

Zofia Fiedorow, Edyta Szlachetka-Wawrzyniak

Department of Phytopathology, Agricultural University of Poznań,
ul. Dąbrowskiego 159, 60-594 Poznań, Poland

TRANSMISSION OF *BROAD BEAN STAIN VIRUS (BBSV)*
BY SEEDS OF PEA (*PISUM SATIVUM* L.)

ABSTRACT

The studies carried out in 1997–1998 were aimed at evaluating the incidence of *Broad Bean Stain Virus (BBSV)* in pea seed material, susceptibility of pea cultivars to the virus and transmission of *BBSV* by seeds of selected pea cultivars. Experiments were performed in field conditions and in a greenhouse. The presence of the virus in pea plants and seeds was determined by isolation onto *Chenopodium murale* or serologically by ELISA test.

In the seed material, the virus was detected only in 0.1% of the seeds. The pea cultivars: Cud Kelvedonu, Delisa II, Hejga, Iłowiecki, Konserwowy IHAR, Maraton, Nefryt, Pegaz, Sześciotygodniowy and Telefon, appeared to be susceptible to the infection by *BBSV* and percentage of infected plants ranged from 6.6 to 35%. Transmission of the virus by seeds occurred in all cultivars mentioned above except for cv. Maraton and percentage of infected seeds ranged from 2.9 to 50.0, depending on cultivar of pea.

Key words: *Broad Bean Stain Virus*, pea, susceptibility, seed transmission

INTRODUCTION

Among the most important pea seed-borne viruses are *Pea seed-borne mosaic virus (PSbMV)*, *Pea early browning virus (PEBV)* and *Broad bean stain virus (BBSV)*. The latter usually does not cause clear disease symptoms and that is why its presence on seed plantations could escape visual observation. In Poland, for the first time, *BBSV* was isolated from an infected horse bean seedling and identified in 1981 (Pospieszny 1983). Earlier, Kowalska and Beczner (1980) have observed in pea plants grown in Poland a virus temporarily called by them “pea seed-borne symptomless virus”, which most likely was the same virus as that detected by Pospieszny and Fiedorow (1984) also isolated *BBSV* from an infected horse bean seedling and evaluated its transmission through horse bean seeds and susceptibility of 20 pea cultivars to infection by this virus. Moreover, she has established that

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the virus isolated by her was serologically related to isolates investigated by Pospieszny (1983) and Kowalska and Beczner (1980).

Our knowledge of the incidence of *BBSV* in Poland, both on crop plantations and in qualified seed material, is poor. All virus isolates mentioned above were obtained incidentally. Also Malinowski [1998] found incidentally that pea seeds of cv. *Łówiecki* were highly infected by *BBSV*. In order to fill the gap, two-year studies (1997–1998) of the infection of pea seed material by *BBSV*, the transmission of the virus by seeds of different pea cultivars and the effect of infection on growth and crop of pea, were carried out in the Department of Phytopathology, Agricultural University of Poznań.

MATERIAL AND METHODS

Seeds of 10 pea cultivars were investigated. Seeds of cultivars Hejga, Konserwowy IHAR, Maraton, Nefryt, and Sześciotygodniowy were of Elite quality and seeds of remaining cultivars, i.e. Cud Kelvedonu, Delisa II, *Łówiecki*, Pegaz and Telefon were of original quality. Experiments were carried out in an insect-free greenhouse. Pea seeds, 120 pieces of each cultivar, were sown on 8 April into pots filled with steamed soil. Beginning from the second week after germination, observations of disease symptoms on pea plants were conducted for 8 weeks at 14-day intervals. Irrespective of observation results, isolations were made from all pea plants onto the test species of *Chenopodium murale*. From plants showing virus-like symptoms, additional isolations were performed onto a wider set of plants: *C. amaranticolor*, *C. quinoa* and *Phaseolus vulgaris*. cv. *Złota Saxa* on purpose of identification of seed transmitting viruses. Virus was isolated only from one seedling of pea cv. *Łówiecki*. The isolated virus caused local and systemic infection of *C. murale* and *C. quinoa*, local infection of *P. vulgaris* and did not infect of *C. amaranticolor*. The range of host plants and symptoms caused by isolated virus pointed out the probability of infection with *BBSV*. Viruses, which were isolated onto test plants were then identified serologically by using the method of double diffusion in agar gel and an antiserum sensitive to *BBSV*.

