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Studies on Natural and Experimental Nucleopolyhedrovirus Infection of the Silkworm, *Bombyx mori* (Race: PMCSR2(SL)) by PCR

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Abstract: The silkworm, *Bombyx mori* L. (Bombicidae: Lepidoptera), basically a nocturnal moth, is a native of China but has long been domesticated throughout the world. The life cycle of silkworms includes various stages, viz. eggs, larval instars, pupa within silky cocoons, and adult moths. *Bombyx mori* nucleopolyhedrovirus (BmNPV), one of the best characterized baculoviruses, is an important pathogen in silkworms. The viral diseases are difficult to manage due to the very short life cycle of silkworms. One of the most effective solutions is the timely detection of such infections so as to stop the spread of the viral disease. In the present study, we determined the specific nucleopolyhedrovirus ~738 base pair amplified from isolated viral DNA by the polymerase chain reaction (PCR) technique.

Keywords: Silkworm, *Bombyx mori,* Grasserie, Nucleopolyhedrovirus, Polyhedrin gene, PCR

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Introduction

Bombyx mori is popularly called the Mulberry silkworm moth. *Bombyx mori* is a completely domesticated (for over 5000 years) and economically important insect that is important for silk production (sericulture). Improvements to sericulture could improve the incomes of farmers in several developing countries, such as China, Thailand, and India. It is considered to be a lepidopteron model insect for basic and applied research and is key for increasing knowledge about other insects and organisms (Xia *et al.,* 2004; Goldsmith *et al.,* 2005). In addition, this silkworm is used as a model insect for studies in entomology, molecular biology, and biotechnology (Nagaraju and Goldsmith, 2002; Zhou *et al.,* 2008). The Mulberry silkworm is a purely domesticated insect. The silk moth is dioecious, i.e. the sexes are separate. Fertilization is internal, preceded by

copulation. The development includes a complicated complete metamorphosis, consisting of four stages viz egg, larva, pupa, and adult (Krishnaswami *et al.,* 1973; Vasantharaj and Ramamurthy, 2015).

A major part of crop loss in sericulture is due to the occurrence of diseases in silkworm, which results in failure of the crop and low harvest rate. There are many microorganisms responsible for the occurrence of diseases, such as bacteria, viruses, protozoa, fungi, etc. Domestic mass rearing of silk moths is highly labor-intensive and usually handled by local farmers, who continuously rear larvae from hatching to pupation with the only available food source, the leaves of the mulberry plant (Hamamura, 1959; Zhang *et al.,* 2019). The pupating larvae produce cocoons from which silk thread can be harvested. The major constraints for increased cocoon yield in sericulture are the occurrence of diseases such as grasserie, flacherie, muscardine, and pebrine caused by viruses, bacteria, fungi, and microsporidia infection, respectively (Khurad *et al.,* 2004; Ravikumar *et al.,* 2011; Gani *et al.,* 2018b). Viruses are considered the most important pathogens in silk moth rearing, causing serious and significant economic damage and thus posing a serious threat to global sericulture (Kumar *et al.,* 1990). Silkworm losses due to diseases in India are about 15–20%, of which more than 50% are attributed to viral infection only (Reddy and Rao, 2009).

