Phytopathol. Mediterr. (2010) 49, 249–252

NEW OR UNUSUAL DISEASE REPORTS

First report of natural infection of *Vigna mungo* var. *silvestris* L. by *Groundnut bud necrosis virus*, a tospovirus

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Summary. In the autumn of 2008, *Vigna mungo* var. *silvestris* growing in the experimental field of the Indian Institute of Pulses Research, Kanpur, India, showed chlorosis around some lateral veins and vein branches (mainly near the leaflet margin), downward curling of the leaf margins, necrosis of the stems and petioles, and twisting of the leaflets. Disease incidence was 20%. Symptoms indicated that the cause was *Groundnut bud necrosis virus*. The virus was identified on the basis of the symptoms on the diagnostic host, and the reverse transcription polymerase chain reaction (RT-PCR) using specific primers of the NSm and NP genes. To our knowledge this is the first report of *Groundnut bud necrosis virus* on *V. mungo* var. *silvestris*.

Key words: GBNV, cowpea, *Vigna mungo*, *Vigna unguiculata*.

Introduction

Tospoviruses have a wide host range and are emerging as serious pathogens affecting the cultivation of several crops on the Indian subcontinent (Akram *et al*., 2004). So far, four tospovirus species have been identified in India, of which *Groundnut bud necrosis virus* (GBNV) is the most prevalent. At the Indian Institute of Pulses Research, Kanpur, India, *Vigna mungo* var. *silvestris* (accession No. IPUW02), a wild relative of *V. mungo,* was grown in an experimental field in 2008. In August–September 2008, symptoms indicative of *Groundnut bud necrosis virus* infection were seen, including chlorosis along some lateral veins and vein branches, mainly near the leaflet margins, downward curling of the leaf margins, stem necrosis, and twisting of the leaflets (Figure 1). Symptomatic leaves were brittle and the whole trifoliate leaf would drop if shaken slightly. Vein necrosis was more pronounced on the lower leaf surface, and also extended to the petiole. Plants infected at an early stage remained stunted and finally died due to top necrosis. Disease incidence was 20% (87 out of 434 plants in the field). Similar symptoms in cultivated species of *Vigna,* also due to GBNV, were reported in an earlier study (Jain *et al*., 2006). Samples from field infected plants were analyzed for GBNV by mechanical inoculation on cowpea (*V. unguiculata* cv. Pusa Komal), a diagnostic host for GBNV. The characteristic symptoms of GBNV appeared on the cowpea plants 4–5

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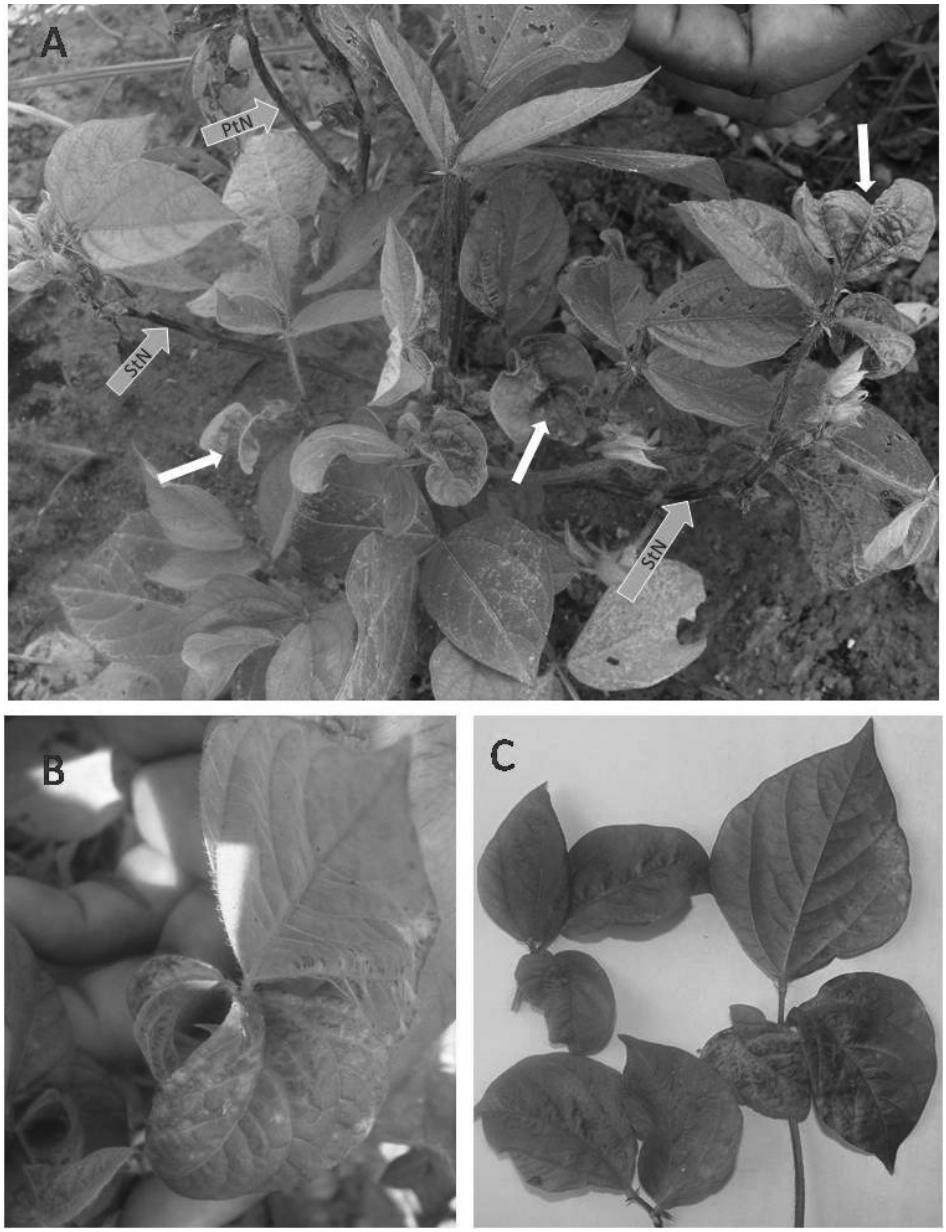

