Evidence for the role of an invasive weed in widespread occurrence of phytoplasma diseases in diverse vegetable crops: implications from lineage-specific molecular markers

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Key Words: phytoplasma, subgroup 16SrII-A, parthenium weed, immunodominant membrane protein

Abstract

During the period from 2011 to 2013, several plant diseases repeatedly occurred in vegetable crops grown in Yuanmou County, Yunnan Province, China. Affected plants included cowpea, sword bean, string bean, tomato, lettuce, and water spinach. The diseased plants exhibited symptoms of witches'-broom growth and floral deformations, linking each disease to phytoplasmal infection. Phylogenetic and virtual RFLP analyses of the phytoplasmal 16S rRNA gene sequences amplified from DNA of diseased plants revealed that all of the individual strains present in the diverse vegetable plants were affiliated with a single '*Candidatus* Phytoplasma' species ('*Ca. Phytoplasma aurantifolia*') and a single ribosomal subgroup (16SrII-A). While presence of subgroup 16SrII-A phytoplasma in this geographic region was reported previously, such widespread infections in diverse plant hosts are unveiled for the first time in this study. In pursuing the source of the infections, we found that areas surrounding the affected vegetable fields were extensively invaded by parthenium weeds (*Parthenium hysterophorus*); and many of the weed plants exhibited abnormal morphologies that were suspicious of, and later diagnosed with, phytoplasmal infections. Results from genotyping of 16S rRNA and lineage-specific immunodominant membrane protein genes revealed that the vegetable-infecting phytoplasmas and the parthenium weed phytoplasma belong to the same genetic lineage. The findings indicate that parthenium weed poses a substantial risk as a reservoir of phytoplasmal infection of nearby agricultural crops in the geographic region since the ecosystems of Yuanmou are insect-rich, and parthenium weed is known to attract diverse leafhoppers. Further studies are warranted to assess the impact of farmland invasions by the noxious weed and to devise practical measures for improved weed control.

1. Introduction

Inhabiting plant sieve cells and being transmitted by phloem-feeding insects, phytoplasmas are a group of cell wall-less bacteria that infect diverse plant species and are responsible for numerous diseases in agriculturally important crops worldwide (Lee *et al.*, 2000; Bertaccini *et al.* 2014). As the genomes of phytoplasmas lack many metabolic and regulatory genes known to be essential in free-living bacteria (Davis *et al.*, 2005; Oshima *et al.*, 2004; Zhao *et al.*, 2014), phytoplasmas are obligate parasites of their host plants and insect vectors. Absence of genes critical for maintaining intracellular redox homeostasis, of genes essential for cell membrane genesis, and of genes encoding sugar phosphotransferase system is among key factors that render phytoplasmas' inability to survive outside their host cells (Zhao *et al.*, 2014; 2015). Due to difficulties in establishing phytoplasma axenic culture, identification and characterization of phytoplasmas rely on molecular analysis of genes having different degrees of nucleotide sequence conservation, and their classification is based on actual enzymatic or computer-simulated RFLP analysis of 16S rRNA gene sequences (Lee *et al.*, 1998; Wei *et al.*, 2007).

China's Yunnan Province is a geographic area where phytoplasma diseases are prevalent as the region has a vegetation- and insect-rich ecosystem (He and Zhang, 2009; Wu *et al.*, 2009) highly favorable for phytoplasma niche adaptation and spread (Cai 2007). Thus far, more than 42 phytoplasma strains belonging to six ribosomal (16Sr) groups and 15 subgroups have been identified from diverse plant species grown in this province; the level of phytoplasma genetic diversity is especially extensive among strains affiliated with the peanut witches'-broom group (16SrII) (Cai, 2007; Cai *et al.*, 2008; 2016; Kuai *et al.*, 2000; Shi, 2014; Wan, 2013; Wan *et al.*, 2014; Wei *et al.*, 2011; Wu *et al.*, 2012). We postulated

