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Distribution and identification of main viruses infecting pepper in Qinghai

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ABSTRACT

From 2019 to 2021, pepper viruses were investigated in pepper planting areas and collected a total of 333 samples were in Qinghai (The central district, Datong, Huangzhong in Xining; Ledu district, Pingan, Huzhu, Minhe in Haidong, and Jianzha in Huangnan). RT-PCR and molecular cloning were conducted for virus detection in 333 suspected viral samples, the results revealed that viruses infecting pepper mainly included 11 capsicum viruses. Tomato spotted wilt tospovirus (TSWV) has the highest detection rate (36%) in Datong County, and Pepper cryptic virus 1 (PCV1) has the highest detection rate (57%) in Huangzhong County. In the Haidong, 86.3% of the peppers were Broad bean wilt virus 2 (BBWV2), virus-infected Pepper cryptic virus 2 (PCV2), TSWV and Cucumber mosaic virus (CMV) were detected in Xunhua, among which PCV1 and CMV had the highest detection rate (30.4%); PCV1, TSWV, and PCV2 were detected in Ledu and PCV2 had the highest detection rate (50%). There were 17 kinds of co-infection and the co-infection of two viruses occurred often. There were only 5 kinds of co-infection of three. Combined infection contained PCV1 and TMV was the most common. The distribution and species of pepper viruses from the pepper planting areas were clarified and it laid the foundation for preventing and controlling pepper viruses across Qinghai province.

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Introduction

Capsicum (*Capsicum* spp.) originated in tropical Latin America and contained five cultivars (*C. annuum*, *C. baccatum, C. chinense*, *C. frutescens*, and *C. pubescens*) and was one of the oldest and most frequently produced vegetable crops globally (1). Virus disease was a serious disease in pepper production, affecting yield and quality of peppers in pepper production areas all over the world (2-5). More than 70 plant viruses have been documented to infect peppers in various parts of the world since Doolittle initially identified the *Cucumber Mosaic Virus* (CMV) on peppers in 1923 (6–9). In most nations, the three main viral infections that affect peppers were *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY), and CMV (11-14). 35 viruses from 14 different genera, including *Potyvirus, Tobamovirus, Polerovirus, Endornavirus*, and other genera, were found in peppers in China (15). The viruses that caused serious damage included *Tomato spotted wilt tospovirus* (TSWV), *Pepper mild mottle virus* (PMMoV), CMV, *Broad bean wilt virus* 2 (BBWV2), *Pepper cryptic virus* 1 (PCV1), *Pepper cryptic virus 2* (PCV2), *Cucurbit aphid-borne yellow virus* (CABYV), *Melon aphidborne yellows virus* (MABYV), and *Tomato yellow leaf curl virus* (TYLCV) (15-18).

Qinghai is located in the northeastern part of the Qinghai-Tibet Plateau. Its distinctively mild environment and lengthy sunny hours were ideal for the ecological industry of plateau summer vegetables. Pepper, a significant plateau specialty vegetable in Qinghai, was well-known for its distinctive flavour and had a very bright future. It had resulted in a high incidence of pepper viral infections due to the expanding pepper crop area and frequent exchange of germplasm resources. In 2017 Li et al. (19) discovered the *Pepper Mild Mottle Virus* (PMMoV) in Haidong City, Qinghai Province. In 2019 in Qinghai, Liu et al. discovered CMV, *Turnip Mosaic Virus* (TuMV), TMV, and BBWV2 in peppers (15).TSWV was discovered on Qinghai peppers by Wu et al (20). Most of the samples from earlier studies were localised, and the sampling regions did not encompass all of the Qinghai pepper growing fields. It was therefore unable to identify the primary species causing damage in the current pepper production to effectively prevent and control pepper virus infections. In this work, we used a systematic collecting method to gather samples of a viral illness from pepper-growing areas in Qinghai Province. The application of the RT-PCR assay allowed for the detection and identification of the virus species present infesting Qinghai peppers, providing a scientific foundation for future prevention and management of pepper viral infections. This study aimed to investigate of distribution and identification of the main viruses infecting Pepper in Qinghai.

Materials and Methods

Sample collection

From 2019 to 2021, a total of 333 suspected leaf samples of pepper viruses were collected from Datong (75 copies), Xunhua (79 copies), Ledu (56 copies), Huzhu (43 copies), Ping An (25 copies), Minhe (9 copies), Huang-

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Table 1. Sample collection information.

