

UDC 576.858.8:582.579(497.1) = 20

VIRUS INFECTION OF FREESIAS IN CROATIA

NADA PLEŠE

(Department of Botany, Faculty of Science, University of Zagreb)

Received January 4, 1985

Glasshouse-grown freesias cultivated for commercial purposes at the HC Žitnjak (Zagreb) exhibited pronounced symptoms of virus infection. Orientational investigations concerning the virus or viruses involved were performed by using bioassay and serological tests. It was established that about 70% of freesia plants examined were infected with bean yellow mosaic virus (BYMV). The appearance of the symptoms of flower colour-breaking has indicated that freesia mosaic virus is also involved in this infection of freesias alone or in combination with BYMV.

Introduction

According to the present knowledge, virus diseases of freesias are caused by freesia streak virus (FSV), freesia mosaic virus (FMV) and bean yellow mosaic virus (BYMV). A filamentous FSV was reported up to now only from England (Brunt 1968, 1969, 1970) and from Germany (Casper and Brunt 1971). Freesia cultivars are affected mainly by FMV and/or BYMV (van Koot et al. 1954 etc.) which provoke various alterations on the leaves, flowers and corms leading gradually to the degeneration of the plants and their death. In addition to the virus diseases mentioned, a harmful disorder, freesia leaf necrosis (FLN) was also described (Hakkaart 1973, van Dorst 1973). It seems to be a virus disease of soilborne nature (van Dorst 1975).

While FMV is a specific virus of freesias, BYMV has often been detected within the family *Iridaceae*. Besides freesia it was established in iris (Brierley and Smith 1948, Brunt and Phillips 1980, Alper and Loebenstein 1981), gladiolus (Brierley and Smith

1947, McWhorter et al. 1947, Zettler and Abo El-Nil 1977), saffron (Russo et al. 1979), *Crocoshmia* and *Tritonia* (Derks et al. 1980) and others. The symptoms it provokes depend on the susceptibility of the species or cultivar affected ranging from spotting, mottling or striped mosaic pattern to necrotic alterations and from flower colour-breaking to flower deformation.

In order to obtain some information about the virus disorders on freesias in our country, orientational investigations were undertaken. This paper is a short report of our investigations on freesias in Croatia.

Material and Methods

Glasshouse-grown freesias at the HC Žitnjak (Zagreb) showed prominent symptoms of virus infection leading to the loss of growth vigour and to progressive degeneration of the plants.

Twenty-two specimens of freesias belonging to various cultivars (Aurora, Carmen, Fantasy, Miranda, etc.) were sampled for virus testing. From this group 17 freesia samples exhibited symptoms of light-green or yellow-striped mosaic and white to reddish-brown necrotic stripes and spots on the leaves (Fig. 1 B, C). In addition, some of those plants showed flower colour-breaking (Fig. 1 A). The remaining 5 freesias from this group appeared healthy, i.e. without visible symptoms.

In biological test *Chenopodium quinoa* and *C. amaranticolor* were used. For mechanical inoculation the leaves of freesias were homogenized in 0,06 M phosphate buffer (pH 7.1) containing 0,10% (v/v) TGA. Leaf dip preparation from infected *C. quinoa* was negatively stained with 2% PTA and layered on formvar-coated grids.

Serological tests were made by double-diffusion method in agar gel containing SDS. The agar medium was prepared according to Hunst and Tolin (1982) by replacing the Ionagar with the 0,7% Bacto-Agar. The serum against BYMV (type strain B25), kindly supplied by Dr. D. Z. Maat (Wageningen), was at our disposal for serological tests. This serum (titre 1/256) was used undiluted and diluted 1/2. A crude sap of the leaves of test plant *C. quinoa* with chlorotic local lesions and the leaf sap of freesias were prepared by adding some drops of distilled water. The leaf extract of healthy *C. quinoa* served as control.

Results and Discussion

The investigations of freesias comprised the detection of virus infection by bioassay and by serological test.

From 22 freesias tested, 15 inocula provoked chlorotic local lesions on inoculated leaves of *C. quinoa* and *C. amaranticolor*. Fourteen virus isolates originated from freesias with virus symptoms and 1 from a plant without symptoms. The remaining 7 inocula extracted from 3 symptoms bearing freesias and 4 from those which appeared healthy provoked no symptoms on test plants.

This analysis showed that the freesias checked contained a virus which is quite easily mechanically transmissible to both test plants. We supposed that we isolated BYMV, because, as known, BYMV is wide spread and quite common on freesias (van Koot et al. 1954, Derks et al. 1980, Oertel 1980 a, b and others). Moreover, the morphology

