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The Occurrence of Grapevine Rugose Wood Disease in Algeria

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Rugose wood disease constitutes one of the major grapevine disease complexes causing significant economic damage worldwide. It is widely distributed in all grapevine growing areas of the world and comprised of four individual syndromes, which may be caused by different viruses. These syndromes are Corky bark, LN 33 stem grooving, Kober stem grooving and Rupestris stem pitting (RSP). The present study focuses on the prevalence of three viruses associated with rugose wood complex (RWC) in Algeria.

Field inspections and collection of symptomatic samples were conducted on autumn 2012 in the table wine and autochthone accession in the western and central regions of Algeria. A total of 202 samples were tested by RT-PCR using specific primers for *Grapevine virus A* (GVA), *Grapevine virus D* (GVD) and *Grapevine rupestris stem pitting associated viru*s (GRSPaV).

The results of RT-PCR indicated the presence of the viruses GVA, GVD and GRSPaV with 68,81% (139 out of 202 infected samples) total average infection rate. The results also indicated the predominance of GRSPaV compared to the prevalence of GVA and GVD with an infection rate of 57,92% vs. 36,63% (74 out of 202) and 2,97% (6 out of 202), respectively. Mixed infections of these three viruses were not observed in any of the samples analysed, however the mixed infection of GVA and GRSPaV was noted with a high rate of 26.73%. The grapevine cultivars; Kings Rubi, Carignan and Mersguerra were the most infected, while the Alicante Bouschet cultivar presented the lowest infection rate. To the best of our knowledge, the present study reports for the first time on the presence of GVD in Algeria.

Keywords: GRSPaV, GVA, GVD, RT-PCR.

Rugose wood complex (RWC) is one of the most widespread graft-transmissible diseases of grapevines (Martelli, 1993), it is comprised of several disease syndromes (Grapevine Rupestris stem pitting, Kober stem grooving, Corky bark, LN33 stem grooving) (Martelli, 2014, 2017). It is caused by a complex composed of six viruses belonging to the family of *Betaflexiviridae*; *Grapevine virus* A (GVA), *Grapevine virus* B (GVB), *Grapevine virus* D (GVD), *Grapevine virus* E (GVE), *Grapevine virus* F (GVF) and *Grapevine rupestris stem pitting-associated virus* (GRSPaV) (Nakaune et al., 2008; Maher Al Rwahnih et al., 2012; Alabi et al., 2013). It causes a delayed bud opening in spring, after a few years of planting some grapes decline and die and others present a

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swelling (Martelli, 2014). Some of these viruses were found to be transmissible by mealybugs (La Notte et al., 1997).

In several cases, plant material infected by viruses is the most effective way of disease propagation at short and long distances. Indeed, the vegetative propagation of shoots taken from infected cultivars plays an important role in the spread of the RWC disease. For this reason, it is important to use healthy mother vines, rootstocks and grafts (Galet, 1977).

In addition, studies on the vectoring of rugose wood disease in vineyards have led to the identification of several species of mealybugs, insects which belong to the families of *Pseudococcidae* and *Coccidae* and involved in the transmission of GVA, GVB and GVE : GVA and GVB are transmitted by *Phenacoccus aceris, Planococcus citri, Planococcus ficus* (Rosciglione and Castellano, 1985; Tanne et al., 1989)*, Pseudococcus affinis, Pseudococcus longispinus* (Rosciglione et al., 1983; La Notte et al., 1997) *Pseudococcus viburni* (Garau et al., 1994). *Pseudococcus comstocki, Heliococcus bohemicus* (Zorloni et al., 2006*), Parthenolecanium corni (Coccidae)* (Hommay et al., 2008), and *Neopulvinaria innumerabilis (Coccidae)* (Zorloni et al., 2006). Often, GVA, GVB and GVE transmission occurs simultaneously with *Grapevine leafroll-associated virus-1* and *Grapevine leafroll-associated virus-3* (GLRaV-1 and GLRaV-3) (Herrbach et al., 2016). No vector has been identified for GVD, GVF and GRSPaV (Le Maguet et al., 2012).

