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Seed Transmission of Como and Potyviruses in Fababean and Vetch Cultivars Introduced into Slovakia

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Two each of como (broad bean stain, BBSV; broad bean true mosaic, BBTMV) and potyviruses (bean yellow mosaic, BYMV; pea seed-bome mosaic, PSbMV) were seed transmitted by test fababean and vetch cultivars. Number of cultivars supporting seed transmission of BBSV (100%) were highest compared to those of BBTMV, BYMV and PSbMV (86%). Seed transmission levels of BBSV (3–20%) and BBTMV (0–28%) were higher than those of BYMV (0–17%) and PSbMV (0–11%) in these cultivars. Common bean cultivars were infected by BBSV (36%) and BYMV (9%) but not by BBTMV or PSbMV. In reactions of test common bean cultivars, the BYMV-fababean isolate was more similar to pea mosaic (PMV) than to other BYMV strains.

Keywords: Fababean, vetch, seed transmission, BBSV, BBTMV, BYMV, PSbMV.

Considerable number of viruses are known to infect naturally the fababean (*Faba vulgaris* Moench, syn = broad bean *Vicia faba* L.) and some *Vicia* species worldwide (Boswell and Gibbs, 1983; Bos et al., 1988; Brunt et al., 1996). Of which at-least four viruses including BBSV (Gibbs et al., 1968; Gibbs and Smith, 1970; Cockbain et al., 1976; Fischer and Lockhart, 1976; Vorra-Ural and Cockbain, 1977; Musil et al., 1978), BBTMV (syn. Echtes Ackerbohnemosaik-Virus, EAMV: Gibbs and Paul, 1970; Bruening, 1978), BYMV (Bos, 1970; Kaiser, 1972; Evans, 1973; Murant et al., 1973; Nitzany, 1975), pea mosaic potyvirus (PMV, a strain of BYMV: Corbett, 1958; Bos, 1970; Bowyer, 1996) and PSbMV (Hampton and Mink, 1975; Hampton et al., 1981; Fry, 1996) are seed transmitted by fababean and some *Vicia* species. Number of improved fababean and vetch varieties developed in other countries have been introduced into Slovakia for raising as vegetable or pulse (food legumes) crops (Vestník, 1999). In such cases, seed-borne viruses can be introduced inadvertently through seed imports when seed certification standard, phytosanitary and quarantine measures are not strictly adhered to (Stace-Smith and Hamilton, 1988; Hampton et al., 1993). Present investigations were therefore, made to check the health status of fababean and vetch varieties registered in Slovakia (Vestník, 1999) especially for their infections with seed-borne viruses using seedling tests, infectivity and serological assays.

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Materials and Methods

Seed transmission tests

Seed lots of 8 fababean, 6-vetch (*Vicia sativa* L., *V. villosa* Roth.) cultivars were sown in a suitable earthen pots containing steam sterilized soil, sand and compost mixture (2 : 1 : 1 proportion) in an insect free glasshouse at a temperature range of 22–32 °C and seedlings were observed for seed transmitted virus symptoms for a period of 4–6 weeks after seed germination. Symptomatic as well as symptom-free seedlings were labeled and their infections with seed transmitted viruses were confirmed using serological assays. The test cultivars were obtained from the following sources:

1. Breeding Station, Horna Streda, Slovakia: Fababean cultivars = Aštar, Brok, Inovec, Liber, Omar and Stredan. *V. sativa* cvs. Fatima, Medea, Slovena, Telma and Toplesa. *V. villosa* cv. Arida.
2. ZelSeed Private Limited, Slovakia = ZelSeed fababean cultivar.
3. Mrs. Maria Mučková, Ivanka pri Dunaji, Slovakia = Ivanka fababean cultivar.

Serological assays

DAS-ELISA (double antibody sandwich form of enzyme-linked immunosorbent assay, Clark and Adams, 1977) and commercial kits comprising of IgG antisera of BYMV-pea isolate and PSbMV (Loewe, Phytodiagnostics, Germany) were used for the detection of potyviruses. Manufacturer's directives were followed for buffer preparation and reagent dilutions. Symptomatic and symptom-free tissue samples ground in extraction buffer (PBS, Tween-PVP) at 1 : 10 proportion were applied to flat-bottomed microplates (Sarstedt) and absorbance readings were taken at 405 nm with an ELISA microplate reader (Multiskan-Ex, Labsystems) 30–60 minutes after substrate deposition. Negative (healthy fababean leaf extracts) and positive (virus) controls were also included in the assay. Readings thrice those of healthy control threshold were considered as positive.