The grasserie disease of silkworm caused by the *Bombyx mori* nucleopolyhedrovirus (BmNPV) (genus *Alphabaculovirus*, family Baculoviridae) is the most abundant and well described pathogen in the history of the silk industry and has had a high impact on the sericulture agrosystem in India and worldwide (Acharya *et al.,* 2002; Ardisson-Araújo *et al.,* 2014). BmNPV infects larval stages of the silk moth, and symptoms of a BmNPV infection include shining and fragile skin, swollen inter-segmental regions, the appearance of milky white haemolymph, death, and the hanging of larvae by their prolegs (Gani *et al.,* 2018a; McCook *et al.,* 2021). The usually high temperatures and humidity prevalent in tropical regions are conducive to the proliferation of polyhedrosis disease. It is known to occur in all larval instars and more commonly in fourth and fifth instars during all seasons, causing 20–50% cocoon crop losses in India (Vidya, 1960; Chitra *et al.,* 1975; Samson *et al.,* 1990; Shivaprakasam and Rabindra, 1995; Nataraju *et al.,* 1998). Baculoviridae is a family of insect viruses with a large, super-coiled double-stranded DNA genome packaged in rod-shaped virions. Baculoviruses are morphologically divided into nucleopolyhedrovirus (NPV) and granulovirus (GV). Lepidopteran NPVs (alphabaculoviruses) are further subdivided into Groups I and II (Herniou *et al.,* 2001). The baculovirus life cycle typically involves the production of two virion phenotypes, budded virions (BVs) and occlusion-derived virions (ODVs). The BVs and ODVs have different functions in the baculoviridae life cycle. BV is required for the dissemination of a viral infection throughout the tissues of an infected host. ODV is required for inter-host transmission (Zhou *et al.,* 2010). NPVs require interaction with their hosts to accomplish virus replication in the host insect cells (Bao *et al.,* 2009).

Earlier, techniques have been developed to detect this viral disease, such as the enzyme-link immunosorbent assay (ELISA) (Vanapruk *et al.,* 1992), DNA hybridization (Attathom *et al.,* 1994), colloidal textile dye-based dipstick immunoassay (Nataraju *et al.,* 1994), and western blot analysis. PCR is an extremely sensitive technique that amplifies target DNA sequences, and PCR amplification of conserved fragments enables the detection of low levels of viral DNA (Mallika, 2006). So far, no such detection study for Grass virus in silkworms (race: PM CSR2(SL) from Athiyanur village, Tiruvannamalai District, Tamil Nadu has been conducted. In this study, the specific nucleopolyhedrovirus 738 base pairs were amplified from isolated viral DNA by the polymerase chain reaction technique (PCR).

Materials and Methods

Collection of infected silkworms:

Grasserie infected silkworms were collected from Silkworm (Race: PM × CSR2(SL) farm, Athiyanur village, Vandavasi Taulk, Tiruvannamalai District.

Diagnosis of disease:

The identification of diseased worms infected with Grasserie in the fields was initially made on the basis of gross pathology. Initially, the skin showed an oily and shiny appearance. With the progressing stage of infection, the entire skin became thin and fragile and the midgut appeared milky white with inter-segmental swelling. The larvae infected with Grasserie in the rearing centres were found to be slightly sluggish (Rashmi *et al.,* 2016).

Purification of OBs from infected silkworms:

Insect cadavers were dissected in water to collect the polyhedral occlusion bodies rich hemolymph. The solution was sonicated and filtrate was centrifuged at 5000g at 4°C for 10 min in a C-24BL refrigerated centrifuge (Remi, Mumbai, India). The pellet was suspended in sterile water and made homogenous using a micropipette. The resulting suspension was also filtered through a muslin cloth. This centrifugation step has been repeated 10 to 15 times to remove residual cell debris and fetid odour. The pellet again suspended in 10 volumes of SDS (2.0%) (Himedia, Mumbai, India) and stirred on an orbital shaker (Jeiotech, Seoul, Korea) for 30 min at 110 rpm (Ke *et al.,* 2014). It was followed by centrifugation by adding sterile distilled water at 6400 g at 4°C for 10 min and this process was repeated 3 times to remove any residual SDS present in the solution. The pellets were suspended in 1 ml of sterile water and the purity and integrity of the OBs were checked through observations of the aliquots under a compound microscope. Then the OB sample was also lyophilized and stored at 4°C until use (Sajjan Dayanand *et al.,* 2015).