Some vectors of GVA and GVB were described in Algeria in several vineyards. Thus, the dynamics of *Planococcus ficus* was studied in vineyards from the west of Algeria (Bissaad et al., 2017).

Few studies were focused on the importance and widespread of this disease. It is essential to know whether this disease is prevalent in Algeria, in order to set up research on the diversity of the causal viruses and their vectors in Algeria. Viruses associated with the rugose wood disease were reported previously in Algeria, based on analyses of samples collected from a limited geographical area (Lekikot et al., 2012). Thus the main objective of this study is the description of the occurrence of several viruses implicated in the RWC disease (GRSPaV, GVA and GVD) and the search of the presence of additional RWC-associated viruses.

Materials and Methods

Field surveys and sample collection

In order to study the occurrence of Rugose Wood virus in Algeria, a total of 202 samples were used to study the occurrence of GVA, GVD and GRSPaV. Sample collection was conducted during the autumn of 2012 from the center and western regions of Algeria containing commercial and autochthonous cultivars (Table 2).

Molecular analyses
Total nucleic acid extraction

Total nucleic acids (TNA) were extracted using 0.2 g of phloem tissues (cortical scrapings) from each sample according to Foissac et al. (2001). The samples were ground

in 1 ml extraction buffer (4 M guanidine thiocyanate, 0.2 M NaOAc pH 5.2, 25 mM EDTA, 1.0 M KOAc pH 5.0 and 2.5% w/v PVP-40) and mixed with 2% sodium metabisulfite as antioxidant. The mixture was transferred into an Eppendorf tube containing 100 µl Sodium Lauryl Sarkosyl (NLS 10%) and incubated at 70 °C for 10 min, then placed on ice for 5 min. After centrifugation at 13,000 rpm for 10 min, 300 µl of supernatant were transferred to an Eppendorf tube to which 150 µl absolute ethanol, 300 µl 6 M Nal and 50 µl SiO_2 (12% with PH 2) were added. The mixture was stirred for 30 min at room temperature and then centrifuged at 6,000 rpm for 1 min. The pellet was recovered and washed with 500 µl of washing buffer (50% STE 1X with PH 7,5, 50% absolute ethanol), re-suspended in 120 µl of sterile distilled water, incubated for 3 min at 70 °C and then centrifuged at 13,000 rpm for 3 min. The supernatant containing the total nucleic acids was transferred to new Eppendorf tubes and stored at –20 °C.

Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed for the detection of *Grapevine virus A* (GVA), *Grapevine virus D* (GVD) and *Grapevine rupestris stem pitting-associated virus* (GRSPaV) by using the specific sets of primers listed in (Table 1).

Table 1

Primers used for the detection of the viruses associated with rugose wood disease

Virus	Primers	Sequences	Amplified product (bp)	Reference
GVA	H7038 C7273	AGGTCCACGTTTGCTAAG CATCGTCTGAGGTTTCTACTAT	236	Mackenzie (1997)
GVD	CP7V CP471C	CTTAGGACGCTCTTCGGGTACA CTGCTCTCCAACCGACGACT	474	Abou-Ghanem et al. (1997)
GRSPaV	RSP-H48 RSP-C49	AGCTGGGATTATAAGGGAGGT CCAGCCGTTCCACCACTAAT	331	Lima et al. (2006)

