Pak. J. Phytopathol., Vol. 34 (01) 2022. 127-133 DOI: 10.33866/phytopathol.034.01.0759

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

SURVEY OF BARLEY STRIPE MOSAIC VIRUS (BSMV) IN MULTIPLE CEREAL VARIETIES CULTIVATED IN CHLEF PROVINCE, NORTHWEST OF ALGERIA

[a]Medjahed Khaldia, [b]Belkahla Hadjira, [c]Abdelaziz Merouane*

[a]*Laboratory of production and protection of field crops. Hassiba Benbouali University of Chlef, Algeria.*
[b] *Department of Biotechnology, Faculty of Natural and Life Sciences, University of Blida 1, Blida, Algeria.*
[c]*Laboratory of Natural Bioressources, Hassiba Benbouali University of Chlef, Algeria.*

A B S T R A C T

This study was designed to survey Barley Stripe Mosaic Virus (BSMV) in various cereal varieties cultivated in 28 fields cultivated in seven districts namely Boukadir, Sobha, Ouled Fares, Tadjena, Kaloul, Abou Elhacene and El Karimia located in Chlef province, North West of Algeria. The survey covered 2 barley, 2 durum wheat, 4 common wheat and one oat varieties and conducted from February to the May 2017. The main objectives of this investigation were to determine the incidence of Barley Stripe Mosaic Virus in crop cereals cultivated in Algeria as well as to verify the resistant varieties. The Barley Stripe Mosaic Virus was examined in plants and seeds symptomatically by mechanical inoculation and serologically by TAS-ELISA technique using specific monoclonal antibodies. The symptoms surveying noticed more expression in spike cereals. The results of serological analysis revealed infection of 27 fields that present 96.24%. The mechanical inoculation test proved infection of totality of seed lots and seeds transmission test showed efficiency of seed transmission with a rate of 77%. In terms of species susceptibility, barley was the most susceptible crop followed by durum wheat and common wheat, whereas the oat was not infected. This investigation is the first report on Barley Stripe Mosaic Virus naturally infecting cereals in the cereal producer zone of Chlef. The findings provide useful information on viral infection of cereal fields, thus, as a priority measurement there is a need to reinforce control of cereal seeds.

Keywords: Barley Stripe Mosaic Virus, Hordeïvirus, Cereals, Transmission, TAS-ELISA

INTRODUCTION

Cereals are subjected to several pathogens including viruses. These pathogens can cause two types of diseases: the first is barley yellow dwarfing virus (BYDV) (Belkahla and Lappiere, 2005). Whereas, the second type is the mosaic viruses named wheat streak mosaic virus (WSMV) represented by wheat streak mosaic virus (WSMV) and barley stripe mosaic virus (BSMV) (Jones *et al*., 2021).

The BSMV pathogen, considered as the type member of the *Hordeivirus* genus, constitute a serious cereal pathogen, due to its efficient seed transmission and worldwide occurrence from United States (Carroll, 1980), Europe countries (Zarzyńska-Nowak *et al*., 2018), South Korea (Lim *et al*., 2016), Zambia (Kapooria and Ndunguru, 2004), Turkey (Köklü, 2004), Chile (Herrera, 2001) and Egypt (Hafez, 2008). The BSMV, characterized with rod-shape, 20 nm diameter and from 110 to 160 nm in length, prefer barley as main host, but it can infect other *Poaceae* members as well as dicotyledonous species (Adams *et al*., 2012). This barley virus cause enormous damages in cereal fields resulting in significant economic losses justifying its quarantine classification (until 1999) by the European and Mediterranean Plant Protection Organization (EPPO, 1983).

The barley stripe mosaic virus (BSMV) is an important seed-transmitted pathogen distributed in all barley-producing regions worldwide. The conventional occurrence of this virus was reported in United States,

Submitted: April 20, 2022
Revised: June 09, 2022
Accepted for Publication: June 15, 2022
* Corresponding Author:
Email: a.merouane@univ-chlef.dz

West Europe, Japan, the ex-URSS republics, China and Australia (Weise, 1987). However, new outbreaks have been observed in African (Egypt and Zambia), South American (Chile), Asian (Turkey and South Korea) and East European countries (Poland) (Zarzyńska-Nowak *et al*., 2018).