previously that an epicenter of group 16SrII phytoplasma evolution may exist in Yunnan (Cai *et al.*, 2008). Presence of closely-related phytoplasma strains in the same geographic location is likely to increase opportunities for phytoplasma-phytoplasma genetic exchanges leading to modifications in phytoplasmal host specificity, vectorship, and virulence (Cai *et al.*, 2016). In the present communication, we report molecular identification of a broad host range subgroup 16SII-A phytoplasma lineage associated with witches'-broom, virescence, and phyllody diseases of diverse agricultural crops grown in Yuanmou County, Yunnan Province, and we suggest that parthenium weed is a likely reservoir of the phytoplasma lineage infecting these crops.

Parthenium weed is an annual or tender perennial herbaceous plant species native to Central and South America. Due to its vigorous growth habit and its ability to adapt to a variety of soil conditions, the weed is highly invasive. Thus far, parthenium weed has been naturalized in subtropical and tropical regions of all Continents except for Europe (Adkins and Shabbir, 2014). While this noxious weed's invasion of agricultural fields alone is already a serious threat to crop production, its ability to host a broad host range phytoplasma, and to serve as a potential reservoir for spread of the phytoplasma, raises an alarming signal further underscoring the need for control of this weed species.

2. Materials and Methods

2.1. Sample collection, DNA extraction, and phytoplasma detection

Diseased vegetable plants exhibiting witches'-broom, phyllody, and virescence symptoms were identified from production fields located in Yuanmou County, Yunnan Province. Leaf samples were collected

from plants displaying witches'-broom symptoms; and deformed floral organ samples were collected from plants showing phyllody and virescence symptoms. Samples were collected over the summer and fall of 2012, and the summer of 2013. Leaf samples of parthenium weed (*Parthenium hysterophorus*) plants showing witches'-broom symptoms were collected from six different locations in the county. As negative controls, leaves were collected from healthy-looking vegetable plants and parthenium weeds. Total DNA was extracted from 0.3g of each sample using DNeasy Plant Mini Kit (QIAGEN, Valencia, CA) as described previously (Cai *et al.*, 2008). Polymerase chain reaction (PCR) assays were performed for amplifications of phytoplasma-unique genomic sequences. Primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) was used for amplification of a partial *rrn* operon; and primer pair R16F2n/R16R2 (Gundersen and Lee, 1996) was used in a subsequent nested PCR for amplification of the 16S rDNA F2nR2 fragment. Primer pair IMP(II)F/IMP(II)R (Wan, 2013; Yu *et al.*, 1998) was used for amplification of immunodominant membrane protein gene (*imp*). PCR thermal cycling conditions for partial *rrn* operon and *imp* gene were as described previously by Wan (2013) and Wei *et al.* (2011), respectively.

2.2. DNA sequence comparative, phylogenetic, and virtual RFLP analyses

The phytoplasmal partial *rrn* operon and *imp* gene amplified above were cloned into pMD18-T vector (TaKaRa Bio Inc, Dalian, China). Both strands of each cloned amplicons were sequenced to achieve a minimal of 4× coverage per base position. DNA sequence reads were assembled using software DNAMAN (Lynnon Biosoft, San Ramon, CA). The sequences were compared to the current GenBank database entries using the nucleotide BLAST program online at <http://www.ncbi.nlm.nih.gov/BLAST>. Phylogenetic analysis was conducted with the software MEGA6 using Maximum Likelihood method

(Tamura *et al.*, 2013). Virtual RFLP analysis was conducted using *iPhyClassifier* (Zhao *et al.*, 2009) online at <http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>.

2.3. Protein secondary structure prediction

The secondary structures of the phytoplasmal immunodominant proteins were predicted using the JPred4 protein secondary structure prediction server online (<http://www.compbio.dundee.ac.uk/jpred4>) with the JNet algorithm (Drozdetskiy *et al.*, 2015).

