Phytopathologia Mediterranea (2013) 52, 3, 532–540

NEW OR UNUSUAL DISEASE REPORT

First report of *Groundnut bud necrosis virus* infecting wild species of *Vigna*, based on *NP* gene sequence characteristics

Mohammad AKRAM[1,*], NAIMUDDIN[1], Aditya PRATAP[2], Nupur MALVIYA[2] and Prashant YADAV[1]

[1] Division of Crop Protection, Indian Institute of Pulses Research, Kanpur 208024, India
[2] Division of Crop Improvement, Indian Institute of Pulses Research, Kanpur 208024, India

Summary. Symptoms such as chlorotic and necrotic spots on leaves, and necrosis of the stems and petioles, were observed in twelve accessions of wild species/sub-species of *Vigna* (*V. umbellata, V. glabrescens, V. hainiana, V. mungo* var. *mungo, V. radiata* var. *radiata* and *V. radiata* var. *sublobata*) grown at the Indian Institute of Pulses Research, Kanpur, India during August-November 2011. Incidence of symptomatic plants ranged between 7–40%. Based on symptoms appearing on diagnostic host cowpea (*Vigna unguiculata* cv. Pusa Komal) after mechanical inoculation, RT-PCR and nucleocapsid protein (*NP*) gene sequence characteristics, *Groundnut bud necrosis virus* (GBNV) was identified as the causal agent. Analysis of the *NP* gene from intra-field (within a field) isolates of GBNV revealed 1–4% diversity in nucleotide and 0–4% in amino acids. However, diversity among intra-field isolates and isolates from cultivated *Vigna* ranged from 2–7% at nucleotide level and 0–6% at amino acids level. This is the first report of GBNV infection in these wild species/sub-species of *Vigna*, and analysis of intra-field diversity in the *NP* gene of 12 GBNV isolates is also outlined.

Key words: tospovirus, cowpea.

Introduction

Cultivated legumes are known to have very narrow genetic bases. Many wild relatives are used in breeding programmes to create variability in different plant characters and to exploit them in developing new plant types for wider adaptability and higher productivity. The importance of wild relatives in crop improvement for developing new genotypes has been reviewed (Singh, 1994; Hajjar and Hodgkin, 2007). Since accessions of wild *Vigna* spp. are reported to vary in their response to particular diseases (Bisht *et al.*, 2005), use of wild accessions of *Vigna* spp. in breeding for disease resistance needs careful assessment against the target diseases. This is especially the case for diseases caused by viruses, as the symptoms caused by two distinct viruses may often appear similar.

Tospoviruses (family: Bunyaviridae) are known to be one of the most important genera infecting a number of economically important crops. To date, five tospovirus species have been identified in India, of which *Groundnut bud necrosis virus* (GBNV) is the most prevalent. This virus has a wide host range and has emerged as an important pathogen of many crops including groundnut, mungbean, urdbean, soybean, cowpea, potato, tomato, and chilli (Sataynarayana *et al.*, 1996; Bhat *et al*., 2001; Varma *et al*., 2002; Jain *et al.*, 2002; Thien *et al.*, 2003; Akram *et al*., 2004, Jain *et al.*, 2007; Saritha and Jain 2007), not only in India but also in other Asian countries including Iran (Golnaraghi *et al.*, 2002), Bangladesh, Pakistan, and Srilanka (reviewed by Mandal *et al.*, 2012). Recently, GBNV has been reported on pea (Akram and Naimuddin 2010, 2012), rajmash (Akram and Naimuddin, 2012) and jute (Sivaprasad *et al.*, 2011). GBNV is known to cause

Corresponding author: M. Akram
Fax: +91 512 2572582
E-mail: akram23859@gmail.com

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

an estimated annual loss of US$89 million in Asia (Reddy *et al*., 1995). In India, GBNV causes 70–90% loss of groundnut (Singh and Srivatava, 1995). Outbreaks of disease caused by GBNV in different tomato growing regions in Maharashtra, Karnataka and Andhra Pradesh in India are common with up to 100% disease incidence (Kunkalikar *el al.,* 2011). GBNV has also emerged as a serious pathogen of potato in which it causes stem necrosis that is often confused with late blight (Pundhir *et al.,* 2012) causing crop losses up to 29% (Singh *et al*., 1997).

The nucleocapsid protein (*NP*) gene and its deduced amino acid sequence have been used to establish identity of tospoviruses and phylogenetic relationships (de Avila *et al.,* 1993; Goldbach and Peters 1996). Genetic diversity in GBNV isolates infecting different crops in different regions of India has been identified based on the *NP* gene (Kunkalikar *et al.,* 2011). However, genetic diversity in the virus population present within a field (intra-field) has not been investigated. Symptoms typical of tospovirus infection were observed in different wild accessions of *Vigna* growing in an experimental field of the Indian Institute of Pulses Research (IIPR), Kanpur. The present study deals with the identification of the virus causal agent of the disease that occurred in different wild accessions of *Vigna* species and sub-species in a field, and analyzed intra-field diversity of the virus.

