

## SURVEY OF VIRUS PATHOGENS IN GLADIOLUS, IRIS, AND TULIP IN THE CZECH REPUBLIC

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### Abstract

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The occurrence of *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tobacco rattle virus* (TRV) in gladiolus, iris, tulip and *Iris yellow spot virus* (IYSV) in iris was investigated by examining the plants by the means of serological techniques (ELISA). ELISA was applied to determine the presence of BYMV, CMV, TRV infections in both aerial and underground parts of gladiolus, iris, and tulip, and IYSV on the aerial parts of iris, respectively. 262 gladiolus plants were tested. 63.7% was infected by BYMV, 29.4% by CMV, and 2.7% by TRV. Out of 180 plants of iris, 1.1% was infected by BYMV, 6.7% by CMV, 2.8% by TRV, and 0% by IYSV. Out of 28 plants of tulip, 28.6% was infected by CMV, and 7.1% by TRV. ELISA proved to be a suitable method for detection of viruses in leaves of these ornamental plants, but it often failed to detect viruses in flowers and corms. A high transmission of BYMV by gladiolus cormlets was also found.

*Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tobacco rattle virus* (TRV), *Iris yellow spot virus* (IYSV) gladiolus, iris, tulip, biological test, ELISA

Gladiolus, iris, and tulip are three of the most important ornamental plants in the world. The magnificent appearance and a wide variety of colour have enhanced their cultivation throughout the world. Flower crops are attacked by a wide array of diseases of biological origin which results in corm rot, leaf and neck rot, leaf chlorosis and necrosis, flower mottle, and other diseases. Viral diseases have an important status because not only do they cause direct damage to the host plant but they also predispose the plant to secondary invaders (Beute, 1970). The majority of viral diseases lead to overall stunting, colour break, flower distortion, and reduced flower and cormlet production (Magie and Poe, 1972). In order to improve the crop productivity and minimize viral infection in different cultivars, a proper diagnosis and control is essential. In addition, a diagnosis is also useful when exporting healthy plants to countries where strict quarantine conditions have been imposed. In this study, the status of different viruses in gladiolus, iris, and tulip was determined based on the ELISA method.

### MATERIALS AND METHODS

#### Occurrence of viral infections in gladiolus, iris, and tulip

For analysis of *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), and *Tobacco rattle virus* (TRV) occurrence, these materials were used (2007):

1. The corms were collected at a market and grown under green house conditions at Mendel University of Agriculture and Forestry, Brno.
2. The cormlets were collected in Jestřabí (near Velká Bíteš – 49° 16' 18" N, 16° 11' 28" E) and grown under green house conditions at Mendel University of Agriculture and Forestry, Brno.
3. Gladiolus leaves were collected from garden of private breeder in the Jestřabí field (near Velká Bíteš – 49° 16' 18" N, 16° 11' 28" E).
4. Gladiolus leaves were collected from private garden in Nedvědice (49° 12' 51" N, 16° 36' 56" E).
5. Unknown gladiolus leaves and flowers were collected in local shops.

6. Leaves and flowers of iris were collected in the arboretum at Mendel University of Agriculture and Forestry, Brno (49° 27' 41" N, 16° 19' 48" E).
7. Tulip leaves were collected in the arboretum at Mendel University of Agriculture and Forestry, Brno (49° 27' 41" N, 16° 19' 48" E).

For the *Iris yellow spot virus* (IYSV) analysis, these materials were used (2008):

8. Iris leaves were collected in the arboretum at Mendel University of Agriculture and Forestry, Brno (49° 27' 41" N, 16° 19' 48" E).

