J. Trop. Plant Pests Dis.
Vol. 24, No. 2, September 2024
Pages: 243–250

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.224243-250

RESEARCH PAPER

Molecular characterization of *Helicoverpa armigera Nucleopolyhedrovirus* (*Hear*NPV) on *Helicoverpa armigera* Hübner larvae (Lepidoptera: Noctuidae)

Yayi Munara Kusumah, Fitrianingrum Kurniawati, Pajar Pramudita, & Michael Christian

Manuscript received: 23 June 2023. Revision accepted: 14 June 2024. Available online: 21 August 2024.

ABSTRACT

Nucleopolyhedrovirus (NPV) is a type of biological agent that can be effectively used as a bioinsecticide to control the cob borer caterpillar in corn. Among these viruses, *Hear*NPV (*Helicoverpa armigera* Nucleopolyhedrovirus) stands out due to its host specificity, environmentally safety, and lack of harm to non-target organisms. The primary objective of this study was to elucidate the molecular characteristics of the DNA polymerase gene of *Hear*NPV. To achieve this, caterpillars were collected from the field, then reared and inoculated in the laboratory under controlled conditions. Following propagation, viral genomic DNA was extracted using a modified CTAB (Cetyltrimethylammonium Bromide) protocol, which is commonly employed for its efficiency in isolating high-quality DNA from various sources. Subsequently, DNA amplification was performed using specific primers: forward primer *Hear*NPV F and reverse primer *Hear*NPV R. These primers were designed to target the DNA polymerase gene, a crucial component in viral replication and a key marker for phylogenetic studies. The results of the phylogenetic analysis indicated that the Bogor strains of *Hear*NPV shared a high degree of similarity with NPVs that infect other species within the *Helicoverpa* genus, including strains from Spain, Australia, Brazil, Russia, and Japan. Specifically, the nucleotide sequence homology ranged from 98.4% to 99.4%, while the amino acid sequence homology ranged from 98.2% to 99.2%. This high level of homology suggests a close evolutionary relationship and potentially similar biological characteristics among these NPV strains.

Key words: biological control, DNA polymerase, identification, NPV

INTRODUCTION

The cotton bollworm caterpillar (*Helicoverpa armigera* Hübner) is a significant pest of various cultivated plants, with corn being one of its primary targets. This pest is particularly destructive during its larval stage, which is known to be the most damaging phase of its life cycle (Kalshoven, 1981). During this stage, the larvae feed voraciously on plant tissues, leading to substantial losses in crop yield and quality.

According to Ginting et al. (2023), the intensity of *H. armigera* attacks varies with altitude, with the midland region (600 masl) experiencing a 100% higher attack intensity compared to both the lowland (30 masl) and highland (1000 masl) regions. Specifically, the attack intensities were recorded at 83% and 92% in the lowland and highland regions, respectively. This suggests that environmental factors linked to altitude may influence the prevalence and severity of *H. armigera* infestations. Furthermore, Susilo & Swibawa (2002) reported that *Helicoverpa* not only targets the cob but also attacks the leaves of corn plants. This dual feeding behavior can exacerbate the damage.

Integrated Pest Management (IPM) is a recommended approach for controlling cotton bollworm caterpillars. One effective pathogen against this pest is the Nucleopolyhedrovirus (NPV) (Arifin, 2006). This virus is host-specific, posing no threat to other organisms, and repeated applications do not induce an immune response in H. armigera (Sanjaya, 2000).

NPV is a virus from the *Baculoviridae* family. More than 700 species of insects have been found to be naturally infected by baculoviruses, with 90% isolated from lepidopteran insects (Raj et al., 2022). NPV has a relatively high virulence and can cause the death of insects within 4 to 7 days after application (Grzywacz, 2000). Several factors can influence the success of baculovirus infection, including host biology, viral virulence, and ecology (Jackson et al., 2005).

