Crop Protection 92 (2017) 99–106

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

‘*Candidatus* Phytoplasma brasiliense’-related strains associated with papaya bunchy top disease in northern Peru represent a distinct geographic lineage

W. Wei [a, 1], E. Pérez-López [b, 1], R.E. Davis [a], L. Bermúdez-Díaz [c], C. Granda-Wong [d], J. Wang [a], Y. Zhao [a, *]

[a] Molecular Plant Pathology Laboratory, USDA-Agricultural Research Service, Beltsville, MD 20705, USA
[b] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, USA
[c] Universidad Privada Atenor Orrego, Av América Sur 3145, Trujillo 1075, Peru
[d] Department of Plant Pathology and Agricultural Engineering, Universidad Nacional de Piura, Piura, Peru

ARTICLE INFO

Article history:
Received 9 September 2016
Received in revised form
25 October 2016
Accepted 29 October 2016

Keywords:
Phytoplasma
Genetic diversity
Geographic lineage
Single nucleotide polymorphism

ABSTRACT

During the 2015–2016 fruit production season, a papaya bunchy top (PBT) disease was observed in a papaya production farm located at Region de Piura, northern Peru. Affected plants exhibited symptoms of excessive proliferation of auxiliary shoots at the top or near top of the main stem, shortening of internodes, leaf yellowing, and necrosis of leaf veins. The symptom syndrome suggested possible infection by a phytoplasma, and the recent discovery of a phytoplasmal disease in a nearby vineyard also indicated that a phytoplasma reservoir and potential vector(s) were present in the area. Molecular diagnostic assays for phytoplasma detection were therefore employed. Nested polymerase chain reactions and subsequent nucleotide sequence analysis confirmed that phytoplasma infection did occur in the papaya production farm. The phytoplasma strains found to be associated with the PBT disease are affiliated with ‘*Candidatus* Phytoplasma brasiliense’, a species that had previously never been implicated in a papaya disease anywhere in the world. We provide molecular evidence to suggest that the papaya-infecting phytoplasma strains identified in this study are closely related to a phytoplasma associated with the first grapevine yellows (GY) disease that we recently identified in Peru. The results of our study indicate that these PBT and GY phytoplasma strains in Peru represent an emerging geographic lineage that is distinct from those of all other known ‘*Ca.* Phytoplasma brasiliense’ strains in the Americas.

Published by Elsevier Ltd.

1. Introduction

Phytoplasmas are a very large group of phloem-inhabiting, insect-transmitted cell wall-less bacteria that infect diverse plant species and cause numerous diseases globally (Maejima et al., 2014). Phylogenetic studies of evolutionarily conserved genes encoding 16S rRNA and house-keeping proteins suggested that extant phytoplasmas descended from a Gram-positive, low G + C walled bacterium (Gundersen et al., 1994) and shared a common ancestor with acholeplasmas (Davis et al., 1997; Zhao et al., 2005). Molecular evidence favors the hypothesis that phage predation and subsequent genomic fusion events, i.e. the formation of sequence variable mosaics (SVMs; Jomantiene and Davis 2006; Jomantiene et al., 2007) or phage-derived pathogenetic islands, launched the phytoplasma clade (Wei et al., 2008a), distinguishing the new taxon from acholeplasmas and all other taxa in the class *Mollicutes* (Zhao et al., 2015). Despite having a monophyletic origin, extant phytoplasmas are genetically diverse, reflecting their evolutionary paths of lineage-specific niche adaptation (Davis et al., 2005). While new phytoplasmas are continuously discovered at a rapid pace in emerging plant diseases worldwide, a formal taxonomy of phytoplasmas is still lacking, largely due to difficulties in isolation of phytoplasmas in axenic culture and the consequent inaccessibility of measurable phenotypic characters suitable for polyphasic strain characterizations. Presently, phytoplasmas are accommodated by a provisional genus, ‘*Candidatus* Phytoplasma’ (IRPCM, 2004), according to conventions formed for recording properties of

* Corresponding author.
E-mail address: yan.zhao@ars.usda.gov (Y. Zhao).
[1] These authors have contributed equally to the study and therefore share first authorship.

http://dx.doi.org/10.1016/j.cropro.2016.10.024
0261-2194/Published by Elsevier Ltd.

uncultured organisms (Murray and Schleifer, 1994; Murray and Stackebrandt, 1995). Thus far, 39 '*Candidatus* Phytoplasma' species have been formally described (Davis et al., 2016), and an additional eight potentially new species have been incidentally cited or suggested (IRPCM, 2004; Wei et al., 2007). Diverse phytoplasma strains are also classified into groups and subgroups based on collective restriction fragment length polymorphism (RFLP) profiles resulting from either actual enzymatic (Lee et al., 1993) or computer-simulated (Wei et al., 2007; 2008b) digestions of the 1.25 kb F2nR2 segment of 16S rRNA gene by a defined set of 17 restriction enzymes. To date, 36 ribosomal (16Sr) groups and more than 150 subgroups have been delineated (Bertaccini et al., 2014; Pérez-López et al., 2016a), highlighting the breadth of the phytoplasmal genetic diversity.

