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Genetic diversity among different geographical isolates of the gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) nucleopolyhedrosis virus (*Hear*NPV)

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Abstract

The gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is infected by nucleopolyhedrosis virus (*Hear*NPV), which is the most promising microbial biocontrol agent of the pest. A genetic diversity analysis of geographically distinct isolates of *Hear*NPV was done, using the *polyhedrin* (*polh*) gene of the viruses that encodes a major structural protein of the occlusion bodies. The gene was amplified and isolated from eight Indian isolates, using the polymerase chain reaction (PCR). These sequences were compared with the *polh* genes of other *Hear*NPV from different geographical regions of the world. A phylogenetic tree was constructed, using *polh* nucleotide/deduced amino acid sequences to know their genetic relatedness. The *polh* gene of isolates originating from nearby locations clustered together with the gene of isolates in the present study; however, some showed relatedness with gene isolate from other geographically distinct isolates, with respect to the genetic distances among them. The Indian isolate (Ban-PDBC-In) shared lower genetic distance of 0.0020 to 0.0040 substitutions per site with the Spanish isolates SP1A-Sp and SP1B-Sp and clustered in one group based on nucleotide sequences. The isolates showed different a clustering pattern in phylogenetic tree based on deduced amino acid sequences than that of the nucleotide sequences. The overall genetic distances between *polh* nucleotides ranged from 0.0000 to 0.0203 substitutions per site, while it was 0.0000 to 0.0121 between deduced amino acid sequences. Among different geographical groups of isolates, the Indian group showed the highest genetic diversity based on both *polh* nucleotide (0.0070 ± 0.0002 substitutions per site) and deduced amino acid (0.0057 ± 0.0003 substitutions per site) sequences among different groups of geographical isolates. A diversity analysis of virus isolates can aid in the selection and identification of virulent virus isolates for the development of a virus-based bio-pesticide formulation.

Keywords: *Helicoverpa armigera*, nucleopolyhedrosis virus, isolates, phylogeny, *polyhedrin*

Background

The gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a cosmopolitan and polyphagous insect pest (Fitt 1989). It has been reported to feeding on 60 cultivated and 67 wild host plants in 36 families (Reed and Pawar 1982). The pest is known to cause an estimated loss of more than US $2 billion annually in semi-arid tropics (Sharma et al. 2005). Several entomopathogenic microbes were exploited for the management of this pest (Swami et al. 2012; Pugalenthi et al. 2013; Bajya et al. 2015). Among these, *H. armigera* nucleopolyhedrosis virus (*Hear*NPV) has attracted much attention because of its host specificity and safety to non-target organisms (Moore et al. 2004; Gupta et al. 2010). However, *Hear*NPV isolates collected from different geographical regions showed considerable variations both in genetics and in virulence (Ogembo et al. 2007; Mehrvar et al. 2008; Figueiredo et al. 2009). Thus, the

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study of the diversity among geographical isolates of *Hear*NPV, at molecular level, is a key research area to generate information on molecular fingerprinting for selecting virulent virus isolates (Kaur et al. 2014).

*Hear*NPV belongs to the family baculoviridae of the insect viruses. They have rod-shaped virions, enveloped in proteinaceous occlusion bodies with supercoiled double-stranded circular DNA genome ranging from 90 to 160 kb (Blissard and Rohrmann 1990; Zhang et al. 2005). In earlier studies, the baculoviruses were differentiated based on restriction enzyme analysis of whole genome (Gettig and McCarthy 1982; Takatsuka et al. 2003). Highly conserved genes such as *polyhedrin* (*polh*) (Rohrmann 1986), *late expression factors* (Hefferon 2004), and *chitinase* (Wang et al. 2004) have been identified in baculoviruses. *Polyhedrin* gene among these encodes major matrix protein of occlusion bodies of baculoviruses (Blissard and Rohrmann 1990). PCR-based amplified sequence of these genes have been used for diversity studies by many scientific groups (Jakubowska et al. 2005; Arrizubi et al. 2013; Kaur et al. 2014) and classification of baculoviruses (Lange et al. 2004; Jehle et al. 2006).

In the present study, genetic diversity of *Hear*NPV from different geographical regions (*Hear*NPV isolates) was studied based on the variations in the sequence of *polh* gene sequence.

Materials and methods

Insect rearing

H. armigera was reared on a semisynthetic chickpea diet at 25 °C and 16-h photoperiod (Wakil et al. 2011).

Multiplication of virus isolates

The crude extracts of polyhedral inclusion bodies (PIBs) were obtained from various institutions located in different regions of India as listed in Table 1.

The virus isolates were multiplied in fourth-instar larvae of *H. armigera*. The larvae were fed on a semisynthetic diet mixed with polyhedral suspension (6.5×10^9 PIBs/ml) of virus and incubated at 25 °C. The infected larvae were collected based on typical disease symptom and homogenized in distilled water. The mixture was filtered through a clean muslin cloth, and the polyhedra were ultrapurified on a 40 to 60% sucrose gradient in a Beckmann centrifuge at 32000 rpm. The isolated polyhedral were stored in distilled water at − 20 °C. The concentration of polyhedra in each virus suspension was determined, using Neubauer Haemocytometer under phase contrast light microscope at × 400 (Grzywacz et al. 2004).