DAC-ELISA (direct antigen coating form of ELISA, Hobbs et al., 1987) and unfractionated polyclonal antisera of BBSV and BBTMV (gifted by Sven Erik Albrechtsen, DGISP, Copenhagen, Denmark) were used for the detection of comoviruses. DGISP's (Danish Government Institute for Seed Pathology) directives were followed for buffer preparation and reagent dilutions. Symptomatic and symptom-free leaf tissue samples ground in carbonate (coating) buffer (0.05 M sodium carbonate, pH 9.6) at 1 : 100 proportion were applied to flat-bottomed microplates and absorbance readings were taken at 405 nm 30–60 min after substrate deposition. Unfractionated antisera cross-absorbed with healthy fababean leaf extracts (in 1 : 20 proportion) were diluted to 1 : 500 in antibody buffer. Enzyme conjugate (alkaline phosphatase-linked anti-rabbit IgG in goat) were diluted 1 : 1000 in conjugate buffer. The positive (virus) and negative (healthy fababean leaf extracts) controls were also included. Readings thrice those of healthy control threshold were considered as positive.

Sap transmission

Four virus isolates (BBSV, BBTMV, BYMV and PSbMV) from fababean were sap transmitted to 11 glasshouse-grown common bean (*Phaseolus vulgaris* L.) cultivars using conventional leaf rub method. Carborundum (500 mesh) was used as an abrasive. Inocula were prepared by triturating virus-infected tissue in a cold 0.05 M phosphate buffer, pH 7.4 at 1 : 10 proportion (w/v). Inoculated plants were observed for 4–6 weeks for symptom development and were subsequently assessed for virus infectivity by DAS-ELISA (for BYMV and PSbMV) and DAC-ELISA (for BBSV and BBTMV).

Results and Discussion

Seed transmission

AII the fababean test cultivars supported the seed transmission of three viruses (BBSV, BYMV and PSbMV) whereas only 75% cultivars supported seed transmission of BBTMV. Infected seeds did not produce symptomatic seedlings in all but two cultivars (Ivanka and ZeISeed). As such seedling tests were of less value in detecting seed transmission of test viruses in a number of fababean cultivars. Detection in such cases had to be resorted to serological assays. Fababean cultivars also differed in supporting level of seed transmission of test viruses. Comoviruses (BBSV and BBTMV) recorded comparatively higher level of seed transmission in fababean cultivars than those of potyviruses (BYMV and PSbMV) *(Table 1a, b, c).*

In case of vetch *(V. sativa* and *V. villosa)* cultivars, seed transmission of comoviruses (BBSV and BBTMV) was supported by all the cultivars whereas only 67% cultivars supported the seed transmission of potyviruses (BYMV and PSbMV). Infected seeds did not produce symptomatic seedlings in all but one cultivar (Medea). Lower level of seed transmission in vetch cultivars was recorded by PSbMV (0–9%) than those of BBSV (3–13%), BYMV (0–14%) and BBTMV (3–15%) *(Table 1a, b, c).*

Commercial seed lots derived from ZelSeed fababean cultivar were tested earlier for the seed transmission of 3 viruses (BBSV, BBTMV and BYMV) but not for PSbMV (Mali, 2000). In the present work, such studies were extended to 8 fababean (including ZelSeed) and 6 vetch cultivars for testing seed transmission of 4 viruses (BBSV, BBTMV, BYMV and PSbMV). Earlier ZelSeed was not found to support seed transmission of BBTMV (Mali, 2000) unlike present studies. Probably the seed lots of ZelSeed tested earlier did not contain BBTMV.

Fababean has been reported earlier to support seed transmissions in a low percentage of PSbMV (Fry, 1996) and BYMV (Nitzany and Cohen, 1962; Kaiser et al., 1971; Kaiser, 1972; Evans, 1973; Murant et al., 1973; Nitzany, 1975) and relatively in high percentage of BBSV and BBTMV (Quantz, 1953; Lloyd et al., 1965; Gibbs et al., 1968; Cockbain et al., 1976; Vorra-Ural and Cockbain, 1977). In the present work, test fababean

Table 1a

Seed transmission of comoviruses in fababean and vetch cultivars as determined by DAC-ELISA