The different organs originating from same plants (leaves, flowers or corms, respectively) were tested by serological methods (ELISA), according to Clark and Adams (1977) for the identification of BYMV, CMV, TRV and IYSV. The ELISA test was carried out according to the manufacture kits (DSMZ). A purified IgG was diluted in coating buffer, then 200 µl of it was added to each well of a microtitre plate and incubated at 37 °C for 2–4 h. The test sample was extracted in sample extraction buffer and 200 µl aliquots were added to each well and incubated overnight at 4 °C. IgG was diluted in conjugate buffer, then 200 µl was added to each well and incubated at 37 °C for 2–4 h. Finally, 200 µl of substrate (p – nitro phenyl phosphate, 10mg /10ml) was added to each well and incubated for half an hour at room temperature. Absorbance at 405 nm was measured for the complete ELISA plate by the means of a flow ELISA microplate reader. The reaction was considered positive for BYMV, CMV, TRV, and IYSV infections if absorbance was > 0.1, which was at least three times the background mean of the healthy control.

Some isolates of BYMV from the market leaf samples and the Jestřabí cultivars were tested by a biological method with an indicator plant of *Pisum sativum* cultivars (Merkur and Jackpot) by the means of mechanical inoculation. The inoculum for the mechanical inoculation was prepared by homogenizing the infected leaves with 0.1M of phosphate buffer (pH 7.2). A visual symptom observation was conducted after three weeks in the host plants.

### The transmission of BYMV from gladiolus corms and cormlets

1. The corms from plants infected by BYMV, which were acquired at the market, Jestřabí, and Nedvědice were grown under greenhouse conditions at Mendel University of Agriculture and Forestry, Brno.
2. The cormlets from plants infected by BYMV from Jestřabí and Nedvědice were grown under greenhouse conditions at Mendel University of Agriculture and Forestry, Brno.

Leaves were collected from plants and tested by ELISA for BYMV as mentioned above.

## RESULTS

### Occurrence of viruses in gladiolus, iris, and tulips

Among the different gladiolus cultivars collected at the market (Tab. I), the BYMV infection was identified in leaves of all gladiolus cultivars. In flowers, the BYMV infections were highly determined only in one cultivar, 3 cultivars were infected moderately, and 2 cultivars were probably infected by BYMV. In corms, the BYMV infection was identified only in one cultivar. In all those cultivars, the CMV and TRV infections were not determined.

In the samples collected in Jestřabí (Tab. II), the BYMV infection was identified in leaves of all gladiolus cultivars and the rate of the cultivar BYMV infection was not different among those individual cultivars, whereas in cormlets, the infection was not found. As far as CMV was concerned, 1 cultivar was moderately infected, 3 cultivars were less infected, and 5 cultivars were not infected at all. The TRV infection was not determined in leaves of those cultivars. In case of cormlets, the CMV and TRV infections were not determined in any of those cultivars.

When analyzing the gladiolus leaves collected in the Jestřabí field (Tab. III), we found that the BYMV infection was highly present in 4 cultivars, moderate

I: Identification of virus on gladiolus leaves, flowers and corms collected from Market (ELISA)

| Name of the Cultivars   | BYMV    |         |        | CMV    |         | TRV    |         |
|-------------------------|---------|---------|--------|--------|---------|--------|---------|
|                         | Leaves  | Flowers | Corms  | Leaves | Flowers | Leaves | Flowers |
| Blue Frost              | 5 / 5   | 3 / 2   | 2 / 0  | 5 / 0  | 3 / 0   | 5 / 0  | NT      |
| Drama                   | 5 / 5   | NT      | 3 / 0  | 5 / 0  | NT      | 5 / 0  | NT      |
| Nova Lux                | 5 / 5   | 2 / 1   | 3 / 0  | 5 / 0  | 2 / 0   | 5 / 0  | NT      |
| Priscilla               | 5 / 5   | 3 / 2   | 2 / 0  | 5 / 0  | 3 / 0   | 5 / 0  | NT      |
| Victor Borge            | 5 / 5   | 3 / 2   | 3 / 1  | 5 / 0  | 3 / 0   | 5 / 0  | NT      |
| Zorro purple/Paara/lila | 5 / 5   | NT      | NT     | 5 / 0  | NT      | 5 / 0  | NT      |
| Topaze Orange           | 5 / 5   | 1 / 0   | 1 / 0  | 5 / 0  | 1 / 0   | 5 / 0  | NT      |
| Sancerre White / Weiss  | 5 / 5   | 1 / 0   | 2 / 0  | 5 / 0  | 1 / 0   | 5 / 0  | NT      |
| Madona Light blue       | 5 / 5   | 3 / 1   | 2 / 0  | 5 / 0  | 3 / 0   | 5 / 0  | NT      |
| Pr.Marg.Rose            | 5 / 5   | 3 / 3   | 2 / 0  | 5 / 0  | 3 / 0   | 5 / 0  | NT      |
| Total No. of samples    | 50 / 50 | 19 / 11 | 20 / 1 | 50 / 0 | 19 / 0  | 50 / 0 | -       |