Variations in NPV virus strains can be influenced by climatic conditions affecting a region; host ecology also plays an important role in different Baculovirus genotypes (Hodgson et al., 2001). This genetic diversity

Corresponding author:
Yayi Munara Kusumah (yayiku@apps.ipb.ac.id)

Department of Plant Protection, Faculty Agriculture, IPB University. Jl. Kamper Kampus IPB Dramaga, Bogor, Jawa Barat, Indonesia 16680

is valuable for identifying strains with superior traits, such as high virulence. Differences in the genome sequences of baculoviruses can be identified using core genes, which are crucial markers. This method involves targeting conserved regions of the genome through oligonucleotide amplification, allowing for easy analysis with digital support programs (Friesen & Miller, 1986).

Polymerase Chain Reaction (PCR) and DNA sequencing are methods used to determine molecular characteristics and identify viruses molecularly by using primers designed to amplify DNA polymerase sequences of NPV in Lepidoptera. This allows for the identification of the molecular characteristics of viruses in *Helicoverpa armigera* can be identified. This study aims to determine the molecular characteristics of NPV in *H. armigera* larvae using DNA polymerase sequences.

MATERIALS AND METHODS

Research Site. The research was conducted at the Insect Pathology Laboratory, Plant Virology Laboratory, and Plant Bacteriology Laboratory in the Department of Plant Protection, Faculty of Agriculture, IPB University. NPV strains were obtained from corn plants in the area around the campus of Bogor Agricultural University.

Isolation *Helicoverpa armigera* NPV (*Hear*NPV). 0.1 g of larval cadaver infected with NPV was crushed in 0.1% SDS buffer until smooth using a sterile mortar. Grinding was carried out until a homogeneous suspension of polyhedra was obtained, and additional 0.1% SDS was added to achieve the desired level of dilution. Once the NPV polyhedra suspension was ready for use, 1 mL of the suspension was transferred into a 1.5 mL micro-tube, and then centrifuged for 1 minute at 2000 rpm. The pellet formed was discarded, and the supernatant was collected. The supernatant was then centrifuged again at 5000 rpm for 20 minutes. The resulting pellets were resuspended using 500 µL of aquabides and centrifuged again at 2000 rpm for 1 minute to obtain relatively pure suspension (Cheng, 1998).

Propagation of *Hear*NPV. NPV propagation was carried out at the Insect Pathology Laboratory. larvae used were second to fourth-instar larvae. The larvae prepared for propagation were fed with baby corn, which had been inoculated with the NPV by dipping the diet into the NPV suspension, which was then air-dried. Caterpillars were placed individually in 90 mL plastic cups, each containing one larva, which was fed with baby corn. The containers was cleaned daily, and the diet was replaced when it ran out. Observations were made every day until the larvae died. Dead larvae were harvested, stored in tubes, and placed in a refrigerator to keep the NPV active. The harvested dead larvae are dark, soft, and watery.

DNA extraction. DNA extraction was carried out using a modified Cetyl-Trimethyl Ammonium Bromide (CTAB) method (Doyle & Doyle, 1990). The extracted samples consisted of 0.1 g and 0.5 g of NPV-infected caterpillars and 50 µL and 100 µL of purified polyhedra pellets. The caterpillar samples were finely ground in 0.1% SDS buffer. The sample in the form of polyhedra pellets was put into a 1.5 mL Eppendorf tube, and then 500 µL of CTAB buffer, which had been supplemented with 1% mercaptoethanol, was added.

The samples were then incubated for 2 hours at 60 ºC, with the tubes being inverted every 10 minutes. After that, the samples were incubated for 3 minutes at 28 ºC, and then 500 µL of Chloroform-Isoamyl alcohol (24:1) was added and centrifuged at 11,000 rpm for 20 minutes. The supernatant on the top surface was collected, using approximately half the volume of the solution without disturbing the pellet. Sodium acetate was added to 1/10 of the collected solution, and isopropanol was added to 2/3 of the collected volume. The supernatant was then incubated overnight at -20 ºC. The incubated supernatant was centrifuged at 12,000 rpm for 15 minutes. The supernatant was carefully removed, and then 500 µL of 80% ethanol was added to the tube, which was centrifuged again at 12,000 rpm for 15 minutes. The resulting pellets were dried by inverting the tube onto tissue paper for two hours. The dry pellets were then suspended in 50 µL of TE buffer (pH 8) and stored in a refrigerator at -20 ºC.