The natural spreading of BSMV is primarily a result of mechanical contact between the leaves (Jackson *et al*., 2009) supported with wind and rain (Slack *et al*., 1975). On the other hand, seeds are regarded as the main transmitter factor. In barley, for example, the seed transmission is responsible on more than 90% of infection (CGKB, 2022).

The control of cereal fields to survey the presence of BSMV is a necessary measurement to avoid the loss of an area sown. The first step consists on the selection and collection of symptomatic plants, preferably in a W pattern which covers the whole field, followed by inoculation test on known sensible varieties to better express the infection. The ELISA test is the efficient tools that can be consistently detect the virus in infected plant tissues at low dilution (Abd El-Aziz, 2020). Additionally, the specificity of the test can detect variable strains of the BSMV (Younes *et al*., 2018).

The region of Chlef, situated North West of Algeria, is known as an important cereal crops producer zone. To the best of our knowledge, there is no report on viral diseases of cereal crops in this region especially BSMV described as a causal factor in considerable losses of yields and no information is available on their incidence.

The viruses are classed among the most important phytopathogens that affect cereal production and cause economic losses. BSMV pathogen can affect plant height and weight and causing a yield loss that can reach up to 75% for (Kapooria and Ndunguru, 2004). In USA, the yield losses reduced by 35-40% in barley culture. The same virus caused 62% grain loss in barley production and 19% yield decrease in winter wheat. In terms of economy, the total loss in barley reached exceeded US$ 30 million between1953-1970 (Sastry, 2013). Unfortunately, the damages of this virus are not documented in Algeria.

Therefore, the present study consists to survey BSMV in various cereal fields distributed in Chlef province and to determine their natural occurrence in plants and seeds.

MATERIALS AND METHODS

Samples collection: The current study was carried out in 28 surveyed fields of diverse varieties of common wheat, durum wheat, barley and oats (Table 1) distributed in seven sites named Boukadir, Sobha, Oueld Fares, Tadjena, Kaloul, Abou Elhacene and El Karimia located in Chlef province, Northern Algeria (Fig.1). The survey time of cereal fields ranged from February to May 2017 between leaf development and inflorescence emergence stages where the symptoms were most expressed.

The sampling was done following the protocol proposed by Ilbagı *et al*. (2006). One hundred cereal leaves were collected randomly showing infection symptoms in a W pattern which covers the whole cereal field. The collected samples were stored in sterile labeled bags and transmitted to the laboratory within 24 hours.

Figure 1. Map of Algeria showing the surveyed fields location.