DNA extraction:

PCR analysis for the viral DNA which was completely isolated from the polyhedral occlusion bodies (OBs) was performed following standard laboratory protocols (Alletti *et al.,* 2017; Gani *et al.,* 2021). The natural and experimental infected fifth instar larvae of silkworm were homogenized separately in NTE buffer (0.2 M NaCl, 0.02 M Tris–HCl and 0.02 M EDTA, pH 7.4), and 10% tissue suspension was made. The suspension was centrifuged at 3000 g for 15 min at 4°C, and supernatant was collected. Then, the tissue suspension was mixed with an appropriate amount of digestion buffer (100 mM NaCl, 10 mM Tris–HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulphate and 0.1 mg ml^{-1} proteinase K) and incubated for 2 h at 65°C to extract the DNA. After incubation, the digests were deproteinized by successive phenol/chloroform/isoamyl alcohol extraction and DNA was recovered by ethanol precipitation and dried. The dried DNA pellet was suspended in TE buffer and used as a template for PCR amplification to detect NPV. Protein debris and DNA were separated by phenol/chloroform/ isoamylalcohol (25:24:1, v:v:v) extraction, and DNA precipitation was occurred and performed with ethanol at -20°C overnight (Sambrook and Russel, 2001). The DNA pellet was dissolved in ultra-pure water and the DNA concentration and purity were determined with a NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA) spectrophotometer.

Amplification of polyhedrin gene of NPV by PCR:

The gene coding for polyhedrin was amplified from genomic DNA of NPV virus by the polymerase chain reaction (PCR) with specific primer set (Table 1) designed for NPV. Forward and reverse primers were designed based on the nucleotide sequence of polyhedrin gene of NPV. The sequence of the primers with the corresponding annealing temperature are given in Table 2. The reaction components of PCR includes 10X PCR Buffer - 1.0 µl, Primer F - 0.5 µl, Primer R - 0.5 µl, dNTPs mix - 1.0 µl, PCR Mastermix - 5 µl, Template - 1.0 µl , Core H2O - 6.0 µl, Total - 15.0 µl/Reaction.

Table 1: Sequence of the primers of polyhedrin gene of NPV

Primer	Product size	Sequence (5'-3')	Annealing temp.
BM NPV - F	738 bp	CGCGGATCCATGCCGAATTATTCATACACCCC	58°C
BM NPV - F		CCGGAATTCTTAATACGCCGGACCAGTGA	

Table 2: The optimized PCR parameters for amplification of polyhedrin gene of NPV

PCR parameters	Temperature (ºC)	Minutes
1. Initial denaturation	95	5
2. 35 cycles of		
Denaturation	**95**	**1**
Annealing	**58**	**1**
Extension	**72**	**1**
3. Final extension	**72**	**10**

Results

Diagnosis of disease:

The naturally infected fifth instar larvae were collected from Athiyanur village, Vandavasi Taulk, Tiruvannamalai District. The infected larvae become obvious in the rearing trays and on montage during cocoon sinning due to shiny white transluscent skin, elevated intersegment membrane and swelled body segments. In later infections stage the body wall rupture and white turbid haemolymh containing a large number of viral polyhedral occlusion bodies (OBs) were observed. The cadaver was white at the time of death and became dark within 3–5 h as they putrefied (Data not shown).

PCR amplification and sequencing of polyhedrin gene of BmNPV:

In the present study, PCR amplification and sequencing of BmNPVPolyhedrin gene have been performed from natural and experimental infected fifth instar larvae silkworm. Figure 1 shows that the DNA extracted from GrasserieBmNPV naturally infected silkworm yielded the amplification product of ~738 bp. The Lane 2 PCR product obtained was ~738 bp for Grasserie as expected and were in accordance to that obtained from the DNA extracted from naturally infected BmNPV (polh gene), in Lane M. PCR amplification of BmNPV DNA was performed by using 40 mg of each sample. Figure 2 shows that the DNA extracted from experimentally infected BmNPV silkworm yielded the amplification product of ~738 bp. The Lane 2 PCR product were obtained high level of expression was ~738 bp for polyhedrin gene when compared to DNA ladder (Lane M). PCR amplification of BmNPV DNA was performed by using 40 mg of each sample.

