

First report of *Groundnut Bud Necrosis Virus* infecting Taro (*Colocasia esculenta*)

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Abstract The natural occurrence of *Groundnut Bud Necrosis Virus* (GBNV) on *Colocasia esculenta* was detected by enzyme linked immunosorbent assay using an antiserum raised against GBNV and reverse transcription polymerase chain reaction using coat protein specific primers. Sequence analysis showed 93–99% and 95–99% identity at nucleotide and amino acid levels respectively with other reported GBNV isolates.

Keywords Groundnut bud necrosis virus · Taro · DAC · ELISA · RT · PCR

Taro (*Colocasia esculenta*) a member of the *Araceae* family, is a traditional root crop of the tropics grown for its edible corms and leaves, and is believed to be one of the earliest cultivated root crops in the world (Plucknett 1976; Kuruvilla and Singh 1981). Corms and leaves of taro are also accredited to have medicinal importance and are used to reduce tuberculosis, ulcers, pulmonary congestion and fungal infections (Misra and Sriram, 2002). Taro is the second most important staple crop in Papua New Guinea; 14th in the world (Singh et al. 2006). The food and agricultural organization estimates that 9.1 million metric tons of corms are produced annually over an area of 2 million ha, but this largely under estimates its production as

few countries keep reliable figures (Mishra et al. 2009). To date five viruses have been reported to infect taro [*Dasheen mosaic virus* (DSMV), *Taro Bacilliform virus* (TaBV), *Colocasia bobone disease virus* (CBDV), *Taro vein chlorosis virus* (TaVcv) and *Taro reovirus* (TaRV)]. *Tospoviruses* are among the most damaging and economically important group of plant viruses causing significant crop losses in wide range of ornamental and food crops in many regions of the world (Mumford et al. 1996). *Groundnut Bud Necrosis Virus* (GBNV) a member of the genus *Tospovirus*, family *Bunyaviridae* consists of enveloped, quasi spherical particles, approximately 80–120 nm in diameter. GBNV has a wide host range (Groundnut, Tomato, Chilies, Potato, Peas, Sunflower, Green gram, Black gram, Cowpea, Cotton, Soybean, Field bean, Carrot, Brinjal, Ornamentals



Fig. 1 Symptoms associated with natural occurrence of *Groundnut Bud Necrosis virus* on colocasia: mosaic, chlorotic spotting and necrotic flecking of leaves

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Table 1 Sequence identity of present isolate (HQ199845) at nucleotide (above the diagonal) and amino acid (below the diagonal) levels respectively with other reported GBNV isolates

