MOLECULAR CHARACTERIZATION AND TISSUE-SPECIFIC COPY NUMBER OF THREE PLASMIDS FROM WHEAT BLUE DWARF PHYTOPLASMA

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SUMMARY

Three plasmids of wheat blue dwarf (WBD) phytoplasma, named pWBD1, pWBD2 and pWBD3, were cloned and confirmed by southern blot. They were 3449 bp, 3601 bp and 3844 bp in length, respectively, and encoded fifteen proteins in all, which included five secreted proteins and five membrane proteins, as well as the replication-associated protein (Rep), copy number control protein (Cop) and single-stranded DNA-binding protein (SSB). Phylogenetic analysis based on the sequences of Rep proteins from 16 phytoplasma type II extrachromosomal DNA molecules (EC-DNAs) and geminiviruses indicated that pWBD1 and pWBD2 were most closely related to pPaWBNy-1 from paulownia witches’ broom phytoplasma Nanyang strain, while pWBD3 was most closely related to pPaWBNy-2. Real time PCR analysis revealed that the copy numbers of WBD phytoplasma plasmids had tissue specificity in infected periwinkle and changed in the course of infection. The copy numbers of plasmids were higher in the tissues below the graft union, especially in the roots. pWBD1 copy number changed little during infection, whilst pWBD2 and pWBD3 copy number peaked three months after symptom appearance.

Key words: southern blot; secreted proteins; membrane proteins; phylogenetics; real-time PCR

INTRODUCTION

Wheat blue dwarf (WBD) disease is one of the most important crop diseases affecting winter wheat (Triticum aestivum L.) in northwestern China. Epidemics of this disease have occurred more than 10 times in Shaanxi province since 1960. The outbreak was estimated to cover 900,000 ha and to cause a total loss of about 50,000 metric tons of wheat yield during an average epidemic year (Zhang et al., 1996). The disease is caused by the WBD phytoplasma, which is transmitted by Psammotettix striatus (Hemiptera: Cicadellidae). Based on a comprehensive classification scheme for phytoplasmas (Lee et al., 1993, 1998, 2000), WBD phytoplasma was recognized as a member of the 16Sr I group (Wu et al., 2010).

So far, a number of plasmids from phytoplasmas have been sequenced. Seven plasmids, ranging from 3.1 to 7 kb in size, were identified in three strains of Onion yellows (OY) phytoplasma (Nishigawa et al., 2003). Four plasmids, 3.8 to 5.1 kb in size, were found in Aster yellows witches’ broom (AY-WB) phytoplasma (Bai et al., 2006), and two plasmids existed in ‘Candidatus (Ca.) Phytoplasma (P.) austrianiense’ (Liefting et al., 2006). Two plasmids, 4.4 kb and 4.8 kb in size, were cloned from paulownia witches’ broom phytoplasma Nanyang strain (PaWBNy) (Lin et al., 2009). In addition, plasmid pNJAY from New Jersey aster yellows phytoplasma (Saccardo et al., 2011), plasmid pCWBFq from Chinaberry witches’ broom phytoplasma-Fuqing strain (CWBFq) (Song et al., 2011) and plasmid pPLLHn-1 from Periwinkle little leaf phytoplasma (PLL-Hn) (Zheng et al., 2012) were also sequenced. Five of these 18 plasmids are type I EC-DNAs, the remaining 13 plasmids are type II EC-DNAs. Type I EC-DNAs are true plasmids, as they possess an expressed gene with significant similarity to the replication initiator (Rap) of the plasmids of the pLS1/pMV158 family. Type II EC-DNAs are unique to phytoplasmas, and characterized by a gene with sequence similarity to the replication associated protein (Rep) of the geminiviruses. The relatively modest similarity between the replication associated proteins of type I EC-DNAs and type II EC-DNAs suggest an independent origin (Firrao et al., 2007). Up to now, 20 type I EC-DNAs and 13 type II EC-DNAs are reported in GenBank database.

