Journal of Biological Control, 26 (2): 131-137, 2012

Research Article

Molecular characterization of *Pieris brassicae* Granulosis virus (PbGV) from the Himalayan region of India

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ABSTRACT: A strain of granulosis virus from *Pieris brassicae* was isolated from the dry temperate region of Himachal Pradesh, India situated at an altitude of 2580 m above msl. The molecular characterization of this strain of PbGV was carried out with granulin gene nucleotide sequence analysis. The nucleotide sequence of 404 bp of PbGV was submitted to GenBank, NCBI with accession number FJ151541. Nucleotide and phylogenetic analysis confirm this isolate as Pieridae (insect family) infecting granulovirus with lowest genetic distance of 0.012, 0.015, 0.016 with other *Pieris rapae* and *Pieris brassicae* granulosis viruses. More number of isolates and other molecular markers, however, would be useful to understand the phylogenetic relationship of this Indian isolate of PbGV.

KEY WORDS: Granulosis virus, *Pieris brassicae,* granulin, phylogeny

(Article chronicle: Received:20-1-2012 Revised: 31-5-2012 Accepted: 3-6-2012)

INTRODUCTION

The cabbage butterfly, *Pieris brassicae* Linnaeus, is a serious pest of cole crops and other crops in the temperate, tropical and subtropical regions of the world (Feltwell, 1978). In India, it causes extensive damage at all the growing stages of cole crops *viz*. seedling, vegetative and flowering stage (Sachan and Gangwar, 1980; Younas *et al.*, 2004; Ali and Rizvi, 2007; Bhandari *et al.*, 2009). Currently, broad-spectrum chemical insecticides are used to control this caterpillar, delaying or suppressing field colonization by natural enemies. The implementation of a proper integrated pest management scheme for the cole crops warrants in areas where the incidence of *P. brassicae* reaches high population levels. Among pathogens, the potential of baculoviruses for pest control has been well documented and they have proven to be effective against many pests of agricultural importance (Entwistle, 1998; Huber, 1986; Moscardi, 1999). In India, the incidence of granulovirus (GV) on *P. brassicae* was first reported by Sood (2004) from the dry temperate region of India and was noticed as the main mortality factor of *P. brassicae* in Sangla Valley of Himachal Pradesh, India (situated at an altitude of 2590m above msl, 31°25′56″ N latitude and 78°15′ 4″ E longitude) (Bhandari *et al.*, 2009; Sood *et al.*, 2010). Therefore, in the present study we compared the sequences of local PbGV (Isolate S1) strain with other granuloviruses for which the granuline gene sequences are available to draw inferences on phylogenetic relationship of the *Pieris brassicae* granulovirus (PbGV).

MATERIALS AND METHODS

Rearing of experimental insect

The initial culture of *P. brassicae* was started from field collected eggs of the pest from the cabbage (*Brassica oleracea* var. *botrytis*) crop. The eggs were kept in sterilized Petri-plates (7.5 cm diameter) over an UV irradiated filter paper moistened with sterile distilled water (SDW) to prevent desiccation under laboratory conditions (Temp. 25 ± 2°C and RH 75-80%). Newly hatched larvae were transferred to fresh cabbage leaves surface sterilized with aqueous solution of sodium hypochlorite (0.05%) followed by three washings with SDW. The cabbage leaves were kept in ethanol washed and UV sterilized cages (15 x 15 x 15 cm^3). The first three larval instars were reared in the small cages (15 x 15 x 15 cm^3) while, the later instars were reared in large

cages (45 x 45 x 55 cm^3). Caterpillars in cages were provided with surface sterilized fresh cabbage leaves daily. The full grown caterpillars were transferred to the new cages for pupation. Two day old pupae were detached from the walls of cage and kept in a batch of 20 pupa in each cage (60 x 60 x 70 cm^3) over a thick layer of UV irradiated filter paper for adult emergence. The adults were held in cages (60 x 60 x 70 cm^3) provided with cotton swabs soaked in honey solution, SDW and some flowering shoots of mustard as pollen source. Potted cabbage plants were kept in each cage for egg laying whenever needed.