Address of sample collection	*Northern* Latitude	East longitude	Altitude	Sample Collection Date	Number of samples
Hezhouzhuang, Tal Town, Datong County, Xining City	36.981645	101.664438	2500	2020.07.01	8
Talwan Village, Tal Town, Datong County, Xining City	36.724184	101.750103	2509	2020.07.01	7
Xinzhuang Town, Datong County, Xining City	37.001944	101.620555	2530	2020.07.01	9
Xinjian Village, Xunhua County, Haidong City	35.855833	102.269722	1919	2020.07.02	7
Xincun Village, Xunhua County, Haidong City	35.854444	102.323055	1925	2020.07.02	13
Modern agricultural demonstration park in Xunhua County, Haidong City	35.868333	102.361388	1870	2020.07.02	8
Dazhuang Village, Xunhua County, Haidong City	35.882500	102.459166	1800	2020.07.02	8
Jishi Town, Xunhua County, Haidong City	35.832500	102.525277	2200	2020.07.02	6
Kambura Town, Jianzha County, Huangnan Prefecture	36.100277	101.879444	2010	2020.07.02	4
Zhiganglaka Village, Kambula Town, Jianzha County, Huangnan Prefecture	36.113888	102.027777	2325	2020.07.02	5
Modern Agricultural Demonstration Park, Ledu District, Haidong City	36.475863	102.308224	1900	2020.07.16	16
Baishengou Village, Ping'an Town, Ping'an District, Haidong City	36.517185	102.992187	2183	2020.06.18	25
Modern Agricultural Demonstration Zone, Zunhua County, Haidong City	35.86679	102.35883	1903	2021.08.19	21
Xinjian Village, Jishi Town, Xunhua County, Haidong City	35.87889	102.46572	1920	2021.08.19	9
Xiazhuang Village, Qingshui Town, Xunhua County, Haidong City	35.83422	102.63479	1861	2021.08.19	7
Modern Agricultural Demonstration Park, Ledu District, Haidong City	36.47586	102.308224	2313	2021.06.23	35
Xinbaozi Village, Shoule Town, Ledu District, Haidong City	36.72449	101.75058	2313	2021.06.23	24
Qiaotou Village, Longzhi Township, Minhe County, Haidong	36.18094	102.908592	2313	2021.06.23	9
Xiajiuzhuang Village, Tal Town, Datong County, Xining City	37.01485	101.601413	2529	2021.06.24	47
Xinzhuang Village, Xinzhuang Town, Datong County, Xining City	37.06307	101.585726	2500	2021.06.24	35
Heerying Village, Duoba Town, Huanzhong County, Xining City	36.70048	101.507823	2408	2021.07.05	8
Yula Village, Duoba Town, Huanzhong County, Xining City	36.70906	101.530022	2416	2021.07.05	17
Xiaxihe Village, Lijiashan Town, Huanzhong County, Xining City	36.756559	101.562809	2345	2021.07.05	9
Yuantai Village,Chengzhong District, Xining City	36.527247	101.677120	2518	2021.07.05	9

zhong (28 copies) Jianzha (9 copies) and Xining Urban District (9 copies) in Qinghai Province, China. Healthy plants were collected as a negative control. Each sample was preserved under −80°C. Sample collection information has been shown in Table 1.

RNA isolation as well as cDNA preparation

The leaf sample (about 0.1 g) was subject to homogenization with TRIzol (Tiangen, Beijing, China) to extract total RNA in line with specific protocols (Sun Guosheng et al. 2016). Thereafter, 1% TBE agarose gel electrophoresis (AGE) was conducted to evaluate RNA integrity, while the NanoDrop OneC spectrophotometer (Gene Company Limited, China) was utilized to determine RNA quality and content. Total RNA extracted (2 μg) was collected for preparation into first-strand cDNA using FastKing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China) in line with specific protocols. cDNA was preserved at −20 °C prior to use.