3. Results and Discussion

3.1. Occurrence of witches'-broom, virescence, and phyllogly diseases in diverse vegetable crops

During the period from 2011 to 2013, several plant diseases repeatedly occurred in various vegetable crops grown in Yuanmou County, Yunnan Province, China. Affected plant species included cowpea (*Vigna unguiculata*), sword bean (*Canavalia gladiata*), string bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), and water spinach (*Ipomoea aquatica*). Since each disease was characterized by a predominant symptom type (Fig. 1), a name reflecting the predominant symptom was given to each respective disease, i.e., cowpea phyllody, sword bean witches'-broom, tomato big bud, or tomato witches'-broom, string bean virescence, lettuce witches'-broom, and water spinach witches'-broom. Diseased cowpea, string bean, sword bean, and tomato plants formed very few fruits/seed pots, and rarely produced seeds, resulting in significant commodity losses; the leaves of diseased lettuce and water spinach were much smaller, reducing commercial values. All diseased plants exhibited a unifying morphological feature, vegetative proliferation (witches'-broom growth) and floral deformation (virescence, phyllody, and

big bud). Such characteristics pointed to possible phytoplasmal infection, as phytoplasma is among very few pathogens known to derail plant meristems from their genetically predetermined destiny, altering the pattern of host plant development and reproduction (Wei *et al.*, 2013).

Molecular diagnostics was deployed to study these vegetable diseases. Three to five symptomatic plants were sampled for each vegetable disease per location. When DNAs from cowpea phyllody, sword bean witches'-broom, tomato big bud, or tomato witches'-broom diseased plants served as template, direct PCR primed by phytoplasmal *rrn*-universal primer pair P1/P7 yielded approximately 1.8 kb amplicons, indicating that the titers of the target phytoplasmal agent in these samples were relatively high (Table 1). On the other hand, when DNAs from string bean virescence, lettuce witches'-broom, or water spinach witches'-broom diseased plants served as template, no amplicon was detectable in direct PCR, and an 1.25 kb amplicon was detectable after a subsequent, nested PCR primed by primer pair R16F2n/R16R2 (Table 1). While all symptomatic vegetable plants analyzed were PCR positive, no amplicon was detected in direct or nested PCRs where DNA extracts from nearby healthy plants served as templates. Results from nucleotide sequencing of the cloned amplicons revealed that the 1.8 kb amplicons from diseased cowpea, sword bean, and tomato plants spanned a near-full length 16S rRNA gene, a 16S-23S rRNA intergenic spacer, a tRNA-Ile gene, and a partial 23S rRNA gene; DNA sequencing results also showed that the 1.25 kb amplicons from diseased string bean, lettuce, and water spinach plants contained a partial 16S rRNA gene. All the amplicons possessed a sequence block that matched the signature sequence (5'-CAAGAYBATKATGKTAGCYGGDCT-3') of phytoplasmas (IRPCM, 2004), proving that the diseases were associated with phytoplasmal infections. The 16S rRNA gene sequences of the

phytoplasma strains associated with each given disease were identical or nearly identical (differed by one or two bases). Nucleotide sequences of representative phytoplasma strains, one for each disease, were deposited in the GenBank (Table 1).

3.2. Identification of the phytoplasma lineage associated with the vegetable diseases

Comparative and phylogenetic analyses of 16S rRNA gene (16SrDNA) sequences were performed to determine the genetic relationship among the seven vegetable-infecting phytoplasma strains and reference strains of formally established '*Ca. Phytoplasma*' species. Results revealed that all the seven strains were closely related to the reference strains of '*Ca. Phytoplasma australasiae*' (a revised nomenclature of '*Ca. Phytoplasma australasia*', 99.5% - 99.8 % sequence identity) (IRPCM, 2004; White *et al.*, 1998) and '*Ca. Phytoplasma aurantifolia*' (97.9% - 98.3% sequence identity) (Zreik *et al.*, 1995) (Fig. 3). Since assignment of strains to '*Ca. Phytoplasma australasiae*' is limited to phytoplasmas that infect papaya only (IRPCM, 2004), it is appropriate to assign the Yuanmou vegetable-infecting phytoplasmas as '*Ca. Phytoplasma aurantifolia*'-related strains.

