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Comparative study of hot-start PCR characterized species specific conserved gene regions of a biocontrol agent *Helicoverpa armigera* nucleopolyhedrovirus with its whole genome

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Abstract
The present investigation was aimed to isolate the strain of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) from virus infected larvae collected from Ludhiana, India during October 2015. Four conserved gene regions of *Helicoverpa armigera* NPV (HearNPV) genome, *viz*, polyhedrin (*polh*), inhibitor of apoptosis-2 (*iap2*), DNA binding protein (*dbp*) and *per os* infectivity factor-1 (*pif*1) genes were characterized by species-specific primers, based on a Hot-Start PCR approach, for an Indian strain of HearNPV. The species-specific primers designed for these genes enabled a successful amplification with the expected base pair size. In search of any insertion / deletion/ difference in the nucleotide sequences, a comparative analysis was studied between these PCR characterized genes and the respective ORFs from the recently sequenced genomes of HearNPV L1 (India), HearNPV SP1A (Spain), HearNPV NNg1 (Kenya) and HearNPV G4 (China). Newly designed species specific primers will also help in quick and reliable identification of HearNPV in the baculovirus sample.

Keywords: *Helicoverpa armigera* NPV (HearNPV), Baculovirus, Hot-Start PCR, Insect virus, Lepidoptera, Species specific primers

1. Introduction
Interests in the investigation on baculovirus has increased due to its ability to infect over 600 insect species of different insect orders such as Lepidoptera, Coleoptera, Hymenoptera, Diptera and Neuroptera as well as due to its specificity towards its host [1]. In the year 2009, the International Committee on Taxonomy of viruses has reported that viruses derived only from the insect orders Lepidoptera, Hymenoptera and Diptera are the members of the family baculoviridae, which facilitated the way to the potential use of baculoviruses in insect pest management [2]. Even with its limitation, of the extensive time period required to kill the insect host, researchers investigate to improve the use of baculovirus as a better biocontrol agent over the pesticide [3]. This showed the way for detailed research on baculovirus genome and characterization of its conserved gene regions. In the last decade, studies have been conducted to characterize several conserved gene regions of baculovirus, specifically the NPV infecting *Helicoverpa armigera*. [4]
H. armigera (Hübner) is the major cause in the low production of commercial crops viz, pigeon pea, cotton, maize and vegetable crops like tomatoes.[5] It is a very serious pest causing 25-70% crop losses in pigeon pea, chickpea and several vegetable crops leading to its status asan agriculturally important pest in India [6]. Due to its highly adaptive nature, it sustains in various agro-climatic conditions and has developed resistance to various insecticides. Since 1970s, efforts have been made for biological control of this pest through several potential isolates of nucleopolyhedrovirus (NPVs) but most of these formulations used are considered to be based on some virus strain [7]. Since most of the isolates of NPV remain uncharacterized, it is necessary to identify specific strains through recent molecular techniques.
The molecular characterization of several NPV strains highlight the fluid nature of its genome with the evidence of frequent gene arrangements leading to the evolution of the new strain and characterization of conserved gene regions.[8] In the last decade, studies have been conducted on characterizing several conserved gene regions of baculoviruses, specifically *H. armigera* NPV (HearNPV). Till date, researchers, although, have characterized the polyhedrin (*polh*) [9], per os infectivity factor-1 (*pif*1) [10, 11], inhibitor of apoptosis-2 (*iap*2) [12, 13, 14] late expression

factor (*lef-8*) by [9] and DNA binding protein (*dbp*) by [15], which was mainly based on the insertion-deletion of individual gene from the complete genome and producing recombinant viral clones or with the use of degenerate primers. Nevertheless, in the current study, four conserved gene regions were characterized using species specific primers and validated four genes by adopting a Hot-Start PCR in HearNPV L1 isolate of India. We analyzed their protein data and explained the comparative study with the whole genome. These chosen genes play a crucial role in insecticidal activity and establish successful infection to target insects, their conserved sequences not only will help in designing robust primers but also in the reliable identification of HearNPV in the baculovirus sample.