Figure 1. A, field-infected *Vigna mungo* var. *silvestris* plant showing necrosis of the stem (StN) and a petiole (PtN) and twisting of the leaflets (arrow); B and C, chlorosis and necrosis along the lateral veins and vein branches, downward curling of the leaf margins and twisting of leaflets.

days after inoculation. The virus produced local lesions as well as systemic symptoms on cowpea plants (Figure 2).

The causal virus was further identified with a reverse transcription- polymerase chain reaction (RT-PCR) to amplify the NSm and NP genes of GBNV. Samples were collected from plants with the characteristic symptoms, and from healthy plants. Four samples were used to detect GBNV using primers specific to the NSm gene and four samples were used to detect the virus using primers specific to the NP gene. The virus was also confirmed in cowpea leaves showing local lesions (inoculated primary leaves) and in systemically infected leaves.

Total RNA was extracted from inoculated and uninoculated healthy cowpea plants, and from both field-infected and healthy *V. mungo* var. *silvestris* plants using an RNeasy kit (Qiagen Inc., Chatsworth, CA, USA). A one-step RT-PCR mix was prepared using a PrimScript™ One step RT-PCR kit (TaKaRa Bio. Inc., Ostu, Siga, Japan), following manufacturer's instructions. RNA was amplified in an automated thermal cycler (Eppendorf Master Cycler Gradient, Hamburg, Germany) programmed for one cycle of 50°C for 30 minutes for cDNA synthesis, and 35 cycles of amplification with the following parameters: 30 s of denaturation at 94°C, 1 min of annealing at 48°C for the NSm gene and 62°C for the NP gene, and 1 min of extension at 72°C, followed by a final extension of 10 min at 72°C. Following RT-PCR, the amplified products were analyzed by 1% agarose gel electrophoresis at 60 V. The gels were inspected in an UV transilluminator and photographed using a digital camera (DSC-H3, Sony Corporation, Tokyo, Japan). The primer-pair specific to the GBNV-NSm gene (5'ATGTCTCGCTTDTCTAAHGTB 3' and 5' TTATATTTCAAGAAGATTATC 3') derived

Figure 2. Symptoms on *Vigna unguiculata* cv. Pusa Komal. A, local chlorotic and necrotic lesions on an inoculated primary leaf; B, systemic symptoms, mainly chlorotic rings, and veinal and interveinal necrosis.