***Hear*NPV DNA Amplification Using PCR.** *Hear*NPV DNA amplification was performed using primers designed at the Insect Pathology Laboratory, Bogor Agricultural University, with *Hear*NPV F 5'- GTT TAG GCA AGT GGT GGA CT -3' (forward) and *Hear*NPV R 5'- TGT AAC TGC AGA TGA CTA CAA AGA -3' (reverse). The target gene was the DNA polymerase gene, with an expected size of approximately 1200 bp. Each amplification reaction used 2 µL of *Hear*NPV DNA (± 40 ng/µL), 12.5 µL of Taq Polymerase, 1 µL of forward primer, 1 µL of reverse primer, and 8.5 µL of ddH2O.

DNA amplification involved several stages: predenaturation at 94 ºC for 4 minutes, denaturation

at 94 ºC for 1 minute, annealing at 50 ºC for 1 minute, elongation at 72 ºC for 2 minutes, and final elongation at 72 ºC for 10 minutes, with a final storage temperature of 4 ºC. This cycle was repeated 30 times. The electrophoresis process was carried out by loading 5 µL of the PCR product into the wells of a 1% agarose gel immersed in a Tris Borate EDTA (TBE) solution, and then running it at 50 V for 50 minutes. Afterward, the electrophoretic gel was stained in an EtBr solution for 20 minutes. DNA visualization was performed by placing the gel on a UV-transilluminator.

Analysis of DNA Fragment Sequencing and Homology Based on Nucleotides and Amino Acids. The amplified DNA fragments were sequenced by 1st BASE Malaysia and sent via PT Genetika Science Indonesia using the sanger sequencing method. The fragment was then trimmed before being compared with the DNA polymerase gene sequence published on the National Center for Biotechnology Information (NCBI) website using the Basic Local Alignment Search Tools (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The selected nucleotide sequence data were aligned using the BioEdit ver 7.2.6 program (Hall, 1999) to analyze nucleotide and amino acid homology. Amino acid homology analysis was carried out by translating the nucleotides using the website.expassy.org/translate/.

***Hear*NPV Phylogeny Analysis**. Phylogenetic analysis was carried out using the Maximum Likelihood approach with 1000× Bootstrap, Kimura-2 parameters, in the MEGA 11 program (Tamura et al., 2021). The Neighbor-Joining approach is a simple algorithm that has a very fast minimum evolution in constructing trees and ignores mutated nucleotide bases. Neighbor-joining selects sequences that, when combined, provide the best estimate of branch length, which most closely reflects the distance between sequences (Dharmayanti, 2011).

RESULTS AND DISCUSSION

Symptoms of NPV Infection and *H. armigera* Larvae Collection. *Helicoverpa armigera* larvae found on corn plants were collected and stored in the laboratory for inoculation (Figure 1). Caterpillars infected with NPV will move toward the plant shoots, their appetite decreases, and their color changes to red. The body then dark, becomes soft, and eventually turns crushed and watery (Samsudin, 2011). The infected larvae are then collected for further processing.

***Hear*NPV Polyhedra Purification.** Purification is done repeatedly to obtain cleaner results, typically requiring 4 to 6 repetitions. The results of *Hear*NPV purification are visible as a clean, clear liquid, with

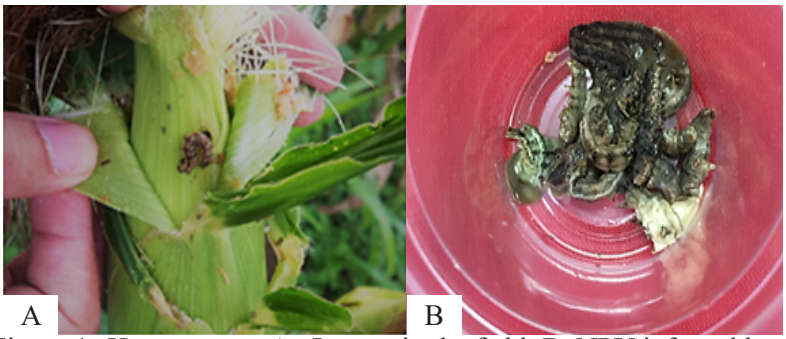

Figure 1. *H. armigera*. A. Larvae in the field; B. NPV-infected larvae.