Papaya (*Carica papaya* L.) is a widely cultivated tropical fruit tree with high nutritional and medicinal value: the papaya fruit is a rich source of vitamins, minerals, and dietary fiber (USDA-ARS Nutrient Data Laboratory, 2014); the seed, leaf, and root of the papaya plant contain anti-inflammatory, anti-parasitic, and analgesic compounds that offer natural remedies for various ailments (Bamisaye et al., 2013). Gaining in consumer popularity, papaya has become the fourth most traded tropical fruit in the world after banana, mango, and pineapple; and global papaya production reached 11.22 million metric tons in 2010 (Evans et al., 2015). Phytoplasmal diseases are among the major factors that limit papaya production, especially in South and Central Americas. Papaya bushy top, papaya yellow crinkle, papaya mosaic, papaya dieback, and papaya apical curl necrosis are a few examples of reported phytoplasmal diseases in papaya (White et al., 1997; Acosta et al., 2011; Melo et al., 2013). Prior to this study, various papaya phytoplasmal diseases have been attributed to strains related to at least six '*Candidatus* Phytoplasma' species and affiliated with five ribosomal (16Sr) groups; these include '*Ca.* Phytoplasma asteris' (group 16SrI) (Acosta et al., 2011), '*Ca.* Phytoplasma aurantifolia' (group 16SrII) (Rao et al., 2011; Verma et al., 2012), '*Ca.* Phytoplasma solani' (group 16SrXII) (Bau et al., 2011), '*Ca.* Phytoplasma australiense' (group 16SrXII) (Elder et al., 2002; Gera et al., 2005), '*Ca.* Phytoplasma hispanicum' (group 16SrXIII) (Melo et al., 2013) and '*Ca.* Phytoplasma caricae' (group 16SrXVII) (Arocha et al., 2005).

Peru is one of the major papaya producing countries, with more than twelve thousand hectares of papaya plantation and a steady increase in exportation of the fruit in recent years (Daga-Avalos, 2015). United States Department of Agriculture recently added papaya to the list of admissible commodities from Peru (USDA-APHIS, 2015). Prior to the present study, there had been only one report of papaya phytoplasmal disease in southern Peru; the etiological agent of that disease was reported as a '*Ca*. Phytoplasma asteris'-related strain belonging to group 16SrI, subgroup B (Hodgetts et al., 2009). In the present communication, we report a papaya phytoplasmal disease in northern Peru and its association with infection by strains closely related to '*Ca.* Phytoplasma brasiliense', a species that had previously never been implicated in a papaya disease anywhere in the world. We also provide molecular evidence to suggest that this papaya-infecting phytoplasma is closely related to a phytoplasma associated with the first known grapevine yellows disease in Peru (Wei et al., 2016). Together, these papaya- and grapevine-infecting strains in Peru represent an emerging geographic lineage.

2. Materials and methods

2.1. Sample collection, DNA extraction, and phytoplasma detection

During the 2015–2016 fruit production season, papaya plants exhibiting bunchy top symptoms were identified in a papaya farm located at Region de Piura, Piura, northern Peru. Leaf samples were collected from five plants displaying various degrees of proliferation of auxiliary shoots at the top of the main stem. Total DNA was extracted from 0.3 g of each sample using a CTAB-based DNA extraction method as described previously (Pérez-López et al., 2016b). 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 amplication of a partial *rrn* operon; and primer pair P1A/16S-SR (Lee et al., 2004) was used in a subsequent nested PCR for amplification of a near-full length 16S rRNA gene and a partial 16S-23S intergenic spacer. PCR thermal cycling conditions were as described previously by Wei et al. (2011).

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

The PCR amplicons obtained above were cloned into pCR®II-TOPO® TA vector (Invitrogen, Carlsbad, CA, USA). Both strands of each cloned amplicons were sequenced to achieve a minimal of 4×coverage per base position. DNA sequence reads assembly and multi-sequence alignment/comparison were conducted using the Lasergene software package (DNASTAR, Madison, WI, USA). Single nucleotide polymorphisms (SNPs) were identified based on the alignment reported generated by the MegAlign program of the Lasergene software package. Phylogenetic analysis was conducted with the software MEGA6 using Minimum Evolution method (Tamura et al., 2013). Virtual RFLP analysis was conducted using *i*PhyClassifier (Zhao et al., 2009) online at http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi.

3. Results and discussion

3.1. Papaya bunchy top disease incidence in northern Peru and its association with infection by 'Ca. Phytoplasma brasiliense'-related strains

During the 2015–2016 fruit production season, in a farm located at Region de Piura, Piura, northern Peru, several papaya plants exhibited abnormal growth pattern that included excessive proliferation of auxiliary shoots at the top or near top of the main stem, shortening of internodes, leaf yellowing, and necrosis of leaf veins (Fig. 1a and b). While such symptom syndrome, referred as papaya bunchy top (PBT), could possibly be attributed to infections by virus, phytoplasma, or reportedly rickettsia (Arocha et al., 2009; Acosta et al., 2013; Luis-Pantoja et al., 2015), we directed our attention to a possible phytoplasmal infection in this case for the following two reasons. First, a new grapevine yellows phytoplasmal disease had recently been discovered in a vineyard nearby the papaya field (Wei et al., 2016), indicating that a phytoplasma reservoir was present in the area. Second, we observed feeding activities of potentially phytoplasma-transmitting leafhoppers (*Empoasca* sp.) over neighboring papaya plants (Fig. 1c). Therefore, we carried out molecular diagnostic assays that probe phytoplasma infections. When DNA samples from three papaya trees with severe symptoms of excessive auxiliary shoots proliferation and leaf yellowing served as templates, a 1.5 kb amplicon was obtained in nested PCRs primed by phytoplasmal universal primers P1/P7 followed by P1A/16S-SR. No amplification product was observed in control reactions containing healthy plant DNA as template. The amplicons obtained independently in reactions containing DNAs extracted from three separate symptomatic papaya trees were cloned, and their nucleotide sequences determined (GenBank Accessions KX810334, KX810335, and KX810336). All three sequences covered a near-full length 16S rRNA gene and a partial 16S-23S