2. Materials and Methods

2.1 Insect culture: Virus: DNA isolation

The larvae of *H. armigera* were collected from the tomato field in Malur, Kolar district, Karnataka, India (13° 01'16 N, 77°56' 17 E) during October 2015. The colonies were maintained on artificial diet [16]. The entire rearing protocol as described by Hamed and Nadeem (2008) [17],was carried out under controlled conditions in Biological Oxygen Demand (BOD) incubators maintained at 27±0.5°C and 60% RH. These were reared for two generations before multiplying virus on them. The HearNPV L1 was isolated from diseases larvae of *H. armigera* collected from Ludhiana, India (30° 90'10 N, 75° 85' 73 E) and virus thus obtained was multiplied in the laboratory raised colonies of *H. armigera* at ICAR-NBAIR, Bengaluru, India. The Occlusion bodies (polyhedra) from the infected larvae were purified by centrifugation at 2500 rpm once for 1 minute. The debris was pelleted, whereas the OBs remained in the supernatant. For the isolation of the virus, the suspension was again subjected to centrifugation at 10000 rpm twice for 5 minutes, each time. The OB settled on the sides of the walls of the centrifuge vial. This process was repeated to isolate only the OBs leaving behind the contaminants. This pellet was then suspended in 1 ml of double distilled water and stored at -20°C for further use. [4]

DNA extractions were made using the method described by [18]. Diseased larvae were crushed in 0.1M sodium carbonate solution. The final suspension included 0.5 $mg.ml^{-1}$ of proteinase K and 1% SDS solution. This was followed by phenol chloroform: isoamylalcohol extraction with the final ethanol precipitation (ice cold 90% ethanol). The DNA was resuspended in a Tris-EDTA buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The extracted DNA was visualized in 0.8% agarose gel.

2.2 Primer designing

Primer sets for the amplification of the conserved gene regions were designed as species-specific primers (Table 1) according to the respective HearNPV sequences in the GenBank database of National Centre for Biotechnology Information (NCBI). Sequences for polyhedrin were available into the database with accession numbers FJ157294, FJ157292, FJ157295, FJ157293, FJ157291; but *iap*2, *pif*1 and *dbp* genes were not available, for which the sequences were traced from the whole genome submitted in the database. Alignment of the each gene group was used as a guide to enable identification of conserved nucleic acid sequences of the group to be used in the design of species specific primer for PCR using Primer 3 online software [19]. The major steps in Primer 3 were the structure and the melting temperature. Having the parameters of length 20-25bp, melting temperature of 55-65 ºC, sequence linguistic complexity >80%, purine and pyrimidine linguistic complexity >80% and the primer quality >90%.

Table 1: List of species-specific primers designed to characterize the genes, accompanied with the respective annealing temperature at which the genes were amplified.

Sl. No	Protein coding Gene	Primer sequence	Base pair Size	Annealing temperature*
1.	Polyhedrin (*polh*)	Forward 5'ACTCGTTACAGTTACAGCCCTACT 3' Reverse 5'CAGTGTATAGCGGAGCGTCA 3'	659bp	54C
2.	Inhibitor of Apoptosis-2 (*iap*2)	Forward 5'CGTCGATTGATCTACGACACA 3' Reverse 5'ATGTGGAATCCGATTTGGAA 3'	804bp	52C
3.	DNA binding Protein(*dbp*)	Forward 5'TGATCGACTCCTTCGATGC 3' Reverse 5'AACGATCTATAAACTGCGAGGAG 3'	972bp	50C
4.	Per os infectivity Factor-1 (*pif*1)	Forward 5'CCGCTACCAATTCACGAAG3' Reverse 5' TCATGTGTACGTGGAATAATTGG3'	1392bp	48C

*The cycling parameters were same for amplification of all the genes except for the annealing temperature. Annealing temperatures below and above the mentioned figures (Fig. 1) gave low yield amplicons or co-amplifications.

2.3 PCR amplification and sequencing

Each PCR reaction mixture consisted of a 50ng DNA sample, 0.25 unit of Taq DNA polymerase, 10x Taq buffer, 2.5 mM $MgCl_2$, 2.5mM each of four dNTPs and 1µl (Fermentas, Germany) of each forward and reverse primer in a final volume of 50 µl. Since a Hot-Start PCR approach, which is a modified form of PCR that avoids a non-specific amplification of DNA by inactivating the Taq polymerase at lower temperatures, was made. Taq DNA polymerase was added to the final volume after the denaturation temperature in the PCR cycle. The cycling parameters for PCR were as follows: cycle 1of denaturation, 4 min at 95°C, cycle 2-35, 2 min at 95°C, 2 min 46°C and 1 min at 72°C, elongation for 10 min at 72°C [20]. Amplification was accomplished using a DNA thermal cycler (Biorad C 1000 Thermal cycler). Each time, each gene was amplified, the annealing temperature varied based on their T_m value. The corresponding annealing temperatures that gave a maximal yield of the PCR amplicons for *polh*, *iap, pif* and *dbp* is given in Table 1. The PCR products were analyzed by a 1.5% agarose gel along with the MassRuler™ Low Range DNA Ruler (Fermentas, Germany). After the PCR, the gel was stained with ethidium bromide (10mg/mL) and photographed using Vilber Loumart™ Gel Documentation System.