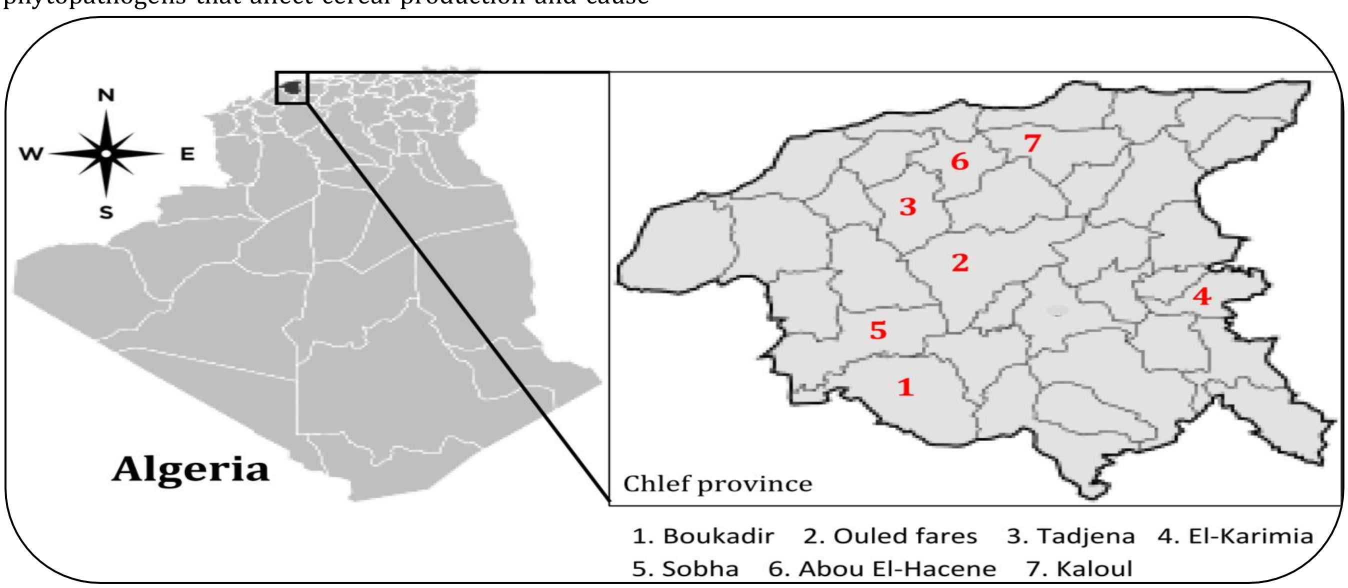

Table1. Cereal varieties used in the study and their collection sites.

Cereal	Variety	Sites of collection						
		Boukadir	OuledFares	Tadjena	El-Karimia	Sobha	Abou Elhacene	Kaloul
Triticumaestivum	AinAbid	×						×
	ARZ	×	×		×	×		×
	Simito	×		×	×	×	×	
	Anapo						×	×
Triticum durum	Acsad						×	
	Ofanto		×	×	×			×
Hordeumvulgare	Rihane	×		×	×	×	×	×
	Saida		×		×			
Avena sativa	n.d					×		

n.d: not defined

Identification tests: Mechanical Transmission Test: The BSMV mechanical transmission test was performed according to Jezewska, (2001). 1 mg of fresh leaves of barley (variety: Rihane) were cold ground in 4 ml of phosphate buffer (PH 7.4) mixed with carborundum (1mg/L), the obtained juice was filtered and served as inoculum.

The inoculum was applied to the upper surface of 4 healthy leaves of tomato (variety: Rio Grande) and 2 healthy leaves of barley (variety: Rihane). The tomato plants were planted in June 2017 and inoculated at vegetative stage growth after 25 days of transplanting. The inoculated plants were then incubated at 18-22 °C during 30 days. After incubation period, the plants were serologically tested.

Test for virus identification in seed: The identification of BSMV in seed was confirmed using ELISA technique (Kılıç*et al*., 2012). From each variety (Rihane, Simito, AinAbid, Anapo and ARZ), 1000 seeds ,with clear symptoms, were finely powdered then dissolved in 10 mL of PBS-Teween Buffer solution. The solutions were held for one hour at room temperature to allow the separation of liquid phase from the sediment. The procedure for analysis of the seed samples (plate washing, adding samples, addition of the enzyme conjugate and plate reveal) was made as described in the instructions of ELISA kit.

Seed transmission test: The seed transmission test aims to reproduce the symptoms of BSMV. For this test, 200 seeds of barley (variety: Rihane) were sown in pots and incubated in a greenhouse conditions. Observation of symptoms (whitish spots on the leaves, browning along the veins of leaves, chlorosis, and emergence of pale yellow) started at day 30.

Serological test: For the serological detection of BSMV, the TAS-ELISA technique was used following the protocol described by Seepiban *et al*. 2017. The specific monoclonal antibodies are diluted in 100 µL CA/10mL fixing buffer and fixed on the micro-plate, and then the plant extract was added. The BSMV particles were then contacted with two types of conjugated antibodies (A and B) mixed and diluted in buffer at a rate of 100µL conjugated CA/10 mL of buffer and coupled to the alkaline phosphate enzyme. The double serological reaction is demonstrated by the reaction of enzyme substrate paranitrophenyl phosphate (1mg/mL), leading to a colored reaction, where the absorbance were read spectrophotometerically at 405 nm.