Materials and methods

Disease status and mechanical inoculation

Disease incidence was recorded in 43 accessions of the wild species and sub-species of *Vigna* grown at the Main Research Farm of the IIPR during 2011. Per cent disease incidence was determined for each accession from total numbers of plants and infected plants recorded for each accession (Table 2).

Symptomatic leaves (2–3) from each accession were collected, separately macerated in chilled 0.1M phosphate buffer containing 0.1% beta mercaptoethanol. The extract was applied onto celite dusted healthy plants of respective accessions and cowpea (*Vigna unguiculata* cv. Pusa Komal) at the primary leaf stage, as described by Noordam (1973). All plants were maintained in an insect-proof greenhouse where temperature ranged 25–32°C. Infected leaves randomly selected from three plants of each accession were used as the source of inoculum.

Total RNA isolation

RNA was isolated from two field-infected leaf samples of each accession collected randomly, and one sample each from artificially inoculated plants of same accessions and cowpea. RNA from corresponding healthy samples was also extracted and used as controls. Total RNA extracted from the above samples using RNAsure Plant Mini Kit (Genetix, New Delhi, India) according to manufacturer's instructions was used as a template in the reverse transcription-polymerase chain reaction (RT-PCR).

RT-PCR for *NP* gene amplification

The primers pair HRP 26 (5′ ATG TCT AAC GT(C/T) AAG CA(A/G) CTC 3′ and HRP 28 (5′ TAC AAT TCC AGC GAA GGA CC 3′) (Jain *et al.,* 2007) was used for amplification of the *NP* gene of GBNV using the TITANIUM One Step RT-PCR kit (Clontech Laboratories Inc., Mountain View, CA, USA) following manufacturer's instructions. Amplification was performed in an automated Thermocycler (Biometra, Germany) programmed for one cycle of 50°C for 30 min for cDNA synthesis, 5 min as initial denaturation at 94°C and 35 cycles involving 30 s of denaturation at 94°C, 1 min annealing at 62°C, 1 min for extension at 68°C followed by one cycle of final extension for 10 min at 68°C. RT-PCR amplified products were subjected to electrophoresis in 1% agarose gel at 60V for 1h and analyzed by staining with ethidium bromide. The cDNA fragments corresponding to the *NP* gene (~800bp) were excised and purified using HiYield™ Gel/PCR DNA Mini Kit (RBC, Banqiao City, Taipei County 220, Taiwan). One RT-PCR positive amplicon from each accession was taken and cloned.

Cloning and sequence analysis

The purified product of the *NP* gene was ligated into RBC T & A Cloning Vector (Real Biotech Corporation, Banqiao City, Taipei County 220, Taiwan,) following manufacturer's instructions. The recombinant vector was cloned in *E. coli* (DH5 alpha). The positive clones were confirmed by colony-PCR. The recombinant plasmid DNA was extracted by GenJET Plasmid Miniprep Kit (Fermentas Life Sciences, New Delhi, India) and the insert was sequenced commercially by 1st BASE (Malaysia).

The nucleotides sequence of the *NP* gene of GBNV isolates was extracted from the sequence data

and the complete *NP* gene of each GBNV isolate was submitted to NCBI database (Table 1). The *NP* gene sequences of GBNV isolates from wild *Vigna* species and sub-species were compared with *NP* gene sequences of the GBNV isolates from the *Vigna* species (Table 1) available in the database. Multiple sequence alignment was conducted by CLUSTALW (http://www.genome.jp/tools/clustalw/) to obtain nucleotide and amino acid identities. The phylogram was constructed using Neighbor-Joining method (Saitou and Nei, 1987) with bootstrapping (1000 replicates) (Felsenstein, 1985). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000). Evolutionary analyses were conducted using MEGA5 software (Tamura *et al.*, 2011).

Results

Disease status and symptomatology

During the season of 2011, 12 of the 43 wild accessions of *Vigna* growing at the IIPR, exhibited chlorotic spots surrounded by irregular brown necrotic margins on leaves, and necrosis of petioles and stems (Figure 1, Table 2). However, necrosis was more prominent in accessions of *Vigna radiata* var. *radiata* and *Vigna radiata* var. *sublobata* (Figure 1). Disease incidence recorded at 45–55 days after sowing ranged between 7–40% (Table 2). The above described symptoms were similar to those caused by GBNV.

Table 1. GBNV isolates of *Vigna* species obtained in this study, and other isolates used for comparisons.

