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Detection and molecular characterization of Egyptian isolates of grapevine viruses

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Summary. – Selected commercial and/or local vineyards and nurseries in three different governorates of Egypt (Alexandria, El-Beheira and El-Menofia) were surveyed for symptoms indicative of infection by grapevine viruses. Leaf samples from red-fruited and white-fruited *Vitis vinefera* were tested for grapevine leafroll associated viruses (GLRaV-1, GLRaV-2, and GLRaV-3), grapevine viruses A and B (GVA, GVB), grapevine rupestris stem pitting virus (GRSPaV), grapevine fanleaf virus (GFLV), and grapevine fleck virus (GFKV) from early April to late October 2010. Incidence of these viruses was assessed by RT-PCR in 60 different samples. Selected amplicons were sequenced. While GVA was the most wide spread (30%), GLRaV-1, GVB, GFLV, and GFKV were not detected during the survey. However, GVA, GLRaV-2, GLRaV-3, and GRSPaV were detected in the form of single infection or in mixed infections of 2 to 4 viruses. Phylogenetic analysis was performed on all Egyptian isolates of GLRaV-2 (4), GLRaV-3 (7), GVA (3), and GRSPaV (6). GRSPaV was detected for the first time in Egypt. Phylogenetic analysis provided insights into the evolutionary relationship between the reported Egyptian isolates and other previously reported isolates.

Keywords: Egypt; grapevine viruses; ELISA; RT-PCR; detection; sequence analysis

Introduction

Grapevine (*Vitis* spp.) is economically one of the most important cultivated fruit species in the world, is one of the oldest horticultural crops widely grown in temperate climates and represents a highly valuable agricultural commodity (Engel *et al*., 2010).

In Egypt, grapevine (*Vitis vinefera*) is of great importance and plays an important role in agricultural economy; the annual production is 1.1 million tones of mainly table grapes and dried fruit (Youssef *et al*., 2009). With an area of about 62,000 ha, which yielded 1,104,000 tons in 2004, and the total area of production of approximately 65,000 ha in 2005, the grapevine (*V. vinifera*) ranks second among the fruit crops of Egypt (Ahmed *et al*., 2004; Youssef *et al*., 2009; Fayek *et al*., 2009). Vineyards are concentrated along the Nile valley and in recently reclaimed desert land. The most important governorates cultivating grapes are Beni-Swef, El-Beheira, Giza, Minofia, and Qualubia (Youssef *et al*., 2007). Table-grape cultivars are the most widely grown, with a prevalence of the traditional local cultivars (Banaty Abiad and Romy Ahmer), although a significant introduction of foreign cultivars, mainly seedless (Fantasy, Flame, King's Ruby and Superior), has taken place in recent years. There are more than 70 infectious agents including viruses, viroids and phytoplasmas that have been reported in grapevines. Among them, at least 58 are viral pathogens, some with extremely high incidences (Martelli and Boudon-Padieu, 2006). Some important virus diseases are caused by GFLV, GFkV, GVA, GVB, and grapevine leafroll-associated viruses. ELISA method can be used for testing multiple plants for

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Abbreviations: GFLV = grapevine fanleaf virus; GFKV = grapevine fleck virus; GLRaV-1, GLRaV-2, and GLRaV-3 = grapevine leafroll associated viruses; GRSPaV = grapevine rupestris stem pitting virus; GVA, GVB = grapevine viruses A and B; HSP70 gene = heat shock protein 70 gene; LRD = leaf roll disease

a single virus using one well per plant sample, or alternatively a single plant can be simultaneously tested for many viruses on a single plate with different antibodies coated to each well in duplicate or triplicate for reproducibility (Craig *et al*., 2004). PCR technology permits the detection of viruses at the levels several orders of magnitude lower than is possible by other methods. This high sensitivity facilitates virus detection during early stages of infection of plants, in soil and in their vector samples (Fenby *et al*., 1995). RT-PCR is a very sensitive and reliable molecular methodology, which has been developed and used for the detection of many plant RNA viruses (Rowhani *et al.,* 2005). In this study, we developed RT-PCR detection methodology for the detection of GLRaV-1, GLRaV-2, GLRaV-3, GVA, GVB, GRSPaV, GFLV, and GFKV, in addition to partial sequence characterization of the Egyptian isolates of the detected viruses.

