

DETECTION OF VIRUS PATHOGENS OF GLADIOLUS IN THE CZECH REPUBLIC BY ELISA

DETEKCE VIROVÝCH PATOGENŮ MEČÍKŮ V ČESKÉ REPUBLICE METODOU ELISA

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ABSTRACT

The gladiolus plants of different origin were tested for presence of *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tobacco rattle virus* (TRV) by enzyme-linked immunosorbent assay (ELISA). *Bean yellow mosaic virus* was the most prevalent one, this pathogen was found in leaves of 138 plants from 226 tested. *Cucumber mosaic virus* was also widespread, it was determined in leaves of 70 plants from 226 tested. *Tobacco rattle virus* was determined only in leaf samples of 7 plants from 199 tested. Enzyme-linked immunosorbent assay was sufficient for detection of BYMV in leaves but sometimes failed to detect it in flowers. This virus was detected only in 11 flowers from 19 tested, which were taken from infected plants. ELISA was totally insufficient for detection BYMV and CMV in corms and cormlets. BYMV was detected only in one corm from 69 tested and CMV in none corm. These corms were taken from plants in which viruses were determined in leaves.

Key words: gladiolus, *Bean yellow mosaic virus*, *Cucumber mosaic virus*, *Tobacco rattle virus*

INTRODUCTION

Gladiolus is an important component of the world floriculture industry and ranks amongst the top six flowers of export market (ANONYMOUS, 1997). In the recent years, in spite of its high demand, decline in production has been observed. The major factor contributing to this is a wide array of diseases of the biological origin. Viral diseases attain important status because they not only cause direct damage to the host but also predispose it to secondary invaders (BEUTE, 1970). Many viruses have been reported to infect gladiolus cultivars out of which *Bean yellow mosaic virus* (BYMV) and *Cucumber mosaic virus* (CMV) are the most prevalent ones (BRIDGMON and WALKER, 1952; FRY, 1953; ZAIDI et al., 1993). The majority of viral diseases leads to overall stunting, colour break, flower distortion, reduced flower and cormel production (BRIDGMON and WALKER, 1952; MAGIE and POE, 1972; RAIZADA et al., 1989) but sometimes viruses may not cause any symptoms (NAGEL et al., 1983). Since gladiolus is being vegetatively propagated, viral infection is carried from generation to generation by corms. In order to improve the crop productivity, quality of germplasm and minimizing the infection by several viruses in different cultivars, its proper diagnosis and control is essential. Thus detection of the masked infection particularly in the propagating material will better prospects for gladiolus crop. In the present study, the emphasis has been given to diagnosis the viral infection in gladiolus plants by visual symptom and enzyme-linked immunosorbent assay (ELISA) method.

MATERIAL AND METHODS

The corm and cormlets of gladiolus, which were collected from markets and locality Jestřabí (near Velká Bíteš, 49°16'18"N, 16°11'28"E) were grown under greenhouse conditions at Mendel University of Agriculture and Forestry, Brno. The leaves and flowers samples were collected during growth stage whereas corms and cormlets were collected during dormant stage. The gladiolus leaves were also collected from Nedvědice (49° 12'51" N, 16°36'56"E) as well from Jestřabí field (near Velká Bíteš) and they were also tested. Similarly, the gladiolus leaves and flowers, which were collected from the local shops in Brno, were tested. All collected samples were tested by ELISA methods according to CLARK and ADAMS (1977). The diagnostic kits for BYMV, CMV and *Tobacco rattle virus* (TRV) detection were used. The ELISA test was done according to the manufacture kits instructions (DSMZ, Germany). Experimental work was carried out in the diagnostic laboratory of the Department of crop science, breeding and plant medicine at Mendel University of Agriculture and Forestry, Brno.

RESULTS AND DISCUSSION

Among the different cultivars of gladiolus, which were collected from markets (Table 1), the BYMV infection was noticed in leaves of all the cultivars. As for detection of BYMV in flowers, the infection was determined in some plants of Blue Frost, Nova Lux, Priscilla, Victor Borge, Madonna Light blue and Pr.marg.Rose varieties, but in Topaze Orange and

Sancerre White/Weiss infection was not found. The corms were also tested for virus infection by means of ELISA, the BYMV infection was noticed only in one Victor Borge cultivar corm. Similarly, the leaves and flowers were tested for CMV infection, in none of the cultivars the infection was noticed. The gladiolus leaves were also tested for TRV infection, no one cultivars infection was found.

The leaves and cormlets which were collected from Jestřabí (Table 2) were tested for BYMV infection and in all cultivars the infection was determined in leaves, but the BYMV infection were not determined in any cormlets of all the cultivars. Similarly, the leaves of these cultivars were also tested for CMV infection, out of which Jarní Louka, Májový Květ, Poušováček, Rachele, Bambino, JoAnn infection was determined whereas in Bombay, Noe, El type, Radyne and Jungle Flower infection by CMV was not found. CMV infection was not noticed in cormlets of these varieties. The leaves and cormlets were also tested for TRV infection, the infection was not determined in all plants.

