IMPACT OF SEED-TRANSMITTED VIRUSES ON QUALITY OF CEREAL SEEDS

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Abstract: Among seed-transmitted cereal viruses the most important is *Barley stripe mosaic virus* (BSMV), infecting barley. Investigations on the occurrence of BSMV in Poland have been carried out in the Institute of Plant Protection in Poznań since 2000. The studies aimed at the evaluation of the BSMV distribution in plant material, the rate of its seed transmission in different barley cultivars and the assessment of the effect of barley infection on the yield. The potential risk of BSMV occurrence for barley crops was discussed. The rate of infection by two seed-transmitted viruses, *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV), in Poland was investigated in view of the risk assessment.

Key words: seed-transmitted viruses, barley, maize, *Barley stripe mosaic virus*, *Maize dwarf mosaic virus*, *Sugarcane mosaic virus*

INTRODUCTION

Seed transmission in the epidemiology of cereal viruses is believed to be not a serious problem. However as much as five viruses infecting cereals in Poland are seed-transmitted: *Barley stripe mosaic virus* (BSMV), *Soil-borne cereal mosaic virus* (SBCMV), *Wheat streak mosaic virus* (WSMV), *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) (Jeżewska 2002, 2006, Jeżewska and Trzmiel 2008, Trzmiel 2009). It is noteworthy that besides typical cereal viruses, also *Tobacco mosaic virus* (TMV) was found to be transmitted with seeds of barley, wheat, rye, triticale and oat (Jeżewska and Trzmiel 2005, 2007).

BSMV is the type member of the genus *Hordeivirus*, with a wide geographic distribution. Virions are rod-shaped, 20 nm in diameter, with a central canal, and usually have three size classes with modal values of 110–160 nm. Barley is the main host of BSMV but the virus can infect also wheat. The virus developed a broad diversity of strains, causing in barley a variety of disease symptoms, differing in severity (Bϋchen-Osmond 1996). BSMV is primarily dependent on seed transmission in barley for its survival in nature, as there are no known vectors involved in the virus transmission. However, BSMV can be efficiently transmitted mechanically, e.g. by foliage contact in a field. The disease is of major importance only in barley and substantial yield reductions have been reported.

BSMV was included into the EPPO List A2 of quarantine organisms. However this status changed when there was admitted that the virus was eradicated from barley seeds in Europe. Nevertheless results of our investigations clearly indicated that only aggressive isolates of the virus have been eliminated. Mild and latent isolates have been commonly detected in different barley materials, including cultivars from breeding collections, breeding lines and commercial seeds.

Investigations on the occurrence of BSMV in Poland have been carried out in the Institute of Plant Protection in Poznań since 2000. Our studies aimed at the evaluation of the range of BSMV distribution in barley seeds, the rate of its seed-transmission in different cultivars and the assessment of the effect of infection of barley with mild isolates of BSMV on the yield.

Two maize mosaic viruses, MDMV and SCMV, recently identified in Poland, are known also to be seed-transmitted, on a very low level of efficiency. However, in contrast to BSMV the viruses have efficient vectors, i.e. cereal aphids (Ford *et al.* 1989; Fuchs 2004; Gordon 2004; Hohmann *et al.* 1999; Persley 1996). The potential of spread of these viruses is therefore serious. Considering such risk, the rate of seed-transmission of MDMV and SCMV in two maize cultivars was also investigated.

MATERIALS AND METHODS

Plant material

Barley seeds from collections of breeders were kindly provided by Hodowla Roślin Smolice, Breeding Station in Baków and The Research Centre for Varieties of Cultivated Plants (COBORU). Following cultivars were investigated:

Spring barley

Annabell, Antek, Barke, Beryl, Blask, Boss, Brenda, Edgar, Granal, Johann, Justina, Orlik, Orthega, Rabel, Rasbet, Rataj, Riviera, Rudzik, Scarlett, Sezam and Stratus.

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Winter barley

Bartosz, Bażant, Bursztyn, Epoque, Fridericus, Gil, Gregor, Horus, Karakan, Kos, Kroton, Lomerit, Maybrit, Merlot, Nickela, Rosita, Tiffany, Tramp and Traminer.

In total 38 barley cultivars were analysed.