Materials and Methods

Field surveys. A survey and collection of samples from different commercial or local vineyards and nurseries in different selected governorates of Egypt, namely Alexandria, El-Beheira, and El-Menofia, representing old and recently reclaimed land, was conducted during the period between early April and late October 2010. Survey was for the detection and assessment of the incidence of major grapevine viruses: GVA, GVB, (GLRaV-1, -2 and -3), GRSPaV, GFLV, and GFKV. Leaf samples, including the petioles and midribs showing various types of virus and virus-like symptoms related to leafroll disease symptoms (LRD) were collected for laboratory testing. A total of 60 *V. vinifera* samples, representing cultivars Flame seedless (red-fruited cultivar), Thompson seedless and Superior (white-fruited cultivars), and rootstocks (Freedom) were collected. All samples were labeled, wrapped in plastic bag and stored at +4°C until use for laboratory analysis after the collection time (April to late October). Detection was performed on two levels: symptomatology and molecular identification.

Preparation of total RNA extracts. Total RNA was extracted from leaf samples using ILLUSTRA RNAspin Mini RNA isolation kit (GE Healthcare, UK), according to the manufacturer's instructions and the procedure was as described by MacKenzie *et al.* (1997). The total RNA was stored at -20°C for one day.

RT-PCR analyses. For the synthesis of the first-strand cDNA of all detected grapevine viruses, 1 µl total RNA was added to 4 µl reverse transcriptase mix containing 1x M-MLV buffer (Promega), 0.5 µg random primer (Roche), 1 mmol/l dNTP mix (Promega) and 50U M-MLV reverse transcriptase (Promega). The mixture was incubated at 42°C for 1 hr, then at 70°C for 10 min, and held at +4°C in the PCR thermocycler (Gene Amp 9700 thermocycler, Applied Biosystem ABI, USA). cDNA was amplified by PCR in a reaction mixture (25 µl final volume) containing 1x Mg-free Buffer (Promega), 2 mmol/l $MgCl_2$ (Promega), 5 pmol of each primer, 0.2 mmol/l dNTP mix (Promega), 1.25 U thermostable Taq DNA polymerase (Promega) and 5 µl of the RT mixture. The list of viruses, primers used, region amplified, expected product size and reference for RT-PCR used to detect grapevine viruses under investigation are shown in Table 1. Thermal cycling conditions tested for the optimization of PCR for the detection of grapevine viruses are shown in Table 2.

Table 1. RT-PCR primers used for detection of grapevine viruses

Virus	Primer name	Primer sequence (5'-3')	Region amplified	Expected product size (bp)	Reference
GVA	GVA659 1F	GAGGTAGATATAGTAGGACCTA	Coat protein (ORF4)	271	Goszczynski and Jooste (2003)
	GVA686 2R	TCGAACATAACCTGTGGCTC			
GVB	H28	GTGCTAAGAACGTCTTCACAGC	Coat protein-RNA binding protein-like (ORF5)	459	Minafra and Hadidi (1994)
	C410	ATCAGCAAACACGCTTGAACCG			
GLRaV-1	HSP70-149f	ACCTGGTTGAACGAGATCGCTT	Heat shock protein 70-like protein (ORF3)	168	Osman *et al.* (2007)
	HSP70-293r	GTAAACGGGTGTTCTTCAATTCTCT			
GLRaV-2	P19qtF4	CTAACAATTTCTTCTTTGGATCGCAT	ORF7-ORF8	202	Beuve *et al.* (2007)
	P24qtR	AGAATGTCTTCAGCTTCATAAGGAG			
GLRaV-3	56F	AAGTGCTCTAGTTAAGGTCAGGAGTGA	Heat shock protein 70-like protein (ORF4)	254	Osman *et al.* (2007)
	285R	GTATTGGACTACCTTTCGGGAAAAT			
GFLV	M2	(C/T)T(A/G)GATTTTAGGCTCAATGG	Movement protein	290	Wetzel *et al.* (2002)
	M3	TG(C/T)AA(A/G)CCAGG(A/G)AAGAAAAT			
GFKV	FkV1	AGTACCTCCTCCACCGCACC	Replicase (ORF1)	243	Sabanadzovic *et al.* (1996)
	FkV2	TTTCTTCGGGCAGAGAGCCGTCC			
GRSPaV	RSP35	AG(A/G)(C/T)TTAG(A/G)GT(A/G)GCTAA (A/G)GC	RNA-dependent RNA polymerase (ORF1)	476	Lunden *et al.* (2010)
	RSP36	CACAT(A/G)TCATG(A/C/G)CC(C/T)GCAAA			

Table 2. Thermal cycling conditions tested for the optimization of PCR for the detection of grapevine viruses

