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Short Communication

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Sequence Diversity in the *NSm* Gene of *Groundnut Bud Necrosis Virus* Isolates Originating from Different Hosts and Locations in India

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Abstract

The movement protein (*NSm*) gene of *Groundnut bud necrosis virus* (GBNV) isolates from pea, mungbean, cowpea, French bean, tomato and potato collected from different locations of India were compared to study their diversity. The *NSm* gene sequences of all the GBNV isolates were highly conserved and had only 0–3% diversity in amino acids and 0–10% in nucleotides. Comparison of amino acid sequence of *NSm* gene of 25 GBNV isolates revealed the presence of many conserved regions. Both 'D-motif' and 'G-residue', the conserved regions of '30K superfamily' of virus movement protein, were present in all the isolates. Clustering of the GBNV isolates does not appear to be based on their place of origin and host plant species.

Introduction

Groundnut bud necrosis virus (GBNV), a tospovirus of the family *Bunyaviridae* and transmitted by thrips, is the most important virus economically (Pappu et al. 2009). It affects a range of crops and causes substantial losses (Kunkalikar et al. 2011). The non-structural movement (*NSm*) protein gene has been shown to be equally important in characterization and classification of tospovirus species (Silva et al. 2001). A fragment of the *NSm* gene has been used to analyse the sequence diversity in TSWV populations from southern Italy (Finetti-Sialer et al. 2002). We aimed to analyse the *NSm* gene sequences available so far to decipher the diversity in GBNV population in India and its relation with host and location, if any. A total of 25 *NSm* gene sequences including ten from this study have been used.

Materials and Methods

Samples with suspected symptoms of GBNV infection from pea, mungbean, cowpea, French bean, potato and tomato collected during 2009–2011 from different locations were ground in prechilled 0.1 M phosphate buffer containing 0.1% beta-mercaptoethanol and inoculated on to host plants and cowpea (*V. unguiculata* cv. Pusa Komal) at the primary leaf stage using Celite as an abrasive.

Total RNA from leaf tissues of the naturally infected, artificially inoculated and healthy leaf tissues of hosts as well as cowpea extracted using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA) was used as a template in the reverse transcription polymerase chain reaction (RT-PCR). The primer (RKJ5/RKJ6) pair (Akram et al. 2004) was used for the amplification of *NSm* gene using TITANIUM One Step RT-PCR kit (Clontech Laboratories Inc., Mountain View, CA, USA) and the annealing temperature of 48°C for 1 min. Amplified products from pea from 4 locations (Udham Singh Nagar, Shahjahanpur, Bareilly and Kanpur), one each of mungbean, cowpea, French bean and tomato from Kanpur, and one from each tomato and potato from Udham Singh Nagar were cloned and sequenced.

Sequence data were edited using Bioedit (Hall 1999). All the complete *NSm* gene sequences available as on Oct 22, 2011 were retrieved from the NCBI database for analysis. Multiple Alignments were conducted by CLUSTALW (http://www.genome.jp/tools/clustalw). The phylogram was constructed using Neighbor-Joining method with bootstrapping (1000 replicates) in MEGA5 (Tamura et al. 2011) software.

Results and Discussion

Groundnut bud necrosis virus induced chlorotic and necrotic spots on leaves, browning in veins, stem necrosis, ring spots on fruits and pods (Fig. 1). Disease incidence was 2–10% in pea, 0–10% in mungbean, 4–5% in French bean, 1–50% in tomato and potato in different fields. In tomato and potato, necrosis of the

