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Short Communication

Detection of sugarcane grassy shoot phytoplasma (16SrXI-B subgroup) in *Pyrilla perpusilla* Walker in Uttar Pradesh, India

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Abstract

Heavy infestation of *Pyrilla perpusilla* was recorded in sugarcane fields on varieties Co0238, CoS767, CoS07250 infected with sugarcane grassy shoot (SCGS) disease showing white leaf symptoms at Shahjahanpur district of Uttar Pradesh. To confirm the possibility that *P. perpusilla* could carry SCGS phytoplasmas DNA was extracted from specimens collected from symptomatic sugarcane fields and from SCGS symptomatic leaf samples. PCR assay was performed with phytoplasma specific universal P1/P7 primers and nested PCR assay with 3for/3rev primers, which yielded about 1.3 kb amplicons in both the *P. perpusilla* and white leaf symptomatic samples. Phytoplasma amplicons from three samples each from *P. perpusilla* and SCGS samples were sequenced. After alignment, the obtained sequence were analyzed through pair wise comparison and showed 100% identity among themselves and 99% with strains of 16SrXI group. RFLP analysis assigned both the phytoplasma strains from *P. perpusilla* and SCGS to the 16SrXI-B subgroup. The study confirmed that *P. perpusilla* carry the same phytoplasma detected in SCGS plants therefore it may play role as vector in transmission of these phytoplasmas in sugarcane crops.

Keywords: *Pyrilla perpusilla*, sugarcane, sugarcane grassy shoot phytoplasma, India

Pyrilla perpusilla Walker (Homoptera: Lophopidae) is one of the most important sucking pest of sugarcane. This is the major pest in Punjab, Uttar Pradesh, Bihar, Haryana, Madhya Pradesh, Gujarat, Maharashtra and it widely distributed in sub tropical regions of India (Singh *et al*., 2006). Many infestations of *P. perpusilla* were reported in Uttar Pradesh and in 2014-2015 an infestation of this pest was recorded from Eastern Uttar Pradesh (Kesav Prasad Pandey *et al*., unpublished). Besides sugarcane, *P. perpusilla* is also reported from other crops, viz. maize, sorghum, pearl millet and rice (Jotwani *et al*., 1969). *P. perpusilla* sucks phloem sap from leaves and excretes honeydew onto foliage leading to the development of a sooty-mould which affects photosynthesis and yield of the crop (Pandey *et al*., 2008). The effect of *P. perpusilla* infestation on sugarcane juice and purity was also reported with yield losses up to 50% (Gupta and Gupta, 1969).

The sugarcane grassy shoot is a major phytoplasma disease of sugarcane in Asian countries (Rao *et al*., 2012). In India, SCGS phytoplasma is spreading at an alarming rate, severely affecting the yield of sugarcane crops. In Uttar Pradesh, the disease appearance in fields is a regular feature and it is the major cause of yield loss in ratoon crops (Tiwari *et al*., 2012). Sugarcane is a vegetatively propagated crop and phytoplasmas infecting sugarcane are known to be transmitted by propagation material and by phloem-feeding leafhopper vectors in India (Srivastava *et al*., 2006; Rao *et al*., 2014; Tiwari *et al*., 2016).

During 2015 (May to July), heavy infestation of *P. perpusilla* was observed in sugarcane fields of three important commercial varieties (Co0238, CoS767, CoS07250) in farmer fields at Shahjahnapur, UP. Since maximum infestation of *P. perpusilla* was noticed in SCGS affected fields showing sugarcane white leaf

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symptoms, an attempt was made to detect phytoplasma presence in *P. perpusilla* samples collected from SCGS infected fields and from non-symptomatic sugarcane fields of the same varieties at distance.

DNA was extracted from leaf midrib of healthy and symptomatic sugarcane leaves of three varieties (Co0238, CoS767, CoS07250) and *P. perpusilla* (10 insects) by CTAB method (Ahrens and Seemuller, 1992) and was used as template in PCR assays by phytoplasma universal primer pair P1/P7 (Deng and Hiruki 1991; Schneider *et al*., 1995) followed by 3for/3rev in nested PCR assays (Manimekalai *et al*., 2010). PCR and nested PCR assays were performed in a Mastercycler (Eppendorf, Germany), and the cycling protocol and reaction mixture were as described by Rao *et al*. (2014). Amplified products were purified, directly sequenced from both directions and aligned sequences were submitted to GenBank. The sequence generated from the present study and the reference phytoplasma strains sequence retrieved from GenBank were used to construct phylogenetic trees by neighbor-joining method with 1,000 replications for each bootstrap value using MEGA 5.0 software version (Tamura *et al*., 2011). *Acholeplasma laidlawii* was used as out group to root the tree.