2.4 Gene Sequence, whole genome comparison and phylogenetic analysis

The PCR products were purified using the MinElute PCR purification kit (M/s Qiagen, Germany) and DNA sequencing was performed by M/s. Eurofins India Pvt. Ltd., Bengaluru, India. The sequences obtained through our studies were subjected to an online bioinformatics suit JustBio

(http://www.justbio.com/hosted-tools.html), for analysis of DNA/RNA, proteins and arrays, to obtain its deduced amino acid. DNA and protein comparisons with entries in the sequence databases were performed using selected Basic Local Alignment Search Tool (BLAST v2.2.0, https://blast.ncbi.nlm.nih.gov/Blast.cgi). The comparison was studied based on the base pair size of the amplified PCR product against the ORFs in the genome, aligned nucleotide (nt) span, difference in the number of nucleotides and presence of gaps within the sequences. The same attributes were used for comparison at the amino acid level also. Further sequences amplified by species specific primers were subjected to phylogenetic analysis with their counterparts from four different genomes viz, L1, SP1A, NNg1, and G4 respectively. The sequences were aligned using ClustalW with default parameters of gap opening penalty 15, and a gap-extension 6.06 in pairwise and 6.06 in multiple alignments and phylogenetic analysis was performed using MEGA v7.0 software. For each gene, a Neighbour-joining (NJ) tree was constructed [21] by applying 1000 bootstrap support [22] using p-distance parameter under general time reversible (GTR) model.

3. Results and Discussion

The species-specific primer successfully amplified the expected DNA fragments from Hear NPV without any non-specific amplification. All the amplified products were successfully sequenced and analysed through several online bioinformatics tools.

3.1 Analysis of polyhedrin (polh) gene

Polyhedrin gene was amplified at 659bp (GenBank Accession No. KY432399) encoding a peptide sequence of 219 amino acids (Fig. 1A). The polyhedrin sequence subjected to the BLAST program revealed 99% of homology with 20 isolates of HearNPV and 99% of homology with 6 isolates of *H. zea* (HzNPV) The nucleotide sequence contained the conserved TAAG motif at the immediate upstream (at the 6th position) which is considered the polyhedrin gene promoter (Fig. 2A). At the amino acids level, comparison study with BLAST program yield similar results. The polypeptide had 99% homology with corresponding HearNPVpolyhedrin homologs. The BLAST tool detected 2 putative conserved domains, one upstream and another downstream the translated polypeptide sequence. The deduced polypeptide contained 35 strongly basic, 35 strongly acidic, 104 hydrophobic and 45 polar amino acid (Fig. 3A); with a calculated molecular mass of 25.649 kDa and an isoelectric point of 5.61.

3.2 Analysis of inhibitor of apoptosis-2 (iap2) gene

The PCR amplicons for *iap*2 gene produced a nucleotide sequence of 804bp (GenBank Accession No. KY432400) encoding a 268 amino acid sequence (Fig. 1B). The sequence had 99% similarity with its homolgous HearNPV *iap* gene and the deduced amino acid, when subjected to % similarity test showed similarity in the range of 98-100% with other HearNPV*iap*2 protein homolog. This ORF was preceded by a putative TATA box element at 9th position, upstream of the gene (Fig. 2B). The polypeptide contained two "Baculovirus IAP Repeats" (BIR) domains, BIR 1 and BIR2, followed by the RING- domain in the C- terminus. BIR 1 included a nucleotide span between 18-83 bp and BIR 2 included a nucleotide span between 118-186 bp with the conserved presence and spacing of cysteine and histidine residue of $CX_2CX_6WX_9HX_6C$. The ring domain, followed by with a nucleotide span between 217-262 bp, contained the C_3HC_4 motif. The polypeptide contained 39 basic, 38 acidic, 120 hydrophobic and 71 polar amino acids (Fig. 3B). The theoretical molecular weight calculated was 31.52 kDa and calculated isoelectric point was 5.66.