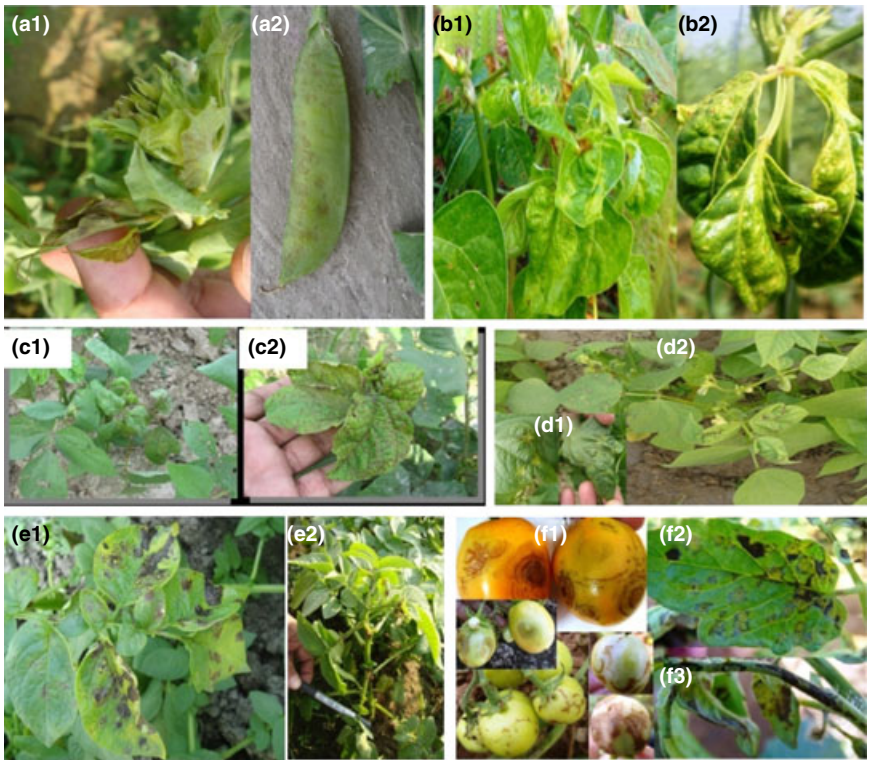

Fig. 1 Symptoms of *Groundnut bud necrosis virus* on pea (a), cowpea (b), mungbean (c), French bean (d), potato (e), tomato (f)

foliage often led to collapse of a stem or of the whole plant resembling symptoms of blight. In general, plants infected at an early stage often collapsed and died. Mechanically inoculated plants developed symptoms that broadly resembled with the symptoms observed in the field.

RT-PCR products revealed the presence of amplicons of expected size (~ 900 bp). *Groundnut bud necrosis virus*

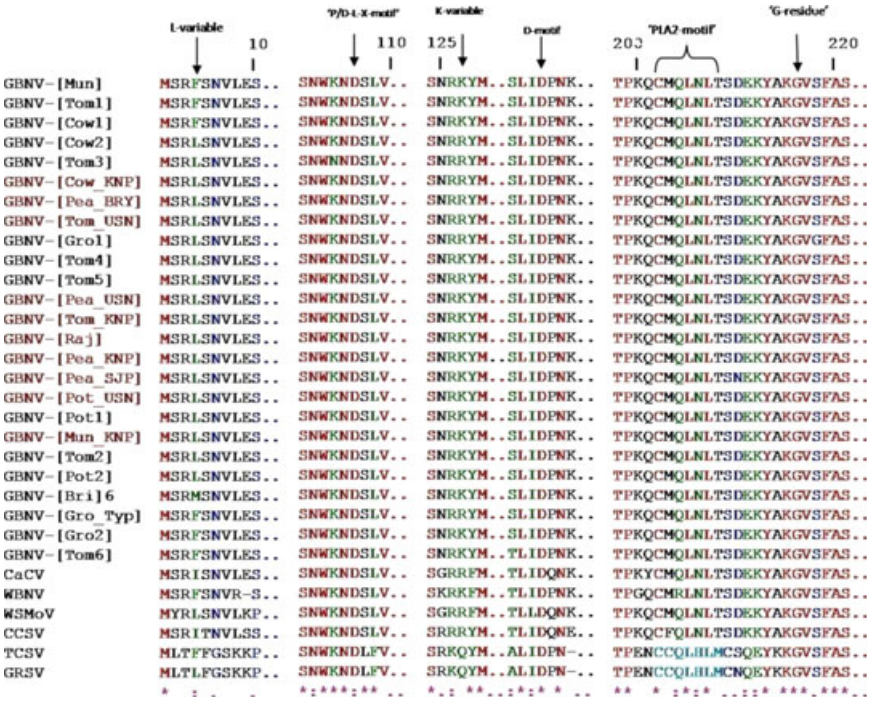

Fig. 2 Multiple alignment of *NSm* gene sequences of *Groundnut bud necrosis virus* isolates and other tospoviruses. GRSV = *Groundnut ringspot virus,* TCSV = *Tomato chlorotic spot virus,* WSMoV = *Watermelon silver mottle virus,* CaCV = Capsicum chlorosis virus, WBNV = Watermelon bud necrosis virus, CCSV = Calla lily chlorotic spot virus