Primer name	Initial denaturation step	Number of cycles	Denaturation step	Annealing step	Elongation step	Final extension step
GVA659 1F/ 686 2R	94°C/5min	30	94°C/10 s	56°C/10 s	72°C/30 s	72°C/10 min
H28/ C410	94°C/5 min	35	94°C/10 s	54°C/10 s	72°C/45 s	72°C/10 min
LR1HSP70-149f/293r	94°C/5 min	30	94°C/10 s	59°C/10 s	72°C/10 s	72°C/10min
P19qtF4/ P24qtR	94°C/5 min	35	94°C/10 s	54°C/10 s	72°C/45 s	72°C/10 min
56F/ 285R	94°C/5 min	30	94°C/10 s	59°C/10s	72°C/10 s	72°C/10 min
M2/ M3	94°C/5 min	35	94°C/10 s	52°C/10 s	72°C/30 s	72°C/10 min
FkV1/FkV2	94°C/5 min	35	94°C/10 s	54°C/10 s	72°C/45 s	72°C/7 min
RSP35/ RSP36	94°C/5 min	35	94°C/10 s	54°C/10 s	72°C/45 s	72°C/7 min

The PCR products (10 µl) were analysed by gel electrophoresis on a 1.2% agarose gel and visualized on a UV-transilluminator after staining with ethidium bromide. The 1Kb DNA ladder (Promega) was included to determine the size of amplified products.

Cloning, sequencing and phylogenetic analyses. The RT-PCR products were purified according to manufacturer's instructions using Wizard SV Gel and Clean-Up System Kit (Promega, USA), ligated into pGEM-T Easy vector (Promega) and transformed into *Escherichia coli* strain M1022 (Promega). Recombinant plasmids containing inserts of the expected size, identified by direct PCR amplification, were purified using a Wizard plus SV miniprep kit (Promega). The identity of the plasmid insert was verified by sequencing (MWG-Biotech AG, Germany) and the sequences obtained were submitted to GenBank. Computer-based comparison of partial nucleotide sequences of the Egyptian isolates of viruses to that of other geographical isolates present in GenBank, by multiple sequence alignments, were generated using Workbench version 3.2, CLUSTALW program (Thompson *et al*., 1994). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Bootstrap test of phylogeny (1000 replicates), Neighor-Joining method) (Tamura *et al*., 2007).

Results

Virus symptoms observed in vineyards

During the period of the survey, only leafroll disease symptoms, in the form of typical leaf symptoms including cupping and reddening of leaves between major veins in red-fruited *V. vinifera* cultivars and cupping and a slight chlorosis of leaves between major veins in white-fruited *V. vinifera* cultivars, were observed by visual inspection. A low field incidence of leaf roll symptoms was observed among vineyards as assessed by visual inspection. Characteristic symptoms of other grapevine viruses were not observed in visited vineyards during the survey. Symptoms were manifested in the margins of upper leaves, which were rolled, and by leaves turning dark yellow with green main veins (Fig. 1a and b). In red-fruited *V. vinifera* cultivars, the leaf blades turned into bright red and leaf margins rolled downward. Symptoms were also manifested in the margins of upper leaves, which were rolled, and by leaves turning dark red with green main veins (Fig. 1c). Leaf roll disease causes also significant losses and delays in fruit ripening (Fig. 1d). The major observation in the survey was that heavy infestations of unidentified mealybugs were common in most of the surveyed vineyards.

RT-PCR for detection of grapevine viruses

A total of 60 symptomatic samples were processed by RT-PCR for the detection of grapevine viruses (GLRaV-1, GLRaV-2, GLRaV-3, GVA, GVB, GRSPaV, GFLV, and GFKV). Results indicated that the overall infection in the surveyed Egyptian grapevines was 46.6% (28 out of 60). 20% of the samples were infected with one virus and 26.6% were infected with more than one virus (mixed infection). GVA was the most widespread virus (30%). GLRaV-3 (28.8%), GRSPaV (16.6%), and GLRaV-2 (10%) were also detected during the survey, while GLRaV-1, GVB, GFLV, and GFKV were not detected in any of the samples tested.

Grapevine viruses GVA, GLRaV-2, GLRaV-3, and GRSPaV were found individually and in various combinations as shown in Table 3. The highest incidence was recorded in El-Menofia (50%), followed by El-Beheira (El-Nobaria) (35.7%). The sanitary status of native Egyptian cultivar Thompson seedless (92.8%) was poorer than that of imported cultivars Superior and Flame seedless (35.8%).

