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Occurrence and diversity of barley yellow dwarf virus in Algeria

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RESEARCH ARTICLE

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ABSTRACT

Cereals are prone to viral infections and the economic impact of these has increased in recent years. Among these diseases barley yellow dwarf (BYD) is one of the most destructive diseases of cereals today. For three consecutive years (2014–2015–2016) surveys were carried out in order to search for BYDV species (BYDV-PAV and -MAV) as well as other cereal viruses, wheat spindle streak mosaic virus (WSSMV), southern bean mosaic virus (SBMV) and barley stripe mosaic virus (BSMV) in seven regions of Algeria (Algiers, Boumerdes, Tipaza Médéa, Adrar, Khenchla and Batna).

Targeted samples were taken randomly from plants of different cereal species (wheat, barley, oats). The sample were analyzed by DAS-ELISA and RT-PCR.

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The results of ELISA and PCR tests showed the presence of BYDV-PAV in barley, durum wheat, soft wheat and oats. Thus, this viral species were found in all the cereal regions surveyed (North, South, East and West).

Seven samples (durum wheat, barley and oats) were sequenced and phylogenetic analyses performed revealed that the Algerian sequences clustered in group I and group II.

KEYWORDS

cereals, DAS-ELISA, RT-PCR, BYDV-PAV

INTRODUCTION

Cereal farming in Algeria occupies a strategic place in the national economy and constitutes the backbone of the Algerian food system. The variability of production can be attributed to several abiotic (Feliachi, 2000) and biotic factors such as plant pathological agents in general and viruses whose economic impact has increased in importance in recent years (Astier et al., 2001).

Therefore, a study on the identification of the main viral diseases of cereals is important, in particular the dwarf yellows disease of barley (BYD). It is the most important viral disease of cereals worldwide and is one of the most destructive diseases of cereals today. The causative agent is barley yellow dwarf virus (BYDV), which belongs to the *Luteovirus* genus in the *Luteoviridae* family. These viruses have viral particles composed of isometric capsids of 24–30 nm. The viral genome is a positive-sense single-stranded RNA 5.5–6 kilobases (kb) long. Virions are transmitted by aphids in a persistent circulating non-propagating manner (Kaddachi et al., 2014).

Based on transmission assays the International Committee on Taxonomy of Viruses describe ten virus species of the barley yellow dwarf group belonging to the family *Luteoviridae*. The species BYDV-PAV, BYDV-MAV, BYDV-PAS, BYDV-kerII, BYDV-kerIII belong to the genus *Luteovirus*, The species in the genus *Polerovirus* are cereal yellow dwarf virus (CYDV-RPV, CYDV-RPS). The species maize yellow dwarf virus (MYDV-RMV), BYDV-SGV, and BYDV-GPV are not yet assigned to a genus (Walls et al., 2019).

Several studies have highlighted the predominance of the BYDV-PAV virus on cereals compared to other viruses in several countries: Algeria, Morocco, Tunisia, Turkey and China (Belkahla and Lapierre, 1999; El Yamani and Bencharki, 1996; Najar et al., 2017; Karaozan and Usta, 2020; Khine et al., 2020).

BYDV –PAV is transmitted efficiently by the aphids *Rhopalosiphum padi* and *Sitobion avenae* (Namara et al., 2020; Parizoto et al., 2013).

Barley stripe mosaic virus (BSMV), of genus *Hordevirus*, is a plant pathogen whose main hosts are barley and wheat. Virions normally contain three positive sense ssRNAs. The RNAs are designated α, β and γ (Hull, 2014). Wheat streak mosaic virus (WSMV) causing wheat streak mosaic, is a monopartite, positive-sense, single-stranded RNA virus and the type member of the genus *Tritimovirus* in the family *Potyviridae* (Singh et al., 2018). SBMV is a member of *Sobemoviridae* family with single-stranded positive-sense genomic RNA of approximately 4,100 nucleotides (Verhoeven et al., 2003).

BYDV and the other BYD viruses cause symptoms of leaf yellowing on barley, stunting, reduction of internodes (Belkahla, 2001) and variation in leaf color depending on the species and varieties of cereals infected (Adhikari et al., 2020).

Several diagnostic tools have been applied for detection of these viruses, the most common are ELISA (Belkahla and Lapierre, 1999) and RT-PCR of the coat protein (CP) gene (Malmstrom and Shu, 2004). The objective of this study is to evaluate the occurrence of the BYD disease in different cultivated species (barley, wheat and oat) from seven different regions of Algeria using serological test and molecular tools to assess the genetic diversity of the viruses causing BYD.

