

# Medjahed et al.,

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1 **Survey of Barley Stripe Mosaic Virus (BSMV) in multiple cereal varieties cultivated in**  
2 **Chlef province, Northwest of Algeria**

3 **Abstract**

4 This study was designed to survey Barley Stripe Mosaic Virus (BSMV) in various cereal  
5 varieties cultivated in 28 fields cultivated in seven districts namely Boukadir, Sobha, Ouled  
6 Fares, Tadjena, Kaloul, Abou Elhacene and El Karimia located in Chlef province, North  
7 West of Algeria. The survey covered 2 barley, 2 durum wheat, 4 common wheat and one oat  
8 varieties and conducted from February to the May 2017. The BSMV was examined in plants  
9 and seeds symptomatically by mechanical inoculation and serologically by TAS-ELISA  
10 technique using specific monoclonal antibodies. The symptoms surveying noticed more  
11 expression in spike cereals. Results of serological analysis revealed 96.24% of field infection  
12 (27 from 28). The mechanical inoculation test proved infection of totality of seed lots and  
13 seeds transmission test showed efficiency of seed transmission for BSMV with a rate of 77%.  
14 In terms of species susceptibility, barley was the most susceptible followed by durum wheat  
15 and common wheat, whereas the oat was not infected. This survey investigation is the first  
16 report on BSMV naturally infecting cereals in the cereal producer zone of Chlef, the findings  
17 provide useful information on viral infection of cereal fields, thus, as a priority measurement,  
18 there is a need to reinforce control of cereal seeds.

19 **Keywords:** BSMV, Hordeivirus, Cereals, Transmission, TAS-ELISA.

20 **Introduction**

21 Cereals are subjected to several pathogens, including viruses that cause two types of diseases:  
22 yellowing viruses, represented by barley yellow dwarfing and caused by barley yellow  
23 dwarfing virus (BYDV) (Belkahla and Lappiere, 2005); The second type is the mosaic viruses

24 named: wheat streak <sup>1</sup> mosaic virus (WSMV), wheat streak mosaic virus (WSMV) and barley  
25 stripe mosaic virus (BSMV) (Jones et al., 2021).

26 The BSMV pathogen, considered as <sup>2</sup> the type member of the *Hordeivirus* genus, constitute a  
27 serious cereal pathogen, due to its efficient seed transmission and worldwide occurrence from  
28 United States (Carroll, 1980), Europe countries (Zarzyńska-Nowak et al., 2018), South Korea  
29 (Lim et al., 2016), Zambia (Kapooria and Ndunguru, 2004), Turkey (Köklü, 2004), Chile  
30 (Herrera, 2001) and Egypt (Hafez, 2008). The BSMV, characterized with rod-shape, 20 nm  
31 diameter and from 110 to 160 nm in length, prefer barley as main host, but it can infect other  
32 *Poaceae* members as well as dicotyledonous species (Adams et al., 2011). This barley virus  
33 cause enormous damages in cereal fields resulting in significant economic losses justifying its  
34 quarantine classification (until 1999) by the European and Mediterranean Plant Protection  
35 Organization (EPPO, 1983).

36 The natural spreading of BSMV is primarily a result of mechanical contact between the leaves  
37 (Jackson et al., 2009) supported with wind and rain (Slack et al., 1975). On the other hand,  
38 seeds are regarded as an important transmitter factor. In barley, for example, the seed  
39 transmission is responsible on more than 90% of infection (Maramorosch, 2012).

40 The region of Chlef, situated North West of Algeria, is known as an important cereal crops  
41 producer zone. <sup>1</sup> To the best of our knowledge, there is no report on viral diseases of cereal  
42 crops in this region especially BSMV described as a causal factor in considerable losses of  
43 yields and no information is available on their incidence. Therefore, the present study consists  
44 to survey BSMV in various cereal fields distributed in Chlef province and to determine their  
45 natural occurrence in plants and seeds.

## 46 **Materials and methods**

### 47 **Samples collection**

48 The current study was carried out in 28 surveyed fields of diverse varieties of common wheat,  
49 durum wheat, barley and oats (Table 1) distributed in seven sites named Boukadir, Sobha,  
50 Oueld Fares, Tadjena, Kaloul, Abou Elhacene and El Karimia located in Chlef province,  
51 Northern Algeria (Fig.1). The survey time of cereal fields ranged from February to the May  
52 2017.

53 At each site, random samplings of infected leaves with clear symptoms were done from cereal  
54 fields. The collected samples were stored in sterile labeled bags and transmitted to the  
55 laboratory within 24 hours.

