Potato J (2012) 39 (1): 81-83

SHORT NOTE

OCCURRENCE OF STEM NECROSIS DISEASE IN POTATO CAUSED BY GROUNDNUT BUD NECROSIS VIRUS IN UTTARAKHAND

VS Pundhir[1], M Akram[2], Mohammad Ansar[1] and Rajshekhara H[3]

KEYWORDS: stem necrosis, potato, groundnut bud necrosis virus, occurrence

Potato crop is susceptible to many biotic stresses, among them stem necrosis caused by groundnut bud necrosis virus (GBNV) is a very serious problem in many parts of India. The stem necrosis disease was first reported in India and based on the morphological and serological studies the causal virus was confirmed as GBNV (Jain *et al.*, 2004). Analysis of nucleocapsid protein (NP) gene sequence was used to determine the extent of GBNV infection in various crops such as cowpea, mungbean, soybean, potato and tomato (Bhat *et al.*, 2002; Jain *et al.*, 2002). The association of GBNV on potato from Madhya Pradesh and Rajasthan has been characterized based on NSm gene analysis (Akram *et al.*, 2004). Stem necrosis incidence was recorded up to 90% in some parts of Madhya Pradesh and Rajasthan (Khurana *et al.*, 2001). The management approaches of stem necrosis disease have been described by Somani *et al.*, (2007).

At Pantnagar symptoms of stem necrosis in potato were observed in 2008 and confused with late blight infection. However, the fungus could not be isolated from such lesions. The typical symptoms indicative of GBNV such as downward curling of leaves with dark brown necrotic ring spots on upper surface and veinal necrosis on lower surface, stem necrosis, brown necrosis at nodes **(Fig. 1A and 1B)**, were observed and the disease incidence up to 50% in potato variety Kufri Bahar were observed in certain plots at Pantnagar.

For confirmation sap transmission was tried from infected potato plant to cowpea which is an indicative host of GBNV. This resulted in development of characteristics chlorotic and necrotic local lesions on the inoculated cowpea plants **(Fig. 1E)**. The stem necrosis associated virus was successfully transmitted by insect vector (*Thrips palmi*) on potato cv. Kufri Bahar under fabricated glasshouse conditions and produced typical stem necrosis symptoms **(Fig. 1C)**. Sap inoculated potato plants developed the spots **(Fig. 1D)** on leaves, but such symptoms were not exactly similar to those as observed in fields. Successful transmission of stem necrosis associated virus on indicator plant and on potato plant by the insect vector indicated the involvement of GBNV in causing stem necrosis in potato plants.

The causal virus was further confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) using primer pairs, HRP 26-5′ ATG TCT AAC GT(C/T) AAG CA(A/G)

[1]GB Pant UA&T Pantnagar-263 145, Uttarakhand, India.
E-mail: ansar.pantversity@gmail.com
[2]Indian Institute of Pulses Research, Kanpur-280 024, Uttar Pradesh, India.
[3]Indian Agricultural Research Institute, New Delhi-110 012, India.