Although the existence of EC-DNAs has been reported in numerous phytoplasmas, there is very little information on the function of the encoded proteins. Remarkably, some proteins encoded by plasmids are secreted or membrane proteins. Seven secreted proteins are encoded by four plasmids of AY-WB phytoplasma (Bai et al., 2006). Three hypothetical proteins encoded by pPAPh2 and pPASb11 are putative membrane proteins (Liefting et al., 2006). Membrane proteins are very important for the interaction between spiroplasmas and their hosts (Ye et al., 1997), and secreted proteins are considered as effector candidates (Kakizawa et al., 2004). Phytoplasma plasmids presumably played key roles in pathogen evolution,
and contributed to the diversity of phytoplasma species through horizontal exchange of genes encoding virulence factors, niche adaptation factors, and factors conferring competitive advantages to phytoplasma cells (Davis et al., 1988; Toruno et al., 2010).

For characterizing the plasmids of WBD phytoplasma and understanding their functions, three plasmids from WBD phytoplasma were cloned and confirmed by southern blot. Sequence analysis and phylogenetic analysis were also conducted. Additionally, the copy numbers of the three plasmids in a spatial and temporal way were determined by real time PCR analysis. These results will provide the groundwork for exploring the functions of plasmids from WBD phytoplasma.

MATERIALS AND METHODS

Phytoplasma sources. Wheat seedlings infected by WBD phytoplasma were collected from open fields in Hancheng (Shaanxi province, China). The phytoplasma was transmitted to healthy periwinkles by *P. striatus*, then maintained and propagated in an insect-proof greenhouse by periodic grafting. Periwinkles infected with WBD phytoplasma displayed virescence, phyllody and yellowing. Healthy periwinkles were used as a control.

Plasmid amplification and analysis. Total DNA was extracted from flowers and leaves of healthy periwinkles and tissues of WBD phytoplasma-infected periwinkle using the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) and then diluted to 50 ng/μl for use as a template for PCR amplification and real time PCR analysis. Seven primer pairs (Table 1) were designed to amplify the plasmids. Amplification conditions consisted of 35 cycles of the following steps: denaturation for 45 s at 94°C, annealing for 45 sec at 50°C, 1 min per predicted kb of the product at 72°C. A final extension period of 10 min at 72°C was included to ensure complete extension of the amplified products. The fragments were cloned into pMD18-T simple vector (Takara-Bio, China) and sequenced. Overlapping DNA fragments were assembled using DNAMAN 6.0 software. ORFs were predicted by the ORF finder (www.ncbi.nlm.nih.gov/gorf/gorf.html) with standard genetic code. Transmembrane regions were identified by the TMHMM-2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and signal peptides were predicted by SignalP-3.0 (http://www.cbs.dtu.dk/services/SignalP-3.0/) programs. Phylogenetic analysis was performed with PhyML (Krupovic et al., 2009; Guindon et al., 2010).

Southern blot. Southern blot analyses were performed on genomic DNA extracted from healthy periwinkle and WBD phytoplasma-infected periwinkle which had displayed symptoms for 12 months. Total DNA extracted from infected and healthy periwinkles was digested with *Hpa*I. Restricted total DNA was subjected to electrophoresis on 0.8% agarose gel at 2 V/cm for 6 h at room temperature in 1× TAE buffer and transferred to a nylon membrane. Fragments amplified by ppf/ppr (Table 1) were used as probes to detect these three plasmids (see Results). Southern blot was carried out using a DIG high prime DNA labeling and detection starter kit (Roche Diagnostic, Germany) according to the manufacturer’s instruction and the method of Lin et al. (2009).

Plasmid quantification. According to the sequences of the three plasmids, three primer pairs (Table 1) for real time PCR analysis were designed to quantify the copy number of each plasmid. The 16S rDNA from WBD phytoplasma genome was amplified with primers rt16s1162 and rt16s999 (Table 1) as a reference gene. There are two copies of 16S rDNA in one phytoplasma, so the number of WBD phytoplasma is half of that of 16S rDNA amplicon,
assumed similar amplification efficiency of the four am-
plicons. The real-time PCR reactions were carried out
using SYBR premix Ex Taq™ II kit (Takara-Bio, Dalian,
China) with the following PCR conditions: 95°C for 1 min,
40 cycles at 95°C for 10 s, 50°C for 20 s, 72°C for 40 s.
Melting curves were analyzed at the end of each amplifica-
tion. All samples were tested three times.