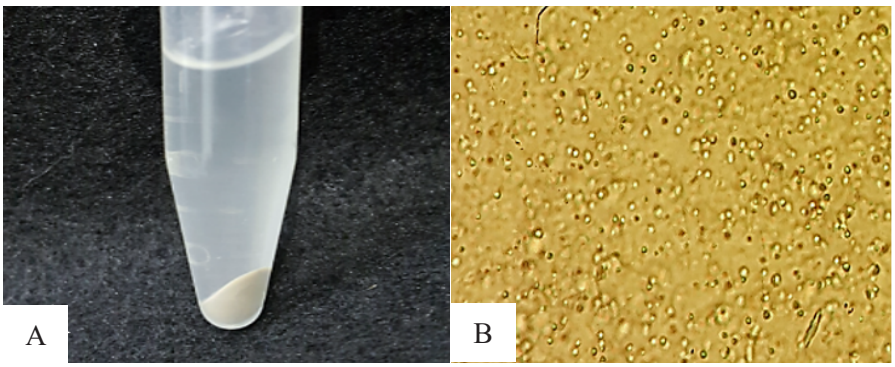

Figure 2. *Hear*NPV polyhedra. A. Pellet in suspension; B. Under a light microscope (400 ×).

a brownish precipitate that consists of polyhedra (Figure 2). The precipitate formed from a collection of *Hear*NPV polyhedra was observed under a microscope at a magnification of 400× (Figure 2). NPV is characterized by the presence of a polyhedral inclusion body (PIB), which contains nucleocapsids or virus particles (virions) with diameters between 20–50 nm and lengths between 200–400 nm (Smith, 1967).

***Hear*NPV DNA Extraction.** The results of DNA extraction from *Hear*NPV from Bogor appear clear. The principle of extraction is to destroy the cell wall without causing damage to the DNA (Sharma et al., 2010). The concentration of *Hear*NPV DNA extracted, as measured using a spectrophotometer, is shown in Table 1.

***Hear*NPV DNA Amplification Using the PCR Method.** Amplification using the PCR technique on the samples showed positive results (Figure 3). The amplification results show a band with the size consistent with the expected target, which is approximately 1200 bp.

***Hear*NPV DNA Fragment Sequencing.** *Hear*NPV DNA fragment sequencing was performed by 1st BASE Singapore and sent via PT. Genetika Science Indonesia. The data received was in the form of a chromatogram, the result of sequencing using the forward and reverse primers used in PCR. The forward and reverse fragments were then compared with other data and aligned to create a consensus sequence using the BioEdit program to obtain the complete *Hear*NPV sequence. The trimming process was carried out because errors can occur at the beginning and end of the PCR process, potentially disrupting the attachment of free nucleotides and causing noise at both ends of the PCR fragment (Criscuolo & Brisse, 2013). The contig results obtained were then compared with the NPV DNA data stored in GenBank to determine the relationship between species using the Basic Local Alignment Search Tool (BLAST) on the website www.ncbi.nlm.nih.gov.

***Hear*NPV Homology Analysis Based on Nucleotides and Amino Acid.** The sequencing results show very good quality with no sequencing errors based on two-sequence alignment analysis (http://blast.ncbi.nlm.nih.gov/). The relationship analysis of Indonesian *Hear*NPV strains compared with ten *Hear*NPV DNA Polymerase gene sequences in Genbank (http://blast.ncbi.nlm.nih.gov/) showed a high level of similarity (98.2–99.5%) (Table 2; Table 3). Fauquet et al. (2005) stated that if there is a similarity in the nucleotide sequence of the CP gene between one virus and another with a value of more than 90%, then the viruses are considered the same species. Indonesian *Hear*NPV strains have similarities (98.2–99.5%) with