3.3 Analysis of DNA binding protein (dbp) gene

Dbp gene was amplified at 972bp (GenBank Accession No. KY432401) and the deduced polypeptide sequence contained 323 amino acids (Fig. 1C). Both the genetic and protein data gave 100 % similarity with the corresponding HearNPV *dbp* homologs. The polypeptide sequence gave a calculated molecular mass of 37.55 kDa and an isoelectric point of 5.81. The ratio of basic/acidic/hydrophobic/polar amino acids was 47/46/142/88 (Fig. 3C). All the characterized genes were submitted in the GenBank and the proteins were submitted simultaneously in the protein databank of NCBI.

3.4 Analysis of per os infectivity iactor-1 (pif1) gene

Pif gene amplified at 1392bp (GenBank Accession No. KY432402) encoded polypeptide sequence of 464 amino acids (Fig. 1D). When subjected to the BLAST search for homology, the sequence was compared with 6 available gene sequences in the database, of which 4 were the sequences derived from the complete genome. Our sequence showed 99% similarity with 4 isolates of HearNPV and 98% similarity with 2 isolates of *Helicoverpa zea* nucleopolyhedrovirus (HzNPV). The putative baculoviral promoter motif, TATA (A) was observed in the upstream of the sequence data (Fig. 2C). The % similarity study in amino acid level aligned the sequence 99% with 4 isolates of HearNPV *pif* homologs and 98% with 2 isolates of HzNPV *pif* sequence. The polypeptide included 53 strongly basic, 48 strongly acidic, 224 hydrophobic and 139 polar amino acid; with a calculated molecular mass of 52.96 kDa and an isoelectric point of 5.80 (Fig. 3D). Within the polypeptide sequence, 79 amino acids were conserved among the most diverse members of baculovirus, of which, 23 were cysteine residues.

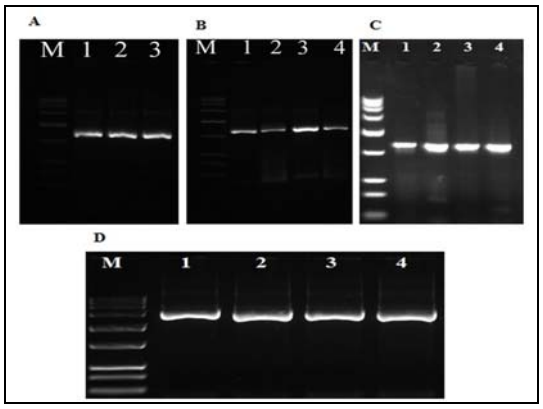

Fig 1: Representative Hot-Start PCR results showing the amplification of (A) *polh* gene at 700 bp (B) *iap*2gene at 800bp (C) *dbp* gene at 900bp (D) *pif*1 gene at 1200bp. M: Genei^{TM} Low Range DNA ruler; Lane 1-3: replicas of *polh* and 1-4 replicas of *iap*2, *dbp* and *pif*1 genes of *H. armigera* NPV (HearNPV).

A 1 TACTT **TAAG** AATTTAGGTGCTGTTATTAAAAATGCCAAACGCAAGAAGCATTTAGA 56

B 1 ATGATGTCC **TATA** TGGAATCCGATTTGGAATTATTAAAAACTGAATCATATCGATA 56

C 95 AGGCTTGGCAAATTGTCAGTACTTTGACGAACAGACCAAACT **TATAA** TGCGCGCG 150

Fig 2: Nucleotide sequence containing the conserved motifs. (A) Presence of the polyhedrin gene promoter, TAAG, at the 6th position in polyhedrin gene. (B) Putative TATA box element at the 10th position, considered to be the transcriptional regulatory signals in *iap*2 gene. (C) TATA (A), putative baculovirus promoter motif in *pif*1 gene located at the 136th position. The motifs are depicted as bold text.