#### 56 **Identification tests**

##### 57 **Mechanical Transmission Test**

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

62 The inoculum was applied to the upper surface of 4 healthy leaves of the tomato (variety: Rio  
63 Grande) and 2 healthy leaves of barley (variety: Rihane). The inoculated plants were then  
64 incubated at 18-22 °C during 30 days. After incubation period, the plants were serologically  
65 tested.

##### 66 **Test for virus identification in seed**

67 The identification of BSMV in seed was confirmed using ELISA technique (Kılıç et al.,  
68 2012). From each variety (Rihane, Simito, Ain Abid, Anapo and ARZ), 1000 seeds ,with clear  
69 symptoms, were finely powdered then dissolved in 10 mL of PBS-Tween Buffer solution.  
70 The solutions were held for one hour at room temperature to allow the separation of liquid  
71 phase from the sediment. The procedure for analysis of the seed samples (plate washing,

72 adding samples, addition of the enzyme conjugate and plate reveal) was made as described in  
73 the instructions of ELISA kit.

#### 74 **Seed transmission test**

75 The seed transmission test aims to reproduce the symptoms of *BSMV*. For this test, 200 seeds  
76 of barley (variety: Rihane) were sown in pots and incubated in a greenhouse conditions.  
77 Observation of symptoms started at day 30.

#### 78 **Serological test**

79 For the serological detection of *BSMV*, the TAS-ELISA technique was used following the  
80 protocol described by Seepiban et al. 2017. The specific monoclonal antibodies are diluted in  
81 100  $\mu$ L CA/10mL fixing buffer and fixed on the micro-plate, and then the plant extract was  
82 added. The *BSMV* particles are then contacted with two types of conjugated antibodies (A and  
83 B) mixed and diluted in buffer at a rate of 100 $\mu$ L conjugated CA/10 mL of buffer and coupled  
84 to the alkaline phosphatase enzyme. The double serological reaction is demonstrated by the  
85 reaction of enzyme substrate paranitrophenyl phosphate (1mg/mL), leading to a colored  
86 reaction, where the absorbance were read spectrophotometrically at 405 nm.

87

### 88 **Results and discussion**

#### 89 **Infection Symptoms**

90 The *BSMV* symptoms observed on barley were severe. These symptoms started as whitish  
91 spots at the base of the leaves which became brown streaks running along the veins to the top  
92 of leaf, followed by general chlorosis, emergence of pale yellow. On wheat varieties,  
93 symptoms were less frequent. Similar to those observed on barley, these symptoms were a  
94 diffuse or mottled yellowing developed later into more or less long brown stripes. During  
95 these surveys it was noticed that the heading stage is the most susceptible to attack where the

96 symptoms were more visible and more important. The results of ELISA test showed that 27  
97 infected samples from a total of 28 cereal fields surveyed (Table 2).

#### 98 **Test for virus identification in seed**

99 The results of the serological test for BSMV in seed showed positive reaction in four varieties  
100 including Rihane, Simito, Ainabid et ARZ. The average OD values ranged from 0.263 to  
101 0.865. The varieties Rihane and Simito showed the highest OD values (Table 3)

#### 102 **Mechanical inoculation test**

103 After four weeks of inoculation, the tomato plants showed symptoms of local and necrotic  
104 lesions. On barley plants, the appearance of whitish spots was observed. The serological test  
105 carried out on inoculated plants revealed the presence of BSMV on 30 samples of tomato and  
106 37 samples of barley. The OD values varied from 0.260 to 0.290 in tomato varieties and  
107 ranged from 0.351 to 0.475 in barley varieties (Table 4)

#### 108 **Seed transmission test**

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

#### 113 **Discussion**

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

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

124 In the mechanical inoculation test, the tomato variety Rio Grande showed symptoms of local  
125 and necrotic lesions due to viral infection stopping and hypersensitivity reaction of the  
126 inoculated species. These findings confirm the high response of *Solanaceae* species to the  
127 mechanical inoculation test and justify their wide use as plant hosts (Jackson et al., 2009). On  
128 the other hand, plants barley of the variety Rihane showed clear whitish and yellowish spots  
129 that resemble to BSMV symptoms in fields. The results of the current study are in agreement  
130 with previous reports mentioning barley as the main host of BSMV with very characteristic  
131 symptoms (Novicov and Atrabecov, 1970).

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

### 138 **Conclusion**

139 The present study, focusing on the serological surveying of BSMV, was a first attempt to  
140 examine the presence of this virus in cereal fields of Chlef province, north Algeria. The  
141 results indicated infection of most cereal varieties, typical symptoms were more expressed in  
142 spikes of plants. The TAS-ELISA revealed high virus susceptibility of barley varieties than

143 wheat ones. The mechanical inoculation test, proved infection of totality of seed lots, and  
144 confirmed that seeds were the principal source of BSMV spreading.

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

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