RESULTS

Infection Symptoms: The BSMV symptoms observed on barley were severe. These symptoms started as whitish spots at the base of the leaves which became brown streaks running along the veins to the top of leaf, followed by general chlorosis, emergence of pale yellow. On wheat varieties, symptoms were less frequent. Similar to those observed on barley, these symptoms were a diffuse or mottled yellowing developed later into more or less long brown stripes. During these surveys it was noticed that the heading stage is the most susceptible to attack where the symptoms were more visible and more important. The results of ELISA test showed that 27 infected samples from a total of 28 cereal fields surveyed (Table 2).

Table 2. Results of the optical density of surveyed cereal varieties.

Site Variety	Boukadkir		Ouled Fares		Tadjna		El-Karimia		PF Si Tayeb		PF Belkhedim		PF Si Mnawer	
	OD	Obs	OD	Obs	OD	Obs	OD	Obs	OD	Obs	OD	Obs	OD	Obs
AinAbid	0.2	+									0.2	+		
ARZ	0.2	+	0.2	+					0.3	+	0.3	+	0.2	+
Simito	0.2	+			0.3	+	0.2	+	0.2	+	0.3	+		
Anapo											0.2	+	0.3	+
Acsad											0.2	+		
Ofanto			0.3	+	0.2	+	0.3	+					0.2	+
Rihane	0.6	+			0.6	+	0.7	+	0.6	+	0.5	+	0.6	+
Saida			0.5	+			0.6	+						
Avon									0.1	-				
Positive control	3.4													
Negative control	0.0													

OD : optical density, Obs : observation, PF: pilot farm

Test for virus identification in seed: The results of the serological test for BSMV in seed showed positive reaction in four varieties including Rihane, Simito, Ain abidet and ARZ. The average OD values ranged from 0.263 to 0.865. The varieties Rihane and Simito showed the highest OD values (Table 3)

Table 3.Optic densities obtained after serological seed test of cereal seeds

N°	Variéties	OD	Observation
1	Rihane	0.7	+
2	Simito	0.8	+
3	Ain Abid	0.2	+
4	Anapo	0.1	-
5	ARZ	0.2	+
6	Positive control	3.4	+
7	Negative control	0.0	-

Mechanical inoculation test: After four weeks of inoculation, the tomato plants showed symptoms of local and necrotic lesions. On barley plants, the appearance of whitish spots was observed. The serological test carried out on inoculated plants revealed the presence of BSMV on 30 samples of tomato and 37 samples of barley. The OD values varied from 0.260 to 0.290 in tomato varieties and ranged from 0.351 to 0.475 in barley varieties (Table 4).

Seed transmission test: The observation of symptoms, after 30 days of transmission test, revealed a discoloration of leaves followed with yellowing diffusion from the base to the top of leaves as well as weak vegetative growth of plants. The serological test showed infection of 77 cereal samples with OD values varying from 0.160 to 0.807 (Table 5).

DISCUSSION

The BSMV is known by a wide diversity of strains, causing diverse disease symptoms with variable degree of aggressiveness in a range of *Poaceae* species especially the common consumed cereals including barley, wheat, rice and oat (Adams *et al.*, 2012). This phytopathogen was included into the EPPO List A2 of quarantine organisms due to its serious impact on the yield reduction as well as weight and length of plant cereals (Jeżewska and Trzmiel, 2009). In addition, this virus can interact synergistically with other viruses to aggravate the disease syndrome and produce substantial losses in cereals (Lommel *et al.*, 1986).

