



## First report of '*Candidatus Phytoplasma aurantifolia*' associated with phyllody of blackgram in India

B.V. Bhaskara Reddy<sup>1\*</sup>, L.Prasanthi<sup>1</sup>, R. Sarada Jayalaxmi<sup>2</sup>, V. Saisruthi<sup>1</sup>, S.M. Shareef<sup>1</sup> and T. Giridhara Krishna<sup>1</sup>

<sup>1</sup> Department of Plant Pathology, Regional Agricultural Research Station, Tirupati- 517501, Andhra Pradesh, India; <sup>2</sup> Department of Plant Pathology, Agricultural College, Tirupati-517501, Andhra Pradesh, India

\*E-mail: [bvbreddy68@gmail.com](mailto:bvbreddy68@gmail.com)

Received: 25 Mar 2014. Published: 24 Nov 2014. Keywords: 16S ribosomal DNA, PCR

Blackgram (*Vigna mungo*) is one of the major pulse crops of the tropics and sub-tropics, and the third-most cultivated in the Indian sub-continent. During a 2013-14 survey at the Regional Agricultural Research Station in Tirupati, Andhra Pradesh, symptoms of yellowing, stunting, witches' broom, little leaves and reduced size of flowers were observed in 2% of blackgram plants (Fig. 1a, b). To investigate the possibility of a phytoplasma association with the symptoms, total DNA was isolated from 100 mg leaf midribs from infected and symptomless plant samples using the CTAB method (Doyle & Doyle, 1990). Total DNA was used as a template for a nested PCR assay with universal primers that target the phytoplasma 16S rRNA gene: P1/P7 (Deng & Hiruki, 1991) and R16F2n/m23sr (Constable *et al.*, 2002). Expected size amplicons of 1.8 kb and 1.6 kb, respectively, were amplified from symptom-bearing blackgram plants (Fig. 2), but not from the symptomless plant samples.

A representative 1.6 kb PCR fragment was cloned into a pTZ57R/T vector (Fermentas, USA) and sequenced, and the consensus sequence was submitted to GenBank (Accession No. KJ540943). BLAST analysis of the partial 16S rDNA sequence of the *Vigna mungo* phytoplasma revealed the highest sequence identity (99%) with phytoplasmas of the 16SrII group '*Candidatus Phytoplasma aurantifolia*', including the phytoplasma associated with blackgram witches' broom and phyllody from Myanmar (AB690304). Phylogenetic analysis (Fig. 3) using MEGA version 4.0 (Tamura *et al.*, 2007) evidenced that the Indian *Vigna mungo* phytoplasma is closely related to phytoplasmas embraced within the '*Ca. P. aurantifolia*' cluster. Virtual RFLP patterns ((AcaClone, <http://www.acaclone.com>) using *Bst*UI, *Hae*III, *Hpa*II and *Mse*I endonucleases were more similar to

those of the blackgram witches' broom and phyllody phytoplasma from Myanmar (AB690304) attributed to '*Ca. P. aurantifolia*' (Win & Jung, 2012). To our knowledge, this is the first report of the association of a '*Ca. P. aurantifolia*' isolate with phyllody and witches' broom in India. The results have significant phytosanitary impact for the epidemiology of phytoplasma diseases of the Fabaceae in the region, particularly in Andhra Pradesh, India.

### References

- Constable FE, Whiting JR, Gibbs KS, Symons RH, 2002. A new grapevine yellows phytoplasma from the Buckland Valley of Victoria, Australia. *Vitis* **41**, 147-153.
- Deng S, Hiruki C, 1991. Amplification of 16 S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* **14**, 53-61. [http://dx.doi.org/10.1016/0167-7012\(91\)90007-D](http://dx.doi.org/10.1016/0167-7012(91)90007-D)
- Doyle JJ, Doyle JL, 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus* **12**, 13-15.
- Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599. <http://dx.doi.org/10.1093/molbev/msm092>
- Win NKK, Jung HY, 2012. Molecular analysis of '*Candidatus Phytoplasma aurantifolia*' associated with phytoplasma diseases in Myanmar. *Journal of General Plant Pathology* **78**, 260-263. <http://dx.doi.org/10.1007/s10327-012-0384-8>



Figure 1

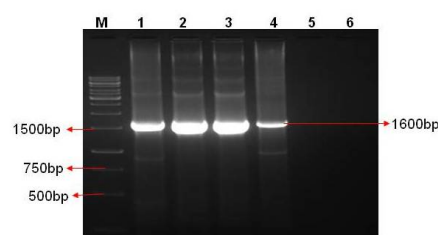


Figure 2

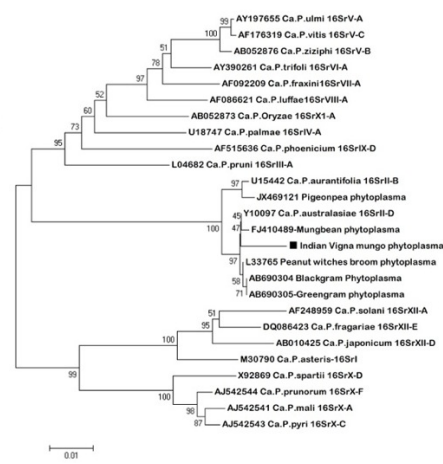


Figure 3

**To cite this report:** Bhaskara Reddy BV, L.Prasanthi, Sarada Jayalaxmi R, Saisruthi V, Shareef SM, Giridhara Krishna T, 2014. First report of '*Candidatus Phytoplasma aurantifolia*' associated with phyllody of blackgram in India. *New Disease Reports* **30**, 25.

<http://dx.doi.org/10.5197/j.2044-0588.2014.030.025>

© 2014 The Authors

This report was published on-line at [www.ndrs.org.uk](http://www.ndrs.org.uk) where high quality versions of the figures can be found.