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Research Papers

Biological characterization and variability of the nucleocapsid protein gene of *Groundnut bud necrosis virus* isolates infecting pea from India

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Summary. A disease of pea characterized by browning in veins, leaves and stems, mostly in growing tips, and brown circular spots on pods, was recorded in four districts of Uttar Pradesh, India. The causal agent of this disease was detected by reverse transcription-polymerase chain reaction (RT-PCR) using primers pair HRP 26/HRP 28 and identified as *Groundnut bud necrosis virus* (GBNV) on the basis of nucleocapsid protein (*NP*) gene sequence. Virus isolates from Bareilly (BRY), Kanpur (KNP), Udham Singh Nagar (USN) and Shahjahanpur (SJP) were designated as GBNV-[Pea_BRY], GBNV-[Pea_KNP], GBNV-[Pea_USN] and GBNV-[Pea_SJP] and their *NP* genes sequenced. The sequence data of each isolate were deposited at NCBI database (JF281101-JF281104). The complete nucleotide sequence of the *NP* genes of all the GBNV isolates had a single open reading frame of 831 nucleotides and 276 amino acids. The isolates had among them 2% variability at amino acid level and 2–3 variability at nucleotide level, but had variability with other GBNV isolates of fabaceous hosts in the range of 0–6% at amino acid level and 1–8% at nucleotide level. Though this variation in nucleotide sequences of GBNV isolates from fabaceous hosts is within the limits of species demarcation for tospoviruses, formation of a separate cluster within the GBNV isolates indicates the possibility of distinct variants in GBNV.

Key words: Tospovirus, fabaceous hosts, nucleocapsid protein gene, thrips.

Introduction

The genus Tospovirus of the family Bunyaviridae is composed of 19 species described so far, and of them 14 have been identified from Asia (Pappu *et al*., 2009). Five tospovirus species have been reported from India viz., *Groundnut bud necrosis* virus (Reddy *et al.*, 1992; Sataynarayana *et al.*, 1996a) from groundnut and many other plant species, *Groundnut yellow spot virus* (Satayanarayana *et al.*, 1998) from groundnut, *Watermelon bud necrosis virus* (Jain *et al.*, 1998) from watermelon, *Iris yellow spot virus* (Ravi *et al.*, 2005) from onion, and *Capsicum chlorosis virus* from tomato and chilli pepper (Kunkalikar *et al.*, 2007, 2010; Krishnareddy *et al.*, 2008), chilli (Kunkalikar *et al.*, 2007, 2010; Krishnareddy *et al.*, 2008). GBNV is the most economically important virus affecting a variety of crops such as peanut, potato, tomato, soybean, urdbean, mungbean and cowpea (Akram *et al*., 2004; Jain *et al*., 2007; Pappu *et al.*, 2009). All the tospoviruses are exclusively transmitted by thrips species (Whitefield *et al.*, 2005). The GBNV particles are enveloped and quasi-spherical in shape, 80–100 nm in diameter with tripartite single stranded RNA genomes. The large (L) RNA (8.9 kb) has only one open reading frame (ORF), and codes for RNA dependent RNA polymerase in viral (v) sense (Gowda *et al.*, 1998). The medium (M) RNA (4.8 kb) has two ORFs in an ambisense replication strategy, and encode the nonstructural movement (NSm) protein in v sense and the glycoprotein precursors (G1 and G2) in viral complementary (vc) sense (Satyanarayana *et*

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al., 1996b). The small (S) RNA (3.05 kb) also has two ORFs in an ambisense replication strategy; and encode the nonstructural (NSs) protein in v sense and Nucleocapsid protein (NP) in vc sense (Satyanarayana *et al.*, 1996a).

GBNV is emerging as an important pathogen of leguminous crops like mungbean (*Vigna radiata*), urdbean (*Vigna mungo*) and cowpea (*Vigna unguiculata*), particularly in peninsular India. This virus was recently reported on pea (*Pisum sativum*) at Kanpur (Akram and Naimuddin, 2010). Annual losses in Asia due to GBNV were estimated at over $89 million (Reddy *et al.*, 1995). In India, GBNV has been reported to cause 70–90% losses in peanut (Singh and Srivastava, 1995) and up to 29% in potato (Singh *et al.*, 1997).