DNA isolation

The DNA was extracted from different isolates according to the protocol of Figueiredo et al. (1999) with slight modifications. The purified polyhedra were re-suspended in 0.1 M sodium carbonate solution [0.1 M sodium carbonate, 0.15 M NaCl, 0.5 M EDTA (pH 10.5)] and incubated at 37 °C for 15 min. Subsequently, 0.5 mg/ml of proteinase K and 1% SDS (sodium dodecyl sulphate) was added and incubated at 37 °C for 15 min. The digested solution was further extracted by phenol and then by phenol to chloroform to isomyl alcohol (25:24:1) solution. The DNA was precipitated by ethanol and re-suspended in 0.1 X TE buffer (pH 8.0).

PCR amplification of polyhedrin (polh) gene

Primers for the specific amplification of *polh* from *Hear*NPV isolates were designed based on *polh* sequence of the Project Directorate of Biological Control, Bangalore (PDBC) isolate (GenBank acc. no. FJ 157293.1), using Primer3 (Version 0.4.0) software. The reaction mixture (20 μl) contained 15 ng DNA template, 3 U *Taq* DNA polymerase, 10 pmol/μl forward primer (5′-ATGTATACTCGTTACAGTTACA GCCCT-3′), 10 pmol/μl reverse primer (5′-TTAATA TGCAGGACCAGTGTATAGC-3′), 2 mM dNTPs, and 10X PCR buffer with 15 mM $MgCl_2$.

The gene amplification was performed, using a thermal cycler (Bio-Rad), with initial denaturation at 94 °C for 4 min followed by another denaturation at

Table 1 *Helicoverpa armigera* nucleopolyhedrosis virus (*Hear*NPV) isolates from various geographical locations in India

Sl. no.	Virus isolate	Abbreviation	Geographic coordinates
1	Dhule, Maharashtra	Dhl-In	20.9042° N, 74.7749° E
2	Akola, Maharashtra	Akl-In	20.7002° N, 77.0082° E
3	Rajanukunte, Karnataka	Rke-In	13.1870° N, 77.5502° E
4	Chandapura, Karnataka	Chan-In	12.8005° N, 77.7136° E
5	Gulbarga, Karnataka	Gul-In	17.3297° N, 76.8343° E
6	Hyderabad, Telangana State	Hyd-In	17.3850° N, 78.4867° E
7	Udaipur, Rajasthan	Udr-In	24.5854° N, 73.7125° E
8	Hosur, Tamil Nadu	Hor-In	12.7409° N, 77.8253° E

94 °C for 1 min, and then annealing at 57 °C for 45 s, followed by extension at 72 °C for 1 min and a final extension at 72 °C for 10 min, and the amplified *polh* gene was subsequently stored at 4 °C until used. There were 35 amplification cycles. The amplified PCR product was electrophoresed in 0.8% agarose gel and visualized in a UV gel documentation system.

Sequencing of amplified product

The agarose gel bands with *polh* gene amplimers were eluted from the agarose block and purified, using

Table 2 Geographical isolates of *Helicoverpa armigera* nucleopolyhedrosis virus (*Hear*NPV) used to compare with the eight isolates from present study

Sl. no.	Origin/group	Isolate	Abbreviation	Accession (nucleotide)	Accession (amino acid)
1	India	Jodhan	Jod-In	FJ157294.1	ACI05105.1
2		Ludhiana PAU	Lud-PAU-In	FJ157291.1	ACI05102.1
3		Ludhiana-I	Lud-I-In	KM268536.1	AIY68501.1
4		Ludhiana-II	Lud-II-In	KY432399.1	ARQ18915.1
5		Bathinda	Bat-In	FJ157292.1	ACI05103.1
6		Faridkot-I	Far-I-In	KC174715.1	AGE92318.1
7		Faridkot-II	Far-II-In	KM357499.1	AIY24932.1
8		Dhule	Dhl-In	MH029114	QCA42461
9		Akola	Akl-In	MH029115	QCA42462
10		Hisar HAU	His-HAU-In	FJ157295.1	ACI05106.1
11		Palampur	Pal-In	LK031772.1	CDR35259.1
12		Hyderabad	Hyd-In	MH029116	QCA42463
13		Udaipur	Udr-In	MH029117	QCA42464
14		Rajanukunte	Rke-In	MH029111	QCA42458
15		Chandapura	Chan-In	MH029112	QCA42459
16		Gulbarga	Gul-In	MH029113	QCA42460
17		Bangalore	Ban-In	JQ612524.1	AFD09505.1
18		Bangalore L1 NBAIR	Ban-L1-NBAIR-In	KT013224.1	ALD88571.1
19		Bangalore PDBC	Ban-PDBC-In	FJ157293.1	ACI05104.1
20		Hosur	Hor-In	MH029118	QCA42465
21		1113 Negamum	1113-Neg-In	HQ246082.1	ADY88204.1
22		2588 Negamum	2588-Neg-In	HQ246093.1	ADY88215.1
23	China	1073 China	1073-Ch	HQ246081.1	ADY88203.1
24		1625 China	1625-Ch	HQ246090.1	ADY88212.1
25		3010 China	3010-Ch	HQ246094.1	ADY88216.1
26	South Africa	1186 South Africa	1186-SA	HQ246085.1	ADY88207.1
27	Sudan	75 Sudan	75-Sud	HQ246071.1	ADY88193.1
28	Spain	LB1 Spain	LB1-Sp	KJ701029.1	AJP07155.1
29		LB3 Spain	LB3-Sp	KJ701030.1	AJP07290.1
30		LB6 Spain	LB6-Sp	KJ701031.1	AJP07425.1
31		SP1A Spain	SP1A-Sp	KJ701032.1	AJP07560.1
32		SP1B Spain	SP1B-Sp	KJ701033.1	AJP07696.1
33	Poland	138 Poland	138-Pol	HQ246073.1	ADY88195.1
34	Australia	Australia	Aus	JN584482.1	AEN03926.1
35		H25EA1 Australia	AC53-Aus	KJ922128.1	AIG63180.1
36		AC53 Australia	H25EA1-Aus	KJ909666.1	AIG63043.1