RT-PCR detection

PCR was completed using the T100 Thermal Cycler PCR system (BioRad, Shanghai, China) under the conditions below, The reaction system (20μl) consisted of 2 × Taq PCR MasterMix II 10 μL (Tiangen, Beijing, China), respective primers (1 μl, Table 2, Sangon, Shanghai, China), first-strand cDNA template (1 μl) and 7μL of ddH_2O under the following conditions, 5min initial denaturation under 94 °C; 45s under 94 °C, 45s under Tm(according to specific primer) °C, and 1 min/kb (depending on the product size) under 72 °C for 34 cycles; followed by 10min eventual extension under 72 °C. Thereafter, 1% TAE AGE was conducted to analyze PCR products, followed by the excision of products with desired size from 1% AGE and purification with TIANgel Midi Purification Kit (Tiangen, Beijing, China). After purification, this assay ligated DNA fragments to a pEASY-Blunt Cloning vector (TransGen, Beijing, China), followed by transfection in *Escherichia coli* Trans-T1 competent cells (Transgen, Beijing, China) to conduct sequencing (Sangon, Shanghai, China).

Sequence as well as phylogenetic analysis

DNAMAN 7.0 was employed for sequence assembly and alignment. Genome sequences were analyzed with BLAST alignment, http://blast.ncbi.nlm.nih.gov/Blast.cgi, to determine virus type. SPSS20.0 statistical software was used for data processing.

Results

Field disease symptoms of pepper in Qinghai province

As suggested by field visits, common pepper viruses significantly induced symptoms in every assayed region, with a virus prevalence rate of 100% within certain fields. The symptoms included slight vein banding or mosaic, serious malformation, mosaic, stunting, mottling, leaf upward cupping, rolling, yellowing, and vein clearing, together with reduced fruit and leaf sizes (Figure 1). Complex infections added to the difficulties in identifying field symptoms induced by one specific virus. As for symptomatic viral infections, the symptom just has one favorable effect on identifying the virus in the distinct difference between normal and virus-affected plants. Nonetheless, identifying the typical virus in zucchini squash according to symptoms only is not easy, since there might be asymptomatic and complex viral infections, or abiotic diseases like a nutrient deficiency. Generally speaking, visual symptom inspection combined with RT-PCR as well as additional tests for validation is important for accurately diagnosing viral infections.

Types and distribution of virus diseases in peppers

In verification tests, 10 typical samples were collected from every virus for positive and negative controls, which were then performed RT-PCR by the use of corresponding primers. Table 3 and Figure 2 display diverse pepper-infecting virus frequencies. Among those 333 samples harvested, 267 were under the infection of one or more viruses, equivalent to the 80.1% infection rate. The results showed that a total of 11 viruses were detected in 333 samples. Single infections accounted for 42.64% (142/333) of plant samples. Complex viral infections that involved 2 and 3 viruses in various combinations were commonly seen.

Infected virus distribution and incidence were different according to the different collection sites. Haidong region displayed the highest infection rate (infections caused by one or more viruses) of 86.3% among those harvested samples, while Xining (71.4%) and Huangnan (44.4%) ranked the second and third places, respectively (Table 3).

Such differences in virus prevalence rates among diverse regions might be associated with the original inocula sources, locations, hosts, as well as early transmission vectors. PCV1, PCV2, MABYV, TSWV, TMV and CABYV could be found within samples at the different counties in Xining, TSWV has the highest detection rate (36%) in Datong County, and PCV1 has the highest detection rate (57%) in Huangzhong County (Figure 2). In the Haidong, 86.3% of the peppers were virus-infected. PCV2, BBWV2, TSWV and CMV were detected in Xunhua, among which PCV1 and CMV had the highest detection rate (30.4%); PCV1, TSWV, and PCV2 were detected in Ledu and PCV2 had the highest detection rate (50%); PCV1, MABYV,

Figure 1. Symptoms of pepper caused by viruses.

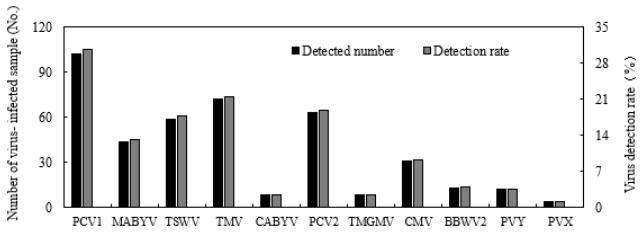

Figure 2. Number of virus-infected pepper sample and their detection rates in Qinghai.

Table 2. Primers used for pepper virus detection.