During surveys of winter season legumes conducted in the January 2009 and 2010, symptoms of browning of veins, leaves and stems, mostly in growing tips, were observed in pea plants at the Indian Institute of Pulses Research, Kanpur, in farmers' fields in Bareilly and Shahjahanpur districts of Uttar Pradesh and at G. B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar, district Udham Singh Nagar, Uttarakhand, India. Pods on affected plants showed brown circular spots and later became completely brown. Affected plants finally became completely dessicated. The incidence of the disease ranged from 1–10% in different fields. In preliminary studies the causal virus was identified as GBNV. Prior to the present study, no information on the *NP* gene sequence of GBNV isolates infecting pea was available. Information generated on variability in *NP* gene may help to develop pathogen-derived resistance strategies to generate GBNV resistant transgenic plants. The present paper outlines the genetic variability of the *NP* gene of GBNV infecting pea in four different districts of North India as well as of other GBNV isolates reported previously from fabaceous crops.

Materials and methods

Sample collection and disease incidence

During January 2009 and 2010 a total of 35 pea fields were surveyed in Kanpur, Bareilly and Shahjahanpur districts of Uttar Pradesh, and Udham Singh Nagar of Uttarakhand, India and 18 samples of pea plants showing tip necrosis symptoms and suspected to carry GBNV infection were randomly collected among the symptomatic ones.

Mechanical transmission

The top portion of each pea (plant) sample was washed with distilled water, blotter dried and macerated in chilled 0.1M phosphate buffer containing 0.1% β mercaptoethanol. The extract obtained from the macerate was inoculated on to pea and cowpea (*Vigna unguiculata* cv. Pusa Komal) at the primary leaf growth stage, using celite powder as an abrasive. The mechanical sap inoculation procedure was as described by Noordam (1973).

Total RNA isolation

Eighteen field samples of affected leaves of pea and five samples each of artificially inoculated leaves of pea and cowpea were taken for RNA isolation. RNA from corresponding healthy samples was also extracted to be used as negative controls. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturer's instructions and was used as templates in the reverse transcription-polymerase chain reaction (RT-PCR).

RT-PCR for amplification of *NP* genes

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 *NP* gene of GBNV using TITANIUM One Step RT-PCR kit (Clontech Laboratories Inc., Mountain View, CA, USA) according to the manufacturer's instructions. Amplification was performed in an automated Thermocycler (Biometra, Gottingen, Germany) programmed for one cycle of 50ºC for 30 min for cDNA synthesis, 5 min as initial denaturation at 94ºC and 35 cycles involving 30 s of denaturation at 94ºC, 1 min annealing at 62ºC, 1 min for extension at 68ºC, followed by one cycle of final extension for 10 min at 68ºC. RT-PCR amplified products were analyzed by electrophoresis in 1% agarose gel at 60V for 1 h and staining with ethidium bromide. The bands corresponding to the *NP* gene RT-PCR products were excised and purified using Wizar SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA). Of the RT-

PCR positive samples, one from each location was randomly selected and cloned.

Cloning and sequence analysis

The purified product of *NP* genes was ligated into RBC T & A Cloning Vector (Real Biotech Corporation, Banqiao City, Taipei County, Taiwan) as per the manufacturer's instructions. The recombinant vector was cloned in the *E. coli* using the Max Efficiency Competent Cells (Invitrogen, Carlsbad, USA) according to the manufacturer's protocols. The positive clones were confirmed by colony PCR and streaked on LB agar containing ampicillin (50 μg mL^{-1}). Single colonies were selected from overnight grown plates and multiplied in 3 mL LB broth supplemented with ampicillin by and maintaining cultures overnight at 37ºC. The cultures were then used to isolate the plasmid using GenJET Plasmid Minipre Kit (Fermentas Life Sciences, Glen Burnie, Maryland, USA). The isolated plasmid DNA was digested with *Bgl*II restriction enzyme following manufacturer's instructions. The presence of insert was confirmed by agarose gel electrophoresis.

The nucleotide sequences of the *NP* gene of GBNV isolates from pea were determined and submitted to GenBank under accession numbers: JF281101-JF281104. *NP* gene sequences of pea isolates of GBNV were compared with *NP* gene sequences of the GBNV isolates from the fabaceous crops (Table 1) available as at 16.05.2011. Sequence data were edited using Bioedit version 5.0.9 (Hall, 1999). Multiple Sequence Alignment by CLUSTALW (http://www.genome.jp/tools/clustalw/) was conducted to obtain nucleotide and amino acid identities. The phylogram was constructed using Neighbor-Joining method (Saitou and Nei, 1987) with bootstrapping (1,000 replicates) (Felsenstein, 1985). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000). Evolutionary analyses were conducted in MEGA5 software (Tamura *et al.*, 2011).

