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‘阳光玫瑰’葡萄病毒小 RNA 测序鉴定及 RT-PCR 检测

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摘要:‘阳光玫瑰’是我国从日本引进的葡萄优良品种。为了明确我国‘阳光玫瑰’葡萄病毒病的病原,本研究采用小 RNA 测序技术对 2 株显症和无症状的‘阳光玫瑰’葡萄样品进行病毒鉴定结果显示:显症样品中测定到 8 种病毒,其中包含葡萄蚕豆萎蔫病毒(Grapevine fabavirus, GFabV)和灰比诺葡萄病毒(Grapevine Pinot gris virus, GPGV);无症状样品中测定到 3 种葡萄病毒。对 46 个‘阳光玫瑰’样品进行 14 种葡萄病毒的 RT-PCR 检测,结果表明:‘阳光玫瑰’葡萄带毒率较高,病毒复合侵染情况普遍;显症样品中,GFabV 检出率为 88.2%,GPGV 和葡萄浆果内坏死病毒(Grapevine berry inner necrosis virus,GINV)检出率为 64.7%和 29.4%,均明显高于无症状样品(13.8%和 10.3%)。本研究旨在探明‘阳光玫瑰’葡萄携带病毒的种类和侵染状况,为其病毒病防控及病毒脱除奠定基础。

关键词:阳光玫瑰; 小 RNA 测序; 葡萄病毒; RT-PCR

Small RNA sequencing and RT-PCR detection of viruses infecting ‘Shine Muscat’ grapevines

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Abstract: ‘Shine-Muscat’ grapevine is a new introduced cultivar in China. To identify the related viruses with the ‘Shine-Muscat’ grapevine virus disease, we adopted small RNA sequencing to detect the viruses in the asymptomatic and symptomatic ‘Shine-Muscat’ grapevine samples. Results showed that the presence of eight viruses in the symptomatic ‘Shine-Muscat’ grapevine including Grapevine fabavirus (GFabV) and Grapevine Pinot gris virus (GPGV), and only three viruses in the asymptomatic grapevine. Forty-six ‘Shine-Muscat’ grapevine samples were tested for 14 viruses by RT-PCR, and the results showed that these samples had high virus detection rate, and mixed-infection of multiple viruses was common. Grapevine fabavirus (GFabV) was only found in the symptomatic samples with a detection rate of 88.2%. There was a higher incidence of GPGV and Grapevine berry inner necrosis virus (GINV) (64.7% and 29.4%) in symptomatic samples than that in asymptomatic samples (13.8% and 10.3%). This study identified the species and infection status of viruses in ‘Shine-Muscat’ grapevines, and provides the theoretical basis for the control of ‘Shine-Muscat’ grapevine virus disease by sanitary treatments for propagation materials.

Key words: Shine-Muscat; small RNA sequencing; grapevine virus; RT-PCR

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‘阳光玫瑰’(Shine-Muscat)葡萄是日本国家果树科学研究所选育的新品种[1],因其外观美、品质佳、耐贮运、栽培效益好而深受我国果农和消费者的喜爱。‘阳光玫瑰’葡萄在我国推广种植过程中,普遍表现叶片变小、褪绿斑驳、畸形等病毒病症状,严重影响果实产量和品质[2]。明确‘阳光玫瑰’葡萄病毒病的病原及其危害的主要病毒种类迫在眉睫。

常用的葡萄病毒快速检测方法(酶联免疫吸附法和反转录聚合酶链式反应)只能鉴定已知的特定病毒,不能鉴定潜在的未知病毒[3]。高通量测序技术可全面检测样品中的核酸分子,对已知和未知病毒均能有效检测[4]。采用小 RNA 测序技术进行病毒鉴定,其原理是植物体内存在 RNA 沉默机制,被病毒侵染的植物组织中产生大量源于病毒的特异小 RNA 分子,这些小 RNA 序列相互重叠,能用来拼接组装病毒的基因组,从而发现和鉴定出相关病毒[5]。小 RNA 测序在植物病毒鉴定方面具有快速、灵敏、经济的优势,在葡萄病毒病的病原鉴定上发挥了重要作用,如与葡萄脉明症状相关的葡萄脉明病毒(Grapevine vein clearing virus, GVCV)、引起葡萄叶片产生褪绿、畸形的灰比诺葡萄病毒(Grapevine Pinot gris virus, GPGV)、引起葡萄红叶病的葡萄红叶相关病毒(Grapevine redleaf-assoicated virus, GRLaV)均是通过该技术鉴定发现[6~8]。对表现葡萄扇叶病类似症状的葡萄样品进行小 RNA 测序,发现葡萄浆果内坏死病毒(Grapevine berry inner necrosis virus, GINV)和葡萄蚕豆病毒组病毒(Grapevine fabavirus, GFabV)与症状发生密切相关[9, 10]。关于感染病毒病的‘阳光玫瑰’葡萄样品的高通量测序研究尚未见报道。