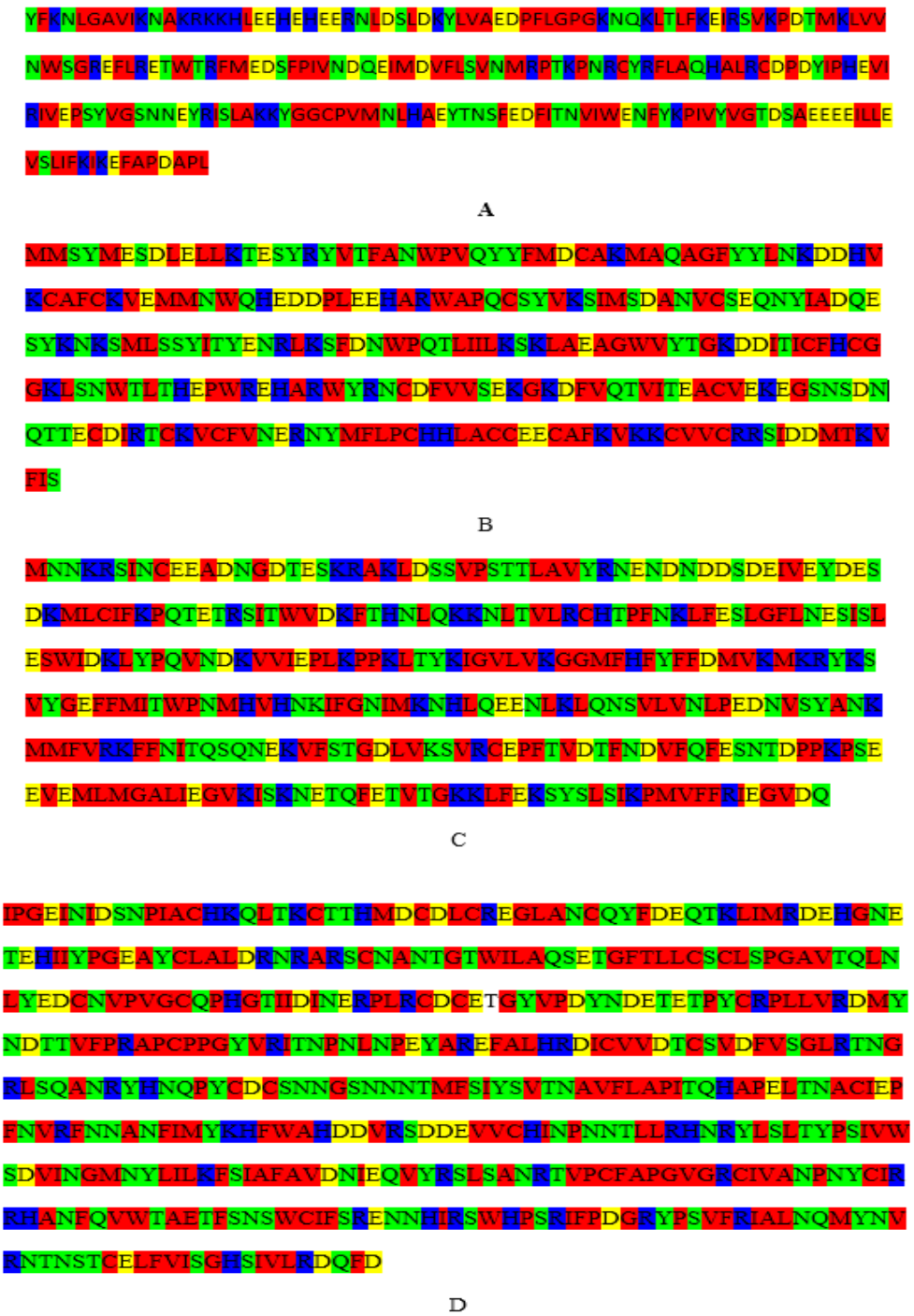

Fig 3: Amino acid sequence of (A) polyhedron (B) *iap*2(C) *dbp*(D) *pif*1. The hydrophobic amino acids are presented in red, polar amino acids in green, basic amino acids in blue and acidic amino acids in yellow. In all the amino acid sequence, red shaded region predominate the most, indicating the hydrophobic nature of the polypeptide.