NT- Not Tested

## II: Identification of virus on gladiolus leaves and cormlets collected from Jestřabí (ELISA)

| Name of the Cultivars | BYMV   |          | CMV    |          | TRV    |          |
|-----------------------|--------|----------|--------|----------|--------|----------|
|                       | Leaves | Cormlets | Leaves | Cormlets | Leaves | Cormlets |
| Bombay                | 5/5    | 5/0      | 5/0    | 5/0      | 5/0    | 5/0      |
| Noe                   | 2/2    | 2/0      | 2/0    | 2/0      | 2/0    | 2/0      |
| EL type               | 5/5    | 5/0      | 5/0    | 5/0      | 5/0    | 5/0      |
| Jarní Louka           | 15/13  | 5/0      | 15/5   | 5/0      | 5/0    | 5/0      |
| Májový Květ           | 12/9   | 4/0      | 12/2   | 4/0      | 4/0    | 4/0      |
| Poušovák              | 8/6    | 5/0      | 8/2    | 5/0      | 5/0    | 5/0      |
| Radyně                | 4/4    | 4/0      | 4/0    | 4/0      | 4/0    | 4/0      |
| Rachelle              | 5/5    | 5/0      | 5/1    | 5/0      | 5/0    | 5/0      |
| Bambino               | 5/5    | 5/0      | 5/1    | 5/0      | 5/0    | 5/0      |
| Jo Ann                | 11/9   | 5/0      | 11/2   | 5/0      | 5/0    | 5/0      |
| Jungle Flower         | 4/4    | 4/0      | 4/0    | 4/0      | 4/0    | 4/0      |
| Total No. of samples  | 76/63  | 49/0     | 76/13  | 49/0     | 49/0   | 49/0     |

## III: Identification of virus on gladiolus leaves collected from Jestřabí field (ELISA)

| Name of the Cultivars | BYMV   | CMV    | TRV    |
|-----------------------|--------|--------|--------|
|                       | Leaves | Leaves | Leaves |
| Noe                   | 4/2    | 4/4    | 4/0    |
| 85-1-82               | 4/4    | 4/4    | 4/0    |
| Amiral                | 4/4    | 4/4    | 4/1    |
| Soumrak               | 4/3    | 4/4    | 4/1    |
| Ostanovis Mgnogenie   | 4/2    | 4/2    | 4/0    |
| Labakan               | 4/0    | 4/0    | 4/0    |
| Safari                | 4/0    | 4/0    | 4/1    |
| Sailor's Delight      | 4/0    | 4/3    | 4/0    |
| Jo Ann                | 4/4    | 4/3    | 4/0    |
| Angelika              | 4/0    | 4/1    | 4/0    |
| El Diablo             | 4/0    | 4/3    | 4/0    |
| Yonan Maru            | 4/0    | 4/0    | 4/0    |
| Gladiris              | 4/2    | 4/4    | 4/1    |
| Dona Maria            | 4/1    | 4/3    | 4/0    |
| Radyně                | 4/0    | 4/1    | 4/0    |
| Kytice                | 4/1    | 4/1    | 4/0    |
| Poušovák              | 4/0    | 4/4    | 4/0    |
| Ladová Socha          | 4/0    | 4/4    | 4/2    |
| Rachelle              | 4/0    | 4/0    | 4/0    |
| Májový Květ           | 4/0    | 4/3    | 4/1    |
| Menuet                | 4/0    | 4/3    | 4/0    |
| Šokoladnica           | 4/0    | 4/1    | 4/0    |
| Modrý Program         | 4/0    | 4/3    | 4/0    |
| Bambino               | 4/2    | 4/2    | 4/0    |
| Marsianka             | 4/0    | 4/0    | 4/0    |
| Total No. of samples  | 100/25 | 100/57 | 100/7  |