Accession	Isolate designation	Host	Place of origin	Submitted at NCBI
*JQ406578	GBNV-[Vmm1-Knp]	*Vigna mungo* var. *mungo*	Uttar Pradesh	2012
*JQ406579	GBNV-[Vmm2-Knp]	*Vigna mungo* var. *mungo*	Uttar Pradesh	2012
*JQ406580	GBNV-[Vmm3-Knp]	*Vigna mungo* var. *mungo*	Uttar Pradesh	2012
*JQ406581	GBNV-[Vmm4-Knp]	*Vigna mungo* var. *mungo*	Uttar Pradesh	2012
*JQ406582	GBNV-[Vum1-Knp]	*Vigna umbellata*	Uttar Pradesh	2012
*JQ406583	GBNV-[Vum2-Knp]	*Vigna umbellata*	Uttar Pradesh	2012
*JQ406584	GBNV-[Vum3-Knp]	*Vigna umbellata*	Uttar Pradesh	2012
*JQ347266	GBNV-[Vrr1-Knp]	*Vigna radiata* var. *radiata*	Uttar Pradesh	2012
*JQ347265	GBNV-[Vrr2-Knp]	*Vigna radiata* var. *radiata*	Uttar Pradesh	2012
*JQ406577	GBNV-[Vha-Knp]	*Vigna hainiana*	Uttar Pradesh	2012
*JQ406576	GBNV-[Vgl-Knp]	*Vigna glabrescens*	Uttar Pradesh	2012
*JQ347264	GBNV-[Vrs-Knp]	*Vigna radiata* var. *sublobata*	Uttar Pradesh	2012
JN662493	GBNV-[Mung-KNP]	*Vigna radiata*	Uttar Pradesh	2011
AY871098	GBNV-[mungbean]	*Vigna radiata*	New Delhi	2005
HQ912024	PBNV-[Mb-Nd.1]	*Vigna radiata* var. *radiata*	New Delhi	2010
HQ324114	PBNV-[BG]	*Vigna mungo*	Andhra Pradesh	2010
JF968415	PBNV-[Cowpea-Nellore]	*Vigna unguiculata*	Andhra Pradesh	2011
FJ749262	PBNV-[AP-gg]	*Vigna radiata*	Andhra Pradesh	2009
FJ749261	PBNV-[AP-bg]	*Vigna mungo*	Andhra Pradesh	2009
DQ058078	PBNV-[Coimbatore]	*Vigna unguiculata*	Tamil Nadu	2005

*This study.

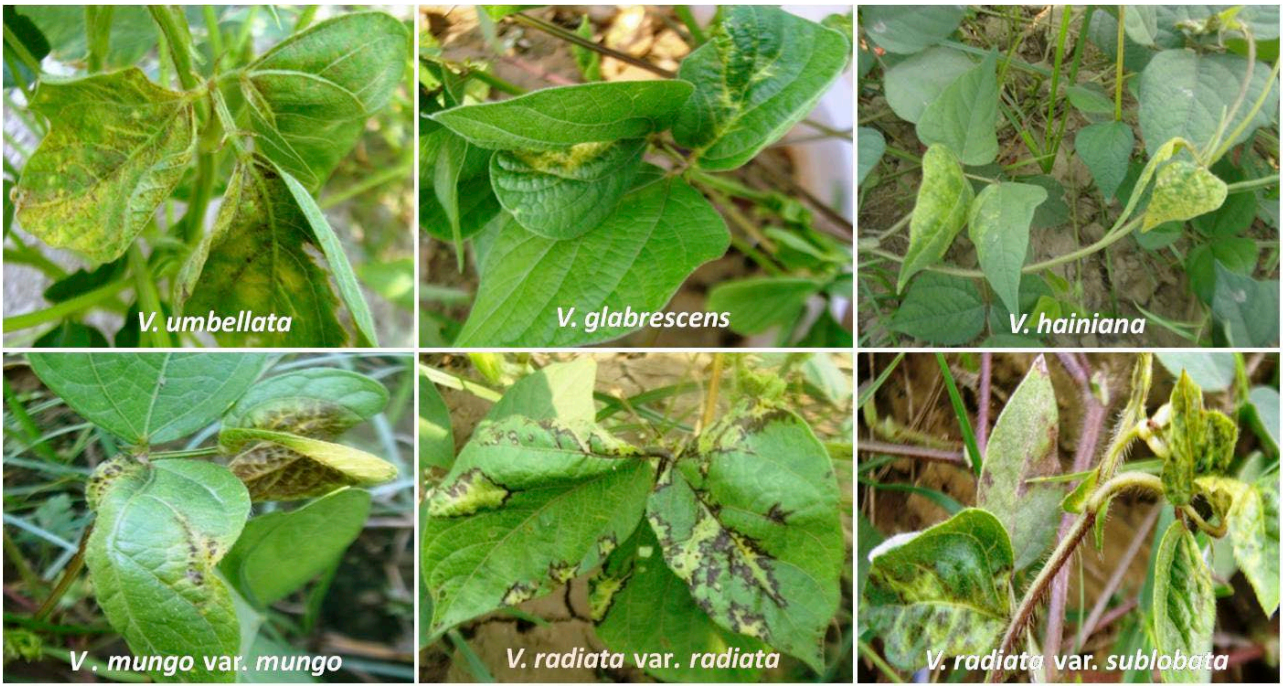

Figure 1. Symptoms of GBNV on naturally infected *Vigna umbellata, Vigna hainiana, Vigna glabrescens, Vigna mungo* var. *mungo, Vigna radiata* var. *radiata* and *Vigna radiata* var. *sublobata*.

Transmission of the causal virus

The virus was easily sap-transmitted from field-infected plants to healthy plants of the same accession causing similar symptoms. Sap inoculation of cowpea cv. Pusa Komal from field-infected leaves of wild accessions also produced characteristic necrotic local lesions on the inoculated primary leaves followed by systemic infection.

