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First Report of *Peanut bud necrosis virus* Infecting Bitter Gourd (*Momordica charantia* L.) in India

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Bitter gourd (*Momordica charantia* L., family Cucurbitaceae) is a major vegetable crop widely cultivated throughout India. Bitter gourd pods are consumed for their nutritional and medicinal value. In 2016, nearly 15% of plants showed virus-like symptoms in a 0.6 ha bitter gourd farm at the Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India. Symptomatic plants were stunted with necrosis of growing tips and yellowing and downward cupping of leaves that became thick and leathery in appearance. Because these symptoms were like those produced by orthotospoviruses in vegetable crops (**Kunkalikar et al. 2011**), leaves from five symptomatic and five nonsymptomatic plants were initially tested by indirect ELISA using polyclonal antibodies raised against *Peanut bud necrosis virus* (PBNV; genus, *Orthotospovirus*; family, Tospoviridae). These antibodies were obtained from the International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India. Only samples from symptomatic plants gave positive reaction, suggesting the presence of PBNV.

Owing to cross-reactivity of PBNV polyclonal antibodies with *Watermelon bud necrosis virus* and *Capsicum chlorosis virus*, two orthotospoviruses infecting vegetables in India (**Kunkalikar et al. 2011**), additional molecular diagnostic assays were performed to confirm the presence of PBNV. For this purpose, total RNA was extracted from leaves of symptomatic and nonsymptomatic plants using TRIzol reagent (Ambion) and subjected to two-step reverse-transcription polymerase chain reaction (RT-PCR) to amplify a portion of the L RNA segment of orthotospoviruses using degenerate primers reported earlier (**Chu et al. 2001**). A DNA fragment of approximately 800 base pairs (bp) was amplified only from symptomatic samples. The amplicons were cloned into pTZ57R/T vector (Fermentas), and two clones per amplicon were sequenced in both orientations. The consensus nucleotide sequence (KY798419) showed 97% identity with the corresponding L RNA sequence of PBNV (AF025538), indicating the presence of PBNV in symptomatic plants. To further confirm these results, total RNA was subjected to RT-PCR using newly designed primer pairs GK PBNV F (5'ATGTCTAACGTYAAGCAGCTC3') and GK PBNV R (5'TTACAATTCCAGCGAAGGAC3') to amplify the nucleocapsid (N) gene encoded by S RNA and GK PBNV MP F (5'ATGTCTCGCTTGTCTAACG3') and GK PBNV MP R (5'CAAGAAGATTATCCATCTC3') to amplify the movement protein (NSm) gene encoded by M RNA of PBNV. The primer pair GK PBNV F and GK PBNV R amplified 830-bp DNA fragment specific to the N gene, and the primer pair GK PBNV MP F and GK PBNV MP R amplified 916-bp fragment specific to the NSm gene from all the five symptomatic samples. The amplicons were sequenced directly, and the derived nucleotide sequence for the N gene (KY798417) exhibited 99% identity with the corresponding sequence of PBNV (JX511967, JX524443, and JX524450), and the NSm gene nucleotide sequence (MF346701) showed 98% identity with the corresponding sequence of PBNV (JN662495, JX535589, and JX535585) reported previously from India. These results further confirmed the presence of PBNV in symptomatic bitter gourd leaves. Previously, PBNV was reported on watermelon from India (**Kunkalikar et al. 2011**). To our knowledge, this study represents the first report of natural PBNV infection of bitter gourd and suggests that PBNV may be more widely distributed in cucurbitaceous vegetable crops in India than previously thought.

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