Partial sequencing of the Egyptian isolates clones of viruses

DNA products of the expected size were obtained from the total RNA from grapevine leaves infected with GLRaV-2 (202 bp), GLRaV-3 (254 bp), GVA (271), and GRSPaV (476 bp). No viral DNA amplicon was obtained from the total

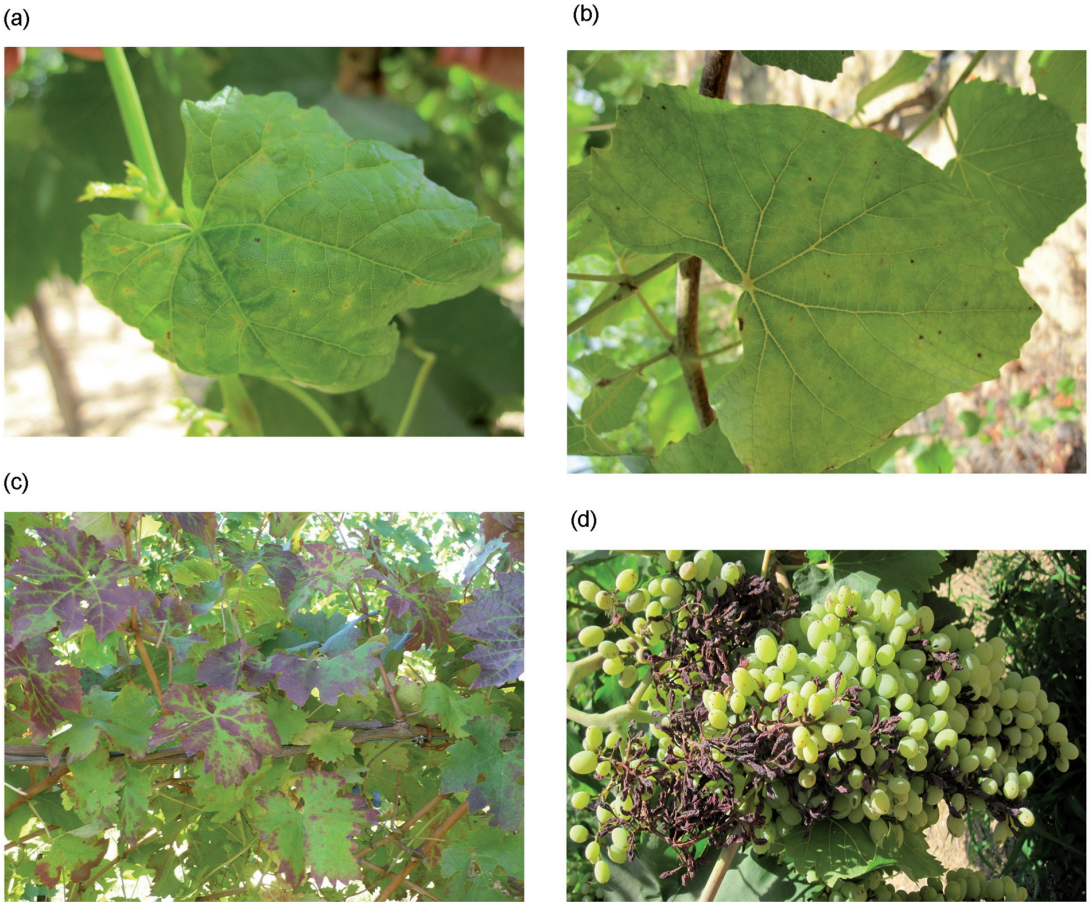

Fig. 1
Suspected leafroll disease symptoms in selected vineyards in different governorates of Egypt from early April to late October 2010
In white-fruited V. *vinifera* cultivars cupping and a slight chlorosis of leaves between major veins (a, b). In red-fruited *Vitis vinifera* cultivars cupping and reddening of leaves between major veins, while main veins remain green (c). Loss, delay and variation in fruit ripening (d).

Table 3. Distribution of viral infection (individually or in various combinations)

Individual infection	
Viral type	Percentage of detection
GVA	10%
GLRaV-3	5%
GRSPaV	3.3%
GLRaV-2	1.6%
Various viral combinations	
Double infection	Percentage of detection
GVA and GLRaV-3	10%
GVA and GLRaV-2	1.6%
GRSPaV and GLRaV-3	1.6%
Triple infection	Percentage of detection
GRSPaV, GLRaV-2 and GLRaV-3	5%
GVA, GRSPaV and GLRaV-3	1.6%
GVA, GLRaV-2 and GLRaV-3	1.6%
Quaternary infection	Percentage of detection
GRSPaV, GVA, GLRaV-2 and GLRaV-3	3.3%