Reverse transcription and amplification

TNA (10 µl) of each sample was mixed with 1 µl random primers (1 µg/µl) and 1.5 µl of sterile water and denatured at 95 °C for 5 min. Reverse transcription was run for 1 h at 39 °C in 1 µl M-MLV (200 u/µl) (In-vitrogen Corporation), 4 µl buffer (5X Fs), 2 µl DTT (0.1 M) and 0.5 µl dNTPs (10 mM) and adjusted to a final volume of 25 µl with sterile distilled water. A volume of 2.5 µl of the synthesized cDNA was used for PCR amplification using a mixture containing 2.5 µl 10X Taq polymerase buffer, 1 µl $MgCl_2$ (50 mM), 1 µl dNTPs (10 mM), 0.5 µl of each primer (20 µM) and 0.25 µl Taq polymerase (5 u/µl) (Invitrogen Corporation, CA, USA) and adjusted to a final volume of 25 µl with sterile distilled water. PCR reactions consisted of one cycle at 94 °C for 5 min, followed by 35 cycles: denaturation at 95 °C for 30 s, annealing at (52 °C/45 s, 54 °C/45 s, and 52 °C/45 s for GVA, GVD and GRSPaV, respectively) and elongation at 72 °C for 1 min, and a final extension step at 72 °C for 7 min. The PCR products were analyzed by electrophoresis in 1.2% agarose gels in 1×TBE buffer and visualized under UV light after staining with Ethidium bromide.

Results

Virus detection and distribution

All samples collected from the central and western regions of Algeria were tested by RT-PCR for the presence of GVA, GVD and GRSPaV. Results revealed the presence of the three viruses in Algerian vineyards. Out of 202 samples, 139 (68.81%) were infected by at least one of the above-mentioned viruses. GRSPaV was the most prevalent with an infection rate of 57.9% (117 out of 202) followed by GVA with 36.6% (74 out of 202) infection rate. GVD was detected for the first time in Algeria in 6 samples out of 202 (2.97% infection rate). Furthermore, cultivar Kin's Rubi was found totally infected by GRSPaV, followed by Carignan with 90%. The lowest rate of GRSPaV infection was found in the cultivar Alicante Bouschet (35.7%).

The highest infection rate was found in the autochthonous grapevine collection of the Institut Technique de l'Arboriculture Fruitière et de la Vigne (ITAF) with 78.3% followed by the wine and table grapes which presented approximately equal prevalence. The table grape King's Rubi was most infected (100%) followed by the wine cultivar Carignan with 90%. The wine cultivar Alicante Bouschet was the least infected (35.7%).

Results also revealed that the mixed infection of the GVA and GRSPaV viruses was the highest with a rate of 26.73%. However, the mixed infection GVA and GVD showed a very low rate (0.1%). Only one sample was reported in mixed infections between GVD and GRSPaV (0.05%). We did not record any mixed infections of the three viruses GVA, GVD and GRSPaV in all samples studied (Table 3).

Discussion

Only a few studies have addressed the presence of the Rugose Wood Complex (RWC) virus disease in Algeria, although GVA and GVB have been reported previously by Lekikot et al. (2012). Therefore, the present study focused on the prevalence of two other grapevine viruses, GVD and GRSPaV. Thus, this is the first study that shows the presence of these two viruses associated with RWC in Algeria.

Previously, Lehad et al. (2015) considered the grapevine leafroll disease (GLD) as the most prevalent grapevine disease in Algeria with an infection rate of 55.7%. However, our results highlight that RWC seems to be more prevalent than GLD with an infection rate of 68.8% (Table 2). The three prospected viruses (GVA, GVD and GRSPaV) were found to occur with different infection rates, with GRSPaV being the most prevalent.

Results obtained revealed the presence of GRSPaV, GVA and GVD in Algeria with differences in their prevalence. For GVD, 6 out of 202 samples in the cultivars Gros Noir, Dattier de Beyrouth, Muscat, Cardinal and the autochthonous grapevine germplasm collection of ITAF were found to be infected (2.97% infection rate). Up to now, no insect vectors for GVD were reported. Several countries have reported the presence of this virus (GVD) but with higher infection rates. In Tunisia, the virus was found to occur with an infection rate of 31.5% (Selmi et al., 2017), in Italy, an infection rate of 31% was documented (Boscia et al., 2001). On the other hand, the GRSPaV was found in this study to be