Test cultivars	Symptoms[a]	Seed transmission levels of comoviruses		Total number
		BBSV	BBTMV	
F. vulgaris				
Aštar	–	8.57 (6/70)[b]	11.43 (8/70)	2[c]
Brok	–	5.26 (4/76)	0 (0/76)	1
Inovec	–	10.71 (6/56)	3.57 (2/56)	2
Ivanka	+	6.06 (4/66)	4.55 (3/66)	2
Liber	–	11.54 (6/52)	9.61 (5/52)	2
Omar	–	20.0 (10/50)	28.0 (14/50)	2
Stredan	–	5.0 (6/120)	0 (0/120)	1
ZelSeed	+	10.0 (6/60)	8.33 (5/60)	2
Range (%)		5.0–20.0	0–28.0	
V. sativa				
Fatima	–	7.29 (7/96)	3.13 (3/96)	2
Medea	+	3.33 (2/60)	6.67 (4/60)	2
Slovena	–	5.0 (4/80)	3.75 (3/80)	2
Telma	–	6.0 (3/50)	12.0 (6/50)	2
Toplesa	–	7.01 (4/57)	5.26 (3/57)	2
Range (%)		3.0–7.0	3.0–13.0	
V. villosa				
Arida	–	12.5 (5/40)	15.0 (6/40)	2

[a] symptoms developed, usually leaflet mottle, distortion, necrotic spots or necrosis (+), symptom free (–)
[b] figures indicating percentage seed transmission followed in parenthesis by number of ELISA positive upon total seedlings indexed
[c] number of viruses seed transmitted in a given cultivar

cultivars supported seed transmission of both como- (BBSV and BBTMV) as well as potyviruses (BYMV and PSbMV) in a high percentage compared to those reported earlier by others *(Table 3).* Such a discrepancy could be attributed to the detection of masked seed transmissions of the test viruses in fababean using ELISA in the present work compared to those detected earlier by others in symptomatic seedlings produced by infected seeds. As such, seedling tests (Phatak, 1974) were of less value for detecting such masked seed transmissions in the present work and serological assays (ELISA) had to be resorted to for this purpose. Earlier, Hamilton and Nichols (1978) also recommended serological assays (ELISA) over seedling tests or infectivity assays for the detection of PSbMV especially in non-Perfection type pea cultivars.

Low percentage of seed transmission of PSbMV has also been reported earlier in some *Vicia* species *(V. articulata, V. narbonensis* and *V. pannonica)* blit not in *V. sativa* or *V. villosa* (Hampton and Mink, 1975). None of the *Vicia* species has been tested earlier for the seed transmission of BYMV although this virus is known to infect *V. sativa* naturally (McCord and Gudauskas, 1968; Boswell and Gibbs, 1983). Similarly, seed transmission of

Table 1b

Seed transmission of potyviruses in fababean and vetch cultivars as determined by DAS-ELISA

Test cultivars	Symptoms[a]	Seed transmission levels of comoviruses		Total number
		BBSV	PSbMV	
F. vulgaris				
Aštar	–	9.33 (7/75)[b]	8.0 (6/75)	2[c]
Brok	–	11.76 (10/85)	10.59 (9/85)	2
Inovec	–	16.67 (7/42)	4.76 (2/42)	2
Ivanka	+	7.58 (5/66)	9.09 (6/66)	2
Liber	–	3.85 (2/52)	7.69 (4/52)	2
Omar	–	4.0 (2/50)	2.0 (1/50)	2
Stredan	–	11.11 (10/90)	2.22 (2/90)	2
ZelSeed	+	15.0 (9/60)	5.0 (3/60)	2
Range (%)		3.85–16.67	2.0–10.59	
V. sativa				
Fatima	–	9.38 (9/96)	6.25 (6/96)	2
Medea	+	0 (0/60)	0 (0/60)	0
Slovena	–	0 (0/80)	0 (0/80)	0
Telma	–	8.0 (4/50)	4.0 (2/50)	2
Toplesa	–	14.0 (8/57)	8.77 (5/57)	2
Range (%)		0–14.0	0–8.77	
V. villosa				
Arida	–	10.0 (4/40)	5.0 (2/40)	2

[a] symptoms developed, usually leaflet mottle, distortion, necrotic spots or necrosis (+), symptom free (–)
[b] figures indicating percentage seed transmission followed in parenthesis by number of ELISA positive upon total seedlings indexed
[c] number of viruses seed transmitted in a given cultivar