Table 4. Optic densities obtained of cereal seeds after mechanical inoculation test

Variety Rio Grande						Variety Rihane					
S	OD	Obs	S	OD	Obs	S	OD	Obs	S	OD	Obs
1	0.2	+	21	0.1	-	1	0.4	+	21	0.4	+
2	0.0	-	22	0.2	+	2	0.4	+	22	0.1	-
3	0.0	-	23	0.2	+	3	0.3	+	23	0.4	+
4	0.0	+	24	0.2	-	4	0.4	+	24	0.4	+
5	0.2	+	25	0.2	+	5	0.4	+	25	0 .3	+
6	0.1	-	26	0.2	+	6	0.4	+	26	0.4	+
7	0.2	+	27	0.2	+	7	0.3	+	27	0.4	+
8	0.2	+	28	0.2	+	8	0.4	+	28	0.4	+
9	0.0	-	29	0.2	+	9	0.4	+	29	0.4	+
10	0.2	+	30	0.2	+	10	0.4	+	30	0.4	+
11	0.1	-	31	0.2	+	11	0.4	+	31	0.4	+
12	0.1	-	32	0.2	+	12	0.4	+	32	0.4	+
13	0.2	+	33	0.2	+	13	0.3	+	33	0.4	+
14	0.2	+	34	0.2	+	14	0.4	+	34	0.4	+
15	0.0	-	35	0.2	+	15	0.4	+	35	0.4	+
16	0.2	+	36	0.2	+	16	0.4	+	36	0.4	+
17	0.2	+	37	0.2	+	17	0.4	+	37	0.4	+
18	0.2	+	38	0.2	+	18	0.4	+	38	0.4	+
19	0.2	+	39	0.2	+	19	0.2	-	39	0.4	+
20	0.2	+	40	0.0	-	20	0.2	-	40	0.4	+
Positive control	3.4										
Negative control	0.0										

OD: optical density, Obs: observation, S: sample.

Table 5.Optic density results obtained after the seed transmission test

Variety Rihane											
S	OD	Obs	E	OD	Obs	S	OD	Obs	S	OD	Obs
1	0.1	+	29	0.2	+	57	0.2	+	85	0.3	+
2	0.1	-	30	0.2	+	58	0.1	+	86	0.2	+
3	0.1	-	31	0.1	+	59	0.1	-	87	0.1	+
4	0.1	-	32	0.2	+	60	0.2	+	88	0.2	-
5	0.2	+	33	0.3	+	61	0.3	+	89	0.1	+
6	0.2	+	34	0.2	+	62	0.2	+	90	0.1	+
7	0.2	+	35	0.3	+	63	0.3	+	91	0.3	-
8	0.2	+	36	0.1	+	64	0.3	+	92	0.1	-
9	0.2	+	37	0.2	+	65	0.1	+	93	0.1	+
10	0.1	-	38	0.1	+	66	0.5	+	94	0.2	-
11	0.2	+	39	0.2	+	67	0.3	+	95	0.1	-
12	0.0	-	40	0.1	-	68	0.2	+	96	0.1	+
13	0.1	+	41	0.2	+	69	0.3	+	97	0.4	+
14	0.2	+	42	0.8	+	70	0.4	+	98	0.3	+
15	0.2	+	43	0.6	+	71	0.3	+	99	0.3	-
16	0.1	-	44	0.2	+	72	0.3	+	100	0.1	
17	0.1	-	45	0.1	-	73	0.1	-	Positive control	3.4	
18	0.2	+	46	0.1	+	74	0.3	+	Negative control	0.0	
19	0.4	+	47	0.1	-	75	0.2	+			
20	0.1	-	48	0.2	+	76	0.2	+			
21	0.3	+	49	0.3	+	77	0.2	+			
22	0.1	+	50	0.3	+	78	0.1	-			
23	0.2	+	51	0.3	+	79	0.1	-			
24	0.1	+	52	0.3	+	80	0.1	-			
25	0.1	-	53	0.1	+	81	0.3	+			
26	0.1	+	54	0.1	+	82	0.3	+			
27	0.2	+	55	0.3	+	83	0.5	+			
28	0.2	+	56	0.1		84	0.3	+			

OD: optical density, Obs: observation, S: sample.

During the present surveys conducted at the different sites in Chlef province, the presence of BSMV was noted in all surveyed fields. The symptoms were more aggressive in barley than wheat. Our results are in agreement with previous literature confirming that barley and wheat are the main cereal hosts of BSMV (Lee *et al*., 2012). Symptoms were more expressed in growing season than other development steps (Nutter *et al*., 1984).