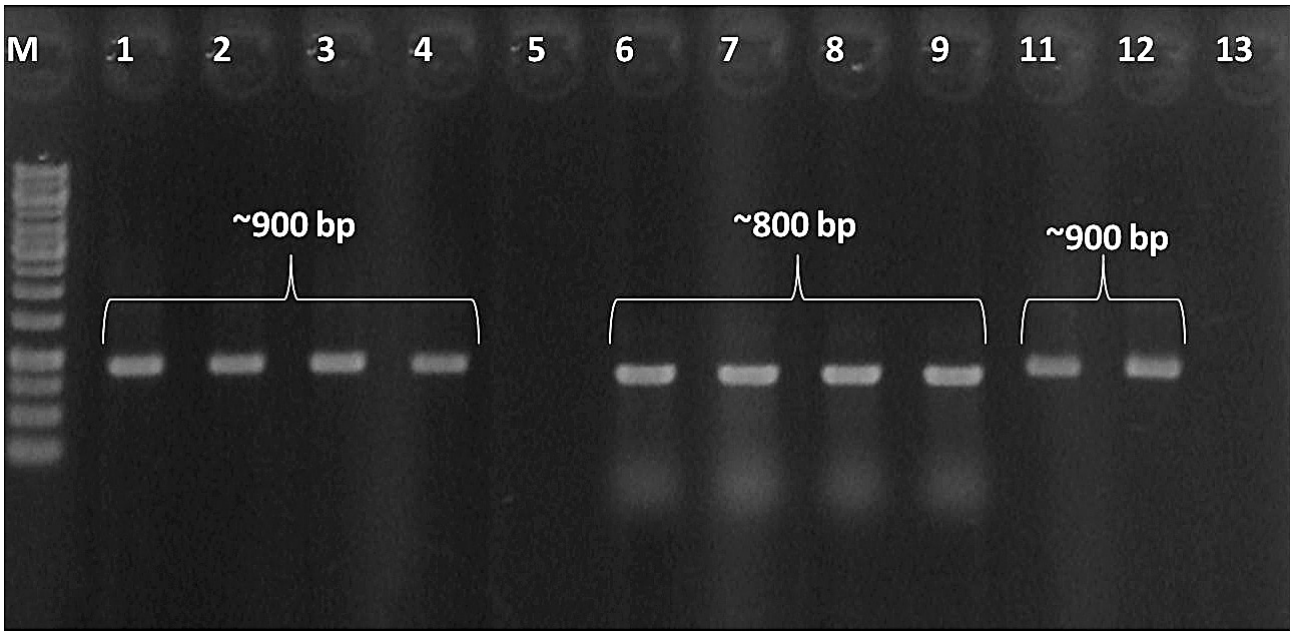

Figure 3. Amplification of a non-structural movement protein (NSm) gene (expected size ~900bp) and a nucleocapsid protein (NP) gene (expected size ~800 bp) of *Groundnut bud necrosis virus* from naturally infected *Vigna mungo* var. *silvestris* and mechanically inoculated *V. unguiculata* cv. Pusa Komal using specific primers. M, 1 kb DNA ladder (Fermentas); lane 1–4 and 6–9, field-infected samples of *V. mungo* var. *silvestris*; lane 5, healthy sample of *V. mungo* var. *silvestris*; lane 11, 12 and 13, inoculated, systemically infected and uninoculated leaf of cowpea (*V. unguiculata* cv. Pusa Komal) respectively.

from the sequences of *Groundnut bud necrosis virus* (U42555), of the non-structural movement protein gene (NSm), amplified a ~900 bp fragment, and the primer pair specific to the GBNV-NP gene (HRP26= 5' ATGTCTAACGTYAAGCARCTC 3' and HRP28=TACAATTCCAGCGAAGGACC 3') amplified an ~800 bp fragment, indicating that GBNV was associated with infected *V. mungo* var. *silvestris*. All the field-infected samples of *V. mungo* var. *silvestris* processed for detection of GBNV using NSm and NP gene specific primers were positive. Using both pairs of primers, GBNV was also confirmed in locally as well as systemically infected leaves of mechanically inoculated cowpea plants (Figure 3). GBNV is known to produce natural infections on leguminous crops, such as cowpea, mungbean, rajmash (*Phaseolus vulgaris*), Groundnut, and vegetables such as tomato and potato, in India (Varma *et al*., 2002; Jain *et al*., 2006). To our knowledge, this is the first report of GBNV naturally infecting *V. mungo* var. *silvestris*.

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Accepted for publication: April 14, 2010