Virus	Primmer Name	Sequence (5′-3′)	Annealing temperature (°C)	Fragment size (bp)
Chilli veinal mottle virus	ChiVMVF	GGAAARGCNCCNTAYAT	49	790
	ChiVMVR	CGCGCTAATGACATATCGGT		
Turnip mosaic virus	TuMVF	TAAACGGAATGTGGGTGATGATGG	62.3	377
	TuMVR	GTCCTCGGTCGTATGCCTTTCC		
Tobacco mosaic virus	TMVF	GATTCGTTTTAAATATGTCTTAC	54.65	600
	TMVR	CTTCGATTTAAGTGGAGGGA		
Tomato mosaic virus	ToMVF	TCTCAAGAATGTTACACGGGAAG	57.3	980
	ToMVR	CGCATTCTCCGTAATTTTGATC		
Tomato mottle mosaic virus	ToMMVF	CTGGAGAAGACTGGGTCTAG	60.2	1193
	ToMMVR	TTCGGTAAGTTCAATGGGACCT		
Pepper mild mottle virus	PMMoVF	CCTCTTCCGAGAGAATCTGAGAC	59	790
	PMMoVR	CGTGTTTCCAAACTTCAGCCAAG		
Tobacco mild green mosaic virus	TMGMVF	GAGGAAATTGAGGATAATGTAAGTG	57.8	700
	TMGMVR	ACGCCATACCACAGTATACAC		
Cucumber green mottle mosaic virus	CGMMVF	ATGGCTTACAATCCGATCAC	59.15	481
	CGMMVR	CTAAGCTTTCGAGGTGGTAGC		
Pepper vein yellows virus	PeVYVF	CGTGGAAGCGTGCTACTCG	61.15	579
	PeVYVR	CTCATCAGTGAAGACTCGACC		
Tobacco vein distorting virus	TVDVF	GCAACAGCGAGACTTTCATCT	57.8	357
	TVDVR	CRTTGCCTTTATAGAGCAGCC		
Tomato spotted wilt tospovirus	TSWVF	TCACTGTAATGTTCCATAGCAA	53.8	861
	TSWVR	AGAGCAATYGTGTCAATTTTATTC		
Cucumber mosaic virus	CMVF1	CCGAAGTAACCCAYGGTCGT	59.15	969
	CMVR1	GATTTGTCCATGACTCGACTC		
	CMVF2	CGCGAGTTAGCGTTTAGTTGT	59.15	762
	CMVR2	TTAACGTCTTCGGACGCCG		
Tomato aspermy virus	TAVF	ATGGCCCAAAACGGTACGG	62	657
	TAVR	TCACACCGGGAGCGTTGAAG		
Tomato chlorosis virus	TOCVF	GCTTCCGAAACTCCGTCTTG	61	439
	TOCVR	TGTCGAAAGTACCGCCACC		
Potato virus X	PVXF	AGTGCGCGAGGTTTACCAATC	61.3	790
	PVXR	GTGGTTTGCCGCGAACGATTC		
Tobacco bushy top virus	TBTVF	TACCACACCTAAACAGCGTTG	60.15	1049
	TBTVR	CTCATCTCCCGCTAAGTCAG		
Alfalfa mosaic virus	AMVF	GTGCGTATAGATGCCGGTTC	60.15	900
	AMVR	GAGCGAATAGGACTTCATACC		
Southern tomato virus	STVF	TGATGGAGGATATCTACTGTCATT	57.8	681
	STVR	ACAAGATGTTTAAAGCCGTGTCC		
Polerovirus	PoleF	GAYTGYTCYGGTTTTGACTGG	50.65	1394
	PoleR	CGTCTACCTATTTSGGRTTN		
Potato virus Y	PVY-CP-F	GGAAATGACACAATCGATGC	52.15	245
	PVY-CP-R	TCAAACTGATTATTAATTATG		
Chilli ringspot virus	ChiRSVF	ATTACAGCAGAGCGTGAAAAGCAG	60.3	600
	ChiRSVR	CTGGAAATCCTGCTATTGTTGACG		
Pepper mottle virus	PepMoVF	ATGAGCAGCTCAAGATCGG	60.15	500
	PepMoVF	CATATTCCTGACCCCAAGCAGG		
Beet western yellows virus	BWYVF	TCAACGGGGAAGCATGGGAATC	60.8	1080
	BWYVR	CATTAGATTTCGCATTTTGGTAGGC		
Cucurbit aphid-borne yellows virus	CABYV F	CARGCACACACGAGTTGCAAGC	60.8	482
	CABYV R	GATYTTATAYTCATGGTAGGCCTTGAG		