BBTMV has also not been tested earlier in *Vicia* species including *V. sativa* or *V. villosa (Table 3).* Nevertheless, BBSV seed transmission has been reported in *V. sativa* (Tapio, 1970; Musil et al., 1978) but not in *V. villosa* or other *Vicia* species. As such, the present work may constitute a first report on the seed transmission of PSbMV, BYMV and BBTMV in *V. sativa* and *V. villosa* and of BBSV in *V. villosa.* Earlier, masked seed transmissions of these viruses might have led other workers to overlook such seed transmissions in vetch. For instance, some workers (Musil and Kowalska, 1993; Musil and Gallo, 1994) could detect earlier the masked seed transmission of pea isolate (Kow 60: Kowalska and Beczner, 1980) of BBSV in pea cultivars as infected seeds did not produce symptomatic seedlings. They however, determined seed transmission of fababean (F1: Valenta et al., 1969) and vetch (VsM: Musil et al., 1978) isolates of BBSV in pea cultivars by indexing symptomatic seedlings produced by infected seeds. These workers also recommended ELISA for the detection of masked seed transmissions of pea isolate of BBSV in pea cultivars.

Ecological and epidemiological significance of PSbMV, BYMV and BBSV, if not of BBTMV is obvious from their seed transmissions in a number of leguminous plant

Table 1c

Seed transmission of como and potyviruses in fababean and vetch cultivars as determined by ELISA

Test cultivars	Symptoms[a]	Seed transmission levels of comoviruses				Total number
		Potyviruses		Comoviruses		
		BYMV	PSbMV	BBSV	BBTMV	
F. vulgaris						
Aštar	–	9.33	8.0	8.57	11.43	4[b]
Brok	–	11.76	10.59	5.26	0	3
Inovec	–	16.67	4.76	10.11	8.57	4
Ivanka	+	7.58	9.09	6.06	4.55	4
Liber	–	3.85	7.69	11.54	9.61	4
Omar	–	4.0	2.0	20.0	28.0	4
Stredan	–	11.11	2.22	5.0	0	3
ZelSeed	+	15.0	5.0	10.0	8.33	4
Range (%)		4–17	2–11	5–20	0–28	
V. sativa						
Fatima	–	9.38	6.25	7.29	3.13	4
Medea	+	0	0	3.33	6.67	2
Slovena	–	0	0	5.0	3.75	2
Telma	–	8.0	4.0	6.0	12.0	4
Toplesa	–	14.0	8.77	7.01	5.26	4
Range (%)		0–14	0–9	3–13	3–15	4
V. villosa						
Arida	–	10.0	5.0	12.5	15.0	
Total		12/14	12/14	14/14	12/14	
(%)		85.75	85.75	100	85.75	

[a] symptoms developed, usually leaflet mottle, distortion, necrotic spots or necrosis (+), symptom free (–)
[b] number of viruses seed transmitted in a given cultivar

species *(Table 3)* and warrants attention in adopting rigorous quarantine measures to check their further spread through infected seeds especially in countries where these viruses are not known to occur (Brunt et al., 1996; Stace-Smith and Hamilton, 1988).

Infectivity test

Of the 11 common bean *(P. vulgaris)* cultivars tested, some were infected by BBSV (36%) and BYMV (9%) but none by BBTMV and PSbMV *(Table 2).* Symptoms produced in common susceptible cultivar (Anka) were not diagnostic enough to differentiate BBSV from that of BYMV and their identity was confirmed by serology. Nevertheless, BBSV could be differentiated from BBTMV on the basis of their infectivity to common bean cultivars; as BBSV is known to infect number of common bean cultivars compared to that of BBTMV (Gibbs and Paul, 1970; Gibbs and Smith, 1970). In the present work, BBTMV

Table 2

Infectivity of fababean seed transmitted viruses to common bean *(P. vulgaris)* cultivars as determined by ELISA

Test cultivars	Symptoms[a]	Seed transmission levels of comoviruses				Total number[c]
		Potyviruses		Comoviruses		
		BBSV	BBTMV	BYMV	PSbMV	
Anka	+(MM)	–	–	+++[b]	–	1
	+(GrVb)	–	–	+++	–	1
	+(Ld)	+++	–	–	–	1
	+(Mt)	–	–	+	–	1
	+(Puck)	+	–	+	–	2
	+(Mt/Ld)	++	–	–	–	1
	+(Nsp)	–	–	++	–	1
Julia	+(Mt/Nsp)	++	–	–	–	1
Dita	+(Mt/Nsp)	+	–	–	–	1
Helia	+(Mt/Nsp)	+	–	–	–	1
Bountiful	–	–	–	–	–	0
Beta	–	–	–	–	–	0
Ida	–	–	–	–	–	0
Kreola	–	–	–	–	–	0
Lucka	–	–	–	–	–	0
Maxidor	–	–	–	–	–	0
Novores	–	–	–	–	–	0
Total		4/11	0/11	1/11	0/11	
(%)		36.4	0	9.1	0	