RT-PCR amplification and cloning of *NP* genes

The RT-PCR amplicon obtained, of about 800bp, corresponded to the *NP* gene of GBNV. All of the samples collected from field-infected and sap-inoculated plants of *Vigna* wild accessions and cowpea gave positive reactions in RT-PCR, confirming the presence of GBNV. No amplification was observed in healthy samples of respective accessions and cowpea. Amplicons were successfully cloned in the RBC T&A cloning vector and sequenced. Sequences of the *NP* gene of 12 isolates were submitted to the GenBank (Table 1).

Nucleocapsid protein gene of GBNV

The *NP* gene of all of the 12 GBNV isolates under study showed 1–4% diversity at nucleotide level and 0–4% at amino acid level. Compared with other isolates (from cultivated *Vigna*), isolates obtained here differed 2–7% at nucleotide level and 0–6% at amino acid level (Table 3).

At the nucleotide level, test isolates GBNV-[Vmm1-Knp], GBNV-[Vmm2-Knp], and GBNV-[Vrs-Knp] had 2–4% diversity, whereas test isolates GBNV-[Vmm3-Knp], GBNV-[Vmm4-Knp], GBNV-[Vum3-Knp], GBNV-[Vrr1-Knp], GBNV-[Vrr2-Knp] and GBNV-[Vha-Knp] had 1–3% diversity, and GBNV-[Vum1-Knp], GBNV-[Vum2-Knp] and GBNV-[Vgl-Knp] had only 1–2% diversity with other test isolates. Tests isolates GBNV-[Vmm2-Knp], GBNV-[Vmm3-Knp], GBNV-[Vrr2-Knp] and GBNV-[Vrs-Knp] had 3–7% diversity, isolates GBNV-[Vmm1-Knp], GBNV-[Vum1-Knp], GBNV-[Vum2-Knp], GBNV-[Vum3-Knp], GBNV-[Vrr1-Knp] and GBNV-[Vgl-Knp] had 2–6% diversity and isolates GBNV-[Vmm4-Knp], GBNV-[Vum3-Knp] and GBNV-[Vha-

Table 2. Wild *Vigna* accessions, disease incidence, symptoms and RT-PCR detection of GBNV.

Wild *Vigna* species/sub-species	Accession No.	Disease Incidence (%)[a]	Symptoms[b]	Results of sap inoculation	RT-PCR results
Vigna umbellata	IC-251442	14	CS, NS, VN, TLm	+	+
Vigna umbellata	IC-251447	7	CS, NS, VN, TLm	+	+
Vigna umbellata	RB-5-1	28	CS, NS, VN, TLm	+	+
Vigna hainiana	IC-251376	15	CS	+	+
Vigna glabrescens	IC-251372	19	CS, TLm	+	+
Vigna mungo var. *mungo*	IC-251383	23	CS, NS, VN, TLm	+	+
Vigna mungo var. *mungo*	IC-251385	23	CS, NS, VN, TLm	+	+
Vigna mungo var. *mungo*	IC-251387	40	CS, NS, VN, TLm	+	+
Vigna mungo var. *mungo*	IC-251390	37	CS, NS, VN, TLm	+	+
Vigna radiata var. *radiata*	IC-251424	33	CS-inb	+	+
Vigna radiata var. *radiata*	IC-251431	27	CS-inb	+	+
Vigna radiata var. *sublobata*	IC-247406	24	CS-inb, LD, NP, NS	+	+
[#]Thirty one accessions	[#]	0	-	-	-

[a] average of two replications
[b] CS = chlorotic spot, NS = necrotis spot, VN = veinal necrosis, TLm = twisting of leaf lamina, CS-inb = chlorotic spots with irregular necrotic border, LD = leaf distortion, NP = necrosis in petiole, NS = necrosis in stem.
[#] [*Vigna umbellata* (PRR-2007-2, PRR-2008-2, IC-251439, IC-251447), *Vigna hainiana* (IC-251381, IC-331448, IC-331450), *Vigna mungo* var. *mungo* (IC-251386), *Vigna radiata* var. *radiata* (IC-251425, IC-251426, IC-251427), *Vigna radiata* var. *sublobata* (IC-251416, IC-253920), *Vigna radiata* (JAM/09-29), *Vigna trilobata* (JAP/10-5, JAP/10-9, IC-349701, IC-276983, IC-331436, IC-331454, IC-331456), *Vigna trineria* (JAP/10-51), *Vigna radiata* var. *setulosa* (IC-251419, IC-251423), *Vigna pilosa* (IC-210580), *Vigna dalzelliana* (IC-349904, IC-331463), *Vigna vexilata* (IC-277007), *Vigna mungo* var. *silvestris* (IC-277014, IC-277021, IC-277031)].

Knp] had 3–6% diversity from the other GBNV isolates at nucleotide level.

At the amino acid level, test isolates GBNV-[Vmm3-Knp] and GBNV-[Vum2-Knp] differed least (0–2%) from other test isolates, whereas test isolates GBNV-[Vrr1-Knp] and GBNV-[Vha-Knp] had maximum diversity (2–4%) from other test isolates. Test isolates GBNV-[Vrr1-Knp] and GBNV-[Vha-Knp] differed most (2–6%) and GBNV-[Vmm3-Knp] and GBNV-[Vum2-Knp] differed least (0–4%) from other GBNV isolates from cultivated *Vigna* (Table 3).