infection was found in 4 cultivars, and 2 cultivars were less infected. The CMV infection was severely noticed in 7 cultivars, moderate infection was determined in 7 cultivars, whereas mild infection was noticed in 6 cultivars. In case of TRV, a moderate infection was determined in one cultivar and mild infection was found in 5 cultivars.

In the 12 unknown gladiolus plants from Nedvědice, the BYMV infection was identified in 5 plants and the CMV infection was found in 7 plants. Both BYMV and CMV infections were found in 4 plants. The TRV infection was determined in none of the plants.

In the 24 unknown plants of gladiolus leaves and flowers collected in a local shop, the BYMV infection was identified in leaves of all 24 unknown plants, however, as far as the flowers were concerned, the infection was identified in 19 flowers. Infections by CMV and TRV were not found in neither the leaves nor the flowers of all 24 plants.

In the biological test on varieties of the *Pisum sativum* plant, all inoculated isolates produced mosaic and few pea plants showed vein clearing symptoms.

In iris (Tab. IV), the BYMV infection was identified only in 2 cultivars. Regarding CMV, the infection was highly present only in one cultivar and mild infection was noticed in 10 cultivars. The presence of the BYMV and CMV was not found in flowers of these cultivars. The TRV infection was determined in 5 cultivars. The same cultivars were tested for IYSV, but none of those were infected.

When testing tulip leaves collected in the arboretum (Tab. V), we found that the CMV infection was highly present in one cultivar, and moderate infection was found in 6 cultivars. However, the TRV infection was identified only in 2 cultivars. In case of BYMV, the infection was not found in any of those cultivars.

## IV: Identification of virus on iris leaves and flowers collected from Arboretum at Mendel University (ELISA)

| Name of the Cultivars   | BYMV   | CMV    |         | TRV    | IYSV   |
|-------------------------|--------|--------|---------|--------|--------|
|                         | Leaves | Leaves | Flowers | Leaves | Leaves |
| Queen of May            | 4/0    | 4/0    | NT      | 4/0    | 2/0    |
| Port Wine               | 4/0    | 4/0    | NT      | 4/0    | 2/0    |
| California Gold         | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Bazaar                  | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Ambroisie               | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Sunny Lilac             | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Curtain Call            | 4/0    | 4/1    | NT      | 4/0    | 2/0    |
| Chappeau                | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Heather Blush           | 4/0    | 4/0    | NT      | 4/0    | 2/0    |
| Metropolitan            | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Jessie Viette           | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Chartreuse Ruffles      | 4/0    | 4/1    | 2/0     | 4/0    | 2/0    |
| High Bit                | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| MRS NeuBronner          | 4/0    | 4/1    | 2/0     | 4/0    | 2/0    |
| Chinquapin              | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Limberick               | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Tiffany                 | 6/0    | 6/0    | 2/0     | 6/0    | 2/0    |
| Tollgate                | 4/1    | 4/1    | NT      | 4/0    | 2/0    |
| Royal Coach             | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Prosper Laugier         | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Dialogue                | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Rebecca Perret          | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Gayhood                 | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Tanya                   | 4/0    | 4/1    | NT      | 4/0    | 2/0    |
| Significant Other       | 5/0    | 5/2    | 2/0     | 5/0    | 2/0    |
| Raspberry Ripples       | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Frieda Mohr             | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Majestic                | 4/0    | 4/0    | NT      | 4/1    | 2/0    |
| Winter Olympus          | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Whole Cloth             | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| El Mohr                 | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Dauntles                | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| FS-00-IC-2              | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Sanguriter              | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Moonlight Sketch        | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Body language           | 4/0    | 4/0    | NT      | 4/0    | 2/0    |
| Zany                    | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Ave maria               | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Harmony                 | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| La beaurte              | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Buffy                   | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Clear water River       | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Perfection              | 6/1    | 6/0    | 2/0     | 6/0    | 2/0    |
| Matimata                | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Basic Black             | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Ice sculpture           | 4/0    | 4/0    | NT      | 4/1    | 2/0    |
| Iris - Dept of Botanica | 2/0    | 2/0    | NT      | 2/0    | NT     |