Virus strain

The *Pieris brassicae* granulovirus (PbGV) used in the study was the local strain isolated from the diseased larvae collected on cabbage crop from the dry temperate region of Himachal Pradesh by Sood (2004). The virus was multiplied in the host larvae in the laboratory conditions of 24 ± 2°C and 50 ± 10% RH.

Extraction of genomic DNA

For the extraction of total genomic DNA, virus infected 5th instar larvae of *P. brassicae* were ground individually in pestle and mortar with liquid nitrogen. About 100 mg powder was transferred to Eppendorf tube containing 680 ìl of CTAB extraction buffer maintained at 60°C and mixed thoroughly by turning the tubes up and down. The tubes were then incubated at 60°C for 1 hour with gentle mixing after every 10 min. To each tube equal volume (680 ìl) of chloroform: isoamyl alcohol (24:1) was added. The contents were mixed gently and centrifuged at 10,000 g for 10 min in high speed refrigerated centrifuge (REMI, India) at 4°C. Aqueous phase was transferred to new tubes, 450 ìl prechilled isopropanol was added and kept at –20°C for 20–30 min to precipitate the DNA. Tubes were then spun at 10,000 g for 10 min and supernatant was decanted. The DNA pellet was washed three times with 70 per cent ethanol, dried and dissolved in 100 ìl of Tris EDTA buffer (10mM Tris HCl and 1mM EDTA, pH 8.0). RNase @ 10 mg/ ml (MBI Fermentas) was added and the emulsion was incubated for half an hour at 37°C. DNA was stored at –20°C for further use (Sood *et al.*, 2010).

Amplification of virus DNA and nucleotide sequencing

The PCR amplification was carried out with granulin gene specific primers pair (forward primer sequence 5' CAAGATCAAGGAATTCGCACCCGACGTA 3' and reverse primer sequence 5' GTTCTAGTTCCTTAAGC GTGGGCTGCAT 3') developed by Burden *et al.* (2002)**.** The PCR reaction was performed in 0.2 ml PCR tubes with 25 ìl reaction volume containing 20 ng of DNA template, 20 pmol of each primer in 25 mM $MgCl_2$, 10 mM of each deoxyribonucleoside triphosphate (Fermentas), 5 units of *Taq* polymerase (Life Technologies India, Pvt. Ltd) and 10x reaction buffer. Amplifications were performed using thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, USA) with an initial denaturation step of 2 min at 94°C followed by 40 cycles at 94°C for 30 sec, 65°C for 1 min, 72°C for 30 sec and a final elongation step at 72°C for 10 min. The product was separated in a 1.2% (w/v) agarose gel in TAE buffer (40 mM Tris-acetate, 1mM EDTA). DNA ladders of 100 bp (Bangalore Genei) and lambda DNA/ *EcoR* I – *Hind* III double digest (MBI Fermentas) were used as molecular weight markers. The gels were run at 80V for 1 h using Bangalore Genei power pac system. The gels were stained with ethidium bromide (0.5 ìg/ ml) for 10 min after electrophoresis. The gels were viewed and images were captured using gel documentation system (Alpha Imager 2200, Alpha Infotech Corporation, USA). PCR products of granulin gene of virus strain obtained through amplification with specific primers were freeze dried (CHRIST *ALPHA I-2LD*) and were custom sequenced (ABI PRISM 310™ Genetic Analyzer, Applied Biosystems, USA) using same upstream and downstream primers (Life Technologies India, Pvt. Ltd., New Delhi, India).

Nucleotide sequence analysis

The sequence of virus strain was blasted using on-line NCBI Blastn program http://www.ncbi.nih.gov/blast. Twenty four sequences of granulin gene of different baculoviruses of high sequence similarity were selected for sequence comparison from GenBank Nucleotide Database, NCBI (Table 1). The selected sequences along with the test sequence were aligned by ClustalW program using website http://www.ebi.ac.uk/clustalw/.

The evolutionary history was inferred using Neighbour Joining (Saitou and Nei, 1987) method with HaNPV as outgroup. Evolutionary distances were calculated using Maximum Composite Likelihood method (Tamura *et al.*, 2004) and in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). A total of 344 positions were detected in the final dataset. Phylogenetic analysis was conducted in MEGA 4.1 Software programme (Tamura *et al.*, 2007).