GeneJET gel extraction kit (Thermo Scientific). The purified DNA fragments were ligated to pTZ57R/T easy cloning plasmid vector, using "InsTAclone™ PCR Cloning Kit" (Fermentas Life Sciences) and sequenced by using M13 forward and reverse primers from Agri-Genome Labs Pvt. Ltd., India. The resulting sequences were extracted, using BioEdit 7.2.1 software. Homology searches were performed, using BLAST program (Altschul et al. 1990) of NCBI website. The *polh* gene sequences were translated to protein, using protein translation tool ExPASy (Expert Protein Analysis System).

Genetic diversity analysis

The *polh* sequences amplified from eight isolates were compared with those from other geographical isolates to infer the genetic diversity. The *polh* sequences belonging to different geographical regions were obtained from the GenBank database (Table 2). Phylogenetic tree was constructed from the aligned *polh* nucleotide/deduced amino sequences with maximum likelihood method by following bootstrap method (replications = 1000) for variance estimation. The *granulin* sequence (PlxyGV-001-gran-SA) (AN: KJ939451.1) was used as an out-group. The pairwise genetic distance, which estimates number of nucleotide/amino acid substitutions per site between a pair of sequences, was determined. The analyses were carried out with MEGA 6.0 software (Tamura et al. 2013). The genetic diversity among different continents was estimated using DIVEIN software, which measures the mean genetic distances between groups of sequences (Deng et al. 2010). Kimura-2-parameter (Kimura 1980) and Jones-Taylor-Thornton substitution models (Jones et al. 1992) estimation were used for nucleotide and amino acid sequences comparison, respectively.

Results and discussion

Amplification with *polh*-specific primers gave an amplimer of approximately of 740 bp from all the eight *Hear*NPV isolates (Fig. 1). BLAST results of sequenced DNA fragments showed 99 to 100 % similarity with the previously deposited *polh* sequences in the GenBank database. This confirmed the identity of the gene in the eight isolates used in the present study as *polh*. The resulting sequences were submitted to the GenBank database, with accession number as, MH029111, MH029112, MH029113, MH029114, MH029115, MH029116, MH029117, and MH029118 for Rajanukunte, Chandapura, Gulbarga, Dhule, Akola, Hyderabad, Udaipur, and Hosur isolates, respectively.

The genetic relatedness of different geographical isolates was determined by phylogenetic analysis of their *polh* sequences. The isolates were clustered into two clades (Clade1and Clade2) based on their *polh* nucleotide sequences (Fig. 2a). Clade2 was further divided into subclades. Most of the isolates were clustered with related geographical isolates, however, some with geographically distant isolates too. The two Indian isolates (2588-Neg-In and 1113-