| Name of the Cultivars       | BYMV   | CMV    |         | TRV    | IYSV   |
|-----------------------------|--------|--------|---------|--------|--------|
|                             | Leaves | Leaves | Flowers | Leaves | Leaves |
| Chezp/1                     | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| FS-00-CE-4                  | 6/0    | 6/1    | 2/0     | 6/0    | 2/0    |
| 96-HT Bheli                 | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| 99-CSMP17                   | 4/0    | 4/0    | NT      | 4/1    | 2/0    |
| Exos.GNG/21                 | 4/0    | 4/0    | NT      | 4/0    | 2/0    |
| S.Poloplick -1              | 6/0    | 6/1    | 2/0     | 6/0    | 2/0    |
| Passion for Pink            | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Border Schnee               | 4/0    | 4/0    | NT      | 4/0    | 2/0    |
| Sunlight Sketch             | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Ledova Saha                 | 3/0    | 3/1    | NT      | 3/0    | 2/0    |
| Unknown 1                   | 2/0    | 2/1    | NT      | 2/0    | NT     |
| Unknown 2                   | 2/0    | 2/0    | NT      | 2/0    | NT     |
| Unknown 3                   | 4/0    | 4/1    | NT      | 4/1    | NT     |
| Unknown 4                   | 2/0    | 2/0    | NT      | 2/0    | NT     |
| Iris variegata alba         | 2/0    | 2/0    | NT      | 2/0    | 2/0    |
| Iris pumila                 | 2/0    | 2/0    | NT      | 2/0    | NT     |
| Iris pallidis spp.cengialte | 4/0    | 4/0    | NT      | 4/1    | 2/0    |
| Total No. of samples        | 180/2  | 180/12 | 14/0    | 180/5  | 116/0  |

NT – Not Tested

V: Identification of virus on tulips leaves collected from Arboretum at Mendel University (ELISA)

| Name of the Cultivars   | BYMV   | CMV    | TRV    |
|-------------------------|--------|--------|--------|
|                         | Leaves | Leaves | Leaves |
| Red wing                | 2/0    | 2/0    | 2/0    |
| Crystal beauty          | 2/0    | 2/0    | 2/0    |
| Pink Impression         | 2/0    | 2/0    | 2/0    |
| Page Polka              | 2/0    | 2/2    | 2/0    |
| Prince                  | 2/0    | 2/0    | 2/0    |
| Jan Recus               | 2/0    | 2/0    | 2/0    |
| Fancy Piraless          | 2/0    | 2/1    | 2/0    |
| Unknown tulip cultivars | 6/0    | 6/1    | 6/1    |
| Lucky Stripe            | 2/0    | 2/1    | 2/0    |
| Banja Lucka             | 2/0    | 2/1    | 2/0    |
| Blushing Beauty         | 2/0    | 2/1    | 2/0    |
| Tulip chrysantha        | 2/0    | 2/1    | 2/1    |
| Total No. of samples    | 28/0   | 28/8   | 28/2   |

#### The transmission of BYMV from gladiolus corms and cormlets

We determined that all the leaf samples originating from last year's infected gladiolus corms and cormlets were infected by BYMV (Tab. VI). We proved that the BYMV infection was easily transmitted from infected corms and cormlets.