RESULTS AND DISCUSSION

Amplification of the fragments from WBD phyto-
plasma plasmids. Using primers deduced from published
plasmid sequences and WBD phytoplasma DNA as tem-
plate, two different DNA fragments were amplified. In
detail, a fragment named Frep was amplified using prim-
ers Rep325 and Rep736, designed to amplify a fragment of
the Rep gene according to the sequences of pPaWBNy-1
(GenBank accession No. ABR08376.1) and pPaWBNy-2
(GenBank accession No. ABR08382.1); and a fragment
named F1 was amplified using Rep325 (described above)
and CDs1-394, designed on CDS1 of pAY-WB I (GenBank
accession No. ABC65789.1) and pOYNIM (GenBank ac-
cession No. BAIH22376.1). Both Frep and F1 (Fig. 1a)
were sequenced and resulted to code for different Rep
and flanking sequences and were therefore further inves-
tigated separately.

Outward primers designed from the F1 sequence per-
mitted the amplification of two major PCR products, namely F2 and F3 (Fig. 1b). Fragment F3 was sequenced
and showed sequence overlap with F1, prompting the
hypothesis that F1 and F3 build up a plasmid, that was
named pWBD1. Fragment F2 was also isolated and se-
quenced and resulted not overlapping with F1, thus being
supposedly part of a second plasmid. Primers developed
from F2 sequence allowed the amplification of fragment
F4 (Fig. 1c), whose sequence was consistent with the hy-
pothesis that F2 and F4 build up plasmid pWBD2.

Furthermore, the sequence of fragment Frep was also
used for the design of outward primers P3F5F and P3F5R,
that permitted the amplification of an amplicon, named
F5 (Fig. 1d), which, however, could not be assembled with
Frep. Outward primers P3F6F and P3F6R were therefore
designed from F5 sequence, and F6 (Fig. 1e) was thus ob-
tained. Sequence overlap between F5 and F6 suggested
that these two fragments may build up a third plasmid,
named pWBD3. The fragment Frep, which could not be
assigned to a plasmid molecule, may have possibly origi-
nated from a sequence located in the chromosome of
WBD phytoplasma.

Sequence analysis of three plasmids. pWBD1 (Gen-
Bank accession No. JX668987), assembled by F2 and F4,
was 3,449 bp in length with a G+C content of 25.4 mol%.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences (5’-3’)</th>
<th>Products (size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep325</td>
<td>GAAGGCACCCCTAGACAT</td>
<td>Frep (412 bp)</td>
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<tr>
<td>Rep736</td>
<td>GGTTACCGAGTCTACTAAG</td>
<td></td>
</tr>
<tr>
<td>CDs1-197</td>
<td>ATTTTAATCTTTATCATTTT</td>
<td></td>
</tr>
<tr>
<td>CDs1-394</td>
<td>AAATCAATCTTCTTCTTCTT</td>
<td></td>
</tr>
<tr>
<td>P2F1F</td>
<td>GAAGGCACCCCTAGACAT</td>
<td></td>
</tr>
<tr>
<td>P2F1R</td>
<td>AAATCAATCTTCTTCTTCTT</td>
<td></td>
</tr>
<tr>
<td>P1F2F</td>
<td>AGCAAGGTAAAAGAGTTAGT</td>
<td></td>
</tr>
<tr>
<td>P1F2R</td>
<td>GGTGGAAGGAAAGATAGGATTTG</td>
<td></td>
</tr>
<tr>
<td>P1F4R</td>
<td>TACCGAAAACCATCAACAG</td>
<td></td>
</tr>
<tr>
<td>P1F4R</td>
<td>TGTCGAATTGTTGGATTA</td>
<td></td>
</tr>
<tr>
<td>P3F5F</td>
<td>CTTCATCTTTACCTTCTCT</td>
<td></td>
</tr>
<tr>
<td>P3F5R</td>
<td>AGTGCTCTAATATCTTTTGTG</td>
<td></td>
</tr>
<tr>
<td>P9F6F</td>
<td>GTGAGGAAAGGAGTTGTCATTTG</td>
<td></td>
</tr>
<tr>
<td>P9F6R</td>
<td>GGGATAGGAGTAAGGAAAAAA</td>
<td></td>
</tr>
<tr>
<td>pf</td>
<td>GAAGGCACCCCAAGAYAT</td>
<td></td>
</tr>
<tr>
<td>prr</td>
<td>GGTGGTTTCCGRAGTCCTACTA</td>
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</tr>
<tr>
<td>RTP1F</td>
<td>GAACCTAAAAATGCTCAA</td>
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</tr>
<tr>
<td>RTP1R</td>
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</tr>
<tr>
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<td>RTP2R</td>
<td>CTGTCTCTTTTTTCTCTGTC</td>
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<tr>
<td>RTP3F</td>
<td>ATGGTGAGTTGCTGAGAA</td>
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<tr>
<td>RTP3R</td>
<td>ATAGGTTTAAAGGATAGG</td>
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</tr>
<tr>
<td>rt16s1162</td>
<td>GCTGGTACATTAGTCTAG</td>
<td></td>
</tr>
<tr>
<td>rt16s999</td>
<td>GCTCGGTACAGGTTTCTT</td>
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Table 1. Primers used in this study.
Four open reading frames (ORF 1-4) longer than 50 amino acids (aa) were oriented in the same direction (Fig. 2a). The overlapped sequences F1 and F3 were assembled as a circular DNA molecule and designated pWBD2 (GenBank accession No. JX668988), which was 3,844 bp with 23.9 mol% G+C, and contains five ORFs encoding products longer than 50 aa, all oriented in the same direction (Fig. 2b). The complete nucleotide sequence of pWBD3 (GenBank accession No. JX668989) was determined to be 3,601 bp with a G+C content of 24.2 mol%. Six ORFs were predicted in pWBD3, five of which (ORF1, 3, 4, 5, 6), longer than 100 aa, were organized on one strand, while ORF2, 52 aa in length, was located on the other strand (Fig. 2c). The size of encoded proteins, their putative functions, and homology with other proteins are summarized in Tables 2 to 4.

