First Report of Plum bark necrosis stem pitting-associated virus Infecting Grapevine in China

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Short report

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Abstract

Background: Virus disease is one of the main diseases in grapevine, and there has been no report on *Plum bark necrosis and stem pitting-associated virus* infecting grapevine in China.

Objective: The leaf samples of grapevine cultivar 'Cabernet Gernischt' were collected from Shandong province, which the leaves suffered from viral-like symptoms with spotting and crinkle.

Methods: Small RNA-seq combined with reverse transcription PCR (RT-PCR) were performed to detect the potential viruses in these field samples. Phylogenetic tree was constructed using the neighbor joining method in MEGA 5.1

Conclusions: This is the first report of PBNSPaV infecting grapevine in China, contributing to a better understanding of the epidemiology and host range distribution of this pathogen.

Synopsis

This study presents the first detection of Plum bark necrosis and stem pitting-associated virus in grapevine from China. The sequences detected (MH371356) have the nearest relationship with the isolate from Australia (LC523035).

Main Text

Grapevine virus diseases affect the yield and quality of grape (Vitis vinifera) [1]. However, with development of grape facility cultivation, especially the seedings were dispatching frequently among different areas, virus diseases have more serious. At present, sixty-five species of viruses were reported to infect grapevine, and some of them can cause serious economic losses worldwide [2]. So far, eleven virus species have been reported in China [3]. The diagnostic methods of the virus mainly including biological assay, serum-testing, molecular biology and election microscopy. Meanwhile, one of the primary detection method of small RNA-seq combined with RT-PCR technique was applied, while the primers were designed according to the species-specific virus sequences.

In September 2017, the leaves with symptoms spotting and crinkle were found in Shandong province from the grapevine (Cabernet Gernischt), which one of the most common wine grape varieties in China. To detect the potential viruses in these samples, total RNA was extracted by TRIzol reagent and small RNA-seq was performed on the field samples. We acquired 23,725,796 clean reads and conducted MEGABLAST using assembled contigs against the viral reference database from NCBI and identified 42 contigs associated with the following seven viral genomes: Grapevine virus A (GVA) (2 contigs), Tomato leaf curl mali virus (ToLCMLV) (1 contigs), Tomato pseudo-curly top virus (TPCTV) (1 contigs), Turnip curly top virus (TCTV) (1 contigs), Prune dwarf virus (PDV) (11 contigs), Prunus necrotic ringspot virus (PNRSV) (22 contigs), and Plum bark necrosis and stem pitting-associated virus (PBNSPaV) (4 contigs) (Table 1).

Table 1
Analysis of the data of virus-derived sRNAs in grapevine

Gene bank NO.	Virus	Transcript counts	Total length	homogeneity analysis
NC_004364.1	PNRSV-RNA 3	9	766	98%~100%
NC_008038.1	PDV	9	596	95%~100%
NC_004363.1	PNRSV- RNA 2	7	474	98%~100%
NC_004362.1	PNRSV- RNA 1	6	453	86%~100%
NC_009992.1	PBNSPaV	4	224	92%~100%
NC_031340.1	GVA	2	150	100%
NC_003825.1	TPCTV	1	79	100%
NC_005348.1	ToLCMLV	1	79	100%
NC_014324.1	TCTV	1	71	97%
NC_008037.1	PDV- RNA 2	1	54	100%
NC_008039.1	PDV- RNA 1	1	54	100%

To confirm the presence of the seven viruses in these grapevines, seven pairs of specific primers were utilized to detect the symptomatic samples by RT-PCR and DNA sequencing (Table 2), which showed that amplified products have the expected sequence size of GVA and PBNSPaV in the samples. And then another pair of primers (PBN-13558-F and PBN-14116-R) (Table 2) was designed to amplify a ~ 550 bp fragment of PBNSPaV. The amplified 550 nt PCR products were cloned and sequenced using the universal primers M13F and M13R. BLAST analysis showed that the 550 bp sequences have 98% identities with PBNSPaV (KC590346.1), which was reported to infect Prunus salicina in France [4]. This ~ 550 bp sequence of PBNSPaV from grapevine in this study was submitted to GenBank with the accession number MH371356. Phylogenetic tree was constructed using MEGA 5.1 software packages and it was suggested that PBNSPaV (MH371356) have the nearest relationship with the isolate from France Australia (LC523035) (Fig. 1). PBNSPaV is a viral species that belongs to the genus Ampelovirus, family Closteroviridae and has been shown to be worldwide distributed, affecting a broad range of Prunus species [5–7]. To our knowledge, this is the first report of PBNSPaV infecting grapevine in China. More attention will be paid on the early detection of PBNSPaV, on performance and quality of the grape.