VI: Transmission of BYMV from corms and cormlets. (ELISA)

| Name of the Cultivars | Corms<br>(Jestřábí,<br>Market and<br>Nedvedce) | Cormlets<br>(Jestřábí and<br>Nedvedce) |
|-----------------------|------------------------------------------------|----------------------------------------|
|                       | BYMV (Leaves)                                  | BYMV (Leaves)                          |
| J 16                  | 1/1                                            | 2/2                                    |
| J 33                  | 1/1                                            | NP                                     |
| J 34                  | 1/1                                            | NP                                     |
| J 49                  | 1/1                                            | 4/4                                    |
| J 95                  | 1/1                                            | 5/5                                    |
| M 63                  | 1/1                                            | NP                                     |
| M 71                  | 1/1                                            | NP                                     |
| M 76                  | 1/1                                            | NP                                     |
| M 78                  | 1/1                                            | NP                                     |
| M 103                 | 1/1                                            | NP                                     |
| N 4                   | 1/1                                            | NP                                     |
| N 5                   | 1/1                                            | NP                                     |
| N 7                   | 1/1                                            | 2/2                                    |
| N 9                   | 1/1                                            | NP                                     |
| J 3 – Noe             | NP                                             | 3/3                                    |
| J 4 – Noe             | NP                                             | 1/1                                    |
| J 14 – Soumrak        | NP                                             | 3/3                                    |
| J 50 Gladiris         | NP                                             | 4/4                                    |
| J 53 – Dona Maria     | NP                                             | 2/2                                    |
| Total No. of Samples  | 14/14                                          | 26/26                                  |

NP – Not Planted

## DISCUSSION

The ELISA test was applied to detect the presence of certain viral infections in both aerial and underground parts of gladiolus, iris, and tulip, respectively. 262 gladiolus plants were tested: 63.7% were infected by BYMV, 29.4% by CMV, and 2.7% by TRV. Out of 180 plants of iris, 1.1% was infected by BYMV, 6.7% by CMV, 2.8% by TRV. No plant of iris was infected by IYSV. Out of 28 plants of tulips, 28.6% was infected by CMV and 7.1% by TRV.

Different cultivars of gladiolus were examined. The BYMV infection was observed in the leaves of the majority of gladiolus cultivars collected at markets, Jestřabí (near Velká Bíteš), Jestřabí field, Nedvědice, and local shops. Similarly, when Selvarajan and Gupta (1996) studied the effects of the BYMV infection on gladiolus by the means of the DAS-ELISA test, the authors identified the most severe viral infection in leaves. The BYMV infection was found in the gladiolus flower collected in markets as well local shops. Similarly, Stein *et al.* (1988) also found the BYMV infection in the flower of gladiolus by using the DAS-ELISA methods. The ELISA test was also applied to reveal the presence of the BYMV in corms and cormlets that were collected in Jestřabí and market, however, this virus was not detected in these organs. Many authors came across the same problem when detecting viral pathogens in corms and cormlets. Bellardi and Vicchi (1995) encountered a problem with the detection of the virus in corms of gladiolus. Nagel *et al.* (1983) also reported that plants tested for the BYMV infection by DAS-ELISA methods showed negative results on cormlets and corms. Stein *et al.* (1994) and Rosner *et al.* (1994) had difficulties to detect the BYMV infection in gladiolus corms and cormlets by the ELISA test, too. Kim *et al.* (1992) observed that ELISA is sensitive for the detection of viral infection on gladiolus plants and its detection reliability of the BYMV infection in leaves and flowers is by 20–80% higher than in cormlets.

ELISA showed the presence of the CMV infection in gladiolus leaves collected in Jestřabí and the Jestřabí field. ELISA is sensitive for the detection of the CMV infection in gladiolus leaves, which has also been reported by Chen *et al.*, (1999), Park *et al.* (1998) and Raj *et al.* (2002). ELISA test was also applied to reveal the presence of the CMV infection in cormlets collected in Jestřabí but it failed to detect it. According to Francki *et al.* (1979) CMV can be easily detected by the ELISA test in gladiolus and its reliability

is higher in leaf samples than cormlets. Stein *et al.* (1988) and Raj *et al.* (1997) also observed that ELISA fails to detect viral infections in cormlets. Additionally, Kim *et al.* (1992) found that the reliability of the CMV detection in leaves and flowers is by 20–80% higher than in cormlets.