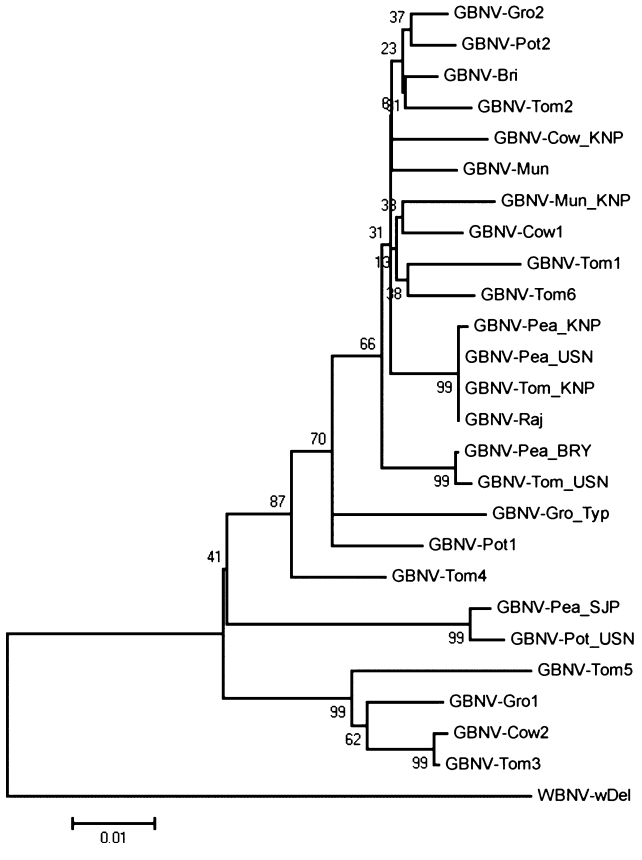

Fig. 3 Phylogenetic relationship between *Groundnut bud necrosis virus* isolates. Abbreviations and GenBank accession numbers: *Pea-BRY(JF281105), *Pea-KNP(JF281106), *Pea-USN(JF281107), *Pea-SJP(JF281108), *Mung-KNP(JN662495), *Cow-KNP(JN662494), *Raj(HQ259244), Cow[1](EU825611), Cow[2](AY221023), Mun (AY871097), Gro[1](AY259524), Gro[2](EU825612), Typ(NC_003620), *Pot-USN(JN575637), *Tom-USN(JN662498), *Tom-KNP (JN662497), Bri(FJ447356), Pot[1](AY259522), Pot[2](AY221024), Tom[1] (FJ705229), Tom[2](FJ447360), Tom[3](AY259523), Tom[4](AY817497), Tom[5](AY817496), Tom[6](AY817495), WBNV(GU474545). *This study

isolates were designated as GBNV-[Pea-BRY], GBNV-[Pea-KNP], GBNV-[Pea-USN] and GBNV-[Pea-SJP], GBNV-[Cow-KNP], GBNV-[Mung-KNP], GBNV-[Pot-USN], GBNV-[Raj], GBNV-[Tom-KNP] and GBNV-[Tom-USN] and their *NSm* gene sequences deposited in the NCBI database. *NSm* gene sequences of GBNV isolates in this study had 0–8% diversity at the nucleotide level, whereas other isolates had 2–9% diversity. Comparison of all the sequences of *NSm* gene of GBNV isolates had nucleotide diversity in the range of 0–10%. The diversity in the *N* gene of 76 GBNV isolates was 0–8.7% (Kunkalikar et al. 2011), indicating that the *N* gene is more conserved than the *NSm* gene. The amino acids sequences of the *NSm* gene of GBNV isolates in this study differed by 0–3%, whereas with other isolates, the difference was 1–3%. All the GBNV isolates reported so far (including ten isolates of this study) had diversity in amino acids sequences in the range of 0–4%.

Comparison of the amino acid sequence of the *NSm* gene of ten isolates with that of the type isolate, GBNV-[Gro-Typ], revealed that the test isolates differed from the latter in having different amino acids at 14 positions. Most of the variations were present in amino acids between 270–300 positions at the C-terminus. However, a significant variation in amino acids was observed in the N-terminus at position no. 4 that was occupied by phenylalanine in the type isolate and five other isolates, whereas in other isolates, the position was occupied by leucine except GBNV-[Bri] in which it was methionine. Both the conserved 'D-motif' and 'G-residue' of '30 K superfamily' (Melcher 2000) of virus movement protein were present not only in all the GBNV isolates but also in other tospoviruses of the *Watermelon silver mottle virus* (WSMoV) sero-group. 'P/D-L-X-motif' known to be present in *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), *Tomato spotted wilt virus*, Chrysanthemum stem necrosis virus, and *Impatiens necrotic spot virus* and a homologous region to the phospholipase A2(PLA2) catalytic site observed in TCSV and GRSV (Silva et al. 2001) were absent from all GBNV isolates including other WSMoV sero-group members (Fig. 2).

The phylogenetic relationship of all the GBNV isolates (Fig. 3) based on amino acids of the *NSm* gene showed segregation into three clusters. The *NSm* protein of tospoviruses is known to play an important role in cell-to-cell movement (Mumford et al. 1996) of the virus and is thought to be involved in host range determination (Silva et al. 2001). Two distinct subgroups in the TSWV populations occurring in southern Italy were identified based on a fragment of *NSm* gene sequence analysis (Finetti-Sialer et al. 2002). Our study of the *NSm* gene of 25 isolates indicates the possible existence of variants or strains in the GBNV populations. This is in agreement with the conclusion drawn from the analysis of the *N* gene sequences of GBNV (Kunkalikar et al. 2011). Studies using the full sequence of the M RNA or of all the three RNAs of GBNV genome may further elucidate the existence of variants or strains. Detailed information on the biological properties of the isolates in the subclusters need to be investigated and compared to settle unequivocally the possible existence of variants or strain.

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