Results

Symptomatology, disease incidence and transmission of causal virus

Naturally infected pea plants showed browning of leaf veins, leaves and stems, mostly in growing tips (Figure 1a, b and d). Pods showed brown circular spots (Figure 1c) and affected plants finally became dessicated. Details of the symptoms have been given in a previous report (Akram and Naimuddin, 2010). The incidence of the disease varied between 0–10% in 35 pea fields surveyed in four districts.

The causal virus was sap transmitted from field infected plants to healthy plants of pea cv. Azad Pea 1 and cowpea cv. Pusa Komal, generally used as diagnostic hosts for tospoviruses. Of the 18 samples used for sap inoculation, 14 produced symptoms on cowpea and 13 produced symptoms on pea. On inoculated leaves of cowpea, chlorotic as well necrotic local lesions appeared within 3–4 days (Figure 1e). On sap inoculated pea plants, within 1 week, local symptoms appeared as necrotic blotches on the inoculated leaves (Figure 1f) and systemic symptoms manifested in the form of necrosis in petioles and stems, and these often led to the collapse of inoculated plants.

Amplification and sequence analysis of *NP* gene

Out of 18 diseased field samples, 17 gave positive results in RT-PCR. RT-PCR products obtained with the primer pair HRP26/HRP28 in agarose gel electrophoresis revealed presence of amplicons of ~ 800bp corresponding to *NP* genes of GBNV. *NP* gene sequences of four GBNV pea isolates collected from Bareilly (BRY), Kanpur (KNP), Udham Singh Nagar (USN) and Shahjahanpur (SJP), designated as GBNV-[Pea_BRY], GBNV-[Pea_KNP], GBNV-[Pea_USN] and GBNV-[Pea_SJP], were deposited at NCBI database (Table 1). The complete nucleotide sequence of the *NP* genes of all four GBNV isolates had single ORFs of 831 nucleotides and 276 amino acids.

Variability in *NP* genes of GBNV

Nucleotide sequences of isolate GBNV-[Pea_SJP] had least variability (1%) with GBNV-[Gro_Typ]. GBNV-[Pea_USN] had minimum 2% variability with GBNV-[Mung_ND], GBNV-[Cow_Ker] and GBNV-[Hya_Mah], whereas GBNV-[Pea_BRY] also had 2% variability with seven isolates of GBNV. GBNV-[Pea_KNP] had minimum 3% variability with ten isolates of GBNV described from different fabaceous crops. All the four GBNV isolates had maximum variability with GBNV-[Cow_Ker], but GBNV-[Pea_KNP] had 8% variability and the other three isolates had 7% variability. GBNV-[Pea_USN] also had maximum

Table 1. Details of the NP gene sequences used in this study.

S. No.	Accession	Host	Designated for this study	Area/Location	Zone
1	*JF281101	Pea	GBNV-[Pea_BRY]	Uttar Pradesh	North Western plan
2	*JF281102	Pea	GBNV-[Pea_KNP]	Uttar Pradesh	North eastern Plan
3	*JF281104	pea	GBNV-[Pea_SJP]	Uttar Pradesh	North Western plan
4	*JF281103	Pea	GBNV-[Pea_USN]	Uttrakhand	North Western plan
5	AF515819	Cowpea	GBNV-[Cow_Ker]	Kerala	South
6	AY512647	Groundnut	GBNV-[Gro_Ker]	Karnataka	South
7	AY871098	Mungbean	GBNV-[Mung_ND]	New Delhi	North Western plan
8	FJ355952	Groundnut	GBNV-[Gro_ND]	New Delhi	North Western plan
9	AF467289	Soybean	GBNV-[Soy_ND]	New Delhi	North Western plan
10	AY529713	Mungbean	GBNV-[Mung_Mah]	Maharashtra	Central
11	AY882006	Soybean	GBNV-[Soy_Mah]	Maharashtra	Central
12	AY882004	Hyacinth bean	GBNV-[Hya_Mah]	Maharashtra	Central
13	AY512650	Black gram	GBNV-[Bg_AP]	Andhra Pradesh	South
14	EF179099	Cowpea	GBNV-[Cow_AP]	Andhra Pradesh	South
15	EF532937	Sem	GBNV-[Sem_AP]	Andhra Pradesh	South
16	HM770021	Groundnut	GBNV-[Gro_AP^1]	Andhra Pradesh	South
17	HM131489	Groundnut	GBNV-[Gro_AP^2]	Andhra Pradesh	South
18	FJ355950	Groundnut	GBNV-[Gro_AP^3]	Andhra Pradesh	South
19	EF179100	Groundnut	GBNV-[Gro_AP^4]	Andhra Pradesh	South
20	FJ355951	Groundnut	GBNV-[Gro_AP^5]	Andhra Pradesh	South
21	AY426318	Sem	GBNV-[Sem_TN]	Tamil Nadu	South
22	DQ058078	Cowpea	GBNV-[Cow_TN]	Tamil Nadu	South
23	HM770022	Groundnut	GBNV-[Gro_TN^1]	Tamil Nadu	South
24	HM770020	Groundnut	GBNV-[Gro_TN^2]	Tamil Nadu	South
25	NC_003619	Groundnut	GBNV-[Gro_Typ]	-	-
26	AF515818	Mungbean	GBNV-[Mung]	-	-
27	NC_008301	CaCV	CaCV	-	-
28	NC_003843	WSMoV	WSMoV	-	-
29	AF045067	WBNV	WBNV	-	-
30	AY867502	CCSV	CCSV	-	-