Melon aphid-borne yellows virus	MABYVF	GGTACCACTACGCTACGCAGCAGCC	64.3	262
	MABYVR	GATYTTATAYTCATGGTAGGCCTTGAG		
Pepper cryptic virus 1	PCV1F	ACATCATCGAGGAGTTCACC	59	245
	PCV1R	GCTGTCCTAGAATTGTCTTC		
Pepper cryptic virus 2	PCV2F	TCATCCGTCCAGCTAACGTA	60	375
	PCV2R	CGTCTCTTTTCTGAGCGGTA		
Pepper mild mottle virus	PMMOVF	AGAACTCGGAGTCATCGGC	54	576
	PMMOVR	GAGTTATCGTACTCCCCACG		
Broad been wilt virus 2	BBWV2F	AGRTATATGCTTGGGCAAGCGCATG	60	490
	BBWV2R	CATGAACATTCCCCATCTCCACGTG		
Pepper veinal mottle virus	PVMVF	AATTAAG CCATTGATTGACCA	56	761
	PVMVR	AGCGCCAATTA TGAAACCGC		
Tomato zonate spot tospovirus	TZSV	CCCGGATCCAGAGCAAT	56	930
	TZSV	CACTGGATCTTTTTTTTTG		
Hot pepper endornavirus	HPEVF	CACGGCAGTAGCAAATAGCA	57	546
	HPEVR	TCCGTGTTAATTTGCGTGAA		

TMV and TMGMV were detected in Huzhu and PCV1 had the highest detection rate (83.7%), followed by TMV (81.4%); PCV1 (12%), TSWV (36%) and PCV2 (48%) were detected in Pinan; Minhe County collected fewer samples, and detected CMV, PVX, MABYV. There are few pepper planting areas in Huangnan Prefecture, mainly concentrated in Jianzha County. BBWV2 was detected in 4 of 9 samples collected from Jianzha County (44.4%). In summary, our assayed peppers were subject to infections by PCV1, TMV, TSWV, MABYV, and PCV2 in Xining and Haidong (Table 3). Consequently, PCV1 was possibly a dominant virus occupying almost 30% of the overall prevalence rate, and its greatest rate was 83.7% in Huzhu (Haidong). TMV ranked second place, occupied 21.6%, and its greatest rate was 81.4% in Huzhu (Haidong). PCV2 and TSWV reported prevalence rates of 18.9 and 17.7% and PCV2 with the highest rate in Ledu (50%), and Pinan (48%), respectively. Among the collected samples, CMV had a low prevalence rate relative to BBWV2, PVY, CABYV, TMGMV, and PVX (Figure 3). Such difference was related to diverse factors, including cultivation environment, geographic factors, viral vectors, or farm management.

Compound infections of pepper virus in Qinghai

Additionally, this work discovered complex infections from those affected pepper plants. Peppers were infected via 2 and 3 distinct viruses (Table 4 and Figure 4). Among those 267 infected pepper samples, complex infections by two viruses could be seen among 112 samples (41.9%), and triple infections were found in 39 samples. Mixed double-infections (PCV1- MABYV, TSWV-MABYV, PCV1-TMV, PVY-TMV, TSWV-PCV1, BBWV2-TSWV,

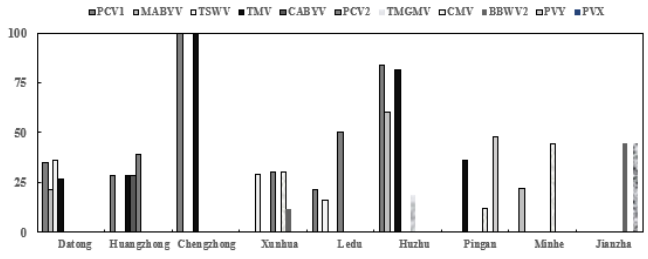

Figure 3. The virus detection rate of pepper samples in Qinghai.

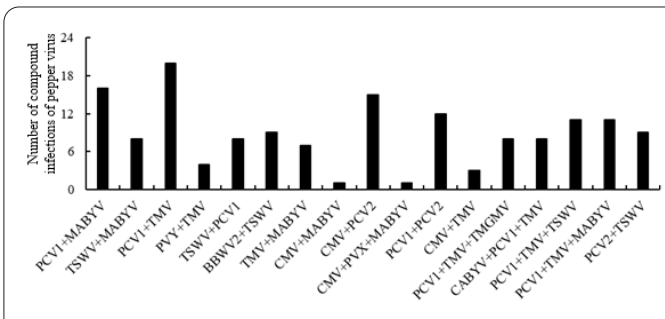

Figure 4. Number of compound infections of pepper virus in Qinghai.