Table 1. The concentration of *Hear*NPV DNA extracted as measured using a spectrophotometer

Sample	Date and time	Nucleid acid conc.	Unit	A260	A280	260/280	260/230	factor
50	16/04/2018	364.8	ng/µL	7.296	3.690	1.98	0.73	50.00
50	16/04/2018	367.0	ng/µL	7.341	3.697	1.99	0.73	50.00
100	16/04/2018	425.4	ng/µL	8.509	4.252	2.00	0.79	50.00
100	16/04/2018	436.8	ng/µL	8.737	4.377	2.00	0.79	50.00

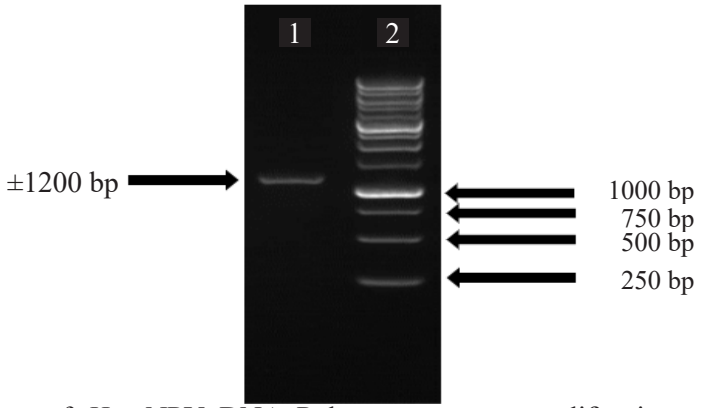

Figure 3. Visualization of *Hear*NPV DNA Polymerase gene amplification on 1% agarose gel using UV-transilluminator. Lane 1 = sample; 2 = 250 DNA Ladder (Thermo Scientific, US).

Table 2. Homology level of *Hear*NPV DNA polymerase strains from several countries based on nucleotide sequence

Strain	Accession No.	Homology level (%)										
		1	2	3	4	5	6	7	8	9	10	11
H. armigera NPV Bogor	Na	ID										
H. armigera NPV strain SP1A	KJ701032	99.5	ID									
H. armigera NPV strain H25EA1	KJ922128	99.5	100	ID								
H. SNPV AC53 strain AC53T5	KU738904	99.5	100	100	ID							
H. zea NPV strain Br/South	KM596835	99.5	100	100	100	ID						
H. armigera NPV strain NNg1	AP010907	99.1	99.5	99.5	99.5	99.5	ID					
H. armigera NPV strain TR	MK507817	99.1	99.5	99.5	99.5	99.5	99.1	ID				
H. armigera NPV strain SP1B	KJ701033	99.1	99.5	99.5	99.5	99.5	100	99.1	ID			
H. armigera NPV strain C1	NC003094	98.2	98.6	98.6	98.6	98.6	98.2	99.1	98.2	ID		
H. armigera NPV strain WV104	MT810812	98.2	98.6	98.6	98.6	98.6	98.2	99.1	98.2	100	ID	
Spodoptera exigua NPV strain HT-SeSP2A	HG425349	56.1	56.1	56.1	56.1	56.1	56.1	56.5	56.1	56.1	56.1	ID

Na (No accession number), NPV (*Nucleopolyhedrovirus*)

Table 3. Homology level of *Hear*NPV DNA polymerase strains from several countries based on amino acid sequence

Strain	Accession No.	Homology level (%)										
		1	2	3	4	5	6	7	8	9	10	11
H. armigera NPV strain Bogor	Na	ID										
H. armigera NPV strain SP1A	KJ701032	99.4	ID									
H. armigera NPV strain H25EA1	KJ922128	99.3	99.3	ID								
Helicoverpa NPV strain AC53T5	KU738904	99.2	99.2	99.9	ID							
H. zea NPV strain Br/South	KM596835	99.2	99.2	99.9	99.8	ID						
H. armigera NPV strain NNg1	AP010907	99.2	99.2	99.5	99.4	99.4	ID					
H. armigera NPV strain TR	MK507817	98.8	99	99.5	99.4	99.4	99	ID				
H. armigera NPV strain SP1B	KJ701033	98.8	99.4	99.3	99.2	99.2	99.4	99	ID			
H. armigera NPV strain C1	NC003094	98.4	98.8	98.9	98.8	98.8	98.8	99.4	99	ID		
H. armigera NPV strain WV104	MT810812	98.4	98.8	98.9	98.8	98.8	98.8	99.4	99	100	ID	
Spodoptera exigua NPV strain HT-SeSP2A	HG425349	57	57.3	56.9	57	57	57.1	56.9	57.3	56.8	56.8	ID