为明确‘阳光玫瑰’病毒病相关的病毒种类,本研究采用小 RNA 测序技术对表现症状和无症状的‘阳光玫瑰’葡萄样品进行病毒鉴定,随后采用 RT-PCR 方法对来源于 8 个省(市)自治区的 46 个‘阳光玫瑰’样品进行 14 种葡萄病毒的检测,以期明确‘阳光玫瑰’葡萄的病毒种类及造成危害的主要病毒,为‘阳光玫瑰’葡萄病毒病的防控和病毒脱除提供依据。

1 材料与方法

1.1 材料

2015 年 6 月于浙江杭州葡萄园中,从邻近表现症状和无症状的 2 年生‘阳光玫瑰’葡萄树上采集叶片,送北京百迈克生物科技有限公司进行小 RNA 深测序。显症样品症状主要表现为叶片褪绿斑驳、畸形及小叶(图 1)。2016-2017 年,从浙江(5)、上海(10)、云南(4)、福建(2)、武汉(2)、山东(12)、江苏(5)、辽宁(6)等地收集 46 个‘阳光玫瑰’样品,其中 17 个样品在春夏季表现不同程度的叶片褪绿斑驳、畸形、小叶等症状,其余 29 个样品未表现明显病毒病症状。

Fig. 1 The symptom on the leaves of ‘Shine-Muscat’ grapevine

1.2 方法

1.2.1 小 RNA 测序及分析 将显症和无症的‘阳光玫瑰’叶片通过干冰运至北京百迈克生物科技有限公司,委托其利用 Illumina HisSeqTM 2000 高通量测序平台进行小 RNA 深测序。获得原始数据后,将长度小于 18 nt 或大于 30 nt、低质量、未插入 3’或 5’接头、带 ployA 或 ployN 的小 RNA 序列及小 RNA 的接头序列去掉,获得过滤后的数据(clean data)。采用 Velvet 软件[11]对 clean data 中的小 RNA 进行拼接,设哈希长度(hash_length)值为 17。将拼接得到的片段(contigs)序列与 NCBI 网站下载的病毒参考数据库(ftp://ftp.ncbi.nlm.nih.gov/refseq/release/viral/)进行本地 blastn 和 blastx,获得病毒的特异性 contigs 序列。

1.2.2 RNA 提取及反转录 采用柱式提取方法[12]进行总 RNA 的提取。反转录在 1.5 mL 灭菌离心管中进行,依次加入灭菌纯水 7.0 μL、50 μmol·L^{-1}随机引物(NNNNNN)(上海生工生物工程技术服务公司合成)1.0 μL,总 RNA 2.0 μL,离心混匀,95℃水浴 5 min,冰上放置 2 min;加入 5 ×

M-MLV buffer 5.0 μL、10 mmol·L^{-1}dNTPs 1.5 μL、200 U·mL^{-1} M-MLV 逆转录酶 0.8 μL、灭菌纯水 2.7 μL,37℃10 min,42℃ 50 min,70℃ 5 min。合成的 cDNA 立即用于 PCR 扩增或置于-20℃冰箱保存。

1.2.3　田间‘阳光玫瑰’样品检测　采用 RT-PCR 方法对 46 个‘阳光玫瑰’葡萄样品进行病毒检测,检测病毒种类为葡萄卷叶相关病毒 1~4、7(Grapevine leafrool-associated virus 1~4、-7, GLRaV-1~4、-7)、葡萄病毒 A(Grapevine virus A, GVA)、葡萄病毒 B (Grapevine virus B, GVB)、葡萄病毒 E (Grapevine virus E, GVE)、沙地葡萄茎痘病毒(Grapevine rupestris stem pitting-associated virus, GRSPaV)、葡萄斑点病毒(Grapevine fleck virus, GFkV)、葡萄扇叶病毒(Grapevine fanleaf virus, GFLV)、GPGV、GINV 和 GFabV 共 14 种。PCR 检测引物及方法见表 1,PCR 扩增均设阴性和阳性对照。