RNA from healthy vine leaves. DNA amplicons obtained by RT-PCR for all isolates of GVA, GLRaV-2, GLRaV-3, and GRSPaV were each cloned and sequenced. Nucleic acid sequences (3 of GVA, 4 of GLRaV-2, 7 of GLRaV-3, and 6 for GRSPaV) determined in this study were made available in GenBank as accession numbers JN688279 to JN688285 for GLRaV-3, JN700905 to JN700908 for GLRaV-2, JN683368 to JN683370 for GVA and JN688271 to JN688276 for GRSPaV. Sequence analysis confirmed the respective viral nature of RT-PCR products.

RT-PCR amplification of different amplicons allowed partial sequences and phylogenetic analysis of different isolates of GLRaV-3 (7), GLRaV-2 (4), GVA (3), and GRSPaV (6). Comparative analysis indicated low to moderate nucleotide sequence identities in the GLRaV-3 heat shock 70 homologue gene (74% to 90%), GLRaV-2 ORF7 and ORF8 protein gene (75% to 97%), GVA coat protein gene (70% to 91%), GRSPaV RNA-dependent RNA polymerase gene (72% to 93%) when compared to corresponding virus reference strains previously reported.

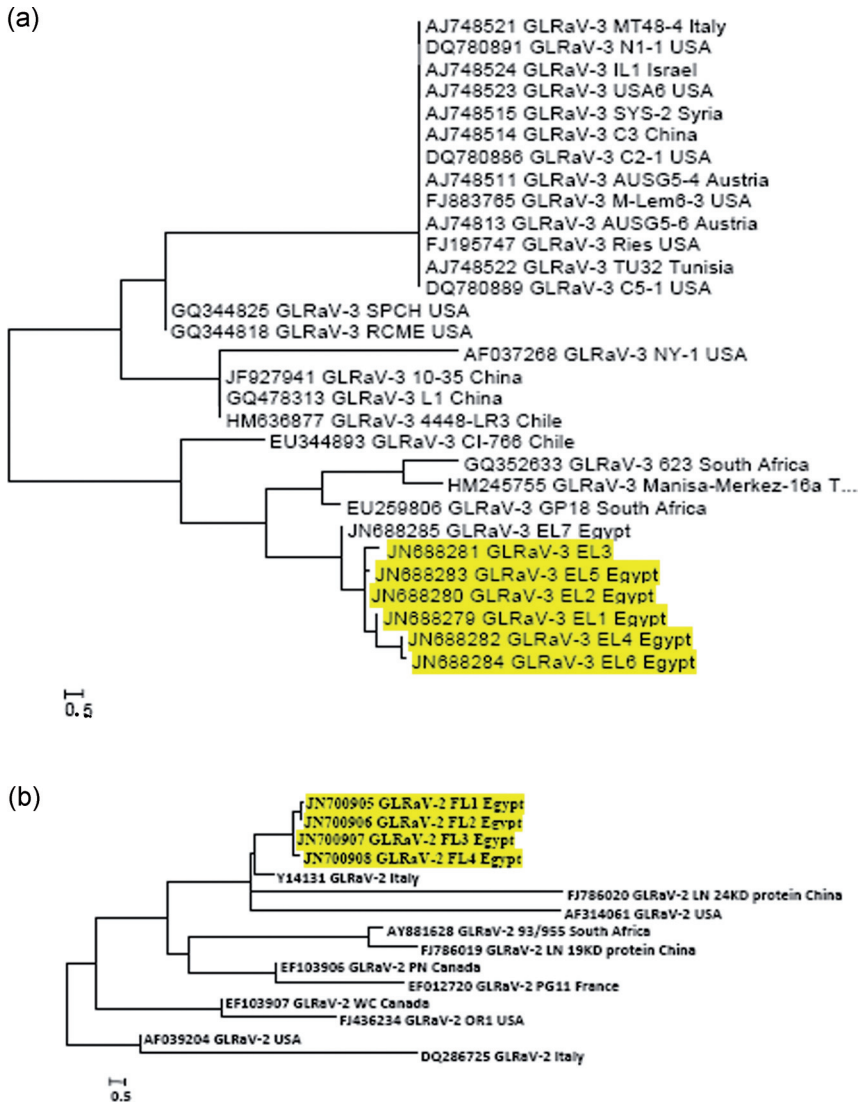

Fig. 2
Phylogenetic trees reconstructed from partial nucleotide sequences of (a) HSP70h gene of grapevine leafroll-associated virus 3 (GLRaV-3) and (b) ORF7 and ORF8 protein gene of grapevine leafroll-associated virus 2 (GLRaV-2) isolates by the neighbor-joining method of MEGA4 Tamura *et al.* (2007)
Bootstrap analysis was performed with 1000 replicates. The scale bar shows the number of substitutions per nucleotide.