Commercial barley seeds originated from Seed Centres in different regions of Poland.

The following barley cultivars were investigated for BSMV seed transmission rate: Annabell, Antek, Blask, Justina, Nagradowicki and Refren. Seeds were collected from plants infected by the virus. The infection was confirmed by ELISA test.

Seeds of maize were provided by Syngenta (cv. Arobase) and by Hodowla Roślin Smolice (Blask).

Virus detection

For virus detection ELISA test was used (Clark and Adams 1977). The indirect variant of the assay, including universal anti-rabbit IgG – alkaline phosphatase conjugate (Sigma-Aldrich Chemie, Germany) was applied. Our own immunoglobulins (IgG) were used, produced on the basis of purified virus preparations of BSMV, MDMV and SCMV, respectively. The worked out procedure of BSMV, MDMV and SCMV diagnostics in seeds consisted of indirect virus detection in young seedlings grown from tested seeds.

Effect of BSMV infection on the yield of barley

Field plot experiment was carried out in 2009 in the Institute of Plant Protection in Poznań. Two spring barley cultivars, Justina and Nagradowicki, were used. Seedlings grown from seeds collected from plants infected with BSMV were tested for the virus presence. Each cultivar was represented by equal number of healthy and BSMV-infected plants. In the case of cv. Justina this number was 77 and for cv. Nagradowicki – 89. In the experiment standard measures and plant protection conditions were applied.

Evaluation of the seed transmission rate of BSMV, MDMV and SCMV

Seeds originated from virus-infected plants were assayed indirectly, as described above, for the virus presence by ELISA test. Barley plants were BSMV-infected from seeds and maize plants mechanically infected with mosaic viruses, MDMV and SCMV.

RESULTS AND DISCUSSION

Detection of BSMV in breeding and commercial barley cultivars

The occurrence of BSMV in barley seeds was studied in cultivars from breeder's collections and in commercial seed stocks.

Results of the virus detection in 40 cultivars obtained from breeders are presented in table 1. Investigations were carried out in 2000–2008. BSMV was found in 24 cultivars:

Table 1. Detection of *Barley stripe mosaic virus* in barley seeds – cultivars from breeding collections

Cultivar	Number of seeds tested	Number of infected seeds	Percentage of infected seeds
Spring barley			
Annabell	200	17	8.5
Antek	100	3	3.0
Barke	100	1	1.0
Beryl	100	0	0
Blask	100	1	1.0
Boss	100	2	2.0
Brenda	100	0	0
Edgar	100	0	0
Granal	100	0	0
Johann	100	0	0
Justina	100	0	0
Orlik	100	0	0
Orthega	100	0	0
Rabel	100	0	0
Rasbet	100	0	0
Rataj	100	0	0
Riviera	100	0	0
Rudzik	100	0	0
Scarlett	110	11	10.0
Sezam	100	0	0
Stratus	110	15	13.6
Winter barley			
Bartosz	288	11	3.8
Bażant	288	2	0.7
Bursztyn	288	7	2.4
Epoque	420	29	6.9
Fridericus	288	1	0.4
Gil	288	13	4.5
Gregor	100	3	3.0
Horus	288	8	2.8
Karakan	288	3	1.0
Kos	100	3	3.0
Kroton	100	2	2.0
Lomerit	288	21	7.3
Maybrit	576	46	8.0
Merlot	288	0	0
Nickela	288	8	2.8
Rosita	288	0	0
Tiffany	100	5	5.0
Tramp	100	1	1.0
Traminer	288	24	8.3

spring barley: Annabell, Antek, Barke, Blask, Boss, Scarlett, Stratus

winter barley: Bartosz, Bażant, Bursztyn, Epoque, Fridericus, Gil, Gregor, Horus, Karakan, Kos, Lomerit, Maybrit, Nickela, Tiffany, Tramp, Traminer.

In total, 237 barley seeds out of 7 172 tested were proved to be virus-infected (3.3%). The highest percentage of seeds infected with BSMV was found in cv. Stratus (13.6%), Scarlett (10.0%), Annabell (8.5%), Traminer (8.3%) and Maybrit (8.0%). In all cases only mild or latent BSMV isolates were found. The virus was not detected in following cultivars: Beryl, Brenda, Edgar, Granal, Johann, Justina, Orlik, Orthega, Rabel, Rasbet, Rataj, Riviera, Rudzik, Sezam, Merlot and Rosita.

