SHORT COMMUNICATION

INCIDENCE OF MAJOR PEACH VIRUSES AND VIROIDS IN CHINA

Y. Yu1,2, Z. Zhao2, L. Qin2, Y. Zhou2, H. Fan2, Z. Zhang2, Z. Wu1 and S. Li2

1Institute of Plant Virology, Fujian Agriculture and Forestry University, Jinshan, Fuzhou, Fujian 350002, P.R. China
2State Key Laboratory of Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Yuanmingyuan West Road No 2, Haidian District, Beijing 100193, P.R. China

SUMMARY

Field surveys were carried out in the main peach-growing areas of China to assess the virus or viroid disease status of peach trees. A total of 505 leaf samples were tested by ELISA or RT-PCR for the presence of nine major peach viruses. The viruses most frequently detected were Apple chlorotic leaf spot virus (ACLSV) (71.8%), followed by Prunus necrotic ringspot virus (PNRSV) (20.2%), Cherry green ring mottle virus (CGRMV) (8.1%), and Apricot pseudo-chlorotic leaf spot virus (APCLSV) (1.6%). The overall average of virus infection level in peach trees was 24.6%. Apple mosaic virus (ApMV), Plum pox virus (PPV), Prune dwarf virus (PDV), Cherry virus A (CVA) and Cherry leaf roll virus (CLRV) were not detected. Of another 583 leaf samples tested for the presence of the known major peach viroids, dot-blot hybridization, 444 (76.2%) were infected. Peach latent mosaic viroid (PLMVd) and Hop stunt viroid (HSVd) were detected in 443 and 18 samples, respectively. This is the first large-scale study on major viruses and viroids that infect peach trees in China.

Key words: Peach, ELISA, RT-PCR, dot-blot hybridization, virus detection, viroids.

Prior to this study, several viruses had been detected in stone fruit trees in China: PDV, PNRSV, ApMV and ACLSV in sweet cherry (Zhou et al., 1996); ACLSV and Apricot pseudo-chlorotic leaf spot virus (APCLSV) in peach (Niu et al., 2012a, 2012b); PPV in apricot (Navratil et al., 2005) and Cherry virus A (CVA) in Prunus mume (Marais et al., 2008). PLMVd and HSVd have been reported from peach, apricot and plum in China (Turturo et al., 1998; Yang et al., 2006; Yang et al., 2007; Zhou et al., 2006). Apple scar skin viroid (ASSVd), which infects pome fruit trees (Hadidi and Barba, 2011), was also detected from naturally infected peach and apricot trees in China (Zhao and Niu, 2008a, 2008b). Subsequently, this viroid was found in sweet cherry in Greece (Kaponi, 2009; Kaponi et al., 2013) and Himalayan wild cherry (P. cerasoides) in India (Walia et al., 2012).

Currently, there is no information on the incidence and distribution of the major peach viruses and viroids in China. Therefore, to gain a better insight into the disease status of peach in this country, we assessed the presence of ACLSV, CGRMV, PNRSV, APCLSV, ApMV, PPV, PDV, CVA, CLRV, PLMVd and HSVd.

From 2011 to 2012, 505 peach leaf samples were randomly collected from 12 provinces (Table 1) where peach trees are grown (Fig. 1). The samples were tested by

<table>
<thead>
<tr>
<th>Province</th>
<th>No. of samples Tested</th>
<th>Infection rate (%)</th>
<th>ACLSV</th>
<th>PNRSV</th>
<th>CGRMV</th>
<th>APCLSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hebei</td>
<td>193</td>
<td>27</td>
<td>14.0</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gansu</td>
<td>90</td>
<td>19</td>
<td>21.1</td>
<td>13</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Shandong</td>
<td>60</td>
<td>35</td>
<td>58.3</td>
<td>28</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Henan</td>
<td>48</td>
<td>15</td>
<td>31.3</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Yunnan</td>
<td>36</td>
<td>13</td>
<td>36.1</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sichuan</td>
<td>22</td>
<td>10</td>
<td>45.4</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Beijing</td>
<td>19</td>
<td>3</td>
<td>15.8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jiangsu</td>
<td>17</td>
<td>1</td>
<td>5.9</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fujian</td>
<td>9</td>
<td>1</td>
<td>11.1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hubei</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liaoning</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shanxi</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>505</td>
<td>124</td>
<td>24.6</td>
<td>89</td>
<td>25</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1. Relative incidence of four major viruses infecting peach trees in China as revealed by DAS-ELISA.

Corresponding author: S.Li; Z.Wu
Fax: +86.10.62890875
E-mail: sfli@ippcaas.cn
double-antibody sandwich ELISA (DAS-ELISA) (Clark and Adams, 1977) using commercial kits for ACLSV (Neogen, USA), PNRSV (Agdia, USA), APCLSV (Yuping, China), and an antiserum to CGRMV (kindly provided by Dr. Zhou Jufang, Huazhong Agriculture University). Table 1 shows the incidence of each of the four viruses infecting peach trees in the 12 provinces as revealed by ELISA. ACLSV, PNRSV, CGRMV and APCLSV were detected in 71.8, 20.2, 8.1 and 1.6% of the samples, respectively. Mixed infections by ACLSV and CGRMV were found in eight samples, ACLSV and PNRSV or ACLSV and APCLSV were detected in one sample.