The deduced aa sequence of ORF1 in pWBD1, pWBD2 and pWBD3 contained conserved motifs present in Rep proteins of other plasmids. The proteins encoded by ORF3 in pWBD1, ORF3 in pWBD2 and ORF4 in pWBD3 had the same motif with the Cop protein of pPLLHn-1 from Periwinkle little leaf phytoplasma. The proteins encoded by the ORF5 in pWBD2 and ORF6 in pWBD3 were considered as SSB protein. Transmembrane domain analysis by the TMHMM V2.0 program revealed that putative proteins deduced from ORF2, ORF3 and ORF4 in pWBD1; ORF2, ORF3 and ORF4 in pWBD2, ORF3, ORF4 and ORF5 in pWBD3 had several transmembrane regions. Putative proteins from ORF3 and ORF4 in pWBD1, ORF3 and ORF4 in pWBD2, ORF4 and ORF5 in pWBD3 had signal peptides, and were therefore regarded as possible secreted proteins. However, the protein encoded by ORF4 in pWBD2,

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### Table 2. Predicted ORFs in pWBD1.

<table>
<thead>
<tr>
<th>Predicted ORFs</th>
<th>Position (nucleotide)</th>
<th>Homologous protein (GenBank accession No.)</th>
<th>Phytoplasma showing best match</th>
<th>Identity (%)</th>
<th>E-value</th>
</tr>
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<tbody>
<tr>
<td>ORF1</td>
<td>1-1179</td>
<td>Rep (YP_001708784.1)</td>
<td>pPaWBBy-1 of PaWB</td>
<td>79.34</td>
<td>0.0</td>
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<td>ORF2</td>
<td>2003-2368</td>
<td>hypothetical protein (YP_456601.1)</td>
<td>Chromosomal gene of AYWB</td>
<td>66.93</td>
<td>9e-44</td>
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<tr>
<td>ORF3</td>
<td>2426-2920</td>
<td>hypothetical protein (YP_002600754.1), Cop (AFA53692.1)</td>
<td>pOYM of OY, pPLLHn-1 of PLL-Hn</td>
<td>50.85</td>
<td>8e-24</td>
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<tr>
<td>ORF4</td>
<td>2923-3258</td>
<td>hypothetical protein (BAH22131.1)</td>
<td>Chromosomal gene of OY-W</td>
<td>43.51</td>
<td>2e-16</td>
</tr>
</tbody>
</table>