Comparison of amino acid sequences of the *NP* gene of test isolates revealed variation at 22 positions. Most striking variation was observed in GBNV-[Vmm1-Knp] which differed from all isolates in having arginine instead of lysine at position number 196 (Figure 2). Similarly, at position number 183 this isolate had valine instead of leucine or serine in the other isolates. Position number 214 was occupied by glutamate in all the isolates except GBNV-[Vmm1-Knp] and GBNV-[Vum3-Knp], in which this position was held by valine. Another difference in amino acids was seen in isolate GBNV-[Vha-Knp] which had lysine, serine, serine and lysine in positions 3, 4, 5 and 6, respectively, instead of asparagines, valine, lysine and glutamine in other isolates. In phylogenetic analysis involving 20 isolates of GBNV including 12 isolates from the present study, two clear clusters formed. Isolate PNBV-[Coimbatore] formed a separate cluster, whereas all the other isolates grouped into one cluster (Figure 3).

Discussion

Field symptoms on wild species/sub-species of *Vigna* resembled those associated with GBNV infection in cultivated *Vigna* species (Bhat *et al.*, 2001), and RT-PCR with primers specific to the *NP* gene of GBNV

Table 3. Similarity of the *NP* gene sequences of GBNV isolates (above diagonal, nucleotide similarity; below diagonal, amino acid similarity).

GBNV isolates [a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	-	96	97	97	98	98	97	98	97	97	98	96	97	98	97	97	98	97	97	94
2	98	-	97	98	98	98	97	98	97	97	98	97	96	97	97	96	96	97	97	93
3	98	98	-	98	98	98	99	98	99	99	98	97	96	96	97	96	96	97	97	93
4	98	98	99	-	99	99	98	98	98	98	99	98	97	97	97	97	97	97	97	94
5	98	100	98	98	-	99	98	98	98	98	99	98	97	97	97	97	97	98	98	94
6	98	98	100	99	98	-	99	99	98	98	99	98	97	97	98	97	98	98	98	94
7	98	98	99	98	98	99	-	98	99	99	98	97	96	96	97	96	97	97	97	94
8	97	97	98	97	97	98	97	-	98	97	98	97	97	98	97	97	97	97	97	94
9	98	98	99	98	98	99	98	97	-	99	98	97	96	96	97	96	96	97	97	93
10	97	97	98	97	97	98	97	96	97	-	98	97	96	96	97	96	96	97	97	94
11	98	98	99	98	98	99	98	97	98	97	-	98	97	97	98	97	97	98	98	94
12	98	98	99	98	98	99	98	97	98	97	98	-	96	96	96	96	96	97	97	93
13	98	98	100	99	98	100	99	98	99	98	99	99	-	97	97	96	96	96	96	93
14	99	98	99	98	98	99	99	97	98	98	98	98	99	-	97	97	97	97	97	94
15	98	98	99	98	98	99	98	97	98	98	98	98	99	99	-	97	97	97	97	94
16	98	98	99	98	98	99	98	97	98	98	98	99	99	99	99	-	98	97	97	94
17	98	98	99	98	98	99	98	97	98	98	98	98	99	99	100	99	-	97	97	94
18	98	98	99	98	98	99	98	97	98	97	100	98	99	98	98	98	98	-	99	94
19	98	98	100	99	98	100	99	98	99	98	99	99	100	99	99	99	99	99	-	94
20	95	95	96	95	95	96	95	94	95	94	95	95	96	96	96	96	96	95	96	-

[a] 1, GBNV-[Vmm1-Knp]; 2, GBNV-[Vmm2-Knp]; 3, GBNV-[Vmm3-Knp]; 4, GBNV-[Vmm4-Knp]; 5, GBNV-[Vum1-Knp]; 6, GBNV-[Vum2-Knp]; 7, GBNV-[Vum3-Knp]; 8, GBNV-[Vrr1-Knp]; 9, GBNV-[Vrr2-Knp]; 10, GBNV-[Vha-Knp]; 11, GBNV-[Vgl-Knp]; 12, GBNV-[Vrs-Knp]; 13, GBNV-[Mung-KNP]; 14, GBNV-[mungbean]; 15, PBNV-[Mb-Nd.1]; 16, PBNV-[BG]; 17, PBNV-[Cowpea-Nellore]; 18, PBNV-[AP-gg]; 19, PBNV-[AP-bg]; 20, PBNV-[Coimbatore].

identified GBNV as the causal virus agent. Diagnosis was further strengthened by inoculating cowpea (*Vigna unguiculata* cv. Pusa Komal), a diagnostic host of GBNV, which reacted with typical symptoms of GBNV infection including necrotic local lesions followed by systemic infection. In one of our studies, five species of thrips *viz*., *Caliothrips indicus*, *Megalurothrips usitatus, Scirtothrips dorsalis, Thrips palmi* and *Plicothrips apicalis*, were identified associated with GBNV infected plants of *Vigna* spp. Among them, *T. palmi* and *S. dorsalis* were found to transmit GBNV (unpublished).