Comparison of partial nucleotide sequences of the Egyptian isolates of grapevine viruses with that of other reported isolates

Phylogenetic relationships among viral isolates were determined for a 254-nt fragment of the GLRaV-3 HSP70h gene, a 202-nt fragment of the GLRaV-2 ORF7 and ORF8 protein gene, a 271-nt fragment of the GVA CP gene, and a 476-nt fragment of the GRSPaV RdRp gene.

The comparison of the HSP70h gene of GLRaV-3 showed that nucleotide sequence identities ranged from 88% to 99% between seven Egyptian isolates (JN688279 to JN688285). Phylogenetic analyses revealed that our 7 Egyptian GLRaV-3 isolates had a distant relationship with other isolates and fell into one clade. The next-closest isolate was GP18 isolate from South Africa (EU259806) as shown in Fig. 2a. This might be an indication that the Egyptian isolates are distinct from other GLRaV-3 isolates reported earlier. Comparison of GLRaV-2 sequences showed that identities of nucleotides between the four Egyptian isolates and other reported isolates were 75% to 97%. Phylogenetic analyses revealed that our 4 Egyptian GLRaV-2 isolates grouped together in the same

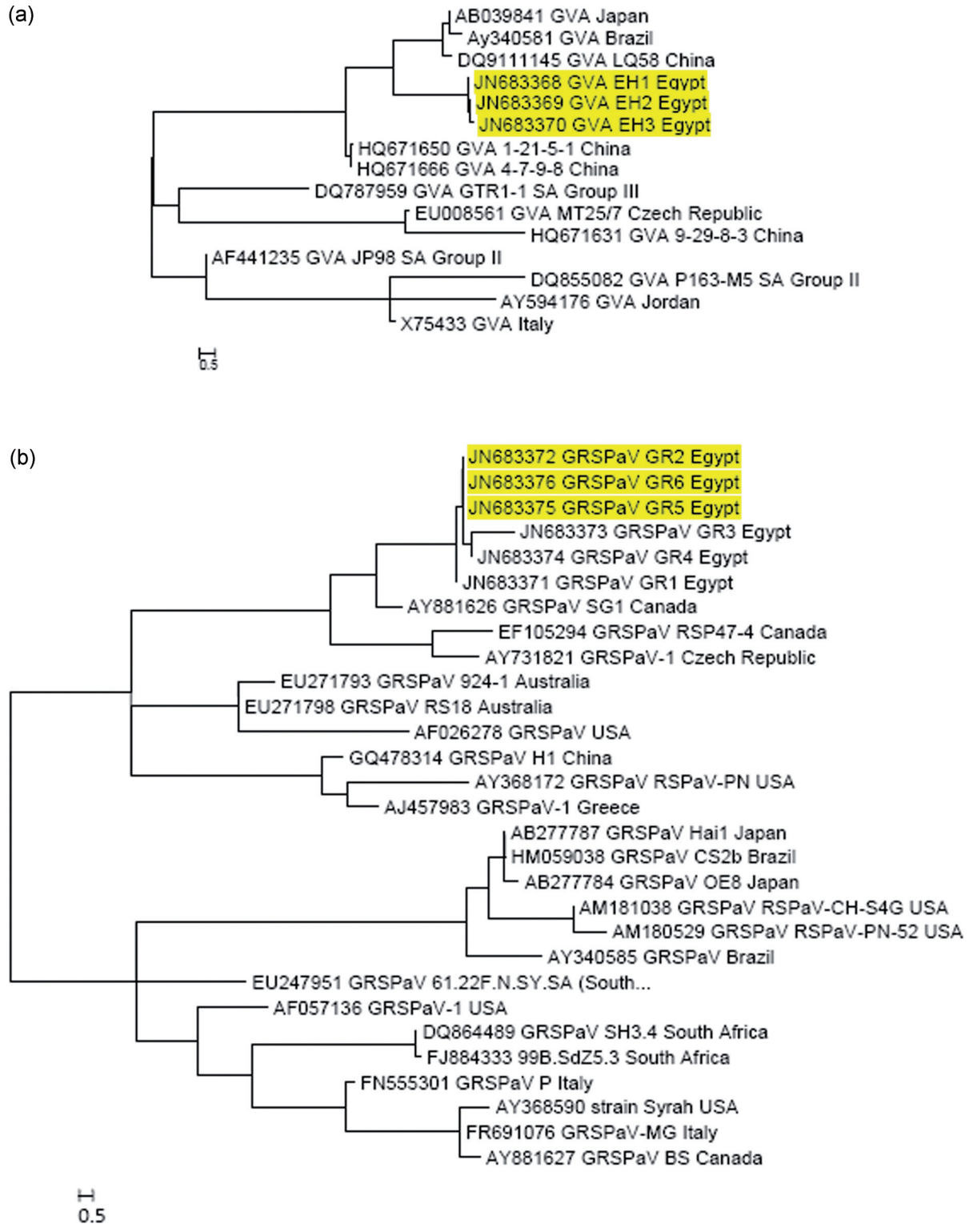

Fig. 3
Phylogenetic trees reconstructed from partial nucleotide sequences of (a) CP gene of grapevine virus A (GVA), and (b)RdRp gene of grapevine rupestris stem pitting virus (GRSPaV) isolates by the neighbor-joining method of MEGA4 Tamura *et al.* (2007)
Bootstrap analysis was performed with 1000 replicates. The scale bar shows the number of substitutions per nucleotide.

branch and appear to be closely related. The next-closest isolate was an Italian isolate (Y14131) as shown in Fig. 2b.