3.5 Comparative and phylogenetic analysis between amplified genes and sequences from the whole genome.

The characterized genes were compared with the recently sequenced genome viz, L1, SP1A, NNg1 and G4 and the previously sequenced. *Helicoverpa armigera* Multicapsid nucleopolyhedrovirus (HearMNPV) genome. Being some of the major genes involved in the successful infection and genetic replication, all the genes were present in L1, NNg1, G4 and SP1A as well as in HearMNPV genome. Polyhedrin gene characterized had 659 bp, which was 82bp short of ORF 1 in NNg1, G4 and SP1A that contained 741bp. When compared with L1 and NNg1, our sequence showed 99% homology, matching a nucleotide span of 61- 719bp of the NNg1 *polh* gene. The results revealed a difference of 8 nucleotides with no gaps. When the same was compared with the G4 and SP1A, similar results were obtained, but with 7 nucleotide difference. At the amino acid level, our sequence encoded a polypeptide sequence of 219 amino acids, where as in NNg1, G4 and SP1A genomes, the *polh* gene encoded a polypeptide sequence of 246 amino acids. Our protein sequence contained 35 strongly basic and acidic amino acids each, 104 hydrophobic and 45 polar amino acids, where in NNg1, G4 and SP1A had 38, 36, 112 and 60 amino acids, respectively. However, this change in the number of amino acid in each group did not reflect in the aligned protein sequence showing a 100% homology without any gaps. The amino acid sequence matched from 22nd amino acid to 240th amino acid with both the protein sequence taken as the reference (protein BLAST, data not shown). Phylogenetic analysis revealed their close association with L1 genome as they fall under the same clade with 96% of bootstrap value (Fig. 4A).

The present study characterized *iap*2gene of 804 bp, 3 nucleotides lesser than NNg1 and SP1A *iap*2 gene (807bp) but 51 nucleotide more than G4 *iap* gene (753bp). Our sequence had 98% similarity with NNg1 because of 12 nucleotide differences, but with no gap and 100% similarity with G4 with only 1 nucleotide difference and no gap. The difference in the bp size (G4) interfered in the number of amino acid of the G4 *iap*2 protein sequence compared, which came up to only 250 amino acid (protein BLAST, data not shown), where in our protein sequence and NNg1 *iap*protein sequence had 268 amino acid. However, comparing the amino acid groups, both NNg1 and our sequence did not share similar figures for strongly basic, hydrophobic and polar amino acids, *i.e*., 40/39, 118/120 and 72/71 but had a similar number of acidic amino acid (38aa). Fig. 4Bshows the relationship of *iap*2 gene of Ludhiana isolate with SP1A *iap*2 gene as they fall under the same clade with 93% of bootstrap value.

In case of *dbp* gene, we amplified 972bp amplicon that coincides with presence of 972bp in L1, NNg1 and SP1A and G4 *dbp* genes. Difference of 4nt as compared to NNg1 was the only difference exhibited which did not even reflect on the amino acid level. The alignment was 100% without any gaps (data not shown). The three sequences contained same number of strongly basic, acidic, hydrophobic and polar amino acids, which were 47, 46, 142 and 88 respectively. Phylogenetic analysis of *dbp* gene of Ludhiana isolate with L1 *dbp* gene revealed their close association as they fall under the same clade with 93% of bootstrap value (Fig. 4C).

Per os infectivity factor-1 gene present in both the genome contained 1587 nt, whereas our sequence was only 1392nt. The difference in the base pair size was also combined with a difference of 19 nt, compared to L1, NNg1 and SP1A were as 16nt, compared to G4. Two gaps were also observed in our sequence in comparison with three genomes. The alignment result (99% homology) showed that our sequence aligned with both genomes matching the nt length between 145-1537nt. The deduced polypeptide encoding 464 amino acids, 64 amino acid lesser than the protein sequence taken as reference (528 amino acid), had aligned from the 49th amino acid to the 512th amino acid of the same (protein BLAST, data not shown). The difference in the nucleotide within the aligned sequence was reflected in the polypeptide sequence also, where in the change was of 3 amino acids. 53 strongly basic, 48 acidic, 224 hydrophobic and 139 polar amino acids comprised our *pif*1 polypeptide whereas, four genomes L1, NNg1, G4 and SP1A contained 57 basic and 262 hydrophobic amino acid but differed with one amino acid in acidic and polar amino acid. Fig. 4D. Shows *pif*1 gene of Ludhiana isolate was falling under the same clade consisting of *pif*1 genes of L1, SP1A and NNg1 genome; however G4 genome was forming an out group as G4 isolate from China was least virulent.

