New Disease Reports

First report of *Groundnut bud necrosis virus* infecting *Phalaenopsis* in India

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Phalaenopsis orchids (*Orchidaceae*) are attractive ornamental plants with a long vase life. Tospoviruses including *Capsicum chlorosis virus*, *Impatiens necrotic spot virus* (INSV) and *Tomato spotted wilt virus* (TSWV) have been reported to infect *Phalaenopsis* (Baker *et al*., 2007; Zheng *et al*., 2008; Cheng *et al*., 2010).

During 2016-17, *Phalaenopsis* plants grown at the National Research Centre for Orchids, Sikkim were observed with symptoms including mild chlorotic spots, mild-mosaic and dark-green patches with light-green/chlorotic margins (Fig. 1). Twelve symptomatic and five non-symptomatic samples were collected and transmission electron microscopy revealed the presence of tospovirus-like particles measuring 80-110 nm in diameter (Fig. 2) in symptomatic samples. The samples were tested by DAC-ELISA using polyclonal antibodies to GBNV (reported to detect all the tospoviruses of Serogroup-IV) (Mandal & Jain, 2010), INSV and TSWV (Agdia Inc., USA). Briefly, 100 mg of leaf tissue was homogenised in coating buffer and 200 µl per well dispensed into ELISA plates. Plates were coated with blocking solution, incubated, coated with virus-specific primary antibodies and incubated again. Plates were washed, coated with secondary antibodies (Goat Anti-Rabbit IgG) and incubated. Finally, plates were coated with substrate solution (p-nitrophenyl phosphate) and incubated. Incubation steps were 37°C for 1 hr and following each incubation, the plates were washed thrice with PBS-T buffer. Absorbance values (A405) were recorded using an ELISA reader (Biotek Instruments, USA) and a result was considered positive if the A405 was twice the mean absorbance value of the healthy control (virus-free *Phalaenopsis* plant). Only symptomatic samples reacted with the polyclonal antibodies of GBNV with absorbance values of 1.97-2.18 compared to 0.38 in the healthy control. The ELISA-positive samples were sap inoculated onto cowpea (*Vigna unguiculata*) cv. Pusa Komal and tobacco (*Nicotiana benthamiana*). Chlorotic local lesions on cowpea and systemic mottling, chlorosis and downward curling of leaves on tobacco were observed four-five days post inoculation (Fig. 3).

To confirm the associated tospovirus species, total RNA was extracted from symptomatic *Phalaenopsis*, cowpea, and tobacco leaves using an RNeasy Kit (Qiagen, Germany), and subjected to RT-PCR using the duplex-PCR primers Gs1F/GWs1R and Ws1F/GWs1R and cycling conditions described by Holkar *et al*. (2017) designed to detect the N gene of GBNV and WBNV respectively. An amplicon of c. 470 bp specific to the GBNV N gene was detected. The amplicons were cloned and sequenced bi-directionally to give a consensus sequence of 477 bp (GenBank Accession No. KY794416). Analysis of the 477 bp sequence of the N gene revealed that it shared identity of 99% nt and 100% aa identity with the corresponding region of other GBNV isolates (JQ406583, EU373784 & EU373779, and AGB58307 for nucleotide and amino acid, respectively). These results confirmed the association of GBNV with the *Phalaenopsis* plants. This is the first report of GBNV infecting *Phalaenopsis* in India and the world.

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Figure 1

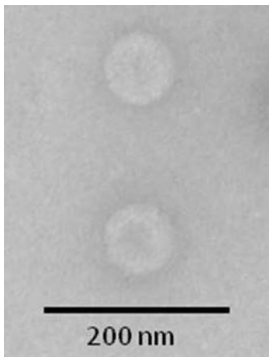

Figure 2

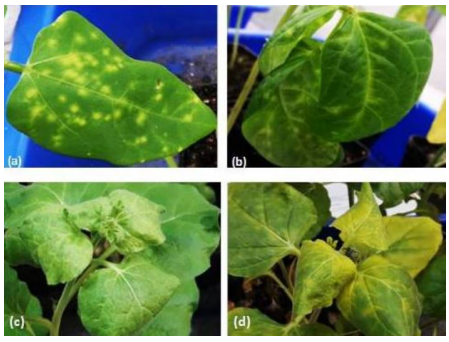

Figure 3

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