*This study.

7% variability with GBNV-[Sem_TN]. A comparison of nucleotide sequences of the four isolates of GBNV with other tospoviruses of *Watermelon silver mottle virus* (WSMoV) serogroup revealed 20–37% variability (Table 2).

The GBNV isolates in this study had among them 2% variability at the amino acid level and 2–3% variability at the nucleotide level. The amino acid sequence of isolate GBNV-[Pea_SJP] was identical to GBNV-[Gro_AP^4], GBNV-[Gro_AP^2] and GB-

NV-[Gro_Typ] and had maximum difference (5%) with GBNV-[Gro_ND]. The amino acid sequence of GBNV-[Pea_USN], GBNV-[Pea_KNP] and GBNV-[Pea_BRY] isolates had minimum (1%) variability with GBNV-[Mung-Mah], GBNV-[Cow_TN[3]] and GBNV-[Soy_Mah]; whereas they had maximum (6%) variability with GBNV-[Gro_ND]. GBNV-[Pea_KNP] isolate also had maximum (6%) variability with GBNV-[Gro_TN[2]]. The GBNV isolates under study had 16–36 % variability in amino acids with other Tospoviruses of WSMoVserogroup (Table 2).

The phylogentic relationship of all the GBNV isolates from fabaceous hosts available in GenBank (including four from the present study), and tospoviruses of the same serogroup were studied based on amino acids sequences of the *NP* gene. The results (Figure 2) showed segregation of all the GBNV isolates into two clusters. Cluster I had most of the GBNV isolates including all the four isolates of the present study, whereas the cluster II consisted of only three GBNV isolates (GBNV-[Gro_ND], GBNV-[Sem_AP] and GBNV-[Gro_TN[2]]). The other tospoviruses, *Capsicum chlorosis virus* (CaCV), WSMoV, *Watermelon bud necrosis virus* (WBNV) and *Calla lily chlorotic spot virus* (CCSV) of the same serogroup formed separate clusters.

Multiple alignment of amino acid sequence of NP gene of four isolates understudy with that of GBNV-[TI] a type isolate (Figure 3) revealed that GBNV-[Pea_SJP] was identical to the type isolate whereas amino acids in rest three isolates differed from GBNV-type isolate at 6 positions.

Discussion

The cause of spotted wilt disease of pea in India was assigned to *Tomato spotted wilt virus* (TSWV) in 1985 (Prasada Rao *et al*., 1985). However, presence