Table 1　Primers used for RT-PCR detection of main viruses of grapevine

Viruses	Primers	Sequences (5′- 3′)	Product/bp	References
GLRaV-1	L1HSP-L	CCGGCKGAGTATAAYTCGT	488	[13]
	L1HSP-R	CGAAGATYTTRATCGCTC		
GLRaV-2	L2HSPL	GGTGATAACCGACGCCTCTA	597	[14]
	L2HSPR	CCTAGCTGACGCAGATTGCT		
GLRaV-3	LC1	CGCTAGGGCTGTGGAAGTATT	546	[15]
	LC2	GTTGTCCCGGGTACCAGATAT		
GLRaV-4	L4F1	ACATTCTCCACCTTGTGCTTTT	319	[16]
	L4R1	CATACAAGCGAGTGCAATTAC		
GLRaV-7	L7F	TATATCCCAACGGAGATGGC	502	[17]
	L7R	ATGTTCCTCCACCAAAATCG		
GRSPaV	RSP52	TGAAGGCTTTAGGGGTTAG	905	[18]
	RSP53	CTTAACCCAGCCTTGAAAT		
GVA	GVA5	CCAGAGGAGTTTGAGACAATA	195	[19]
	GVA6	GTCCCGACCAAGGCGATGTACCC		
GVB	GVBh	ATCAGCAAACACGCTTGAACCG	460	[20]
	GVBc	GTGCTAAGAACGTCTTCACAGC		
GVE	GVEMP1	TGTGGGGTGCATAGTCATAGGTTT	478	[21]
	GVEMP2	GCTTTTGACTCCATTGGCTTTCTC		
GFLV	FL-MP1A	TCNACCAGAGCCAATTACAC	675	[22]
	FL-MP1B	GTGARCGRCTYCTRATAGAG		
	FL-MPn1A	AGGCTYAATGGTATWCCGAC	546	
	FL-MPn1B	KCKTGCYTCAGGVGTTCCAG		
GFkV	GFkV-L630	GGCCAGGTTGTAGTCGGTGTTGTC	352	[23]
	GFkV-U279	TGGTCCTCGGCCCAGTGAAAAAGTA		
GPGV	GPGVCP1A	TGAGATCAACAGTCAGGAGAG	544	[24]
	GPGVCP1B	GAAGCCGTGATAGCATTAGTC		
GINV	GBINMP1A	WMGKGTTTCTGGGAAGATTGC	968	[25]
	GBINMP1B	KACSAGATCCCTCAATTCCTG		
GFabV	NODE1a	GGAAATAACATTCCCAGGAAG	325	[10]
	NODE1b	TCCTGCTGTCAAATTTCATTC		

2 结果

2.1 小 RNA 测序及分析

对小 RNA 测序数据进行分析,结果从无症状和显症的‘阳光玫瑰’样品获得的小 RNA 总数分别为 20 518 812 和 17 216 628,过滤后分别获得 17 212 241和 14 109 655 的 clean reads,占小 RNA 总数的 83.89%和 81.95%。小 RNAs 以 21 nt 和 24 nt 为最多,其次为 22 nt、23 nt 和 25 nt。

2.2 小 RNA 拼接及注释

采用 Velvet 软件对获得的小 RNA 进行拼接,并将拼接得到的 contigs 序列与病毒参考数据库中的病毒基因序列进行比对,结果表明:无症状葡萄样品中存在 GLRaV-3、GRSPaV 和 GFkV 3 种葡萄病毒;显症葡萄样品中存在 GLRaV-3、GRSPaV、GFkV、GVA、GVB、GVE、GPGV 和 GFabV 8 种葡萄病毒(表 2)。拼接得到的病毒序列在病毒基因参考序列的覆盖率高达 72.8%~100%,同源性为 88.14%~100%。

2.3 病毒检测

在 17 个表现症状的‘阳光玫瑰’葡萄样品中,检测到 GLRaV-2、GLRaV-3、GLRaV-4、GRSPaV、GVA、GVB、GVE、GFkV、GINV、GPGV 和 GFabV,检出率依次为 5.9%、35.3%,17.6%、58.8%、52.9%、17.6%、29.4%、47.1%、29.4%、64.7%和 88.2%。在 29 个无症葡萄样品中,检测到 GLRaV-2、GLRaV-3、GLRaV-4、GRSPaV、GVA、GVB、GVE、GFkV、GINV 和 GPGV,检出率分别为 6.9%、62.1%、3.4%、20.7%、31.0%、27.6%、65.5%、27.6%、10.3% 和 13.8%。GFabV 仅在显症的‘阳光玫瑰’葡萄上检测到,检出率为 88.2%。除 GFabV 外,在显症和无症‘阳光玫瑰’葡萄样品上检测出的病毒种类相同,但 GINV 和 GPGV 在显症样品中的检出率(29.4%和 64.7%)明显高于无症样品(10.3%和 13.8%)(表 3)。