Table 1. Details of granulin gene sequences used in analysis

Sl. No.	Name of virus	Host insect name	Country	Accession No.
1	PbGV	*Pieris brassicae*	Germany	DQ235253
2	PbGV	*Pieris brassicae*	–	X02498
3	PrGV	*Pieris rapae*	Germany	AY519252
4	PrGV	*Pieris rapae*	China	AY428513
5	PrGV	*Pieris rapae*	–	AY706673
6	CpGV	*Cydia pomonella*	Germany	AY706670
7	CpGV	*Cydia pomonella*	Germany	AY706667
8	TnGV	*Trichoplusia ni*	Germany	AY519201
9	TnGV	*Trichoplusia ni*	–	K02910
10	AsGV	*Agrotis segetum*	Germany	AY706660
11	PoGV	*Phthorimaea operculella*	France	AF499596
12	PxGV	*Plutella xylostella*	Japan	AF270937
13	ClGV	*Cryptophlebia leucotreta*	Germany	AY229987
14	SlGV	*Spodoptera litura*	South Korea	DQ288858
15	PsGV	*Plathypena scabra*	Germany	AY706675
16	EaGV	*Epinotia aporema*	Argentina	EU723206
17	EaGV	*Epinotia aporema*	Argentina	EU723205
18	EaGV	*Epinotia aporema*	Argentina	AF473703
19	CpGV	*Cydia pomonella*	Germany	EU428824
20	CaGV	*Clostera anachoreta*	China	AY993940
21	CaGV	*Clostera anachoreta*	Germany	AY706664
22	AoGV	*Adoxophyes orana*	Germany	AY519258
23	AoGV	*Adoxophyes orana*	Germany	AY706658
24	XcGV	*Xestia c-nigrum*	Japan	U70069
25	HaNPV	*Helicoverpa armigera*	Thailand	EF095545

Country name not found in the GenBank database (NCBI)

RESULTS AND DISCUSSION

Granulin gene sequencing of *Pieris brassicae* granulovirus (PbGV) using granuline gene specific primers resulted in a sequence of 404bp. This sequence was submitted to NCBI GenBank nucleotide database with accession number FJ151541. This constitutes the first record of granulin gene sequence of PbGV from India. Nucleotide sequence analysis of test virus using clustalW programme revealed that PbGV showed maximum homology with other *Pieris brassicae* granulovirus (X02498, DQ235253) and *Pieris rape* granulovirus (AY519252, AY428513, AY706673). The multiple sequence alignment analysis of granulin gene of the PbGV with twenty four granulin gene sequences available in the GenBank Database (NCBI) revealed genetic distance ranging from 0.012 to 0.139. Test strain of PbGV (FJ151541) showed minimum genetic distance of 0.012, 0.014, and 0.016 with, PrGV (AY519252), PrGV (AY428312) and PbGV (DQ235253), respectively. The genetic distance of PbGV (test strain) with out-group member *i.e.* HaNPV was 0.397 (Table 2).

The genetic distance estimates and phylogenetic tree also confirmed similar relationship pattern of the PbGV. The phylogenetic tree (Figure 1) analysed with Neighbour – Joining method grouped Indian strain of PbGV with other country PbGV strain along with

Table 2. Pair wise genetic distance between PbGV and other granulovirus granulin gene sequences collected from GenBank, NCBI