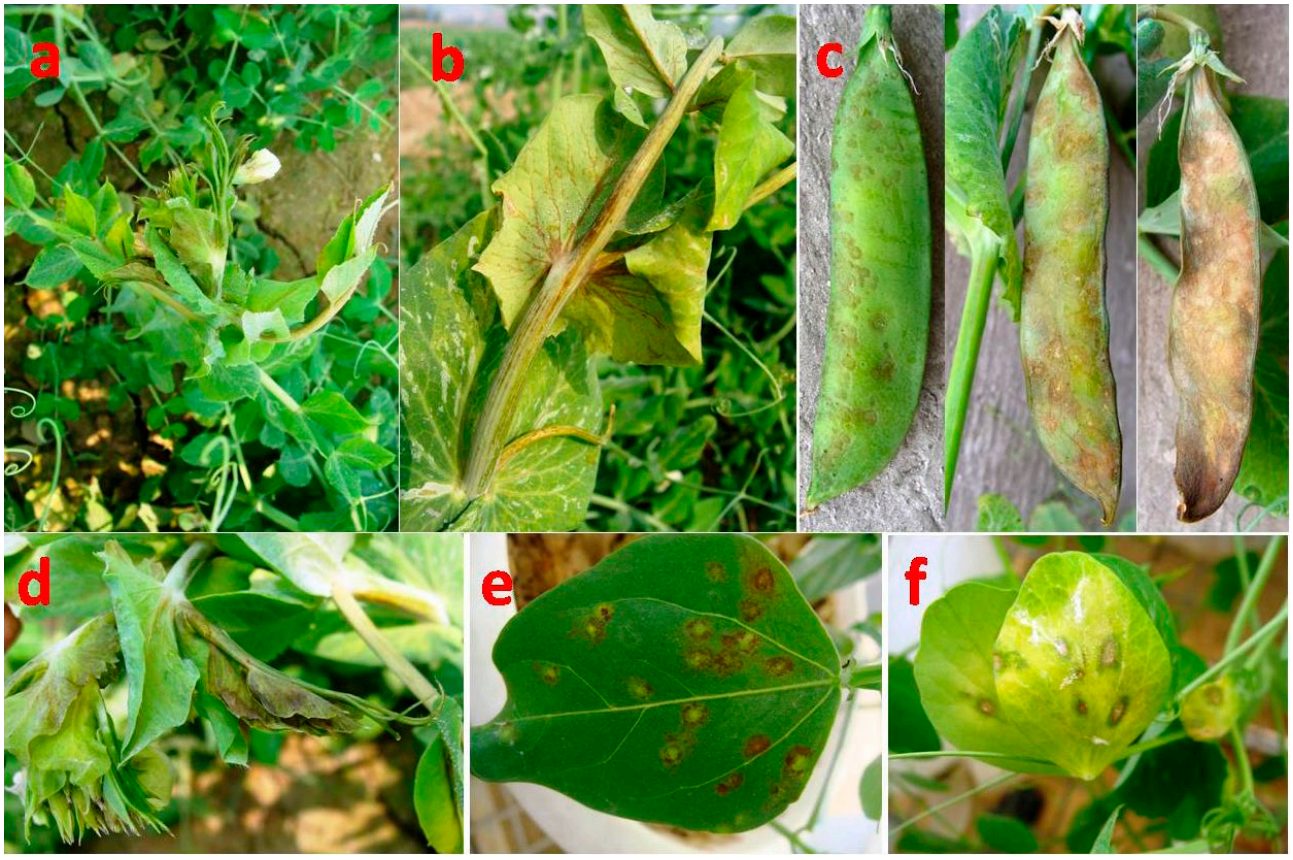

Figure 1. *Groundnut bud necrosis virus* symptoms on pea includes tip necrosis (a & d), veinal necrosis (b), necrotic spots on pods (c), chlorosis and necrotic spots on inoculated leaves of pea (f) and chlorotic/ necrotic spots on cowpea inoculated leaves (e).

Table 2. Details of survey and detection of GBNV in field infected pea samples.

Sampling area No.	Location	Number of fields surveyed	Number of samples collected	Number of samples that gave positive result in mechanical inoculation on		Samples positive in PCR
				cowpea	pea	
1	Kanpur, Uttar Pradesh	6	4	4	4	4
2	Bareilly, Uttar Pradesh	6	6	4	3	6
3	Shahjahanpur, Uttar Pradesh	8	2	2	2	2
4	Udham Singh Nagar, Uttarakhand,	15	6	4	4	5

