New host record of a ‘Candidatus Phytoplasma asteris’-related strain infecting peach in India

Jitender Singh · Anchal Rani · Pankaj Kumar ·
V. K. Baranwal · P. L. Saroj · Anil Sirohi ·
Amar N. Pandey · Peer M. Schenk

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Abstract Phytoplasma-like symptoms such as general decline, leaf reddening, yellowing, shortening of internodes and witches broom were observed in peach trees in the orchards of Meerut, India during May–August 2010–13. Leaf samples from symptomatic peach trees were collected and the presence of phytoplasma was confirmed by amplification of the 16S rRNA gene and *secA* gene by phytoplasma specific primers. Sequencing and BLAST analysis of ~1.25 kb of the 16S rDNA gene and ~840 bp of non-ribosomal *secA* gene fragments indicated that phytoplasma associated with peach disease was identical to the 16SrI Aster yellows group phytoplasma. Phylogenetic analysis of 16S rDNA peach phytoplasma also grouped it with 16SrI-A. This is the first report of association of a phytoplasma of 16SrI-A subgroup with peach trees in India.

Keywords BLAST · PCR · Phytoplasma · *Prunus persica* · Witches broom

In India, peach (*Prunus persica*) is mostly grown in the subtropical region of Uttar Pradesh and neighbouring states. During May 2010–13, symptoms of general decline, leaf reddening, yellowing, shortening of internodes, and witches broom (Fig. 1) were observed in about 40 % of the trees in the peach orchards near Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut in Uttar Pradesh State of India. Remaining trees appeared healthy showing vigorous growth throughout the year. Therefore, a study was undertaken to determine the presence of a phytoplasma in symptomatic and non-symptomatic peach trees. Several groups of phytoplasmas are known to be associated with peach diseases worldwide (Poggi Pollini et al. 1993; Marcone et al. 1996; Blomquist and Kirkpatrick 2002; Anfoka and Fattash 2004; Zunnoon-Khan et al. 2010; Arocha-Rosete et al. 2011). Identification of the phytoplasma associated with this peach disease was carried out by amplifying and sequencing the *secA* gene and 16S rRNA gene.

Total DNA was extracted from leaf midribs of 30 trees in three replicates each (symptomatic and non symptomatic) with DNeasy Plant Mini Kit, Qiagen, Germany. A nested PCR assay to amplify the phytoplasma 16S rRNA gene using universal phytoplasma primers P1/P7 (Deng and Hiruki 1991), R16F2/R16R2 (Gundersen and Lee 1996) and P1/Tint (Smart et al. 1996) was carried out. The characteristic bands of desired size (1.8, 1.26 and 1.6 kb, respectively) were obtained in twelve out of the thirty trees tested

J. Singh (✉) · A. Rani · P. Kumar · A. Sirohi
College of Biotechnology, Sardar Vallabhbhai Patel University of
Agriculture and Technology, Meerut 250110, India
e-mail: jeets_80@rediffmail.com

V. K. Baranwal
Plant Virology Unit, Division of Plant Pathology, Indian Agricultural
Research Institute, New Delhi, India 110012

P. L. Saroj
Indian Council of Agricultural Research, Division of Horticulture,
KAB-II, New Delhi 110 012, India

J. Singh · A. N. Pandey · P. M. Schenk
School of Agriculture and Food Sciences, The University of
Queensland, Brisbane, QLD 4072, Australia

Fig. 1 Symptoms of phytoplasma disease infecting peach trees. **a** and **b**; Typical symptoms of phytoplasmal disease in the peach orchards of Meerut, India. Symptoms include leaf reddening, rosette-type appearance similar to witches broom. **c** Healthy peach trees

for the presence of phytoplasmas from symptom-bearing trees. The twelve positive trees were further tested for phytoplasma with primers *secA1F/secA3R* of non-ribosomal *secA* gene (Hodgetts et al. 2008) and bands of ~840 bp were observed. However, there was no phytoplasma detected in symptomless trees. Three PCR products amplified with P1/Tint samples and three from *secA1F/secA3R* were selected and purified (QIAquick Gel Extraction kit, Qiagen, Germany) and directly sequenced at Bioserve Pvt Limited, Hyderabad. A resultant 1,592 bp 16S rDNA sequence with Accession No. AB858361 and 482 bp *secA* gene sequence AB858360 was deposited in DNA Data Bank

of Japan (<http://www.ddbj.nig.ac.jp/>). Blastn analysis of the 16S rDNA gene sequences revealed that the peach phytoplasma was 99 % identical to phytoplasmas in the aster yellows phytoplasma group (16SrI group). Similarly, Blastn analysis of the *secA* gene sequences also confirmed that the phytoplasma was 95–99 % identical to phytoplasmas in the aster yellows phytoplasma group (16SrI).

Phylogenetic analysis was performed by constructing an evolutionary tree using MEGA version 4.0 (Tamura et al. 2007) using sequences from earlier reported phytoplasmas (Fig. 2). Sequences were aligned by Clustal X (Thompson et al. 1997) and trimmed to the length of the phytoplasma sequences being analysed in the present study. Maximum parsimony method was used for tracing evolutionary relationships. The out group chosen for rooting was *Acholeplasma palmae* (M23932). On comparison with 16S rDNA sequences of phytoplasma groups and subgroups associated with different plant/trees species peach phytoplasma (AB858361) clustered with the 16SrI ‘*Candidatus* Phytoplasma asteris’ group.