The isolate of *Broad Bean Stain Virus*, obtained from naturally infected pea seedling of cv. *Łówiecki* was later used for the infection of pea plants in experiments carried out in 1998. The experiments were performed in a greenhouse from 3 April to 26 June and repeated from 25 May to 19 September and in the field from 17 April to 30 July 1998. In all the experiments 10 pea cultivars, mentioned earlier, were used. In each of both replicates of the greenhouse experiments, 20 seedlings of each cultivar, being at four-leaf stage, were inoculated mechanically with virus isolate reproduced earlier in pea of cv. *Koral*. Observations of symptoms on inoculated pea plants were conducted 2 and 4 weeks after the inoculation. After 4 weeks, isolations were made from

all inoculated pea plants onto test species of *C. murale* or *C. quinoa* in order to detect plants infected symptomlessly. After ripening of pea plants, seeds were collected from them, separately from each plant. Two months after harvesting, when the plants became dry, their weight, length of stems as well as number and weight of seeds were determined. The collected data were processed statistically by means of analysis of variance and comparison of averages using Student's t-test.

In the field experiment, about 200 seeds of each pea cultivar were sown in separate plots. When the plants were at four-leaf stage, mechanical inoculation of 20 plants of each cultivar was performed with the same *BBSV* isolate as that used in the greenhouse experiments. Ten plants in each of two central rows of a plot, were inoculated. The evaluation of infection of pea plants was performed on the grounds of disease symptoms and results of reisolation made from each inoculated plant onto *C. murale*. *BBSV*-infected pea plants were labelled and allowed to grow until ripening. Non inoculated or uninfected plants were left in the field as well.

Seeds harvested from pea plants infected with the virus were investigated in the aspect of their infection by *BBSV*. Seeds harvested from pea plants infected in the greenhouse, where only *Broad Bean Stain Virus* could be taken into consideration, were examined using biological assay, whereas seeds picked up from the field-grown plants, which could be accidentally infected by other viruses, were subject to serological ELISA test using a *BBSV*-sensitive antiserum. Seeds examined by the biological assay on *C. murale* were allowed to germinate on Petri dishes padded with moistened filter paper. Sprouts, ca. 1 cm in length, were crushed in a piece of plastic film after adding a drop of water and the obtained pulp was used for rubbing *Chenopodium* leaves. The occurrence of disease symptoms on *Chenopodium* plants after three weeks upon the inoculation were assumed as a proof of infection by *BBSV*. Number of tested seeds of different cultivars ranged from 6 to 22 in the greenhouse experiments and from 8 to 155 in the field experiments. In total, 134 seeds were examined by using the biological assay and 409 by using the serological method.

The test DAS ELISA was carried out at the Institute of Plant Protection in Poznań, using antiserum prepared in the Laboratory of Virology and Bacteriology. The test was performed on three-days old pea sprouts. Each experiment was repeated twice. The wells were filled with 0.1 ml of purified globulin diluted with buffer in the ratio of 1:500. Plates were subject to incubation for 4 hours at 37°C followed by washing with PBS+Tween buffer. To a sprout ground in a mortar, 0.35 ml of the buffer was added and 0.1 ml of sap was placed in a well. Then it was incubated overnight in a refrigerator, followed by washing once with distilled water and three times with PBS+Tween buffer. Next 0.1 ml of globulin conjugated with enzyme (alkaline phosphatase), diluted with

buffer in the ratio of 1:1000, was added, followed by 4-hour incubation at 37°C. Again washing with PBS+Tween buffer was carried out three times and 0.1 ml of freshly prepared developer was added followed by 45-minute incubation at room temperature. Absorbance was measured at the wavelength of 405 nm on automatic reader Labysystem Uniskan 2. Readings were considered positive, if a value read out on the instrument was higher than that of negative test result by a factor of 2.

RESULTS

Infection of seed material

Only one of 1200 examined seedlings of pea was found infected by *BBSV* as indicated by symptoms observed on test plants and positive serological reaction to antyserum sensitive to this virus. It has made 0.1% of seeds examined. The infected seedling has shown weak symptoms of mosaic which faded away as the plant aged. The biological assay has not indicated the presence of any other viruses in the seed material investigated.