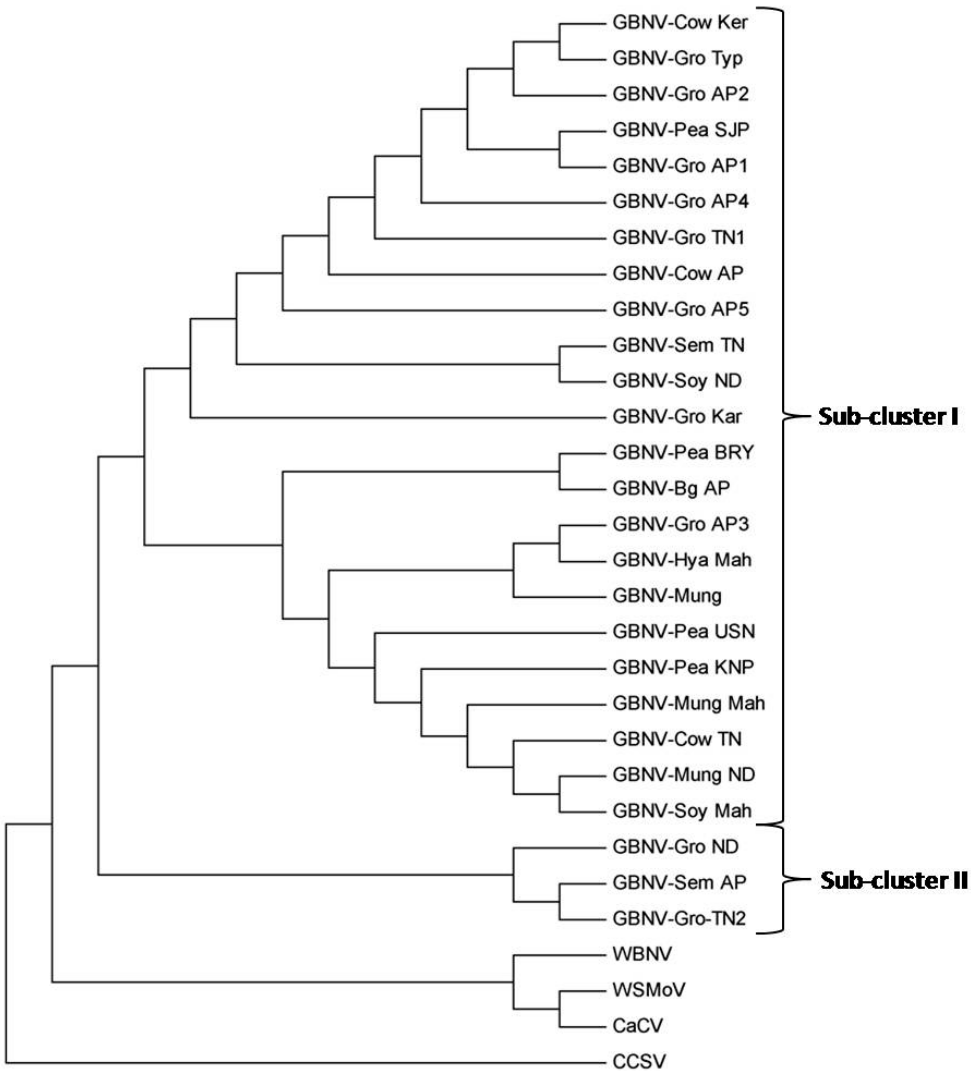

Figure 2. Cluster phylogram illustrating phylogentic relationship based on multiples alignments of amino acids sequences of 26 NP gene of GBNV isolates from Fabaceous hosts and 4 tospoviruses of the WSMoV serogroup.

of TSWV in India has not been confirmed (Pappu *et al.*, 2009). We recently observed a disease of pea with symptoms similar to those described by Prasada Rao *et al*. (1985) and found it to be caused by GBNV based on partial nonstructural movement (NSm) gene sequence analysis (Akram and Naimuddin, 2010).