Granulin gene sequences	FJ151541_PbGV	DQ235253_PbGV	X02498_PbGV	AY519252_PrGV	AY428513_PrGV	AY706673_PrGV	AY706670_CpGV	AY706667_CpGV	AY519201_TnGV	K02910_TnGV	AY706660_AsGV	AF499596_PoGV	AF270937_PxGV	AY229987_ClGV	DQ288858_SlGV	AY706675_PsGV	EU723206_EaGV	EU723205_EaGV	AF473703_EaGV	EU428824_CpGv	AY993940_CaGV	AY706664_CaGV	AY519258_AoGV	AY706658_AoGV	U70069_XcGV	EF095545_HaNPV
FJ151541_PbGV		0.011	0.013	0.009	0.010	0.010	0.049	0.049	0.058	0.058	0.061	0.056	0.062	0.057	0.067	0.050	0.047	0.047	0.047	0.049	0.052	0.052	0.050	0.050	0.057	0.436
DQ235253_PbGV	0.016		0.013	0.008	0.003	0.004	0.048	0.048	0.063	0.063	0.060	0.052	0.063	0.054	0.067	0.052	0.049	0.049	0.049	0.048	0.053	0.053	0.048	0.048	0.062	0.432
X02498_PbGV	0.022	0.019		0.008	0.012	0.011	0.050	0.0550	0.063	0.063	0.063	0.048	0.065	0.053	0.068	0.051	0.052	0.052	0.052	0.050	0.051	0.051	0.049	0.049	0.059	0.450
AY519252_PrGV	0.012	0.009	0.009		0.007	0.006	0.048	0.048	0.060	0.060	0.059	0.050	0.064	0.053	0.066	0.051	0.049	0.049	0.049	0.048	0.050	0.050	0.048	0.048	0.060	0.429
AY428513_PrGV	0.015	0.001	0.018	0.008		0.003	0.047	0.047	0.062	0.062	0.061	0.051	0.062	0.055	0.068	0.052	0.049	0.049	0.049	0.047	0.052	0.052	0.049	0.049	0.0661	0.435
AY706673_PrGV	0.014	0.003	0.016	0.007	0.001		0.048	0.048	0.061	0.061	0.060	0.050	0.063	0.054	0.067	0.051	0.048	0.048	0.048	0.048	0.051	0.051	0.048	0.048	0.060	0.435
AY706670_CpGV	0.106	0.102	0.108	0.104	0.101	0.102		0.000	0.047	0.047	0.045	0.041	0.051	0.038	0.053	0.037	0.034	0.034	0.034	0.000	0.044	0.044	0.0399	0.039	0.048	0.466
AY706667_CpGV	0.106	0.102	0.108	0.104	0.101	0.102	0.000		0.047	0.047	0.045	0.041	0.051	0.038	0.053	0.037	0.034	0.034	0.034	0.000	0.044	0.044	0.039	0.039	0.048	0.466
AY519201_TnGV	0.122	0.133	0.132	0.127	0.131	0.130	0.100	0.100		0.000	0.053	0.055	0.063	0.054	0.052	0.048	0.053	0.053	0.053	0.047	0.048	0.048	0.042	0.042	0.023	0.446
K02910_TnGV	0.122	0.133	0.132	0.127	0.131	0.130	0.100	0.100	0.000		0.053	0.055	0.063	0.054	0.052	0.048	0.053	0.053	0.053	0.047	0.048	0.048	0.042	0.042	0.023	0.446
AY706660_AsGV	0.131	0.129	0.135	0.127	0.131	0.129	0.098	0.098	0.115	0.115		0.049	0.064	0.042	0.063	0.049	0.048	0.048	0.048	0.045	0.051	0.051	0.046	0.046	0.056	0.486
AF499596_PoGV	0.118	0.109	0.101	0.104	0.108	0.106	0.086	0.086	0.116	0.116	0.106		0.060	0.040	0.063	0.048	0.053	0.053	0.053	0.041	0.052	0.052	0.048	0.048	0.058	0.428
AF270937_PxGV	0.133	0.134	0.139	0.138	0.133	0.134	0.107	0.107	0.130	0.130	0.135	0.124		0.061	0.074	0.054	0.055	0.055	0.055	0.051	0.059	0.059	0.057	0.057	0.059	0.498
AY229987_ClGV	0.123	0.118	0.116	0.115	0.119	0.118	0.084	0.084	0.116	0.116	0.091	0.086	0.131		0.058	0.039	0.041	0.041	0.041	0.038	0.048	0.048	0.046	0.046	0.052	0.511
DQ288858_SlGV	0.139	0.141	0.141	0.137	0.143	0.141	0.116	0.116	0.112	0.112	0.132	0.135	0.152	0.127		0.054	0.054	0.054	0.054	0.053	0.061	0.061	0.058	0.058	0.048	0.527

Table 2 (Contd...)