Table 3. Detection results of the major viruses in diseased pepper in Qinghai.

Areas	County	Sample number	Number of virus-infected samples	Virus detection rate (%)	Virus species
Xining	Datong	75	52	69.3	PCV1, MABYV, TSWV, TMV
	Huang Zhong	28	19	67.9	PCV1, TMV, CABYV, PCV2
	Center City	9	9	100	PCV1, TMV
Haidong	Xunhua	79	70	88.6	CMV, BBWV2, TSWV, PCV2
	Ledu	56	37	66.1	PCV1, TSWV, PCV2
	Huzhu	43	43	100	PCV1, MABYV, TMV, TMGMV
	Pingan	25	24	96	TMV, CMV, PVY
	Minhe	9	9	100	CMV, PVX, MABYV
Huangnan	Jianzha	9	4	44.44	BBWV2

Table 4. The detection rate of the composite virus and co-infection types in Qinghai.

County	Detection rate of composite virus (%)	Co-infection types
Datong	71.2	PCV1-MABYV, TSWV-MABYV, PCV1-TMV, PCV1-TMV-TSWV, TSWV-PCV1
Huang Zhong	42.1	CABYV-PCV1-TMV
Center city	100	PCV1-TMV
Xunhua	47.1	CMV-PCV2, BBWV2-TSWV, PCV2-TSWV
Ledu	32.4	PCV1-PCV2
Huzhu	100	PCV1-TMV-TMGMV, PCV1-TMV-MABYV, TMV-MABYV, PCV1-MABYV, PCV1-TMV
Pingan	29.2	CMV-TMV, PVY-TMV
Minhe	25	CMV-PVX-MABYV, CMV-MABYV

TMV-MABYV, CMV-MABYV, CMV-PCV2, PCV1-PCV2, CMV-TMV, PCV2-TSWV), triple-infections (CMV-PVX-MABYV, PCV1-TMV-TMGMV, CABYV-PCV1-TMV, PCV1-TMV-TSWV, PCV1-TMV-MABYV) could be seen at different levels (Figure 4). Typically, PCV1-TMV, PCV1-MABYV, and PCV2-CMV infection was mostly found. Analyzing the compound infection phenomenon in various regions, the compound infection rates of Huzhu (100%), Datong (71.2%), and Chengzhong District of Xining City (100%) were relatively high (Table 3), among which 43 samples were collected in Huzhu County were all compound infection, the number of samples collected in Chengzhong District of Xining City was small, but the 9 samples collected were all co-infected with PCV1 and TMV. In Huangzhong, only CABYV-PCV1-TMV compound infection was detected, and the infection rate was 42%. Xunhua, Ledu, and Ping'an were mainly infected by two kinds of viruses. Among them, the combined infection rate in Xunhua County was 47.1%, and there were mainly three types, namely PCV2-TSWV, CMV-PCV2, and BBWV2-TSWV.

Discussion

In this study, the RT-PCR method was used to detect and evaluate the viral species infecting peppers in Qinghai Province thoroughly. Eleven viruses, including PCV1, MABYV, TSWV, TMV, CABYV, PCV2, TMGMV, CMV, BBWV2, PVY, and PVX were discovered as infecting peppers in Qinghai Province. The detection rates of PCV1, TMV, PCV2, and TSWV among them were all greater than 15%. In the study's pepper production area, the aforementioned four viruses were present in 7 of the study's 9 counties. Accordingly, it was assumed that PCV1, TMV, PCV2, and TSWV were the main viruses infecting peppers in Qinghai at the time.

Researches had been done on the types of viruses that commonly infect peppers in Qinghai Province. Six viruses were found on peppers in Qinghai: CMV, TMV, PMMoV, BBWV2, TuMV, and TSMV (15,19,20). Samples from open-field peppers were not included in the previous study, which was mostly conducted in Haidong City's greenhouses. By methodically gathering virus samples from pepper production sites in Qinghai Province, researchers in this study discovered seven new viruses: TMGMV, MABYV, CABYV, PCV2, PVX, PCV1, and PVY. The detection rate of the MABYV virus was higher at 13.2%. It demonstrated that the prevalence of pepper viral disease species and number appear to alter dramatically with frequent seedling transfers, and the dominant population of virus disease changes noticeably in different years. The surveillance of virus diseases must be done well. Additionally, TMV and TSWV were more frequently found on Qinghai peppers than the other viruses, with detection rates of 21.6% and 17.7%, respectively, among the 11 viruses found. This is in line with the findings of other investigations.