The result of the present work substantiatesour hypothesis that the PCR based approach towards virus detection, identification and characterization is highly sensitive, cost-efficient and reliable. Many published reports on characterization studies depended on southern hybridization using probes or traditional serological methods (ELISA) or even the use of degenerate primers. The technique of southern hybridization requires multiple probes of various NPVs for detection of baculovirus [23] used serological methods, proving it to be effective to identify baculoviruses, but encountered difficulties to interpret antigenic cross reactivity or close antigenic relationships leading to confused results. Even with the use of degenerate primers [18], problem faced was that the concentration of some permutation in the mixture is so small that the amplification might be inhibited. Due to these reasons, PCR, the easy and fast method using species specific primers is now preferred over other techniques. With such a powerful tool, a good set of primers is an obligatory feature for sample screening. Hence, several criteria should be observed while designing primers specific towards a particular species. In the current study, we have determined all the genes without disturbing its conserved sequences and the promoter regions. Comparison with its similar homolog highlighted that the conserved regions were amplified without fail.

Polyhedrin is known to be highly conserved and so far, the major gene used to construct possible phylogenetic relationships among the occluded baculovirus. It has been reported that lepidopteran NPV *polh* are closely related to one another. [24]Asdiscussed the homology analysis of *polh* gene between HearNPV and HzNPV proving its similarity up to 100%. Similarly, comparison of *Autographa californica* Multicapisd nucleopolyhedrovirus (AcMNPV) and HearNPV revealed 85% similarity. We subjected our sequence for homology analysis and the results are in conformity with the above cited studies. Presence of conserved motifs both upstream anddownstream of the gene was well explained in *Perina nuda* nucleopolyhedrovirus (PenuNPV), *Spodoptera litura* nucleopolyhedrovirus (SpliNPV) and HearNPV [25-24]. All these studies were based on southern hybridization technique, however, the interesting fact is that, even with the PCR approach, we were able to amplify the expected conserved DNA fragment. The insecticidal potency of occlusion bodies (OBs) relates to the successful infection determined by the per os infectivity factors, the usual route of

transmission [27]. The interesting mechanism begins with the ingestion of OBs by the susceptible insect larvae followed by the release of occlusion derived virions (ODVs) when OBs dissolve in the alkaline insect midgut. *Pif*1plays its role in the subsequent action, wherein ODV membrane fuses with membranes of midgut epithelial cells, releasing the nucleocapsids that migrate towards the nucleus, leading to infection [28]. Recent reports on characterization and functional investigation of *pif*gene in HearNPV are available specifically on *pif*-2 and the entire *pif* complex [29, 30, 10] worked on *pif*1 gene of SpliNPV, wherein the presence of conserved cysteine residues was mentioned.