### Table 3. Predicted ORFs in pWBD2.

<table>
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<tr>
<th>Predicted ORFs</th>
<th>Position (nucleotide)</th>
<th>Homologous protein (GenBank accession No.)</th>
<th>Phytoplasma showing best match</th>
<th>Identity (%)</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF1</td>
<td>1-1179</td>
<td>Rep (YP_001708784.1)</td>
<td>pPaWBBy-1 of PaWB</td>
<td>79.34</td>
<td>0.0</td>
</tr>
<tr>
<td>ORF2</td>
<td>1913-2146</td>
<td>hypothetical protein (AEA36721.1)</td>
<td>p09PLY-2 of Periwinkle leaf yellowing phytoplasma (PLY)</td>
<td>35.85</td>
<td>1e-21</td>
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<tr>
<td>ORF3</td>
<td>2414-2966</td>
<td>hypothetical protein (AEA36717.1), Cop (AFA53692.1)</td>
<td>p09PLY-1 of PLL-Hn</td>
<td>53.48</td>
<td>1e-50</td>
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<tr>
<td>ORF4</td>
<td>2969-3457</td>
<td>hypothetical protein (BAH22131.1)</td>
<td>Chromosomal gene of OY-W</td>
<td>55.38</td>
<td>2e-36</td>
</tr>
<tr>
<td>ORF5</td>
<td>3481-3795</td>
<td>SSB (YP_456346.1)</td>
<td>Chromosomal gene of AYWB</td>
<td>86.54</td>
<td>6e-60</td>
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</table>
which contained a predicted transmembrane region in addition to a signal peptide, was likely to remain attached to the WBD phytoplasma membrane after secretion.

All three plasmids from WBD phytoplasma belonged to Type II EC-DNA which is unique to phytoplasmas (Firrao et al., 2007). In addition to Rep, SSB and Cop, there were seven unknown proteins in these three plasmids, among which two were secreted proteins and four membrane proteins. A large proportion of membrane proteins identified in genomes and plasmids of phytoplasmas might contribute to phytoplasma survival and interactions with hosts (Suzuki et al., 2006).

**Detection of WBD phytoplasma plasmids.** Three DNA fragments of estimated size between 3.5 and 4.5 kbp were detected in total DNA extracted from WBD-infected periwinkle and digested by HpaI and presumed to be linear forms of the three cloned plasmids (Fig. 3). No band was observed in total DNA extracted from healthy periwinkle. Thus, the three plasmids sequenced in this work appear to be the complete plasmid complement of this isolate of WDB phytoplasma.

**Comparative analysis of phytoplasma plasmids.** Phylogenetic analysis was carried out on the sequences of Rep proteins from 16 phytoplasma Type II Ec-DNAs and geminiviruses. pWBD1 and pWBD2 were more closely related to pPaWBNy-1 from PaWB phytoplasma than to plasmids from other phytoplasmas, while pWBD3 had a closest relationship with pPaWBNy-2 (Fig. 4).

**Distribution of the three plasmids in WBD phytoplasma-infected periwinkle.** To investigate the distribution of WBD phytoplasma plasmids in plants, 11 tissue samples form symptomatic periwinkles were used to quantify the copy numbers of these three plasmids. In all 11 tissue samples, the copy number of pWBD2 was the highest, and the pWBD3 was the lowest (Fig. 5). For pWBD1 and pWBD2, the lowest copy number was found in leaves and flowers, and the highest in the roots, whilst the copy number in the stem was intermediate. For pWBD3 the copy number was very low above the graft union. In the tissues below the graft union the highest copy number of pWBD3 was in the roots, followed by leaves and stem.

Meanwhile, top leaves of WBD phytoplasma-infected periwinkle in different phases of infection (time the symptom appearance, one, three, seven and twelve months after symptom appearance) were used to study the changes of plasmids copy numbers (Fig. 6). It was shown that the copy number of pWBD1 changed little during the whole period of infection. The copy number of pWBD2 peaked three months after symptom appearance, and dropped to the level of that one month after symptom appearance. Twelve months after symptom appearance, it further dropped to the same level of that reached at the time of first symptom appearance. The highest copy number of pWBD3 (67±1) was recorded three months after symptom appearance, and dropped to 60±1 at seven months after symptom appearance. Twelve months after symptom appearance, it was lower than that recorded one month after symptom appearance. Interestingly, Liefting et al. (2004) found that only pBLTVA-1 could be detected in recently infected periwinkle shoots, while the concentration of pBLTVA-2 increased significantly later in the infection process according to Southern blot analysis. Another study found that two high-copy plasmids existed in