Na (No accession number), NPV (*Nucleopolyhedrovirus*)

strains from other countries, so it can be concluded that Indonesian *Hear*NPV strains are the same strain as those from Spain, Australia, Brazil, Kenya, Turkey and China.

Based on nucleotide homology analysis, the *Hear*NPV Bogor strains are classified in the same species as the NPVs that infect *Helicoverpa* because they have a high percentage of nucleotide homology at 99% and amino acids homology at 98–99% (Table 2; Table 3). Differences in nucleotides and amino acids homology percentages are due to geographic location, distribution, and the number of available species. According to Shapiro et al. (1992), the same species from different locations can exhibit different genetic variations.

Based on nucleotide and amino acid sequence alignment, the DNA polymerase gene of *Hear*NPV Bogor has 5 point mutations in the nucleotide sequence. Point mutations at amino acid position 5 and 40 result in translation of different amino acids, while mutations at positions 78, 79, and 148 do not change the translation product, resulting in silent mutations (Table 4). Alteration in amino acid translation indicates the occurrence of missense mutations. A missense mutation is a change in the nucleotide sequence that causes a change in the translated amino acid structure (Miosge et al., 2015). A high frequency of missense mutations can lead to different types of proteins being produced. Changes in protein structure due to missense mutations can affect protein function (Mahmood et al., 2022). Based on amino acid homology analysis, the mutation in the DNA polymerase gene of *Hear*NPV Bogor did not affect protein function because of the relatively high similarity percentage with other *Hear*NPV strain. According to Pearson (2013), amino acids with a similarity level of more than 80% share the same function.

Phylogeny Analysis of *Hear*NPV Based on Nucleotides. The construction of the phylogeny tree based on the nucleotide sequence shows that the *Hear*NPV strains from Bogor are in the same group as those originating from the *Hear*NPV from Spain, Brazil, Australia, Kenya, Turkey and China (Figure 4).

Phylogenetic analysis was carried out to determine the relationship between descendants and their ancestors based on the similarity of characters as the basis for comparison. The diversity observed reflects differences between species as well as variations within the same species in different locations. A phylogenetic

Table 4. Nucleotide and amino acid mutation position in *Hear*NPV Bogor DNA *polymerase* gene Alignment of *Hear*NPV DNA *polymerase* strains from several countries based on amino acid sequence

Amino Acid Type	Codon	Amino acid position	Nucleotide Position	Mutation position		
				1	2	3
L	TTG	5	14		1	
S	AGT	40	118	1		
D	GAT	78	234			1
L	TTG	79	235	1		
F	TTC	148	444			1