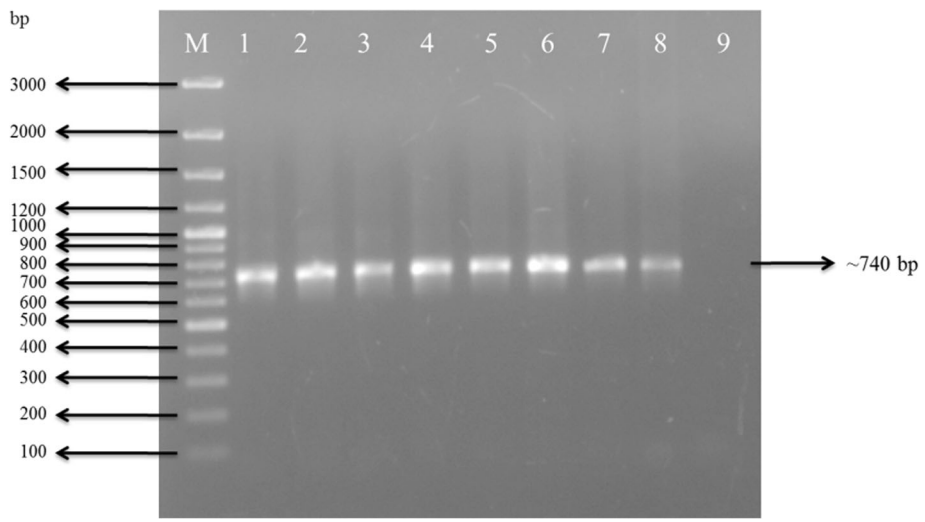

Fig. 1 Electrophoretic profile of *polyhedrin* gene amplified from total DNA of different geographical isolates of *Hear*NPV (M: Marker: 100bp ladder (Thermoscientific); Lane 1: Rke-In; 2: Chan-In; 3: Gul-In; 4: Dhl-In; 5: Akl-In; 6: Hyd-In; 7: Udr-In; 8: Hor-In; 9: Non template control

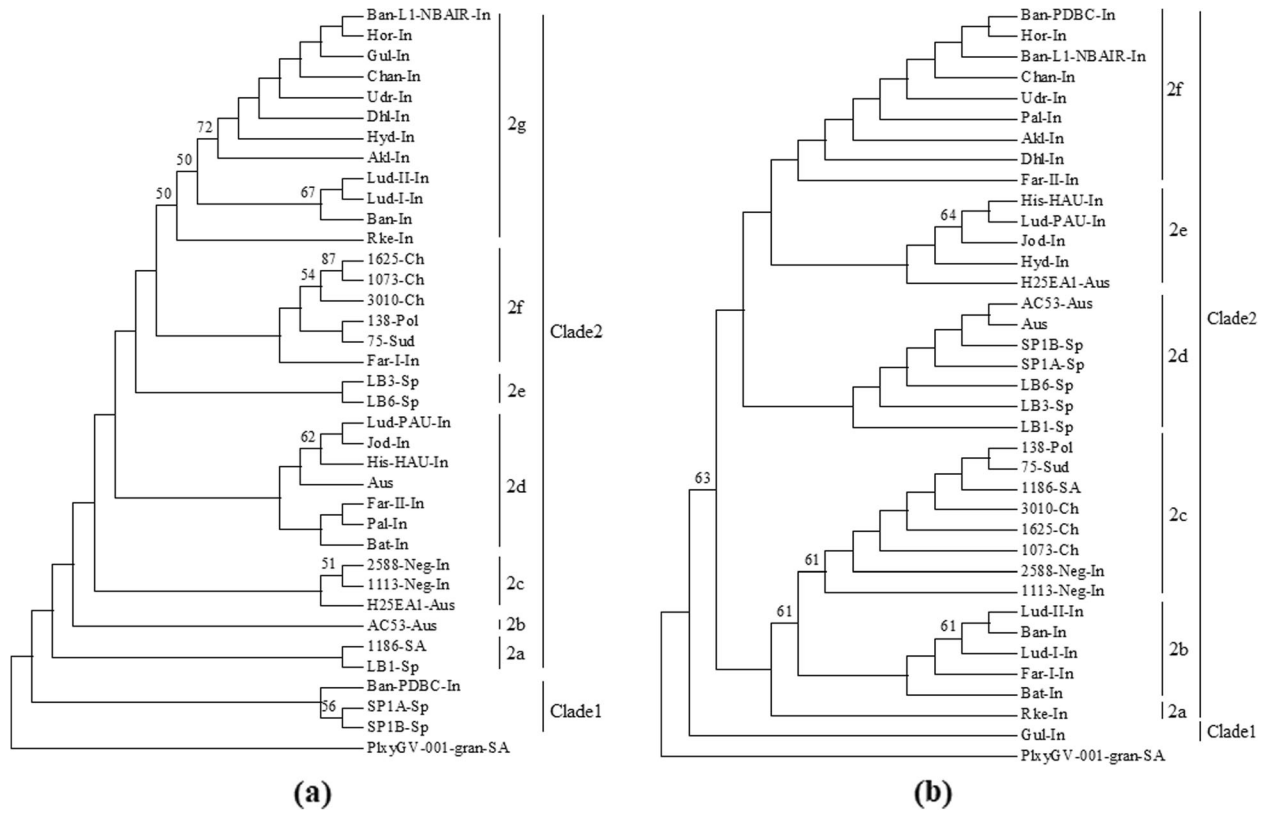

Fig. 2 Phylogenetic tree based on *polyhedrin* nucleotide (**a**)/ deduced amino acid sequences (**b**) of *Hear*NPV isolates from different geographical regions

Neg-In) formed a cluster with Australian isolate (H25EA1-Aus). Likewise, another Indian isolate (Far-1-In) showed relatedness with isolates from Sudan, Poland, and China. The isolates clustered with respect to the genetic distances among them. The Indian isolate (Ban-PDBC-In) shared a lower genetic distance of 0.0020 to 0.0040 substitutions per site (Table 3) with the Spanish isolates SP1A-Sp and SP1B-Sp and clustered in Clade1. It had genetic distance of 0.0040 to 0.0141 substitutions per site with other isolates.