Table 2 BLAST analysis of contigs with reference from NCBI

Reference sequence	Reference length/bp	Asymptomatic sample			Symptomatic sample		
		Contigs	Consensus length/bp	Coverage /%	Contigs	Consensus length/bp	Coverage /%
GLRaV-3 (GQ352631)	18 498	11	18 485	99.90	31	18 166	98.20
GRSPaV (AY368590)	8 744	65	7 975	91.20	49	7 803	89.30
GFkV (AJ309022)	7 564	57	6 846	90.50	60	6 901	91.20
GFabV RNA1 (KX241484)	5 802	0	0	0	4	5 785	99.70
GFabV RNA2 (KX241485)	3 134	0	0	0	6	3 052	97.40
GPGV (FR877530)	7 275	0	0	0	26	6 673	91.70
GVE (GU903012)	7 568	0	0	0	13	7 400	97.80
GVA (HQ671648)	780	0	0	0	10	568	72.80
GVB (KC861198)	722	0	0	0	2	722	100

Table 3　The detection result of grapevine viruses in ‘Shine-Muscat’ grapevines

Origin	Sample	Symptoms	Detection result
Zhejiang	ZJ-1	CM, LD	GRSPaV , GLRaV-3, GVA, GVE, GFkV, GPGV, GFabV
	ZJ-2	CM, LD	GRSPaV, GLRaV-3, GVA, GVB, GVE, GFkV, GPGV, GFabV
	ZJ-3	CM, LD	GRSPaV , GLRaV-3, GVA, GVE, GFabV
	ZJ-4	Symptomless	GRSPaV , GLRaV-3, GVE, GFkV
	ZJ-5	Symptomless	GRSPaV , GLRaV-3, GVA, GVE
Shanghai	SH-1	Symptomless	GVE
	SH-2	Symptomless	-
	SH-3	Symptomless	-
	SH-4	Symptomless	-
	SH-5	Symptomless	-
	SH-6	Symptomless	-
	SH-7	Symptomless	-
	SH-8	Symptomless	GVA, GVE
	SH-9	Symptomless	-
	SH-10	Symptomless	-
Yunnan	YN-1	CM, LD, SM	GLRaV-4,GVA, GINV, GPGV , GFabV
	YN-2	CM	GLRaV-4,GVA,GPGV
	YN-3	CM, LD,	GLRaV-4,GVA, GPGV, GFabV
	YN-4	CM, LD,	GPGV
Fujian	FJ-1	CM, LD, SM	GRSPaV, GFkV, GFabV
	FJ-2	CM, LD, SM	GRSPaV, GFkV, GFabV
Wuhan	WH-1	CM, LD	GFabV
	WH-2	CM, LD	GFabV
Shandong	SD-1	Symptomless	GLRaV-3, GVB, GVE
	SD-2	Symptomless	GLRaV-3, GVE
	SD-3	Symptomless	GLRaV-3,GVA, GVB, GVE
	SD-4	Symptomless	GLRaV-3,GVA, GVE, GFkV
	SD-5	Symptomless	GLRaV-3,GVA, GVB, GVE, GFkV
	SD-6	Symptomless	GLRaV-3, GVE, GFkV
	SD-7	Symptomless	GLRaV-3, GVE, GFkV
	SD-8	Symptomless	GLRaV-3, GVE
	SD-9	Symptomless	GLRaV-3,GVA, GVE
	SD-10	Symptomless	GLRaV-3, GVB, GVE, GFkV
	SD-11	Symptomless	GLRaV-3, GVE, GFkV
	SD-12	Symptomless	GLRaV-3, GVB, GVE
Jiangsu	JS-1	CM, LD, SM	GLRaV-2, GLRaV-3, GRSPaV GVA, GVB, GVE, GPGV, GFabV
	JS-2	CM, LD, SM	GLRaV-3, GRSPaV, GVA, GVB, GVE, GFabV
	JS-3	Symptomless	GLRaV-2, GLRaV-3, GRSPaV, GVA,GVB, GVE, GPGV
	JS-4	Symptomless	GLRaV-3, GLRaV-4, GRSPaV, GVA, GVB, GVE
	JS-5	Symptomless	GLRaV-2, GLRaV-3, GRSPaV, GVA, GVB, GVE, GINV, GPGV
Liaoning	LN-1	CM, LD	GRSPaV, GFkV, GPGV, GINV, GFabV
	LN-2	CM, LD	GRSPaV, GFkV, GPGV, GINV, GFabV
	LN-3	CM, LD	GRSPaV, GLRaV-3, GVA, GFkV, GPGV, GINV, GFabV
	LN-4	CM, LD	GRSPaV, GFkV, GPGV, GINV, GFabV
	LN-5	Symptomless	GRSPaV, GPGV, GINV
	LN-6	Symptomless	GFkV, GPGV, GINV