In addition to '*Ca. Phytoplasma*' species assignment, phytoplasmas are often classified into groups and subgroups on the basis of collective RFLP profiles derived from actual enzymatic or computer-simulated digestions of the 16S rDNA F2nR2 fragment by a defined set of 17 restriction enzymes (Lee *et al.*, 1998; Wei *et al.*, 2007). The F2nR2 fragment refers to the 1.25 kb portion of the 16S rDNA bounded by the two conserved nucleotide blocks corresponding to the annealing sites for primer pair R16F2n/R16R2 (Gundersen and Lee, 1996). An *iPhyClassifier* operation, using the sequences of the F2nR2 fragments of

the seven vegetable phytoplasma strains as queries, resulted in a collective RFLP pattern (Fig. 4A) identical to that of the representative strain (GenBank accession L33765) of the peanut witches'-broom phytoplasma group, subgroup 16SrII-A (Fig. 4B), indicating that all the seven vegetable-infecting phytoplasma strains belong to the same subgroup.

3.3. Prevalence of parthenium weed and its potential as a reservoir of 16SrII-A phytoplasma in the region

While some of the vegetable crops affected in the disease incidence are botanically defined as tender perennials, all were grown in the production fields as annuals directly from seeds. Since phytoplasmas are restricted in phloem sieve cells that lack a direct connection to embryos, phytoplasma transmission *via* seeds has been rarely reported (Bertaccini and Duduk, 2009; Cordova *et al.*, 2003). In this Yuanmou vegetable disease occurrence, the possibility of seed transmission was even slim as a single subgroup lineage of phytoplasma was detected in diverse vegetable crops grown from seeds of different commercial sources. We hypothesized that the widespread diseases were more likely caused by *de novo* phytoplasmal infections from a common reservoir nearby.

In order to identify the possible reservoir of phytoplasma responsible for the *de novo* infections of crops, we surveyed areas surrounding the vegetable production fields where the diseases occurred. We found that hillsides, pasturelands, and roadsides next to the vegetable fields were heavily invaded by parthenium weed, and that many of the weed plants also exhibited abnormal morphologies such as witches'-broom, phyllody, and virescence (Fig. 1H and 1G). We therefore examined parthenium weed plants with witches'-broom symptoms at six different locations. Total DNAs were extracted from the leaf samples

and subjected to PCR assays. With primer pair P1/P7, the PCRs yielded a 1.8 kb partial *rrn* operon amplicon without need for nested PCR. The partial *rrn* operon sequences derived from independent samples collected in six locations were mutually identical or nearly identical. The consensus sequence (GenBank accession KC953002) perfectly matched to the partial *rrn* sequences of the sword bean witches'-broom (KC953003), tomato big bud (KC953004), and tomato witches'-broom (KC953005) phytoplasmas, and differed by two to five bases from that of the cowpea phyllody (KC953001), string bean virescence (KJ735762), water spinach witches'-broom (KJ735782), and lettuce witches'-broom (KJ735770) phytoplasmas, respectively. Result from computer-simulated RFLP analysis of the 16S rDNA F2nR2 sequence revealed that the parthenium weed phytoplasma belongs to the subgroup 16SrII-A (Fig. 4C). While infections of parthenium weed by a subgroup 16SrII-A phytoplasma was reported previously in Hainan Island, China (Li *et al.*, 2011), this is the first time parthenium weed is recognized as a natural host of a subgroup 16SrII-A phytoplasma in Yunnan, and the first time that a link is drawn between the weed and diseases in agricultural crops.