Phylogenetic tree of deduced amino acid sequences showed a different clustering pattern than nucleotide sequences (Fig. 2b). The isolate (Ban-PDBC-In) clustered with geographically related Indian isolates. In contrast, it was placed with Spanish isolates (SP1A-Sp and SP1B-Sp), when analyzed based on nucleotide sequences. The overall genetic distances between *polh* nucleotide sequences ranged from 0.0000 to 0.0203 substitutions per site, while it ranged from 0.0000 to 0.0121 substitutions per site between deduced amino acid sequences (Table 3). This showed that *polh* protein is relatively conserved in the isolates, although there was a slightly higher variation in the nucleotide sequences.

The highest genetic diversity was reported in a group of isolates from India based on both *polh* nucleotide (0.0070 ± 0.0002 substitutions per site) and deduced amino acid (0.0057 ± 0.0003 substitutions per site) sequences (Table 4, Fig. 3). The groups of isolates from other continents showed zero diversity in amino acid sequences, although with some diversity in their nucleotide sequences.

The present findings showed genetic variations in geographically unrelated *Hear*NPV isolates. Similar kinds of results were reported by many scientific groups, in different nucleopolyhedrosis viruses infecting different insects. Kaur et al. (2014) observed that the *Hear*NPV isolates originating from closer locations were placed in one cluster in a phylogenetic tree. However, one Indian isolate (Bangalore) showed that it was closely related with isolates from Kenya, Israel, and South Africa. According to Rowley et al. (2011), some Indian and Chinese isolates were phylogenetically related with geographically distinct isolates. Many studies have reported genetic