[a] symptoms produced: green vein banding (GrVb), leaf distortion (Ld), mild mosaic (MM), mottle (Mt), necrotic spots (Nsp), puckering (Puck), symptom free (–)
[b] OD values <0.010 (–), >0.250 (+), >0.500 (++), >1.500 (+++)
[c] number of viruses infecting a given cultivar

was not found infectious to any of the cultivars tested. BYMV strains are known to differ for their infectivity to common bean cultivars. PMV (pea mosaic virus) strain of BYMV is differentiated from other BYMV strains in its inability to infect number of common bean cultivars (Bos, 1970; Musil et al., 1975; Schroeder and Provvidenti, 1966). In this respect, the fababean isolate of BYMV in the present work resembles PMV rather than other BYMV strains (Jones and Diachum, 1977). Moreover, PMV is also known to be seed transmitted in fababean *(Table 3).* Few PSbMV isolates are known to infect common bean cultivars causing local but not systemic symptoms or infections. The present fababean isolate of PSbMV could not infect any of the common bean cultivar tested. As such it was distinct from other PSbMV isolates such as SL5, WI-1 and P-202 (ST) (Hampton et al., 1981) in this respect. On the basis of infectivity to common bean, BBSV can be distinguished from BBTMV; PMV from BYMV but not from PSbMV. In such cases, their

Table 3

Seed transmission of como- and potyviruses in various plant species

Viruses	Plant species	Seed transmission (%)		Key references
		Reported	Present data	
PSbMV	Peas (*Pisum sativum* L.)	0–100	NT[c]	Hampton et al. (1976) Kohenen et al. (1995) Khetarpal and Maury (1987) Ligat and Randles (1993) Miličič and Plavšič (1978) Musil et al. (1983) Rishi and Hampton (1987) Stevenson and Hagedom (1969) Šubr and Boháčová (1988) Wang et al. (1993)
	Lentil (*Lens culinaris* Med.)	+[a]	NT	Alconero et al. (1986) Goodel and Hampton (1984) Hampton and Muelhbauer (1977) Kumari and Makkouk (1995)
	Chickpea (*Cicer arietinum* L.)	+	NT	Latham and Jones (2001)
	Fababean	+	2–11	Fry (1996)
	V. articulata	+	NT	Hampton and Mink (1975)
	V. narbonensis	+	NT	Hampton and Mink (1975)
	V. pannonica	+	NT	Hampton and Mink (1975)
	V. sativa	NR[b]	0–9	
	V. villosa	NR	5.0	
BYMV	Peas	+	NT	Bos (1970)
	Clovers (*Trifolium* spp.)	+	NT	Bos (1970)
	Lupines (*Lupinus* spp.)	3.6–6.2	NT	Zschau (1962)
	Lentil	+	NT	Kumari et al. (1994)
	Fababean	0.1–2.4	4–17	Evans (1973) Kaiser (1972) Kaiser et al. (1971)
	V. sativa	NR	0–14	
	V. villosa	NR	10.0	
PMV	Fababean	0.5–1.0	4–17	Murant et al. (1973) Nitzany (1975) Nitzany and Cohen (1962)
	V. sativa	NR	0–14	
	V. villosa	NR	10.0	
BBSV	Pea	1–70	NT	Musil and Kowalska (1993) Musil and Gallo (1994)
	Lentil	+	NT	Makkouk and Azzam (1986)
	Fababean	0–10	5–20	Lloyds et al. (1965)
	V. sativa	+	3–7	Musil et al. (1978) Tapio (1970) Plaschke-Jakubík et al. (1996)
	V. villosa	NR	12.5	
BBTMV	Fababean	2.5–15	0–28	Quantz (1953)
	V. sativa	NR	3–13	
	V. villosa	NR	15.0	

[a] seed transmission occurring but in low percentage; [b] not reported; [c] not tested

(PMV and PSbMV) identity can be established by serology as these viruses are serologically distinct from each other (Jones and Diachum, 1977). In the present work, virus (BYMV and PSbMV) specific antisera (Loewe) were used to detect seed transmission of potyviruses in fababean and vetch cultivars.

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