Table 2 presents results of screening tests of commercial seeds. The most serious case was recognised in 2001 in cv. Kos. It was the only case where BSMV infection resulted in severe plant symptoms and important crop losses. Other BSMV isolates detected in seeds were mild or latent.

Table 2. Detection of *Barley stripe mosaic virus* in barley seeds – commercial cultivars

Year	Cultivar	Number of		Percentage of infection
		seeds tested	infected seeds	
2001	Kos	30	18	60
	Lot	100	0	0
	Nagrad	100	0	0
	Rodion	100	0	0
2006	Lomerit	250	7	2.8
	Tiffany	668	8	1.2
2007	Lomerit	192	6	3.1
	Tiffany	192	11	5.7
2008	Antek	288	10	3.5
	Justina	288	23	8.0
	Nagradowicki	288	3	1.0
	Refren	288	22	7.6
	Karakan	420	47	11.7
	Lomerit	288	4	1.4
	Traminer	288	5	1.7

The data presented above indicate that BSMV is widely distributed in barley seeds in Poland. It was found both in original Polish cultivars and in foreign ones.

In 2003–2004 the occurrence of BSMV was also evaluated in breeding materials. BSMV presence was revealed in 37 barley breeding lines out of 105 tested. Pedigree analysis of BSMV bearing lines indicated possible sources of the virus introduction into new cultivars. The most frequent parent form in the pedigrees of the BSMV-infected new lines was old spring barley cultivar Atol (Jeżewska 2006). These results confirm the risk of survival of the virus in the form of mild isolates. The mild type of the virus isolates assures their easy surviving in plant material.

BSMV seed transmission in barley cultivars under experimental conditions

Seed transmission rate was evaluated in the following barley cultivars: Annabell, Antek, Blask, Justina, Kroton, Nagradowicki, Refren, Scarlett, Stratus and Tiffany (Table 3). The most efficient seed transmission was noted in cv. Nagradowicki (15.4%). High efficiency was also observed in cvs. Refren (14.5%), Justina (13.4%) and Kroton (11.7%).

Table 3. Efficiency of *Barley stripe mosaic virus* seed transmission in different barley cultivars

Cultivar	Number of		Efficiency of BSMV seed-transmission
	seeds tested	infected seeds	
Annabell	1530	98	6.4
Antek	472	9	1.9
Blask	162	1	0.6
Justina	574	77	13.4
Kroton	180	21	11.7
Nagradowicki	578	89	15.4
Refren	256	37	14.5
Scarlett	182	12	6.6
Stratus	192	19	9.9
Tiffany	160	6	3.8

BSMV is known for its great diversity of strains differing in the degree of aggressiveness and the rate of seed transmission. Stewart *et al.* (2005) presented a study of the evolution of virulence under both horizontal and vertical transmission. The authors clearly demonstrated that the decrease of aggressiveness results in the increase of the efficiency of seed transmission.

Effect of BSMV infection on the yield of two spring barley cultivars

The effect of BSMV infection on the yield and weight of 1 000 kernels of 2 cultivars, Justina and Nagradowicki, was determined (Table 4). In contrast to previous experiments the plants were not mechanically inoculated but infected from seeds. Such mode of infection required extensive testing work, thus limiting the number of experimental plants. In spite of the fact that BSMV isolate involved in the experiment did not cause typical mosaic symptoms and could be easily neglected as disease factor, its reducing effect on the yield was evident, both in cv. Justina (9.5%) and in cv. Nagradowicki (14.8%). The virus infection influenced less the weight of 1000 kernels (0.5% for Justina and 2.8% for Nagradowicki).