Table 3 Pairwise genetic distance between the *polyhedrin* nucleotide/deduced amino acid sequences of *Hear*NPV isolates from different geographical regions (the numbers above the diagonal line indicate the amino acid genetic distance, while numbers below the diagonal line indicate nucleotide genetic distance)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Jod-In		0.0000	0.0121	0.0121	0.0120	0.0120	0.0060	0.0060	0.0060	0.0000	0.0060	0.0119	0.0060	0.0120	0.0060	0.0119	0.0121
2	Lud-PAU-In	0.0000		0.0121	0.0121	0.0120	0.0120	0.0060	0.0060	0.0060	0.0000	0.0060	0.0119	0.0060	0.0120	0.0060	0.0119	0.0121
3	Lud-I-In	0.0080	0.0080		0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0121	0.0060	0.0120	0.0060	0.0121	0.0060	0.0120	0.0000
4	Lud-II-In	0.0080	0.0080	0.0000		0.0060	0.0060	0.0060	0.0060	0.0060	0.0121	0.0060	0.0120	0.0060	0.0121	0.0060	0.0120	0.0000
5	Bat-In	0.0020	0.0020	0.0060	0.0060		0.0060	0.0060	0.0060	0.0060	0.0120	0.0060	0.0119	0.0060	0.0120	0.0060	0.0119	0.0060
6	Far-I-In	0.0040	0.0040	0.0060	0.0060	0.0020		0.0060	0.0060	0.0060	0.0120	0.0060	0.0120	0.0060	0.0121	0.0060	0.0120	0.0060
7	Far-II-In	0.0020	0.0020	0.0060	0.0060	0.0000	0.0020		0.0000	0.0000	0.0060	0.0000	0.0060	0.0000	0.0060	0.0000	0.0059	0.0060
8	Dhl-In	0.0080	0.0080	0.0040	0.0040	0.0060	0.0080	0.0060		0.0000	0.0060	0.0000	0.0060	0.0000	0.0060	0.0000	0.0059	0.0060
9	Akl-In	0.0100	0.0100	0.0060	0.0060	0.0080	0.0100	0.0080	0.0020		0.0060	0.0000	0.0060	0.0000	0.0060	0.0000	0.0059	0.0060
10	His-HAU-In	0.0000	0.0000	0.0080	0.0080	0.0020	0.0040	0.0020	0.0080	0.0100		0.0060	0.0119	0.0060	0.0120	0.0060	0.0119	0.0121
11	Pal-In	0.0020	0.0020	0.0060	0.0060	0.0000	0.0020	0.0000	0.0060	0.0080	0.0020		0.0060	0.0000	0.0060	0.0000	0.0059	0.0060
12	Hyd-In	0.0121	0.0121	0.0080	0.0080	0.0100	0.0121	0.0100	0.0040	0.0060	0.0121	0.0100		0.0060	0.0120	0.0060	0.0119	0.0120
13	Udr-In	0.0080	0.0080	0.0040	0.0040	0.0060	0.0080	0.0060	0.0000	0.0020	0.0080	0.0060	0.0040		0.0060	0.0000	0.0059	0.0060
14	Rke-In	0.0060	0.0060	0.0060	0.0060	0.0040	0.0060	0.0040	0.0060	0.0080	0.0060	0.0040	0.0100	0.0060		0.0060	0.0120	0.0121
15	Chan-In	0.0080	0.0080	0.0040	0.0040	0.0060	0.0080	0.0060	0.0000	0.0020	0.0080	0.0060	0.0040	0.0000	0.0060		0.0059	0.0060
16	Gul-In	0.0100	0.0100	0.0060	0.0060	0.0080	0.0100	0.0080	0.0020	0.0040	0.0100	0.0080	0.0060	0.0020	0.0080	0.0020		0.0120
17	Ban-In	0.0080	0.0080	0.0000	0.0000	0.0060	0.0060	0.0060	0.0040	0.0060	0.0080	0.0060	0.0080	0.0040	0.0060	0.0040	0.0060	
18	Ban-L1-NBAIR-In	0.0080	0.0080	0.0040	0.0040	0.0060	0.0080	0.0060	0.0000	0.0020	0.0080	0.0060	0.0040	0.0000	0.0060	0.0000	0.0020	0.0040
19	Ban-PDBC-In	0.0060	0.0060	0.0100	0.0100	0.0040	0.0060	0.0040	0.0100	0.0121	0.0060	0.0040	0.0141	0.0100	0.0080	0.0100	0.0121	0.0100
20	Hor-In	0.0080	0.0080	0.0040	0.0040	0.0060	0.0080	0.0060	0.0000	0.0020	0.0080	0.0060	0.0040	0.0000	0.0060	0.0000	0.0020	0.0040
21	1113-Neg-In	0.0060	0.0060	0.0060	0.0060	0.0040	0.0040	0.0040	0.0100	0.0121	0.0060	0.0040	0.0141	0.0100	0.0080	0.0100	0.0121	0.0060
22	2588-Neg-In	0.0060	0.0060	0.0060	0.0060	0.0040	0.0040	0.0040	0.0100	0.0121	0.0060	0.0040	0.0141	0.0100	0.0080	0.0100	0.0121	0.0060
23	1073-Ch	0.0120	0.0120	0.0121	0.0121	0.0100	0.0100	0.0100	0.0162	0.0182	0.0120	0.0100	0.0203	0.0162	0.0141	0.0162	0.0182	0.0121
24	1625-Ch	0.0120	0.0120	0.0121	0.0121	0.0100	0.0100	0.0100	0.0162	0.0182	0.0120	0.0100	0.0203	0.0162	0.0141	0.0162	0.0182	0.0121
25	3010-Ch	0.0080	0.0080	0.0080	0.0080	0.0060	0.0060	0.0060	0.0121	0.0141	0.0080	0.0060	0.0162	0.0121	0.0100	0.0121	0.0141	0.0080
26	1186-SA	0.0120	0.0120	0.0121	0.0121	0.0100	0.0100	0.0100	0.0162	0.0182	0.0120	0.0100	0.0203	0.0162	0.0141	0.0162	0.0182	0.0121
27	75-Sud	0.0040	0.0040	0.0040	0.0040	0.0020	0.0020	0.0020	0.0080	0.0100	0.0040	0.0020	0.0121	0.0080	0.0060	0.0080	0.0100	0.0040
28	LB1-Sp	0.0080	0.0080	0.0121	0.0121	0.0060	0.0080	0.0060	0.0121	0.0141	0.0080	0.0060	0.0162	0.0121	0.0100	0.0121	0.0141	0.0121
29	LB3-Sp	0.0040	0.0040	0.0080	0.0080	0.0020	0.0040	0.0020	0.0080	0.0100	0.0040	0.0020	0.0121	0.0080	0.0060	0.0080	0.0100	0.0080
30	LB6-Sp	0.0040	0.0040	0.0080	0.0080	0.0020	0.0040	0.0020	0.0080	0.0100	0.0040	0.0020	0.0121	0.0080	0.0060	0.0080	0.0100	0.0080
31	SP1A-Sp	0.0100	0.0100	0.0141	0.0141	0.0080	0.0100	0.0080	0.0141	0.0162	0.0100	0.0080	0.0183	0.0141	0.0121	0.0141	0.0162	0.0141
32	SP1B-Sp	0.0080	0.0080	0.0121	0.0121	0.0060	0.0080	0.0060	0.0121	0.0141	0.0080	0.0060	0.0162	0.0121	0.0100	0.0121	0.0141	0.0121

Table 3 Pairwise genetic distance between the *polyhedrin* nucleotide/deduced amino acid sequences of *Hear*NPV isolates from different geographical regions (the numbers above the diagonal line indicate the amino acid genetic distance, while numbers below the diagonal line indicate nucleotide genetic distance) *(Continued)*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
33	138-Pol	0.0040	0.0040	0.0040	0.0040	0.0020	0.0020	0.0020	0.0080	0.0100	0.0040	0.0020	0.0121	0.0080	0.0060	0.0080	0.0100	0.0040
34	Aus	0.0060	0.0060	0.0100	0.0100	0.0040	0.0060	0.0040	0.0100	0.0121	0.0060	0.0040	0.0141	0.0100	0.0080	0.0100	0.0121	0.0100
35	AC53-Aus	0.0060	0.0060	0.0100	0.0100	0.0040	0.0060	0.0040	0.0100	0.0121	0.0060	0.0040	0.0141	0.0100	0.0080	0.0100	0.0121	0.0100
36	H25EA1-Aus	0.0040	0.0040	0.0080	0.0080	0.0020	0.0040	0.0020	0.0080	0.0100	0.0040	0.0020	0.0121	0.0080	0.0060	0.0080	0.0100	0.0080