MATERIALS AND METHODS

Prospections

The survey of viral symptoms in barley, hard wheat, soft wheat and oats, in different regions of Algeria revealed two types of traits: yellowing and reddening. The yellowing symptoms were more important on barley. While, reddening symptoms were detected on wheat (soft and hard) and oats (Fig. 1).

Sampling

Sampling was carried out randomly for three consecutive years 2014–2015–2016 in nine regions of Algeria (East, West, North and South): Algiers, Boumerdes, Tipaza, Adrar, Khenchela, Batna, and Médéa. Samples were taken from several species of cereals: *Triticum durum* (hard wheat),

Fig. 1. Symptom of BYDV on wheat, barley and oat (A: Yellowing symptom on barley, B: Reddening symptom on oat and C: Reddening and yellowing symptom on wheat)

Triticum aestivum (soft wheat), *Hordeum vulgare* (barley), and *Avena sativa* (oats) and during different phenological stages, from tillering to heading.

Symptomatic leaves were kept in a refrigerator at −80 °C and stored until further analysis, which meant that the samples were dried using anhydrous calcium chloride ($CaCl_2$).

A sample is consisted of 10 leaves per plant. They were collected across the grower's farm in all directions randomly. Thus, a total of 2,680 samples were collected and combined per 10 in each field for testing and on different cereal species according to the sampling method described by Cadot et al. (2014) (Supplementary data 1). Some collected samples presented symptoms.

Serological tests

The samples collected were tested by the DAS- ELISA method (Clarck and Adams, 1977) using polyclonal antibody for the viruses BYDV-PAV and BYDV-MAV (AGDIA, Elkhart, IN, USA) and for the viruses WSSM, SBWMV and BSMV the kits were purchased from SEDIAG (France).

The samples taken were analyzed in groups of 10, meaning one leaf from 10 different plant as formerly described by Cadot et al. (2014).

RT-PCR for detection of BYDV-PAV

Nine samples were preserved by the Bos method belonging to different species of cereals (hard wheat, soft wheat, barley and oats) were taken from three different regions (Algiers, Tipaza and Médéa).

The extraction of RNA was carried out by the RNeasy Plant Mini kit. from Qiagen (Qiagen, USA) according to the manufacturer's instructions. The cDNA for RT-PCR was synthesized using a total volume of 16 µL with 2 µL of RNA, 1 µL of random primer (100 µM) and 13 µL nuclease-free water were incubated at 65 °C for 5 min the first step. The cDNA for RT-PCR was synthesized in a total volume of 20 µL with 2 µL of 10 × RT buffer with DTT, 0.5 µL of dNTP MIX (20 mM of each), 1 µL FIREScript RT, 0.5 µL RiboGrip RNase Inhibitor (10 U μL^{-1}) and 16 µL of the mix of the first step according to the manufacturer instruction (Solis BioDyne, Tartu, Estonia). The mixtures were incubated at 37 °C for 60 min and 70 °C for 10 min.

PCR was carried out using the primer pairs BYDVF (5′-GTTCTGCCTCAACATCGGAT -3′) and BYDVR (5′-AATAGGTAGACTCCTCAACA -3′) amplifying a 740 bp fragment of the CP gene (Ratsgou et al., 2005) using a S1000 Thermal Cycler (Bio-Rad, USA). The PCR program was as follows: initial denaturation 95 °C for 12 min; 40 cycles at 95 °C for 30 s, 55 °C for 45 s and 72 °C for 60 s; final extension at 72 °C for 5 min. After the cycles ended the reaction mixture was cooled to 4 °C. Nine isolates were sequenced with Sanger method, the sequencing was done in one direction. The sequence were deposited in NCBI in order to be accessioned.

Nucleotide sequence analyses

A comparison at the nucleotide level was performed for the sequenced isolates and those retrieved from NCBI GenBank (Supplementary data 2) using MEGA11 (Tamura et al., 2021). An alignment was created of 112 sequences of 632 nt length with ClustalW method (Thompson et al., 1994) and nucleotide and amino acid pairwise percent identity was calculated for the Algerian sequences using a program implemented in MEGA 11 (Tamura et al., 2021).

Phylogenetic analyses

The phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstraps (Saitou and Nei, 1987). Percent of identity between and within groups were calculated for the phylogenetic tree. All these analyses were carried out with the software MEGA 11 (Tamura et al., 2021).

RESULTS

Serological test

The serological screening of the collected samples from different cereal species (barley, hard wheat, soft wheat and oat) allowed to demonstrate the presence of BYDV-PAV in all cereal regions (North, South, East and West). Serological tests indicated that samples showing symptoms of yellowing in barley, redness in wheat (hard and soft) and oats were infected with the barley dwarf yellows virus BYD-PAV. All tested samples were negative to BYDV-MAV, BYDV-PAM, BSMV, WSSMV, SBWMV.