CM: Chlorotic mottling; LD: Leaf deformation; SM: Small leaf.

3 讨论

‘阳光玫瑰’的病毒病症状与 GFLV 引起的葡萄扇叶病症状类似,但本研究从表现症状的‘阳光玫瑰’葡萄样品中并未检测到 GFLV,这说明葡萄扇叶病症状并不一定由 GFLV 引起。我们在其他葡萄品种上也发现了类似情况[22, 25]。对于未受 GFLV 侵染的葡萄,该症状的发生与 GINV、GPGV、GFabV 等 3 种葡萄病毒有关[9,10,24,25]。Lou 等[2]对‘阳光玫瑰’葡萄样品中 GPGV、GLRaV-3、GFkV、GFLV、GRSPaV、GVA、GVB 等 7 种葡萄病毒进行了检测,但并未检测 GINV 和 GFabV。

本研究采用小 RNA 测序技术对无症状和表现症状的‘阳光玫瑰’葡萄样品分别进行病毒种类鉴定,无症样品中检测出 3 种葡萄病毒,显症样品中检测到包括 GFabV 和 GPGV 的 8 种葡萄病毒。对 46 个‘阳光玫瑰’样品的病毒 RT-PCR 检测结果显示,GFabV 仅存在于显症样品中,在显症样品中 GINV 和 GPGV 也有较高的检出率。据此推测,这 3 种葡萄病毒,尤其是 GFabV,可能与‘阳光玫瑰’葡萄病毒病发生密切相关。对于多种病毒复合侵染的现象,很难判断到底是哪一种病毒引起的症状。因此,对于上述推测,还需通过单一毒源嫁接传染实验、侵染性克隆接种实验等予以证实。本研究结果显示,除上海的绝大部分样品未检测到病毒外,其他显症和未显症的样品中均检出多种葡萄病毒。究其原因,可能是‘阳光玫瑰’在各地栽培引进过程中,频繁采用携带病毒材料(接穗和砧木)繁殖和昆虫介体传毒所致。一些葡萄病毒通常不会引起葡萄扇叶病类似症状,在无症状的‘阳光玫瑰’中虽能被检测到,但通常表现为潜隐。

葡萄病毒病无有效药剂进行防治,种植脱毒苗木是目前唯一有效的防控措施。探明‘阳光玫瑰’葡萄病毒病的主要致病病毒,对培育脱毒苗木至关重要。本研究明确了感染病毒病的‘阳光玫瑰’上的主要病毒种类及其侵染率,为开展该葡萄品种病毒病致病因子、病毒脱除及其防控技术研究奠定了基础。

参考文献

[1] Yamada M, Yamane H, Sato A, *et al*. New grape cultivar ‘Shine Muscat’ (in Japanese) [J]. Bulletin of the National Institute of Fruit Tree Science, 2008, 7: 21-38.

[2] Lou B H, Bai X J, Song Y Q, *et al*. The detection of main viruses in ‘Shine Muscat’ grape (in Chinese) [J]. Journal of Plant Protection (植物保护学报), 2017, 44(2): 345-346.

[3] Fan X D, Dong Y F, Zhang Z P, *et al*. Progress on molecular detection of grapevine viruses (in Chinese) [J]. Acta Horticulturae Sinica (园艺学报), 2014, 41(5): 1009-1019.

[4] Qian Y J, Xu Y, Zhou Q, *et al*. Application of next-generation sequencing technology for plant virus identification (in Chinese) [J]. Scientia Sinica Vitae(中国科学: 生命科学), 2014, 44(4): 351-363.

[5] Kreuze J F, Perez A, Untiveros M, *et al*. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses [J]. Virology, 2009, 388(1): 1-7.