Susceptibility of pea cultivars to mechanical infection with *BBSV*

Under greenhouse conditions, all investigated pea cultivars were infected with *BBSV*. Under field conditions no infection was found in cultivars Delisa II and Maraton. Mean percentage of plants infected in the greenhouse and in the field ranged from 6.6 in the case of cultivar Maraton to 35.0 in that of the cultivar Konserwowy IHAR (Table 1). On infected with *BBSV* pea plants, no characteristic symptoms were ob-

Table 1
Susceptibility of pea cultivars to *Broad bean stain virus (BBSV)*

Cultivar	Number* of virus-infected plants after sap inoculation			Percentage** of infected plants
	in the greenhouse	in the field	total	
1. Cud Kelvedonu	4	2	6	10.0
2. Delisa II	6	0	6	10.0
3. Hejga	6	2	8	13.3
4. Hówiecki	6	1	7	11.6
5. Konserwowy IHAR	10	11	21	35.0
6. Maraton	4	0	4	6.6
7. Nefryt	7	9	16	26.6
8. Pegaz	11	9	20	33.3
9. Sześciotygodniowy	11	5	16	26.6
10. Telefon	10	2	12	20.0

* - number of plants inoculated with virus: in the greenhouse 40, in the field 20,

** - percentage of infected plants was calculated in relation to 60 inoculated plants

served except for a mild mosaic and inconsiderable dwarfing of some plants, therefore the percentage of plants infected with *BBSV* was evaluated based on biological assay.

Effect of *BBSV* on pea plants growth and yield of seeds under greenhouse conditions

The infection with *BBSV* has caused statistically significant reduction in plant height and mass of the cultivar Konserwowy IHAR as well as seed yield of the cultivar Konserwowy IHAR and Cud Kelvedonu. On the other hand, infected pea plants of the cultivar Hejga were characterized by a higher dry mass and were significantly taller than healthy plants. In other cultivars, the differences between healthy and diseased plants were not statistically significant (Table 2).

The effect of *Broad bean stain virus (BBSV)* on pea plants growth and yield of seeds (greenhouse, pot, experiment)

Table 2

Cultivar	Difference between healthy and infected plants in %			
	weight of dry plants	height	number of seeds	weight of seeds
1. Cud Kelvedonu	22.5	19.3	46.4*	57.1*
2. Delisa II	6.0	0.6	+10.3	+18.2
3. Hejga	+22.7	+ 11.8*	12.5	8.1
4. Hówiecki	+1.5	1.7	11.8	0.0
5. Konserwowy IHAR	18.4*	13.5*	53.8*	43.9*
6. Maraton	+3.8	6.5	+19.6	3.8
7. Nefryt	16.9	12.4	+11.1	10.5
8. Pegaz	+2.8	+18.9	22.7	11.6
9. Sześciotygodniowy	1.4	2.2	10.0	2.4
10. Telefon	+8.4	14.7	+13.6	+8.7

+ difference in favor of infected plants

* significant difference at the 0.05 % level

Transmission of *BBSV* through pea seeds

Under greenhouse conditions, seeds of five pea cultivars: Cud Kelvedonu, Hejga, Konserwowy IHAR, Maraton and Nefryt were free of virus infection, whereas seeds of five remaining cultivars: Delisa II, Hówiecki, Pegaz, Sześciotygodniowy and Telefon were infected by the virus. Under field conditions, the infection of seeds with *BBSV* was detected in seven pea cultivars. Percentage of infected seeds ranged from 2.9 in the cultivar Hejga to 50.0 in the cultivar Hówiecki (Table 3). Only some of infected plants yielded infected seeds and differences in percentage of infected seeds between the plants were considerable (Table 4).

Transmission of *Broad bean stain virus (BBSV)* by pea seeds

Table 3

Cultivar	Number of seeds collected from plants infected by virus			Number of seeds infected by virus			Percentage of virus – infected seeds
	in the green-house	in the field	total	in the green-house	in the field	total	
1. Cud Kelvedonu	6	24	30	0	4	4	13.3
2. Delisa II	18	0	18	1	0	1	5.5
3. Hejga	7	27	34	0	1	1	2.9
4. Hówiecki	16	0	16	8	0	8	50.0
5. Konserwowy IHAR	12	94	106	0	10	10	9.4
6. Maraton	11	0	11	0	0	0	0
7. Nefryt	12	47	59	0	16	16	27.1
8. Pegaz	19	155	174	6	20	26	14.9
9. Sześciotygodniowy	22	54	76	1	33	4	5.2
10. Telefon	11	8	19	2	2	4	21.0

The infection of seeds of different pea plants with *Broad bean stain virus (BBSV)*