The TRV infection was identified in gladiolus leaves collected in the Jestřabí field. Similarly, the TRV occurrence in the gladiolus leaves was also reported from Holland, Israel, Egypt, and Poland (Stein, 1995). The TRV causes only mild symptoms in gladioli, and in most plants there are no visual symptoms at all. The effects of the TRV infection and symptom expression in the presence of other viruses have been noticed in gladiolus leaves (Brunt, 1986; Navalinskiene and Samuitiene, 2000).

The CMV infection was mostly determined in iris leaves rather than flowers collected in arboretum. These findings support an earlier report of Yuang – Mei Fang *et al.* (1998) and Takamatsu *et al.* (1994), who also observed the CMV infection in iris leaves rather than flowers. IYSV was initially identified in onion (*Allium cepa*) in Israel (Gera *et al.*, 1998) and in iris in the Netherlands (Cortes *et al.*, 1998), and it was recently detected in onion in Brazil (Pozzer *et al.*, 1999), but we failed to determine this virus in iris leaves.

Tulips were infected by CMV rather than TRV, and none of the cultivars were infected by BYMV. Similarly, the occurrence and distribution of CMV was determined by DAS-ELISA by many authors and the disease occurred more in leaves of tulip plants (Ploeg *et al.*, 1989; Korbin and Kaminska, 1998; Polak, 1999; Mokra *et al.*, 2002).

In this study, we noticed that BYMV was the most prevalent virus. It infects gladiolus more than CMV and TRV. Therefore, it gives an idea about the status of viral infections in the south Moravian region of the Czech Republic. In addition, the present study also concluded that ELISA is a quick, reliable and sensitive technique to diagnose the BYMV, CMV and TRV infections in gladiolus, iris, and tulip leaves, but it fails to detect these viruses in corms and cormlets. Because we found that BYMV was highly transmitted by infected corms and cormlets, a suitable and reliable method is required to determine viruses in these organs. Suitable detection methods such as the molecular diagnosis by RT-PCR will be needed in order to identify viral infections in corms and cormlets. Furthermore, we are also planning to use RT-PCR to diagnose viruses in corms and cormlets in our successive research work.

## SOUHRN

### Virové patogeny mečíků, kosatců a tulipánů v České republice

V rámci průzkumu byl metodou DAS-ELISA (Clark, Adams; 1977) hodnocen zdravotní stav kosatců, mečíků a tulipánů pěstovaných v jihomoravském regionu. Vzorky byly odebírány z různých lokalit a také z obchodní sítě. Byly testovány různé části rostlin – listy, květy a hlízy. Celkem bylo testováno 262 rostlin mečíků, přičemž 63,7% bylo infikováno *Bean yellow mosaic virus* (BYMV), 29,4% *Cucumber mosaic virus* (CMV) a 2,7% *Tobacco rattle virus* (TRV). Ze 180 rostlin kosatců bylo 1,1% infikováno BYMV, 6,7% CMV, 2,8% TRV a *Iris yellow spot virus* (IYSV) 0%. Z 28 rostlin tulipánů bylo 28,6% infikováno CMV

a 7,1% TRV. Z uvedeného přehledu je zřejmé, že limitujícím faktorem především pro pěstování mečíků je velká promořenost materiálů BYMV, tento virus je přítomen i v hlízách a rostlinách prodávaných v obchodní síti. V našich testech se také prokázal vysoký přenos toho viru hlízami a hlízkami mečíků.

DAS-ELISA byla velmi vhodnou metodou pro detekci BYMV, CMV a TRV v listech mečíků, kosatců a tulipánů. V některých případech tato metoda selhávala v detekci BYMV v květech mečíků a CMV v květech kosatců, zcela nevhodnou pak byla pro determinaci virů v hlízách mečíků. Z hlediska tvorby zdravého rozmnožovacího materiálu je nutné propracovat senzitivnější metody detekce, jednou z nich může být RT-PCR, což bude předmětem dalšího výzkumu.

virus žluté mozaiky fazolu, virus mozaiky okurky, mečíky, kosatce, tulipány

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