Granulin gene sequences	FJ151541_PbGV	DQ235253_PbGV	X02498_PbGV	AY519252_PrGV	AY428513_PrGV	AY706673_PrGV	AY706670_CpGV	AY706667_CpGV	AY519201_TnGV	K02910_TnGV	AY706660_AsGV	AF499596_PoGV	AF270937_PxGV	AY229987_ClGV	DQ288858_SlGV	AY706675_PsGV	EU723206_EaGV	EU723205_EaGV	AF473703_EaGV	EU428824_CpGv	AY993940_CaGV	AY706664_CaGV	AY519258_AoGV	AY706658_AoGV	U70069_XcGV	EF095545_HaNPV
AY706675_PsGV	0.106	0.112	0.110	0.108	0.110	0.109	0.078	0.078	0.101	0.101	0.105	0.103	0.114	0.083	0.115		0.041	0.041	0.041	0.037	0.037	0.037	0.039	0.039	0.051	0.451
EU723206_EaGV	0.100	0.107	0.111	0.105	0.105	0.104	0.074	0.074	0.114	0.114	0.105	0.115	0.116	0.088	0.117	0.086		0.000	0.000	0.034	0.047	0.047	0.045	0.045	0.049	0.450
EU723205_EaGV	0.100	0.107	0.111	0.105	0.105	0.104	0.074	0.074	0.114	0.114	0.105	0.115	0.116	0.088	0.117	0.086	0.000		0.000	0.034	0.047	0.047	0.045	0.045	0.049	0.450
AF473703_EaGV	0.100	0.107	0.111	0.105	0.105	0.104	0.074	0.074	0.114	0.114	0.105	0.115	0.116	0.088	0.117	0.086	0.000	0.000		0.034	0.047	0.047	0.045	0.045	0.049	0.450
EU428824_CpGV	0.106	0.102	0.108	0.104	0.101	0.102	0.000	0.000	0.100	0.100	0.098	0.086	0.107	0.084	0.116	0.078	0.074	0.074	0.074		0.044	0.044	0.039	0.039	0.048	0.464
AY993940_CaGV	0.110	0.113	0.108	0.106	0.112	0.110	0.095	0.095	0.103	0.103	0.111	0.111	0.124	0.106	0.128	0.079	0.100	0.100	0.100	0.095		0.000	0.036	0.036	0.049	0.440
AY706664_CaGV	0.110	0.113	0.108	0.106	0.112	0.110	0.095	0.095	0.103	0.103	0.111	0.111	0.124	0.106	0.128	0.079	0.100	0.100	0.100	0.095	0.000		0.036	0.036	0.049	0.440
AY519258_AoGV	0.106	0.103	0.107	0.103	0.105	0.103	0.083	0.083	0.091	0.091	0.100	0.103	0.119	0.101	0.123	0.079	0.097	0.097	0.097	0.083	0.075	0.075		0.000	0.044	0.460
AY706658_AoGV	0.106	0.103	0.107	0.103	0.105	0.103	0.083	0.083	0.091	0.091	0.100	0.103	0.119	0.101	0.123	0.079	0.097	0.097	0.097	0.083	0.075	0.075	0.000		0.044	0.460
U70069_XcGV	0.120	0.131	0.124	0.125	0.129	0.127	0.104	0.104	0.045	0.045	0.122	0.121	0.123	0.114	0.103	0.108	0.106	0.106	0.106	0.104	0.104	0.104	0.095	0.095		0.461
EF095545_HaNPV	0.397	0.397	0.398	0.393	0.399	0.399	0.403	0.403	0.403	0.403	0.406	0.387	0.416	0.413	0.409	0.401	0.402	0.402	0.402	0.403	0.401	0.401	0.400	0.400	0.409	

Below diagonal and above diagonal values are number of base substitution per site and standard error estimate(s), respectively, and were obtained by a bootstrap procedure (1000 replicates)