Table 2
Primers used for RT-PCR

No.	Virus	Primer	Sequence
1	GVA	GVA-(400–420)-F	5-tggatctgtgagaagggaacg-3
		GVA-(977–1000)-R	5-cgtatacggagtcaattggaacgt-3
2	TPCTV	TPTV-(400–419)-F	5-gtttcgcggagcgaaatccg-3
		TPTV-(880–900)-R	5-ttcttcatcgccggacatcca-3
3	TCTV	TCTV-CP-F	5-ctcccggttacgatttggcc-3
		TCTV-CP-R	5-ccaccaggatcattatcaagaataatcc-3
5	PDV	PD-CP-135-F	5-TTCCGAGTGGATGCTTCACG-3
		PD-CP-564-R	5-CATCGAGTGTTGGAGGTACTGAG-3
6	PNRSV	PN- R2-184-F	5-TGTTTGAAGCGTACATGGTGAC-3
		PN-R2-1096-R	5-GCTTCGCGGAAAGTCGGTAC-3
7	PBNSPaV	PBN-9508-F	5-agttttgttttcactgcatgtag-3
		PBN-10170-R	5-caacctgaaacgagtggaac-3
		PBN-13568-F	5-GGATTAGGTGAGGTGTGGTTGAC-3
		PBN-14139-R	5-GTGCATTGCCGATTCCCGGAC-3

Discussion

As one of the most important grape varieties around the world, 'Cabernet Gernischt' was planted in many wine regions. Shandong is one of the main region of wines, which is the main planting base of the grape. Virus disease is one of the main diseases in grapevine, and may affect the yield and quality of grapevine [3]. PBNSPaV can infect many stone fruit species and causes decline, gummosis, flattening of scaffold branches, and stem necrotic pits in some diseased trees. Up to now, there have been no reports of PBNSPaV infecting grape in China. In this study, the results show that PBNSPaV and the associated disease may occur in main cultivated grape species in China. Given the importance and the devastating symptoms of the disease, our findings contributed to a better understanding of the epidemiology and host range distribution of this pathogen.

Conclusion

In September 2017, the leaf samples of grapevine cultivar 'Cabernet Gernischt' were collected from Shandong province, which the leaves suffered from viral-like symptoms with spotting and crinkle. To detect the potential viruses in these samples, small RNA-seq combined with reverse transcription PCR (RT-

PCR) were performed on the field samples, and Grapevine virus A (GVA) and Plum bark necrosis and stem pitting-associated virus (PBNSPaV) were identified. And, this is the first report of PBNSPaV infecting grapevine in China.

List Of Abbreviations

RT-PCR: reverse transcription PCR; GVA: Grapevine virus A; PBNSPaV: Plum bark necrosis and stem pitting-associated virus; NJ: the neighbor-joining method; ToLCMLV: TPCTV: Tomato leaf curl mali virus; Tomato pseudo-curly top virus; TCTV: Turnip curly top virus; PDV: Prune dwarf virus; PNRSV: Prunus necrotic ringspot virus.

Declarations

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Authors' contributions

DW conceived and designed the study. YS, YY and QL performed the experiments. YF analyzed the epidemiological data.DW wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

This ~550 bp sequence of PBNSPaV from grapevine in this study was submitted to GenBank with the accession number MH371356

Ethics approval and consent to participate

Not applicable

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Consent for publication

The authors declare that they agreed to publish this paper with the permission of the publishing houses

Competing interests

The authors declare that they have no competing interests.

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References

1. Wang, et al. Research progress in grapevine viruses. Northern Horticulture. 2017;09:179–83. (in Chinese).
2. Martelli GP. Directory of virus and virus-like diseases of the grapevine and their agents[J]. Journal of Plant Pathology. 2014;96(1sup):1–136.
3. Fan, et al. Progress on Molecular Detection of Grapevine Viruses. Acta Horticulturae Sinica,2014, 41(5): 1009–1019.
4. Marais, et al. Characterization by deep sequencing of divergent plum bark necrosis stem pitting-associated virus (PBNSPaV) isolates and development of a broad-spectrum PBNSPaV detection assay[J]. Phytopathology. 2014;104(6):660–6.
5. 10.4454/jpp.v92i3.323
   Matic, et al. J. Plant Pathol. 92: 759. http://dx.doi.org/10.4454/jpp.v92i3.323 Uyemoto and Teviotdale 1996, 2010. Phytopathology 86: 111.
6. Candresse, et al. Plant Dis. 2017;101:846. https://doi.org/10.1094/PDIS-09-16-1324-PDN.
7. Zamorano, et al. Plant Dis. 2017;101:1685. https://doi.org/10.1094/PDIS-04-17-0533-PDN.