The *P. perpusilla* and SCGS phytoplasma strain sequences were used in *in silico* RFLP analysis using *i*Phy Classifier online tool and compared with the virtual RFLP gel from 16SrXI phytoplasma subgroups for assigning 16Sr subgroup classification (Zhao *et al*., 2009). RFLP of amplicons from the phytoplasma detected in *P. perpusilla* was also generated with *Alu*I, *Hha*I, *Hpa*II and *EcoR*I restriction enzymes (Fermentas, USA) (Lee *et al*., 1998). The obtained pattern was compared with the one of reference phytoplasma strain of subgroup 16SrXI-B (GenBank accession number, Acc. No. X76432).

Incidence of 5-32% of SCGS disease with chlorotic leaves was observed in different fields of sugarcane varieties Co0238, CoS767, CoS07250 at Shahjahanpur during May to July, 2015 (data not shown). Heavy population of *P. perpusilla* (40-50 individual/leaf) was also observed in the same fields. Amplifications (1.3 kb) were obtained from *P. perpusilla* specimens collected from chlorotic leaf symptomatic sugarcane fields and SCGS samples along with positive control in nested PCR assays. However, no amplification was observed in non

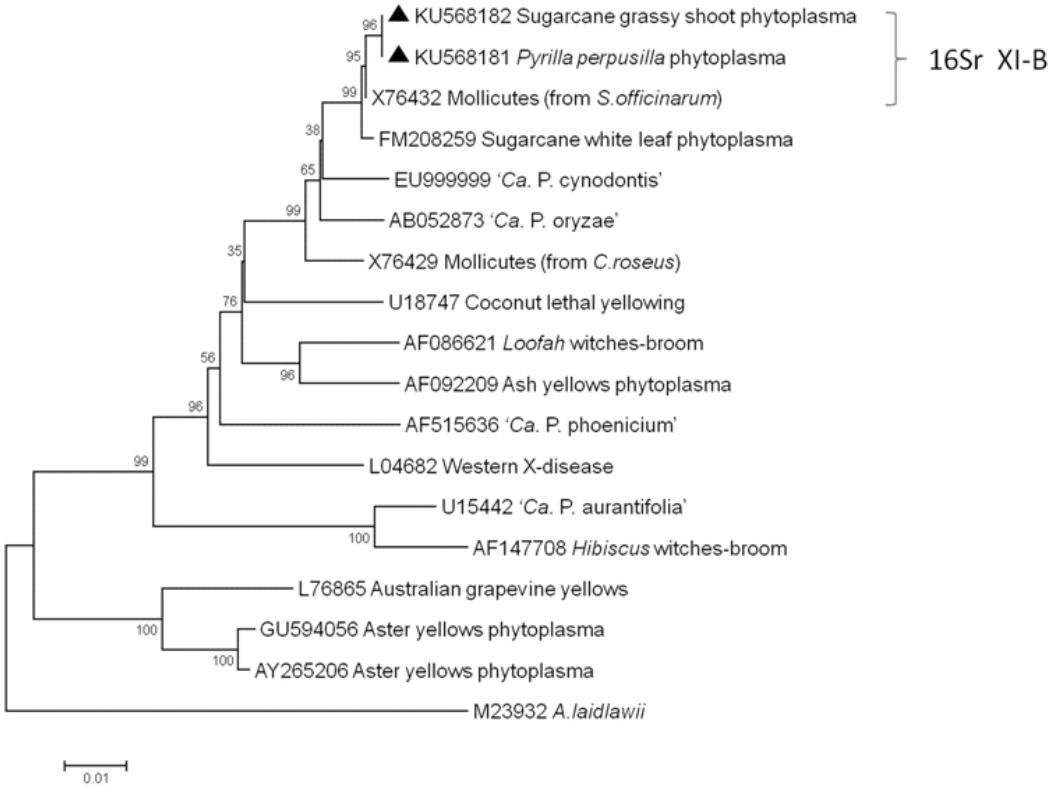

Figure 1. Phylogenetic relationship of phytoplasmas associated with *P. perpusilla* and SCGS (▲ highlighted) to other phytoplasma sequences retrieved from Genbank (Accession numbers are on the left of the phytoplasma strain name). Bootstrap values (1,000 replications) are shown as percentages at the branch points.

