jugoslaviji.

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