### Table 4. Predicted ORFs in pWBD3.

<table>
<thead>
<tr>
<th>Predicted ORFs</th>
<th>Position (nucleotide)</th>
<th>Homologous protein (GenBank accession No.)</th>
<th>Phytoplasma showing best match</th>
<th>Identity (%)</th>
<th>E-value</th>
</tr>
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<tbody>
<tr>
<td>ORF1</td>
<td>1-1101</td>
<td>Rep (YP_004422914.1)</td>
<td>pCWBFq of CWBFq</td>
<td>88.01</td>
<td>0.0</td>
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<td>ORF2</td>
<td>1694-1536</td>
<td>hypothetical protein (YP_003617080.1)</td>
<td>pARG1 p2 of Rehmannia glutinosa phytoplasma</td>
<td>12.28</td>
<td>0.049</td>
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<td>ORF3</td>
<td>1784-2275</td>
<td>hypothetical protein (YP_002600753.1)</td>
<td>pOYM of OY</td>
<td>72.56</td>
<td>1e-43</td>
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<td>ORF4</td>
<td>2282-2734</td>
<td>hypothetical protein (YP_002600754.1), Cop (BAH22368.1)</td>
<td>pOYM, EcOYW1 of OY</td>
<td>51.98</td>
<td>1e-36</td>
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<td>ORF5</td>
<td>2740-3210</td>
<td>hypothetical protein (YP_001708788.1)</td>
<td>pPaWBNy-1 of PaWB</td>
<td>61.39</td>
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<td>ORF6</td>
<td>3237-3548</td>
<td>SSB (YP_214984.1)</td>
<td>pOYM of OY</td>
<td>92.31</td>
<td>1e-62</td>
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</table>

Fig. 3. Southern blot analysis of WBD phytoplasma plasmids. M: 500 bp DNA Ladder (TIANGEN, China); 1. Total DNA extracted from WBD-infected periwinkle and restricted by HpaI. 2. Total DNA extracted from healthy periwinkle and restricted by HpaI. Bands of plasmids from WBD phytoplasma are indicated by white arrows.
Fig. 4. Phylogenetic tree of the RCR Rep proteins. Maximum likelihood tree was obtained using the PhyML program with the WAG evolutionary model. Sequence alignment was constructed using CLUSTALW. Numbers at the relevant branch-points represent bootstrap values (1000 replicates). GenBank accession Nos: EcOYW2 (BAD36752.1), EcOYM (BAD04084.1), EcOYNIM (BAD91321.1), EcOYW1 (BAH2364.1), pAYWB-I (ABC65789.1), pAYWB-III (ABC65798.1), pPAPh2 (ABD04144.1), pPASb11 (ABD04148.1), pPaWBN-1 (ABR08376.1), pPaWBN-2 (ABR08382.1), pNJAY (CBX25033.1), pPLLHn-1 (AFA53690.1), pCWBf (YP 004422914.1), Wheat dwarf virus (CAJ13704), Beet mild curly top virus (AAC54875), Maize streak virus (AAK73446), Beet curly top virus (NP_040557), Tobacco yellow dwarf virus (NP_620726), Macroptilium mosaic Puerto Rico virus (NP_671461), Malvastrum leaf curl virus (YP 459911), Blainvillea yellow spot virus (YP 001960962).

Fig. 5. Copy numbers of the three WBD phytoplasma plasmids in different tissues of infected periwinkle assayed by quantitative real time PCR. Mean expression values were calculated from three independent replications. Vertical bars represent standard errors.
plants associated with strains of ‘Ca. P. asteris’ (Petrzik et al., 2011). Although the copy number of the three WBD phytoplasma plasmids had tissue specificity in infected periwinkle and the copy number changed during the course of infection, it remains to be determined whether one phytoplasma cell harbors all plasmids, or different phytoplasma cells harbor different plasmids(s).

As far as we are aware, this is the first report on tissue distribution and dynamics of phytoplasma plasmids. A major finding was that the copy number of the three WBD phytoplasma plasmids in tissues sampled below the graft union, especially in the root, was strikingly higher than in the other parts of WBD phytoplasma-infected periwinkle.

ACKNOWLEDGEMENTS

Research supported by the National Natural Science Foundation of China (Grant number: 30970133), the Ph.D. Program Foundation and 111 project from the Ministry of Education of China (Grant number: 20100204110004 and B07049).

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Fig. 6. Copy numbers of the three WBD phytoplasma plasmids during the infection time after periwinkle displayed symptoms. Mean expression values were calculated from three independent replications. Vertical bars represent standard errors.


Received March 4, 2013
Accepted August 1, 2013