Table 3 Pairwise genetic distance between the *polyhedrin* nucleotide/deduced amino acid sequences of *Hear*NPV isolates from different geographical regions (the numbers above the diagonal line indicate the amino acid genetic distance, while numbers below the diagonal line indicate nucleotide genetic distance) *(Continued)*

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	0.0060	0.0060	0.0060	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0060	0.0060	0.0060	0.0060	0.0060	0.0120	0.0060	0.0060	0.0060
2	0.0060	0.0060	0.0060	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0060	0.0060	0.0060	0.0060	0.0060	0.0120	0.0060	0.0060	0.0060
3	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060
4	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060
5	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060
6	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060
7	0.0000	0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
8	0.0000	0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
9	0.0000	0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
10	0.0060	0.0060	0.0060	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	00060	0.0060	0.0060	0.0060	0.0060	0.0120	0.0060	0.0060	0.0060
11	0.0000	0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
12	0.0060	0.0060	0.0060	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	00060	0.0060	0.0060	0.0060	0.0060	0.0120	0.0060	0.0060	0.0060
13	0.0000	0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
14	0.0060	0.0060	0.0060	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	0.0120	00060	0.0060	0.0060	0.0060	0.0060	0.0120	0.0060	0.0060	0.0060
15	0.0000	0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
16	0.0059	0.0059	0.0059	0.0119	0.0119	0.0119	0.0119	0.0119	0.0119	0.0119	00059	0.0059	0.0059	0.0059	0.0059	0.0119	0.0059	0.0059	0.0059
17	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060
18		0.0000	0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
19	0.0100		0.0000	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
20	0.0000	0.0100		0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	0.0060	00000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
21	0.0100	0.0080	0.0100		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
22	0.0100	0.0080	0.0100	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
23	0.0162	0.0141	0.0162	0.0100	0.0100		0.0000	0.0000	0.0000	0.0000	00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
24	0.0162	0.0141	0.0162	0.0100	0.0100	0.0000		0.0000	0.0000	0.0000	00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
25	0.0121	0.0100	0.0121	0.0060	0.0060	0.0040	0.0040		0.0000	0.0000	00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
26	0.0162	0.0100	0.0162	0.0100	0.0100	0.0121	0.0121	0.0080		0.0000	00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
27	0.0080	0.0060	0.0080	0.0020	0.0020	0.0080	0.0080	0.0040	0.0080		00060	0.0060	0.0060	0.0060	0.0060	0.0000	0.0060	0.0060	0.0060
28	0.0121	0.0060	0.0121	0.0100	0.0100	0.0162	0.0162	0.0121	0.0080	0.0080		0.0000	0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
29	0.0080	0.0060	0.0080	0.0060	0.0060	0.0121	0.0121	0.0080	0.0121	0.0040	0.0080		0.0000	0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
30	0.0080	0.0060	0.0080	0.0060	0.0060	0.0121	0.0121	0.0080	0.0121	0.0040	0.0080	0.0000		0.0000	0.0000	0.0060	0.0000	0.0000	0.0000
31	0.0141	0.0040	0.0141	0.0121	0.0121	0.0141	0.0141	0.0100	0.0100	0.0100	0.0100	0.0100	0.0100		0.0000	0.0060	0.0000	0.0000	0.0000
32	0.0121	0.0020	0.0121	0.0100	0.0100	0.0121	0.0121	0.0080	0.0080	0.0080	0.0080	0.0080	0.0080	0.0020		0.0060	0.0000	0.0000	0.0000

Table 3 Pairwise genetic distance between the *polyhedrin* nucleotide/deduced amino acid sequences of *Hear*NPV isolates from different geographical regions (the numbers above the diagonal line indicate the amino acid genetic distance, while numbers below the diagonal line indicate nucleotide genetic distance) *(Continued)*

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
33	0.0080	0.0060	0.0080	0.0020	0.0020	0.0080	0.0080	0.0040	0.0080	0.0000	0.0080	0.0040	0.0040	0.0100	0.0080		0.0060	0.0060	0.0060
34	0.0100	0.0080	0.0100	0.0080	0.0080	0.0060	0.0060	0.0100	0.0141	0.0060	0.0100	0.0060	0.0060	0.0121	0.0100	0.0060		0.0000	0.0000
35	0.0100	0.0040	0.0100	0.0040	0.0040	0.0141	0.0141	0.0100	0.0100	0.0060	0.0060	0.0060	0.0060	0.0080	0.0060	0.0060	0.0080		0.0000
36	0.0080	0.0060	0.0080	0.0020	0.0020	0.0121	0.0121	0.0080	0.0121	0.0040	0.0080	0.0040	0.0040	0.0100	0.0080	0.0040	0.0060	0.0020	

Table 4 Pairwise genetic diversity of groups of *HearNPV* isolates from different continents