All the twelve GBNV isolates examined in this study had among them only 0–4% diversity at amino acid and 1–4% variation at the nucleotide level. At the amino acid level, 0–6% diversity has been reported in an earlier study involving 26 isolates of GBNV from fabaceous hosts from different geographical locations of India (Akram and Naimuddin 2012). Comparison of amino acid sequences of 76 GBNV isolates infecting vegetable crops in different parts of India showed 0–9% diversity among them (Kunkalikar *et al.* 2011). In a more recent review by Mandal *et al.* (2012), comparison of amino acid sequences of more than 100 GBNV isolates originating from different hosts and locations in India revealed diversity of up to 8%. All these values of divergence

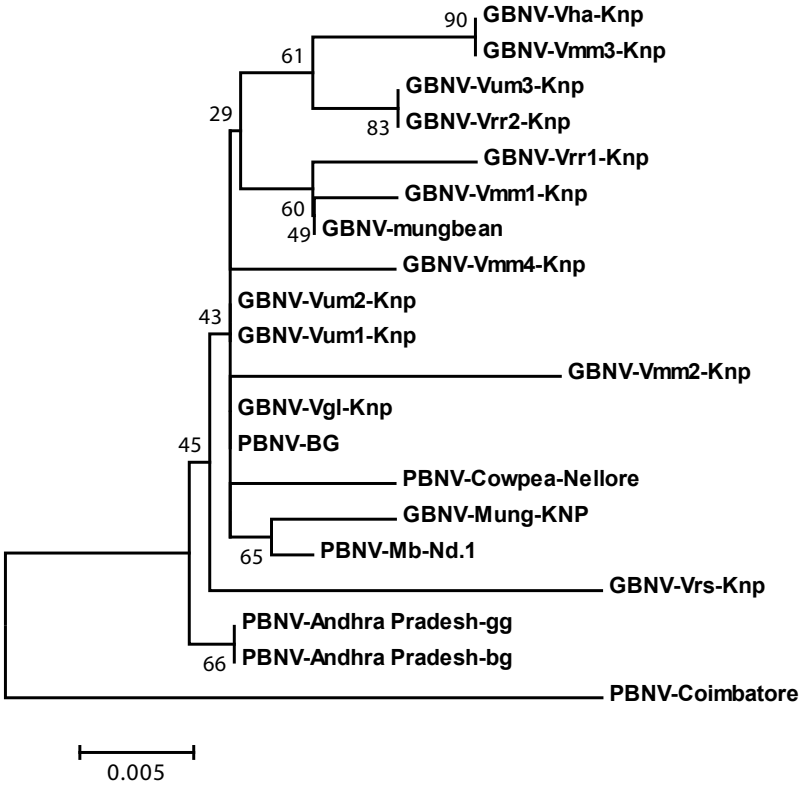

Figure 2. Phylogeny was inferred using Neighbor-Joining method of the GBNV isolates obtained in this study from wild *Vigna* species and sub-species and the GBNV isolates from *Vigna* species already available. The optimal tree with the sum of branch length = 0.12182971 is shown. The evolutionary distances were computed using the p-distance method and are in the units of the number of amino acid differences per site. The analysis involved 20 nucleotide sequences. Evolutionary analyses were conducted in MEGA5. Details of the isolates are given in Table 1.

in amino acid sequences do not extend beyond the species demarcation criterion for members of *Tospovirus* (Nichol *et al.*, 2005). It isconcluded, therefore, that the diversity in amino acid sequence of the *NP* gene of the test isolates collected from one field (intra-field) is very low (0–4%), but a similar low level of diversity is also found between inter-field isolates (Kunkalikar *et al.*, 2011).

The importance of wild relatives of *Vigna* in improving cultivated varieties of *V. radiata* and *V. mungo* has been elaborated by Kumar *et al.* (2011). Their susceptibility to GBNV needs to be taken into consideration before using wild *Vigna* spp. in breeding programmes. The present study is the first demonstration of GBNV infection in the twelve accessions of wild *Vigna* species and sub-species (*V. umbellata, V. glabrescens, V. hainiana, V. mungo* var. *mungo, V. radiata* var. *radiata* and *V. radiata* var. *sublobata*).

Acknowledgements

We thank Dr N. Nadarajan for his constant support and encouragement. Financial assistance to the senior author under the Lal Bahadur Shastri Young Scientist Award Scheme of the Indian Council of Agricultural Research, New Delhi, is gratefully acknowledged.

Literature cited

Akram M., R.K. Jain, V. Chaudhary, Y.S. Ahlawat and S.M.P. Khurana, 2004. Comparison of Groundnut bud necrosis virus isolates based on movement protein (NSm) gene sequences. *Annals of Applied Biology* 145, 285–289.

Akram M. and K. Naimuddin, 2010. First report of natural infection of *Vigna mungo* var. *silvestris* L. by *Groundnut bud necrosis virus*, a tospovirus. *Phytopathologia Mediterrranea* 49, 249–252.