[6] Zhang Y, Singh K, Kaur R, *et al*. Association of a novel DNA virus with the grapevine vein-clearing and vine decline syndrome [J]. Phytopathology, 2011, 101(9): 1081-1090.

[7] Giampetruzzi A, Roumi V, Roberto R *et al*. A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in Cv Pinot gris [J]. Virus Research, 2012, 163(1): 262-268.

[8] Poojari S, Alabi O J, Fofanov V Y, *et al*. A leafhopper-transmissible DNA virus with novel evolutionary lineage in the family *Geminiviridae* implicated in grapevine redleaf disease by next-generation sequencing [J]. PLoS One, 2013, 8(6): E64194.

[9] Fan X D, Hong N, Zhang Z P, *et al*. Identification of a divergent variant of *Grapevine berry inner necrosis virus* in grapevines showing chlorotic mottling and ring spot symptoms [J]. Archives of Virology, 2016b, 161(7): 2025-2027.

[10] Fan X D, Zhang Z P, Ren F, *et al*. First report of *Grapevine fabavirus* in grapevines in China [J]. Plant Disease, 2017a, 101(5): 847.

[11] Zerbino D R, Birney E. Velvet: algorithmsfor de novo short read assembly using de Bruijn graphs [J].

Genome Research, 2008, 18(5): 821-829.

[12] Fan X D, Dong Y F, Zhang Z P, *et al*. Molecular identification and gene sequence analysis of *Grapevine virus E*. (in Chinese) [J]. Acta Phytopathologyica Sinica(植物病理学报), 2014, 44(5): 455-460.

[13] Fan X D, Hong N, Dong Y F, *et al*. Genetic diversity and recombination analysis of *Grapevine leafroll-associated virus* 1 from China [J]. Archives of Virology, 2015a, 160(7): 1669-1678.

[14] Fan X D, Dong Y F, Zhang Z P, *et al*. Detection and Sequence Analysis of *Grapevine leafroll-associated virus* 2 isolates from China [J]. Journal of Phytopathology, 2015b, 163(1): 978-986.

[15] Oman F, Rwhani A. Application of a spotting sample preparation technique for the detection of pathogens in woody plants by RT-PCR and real-time PCR (TaqMan) [J]. Journal of Virological Methods, 2006, 133(2): 130-136.

[16] Pei G Q, Dong Y F, Zhang Z P, *et al*. First Report of *Grapevine leafroll-associated virus* 4 and 5 in grapevines in China [J]. Plant Diseaae, 2010, 94(1): 130.

[17] Manduláné F E, Czotter N, Lózsa R, *et al*. Conventional PCR primers for the detection of grapevine pathogens disseminated by propagating material [J]. International Journal of Horticultural Science, 2014, 20(3-4): 69-80.

[18] Nolasco G, Mansinho A, Teixeira Santos M, *et al*. Large scale evaluation of primers for diagnosis of *Rupestris stems pitting associated virus*-1 [J]. European Journal of Plant Pathology, 2000, 106 (4): 311-318.

[19] De Meyer J, Gaudin M, Bourquin L, *et al*. New primers for the molecular identification and detection of *grapevine virus A*(GVA)[A]. Extended abstracts 13th meeting ICVG [C]. Adelaide: Australia, 2000: 138-140.

[20] Minafra A, Saldarelli P, Grieco F, *et al*. Nucleotide sequence of the 3-terminal region of the RNA of two filamentous grapevine viruses [J]. Archives of Virology, 1994, 137(3-4): 249-261.

[21] Fan X D, Dong Y F, Zhang Z P, *et al*. First report of *Grapevine virus E* from grapevines in China [J]. Journal of Plant Pathology, 2013, 95(3):661.

[22] Zhou J, Fan X, Dong Y F, *et al*. Detection and genetic variation analysis of *Grapevine fanleaf virus* (GFLV) isolates in China [J]. Archives of Virology, 2015, 160(11): 2661-2667.

[23] Shi B J, Habili N, Symons R H. Nucleotide sequence variation in a small region of the *Grapevine fleck virus* replicase provides evidence for two sequence variants of the virus [J]. Annals of Applied Biology, 2003, 142(3): 349-355.

[24] Fan X D, Dong Y F, Zhang Z P, *et al*. First report of *Grapevine Pinot gris virus* from grapevines in China [J]. Plant Disease, 2016a, 100(2): 540.

[25] Fan X D, Zhang Z P, Ren F, *et al*. Occurrence and genetic diversity of *Grapevine berry inner necrosis virus* from grapevines in China [J]. Plant Disease, 2017b, 101(1):144-149.

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