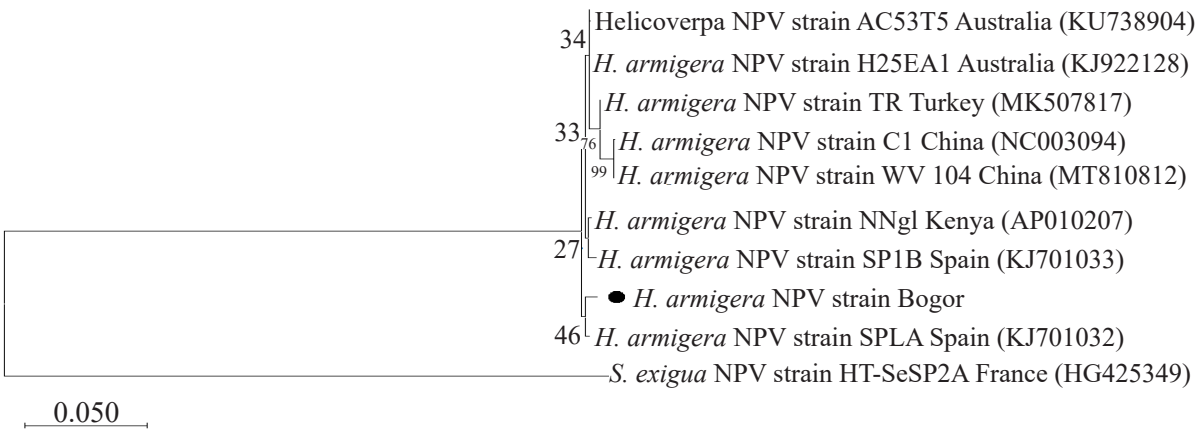

Figure 4. *Hear*NPV DNA phylogeny tree based on the partial nucleotide sequence of DNA Polymerase gene processed using Maximum-likelihood method with Tamura-Nei model (1000× bootstrap).

tree is a method used to illustrate the evolutionary relationships between organisms. Different climatic conditions can lead to differences in virus strains; host ecology also plays an important role in shaping different Baculovirus genotypes (Hodgson et al., 2001).

CONCLUSION

*Hear*NPV Bogor strains can be identified using DNA polymerase sequences. Homology analysis based on nucleotide and amino acid sequences showed that the strain from Bogor has a high degree of homology, 99%, with *Helicoverpa* strains from other regions.

ACKNOWLEDGMENTS

We would like to express our deepest gratitude to all individuals and institutions that contributed to the successful completion of this research endeavor. Special thanks are extended to the Department of Plant Protection at IPB University for providing access to essential resources and facilities. Additionally, we acknowledge the contributions of our colleagues and collaborators who generously shared their expertise. Lastly, we extend our appreciation to the participants who volunteered their time, making this study possible. Each of these contributions has played a crucial role in advancing our understanding in this field.

FUNDING

This research was independently funded.

AUTHORS' CONTRIBUTIONS

YMK conceived and planned the experiment, including sampling and colleting infected larvae. PP carried out the isolation of DNA polymerase gene from *Helicoverpa armigera* NPV and the polyhedra. FNK and MK performed molecular work and data sequencing analysis. YMK and FNK interpreted the sequence data. YMK, FNK, PP, and MC prepared the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

We confirm that we have no competing interests to declare regarding the publication of our work.

REFERENCES

Arifin M. 2006. Kompatibilitas SlNPV dengan *Ha*NPV dalam pengendalian ulat grayak dan ulat pemakan polong kedelai [Compatibility of SlNPV with *Ha*NPV in controlling armyworms and soybean pod eating caterpillars]. *Penelitian Pertanian Tanaman Pangan.* 25: 65–71.

Cheng XW. 1998. Characterization of two nuclear polyhedrosis viruses from *Thysanoplusia orichalcea* (L.) (Lepidoptera: Noctuidae) from Indonesia. *Dissertasion*. Clemson University. South Carolina.

Criscuolo A & Brisse S. 2013. AlienTrimmer: A tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. *Genomics.* 102(5–6): 500–506. https://doi.org/10.1016/j.ygeno.2013.07.011

Dharmayanti NLPI. 2011. Filogenetika molekuler: Metode taksonomi organisme berdasarkan sejarah evolusi [Molecular phylogenetic: Organisms taxonomic method of based on evolution history]. *WARTAZOA.* 21(1): 1–10.

Doyle JJ & Doyle JL. 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus.* 12: 13–15.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, & Ball LA. 2005. *Virus Taxonomy, VIIIth Report of the ICTV*. Elsevier/Academic Press. London.

Friesen PD & Miller LK. 1986. The regulation of baculovirus gene expression. *Curr. Top Microbiol. Immunol*. 131: 31–49. https://doi.org/10.1007/978-3-642-71589-1_3

Ginting S, Chozin M, & Sudjatmiko S. 2023. Infestation of *Helicoverpa armigera* (Hubner) and *Ostrinia furnacalis* Guenee on three tropical altitude variations. *J. Trop. Plant Pests Dis*. 23, (1): 31–37.