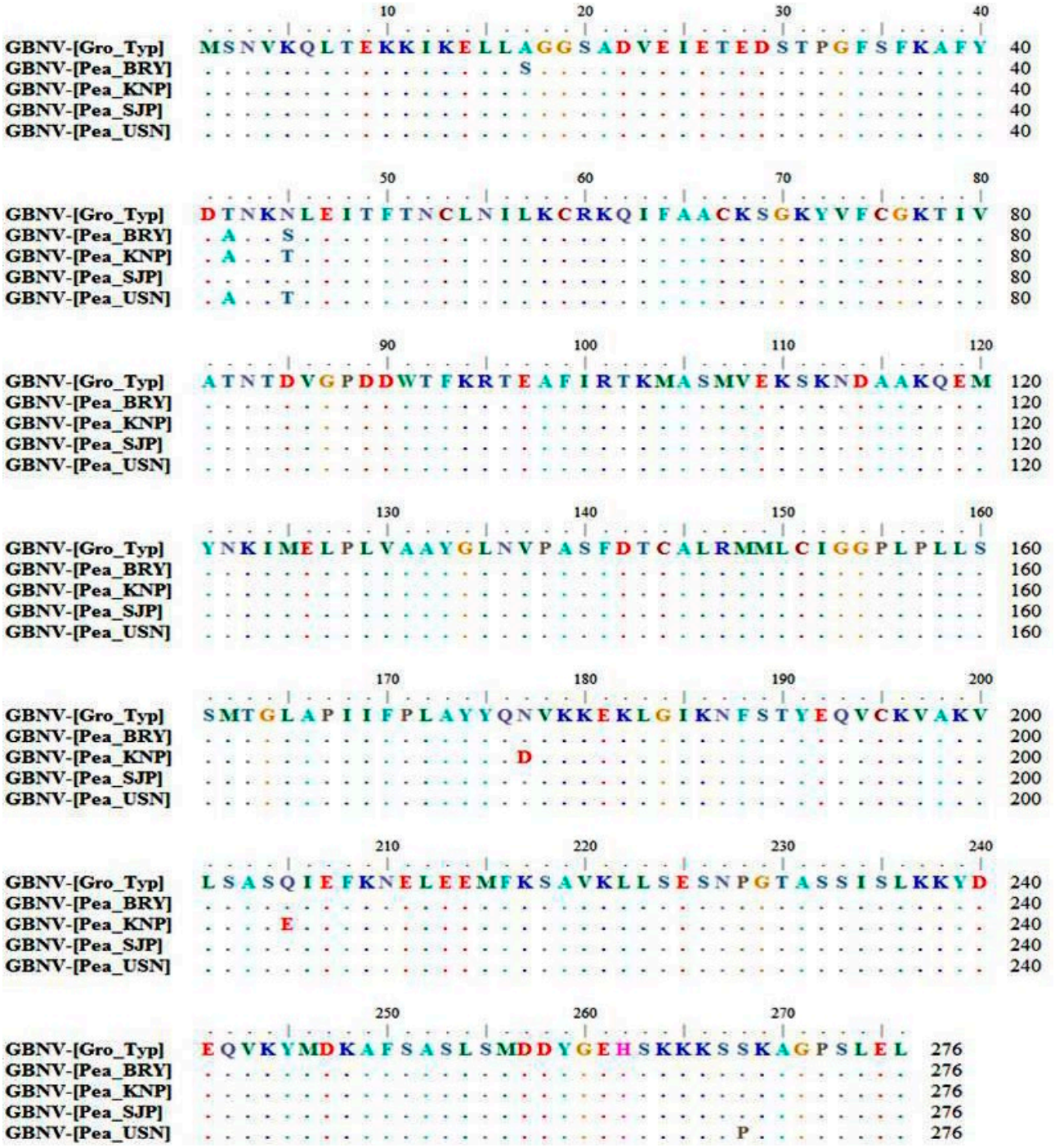

Figure 3. Multiple alignment of NP gene of GBNV isolates of Pea under study and Type isolate of GBNV. Dot indicates the similar amino acids.

Table 3. Per cent homology of nucleotides (below diagonally) and amino acids (above diagonally) of the GBNV isolates and the other tospoviruses of the WSMoV sero-group.