Oats and barley seemed to be the most infected plants with 85 and 86% occurrence, respectively. In comparison to soft wheat and hard wheat with 31 and 44% rate of infection, respectively. The results also revealed that BYDV is present in all cereal regions of Algeria. Thus, for Algiers and Boumerdes located in the central region, the results showed 82 and 64% occurrence, respectively. While Médéa and Tipaza, region situated in the west, showed 23 and 32% occurrence. Khenchla and Batna regions in the east revealed 64 and 67% occurrence, respectively. The region of Adrar in the south presented 67% rate of infection (Table 1). However, the mosaic symptoms observed may be due to other unidentified viruses.

Nucleotide and amino acid comparison

Seven Algerian samples positive in DAS-ELISA test (from Algiers, Tipaza and Médéa on wheat, barley and oat) generated amplicons of the expected size (740 nt) using specific BYDV-PAV primers, they were sequenced and deposed in NCBI under the accession numbers OU595691-OU595697.

BAST search (https://www.ncbi.nlm.nih.gov/) revealed that Algerian sequence presented 88–91% identity to the isolate BYDV-PAV (X07653). Comparison at nucleotide and amino acid identity revealed that the isolate sequenced ranged from 89 to 100% for nucleotide identity and from 96 to 100% for amino acid identity. The results revealed that sequences provided from

Table 1. Occurrence of BYDV-PAV in Analyze samples group

Species	Nbr of samples	Nbr of group analyzed	Nbr of positive group (BYDV-PAV)	Percent of positive groups (BYDV-PAV)
Barley	210	21	18	86%
Hard wheat	1980	198	87	44%
Soft wheat	360	36	11	31%
Oat	130	13	11	85%

different species present a high identity, thus, the isolate ALG1 presented 92% identity to the isolate ALG2 and 97% identity to the isolate ALG3 from wheat. It presented also 97% nucleotide identity with the isolate ALG4 from oat and 90% with the other isolates. These results indicated the absence of specific host variant of BYDV. Therefore, the same variant may infect several species among the family of *Poacea*. The isolate ALG3 and ALG4 revealed to be the more divergent with 89–90% nucleotide percent identity but they presented 96–97% amino-acid identity (Table 2).

Phylogenetic analyses

Phylogenetic tree performed in this study using all sequences of CP and RTD region revealed that the virus BVYD serotype PAV clustered into five distinct clusters. In order to assess the relationship within and between the designed groups, the within percent identity was calculated and revealed 93, 92, 98, 96 and 95% respectively for the Group I, Group II, Group III, Group IV and Group V (Table 3). These results revealed the strong relationship in the same group. However, the between-group percent identity ranged between 79 and 87% revealing a clear demarcation between groups (Table 4).

Table 2. Nucleotide percent identity (lower-left) Amino-acid identity (upper-right) of the Algerian sequence

ALG1	100%	98%	99%	99%	97%	98%	98%
ALG2	92%	100%	97%	97%	100%	100%	100%
ALG3	97%	91%	100%	100%	96%	97%	97%
ALG4	97%	91%	100%	100%	96%	97%	97%
ALG7	90%	97%	89%	89%	100%	100%	100%
ALG12	90%	97%	90%	90%	99%	100%	100%
ALG13	90%	97%	89%	89%	99%	98%	100%

Table 3. The Within group percent identity of the designed tree

I	93%
II	92%
III	98%
IV	96%
V	95%

Table 4. The between group percent identity of the designed tree

I				
II	81%			
III	89%	82%		
IV	81%	79%	82%	
V	87%	80%	88%	82%

The phylogenetic tree constructed with 112 BYD-PAV of a 632 nt fragment from the CP gene region revealed that the Algerian isolates clustered under the Group I with isolate ALG2, ALG7, ALG12 and ALG13 and in the Group III with the isolate ALG1, ALG3 and ALG4.

The tree revealed the absence of correlation between the BYDV- PAV isolates distribution and the natural host (wheat, oat, barley). Hence the cluster presented sequences isolated from wheat, barley and oat collected from different geographical regions. Same result was obtained for the other groups (Fig. 2).

DISCUSSION

The survey of viral symptoms in barley, hard wheat, soft wheat and oats, in different regions of Algeria revealed two types of symptoms: yellowing and reddening. Barley and oat presented the most intense symptoms and affected the majority of plants in the field. Indeed, most of the yellowing symptoms were observed on barley. While, reddening symptoms were observed on wheat (soft and hard) and oats.

Samples presenting a golden yellowing, reddening and dwarf in wheat, barley and oat were tested by DAS ELISA and revealed the presence of BYDV-PAV confirming the presence of this virus in these plants. Oswald et al. (1953) reported the same symptomatic description of BYDV infection by yellowing and/or reddening of leaves, stunted growth, and reduced root biomass.