Table 4. Impact of *Barley stripe mosaic virus* seed infection on the yield of barley in plot experiment (Poznań, 2009)

Cultivar	Plot	Yield [kg/m^2]	Weight of 1000 seeds [g]
Justina	Healthy control	1.27	38.0
	BSMV infected	1.15	37.8
Nagradowicki	Healthy control	1.53	43.3
	BSMV infected	1.31	42.1

In previous field experiment the impact of BSMV infection on the yield and weight of 1 000 kernels was evaluated using two spring barley cultivars, Annabell and Blask, mechanically infected with mild isolate of BSMV. The highest yield loss was noticed in 2004 for cv. Annabell. The yield loss amounted to 17.1% and 1000 kernels weight was lower about 9.2% comparing to the healthy control. These differences were confirmed statistically (Jeżewska 2006). Much data were reported on the serious yield reduction due to the infection of barley with BSMV (Carroll 1980; Chiko and Baker 1978; Eslick 1953). However the authors estimated crop losses for typical or even strongly aggressive BSMV isolates while our results refer to mild ones. When infection develops without visible symptoms, growers are not aware of the reason of decreased yield. However crop losses on the level up to 10% should not be ignored.

Seed transmission rate of MDMV and SCMV in two maize cultivars

Preliminary data concerning the rate of seed transmission of two maize mosaic viruses, MDMV and SCMV, in two maize cultivars, Arobase and Blask, are presented in Table 5. In both cultivars the efficiency of virus seed transmission was higher than expected on the basis of literature data (Fuchs 2004; Hill *et al.* 1974; Mickel *et al.* 1984). In cv. Arobase it was 1.1% for MDMV and 1.7% for SCMV, and in cv. Blask 6.5% and 1.8%, respectively. Taking into account that MDMV and SCMV are readily transmitted by aphids, the demonstrated level of their seed transmission rate should not be ignored.

Table 5. Efficiency of seed transmission of *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) in two maize cultivars

Cultivar	Detection of infection			
	MDMV		SCMV	
	number of infected seeds/ number of seeds tested	rate of seed transmission [%]	number of infected seeds/ number of seeds tested	rate of seed transmission [%]
Arobase	17/1553	1.1	23/1612	1.7
Blask	85/1306	6.5	55/3076	1.8

CONCLUSIONS

In summary, it was demonstrated that BSMV, the principal seed-transmitted cereal virus, is widely distributed in barley seeds in Poland. In spite of the fact that it usually occurs in the form of mild strains and infection is often symptomless the crop losses due to the infections may be important. Therefore some control measures for barley seeds in breeders' collections and for seed cultures should be recommended.

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POLISH SUMMARY

WPŁYW WIRUSÓW PRZENOSZONYCH PRZEZ NASIONA NA JAKOŚĆ ZIARNA ZBÓŻ

Spośród pięciu wirusów porażających zboża występujących w Polsce i przenoszonych przez nasiona, największe zagrożenie stanowi wirus pasiastej mozaiki jęczmienia (*Barley stripe mosaic virus*, BSMV). Prowadzone badania miały na celu określenie występowania BSMV w materiale nasiennym jęczmienia oraz ocenę ryzyka związanego z porażeniem nasion przez ten wirus. BSMV wykrywano powszechnie w nasionach jęczmienia pochodzących zarówno z kolekcji hodowlanych oraz w materiałach wyjściowych do hodowli, jak i w materiale siewnym dostępnym w Centralach Nasiennych. Pomimo, iż w Polsce występowały głównie łagodne izolaty BSMV, nie wywołujące charakterystycznych objawów mozaiki liści, przedstawione wyniki doświadczeń potwierdziły niekorzystny wpływ porażeń na plon roślin jęczmienia w warunkach polowych. Celem zapewnienia dobrej jakości nasion jęczmienia, wskazane byłoby wprowadzenie kontroli zdrowotności nasion jęczmienia w kolekcjach hodowlanych oraz w materiale siewnym przeznaczonych na plantacje nasienne.

Przebadano skuteczność przenoszenia przez nasiona dwóch wirusów wywołujących objawy mozaiki kukurydzy, karłowej mozaiki kukurydzy (*Maize dwarf mosaic virus*, MDMV) oraz mozaiki trzciny cukrowej (*Sugarcane mosaic virus*, SCMV). Stwierdzono, iż tempo rozprzestrzeniania się zarówno MDMV jak i SCMV przez nasiona na przykładzie dwóch odmian kukurydzy, było wyższe, niż można było przewidywać na podstawie danych literaturowych.