Ubiquitous presence of parthenium weed in the vicinity of vegetable production fields and the ability of the weed to host subgroup 16SrII-A phytoplasma pointed to a possibility that the weed plants served as a reservoir for spreading the phytoplasma to nearby vegetable plants by a yet to be identified insect vector in the region. Such a hypothesis was also in line with the following facts: First, in Yunnan Province, parthenium weed is a perennial; therefore, infected weed plants can carry phytoplasmas from one year to the next, making the inoculums available for transmission during acquisition feeding of insect vectors for multiple growing seasons. Second, parthenium weed is an insectary plant of the aster family (*Asteraceae*)

known to attract phytoplasma-transmitting leafhoppers such as aster leaf hoppers (*Macrosteles quadrilineatus*). Third, at least 134 leafhopper species have been found present in Yunnan province, and many of which are polyphagous and feed on annual flowers and vegetables in addition to forest trees (<http://www.zoology.csdb.cn/>; Yang *et al.*, 2010; Zhang *et al.*, 2015).

3.4. Further evidence connecting the parthenium weed phytoplasma and the vegetable diseases

We reasoned that, if parthenium weeds were truly the source of phytoplasmal infections in the diverse vegetable crops, results from molecular characterization of any phytoplasmal non-ribosomal gene, especially evolutionarily less conserved lineage-specific genes, should show unambiguously that the parthenium weed phytoplasma and the phytoplasmas detected in diverse vegetable crops indeed belonged to the same lineage, because such genes would likely afford finer distinctions among closely related lineages. To demonstrate this, we chose to examine the gene (*imp*) that encodes the immunodominant membrane protein (IMP) for the following two reasons. First, previous studies revealed that, among different phytoplasma lineages within the group 16SrII, the *imp* gene sequences were more variable than those of the 16S rRNA gene, and were lineage-specific (Siampour *et al.*, 2013; Yu *et al.*, 1998). Second, immunodominant membrane proteins are abundantly expressed and are located at the exterior surface of phytoplasma cells (Kakizawa *et al.*, 2004; Morton *et al.*, 2002) with significant implications in phytoplasma-vector interactions (Galetto *et al.*, 2011; Rashidi *et al.*, 2015) and phytoplasma transmission (Ishii *et al.*, 2009; Suzuki *et al.*, 2006).

Our result from a phylogenetic analysis of the *imp* gene sequences revealed that all the vegetable-infecting phytoplasmas identified in the present study were indeed clustered together with the parthenium weed phytoplasma (Fig. 5). In fact, the *imp* gene sequences of the seven vegetable phytoplasmas were either identical to, or differed by very few bases from, that of the parthenium weed phytoplasma. In the latter case, the base variations mostly occurred at the “wobble” position of respective codons and did not change the encoded amino acids (synonymous mutations). At the amino acid sequence level, the IMPs of the string bean virescence phytoplasma (SBV-YNym1) and the tomato big bud phytoplasma (TBB-YNym) are identical to that of the parthenium weed phytoplasma (Fig. 6). The IMPs of the other five vegetable phytoplasmas differ by one amino acid from that of the parthenium weed phytoplasma (Fig. 6). Such single amino acid change did not seem to alter the secondary structures, the coiled-coil assemblies, and the solvent accessibility of the IMPs among the phytoplasma strains (data not shown), as assessed by JNet algorithm, one of the most accurate protein secondary structure prediction methods (Drozdetskiy *et al.*, 2015). These results support the notion that the seven vegetable phytoplasmas and the parthenium weed phytoplasma can be derived from the same genetic lineage.