The results of the TAS-ELISA confirmed the presence of the virus in the tested samples. In terms of species susceptibility, barley is the most susceptible followed by durum wheat and finally common wheat as the least affected species. The oat species did not react to the serological test due to its rarely infection by BSMV (Chiko, 1975), in the other hand, the BSMV is less frequently reported on wheat (Lapierre and Hariri, 2008).

In the mechanical inoculation test, the tomato variety Rio Grande showed symptoms of local and necrotic lesions due to viral infection stopping and hypersensitivity reaction of the inoculated species. These findings confirm the high response of *Solanaceae* species to the mechanical inoculation test and justify their wide use as plant hosts (Jackson *et al*., 2009). On the other hand, plants barley of the variety Rihane showed clear whitish and yellowish spots that resemble to BSMV symptoms in fields. The results of the current study are in agreement with previous reports mentioning barley as the main host of BSMV with very characteristic symptoms.

The seed samples transmission test showed similar symptoms to those observed in the field. This assay showed that BSMV is efficiently transmitted through the seed with a percentage attaining 77%. A previous study by Jackson *et al*. (2009) noticed that seeds are the most efficient mean of BSMV transmission. The present study revealed, via serological test, the presence of BSMV in the majority of the seed lots tested and confirms the role played by seeds as the most plant infection transmitter (Kılıç *et al*., 2012).

CONCLUSION

The present study, focusing on the serological surveying of BSMV, was a first attempt to examine the presence of this virus in cereal fields of Chlef province, north Algeria. This pathogen is widely distributed in different cereal fields in Algeria, and responsible for substantial losses in cereal cultures. Their visual symptoms varied from yellow or white chlorotic striping leaf necrosis.

Our findings indicated infection of most cereal varieties, typical symptoms were more expressed in spikes of plants. The TAS-ELISA revealed high virus susceptibility of barley varieties than wheat ones. The mechanical inoculation test, proved infection of totality of seed lots, and confirmed that seeds were the principal source of BSMV spreading.

Further investigations are recommended to extend BSMV surveys in terms of regions and varieties by implication of new molecular tools permitting highly specific and sensitive detection of different BSMV strains in seeds and plant material.

REFERENCES

Abd El-Aziz, M. H. 2019. Three modern serological methods to detect plant viruses. Journal of Plant Science and Phytopathology, 3: 101-106.

Adams, M. J., E. Lefkowitz and E. B. Carstens. 2011. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Elsevier.

Belkahla, H. and H. Lapierre. 2005. Serodetection of viruses associated to barley yellow dwarf (BYD) on cereals in Algeria. Phytoprotection, 80: 169-177.

Carroll, T. W. 1980. Barley Stripe Mosaic Virus: Its Economic Importance and Control. Plant Disease, 64: 136.

CGKB. 2022. Viruses-barley: Barley Stripe Mosaic Virus (BSMV). Crop Genebank Knowledge Base, Montpellier, France.

Chiko, A. W. 1975. Natural occurrence of barley stripe mosaic virus in wild oats (*Avena fatua*). Canadian Journal of Botany, 53: 417-420.

EPPO. 1983. Barley Stripe Mosaic Virus. EPPO Bulletin, 13: 1-5.

Hafez, E. E., E. E. A. Aleemand F. A. Fattouh. 2008. Comparison of Barley Stripe Mosaic Virus Strains. Zeitschriftfür Naturforschung, 63: 271-276.

Herrera, M., M. Edmundo Beratto, V. Orlando Andrade and V. Monica Madariaga. 2001. Identification of barley stripe mosaic virus (BSMV) on barley in Chile. Agricultura Tecnica, 61: 275-280.

Ilbağı, H., F. Rabenstein, A. Habekuss, F. Ordonand A. Çıtır. 2007. Incidence of virus diseases in maize fields in the Trakya region of Turkey. Phytoprotection, 87: 115-122.

Jackson, A. O., H.-S. Lim, J. Bragg, U. Ganesanand M. Y. Lee. 2009. Hordeivirus Replication, Movement, and Pathogenesis. Annual Review of Phytopathology, 47: 385-422.