In the present study, we carried out agarose gel electrophoresis analysis of PCR amplified DNA gene fragment from different organs of natural infected *B. mori* larvae. PCR amplification of BmNPV DNA was performed by using 40 mg of each sample. Lane 1: DNA Markers = 100-1000 bp; Lane 2: Foregut; Lane 3: Midgut; Lane 4: Hemolymph of natural infected fifth instar silkworm larvae. The Lane 3 PCR product were obtained high level of expression was ~738 bp for polh gene when compared to Lane 1 of foregut sample and Lane 2 of midgut sample (Fig. 3). In contrast the different organs of experimental infected *B. mori* larvae. The Lane 1 foregut samples obtained high level of expression was ~738 bp for polh gene when compared to Lane 2

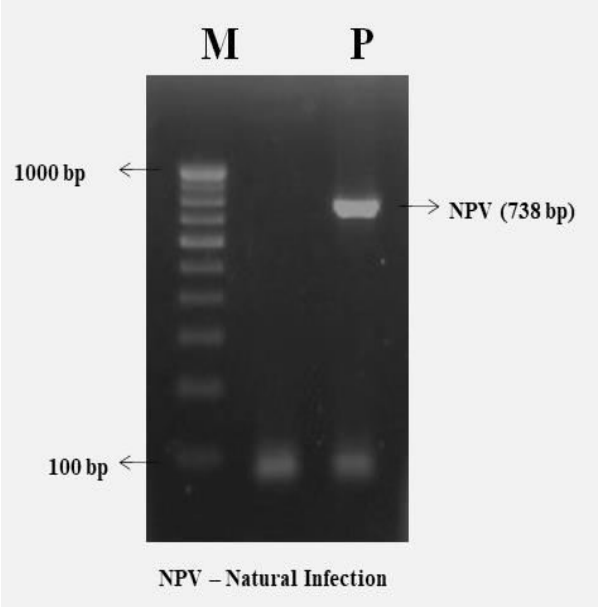

Fig. 1: Agarose gel electrophoresis analysis of PCR amplified DNA gene fragment from natural infected *B. mori* 5th instar larvae. PCR amplification of BmNPV DNA using 40 mg of each sample. Lane 1: DNA Markers = 100-1000 bp; Lane 2: BmNPV DNA positive control.

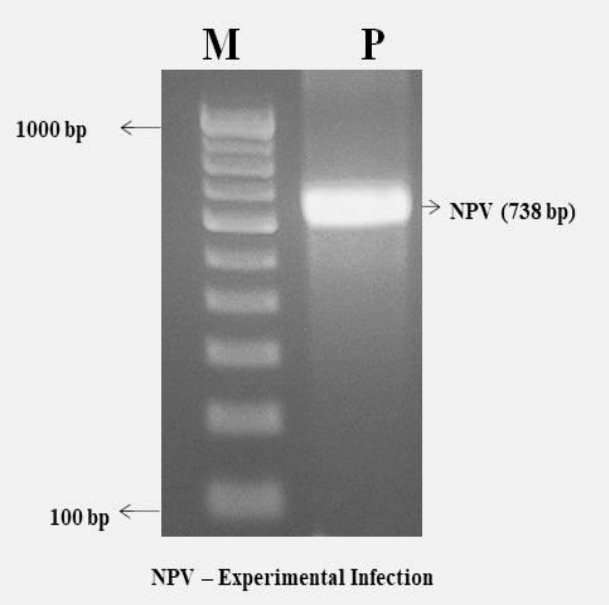

Fig. 2: Agarose gel electrophoresis analysis of PCR amplified DNA gene fragment from experimental infected *B. mori* 5th instar larvae. PCR amplification of BmNPV DNA using 40 mg of each sample. Lane 1: DNA Markers = 100-1000 bp; Lane 2: BmNPV DNA positive control.

of midgut sample and Lane 3 of hemolymph sample (Fig. 4). In this study we confirmed the Clustal W multiple sequence alignment of *Bombyx mori* nucleopolyhedrovirus (NPV), Polyhedrin Gene sequence of Indian isolate of nucleopolyhedrovirus (NPV), Polyhedrin Gene with sequences of nucleopolyhedrovirus (NPV) and Polyhedrin Gene isolates of China and Thailand (Fig. 5). Moreover, the phylogenetic tree was analysed from nucleopolyhedrovirus (Fig. 6).