Group	Number of sequences	Genetic diversity	
		Nucleotide Mean ± S.E.	Amino acid Mean ± S.E.
Australia	3	0.0054 ± 0.0014	0.0000 ± 0.0000
Poland	1	0.0000 ± 0.0000	0.0000 ± 0.0000
Spain	5	0.0065 ± 0.0010	0.0000 ± 0.0000
Sudan	1	0.0000 ± 0.0000	0.0000 ± 0.0000
South Africa	1	0.0000 ± 0.0000	0.0000 ± 0.0000
China	3	0.0039 ± 0.0011	0.0000 ± 0.0000
India	22	0.0070 ± 0.0002	0.0057 ± 0.0003

variation in geographically distinct baculoviruses based on the restriction fragment analysis of whole genomes. *Hear*NPV isolates collected from Spain and Portugal showed genetic variations based on their restriction fragment profile (Figueiredo et al. 1999). Similar kinds of results were found in restriction endonuclease analysis of *Spodoptera litura* NPV and *S. littoralis* NPV from Japan, Vietnam, Malaysia, India, and Egypt (Takatsuka et al. 2003) also revealed similar findings. The regional isolates of *Hear*NPV from India (Patel et al. 2009) and *S. frugiperda* NPV from Brazil (Barreto et al. 2005) showed differences in their RAPD profiling. The *Hear*NPV samples collected from South Africa, Zimbabwe, Thailand, and Kenya were identified as variants of the same baculovirus (Ogembo et al. 2007).

There are several hypotheses for pathogen genotypic variations, including host immune response specificity, trade-offs between pathogen fitness components, interactions between pathogen genotypes within hosts, and differential selection of pathogen genotypes. Pathogen genotypes with low fitness in some hosts may be sustained in populations by having relatively high fitness under different ecological conditions. In other words, genotypic variation in pathogen populations may be promoted by genotype (G), environment (E), and (G*E) interactions acting at the level of the pathogen genotype (Hodgson et al. 2002). The other reasons for these variations could be due to the insertion of host DNA into the viral genome, duplication of virus sequences, insertions, deletions, and point mutations in the viral DNA.

These whole genome or gene-specific genotypic variations can be used as a genetic marker for understanding of the biodiversity of the viruses, their evolution patterns, and recombination. This understanding combined with bioassay studies could lead to develop or engineer potent biological control agents (BCAs) suitable for pests distributed over wide geographical areas or integrate such BCAs with management strategies to make them more efficient, economical, and environmentally safe.

Conclusion

The present study revealed genetic variations among the *Hear*NPV isolates belonging to different geographical regions. This information can be further validated by studying other highly conserved genes. The genetic variations coupled with bioassay studies can be used for the selection of most virulent isolates for the designing commercial formulations.

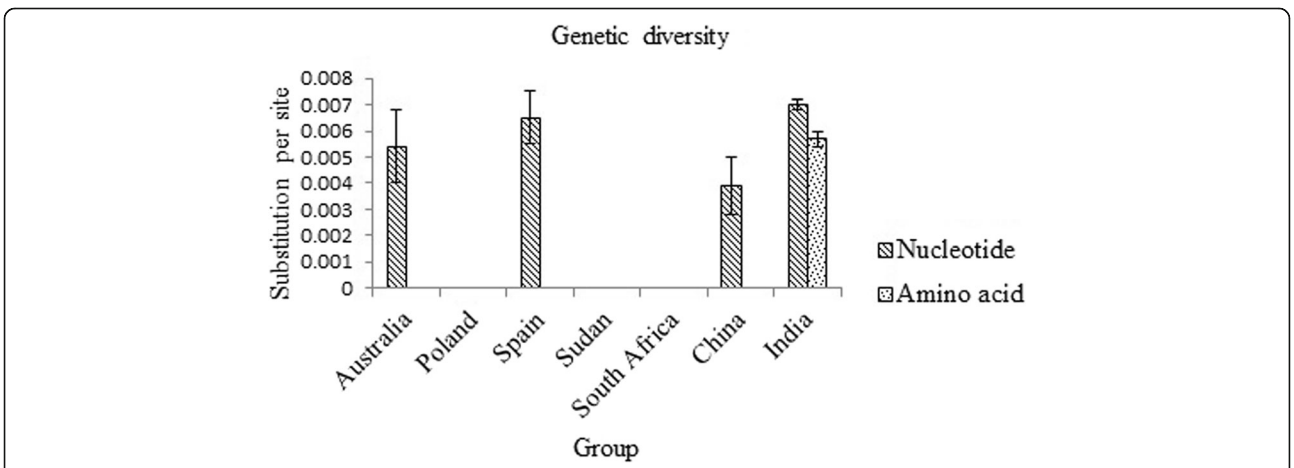

Fig. 3 Pairwise genetic diversity of *Hear*NPV isolates from different geographical regions

Abbreviations
*Hear*NPV: *Helicoverpa armigera* nucleopolyhedrosis virus; *polh*: *polyhedrin*

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Authors' contributions
KSJ and AP planned and directed the experiment. RS and KRT conducted the experiment and prepared the manuscript draft. All authors have read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available in the GenBank database [https://www.ncbi.nlm.nih.gov/nucleotide].

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no competing interests

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