Jezewska, M. 2001. Identification of Barley stripe mosaic virus in Poland. Journal of Plant Protection Research, 41(2): 164-167.

Jeżewska, M. and K. Trzmiel. 2009. Impact of Seed-Transmitted Viruses on Quality of Cereal Seeds. Journal of Plant Protection Research, 49: 460-464.

Jones, R. A. C., M. Sharman, P. Trębicki, S. Mainaand B. S. Congdon. 2021. Virus Diseases of Cereal and Oilseed Crops in Australia: Current Position and Future Challenges. Viruses, 13(10): 2051.

Kapooria, R. G. and J. Ndunguru. 2004. Occurrence of viruses in irrigated wheat in Zambia. EPPO Bulletin, 34: 413-419.

Kılıç, H., D. Altındal, N. Yardımcı and L. Akgün. 2012. Detection of Wheat streak mosaic virus and Barley stripe mosaic virus by DAS-ELISA methods in seeds of different wheat cultivars. Ziraat Fakültesi Dergisi, Uludağ Üniversitesi, 26: 17-25.

Köklü, G. 2004. Occurrence of cereal viruses on wheat in Tekirdag, Turkey. Phytoprotection, 85: 19-25.

Lapierre, H. D. and Hariri, D. 2008. Cereal viruses: wheat and barley, In: B. W. J. Mahy & M. H. V. Van Regenmortel, (eds.), Desk encyclopaedia of plant and fungal virology. Academic Press, London, UK.

Lee, W.-S., K. E. Hammond-Kosackand K. Kanyuka. 2012. Barley stripe mosaic virus-mediated tools for investigating gene function in cereal plants and their pathogens: virus-induced gene silencing, host-mediated gene silencing, and virus-mediated overexpression of heterologous protein. Plant Physiology, 160: 582-590.

Lim, C. H., I. Y. Mohammed, Y. A. Abakr, F. K. Kazi, S. Yusup and H. L. Lam. 2016. Novel input-output prediction approach for biomass pyrolysis. Journal of Cleaner Production, 136: 51-61.

Lommel, S. A. 1986. Identification of Wheat Spindle Streak Mosaic Virus and Its Role in a New Disease of Winter Wheat in Kansas. Plant Disease, 70: 964.

Nutter, D. O. 1984. Access to Care and the Evolution of Corporate, for-Profit Medicine. New England Journal of Medicine, 311: 917-919.

Sastry, K. S. 2013. Seed-borne plant virus diseases. Springer, New Delhi, India.

Seepiban, C., S, Charoenvilaisiri, N. Warin, A. Bhunchoth, N. Phironrit, B. Phuangrat, O. Chatchawankanphanich, S. Attathom and O. Gajanandana. 2017. Development and application of triple antibody sandwich enzyme-linked immunosorbent assays for begomovirus detection using monoclonal antibodies against Tomato yellow leaf curl Thailand virus. Virology Journal, 14(1): 1-14.

Wiese, M. 1987. Compendium of wheat diseases. APS Press, St. Paul, MN. Compendium of wheat diseases. 2nd ed. APS Press, St. Paul, MN, United States.

Younes, H., D. Aseel and M. Abd El-Aziz. 2018. Identification and purification of cowpea mosaic comovirus isolated from infected cowpea (*Vigna unguiculata* L.) in northern Egypt. International Journal of Agricultural Environment and Research, 4: 20.

Zarzyńska-Nowak, A., B. Hasiów-Jaroszewskaand M. Jeżewska. 2018. Molecular analysis of barley stripe mosaic virus isolates differing in their biological properties and the development of reverse transcription loop-mediated isothermal amplification assays for their detection. Archives of Virology, 163: 1163-1170.

Contribution of Authors:

Medjahed Khaldia	:	Conducting the fieldwork and experiments, drafting the manuscript,
Belkahla Hadjira	:	Research designing, conceiving and supervising the research.
Abdelaziz Merouane	:	Literature research, data analysis and discussion, revising the manuscript with input from all authors.