Sequence analysis of CP gene of GVA showed nucleotides identities ranging from 90% to 96% between three Egyptian isolates (JN683368 to JN683370). Phylogenetic analyses revealed that the three Egyptian GVA isolates had a distant relationship with other isolates, fell into one clade and appear to be closely related. The next-closest isolate was LQ58 isolate from China (DQ9111145) as shown in Fig. 3a. The sequence analysis of RdRp gene of GRSPaV, showed nucleotides identities ranging from 87% to 99% between six Egyptian isolates (JN688271 to JN688276). Phylogenetic analyses revealed that our 6 Egyptian GRSPaV isolates had a distant relationship with other isolates and grouped together in the same branch. The next-closest isolate was SG1 isolate from Canada (AY881626) as shown in Fig. 3b. It should be noted that geographical location-associated clustering of Egyptian GLRaV-2, GLRaV-3, GVA, and GRSPaV isolates was observed.

Discussion

In Egypt, grapevine (*V. vinifera*) is an economically important crop in agricultural economy (Youssef *et al*. 2009). Surveyed area covered commercial or local vineyards and nurseries in Alexandria, El Beheira (El Nobaria) and El-Menofia, representing old and recently reclaimed desert land. The only symptoms observed and identified with reasonable confidence in the field were those of leafroll disease, in both red and white-berried *V. vinifera* cultivars, which consist of typical leaf symptoms including cupping and reddening of leaves between major veins in red-berried *V. vinifera* cultivar (Flame Seedless) and cupping and chlorosis of leaves between major veins in white-berried *V. vinifera* cultivars (Thompson seedless and Superior). This is in contrast to previous reports by Ahmed et al. (2004) on the presence of symptoms only on red-berried cultivars and by Salem et al. (2007) on absence of leafroll symptoms and presence of symptoms associated with GFLV and GVA. Heavy infestations of unidentified mealybugs were common in most of the surveyed vineyards, similar to previous report by Ahmed et al. (2004). Based on previous reports, the detection of viral infection was dedicated to viruses GLRaV-1, GLRaV-2, GLRaV-3, GFLV, GFKV, GRSPaV, GVA, and GVB.