Table 4

Cultivar	Number* of plants infected	Number* of seeds in individual plant		Percentage of infected seeds
	with BBSV	tested**	infected	
1. Cud Kelvedonu	2	15; 9	0; 4	0; 44.4
2. Hejga	2	9; 18	0; 1	0; 5.5
3. Konserwowy IHAR	10	2; 11; 7; 18; 15; 7; 11; 5; 14; 8	0; 0; 0; 1; 1; 1; 1; 1; 3; 2	0; 0; 0; 5.5; 6.7; 14.3; 14.3; 20.0 21.4; 25.0
4. Nefryt	6	3; 14; 11; 7; 6; 6	0; 2; 3; 3; 4; 4	0; 14.3; 27.3; 42.9; 66.7; 66.7
5. Pegaz	9	3; 8; 9; 19; 19; 12; 22; 45; 18;	0; 0; 0; 1; 1; 1; 2; 10; 5	0; 0; 0; 5.3; 5.3; 8.3; 9.1; 22.2; 27.8
6. Sześciotygodniowy	5	14; 2; 1; 23; 14	0; 0; 0; 1; 2	0; 0; 0; 4.3; 14.3
7. Telefon	1	8	2	25.0

*tested seeds were collected from all pea plants infected with *BBSV* in the field experiment after mechanical inoculation

** seeds were tested by ELISA method

DISCUSSION

Results of the studies presented in this paper show that the infection of pea seed material with viruses was low. Only one seedling of 1200 tested was infected with virus identified as BBSV. It is worth to add, however, that the studies were performed using a small number of plants obtained from one-year harvest only. Data reported by Malinowski (1998) indicate that the infection of pea seed material with BBSV could be considerably higher.

All pea cultivars investigated were susceptible to infection with BBSV. However, the disease symptoms were not clear, and not made conclusion that a plant was infected. According to Fiedorow (1984) and Pospieszny (1982), the virus causes on some of pea cultivars vein clearing and mild mosaic, but these symptoms could be observed during a short time. In the investigation carried out by Fiedorow (1984), 11 of 20 Polish pea cultivars were susceptible to infection, in that performed by Musil and Kowalska (1993) and Musil and Gallo (1994) seven cultivars: Cud Kelvedonu, Delisa, Iłowiecki, Konserwowy IHAR, Nefryt, Pegaz and Sześciotygodniowy were infected with BBSV. Kowalska and Beczner (1980) reported that 30 pea cultivars investigated by them were subject to infection by so-called pea seed-borne symptomless virus which, as demonstrated by Fiedorow (1984), was serologically closely related to *Broad Bean Stain Virus*. Therefore it is reasonable to suppose that majority of Polish pea cultivars are susceptible to *Broad Bean Stain Virus*.

Results reported in the present study indicate that BBSV retards neither the growth of plants nor the production of green matter or pea seeds. The exception was made by the cultivars Konserwowy IHAR and Cud Kelvedonu in the case of which the above parameters were significantly smaller in infected plants compared to healthy plants. In other leguminous plants, e.g. lentil, BBSV can cause a marked reduction of crop (Mabrouk and Mansour 1998).

Presented results have shown that the transmission of BBSV through seeds of pea could be easy and the percentage of infected seedlings is quite high. Only in one of ten investigated cultivars, namely in Maraton, the virus transmission through seeds was not detected, but it cannot be ruled out that if higher number of seeds from infected plants were investigated, the result would be positive. Considerable differences were found in percentage of infected seeds between cultivars investigated. Seeds of cultivars Iłowiecki, Nefryt and Telefon were more infected with BBSV than seeds of cultivars Hejga, Delisa II and Sześciotygodniowy. However, on the ground of the differences observed, we cannot say that some of the cultivars are characterized by higher or lower capability of transmitting the virus through seeds. Both our results and data reported in the literature (Musil and Gallo 1994) point to a considerable effect of experiment conditions on the transmission of BBSV through seeds. Moreover, a considerable indi-

vidual variability exists among plants of the same cultivar as concerns transmission the virus through seeds, which was demonstrated in this study. Relationships of such a type exist also in the case of other seed-borne viruses (Matthews 1981). The investigation presented in this paper have confirmed earlier reports on the transmission of *BBSV* through seeds of the cultivar Hówiecki (Kowalska and Beczner 1980, Malinowski 1998) and pointed to a possibility of transmitting the virus by other cultivars, namely Cud Kelvedonu, Delisa II, Hejga, Konserwowy IHAR, Nefryt, Pegaz, Sześciotygodniowy and Telefon.

CONCLUSIONS

1. The qualified seed material of pea was infected with *Broad Bean Stain Virus* only in 0.1% and was free of other seed transmitted viruses.
2. All pea cultivars investigated were subject to infection with *Broad Bean Stain Virus*.
3. Cultivars of pea differed in their susceptibility to mechanical inoculation with *BBSV*.
4. *Broad Bean Stain Virus* could be transmitted through a considerable percentage of seeds of commonly cultivated pea cultivars.
5. The virus does not cause symptoms on pea leaves clear enough to evaluate of the extent of the infection of a pea plantation or to carry out a negative selection.

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