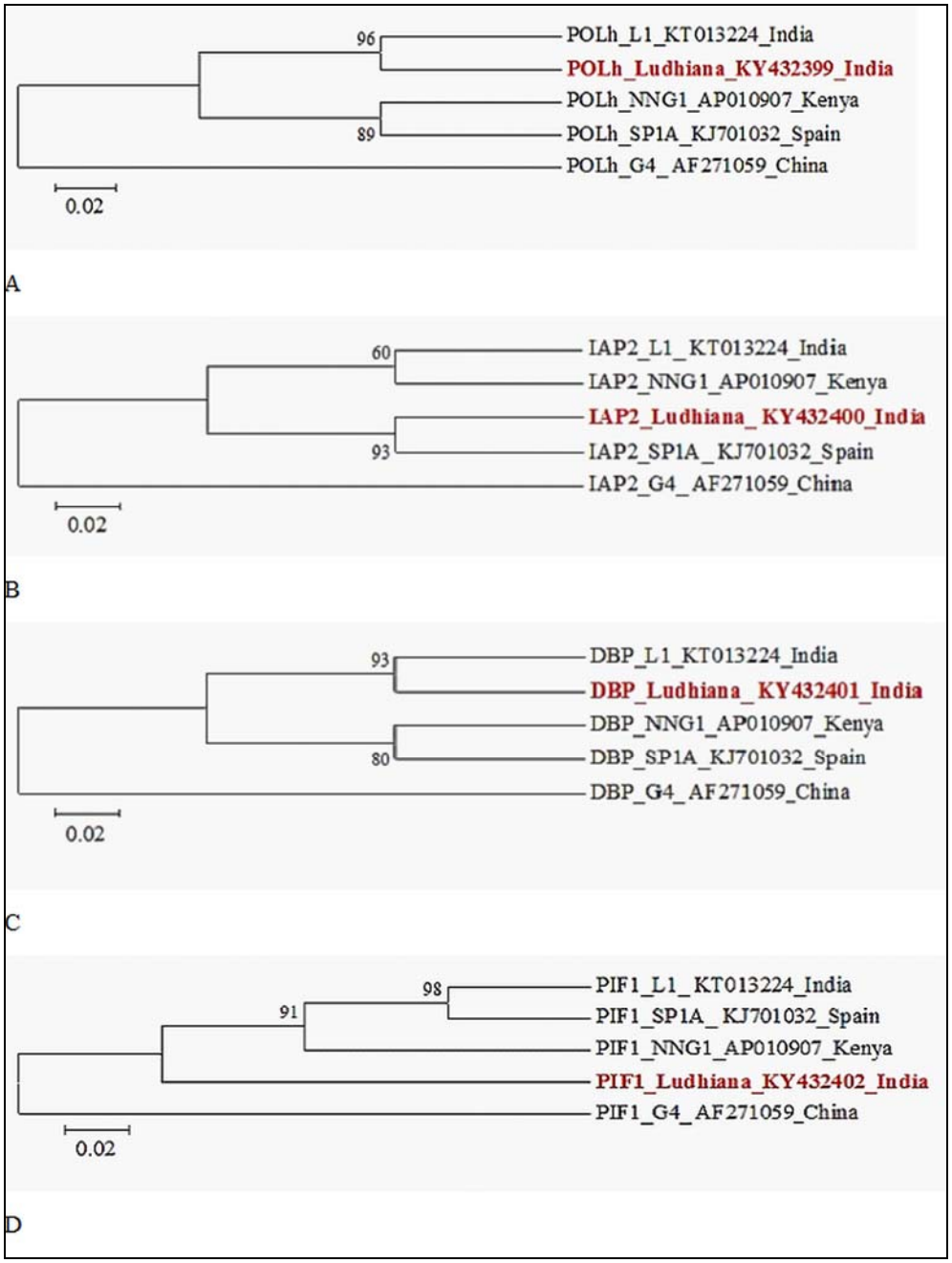

Fig 4: Phylogenetic tree of 4 genes viz, *polh*, *iap*2, *dbp* and *pif*1 with their counterparts in L1, SP1A, NNg1 and G4 genome. (A) NJ tree of polyhedron gene, (B) NJ tree of inhibitor of apoptosis-2 gene, (C) NJ tree of DNA binding protein and (D) NJ tree of Per os infectivity factor-1 gene (number at each node indicates bootstrap values)

We observed a similar pattern in our *pif*1gene, of the 26 cysteine residues, 23 were conserved among the most diverse members of baculovirus. This indicated the protein can form multiple disulfide bonds and might be folded. Since no other *pif* gene was available in the GenBank nucleotide database, the BLAST search compared our gene with the complete genome of HearNPV showing 99% similarity and 98% similarity with HzNPV. With the available data on the characterization of *iap*2 gene, presence of BIR domains, RING- domain and the C_3HC_4 motif has been determined as the conserved gene regions. According to [31] the spacing of cysteines and histidine within the BIR suggests the possibility of metal ion coordination and nucleic acid binding. C_3HC_4 motif is also found in the gene encoding peroxisome activating factor [32] and the neutralized gene involved in restricting the number of neural progenitor cells during embryonic neurogenesis [33]. We were also able to identify these DNA fragments at the expected sites in the PCR

amplified gene. In all the characterized gene comparisons, the close relatedness (up to 98%) of HzNPV and HearNPV was expected, as the former is a sub-strain of later [34,15]investigated the putative basic DNA-Binding protein (BDBP) in *H. armigera* is the only data available so far in that particular gene. However we have characterized the ssDNA–binding protein which differs from BDBP. Wang characterized a 330nt long ORF encoding 109 amino acids with a molecular mass of 11.6kDa where as we have amplified a 972bp amplicons that encodes 323 amino acids and has a calculated molecular mass of 37.55 kDa.

4. Conclusion

Thus, the present study objective was to produce the described genes as a product of the specific, powerful and universal technique of Polymerase chain reaction, employing a set of species- specific primers, enabling to amplify the gene without disturbing the conserved gene regions. The BLAST searches indicated that primers used were successful in amplifying the expected DNA fragment at the precise base pair size. The comparative analysis proved that PCR targeted not just the partial sequence, but the sequence along with its conserved region intact and the variant region. In conclusion, this technique offers a sensitive system for the specific baculovirus detection even with low amounts of DNA.

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