Accession No.	Nucleotide identity										Amino acid identity									
	HQ199845	EF179100	EF532937	EF179099	FJ447355	FJ447359	HM770020	DQ375811	AF467289	AY882003	AF515821	AY184354	AY512650	AY529713	AY512651	AY426317				
HQ199845	100	99.3	97.4	97.4	98.0	98.0	99.6	97.2	97.1	98.3	97.4	97.5	97.1	97.3	93.2					
EF179100	99.2	100	97.5	97.5	98.4	99.7	97.8	97.3	97.7	98.4	97.5	98.0	97.4	97.9	93.3					
EF532937	97.8	98.5	100	98.0	97.7	97.3	97.1	96.8	96.6	97.7	98.1	98.1	97.4	97.8	93.8					
EF179099	98.1	98.9	98.9	100	97.7	97.3	97.1	96.8	97.1	97.7	97.9	98.6	97.7	97.8	94.2					
FJ447355	99.2	100	98.5	98.9	100	98.3	98.1	97.4	97.2	98.0	97.7	98.1	97.8	98.1	93.5					
FJ447359	99.2	100	98.5	98.9	100	100	98.1	97.9	97.2	98.0	97.7	98.0	97.1	97.7	93.5					
HM770020	99.6	99.6	98.1	98.5	99.6	100	100	97.1	97.4	98.1	97.3	97.8	97.2	97.7	93.1					
DQ375811	98.5	99.2	97.8	98.1	99.2	98.9	100	97.1	97.3	97.4	97.1	97.5	96.9	97.5	93.5					
AF467289	98.1	98.9	97.4	97.8	98.9	98.5	98.1	100	96.3	97.2	96.8	96.9	96.5	96.6	92.9					
AY882003	99.2	100	98.5	98.9	100	99.6	99.2	98.9	100	97.2	96.6	97.1	96.7	97.1	93.3					
AF515821	98.9	99.6	98.1	98.5	99.6	99.2	98.9	98.5	99.6	100	97.7	97.8	97.3	97.4	93.5					
AY184354	97.8	98.5	98.1	98.5	98.5	98.1	97.8	97.4	98.5	98.1	100	98.0	97.5	97.7	93.7					
AY512650	97.8	98.5	98.5	98.9	98.5	98.1	97.8	97.4	98.5	98.5	98.1	100	98.1	98.3	93.7					
AY529713	98.1	98.9	98.9	99.2	98.9	98.5	98.1	97.8	98.9	98.5	98.5	98.9	100	97.4	93.5					
AY512651	98.5	99.2	99.2	99.6	99.2	98.9	98.5	98.1	99.2	98.9	98.9	99.2	99.6	100	94.1					
AY426317	95.2	96.0	95.6	96.7	96.0	95.6	95.6	94.9	96.0	95.6	95.2	95.6	96.0	96.3	100					

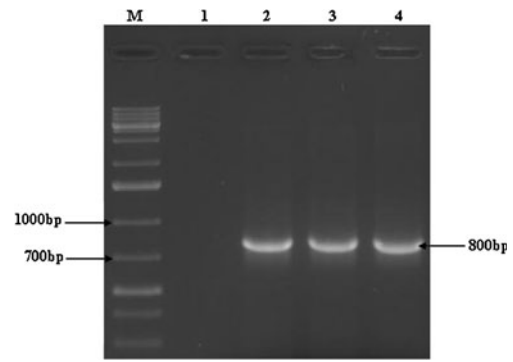


Fig. 2 Agarose gel electrophoresis of RT-PCR products. Lane M, 1Kb DNA ladder. Lane 1, healthy. Lane 2,3,4, infected colocasia samples

and weeds; *Vigna unguiculata*, *Nicotiana glutinosa*, *Petunia hybrida*, *Vinca rosea* and *Trapaolum majus*) (Reddy 1991).

Host range studies for GBNV and TSV have been carried out in our laboratory for the past 4 years. During one survey in 2010 we found GBNV suspected infection in colocasia fields in Nellore district of Andhra Pradesh, India. Mosaic, chlorotic spotting and necrotic flecking of leaves were observed (Fig. 1). Stunted growth was observed in infected plants. The symptomatic leaves tested positive for PBNV by DAC-ELISA (Clark and Joseph 1984) using polyclonal antibodies. The samples were also screened for TSV and were found to be negative. RT-PCR tests of leaf tissue from diseased colocasia plants using primers specific for the nucleocapsid gene of GBNV (Satyanarayana et al. 1996) resulted in an amplicon of the expected size (~800 bp) (Fig. 2). The amplicon was cloned into pTZ57R/T vector (Fermentas, USA), sequenced and deposited in GenBank DNA database (Accession No. HQ199845). Sequence analysis (BioEdit v. 7.05) and comparison with other GBNV isolates (GenBank Accession No. EF179100, EF179099, EF532937, FJ447355, FJ447359, HM770020, DQ375811, AF467289, AY512650, AY882003, AY529713, AY512651, AF515821, AY426317, and AY184354) showed 93–99% and 95–99% identity at nucleotide and amino acid levels respectively (Table 1).

The infected colocasia crop was destroyed in the interest of national biosecurity. Considering the economic importance and global consumption of colocasia, it is important for the impact of GBNV to be studied further. To the best of our knowledge this is the first report of the natural occurrence of GBNV on colocasia.

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