Prior to this study, we identified two 16SrII-A phytoplasma strains from cauliflower plants with phyllody disease (CauPh-YNym, Cai *et al.*, 2016) and peanut plants with witches'-broom disease (PnWB-YNym, Wan *et al.*, 2014), respectively, in Yuanmou County; but the *imp* genes of these two strains were not characterized until the present study. Since the *imp* gene and the IMP amino acid sequences of the CauPh-YNym and PnWB-YNym phytoplasmas are identical to that of the parthenium weed phytoplasma (Fig. 6), the two phytoplasma strains are also members of the same genetic lineage. Such

result implies that the peanut witches'-broom and cauliflower phyllody diseases may also share the same source of infection, and the phytoplasma lineage affects agricultural crops of at least six plant families in Yuanmou County alone.

It appears that this phytoplasma lineage may also include several previously identified subgroup 16SrII-A phytoplasma strains, such as those associated with sweet potato witches'-broom and pear decline diseases in Taiwan and *Crotalaria* witches'-broom disease in Hainan, all of which were present in southern China (Fig. 5). It is worth noting that infection of parthenium weed by a subgroup 16SrII-A phytoplasma was reported previously in Hainan (Li et al., 2011) but the *imp* gene of the Hainan parthenium weed phytoplasma has yet to be characterized and no etiological study has been conducted to link parthenium weed and any other phytoplasmal diseases in Hainan. Findings from the present study may shed light on identification of potential reservoir(s) of subgroup 16SrII-A phytoplasmas in Hainan and Taiwan islands. In-depth study is warranted to assess the extent of farmland invasions of parthenium weed in southern China, to evaluate its impact in spread of phytoplasma diseases, and to devise practical measures for improved weed control.

Acknowledgements

This research was supported by the Program for Innovative Research Team (in Science and Technology) in University of Yunnan Province, IRTSTYN (2014-2022), the National Natural Science Foundation of China (Grant number 31060239) and the US Department of Agriculture, Agricultural Research Service (Project number 1245-22000-282-00D).

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Figure Legends

Figure 1 Symptoms exhibited by diseased vegetable and parthenium weed plants. (A) witches'-broom growth in sword bean. (B) phyllogdy in cowpea. (C) virescence in string bean. (D) witches'-broom growth in lettuce. (E) big bud in tomato. (F) withches'-broom growth in tomato. (G) witches'-broom growth in water spinach. (H) withches'-broom growth and (I) virescence in parthenium weed.

Figure 2 A map of Yunnan Province, China with a close-up view of Yuanmou County, showing sample locations. Open circles and triangles indicate the locations where diseased vegetable plants and parthenum weeds, respectively, were sampled. Numbers in the circles indicate affected vegetable species: 1, lettuce (*Lactuca sativa*); 2, tomato (*Solanum lycopersicum*); 3, sword bean (*Canavalia gladiata*), 4, water spinach (*Ipomoea aquatica*); 5, cowpea (*Vigna unguiculata*); and 6, string bean (*Phaseolus vulgaris*).

Figure 3 Phylogenetic positions of the seven vegetable-infecting phytoplasma strains in the phytoplasma clade. The Phylogenetic relationship of the phytoplasmas was inferred from analysis of 16S rRNA gene sequences by using the Maximum Likelihood algorithm implemented in the Molecular Evolutionary Genetics Analysis program (MEGA 6). The initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The reliability of the analysis was subjected to a bootstrap test with 1000 replicates. The taxa used in the phylogenetic tree reconstruction included the seven phytoplasma strains associated with vegetable diseases in Yuanmou (in bold face), the parthenium weed phytoplasma (in bold face), and the reference strains of 38 formally described '*Candidatus* Phytoplasma' species. The numbers

at the nodes of the branches indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. *Acholeplasma palmae* served as an out-group during the phylogenetic tree reconstruction. The scale bar represents the number of nucleotide substitutions per site.