In the leaves of gladiolus cultivars which were collected from Jestřabí field (Table 3, varieties are given by codes – the list is available in author), the BYMV infection was found in J-51, J-120, J-9, J-135, J-144, J-52, J-27, J-78, J-93 and J-29 whereas in J-94, J-61, J-100, J-88, J-16, J-59, J-127, J-98, J-47, J-79, J-126, J-36, J-17, J-38 and J-13, the infection was not found. For CMV, the infection was noticed in J-51, J-120, J-9, J-135, J-144, J-100, J-52, J-88, J-16, J-27, J-78, J-127, J-93, J-98, J-47, J-126, J-36, J-17, J-38 and J-29 but the remaining cultivars such as J-94, J-61, J-59, J-79 and J-13 the CMV were not infected. The samples were also tested for TRV infection, out of which J-9, J-135, J-61, J-27, J-47 and J-126 were positive for this virus.

From 12 plants of gladiolus which were collected in Nedvědice, the BYMV infection was determined in 5 plants and CMV infection was noticed in 7 plants whereas 4 plants both BYMV and CMV infection were determined. None of the plant was infected by TRV. Similarly, the BYMV infection was found in leaves of all 24 plants of unknown origin, which were collected from local shops, but BYMV was determined only in 20 flowers of these plants. CMV and TRV infection was not noticed in leaves and also flowers of all plants from unknown cultivars.

The different cultivars of gladiolus were examined by the DAS-ELISA tests. BYMV infections were observed in majority of the gladiolus leaves, which were collected from markets, Jestřabí (near Velká Bíteš), Jestřabí field, Nedvědice and local shops. Similarly, SELVARAJAN and GUPTA (1996) studied the characterization of BYMV causing infection on gladiolus by means DAS-ELISA test and the author noticed that the virus infection were severe in leaves. Also BELLARDI and VICCHI (1995) confirmed, that DAS-ELISA is able to detect the high presence of BYMV in leaves and these authors concluded that this technique is the best to apply to the shoot of gladiolus. The BYMV infection was also determined in flowers of gladiolus, which were collected from markets as well as from local shops. Similarly, STEIN *et al.* (1988) also noticed BYMV infection occurred in flowers of gladiolus by DAS-ELISA methods. The ELISA test was applied to reveal the presence of BYMV infection in cormlets and corms, which were collected from Jestřabí as well in markets, but the BYMV infection was not determined in cormlets. Similarly, NAGEL *et al.* (1983),

BELLARDI and VICCHI (1995), SELVARAJAN and GUPTA (1996) and PARK *et al.* (1998) also reported that plants tested for BYMV infection by DAS-ELISA methods showed negative results on cormlets and corms. We also determined CMV infection in leaves of gladiolus, which were collected from Jestřabí corms and Jestřabí field. According to some authors, this virus accumulates in lower older leaves of gladiolus (CHEN *et al.*, 1999; PARK *et al.*, 1998; RAJ *et al.* 2002). The authors identified CMV by means of host range and ELISA and this technique proved to be sensitive for detection of CMV in leaves of gladiolus. We also determined TRV infection in the leaves of gladiolus, which were collected from Jestřabí field. Similarly, TRV occurrence of 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.

CONCLUSION

DAS-ELISA is the one of the suitable technique to detect the various viruses (BYMV, CMV and TRV) in gladiolus leaves. Until now ELISA has been extensively used for diagnosis of viruses infecting gladiolus because it is quick methods but this technique sometimes fail to detect these viruses in dormant corms and cormlets. In order to obtain a sensitive detection of BYMV in leaves, flowers, corms and cormlets a suitable detection methods of molecular diagnosis (RT-PCR) is required to identify the virus in both aerial and underground plant parts where the ELISA test fail to determine the BYMV in gladiolus.

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Table: 1 Identification of virus in leaves, flowers and corms of gladiolus collected from market

Cultivar name	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	50 / 50	19 / 11	20 / 1	50 / 0	19 / 0	50 / 0	-

Table: 2 Identification of virus on leaves and cormlets of gladiolus collected from Jestřabí

Cultivar name	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
Radyne	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	76 / 63	49 / 0	76 / 13	49 / 0	49 / 0	49 / 0

Table: 3 Identification of virus on leaves of gladiolus collected from Jestřabí field (ELISA)

Cultivars code	BYMV	CMV	TRV
	Leaves	Leaves	Leaves
J - 51	4 / 2	4 / 4	4 / 0
J - 120	4 / 4	4 / 4	4 / 0
J - 9	4 / 4	4 / 4	4 / 1
J - 135	4 / 3	4 / 4	4 / 1
J - 144	4 / 2	4 / 2	4 / 0
J - 94	4 / 0	4 / 0	4 / 0
J - 61	4 / 0	4 / 0	4 / 1
J - 100	4 / 0	4 / 3	4 / 0
J - 52	4 / 4	4 / 3	4 / 0
J - 88	4 / 0	4 / 1	4 / 0
J - 16	4 / 0	4 / 3	4 / 0
J - 59	4 / 0	4 / 0	4 / 0
J - 27	4 / 2	4 / 4	4 / 1
J - 78	4 / 1	4 / 3	4 / 0
J - 127	4 / 0	4 / 1	4 / 0
J - 93	4 / 1	4 / 1	4 / 0
J - 98	4 / 0	4 / 4	4 / 0
J - 47	4 / 0	4 / 4	4 / 2
J - 79	4 / 0	4 / 0	4 / 0
J - 126	4 / 0	4 / 3	4 / 1
J - 36	4 / 0	4 / 3	4 / 0
J - 17	4 / 0	4 / 1	4 / 0

J - 38	4 / 0	4 / 3	4 / 0
J - 29	4 / 2	4 / 2	4 / 0
J - 13	4 / 0	4 / 0	4 / 0
Total	100 / 25	100 / 57	100 / 7

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