Several different methods such as biological indexing (Ahmed *et al*., 2004; Salem *et al*., 2007), serological assays (ELISA) (Lunden *et al*., 2010) and/or molecular assays (RT-PCR, multiplex PCR and real time PCR) (Gambino and Gribaudo, 2006; Osman *et al*., 2008; Wang *et al*., 2011) were previously used for detecting grape vine viruses. Our study for virus identification was, however, based solely on their detection by RT-PCR using virus-specific primers. Several studies have confirmed the efficiency of this technique for detection of grapevine viruses (Youssef *et al*., 2007; Eichmeier *et al*., 2011). With the use of RT-PCR for the identification of infection in 60 samples, an overall viral infection was identified in 46.6% of samples (28 out of 60). 20% of the samples were infected with one virus and 26.6% were infected with more than one virus (mixed infection). The most widespread virus in our survey was GVA (30%) followed by GLRaV-3 (28.8%), GRSPaV (16.6%) and GLRaV-2 (10%). GLRaV-1, GVB, GFLV, and GFKV were not detected. Prevalence of GLRaV-2, GLRaV-3, and GVA virus infection was in El Menofia governorate. The low sanitary status of the sampled areas correlated with higher incidence of the virus. However, prevalence of GRSPaV was in El Beheira governorate. The highest incidence was recorded in El-Menofia (old land) (50%), followed by El-Beheira (El-Nobaria) (new land) (35.7%). This is lower incidence than that previously reported for grapevine viruses in Egypt and in other Mediterranean countries (Digiaro *et al*., 2000; Ahmed *et al*., 2004). Despite the high incidence of GLRaV-3 and the presence of GLRaV-2, a low field incidence of leafroll symptoms was observed. This is possibly because most native Egyptian cultivars are white-berried table grapes that show mild or unapparent symptoms. This is in agreement with previous reports, which indicated that GLRD symptoms in white-berried cultivars are less striking, with mild to no chlorosis in the interveinal areas (Rayapati *et al*., 2008; Jarugula *et al*., 2010). The self-rooted condition of Egyptian vineyards accounts for the apparent absence of rugose wood symptoms in the field, notwithstanding the very high incidence of GVA (the putative agent of Kober stem grooving) and the presence of GRSPaV (the agent of rupestris stem pitting, RSP), two of the diseases of the rugose wood complex (Martelli and Boudon-Padieu, 2006). Rugose wood is a disease of the scion/graft combination, thus infection is latent in ungrafted scions and rootstocks, but symptoms develop after grafting (Martelli and Boudon-Padieu, 2006). Also, it was reported that some variants of GRSPaV are latent in *V. vinifera* cultivars (Alabi *et al*., 2010). It should be stressed that heavy widespread mealybug infestations are a major threat to the Egyptian viticultural industry. These are the basis for equally widespread occurrence of GVA and GLRaV-3 in traditional vineyards, and also contaminate newly introduced planting material. Indications of an apparent total absence of GFLV and GFKV in our study show that both viruses may have had little chance to spread, as native grapevine germplasm is likely to have been essentially healthy since the very beginning of cultivation, and own-rooted nature of Egyptian vineyards has favored the preservation of a healthy status by avoiding the contamination that so very frequently occurs in nurseries. In addition to the GFLV, its nematode vectors *Xiphinema index* may not be present in our country. The presented results are in agreement with previous report by

Ahmed *et al.* (2004) on the absence of GFKV and scarce presence of GFKV. Our results are in contrast with previous reports on presence of GFLV in Egypt and GFKV, which occurs at high rates in countries in Mediterranean region (Tolba and El-Kady, 1991; Digiaro *et al.*, 2000; Darwish, 2005; Fiore *et al.*, 2008; Fayek *et al.*, 2009; Youssef *et al.*, 2009).

Analyses of partial gene sequences revealed a close relationship of GLRaV-2, GLRaV-3, GRSPaV, and GVA isolates from Egypt and other isolates from various geographical origins. These data are consistent with the fact that transmission of these viruses occurs predominantly through uncontrolled exchange and propagation of budwood material. Frequent exchanges of propagative grapevine materials and grafting of various combinations of scions and rootstocks create ample opportunities for different viruses and viral isolates to merge into a single vine. Afterwards, a viral complex would perpetuate in the progeny vines via large-scale vegetative propagation. This study provides an example of mixed infection and supports the imperative need to develop a network of virus-tested grapevines for national and international exchange of propagation grapevine materials. Growing certified grapevines can reduce the incidence of mixed infections and thus prevent new disease outbreaks in grape-growing regions with diverse climatic, environmental and soil conditions. It was reported that the genomic diversity of GLRaV-3 has been examined based on the analysis of partial or complete heat shock protein 70 gene (HSP70) (Turturo *et al.*, 2005; Fuchs *et al.*, 2009). It was reported that the genomic diversity of GVA has been examined based on the analysis of partial or complete coat protein gene (CP), because it had a certain degree of variability (Goszczynski and Jooste, 2003; Murolo *et al.*, 2008). It was reported that the genomic diversity of GRSPaV has been examined based on the analysis of partial or complete RNA-dependent RNA polymerase gene (RdRp) (ORF 1), which is one of the most conserved regions of the GRSPaV genome (Meng *et al.*, 1998; Lunden *et al.*, 2010). Characterizing virus isolates is an important aspect of the development of tools for the detection of this virus, the information about sequence diversity among variants may help to select regions of the virus genome targeted for specific virus detection and to design primers for reliable detection of all known virus variants.

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