Sequence similarity matrices generated for 16 s rRNA gene using Bioedit (Hall 1999) ranged between 89–99 %. Phylogenetic analysis supported BLAST comparisons, showing the similarity of the phytoplasma associated with peach disease (AB858361) to subgroup 16SrI-A by clustering in the single distinct branch that encompasses this subgroup.

Our study confirmed a new host recording of 16SrI aster yellows phytoplasma (AY) group with peach disease in India. The 16SrI group phytoplasma has been associated with several phytoplasma diseases in India such as Periwinkle virescence (Azadvar and Baranwal 2009), Sandal spike (Khan et al. 2008), Sesamum phyllody (Khan et al. 2007), Parthenium virescence (Raj et al., 2008b), Achyranthesaspera yellows (Raj et al. 2008c), Little leaf disease of desert rose (Raj et al. 2006a), witches-broom disease of *Cannabis sativa* (Raj et al. 2008a), Pigeon pea little leaf (Raj et al. 2006b), Chilli little leaf (Khan and Raj 2005), witches-broom of *Crotalaria tetragona* (Baiswar et al. 2009) and Black pepper phyllody (Bhat et al. 2006). These results highlight to the diversity of phytoplasma found in peach previously well documented. The molecular identification and characterisation of peach disease should contribute to expanding the knowledge of the genetic diversity and epidemiology of 16SrI phytoplasmas in

Phylogenetic tree showing the relationships between 48 accessions of *P. asteris* and related species. The tree is rooted at the bottom left. Bootstrap values are indicated at the nodes. The accessions are listed on the right, with their IDs, species names, and sample identifiers.

Scale bar: 0.1

Accessions (from top to bottom):

- AY180956 Ca. *P. asteris* 16Srl-A
- HQ589187 Ca. *P. asteris* 16Srl-A
- AY265215 Ca. *P. asteris* 16Srl-A
- AF453416 Ca. *P. asteris* 16Srl-A
- AY265214 Ca. *P. asteris* 16Srl-A
- GU223210 Ca. *P. asteris* 16Srl-A
- EU215424 Ca. *P. asteris* 16Srl-A
- AB858361 Ca. *P. asteris* Peach]
- EU123466 Ca. *P. asteris* 16Srl-A
- DQ174115 Ca. *P. asteris* 16Srl-A
- AY265213 Ca. *P. asteris* 16Srl-E
- AY265209 Ca. *P. asteris* 16Srl-B
- AF322644 Ca. *P. asteris* 16Srl-B
- AY180945 Ca. *P. asteris* 16Srl-B
- CP000061 Ca. *P. asteris* 16Srl-B
- AY566302 Ca. *P. asteris* 16Srl-B
- M30790 Ca. *P. asteris* 16Srl-B
- KC920747 Ca. *P. asteris* 16Srl-B
- AY180947 Ca. *P. asteris* 16Srl-B
- EF176608 Ca. *P. asteris* 16Srl-B
- AF503568 Ca. *P. asteris* 16Srl-P
- U96616 Ca. *P. asteris* 16Srl-K
- AY265212 Ca. *P. asteris* 16Srl-F
- AY265218 Ca. *P. asteris* 16Srl-S
- DQ640502 Ca. *P. asteris* 16Srl-S
- AF200431 Ca. *P. asteris* 16Srl-R
- AF222065 Ca. *P. asteris* 16Srl-R
- AJ539179 SugarCane P. 16SrXXVI-A
- AJ539180 SugarCane P. 16SrXXVI-A
- U18747 Ca. *P. palmae* 16SrlV-A
- X80117 Ca. *P. costanzaniae* 16SrlV-C
- Y14175 Ca. *P. cocosnigeriae* 16SrlXII-A
- AY390261 Ca. *P. trifolii* 16SrlVI-A
- FJ204395 Ca. *P. trifolii* 16SrlVI-A
- AF092209 Ca. *P. fraxini* 16SrlVII-A
- AB052876 Ca. *P. ziziphi* 16SrlV-B
- AF176319 Ca. *P. vitis* 16SrlV-C
- AY197655 Ca. *P. ulmi* 16SrlV-A
- AF086621 Ca. *P. luffae* 16SrlVIII-A
- AB054986 Ca. *P. Castaneae* 16SrlXIX
- AJ632155 Ca. *P. pini* 16SrlXXI
- AB052873 Ca. *P. oryzae* 16SrlXI-A
- AJ550984 Ca. *P. cynodontis* 16SrlXIV
- AF515636 Ca. *P. phoenicium* 16SrlX-D
- EF666051 Ca. *P. omanense* 16SrlXXIX-A
- L04682 Ca. *P. pruni* 16SrlIII-A
- U15442 Ca. *P. aurantifolia* 16SrlI-B
- AF147708 Ca. *P. brasiliense* 16SrlXV
- FJ432664 Ca. *P. tamaricis* 16SrlXXX-A
- X76431 Ca. *P. rhamnii* 16SrlXX
- X92869 Ca. *P. spartii* 16SrlX-D
- AJ542543 Ca. *P. pyri* 16SrlX-C
- AJ542541 Ca. *P. mali* 16SrlX-A
- AJ542544 Ca. *P. prunorum* 16SrlX-F
- M23932 *A. laidlawii*

which could assist in developing effective disease management strategies.

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