Sweet potato little leaf strain V4 phytoplasma associated with snake bean in the Northern Territory, Australia

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Abstract Sweet potato little leaf strain V4 phytoplasma (SPLL-V4) was found to be associated with 12-week old snake bean plants (*Vigna unguiculata* ssp. *sesquipedalis*) from a block with 890 plants in Lambells Lagoon, Northern Territory. Symptoms included witches' broom, big bud, phyllody and little leaf. There was a 19.9 % incidence of symptoms on 366 plants assessed. The same phytoplasma was also detected from a single plant of *Phyllanthus amarus* with witches' broom symptoms growing near the snake bean plants. The SPLL-V4 phytoplasma was identified based upon the 16S ribosomal RNA gene and intergenic spacer region nucleotide sequence and restriction fragment length polymorphism.

Keywords “*Candidatus* Phytoplasma aurantifolia” · *Vigna unguiculata* ssp. *sesquipedalis*

Snake bean (*Vigna unguiculata* ssp. *sesquipedalis*) has been cultivated commercially in the Northern Territory (NT) since the early 1990s. Current production is approximately 537 t, valued at AUD\$2.8 million (Department of Primary Industry and Fisheries 2010). Crops are grown predominantly in the dry season from May to September, supplying markets in population centres in the Eastern states of Australia when local production of beans ceases due to low autumn and winter temperatures. Beans are grown on 3 m high trellis (net) approximately 2 m apart. They are hand harvested, cooled and marketed in 10 kg boxes. Most snake bean consumers in Australia are of Asian origin and most growers in the NT are of Vietnamese or Cambodian ethnicity.

In December 2010, 12-week old snake beans plants grown in a block of 890 (approximate) plants at Lambells Lagoon, NT, were observed with phytoplasma-like symptoms, namely reduced leaf size (little leaf), big bud, phyllody, deformation and proliferation of stems, leaves and flowers (witches' broom) (Fig. 1). The disease incidence was assessed on 366 plants and determined to be 19.9 %. *Phyllanthus amarus* (stonebreaker) growing near the snake bean plants was also showing witches' broom symptoms.

A sample of symptomatic snake bean plants and the *Phyllanthus amarus* plant was collected. DNA extractions were conducted using the DNeasy Plant kit (Qiagen, Australia) following the manufacturer's instructions. The phytoplasma 16S rRNA, intergenic spacer region and the start of the 23S rRNA gene were amplified with the polymerase chain reaction (PCR) using universal primers: fP1 (Deng and Hiruki 1991) and rP7 (Schneider et al. 1995). The 1,800 bp PCR product was sequenced and bioinformatic analyses were conducted using the Geneious Pro software (Drummond et al. 2011). Genetic relatedness between the snake bean and *Phyllanthus amarus* phytoplasmas were determined by restriction fragment length polymorphism (RFLP) using the restriction enzymes, *Alu* I and *Rsa* I (Promega, Australia), following the manufacturer's instructions. The digestion patterns were compared with reference samples tomato big bud (TBB), sweet potato little leaf (SPLL) and SPLL strain-V4 (SPLL-V4) phytoplasmas. The snake bean and *Phyllanthus amarus* phytoplasmas were indistinguishable from the SPLL-V4 phytoplasma (data not shown).

The phylogenetic dendrogram of the 16S rRNA gene sequences from 25 phytoplasmas showed that phytoplasma from snake bean grouped with the SPLL phytoplasma previously detected in snake beans and tomatoes in Western Australia (Saqib et al. 2006), GenBank accession numbers DQ375777 and DQ375778, respectively (Fig. 2). They all clustered together with the TBB phytoplasma (GenBank

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Fig. 1 Snake bean plants associated with sweet potato little leaf strain V4 phytoplasma in the Northern Territory, in December 2010 (images by J.R. Liberato)

accession number EF193359) and SPL-LV4 (GenBank accession number AJ289193). TBB and SPL-LV4 are closely related phytoplasmas within the “*Candidatus* Phytoplasma aurantifolia” 16S rII group (IRPCM 2004). The 16S rRNA gene nucleotide sequence from the phytoplasma in snake bean was submitted to GenBank and assigned the accession number JQ067649. A sub-sample of diseased snake bean associated with the SPL-LV4 phytoplasma was deposited in the Northern Territory Plant Pathology Herbarium (DNAP 4610).

Streten and Gibb (2006) listed 28 genera of plants as hosts of the SPL-LV4 phytoplasma in Australia. In the NT, SPL-LV4 was reported on *Ipomoea batatas* (sweet potato) (Gibb et al. 1995, Davis et al. 1997), *Carica papaya* (papaya) (De La Rue et al. 1999), *Cleoma viscosa* (mustard bush), *Crotalaria brevis* (rattlepod), *Indigofera colutea* (sticky indigo), *Phyllanthus maderaspatensis* and *Senna obtusifolia* (sicklepod) (Schneider et al. 1999); and SPL-LV4 was reported on *Aeschynomene americana* (American jointvetch), *Alysicarpus vaginalis* (alyce clover), *Arachis hypogaea* (peanut), *Centrosema pascuorum* (cavalcade),

Crotalaria goreensis (gambia pea), *Medicago sativa* (lucerne), *Mitracarpus hirtus* (tropical girdlepod), *Rhynchosia minima* (rhynchosia), *Sesamum indicum* (sesame), *Vigna radiata* (mung bean) (Wilson et al. 2001) and *C. papaya* (Padovan and Gibb 2001).

Phytoplasmas are non-culturable, one-celled, wall-less prokaryotes (class Mollicutes) that replicate in insects and plants, where they are phloem-limited. Phytoplasmas are naturally transmitted by phloem-feeding insects (Hogenhout et al. 2008). More than three quarters of the insect vectors of phytoplasmas belong to the subfamily Deltocephalinae (Hemiptera: Cicadellidae, leafhoppers) (Weintraub and Beanland 2006). There is evidence that the leafhoppers, *Orosius* spp., are vectors of SPL-LV4. Padovan & Gibb (2001) found SPL-LV4 phytoplasmas in the bodies of a combined sample of *O. argentatus* and *O. lotophagorum*. Based on the number and origin of specimens of *O. argentatus* (common brown leafhopper) in the Australian collections (APPD 2011), this leafhopper is widely distributed throughout Australia and is the vector of several phytoplasmas in Australia (Hill 1943, referred as *Thamnotettix*

Fig. 2 Phylogenetic dendrogram of the 16S rRNA phytoplasma gene, generated using Geneious Pro software. The phytoplasma abbreviations “*Candidatus* Phytoplasma” represents each of the phytoplasma

argentata, Grylls 1979, Weintraub and Beanland 2006). *Orosius lotophagorum ryukyuensis* has been shown to transmit the phytoplasma associated with little leaf and phyllody symptoms on *Ipomoea plebeia* (bellvine) in Australia (Behncken 1984) and the one associated with witches’ broom on *I. batatas* in Solomon Island (Jackson and Zettler 1983).

This is the first report of the SPLL-V4 phytoplasma in snake beans and *Phyllanthus amarus* in the NT. Phytoplasma-like symptoms were previously observed in snake bean plants in the field in the NT on eight occasions from 1994 to 2005 but occurring in low incidences (Department of Primary Industry and Fisheries, Plant Pathology Section unpublished records; B.D. Condé, pers. comm.). The symptoms expressed in the snake beans

16S groupings. The bootstrapped values were determined from 1,000 replicate trees. *Acholeplasma laidlawii* was used as the outgroup to root the tree

(Fig. 1) were similar to those described in Broome, Western Australia by Saqib et al. (2006).

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