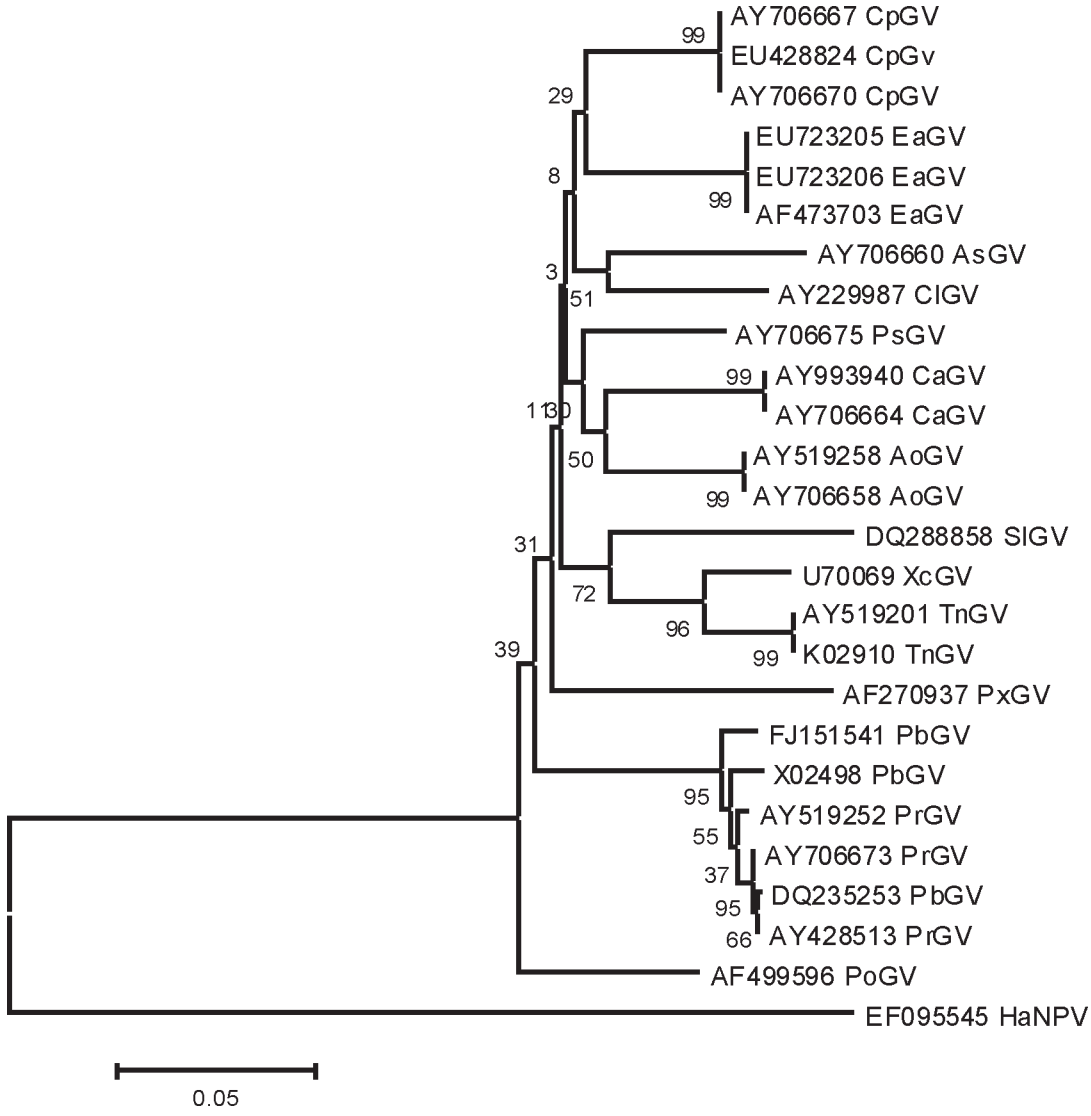

Fig. 1 Phylogenetic tree of *Pieris brassicae* granulosis virus (PbGV) based on granulin gene with HaNPV as outgroups using the Neighbor-Joining method and confidence level was calculated with the bootstrap test (1000 replicates). Arrow denotes the position of test strain of PbGV sequenced in present study

nearest PrGV strain. These (PbGV and PrGV) strains of viruses are infecting the Pieridae group of insects (Crook, 1981). The genetic distance of PbGV and other PrGV were more with other baculoviruses in the present study.