In this study, prospections indicated the presence of BYD symptoms on wheat, barley and oat in different regions of Algeria. This was confirmed by DAS-ELISA tests specifically for the BYDV-PAV. Indeed, the BYDV can infect several species from the *Poacea* family.

Using DAS-ELISA on symptomatic samples allowed the detection of BYDV-PAV in different host species through different regions of Algeria with a large predominance.

However, samples can also be symptomless (Thackray et al., 2005). In our study, some symptomless samples were revealed to be infected by BYDV-PAV.

Results obtained revealed the absence of BYDV-MAV, BSMV, WSSMV and SBWMV indicating that the major symptoms observed are caused by the presence of BYDV-PAV. El Yamani and Bencharki (1996) reported a significant occurrence of BYDV-PAV. Najar et al. (2017) reported that BYDV-PAV is the most predominant in Tunisia with 66% occurrence. In Algeria, BYDV was previously reported as the most predominant serotype (Belkahla and Lapierre, 1999). Adhikari et al. (2020), found that 70% of positive samples to BYD belonged to BYDV-PAV.

The large occurrence of BYDV-PAV may be due to large propagation of its vector, *R. padi* observed during our surveys. The majority of the vector of BYDV were reported in Algeria (Belkahla and Lapierre, 1999; Bakroune et al., 2020).

The comparison at nucleotide and amino acid levels revealed that Algerian sequences had from 89 to 100% nucleotide identity and from 96 to 100% amino acid identity through their assessed regions. The high level of amino acid identity in comparison to nucleotide identity is due to the presence of silent mutations.

The isolate ALG1 presents 92% identity to the isolate ALG2 and 97% identity to the isolate ALG3 from wheat and 97% nucleotide identity to the isolate ALG4 from oat and 90% to the other sequences. These results indicate the absence of BYDV host specificity and the same serotype may infect several species among the family of *Poaceae*. The isolates ALG3 and ALG4 revealed 89–90% nucleotide percent identity but they presented 96–97% amino-acid identity, this difference in nucleotide and amino acid identity is due to the presence of silent mutations.

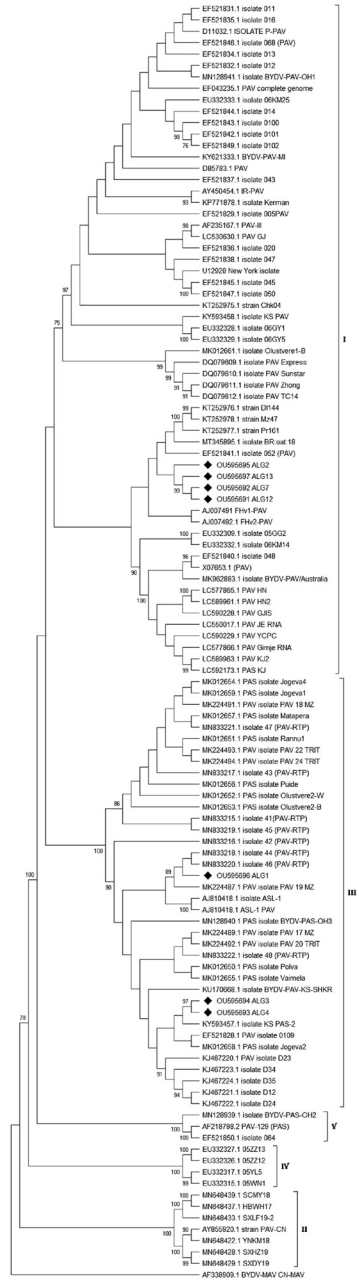

Fig. 2. Phylogenetic tree of 112 Barley Yellow Dwarf Virus-PAV of a 632 nt fragment from the CP gene region, Percentage of bootstrap support (≥75%) from 1,000 replicates. Barley Yellow Dwarf Virus-MAV (GenBank accession number AF338909.1) was used as an outgroup. (Algerian sequences were indicated by ◆)

Phylogenetic tree obtained in this study revealed new groups that were not previously described. Working on all sequences available allowed more visibility on the diversity of this species. Results revealed that isolates from each groups present a great relationship and an important distance between groups.

It is important to make research on local cereal varieties and weed of the *Poacea* family from different geographical regions in order to set more efficient phylogenetic analyses.

The analysis of the CP gene sequences among field isolates collected from different regions showed that Algerian isolates where clustered separately in two clades, independently of their hosts. Our results will be helpful to understand the spread of the disease in Algeria and to provide information for its control.

SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1556/038.2023.00172.

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