Different virus species and dominant viruses were found in various geographical locations. CMV, PMMoV, TMV, PVY, and TEV were among the pepper viruses in the Gansu Hexi region, with TMV and CMV being the predominant virus species (22). CMV was the most prevalent viral species, with BBWV-2, TMV, TuMV, and ToMV also infecting peppers in Chongqing (23). The viruses that infected peppers in Hainan Province were CMV, ChiVMV, PMMoV, ChiRSV, and PVMV. CMV was the predominant virus, but three viruses—ChiRSV, PMMoV, and PVMV—were found at levels greater than 30% and have since grown to be significant pathogens on peppers in Hainan Province (24). Ten types of viruses, including BBWV-2, CMV, PMMoV, TMV, PVY, ToMV, TuMV, TSWV, AMV, and PepMoV, infected peppers in Guizhou province (25). There were several viruses affecting peppers in Tianjin, with CMV predominating among them. These viruses included BBWV, CMV, TMV, ToMV, TSWV, and TYLCV (26). The findings of this study revealed that among the 11 viruses affecting peppers in Qinghai, PCV1, TMV, PCV2, and TSWV were the prevalent viruses. These viruses differed noticeably from the dominant viruses on peppers in other Chinese provinces.

Additionally, it was discovered that the predominant virus kinds in various parts of Qinghai Province varied significantly. Datong County had the highest rate of TSWV detection, Huanzhong County had the highest rate of PCV1 detection, Xunhua County had the highest rate of CMV and PCV2 detection, Ledu District had the highest rate of PCV2 detection, Huzhu County had the highest rate of TMV and PCV1 detection, Ping'an District had the highest rate of PVY detection, and Jianzha County had the highest rate of BBWV2 detection. The predominant virus affecting peppers in Qinghai Province did not correlate with the predominant virus in Jianzha and Zhuanhua counties. The local farming system, cultivars, and environment may all play a role in this phenomenon. For instance, the primary cultivar in Xunhua County is line pepper, which has been grown there since the 1960s. This cultivar has a long growing season and a small number of varieties, which is distinct from the cultivation pattern in other parts

of Qinghai Province.

Compound plant virus invasion was a typical natural occurrence. In nine Chinese provinces, Zhang (27) found that 79.33% of the samples tested were simultaneously infected by two or more viruses. According to Yao et al. (28), CMV and PMMoV compound infection of pepper was widespread in fields in the regions of Hainan and Guangdong. In the Chongqing region, Guo et al. (29) observed that the compound infestation rate of the pepper viral disease was 66.10%, with the compound infestation rates of two, three, four, or more viruses being 30.51%, 26.27%, and 9.32%, respectively. In Guizhou, peppers were infected by 32.66% of two or three viruses, according to Wang et al. (25). In this investigation, we discovered that Qinghai peppers frequently have virus complex infestation. There were more complicated PCV1 and TMV infestation types among the 267 positive samples, with 125 samples having two or three viral complex infestations. Thus, it was clear that viruses two and three made up the majority of the invasion. This study clarified the main virus species on Qinghai pepper through systematic investigation, which laid the foundation for further research on the pathogenic mechanism of these viruses.

In 7 out of the 9 counties and cities in Qinghai, PCV1, TMV, TSWV, TMV, CABYV, PCV2, TMGMV, CMV, BBWV2, PVY, and PVX all had detection rates above 15%. They were the predominant viruses that affect pepper in Qinghai and are very dissimilar from the predominant viruses that affect pepper in other Chinese provinces. Furthermore, there was a significant infestation of two and three virus complexes, which were the most common types of infestation, on the Qinghai pepper at the same time.

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Conflicts of interest
The authors declare that they have no competing interests.

Data availability statement
The data used to support the findings of this study are included in the article.

Author contributions
Conceptualization, JH.Y. and T.H.; methodology, JH.Y., and L.H.; software, T.H., H.W.; formal analysis, JH.Y.; investigation, T.H.; resources, L.H.; writing–original draft preparation, T.H.; writing–review and editing, JH.Y.; supervision, JH.Y., and T.H.; project administration, H.W.; All authors have read and agreed to the published version of the manuscript.

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