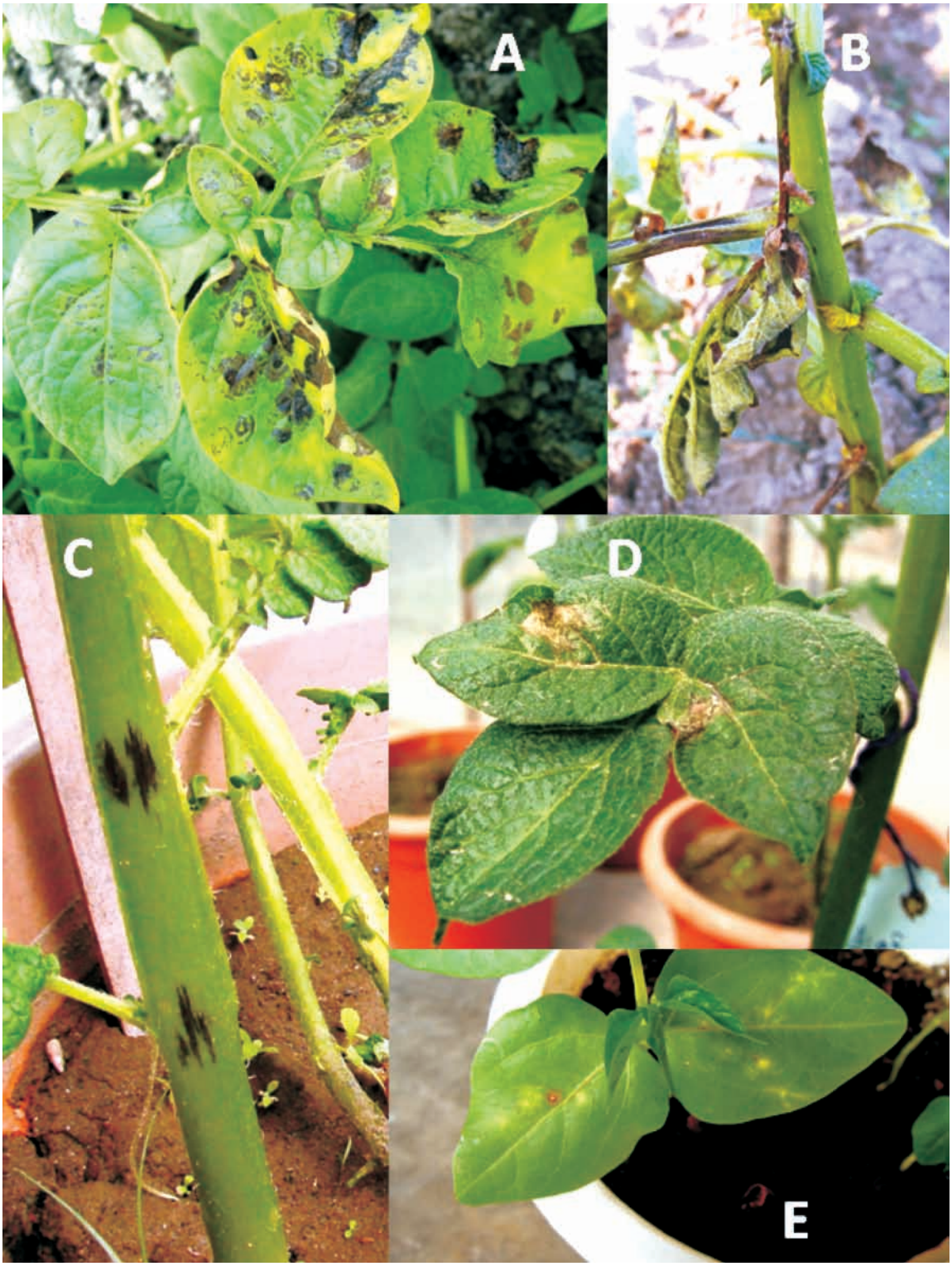

Fig. 1. Symptoms on naturally infected potato plants and on artificially inoculated potato and cowpea plants. A- Dark brown necrotic and chlorotic ring spots on upper leaf surface. B- Stem necrosis; C- Stem necrosis on thrips inoculated potato plants; D- Brown necrotic spots on sap inoculated plants; E- Chlorotic/ necrotic spots on sap inoculated cowpea plants.

CTC-3′/HRP 28-5′TAC AAT TCC AGC GAA GGA CC-3′ targeting nucleocapsid protein gene (NP) of GBNV. In agarose gel electrophoresis, amplified products of RT-PCR, out of 22 diseased samples, 17 samples yielded DNA fragment of about ~800 bp corresponding to NP gene of GBNV. The purified product of NP gene was cloned into RBC T & A Cloning Vector and *E. coli* cells (Max Efficiency Competent Cells-Invitrogen, Carisbad, USA) as per the manufacturer instructions and the positive clones were sequenced. The sequence data of NP gene of potato isolate was submitted to NCBI under the accession (JN575637).

The sequence analysis revealed that the complete nucleotide sequence of the NP gene of GBNV potato isolates had single open reading frame (ORF) of 831 nucleotide and 276 amino acids. The blast results of NP gene sequence of present GBNV-[Pot_USN] isolate (JN575637) showed (99%) identity with known GBNV isolate (JF281102) followed by 98% identity with other known GBNV isolates (JF281103, DQ375811, DQ375812, AY882004, EU373772 and EU373768) at nucleotide level. To distinguish tospovirus species the variation in nucleotide sequences of NP gene of a tospovirus should be <90% (Fauquet, *et al.*, 2005). If the nucleotide sequence homology of NP gene is >90% with a particular tospovirus that means the virus species will be an isolate of that particular virus.

The NP gene nucleotide sequence had 99% similarity with known GBNV isolate (JF281102) indicating the tested disease samples showing various types of symptoms including of stem necrosis in potato at Pantnagar and it adjoining areas was an isolate of GBNV and designated as GBNV-[Pot_USN]. Our results explicit the evidence of first report of GBNV, a tospovirus infection in potato from Uttarakhand. Looking at potential of the virus for causing damage to potato crop, serious efforts should be made for further studies to protect potato from stem necrosis disease, an important crop of the area.

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MS received: 17 September 2011; Accepted: 06 January 2012