Grzywacz D. 2000. Control of insect pest with entomopathogenic viruses. *Arab J. Pl. Prot.* 18(2): 128–132.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series.* 41: 95–98.

Hodgson DJ, Vanbergen AJ, Watt AD, Hails RS, & Cory JS. 2001. Phenotypic variation between naturally co-existing genotypes of a Lepidopteran *Baculovirus*. *Evol Ecol Res*. 3: 687–701.

Jackson TA, Crawford AM, & Glare TR. 2005. *Oryctes* virus—Time for a new look at a useful biocontrol agent. *J. Invertebr. Pathol.* 89(1): 91–94. https://doi.org/10.1016/j.jip.2005.03.009

Kalshoven LGE. 1981. *The Pests of Crops in Indonesia*. Translated by P. A. van der Laan. PT. Ichtiar Baru-Van Hoeve, Jakarta.

Mahmood TB, Hossan MI, Mahmud S, Shimu MS, Alam MJ, Bhuyan MMR, & Emran TB. 2022. Missense mutations in spike protein of SARS-CoV-2 delta variant contribute to the alteration in viral structure and interaction with hACE2 receptor. *Immun. Inflamm. Dis.* 10(9): 1–18. https://doi.org/10.1002/iid3.683

Miosge LA, Field MA, Sontani Y, Cho V, Johnson S, Palkova A, Balakishnan B, Liang R, Zhang Y, Lyon S, Beutler B, Whittle B, Bertram EM, Enders A, Goodnow CC, & Andrews TD. 2015. Comparison of predicted and actual consequences of missense mutations. *PNAS.* 112(37): 189–198. https://doi.org/10.1073/pnas.1511585112

[NCBI] National Center for Biotechnology Information. 2023. *Basic Local Alignment Search Tool*. http://www.ncbi.nlm.nih.gov/BLAST. Accessed 15 July 2023.

Raj MN, Ipsita Samal, Amit Paschapur, Subbanna, A.R.N.S. 2022. Entomopathogenic viruses and their potential role in sustainable pest management. In: Singh HB & Vaishnav A (Eds.). *New and Future Developments in Microbial Biotechnology and Bioengineering*. pp. 47–72. Elsevier. https://doi.org/10.1016/B978-0-323-85579-2.00015-0

Pearson WR. 2013. An introduction to sequence similarity ("homology") searching. *Curr. Protoc. Bioinformatics.* 42(1): 311–317. https://doi.org/10.1002/0471250953.bi0301s42

Samsudin. 2011. Uji Patologi dan Perbaikan Kinerja *Spodoptera exigua* nucleopolyherdovirus (SeNPV) [Pathology Test and Performance improvement of *Spodoptera exigua nucleopolyherdovirus* (SeNPV)]. *Dissertation*. IPB University. Bogor.

Sanjaya Y. 2000. Perubahan Tingkat Toleransi *Helicoverpa armigera* HÜBNER yang Terinfeksi *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV) [Changes in the tolerance level of *Helicoverpa armigera* HÜBNER infected with *Helicoverpa armigera* Nuclear Polyhedrosis Virus (*Ha*NPV)]. *Thesis*. Bandung Technology Institute. Bandung.

Shapiro JA. 1992. Natural genetic engineering in evolution. *Genetica.* 86: 99–111. https://doi.org/10.1007/bf00133714

Sharma P, Joshi N, & Sharma A. 2010. Isolation of genomic DNA from medicinal plants without liquid nitrogen. *Indian J. Exp. Biol.* 48(6): 610–614.

Smith KM. 1967. *Insect Virology*. CPC Press, San Diego.

Susilo FX & Swibawa IG. 2002. Insect pests in agroecosystem where three corn varieties were grown under conservation versus full tillage system in natar, south lampung in 2001 growing season. *J. Trop. Plant Pests Dis*. 2(1): 8 –14.

Tamura K, Stecher G, & Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol Biol Evol.* 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120