The samples were also tested singly by RT-PCR, using protocols and primers designed for ACLSV, ApMV, PDV (Sanchez-Navarro et al., 2005), and CVA (Marais et al., 2012). An additional five pairs of primers were designed in this study and evaluated for each virus based on available sequences in GenBank using the Primer Select program in DNAStar 5.01 (Lynnon Corporation, Canada) (Table 2). No infections by ApMV, PPV, PDV, CVA and CLRV were detected by RT-PCR assays. All samples that tested positive by ELISA were also positive by RT-PCR, however, three samples positive for ACLSV in the RT-PCR assay were negative in ELISA.

Some of the surveyed leaf samples showed symptoms ranging from yellowing, mottling and necrosis/chlorosis, which were associated with the presence of viruses of the genus Ilarvirus.

Another 583 peach leaf samples were collected from nine Chinese provinces (Table 3). Dot-blot hybridization experiments were carried out using digoxigenin (DIG)-labeled cRNA probes for PLMVd and HSVd. Probes were prepared from linearized recombinant plasmids containing individual full-length cDNA of each viroid by in vitro transcription using T7 RNA polymerase according to the instructions in the DIG RNA labeling kit (Roche, Switzerland). Pre-hybridization and hybridization were performed according to instructions of DIG RNA Labeling Kit and Detection Starter Kit (Roche, Switzerland).

Dot-blot hybridization assays showed that 444 of 583 samples (76.2%) tested positive for viroids (PLMVd or HSVd) (Table 3). Mixed infections by PLMVd and HSVd were detected in 17 samples. PLMVd was detected in peach trees from all provinces surveyed with incidence up to 100%, except for Fujian in which only one sample was collected. HSVd was mostly restricted to northern China, the infection rates of individual provinces being: Beijing, 19.6%, Gansu, 20.0%, Shaanxi, 21.1%, Xinjiang, 33.3%. The viroid, however, was not detected in peach trees from Henan, Hebei, Shandong and Guangxi provinces. RT-PCR using primers designed for PLMVd (Loreti et al., 1999) and HSVd (Zhou et al., 2006) was carried out on weakly positive samples, and results confirmed the hybridization analysis (data not shown).

It is interesting to note that the four peach viruses ACLSV, PNRSV, CGRMV, and APCLSV were found in distinct geographical regions (Fig. 2A). ACLSV prevailed in Hebei (90%), Yunnan (84.6%) and Shandong (77.8%) provinces, but it was not present in Jiangsu province. In contrast, PNRSV had the highest infection level in Jiangsu (100%), but was not detected in Hebei and Shandong. CGRMV was restricted to Shandong (19.4%), Gansu (4.2%) and Fujian (50%). APCLSV which has recently been reported from China (Niu et al., 2012b), was detected in 5.6% of Shandong peach trees. The geographical...
isolation of the virus could be due to different modes of its disseminations, although other explanations are also possible, such as the impact of cultural practices (top grafting, rootstock and scion infection, etc.).

Significant differences in the incidence of the same virus were observed in different regions (Fig. 2B). The infection rate of ACLSV in the surveyed provinces varied from 0.5% (Beijing) to 4.9% (Shandong). No significant differences of PNRSV, CGRMV and HSVd incidence were observed between different provinces. In contrast, a clear difference in PLMVd incidence was observed: 62.5% in Henan and 0.2% in Shandong and Guangxi.

This study has shown a virus disease incidence, especially in northern China, at a rate higher than that reported from other countries like Algeria (Rouag et al., 2008) and Egypt (El-Maghraby et al., 2007). ApMV, PPV, PDV, CVA and CLRV were not detected, thus more samples need to be tested to establish whether or not these viruses occur in the country. No viruses were detected in Hubei, Liaoning and Shanxi by ELISA and RT-PCR. However, since only 11 samples were analyzed, additional testing from these regions is desirable.

The incidence of PLMVd in peach was higher than that found in other countries: Spain (Badenes and Llacer, 1998), Syria (Ismaiel et al., 2001), western Turkey (Torres et al., 2004), Jordan (Al-Rwahnih et al., 2001), Algeria (Rouag et al., 2008), and Italy (Barba and Faggioli, 1999). The incidence of HSVd was relatively lower than that that recorded from other countries like Algeria (Rouag et al., 2008), western Turkey (Torres et al., 2004), and Egypt (El-Maghraby et al., 2007). No attempts were made in this study to test for ASSVd in peach trees.
To the best of our knowledge, this is the first large-scale survey of the major viruses and viroids that infect peach trees in China. Although further and more detailed investigations are desirable, we recognize that the results of the present study provide a basis for the establishment of a national certification program for the phytosanitary improvement of the Chinese peach industry, that would limit the dissemination of graft-transmissible diseases within the country.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ear-marked Fund for China Agriculture Research System (CARS-31), the National Basic Research and Development Program of China (973 Program) (No. 2009CB119200), the Special Fund for Agro-scientific Research in the Public Interest (Nos. 201203076 and 200903004) and the National Natural Science Foundation of China (Nos. 31171819 and 31000842).

REFERENCES