Figures

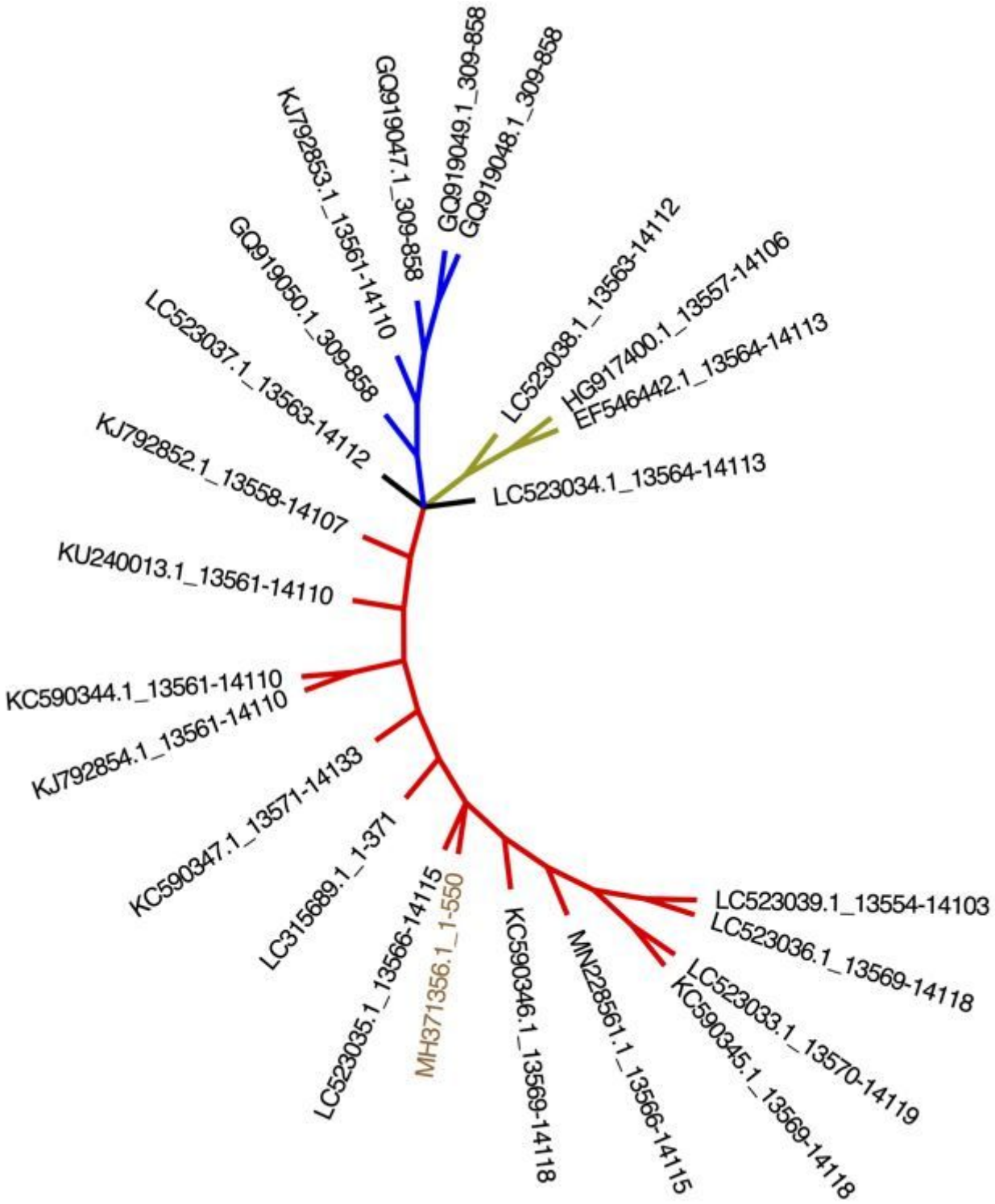

Figure 1

Phylogenetic tree was constructed based on the 13568 to 14139 of PBNSPaV using the neighbor joining method in MEGA 5.1. Bootstrap analysis with 1000 replicates. The new isolate was highlighted with dots