Table 2
Infection rates of samples tested for the presence of GRSPaV, GVA and GVD

	Cultivar	No. of samples	Regions	Infection %	GVA %	GVD %	GRSPaV %
Table cultivars	Gros noir	50	Algiers, Mascara, Tizi-Ouzou	64 (32/50)	38	4	52
	Dattier	35	Algiers, Mascara, Ain Temouchent, Boumerdes	62.86 (22/35)	25.71	2.86	57.14
	Muscat	25	Tizi Ouzou	76 (19/25)	36	4	68
	Cardinal	22	Boumerdes	63.63 (14/22)	22.73	4.55	59.09
	Kings Rubi	8	Boumerdes	100 (8/8)	100	0	62.5
Wine cultivars	Alicante Bouschet	14	Ain Temouchent	35.71 (5/14)	35.71	0	35.71
	Carignan	10	Mascara	90 (9/10)	20	0	80
	Valensi	8	Ain Temouchent, Mascara	76 (6/8)	0	0	75
	Mersguerra	7	Ain Temouchent	85.71 (6/7)	71.43	0	57.14
Autochthones	autochthone	23	Mascara	78.26 (18/23)	52.17	4.35	56.52
Total		202		68.81% (139/202)	36.63% (74/202)	2.97% (6/202)	57.92% (117/202)

Table 3
Rates (%) of the mixed infections of vine samples by the different viruses associated with rugose wood disease

Vitivirus	GVD	GRSPaV	GVA
GVD	/	0.05% (1/202)	0.1% (2/202)
GRSPaV		/	26.73% (54/202)
GVA			/

the more prevalent virus in Algeria with an infection rate of 68.8%. In Tunisia, this virus (GRSPaV) was found to be also prevalent with an infection rate of 51.3%, while for South Africa an infection rate of 36.8% was reported (Jooste et al., 2015). In addition, infection rates for GRSPaV previously documented for different countries are as follows: Portugal (44%) (Digiaro et al., 1999), Spain (49%) (Fiore et al., 2016), Italy (74%) (Digiaro et al., 1999), Kosovo (80.4%) (Dida et al., 2012). Thus the virus (GRSPaV) may be considered as the most predominant grapevine virus reported up to now in Algeria (infection rate: 68.8%.). In contrast, Lehad et al. (2015) reported that in Algeria GLRaV-3 has a prevalence (infection rate) of only 44%.

GVA was reported to be significantly prevalent in Tunisia (infection rate: 47.9%) (Selmi et al., 2017), Lebanon (32.4%) (Haidar et al., 1996), Italy (41%) (Digiaro et al., 1999), Turkey (55%) (Digiaro et al., 1999), Palestine (66.1%) (Alkowni et al., 1998) and Egypt (67.9%) (Ahmed et al., 2004). On the other hand, the virus was reported to be less prevalent in other countries, e.g. in South Africa (infection rate: 19.3%) (Jooste et al., 2015), Malta (12%) (Digiaro et al., 1999), Kosovo (11.1%) (Dida et al., 2012), Portugal (6%) (Digiaro et al., 1999), Russia (6%) (Porotikova et al., 2016) and China (4.7%) (Fan et al., 2013).

Importantly, the mixed infection by GVA and GRSPaV was quite considerable with an infection rate of 26.73%. Similarly, previous research has reported the association of

GVA and GRSPaV in several other countries. For example, in Tunisia, this mixed infection was found to occur with an infection rate of 56.8% (Selmi et al., 2017). In Italy and Spain mixed infections between these two viruses were also reported (Fiore et al., 2016; Sabella et al., 2018). These results showed that there is a positive correlation of prevalence between these two viruses.

The mealybug *Planococcus ficus* (Signoret, 1875) reported as a vector for RWC viruses was signalled in Algeria (Bissaad et al., 2017). The presence of vectors of this disease may explain the large distribution of this disease in Algeria.

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