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	-	98	98	98	99	98	98	98	99	99	99	96	99	99	100	99	99	96	100	95	99	98	100	99	99	98	82	84	83	64
2	98	-	98	98	99	98	98	98	98	99	98	96	98	98	98	98	98	95	98	94	98	98	98	98	99	98	82	84	83	65
3	97	98	-	98	99	98	98	98	97	99	98	95	97	98	98	97	97	94	98	94	97	98	98	97	99	98	82	83	82	64
4	98	97	97	-	99	98	98	98	98	99	98	96	98	98	98	98	98	95	98	94	98	98	98	98	99	98	82	83	83	64
5	98	97	96	97	-	99	99	98	98	100	98	96	98	98	99	98	98	95	99	95	98	98	99	98	100	98	82	84	83	65
6	96	96	96	96	96	-	98	98	97	99	98	95	97	98	98	97	97	94	98	94	97	98	98	97	99	98	82	84	82	64
7	97	98	97	97	96	96	-	98	97	99	98	95	97	98	98	97	97	94	98	94	97	98	98	97	99	98	82	83	82	64
8	98	97	97	98	98	96	97	-	97	98	98	95	97	98	98	97	97	94	98	94	97	98	98	97	98	98	82	82	82	64
9	93	93	92	93	93	93	93	93	-	98	98	96	98	98	99	98	98	95	99	94	98	97	99	98	98	97	82	84	82	64
10	94	94	93	94	94	94	94	94	97	-	98	96	98	98	99	98	98	95	99	95	98	98	99	98	100	98	82	84	83	65
11	98	97	96	97	97	96	96	98	93	95	-	97	98	99	99	98	98	96	99	96	98	97	99	98	98	97	82	84	83	65
12	98	97	96	97	97	96	97	98	93	94	98	-	96	96	96	96	98	99	96	98	96	95	96	96	96	95	84	85	84	65
13	94	93	93	93	94	94	93	94	97	99	94	94	-	98	99	98	98	95	99	94	98	97	99	100	98	97	82	83	82	64
14	98	97	97	98	97	96	97	98	93	94	97	97	93	-	99	98	98	95	99	95	98	97	99	98	98	97	83	84	82	65
15	98	97	97	98	97	96	97	98	93	94	97	97	93	97	-	99	99	96	100	95	99	98	100	99	99	98	83	84	82	65
16	97	97	97	97	97	96	97	97	93	94	97	97	93	97	99	-	98	95	99	94	98	97	99	98	98	97	82	84	82	64
17	97	97	96	97	97	96	97	97	93	94	97	97	93	97	99	99	-	95	99	94	98	97	99	98	98	97	82	83	82	64
18	98	97	97	97	97	96	97	97	92	94	97	97	93	97	99	99	99	-	96	97	96	94	96	95	95	94	83	84	83	64
19	98	97	97	98	97	96	97	98	93	94	98	97	94	97	98	98	98	98	-	95	99	98	100	99	99	98	82	84	83	64
20	97	97	97	97	96	96	97	97	92	93	97	97	93	96	97	97	97	97	98	-	95	94	95	94	95	94	83	84	83	65
21	97	97	96	97	96	96	96	97	93	94	97	97	94	97	98	97	98	97	98	97	-	97	99	98	98	97	82	84	82	64
22	98	97	96	98	97	96	97	98	93	94	97	97	93	97	97	97	97	97	98	97	97	-	98	97	98	100	82	84	83	64
23	99	97	97	98	97	97	97	98	93	94	98	98	94	98	98	97	97	98	98	97	97	98	-	99	99	98	82	84	83	64
24	97	97	96	96	96	96	97	96	92	93	96	96	93	96	97	97	97	97	97	97	97	96	97	-	98	97	82	83	82	64
25	98	97	96	97	97	96	97	98	93	94	97	97	93	97	97	97	97	97	97	97	96	97	98	97	-	98	82	84	83	65
26	98	98	97	98	97	97	98	98	93	94	98	98	94	98	99	98	98	99	98	98	98	98	98	97	98	-	82	84	83	64
27	80	80	80	80	81	81	80	80	79	80	80	80	80	81	80	80	80	80	80	80	80	80	81	79	80	80	-	84	81	65
28	80	80	80	79	81	80	81	81	80	81	80	80	81	80	80	80	80	80	80	80	80	80	81	80	79	80	79	-	85	63
29	80	80	80	80	81	80	80	80	79	80	80	80	80	80	80	80	80	80	80	80	80	80	80	79	80	80	78	78	-	63
30	63	63	63	63	62	62	63	63	64	64	63	62	63	63	63	63	63	63	63	62	63	63	63	62	63	63	63	64	64	-

1= GBNV-[Pea_SJP], 2= GBNV-[Pea_USN], 3= GBNV-[Pea_KNP], 4= GBNV-[Pea_BRY], 5=GBNV-[Mung_Mah], 6=GBNV-[Mung], 7=GBNV-[Mung_ND], 8=GBNV-[Bg_AP], 9=GBNV-[Cow_Ker], 10=GBNV-[Cow_TN[3]], 11=GBNV-[Cow_AP], 12=GBNV-[Sem_AP], 13=GBNV-[Sem_TN], 14=GBNV-[Gro_Ker], 15=GBNV-[Gro_AP[4]], 16=GBNV-[Gro_TN[1]], 17=GBNV-[Gro_AP[1]], 18=GBNV-[Gro_TN[2]], 19=GBNV-[Gro_AP[2]], 20=GBNV-[Gro_ND], 21=GBNV-[Gro_AP[5]], 22=GBNV-[Gro_AP[3]], 23=GBNV-[Gro_Typ], 24=GBNV-[Soy_ND], 25=GBNV-[Soy_Mah], 26=GBNV-[Hya_Mah], 27= WBNV, 28=WSMoV,29=CaCV, 30=CCSV

Variability in 76 GBNV isolates infecting different vegetable crops has been studied recently by Kunkalikar *et al*. (2011), who reported 91.3 to 100% identities at the amino acid level. We found 94 to 100% identity at the amino acid level, and 92–99% identity at the nucleotide level, in sequences of *NP* gene of 26 GBNV isolates including four pea isolates (present study) infecting Fabaceous hosts in different agro-ecological zones across the India. There was no correlation between the variation in the sequences of GBNV isolates either with geographical locations or with hosts.

Though the variation in nucleotide sequences of GBNV isolates from fabaceous crops compared here are within the limits of species demarcation for tospoviruses (Fauquet, *et al*., 2005), formation of a separate cluster within the GBNV isolates indicates the possibility of distinct variants in GBNV, as has been indicated by Kunkalikar *et al*. (2011).

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