Figure 4 Computer-simulated restriction fragment length polymorphism (RFLP) analysis of the phytoplasmal 16S rDNA F2nR2 sequence by 17 restriction enzymes. (A) virtual RFLP profile of the seven Yuanmou vegetable-infecting phytoplasma strains as represented by the string bean virescence phytoplasma SBV-YNym1. (B) virtual RFLP profile of the subgroup 16SrII-A representative strain PnWB-Taiwan. (C) virtual RFLP profile of the parthenium weed witches'-broom phytoplasma ParWWB-YNym1. The RFLP profiles exhibited by the three panels (A, B & C) are identical. MW: ϕ X174DNA *Hae*III digests.

Figure 5 Phylogenetic relationship among different lineages of the peanut witches'-broom phytoplasma group. The evolutionary history was inferred from analysis of the imminodominant protein gene sequences by using the Maximum Likelihood algorithm implemented in the Molecular Evolutionary Genetics Analysis program (MEGA 6). The initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The numbers at the nodes of the branches indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test with 1000 replicates. The analysis involved 30 strains in the peanut witches'-broom phytoplasma group (16SrII). The eight phytoplasma strains identified in the present study are in bold face (see Table 1 for strain designations).

398 AP1, a representative strain of the apple proliferation group (16SrX) served as an out-group during the
399 phylogenetic reconstruction. The scale bar represents the number of nucleotide substitutions per site.

400 **Figure 6** Alignment of the amino acid sequences of the immunodominant membrane proteins (IMPs) of ten
401 Yuanmou 16SrII-A phytoplasma strains. A dash (-) in the alignment indicates the amino acid residue is
402 identical to that of the parthenium weed phytoplasma (ParWWb-YNym1) IMP at the corresponding
403 position.

Table 1 Vegetable-infecting phytoplasmas identified in this study

Strain	Plant host	Predominant symptom	GenBank accession no.	
			<i>rrn</i>	<i>imp</i>
SBWB-YNym1	Sword bean	Witches'-broom growth	KC953003	KC953022
CowP-YNym	Cowpea	Phyllody	KC953001	KC953019
SBV-YNym1	String bean	Virescence	KJ735762	KJ735765
LeWB-YNym	Lettuce	Witches'-broom growth	KJ735770	KJ735773
TBB-YNym	Tomato	Big bud	KC953004	KC953023
TWB-YNym	Tomato	Witches'-broom growth	KC953005	KC953024
WSWB-YNym	Water spinach	Witches'-broom growth	KJ735782	KJ735785
ParWWB-YNym1	Parthenium weed	Witches'-broom growth/virescence	KC953002	KC953020

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

ParWB-YNym1	MNDRENIQTKNCKITVGVIASAGIALVVYLITAKLLMPPFTIKTLTTKDIDMLKVEIK	60
SBV-YNym1	-----	60
TBB-YNym	-----	60
CowP-YNym	-----	60
SBWB-YNym1	-----	60
LeWB-YNym	-----	60
WSWB-YNym	-----	60
TWB-YNym	-----	60
CauPh-YNym	-----	60
PnWB-YNym	-----	60
ParWB-YNym1	DFTGVNTKDKLSSDDAKQESQKAFDAINKIVDAFAENNKADIKDKKISDSTIAAANMLKT	120
SBV-YNym1	-----	120
TBB-YNym	-----	120
CowP-YNym	----D-----	120
SBWB-YNym1	---D-----	120
LeWB-YNym	----D-----	120
WSWB-YNym	-----	120
TWB-YNym	-----	120
CauPh-YNym	-----	120
PnWB-YNym	-----	120
ParWB-YNym1	KADNAUKFVWENASVTNWTDDEVQDFVWVKVVKTKIINDILSQAKTDLKVQS	172
SBV-YNym1	-----	172
TBB-YNym	-----	172
CowP-YNym	-----	172
SBWB-YNym1	-----	172
LeWB-YNym	-----	172
WSWB-YNym	-----S-----	172
TWB-YNym	-----S-----	172
CauPh-YNym	-----	172
PnWB-YNym	-----	172

Fig. 6