Akram M. and K. Naimuddin, 2012. Biological characteriza-

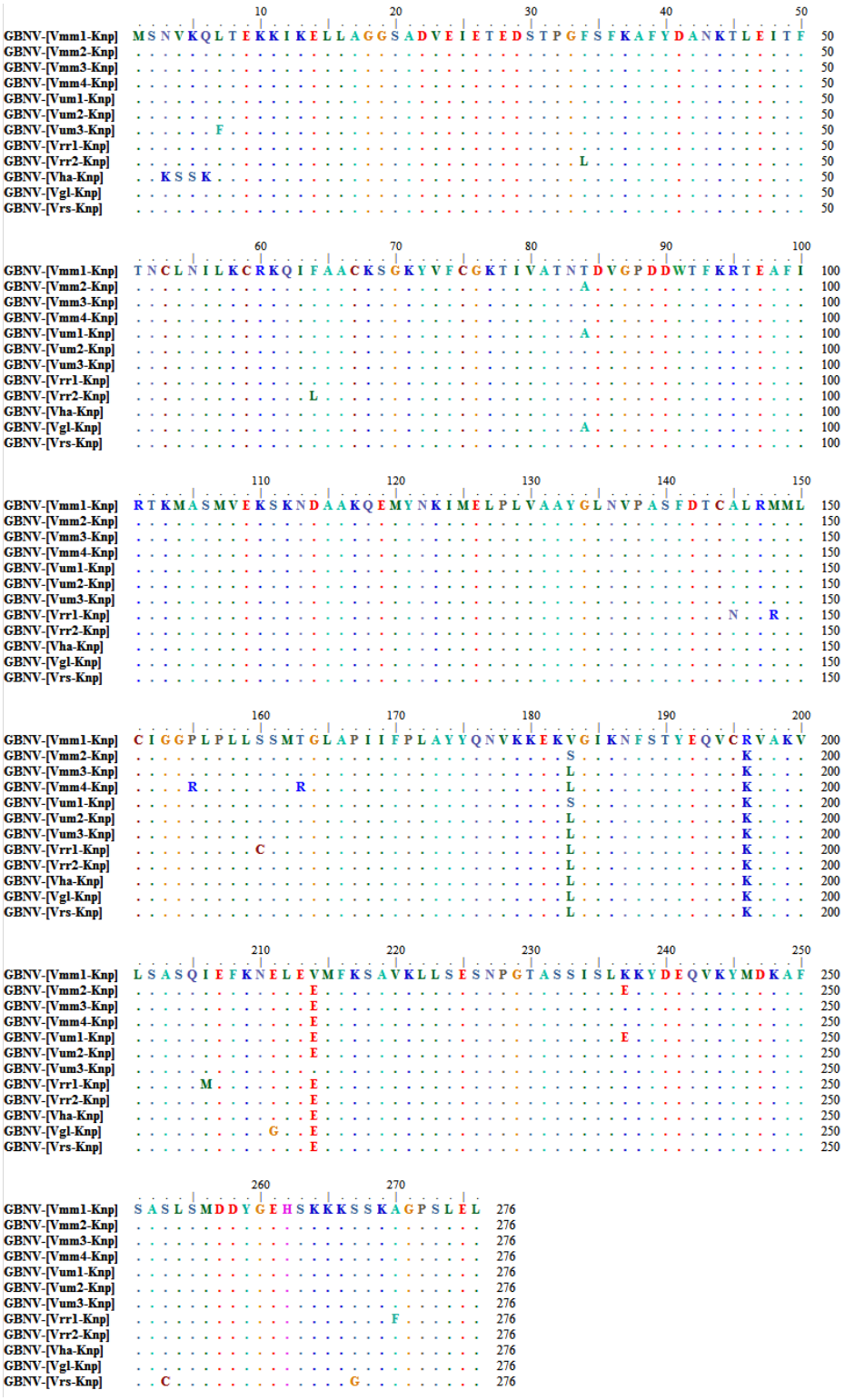

Figure 3. Multiple alignments of *NP* gene sequences of GBNV isolates collected from one field. Dot indicates identical amino acids.

tion and variability of the nucleocapsid protein gene of *Groundnut bud necrosis virus* isolates infecting pea from India. *Phytopathologia Mediterranea* 51, 266–275.

Bhat A.I., R.K. Jain, A. Varma, Naresh Chandra and S.K. Lal, 2001. Tospovirus(es) infecting grain legumes in Delhi, their identification by serology and nucleic acid hybridization. *Indian Phytopathology* 54**,** 112–116.

Bisht I.S., K.V. Bhat, S. Lakhanpaul, M. Latha, P.K. Jayan, B.K. Biswas and A.K. Singh, 2005. Diversity and genetic resources of wild Vigna species in India. *Genetic Resources and Crop Evolution* 52, 53–68.

de Avila A.C., P. de Haan, R. Kormelink, R. de O. Resende, R.W. Goldbach and D. Peters, 1993. Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. *Journal of General Virology* 74, 153–159.

Felsenstein J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39, 783–791.

Goldbach R. and D. Peters, 1996. Molecular and Biological Aspects of Tospoviruses. In: *The Bunyaviridae* (R.M. Elliott, ed.), Plenum Press, New York, NY, USA, 129–157.