Phylogenetic analysis of the available granulin gene sequences of *Pieris* spp. granuloviruses showed the distinct clustering of *Pieris* spp. granulin gene sequences. PbGV isolate S1 sequenced in this study is closer to PbGV (X02498). PbGV and PrGV from different locations available in GenBank (NCBI) were closer to TnGV and PxGV in the present study. The grouping of PbGVs and PrGVs in one clade in the present analysis is also supported by the findings of Crook (1981), who reported that the homology between the DNAs of PbGV and PrGV was 97.7 per cent. He also suggested that there were no differences between virus capsules when examined by immune diffusion, ELISA, or SDS-polyacrylamide gel electrophoresis. The findings also suggested that the PbGV and PrGV may infect *Pieris rapae* and *P. brassicae* in nature, respectively. The cross infectivity of the PbGV isolate could not be evaluated against *P. rapae* as this pest was not prevalent in the region, however the present isolate when evaluated against *Plutcella xylostella* Linn. and *Trichoplusia* species was not able to cause any mortality in these insect larvae in spite of the close genetic distances. Moreover, baculovirus relationships based solely on occlusion body sequences can be inaccurate (Zanotto *et al.*, 1993; Bulach *et al.*, 1999; Bideshi *et al.*, 2000) analysis of other genes, such as

those encoding helicases and DNA polymerases, as well as more granulin genes, are required to determine the distance between *Pieris brassicae* GV and the other granulins.

ACKNOWLEDGEMENT

Authors are thankful to Indian Council of Agricultural Research (ICAR), New Delhi, India for financial support in the form of *adhoc* Research Project (F. No. 1-30 / 2004–PP / ICAR – 196–19).

REFERENCES

Ali A, Rizvi PQ. 2007. Developmental response of cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) on different cole crops under laboratory and field condition. *Asian J Pl Sci.* **6**: 1241–1245.

Bhandari K, Sood P, Mehta PK, Choudhary A, Prabhakar CS. 2009. Effect of botanical extracts on the biological activity of granulosis virus against *Pieris brassicae.* *Phytoparasitica* **37**: 317–322.

Bideshi DK, Bigot Y, Federici BA. 2000. Molecular characterization and phylogenetic analysis of the *Harrisina brillians* granulovirus granulin gene. *Arch Virol.* **145**: 1933–1945.

Bulach DM, Kumar AA, Zaia A, Liang B, Tribe DE. 1999. Group II nucleopolyhedrovirus subgroups revealed by phylogenetic analysis of polyhedrin and DNA polymerase gene sequences. *J Inv Pathol.* **73**: 59–73.

Burden P, Griffiths CM, Corry JS, Smith P, Sact SM. 2002. Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. *Molecular Ecol.* **11**: 547–555.

Crook NE. 1981. A comparison of the granulosis viruses from *Pieris brassicae* and *Pieris rapae*. *Virology* **115**(1): 173–181.

Entwistle PF. 1998. A world survey of virus control of insect pests, In: Hunter-Fujita, F. R. *et al.* (Eds.). *Insect Viruses and Pest Management.* John Wiley & Sons Ltd., Chichester, England, pp. 189–200 .

Feltwell J. 1978. The depredations of the large white butterfly (*Pieris brassicae*) (Pieridae). *J Res Lepidoptera* **17**: 218–225.

Huber J. 1986. Use of baculoviruses in pest management programs, pp. 181–202. **In:** Granados, R. R. and Federici, B. A. (Eds.). *The Biology of Baculoviruses*, Vol. I. CRC Press, Inc., Boca Raton.

Moscardi F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. *Ann Rev Ent.* **44**: 257–489.

Sachan JN, Gangwar SK. 1980. Vertical distribution of important pest of cole crop in Meghalya as influenced by environment factors. *Indian J Ent.* **42**: 414–421.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mole Biol Evol.* **4**: 406–425.

Sood P. 2004. New record of granulovirus on cabbage white butterfly, *Pieris brassicae* Linn. from dry temperate regions of Himachal Pradesh. *Himachal J Agric Res.* **30**: 146–148.

Sood P, Mehta PK, Bhandari K, Prabhakar CS. 2010. Transmission and effect of sublethal infection of granulosis virus (PbGV) on *Pieris brassicae* Linn. (Pieridae: Lepidoptera). *J Appl Ent.* **134** (9–10): 774–780.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol.* **24**: 1596– 1599.

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc National Acad Sci USA* **101**: 11030–11035.

Younas M, Naeem M, Rquib A, Masud S. 2004. Population dynamics of *Pieris brassicae* on five cauliflower at Peshawar. *Asian J Pl Sci.* **3**: 391–393.

Zanotto PM, de A, Kessing BD, Maruniak JE. 1993. Phylogenetic interrelationships among baculoviruses, evolutionary rates and host associations. *J Inv Pathol.* **62**: 147–164.