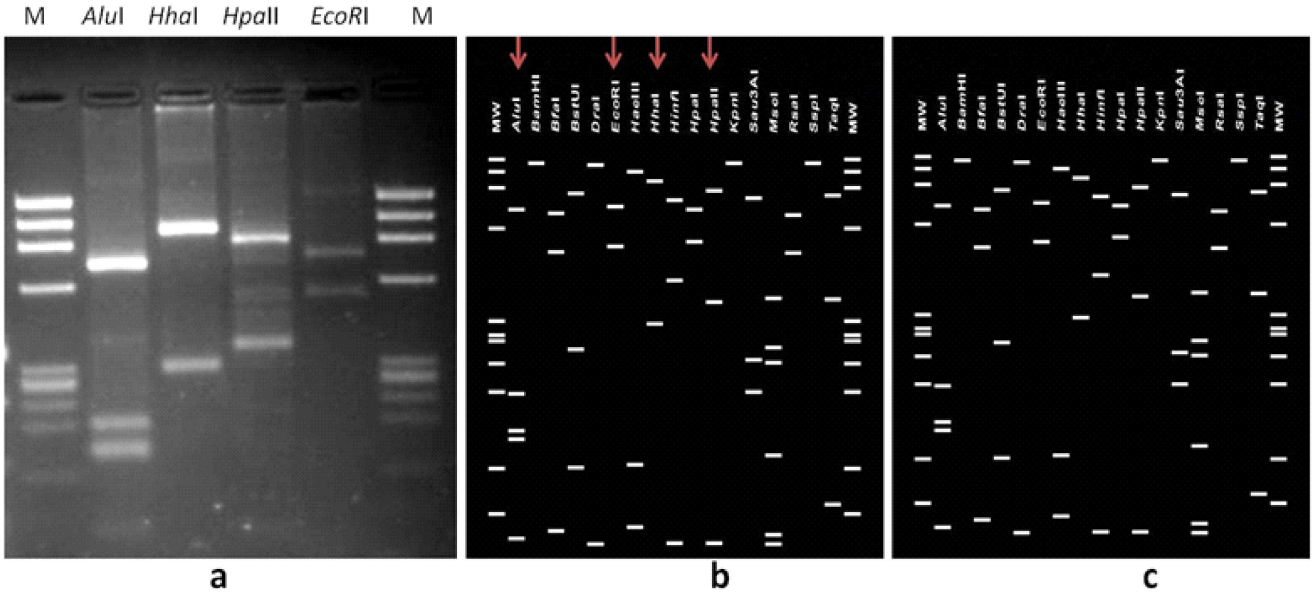

Figure 2. RFLP analysis in agarose gel of 1.2 kb PCR products amplified from *P. perpusilla* with restriction enzymes *Alu*I, *Hha*I, *Hpa*II, *Eco*RI (a) compared with virtual RFLP patterns (*i*Phyclassifier) of 16Sr DNA sequences of *P. perpusilla* (b) and a phytoplasma strains in 16SrXI-B subgroup (Accession Number X76432) (c).

symptomatic sugarcane leaf and *P. perpusilla* insects collected from sugarcane fields without chlorotic symptoms.

Pair wise alignment of 16S rDNA sequences of SCGS and *P. perpusilla* phytoplasma strains showed 100% sequence identity among themselves and 99% identities with '*Candidatus* Phytoplasma orzyae'-related phytoplasmas. Phylogenetic analysis also supported the above results and SCGS along with *P. perpusilla* strains clustered with strains classified in 16SrXI group (Figure 1). The *insilico* RFLP pattern of 16S rDNA gene of both the SCGS (Acc. No. KU568182) and *P. perpusilla* (Acc. No. KU568181) was analyzed by using *i*Phyclassifier software and showed the same restriction profile patterns of strains in 16SrXI-B subgroup ('*Ca.* P. orzyae', Acc. No. X76432; 16SrXI-B subgroup) (Figures 2b and c), which was in agreement with RFLP results (Figure 2a). Based on sequence comparison analysis, phylogenetic relationship and *in silico* and RFLP profilings, the phytoplasma strains associated with *P. perpusilla* and SCGS were identified as belonging to 16SrXI-B subgroup.

Earlier, different leafhopper species i.e. *Deltocephalus vulgaris* (Srivastava *et al*., 2006), *Exitianus indicus* (Rao *et al*., 2014), *Majiestas portica* and *Cofana unimaculata* (Tiwari *et al*., 2016) were reported as putative vectors of SCGS phytoplasma. In the present study, the role of *P. perpusilla* was suggested as a putative vector for '*Ca* P. oryzae' strains of 16SrXI group of phytoplasmas associated with SCGS disease in India. Since *P. perpusilla* is a serious sucking pest of sugarcane on important commercial varieties in Uttar Pradesh, and phytoplasma is found associated with *P. perpusilla*, it's role in field transmission of the phytoplasma cannot be ruled out. Further experiments are in progress to prove the role of *P. perpusilla* vector of SCGS phytoplasmas in India.

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