Golnaraghi A.R., R. Pourrahim, N. Shahraeen and S. Farzadfar, 2002. First report of Groundnut bud necrosis virus in Iran. *Plant Disease* 86, 561.

Hajjar R. and T. Hodgkin, 2007. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica* 156, 1–13.

Jain R.K., K. Umamaheswaran, A.I. Bhat, H.X. Thien and Y.S. Ahlawat, 2002. Necrosis disease on cowpea, mungbean and tomato is caused by *Groundnut bud necrosis virus*. *Indian Phytopathology* 55, 354.

Jain R.K., S. Bag, K. Umamaheshwaran and B. Mandal, 2007. Natural infection by Tospoviruses of cucurbitaceous and fabaceous vegetable crops in India. *Journal of Phytopathology* 155, 22–25.

Kumar S., M. Imtiaz, S. Gupta and A. Pratap, 2011. Distant Hybridization and Alien Gene Introgression. *In*: *Biology and Breeding of Food Legumes* (A. Pratap and J. Kumar, ed.), Oxfordshire, UK CAB, International, 81–110.

Kunkalikar S.R., P. Sudarsana, B.M. Arun, P. Rajagopalan, T.C. Chen, S.D. Yeh, R.A. Naidu, U.B. Zehr and K.S. Ravi, 2011. Importance and genetic diversity of vegetable-infecting tospoviruses in India. *Phytopathology* 101, 367–376.

Mandal B., R.K. Jain, M. Krishnareddy, N.K. Krishna Kumar, K.S. Ravi and H.R. Pappu, 2012. Emerging Problems of Tospoviruses (*Bunyaviridae*) and their Management in the Indian Subcontinent. *Plant Disease* 96, 468–479.

Nei M. and S. Kumar, 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, NY, USA.

Nichol S.T., B.J. Beaty, R.M. Elliott, R. Goldbach, A. Plyusnin, C.S. Schmaljohn and R.B. Tesh, 2005. Family Bunyaviridae. In: *Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses* (C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball, ed.), Elsevier, San Diego, CA, USA, 695–716.

Noordam D., 1973. *Identification of Plant Viruses: Methods & Experiments*. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands, 207 pp.

Pundhir V.S., M. Akram, M. Ansar and H. Rajshekhara, 2012. Occurrence of stem necrosis disease in potato caused by groundnut bud necrosis virus in Uttarakhand. *Potato Journal* 39, 81–83.

Reddy D.V.R., A.A.M.T. Buiel, S.L. Satyanarayana, A.S. Dwivedi, A.S. Reddy, K. Ratna, G.V. Vijaylakshmi, Ranga Rao, R.A. Naidu and J.A. Wightman, 1995. Peanut bud necrosis virus disease: an overview. In: *Recent Studies on Peanut Bud Necrosis Disease* (A.A.M. Buiel, J.E. Parlevliet, J.M. Lenne, ed.). Patancheru, India, 20 March 1995, ICRISAT Asia Centre, 3–7.

Saitou N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406–425.

Saritha R.K. and R.K. Jain, 2007. Nucleotide sequence of the S and M- RNA segments of a groundnut bud necrosis virus isolate from *Vigna radiata*. *Archives of Virology* 152, 1195–1200.

Satayanarayana T., S.E. Mitchell, D.V.R. Reddy, S. Brown, S. Kresovich, R. Jarret, R.A. Naidu, and J.W. Demski, 1996. Peanut bud necrosis tospovirus S RNA: complete nucleotide sequence, genome organization and homology to other Tospoviruses. *Archives of Virology* 141, 85–98.

Singh A.B. and S. K. Srivatava, 1995. Status and control strategy of peanut bud necrosis disease in Uttar Pradesh. In: *Recent studies on peanut bud necrosis disease* (A.A.M. Buiel, J.E. Parevliet and J.M. Lenne, ed.). Patancheru, India, 20 March 1995, ICRISAT Asia Centre, 65–68.

Singh D.P., 1994. Breeding for resistance to diseases in mungbean: Problems and prospects. In: *Recent Advances in Mungbean Research* (A.N. Asthana and D.H. Kim ed.), Indian Society of Pulses Research (IIPR), Kanpur, India, 152–164.

Singh R. B., K.K. Srivastava, S.M.P. Khurana and S.K. Pandey, 1997. Assessment of yield losses due to potato stem necrosis disease. *Indian Journal of Virology,* 3, 135–137.

Sivaprasad Y., B.V.B. Reddy, C.V.M.N. Kumar, K.R. Reddy and D.V.R.S. Gopal, 2011. Jute (*Corchorus capsularis*): a new host of Peanut bud necrosis virus. *New Disease Reports* 23**,** 33.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28, 2731–2739.

Thien H.X., A.I. Bhat and R.K. Jain, 2003. Mungbean necrosis disease caused by a strain of *Groundnut bud necrosis virus*. *Indian Phytopathology* 56, 54–60.

Varma A., R.K. Jain and A.I. Bhat, 2002. Virus resistant transgenic plants for environmentally safe management of viral diseases. *Indian Journal of Biotechnology* 1, 73–86.

Accepted for publication: March 15, 2013