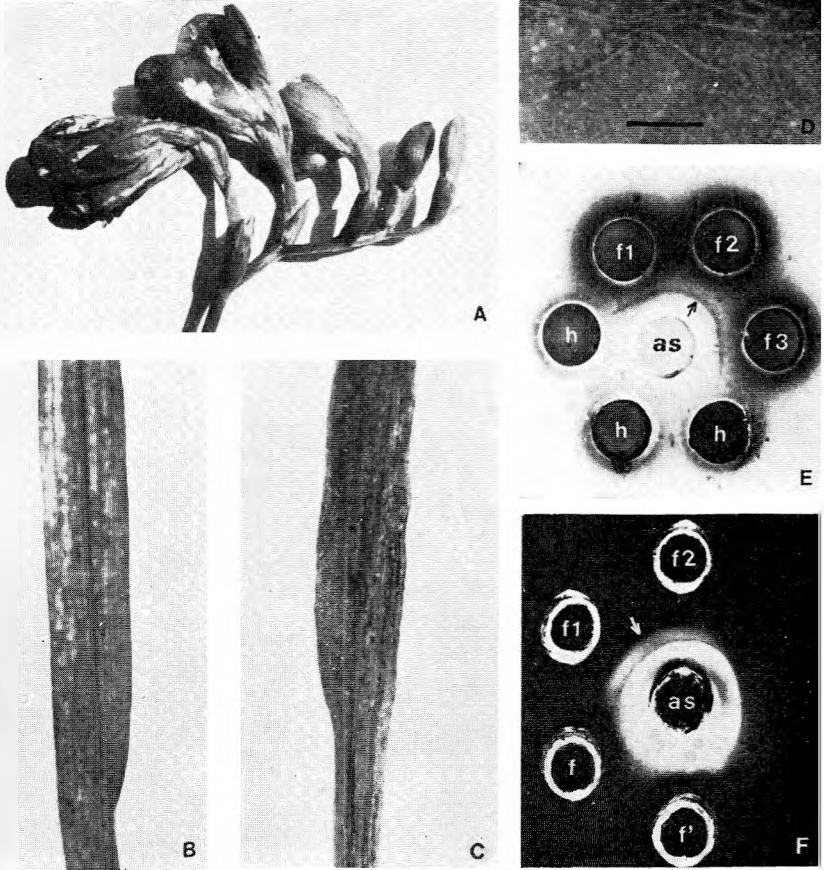


Fig. 1. **A** Symptom of flower colour-breaking in virus infected freesia plant. **B** Light-green- to yellow-striped mosaic pattern and **C** reddish-brown necrotic stripes on the leaves of virus infected freesia samples. **D** Virus particles of a freesia isolate of BYMV in leaf dip preparation of infected *C. quinoa*; bar represents 250 nm. Gel-diffusion serological tests with SDS: in **E** peripheral wells — crude leaf sap of *C. quinoa* plants infected with freesia isolates (f1, f2, f3) of BYMV and sap of healthy plant (h); in **F** peripheral wells — crude leaf sap of 2 freesia samples with virus symptoms (f1, f2) and sap from 2 plants without symptoms (f, f'); central wells (as) — antiserum to BYMV-B25; the virus precipitation lines are marked with arrow.

of virus particles of about 750 nm length (Fig. 1 D) suggested that this virus was in question.

Our assumption was confirmed by serological investigations. In serological tests the isolated virus reacted with the serum against BYMV forming in the gel a precipitation line shaped characteristically for degraded virus particles (Fig. 1 E, F). Only 10 isolates were tested serologically because the symptoms in *Chenopodium* provoked by all 15 isolates indicated infection with BYMV. Two isolates were also proved serologically directly in the leaf sap of freesias (Fig. 1 F). BYMV could not be detected serologically in the leaves of 4 freesias which appeared healthy by plant test (Fig. 1 F).

On the basis of examinations described, it was possible to establish that in virus disorders of freesias in our market-gardening BYMV was involved. We established its presence in 68% of tested freesias. It is also discernible from the literature data that BYMV plays an important role in virus diseases of freesias because of the frequency of infections and the injuries it produces (van Koot et al. 1954, Derks et al. 1980, Oertel 1980 a, b). Although the serological properties of our BYMV isolates were not investigated, considering the symptoms on assay species, it is very likely that they all belong to the same strain.

The symptoms of flower colour-breaking and flower distortion characteristic of infection with FMV were also observed on some exemplars in flowering stage (Fig. 1 A). In addition, our attempts to isolate a virus from some freesia samples exhibiting evident virus symptoms were unsuccessful. As known, it is very difficult to transmit FMV by sap inoculation. Both these facts indicated that at least in some freesias tested, FMV was also present alone or in combination with BYMV. Unfortunately, the serum against FMV was not available.

The appearance of special FSV is altogether unclear. Its identification seems uncertain because the particle length reported for this virus corresponds to that of FMV (cf. Casper and Brandes 1971, Hakkaart 1973, van Dorst 1973).

The presence of disorder FLN in investigated freesia plantation is doubtful, but we observed that some freesia plots were nearly completely destroyed.

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Thanks are due to Mr. Jakov Biličić of the Horticultural Cooperative Žitnjak for kindly supplying freesia samples and helpful information.

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S A Ž E T A K

VIRUSNA INFEKCIJA FREZIJA U HRVATSKOJ

Nada Pleše

(Botanički zavod, Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu)

Frezije koje se uzgajaju u staklenicima VK »Žitnjak« (Zagreb) u komercijalne svrhe pokazuju jasne simptome virusne infekcije koja doводи do oslabljenog rasta i postupne degeneracije zaraženih biljaka. Orientacijska istraživanja o virusu ili virusima uzročnicima te infekcije frezija izvršena su s pomoću biotesta i serološkim testiranjem. Utvrđeno je da je oko 70% ispitanih primjeraka frezije zaraženo virusom žutog mozaika graha (bean yellow mosaic virus, BYMV). Prisutnost karakterističnih simptoma šarenila cvijeta (flower colour-breaking) upozorava na to da je u tu virusnu infekciju frezija uključen i virus mozaika frezije (freesia mosaic virus), i to sam ili u smjesnoj infekciji s BYMV.

Doc. dr. Nada Pleše
Prirodoslovno-matematički fakultet
Marulićev trg 20/II
YU-41000 Zagreb (Jugoslavija)