Discussion

Silkworm *Bombyx mori*(Lepidoptera: Bombycidae) is a domesticated silk moth. It is a typical

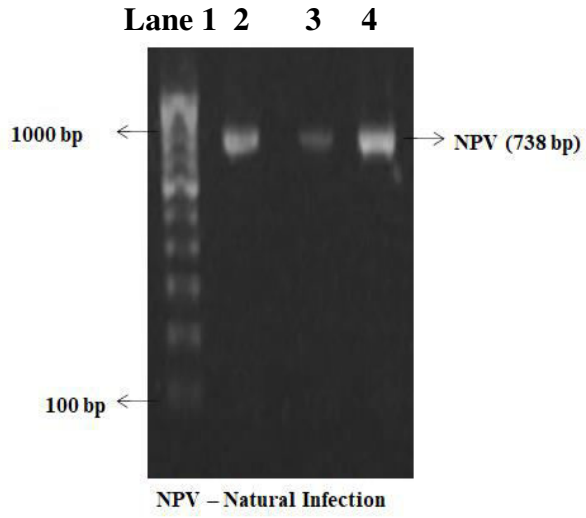

Fig. 3: Agarose gel electrophoresis analysis of PCR amplified DNA gene fragment from different organs of natural infected *B. mori* larvae. PCR amplification of BmNPV DNA using 40 mg of each sample. Lane 1: DNA Markers = 100-1000 bp; Lane 2: Foregut Lane 3: Midgut; Lane 4: Hemolymph of natural infected fifth instar silkworm larvae.

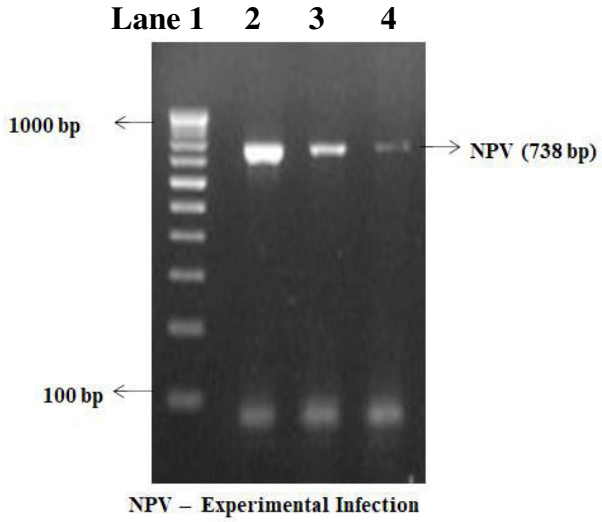

Fig. 4: Agarose gel electrophoresis analysis of PCR amplified DNA gene fragment from different organs of experimental infected *B. mori* larvae. PCR amplification of BmNPV DNA using 40 mg of each sample. Lane 1: DNA Markers = 100-1000 bp; Lane 2: Foregut; Lane 3: Midgut; Lane 4: Hemolymph of experimental infected fifth instar silkworm larvae.

representative of the lepidopteran insects and has great importance in agriculture and the economy. Xia *et al.* (2004) termed the silkworm *B. mori* "economically important" due to its use in silk industry to maximize silk fibre productivity. The silkworm is a promising model organism in life science due to its short generation time, rich genetic resources, clearly sequenced genetic background and a considerable number of genes that are homologous to silkworm that makes it suitable for various life science studies (Meng *et al.,* 2017). It is a powerful insect model in research owing to its relatively large size and easy maintenance and rearing (Xia *et al.,* 2014).

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India       ATGCCGAATTATTCATACACCCCCACCATCGGGCGTACTTACGTGTACGACAATAAATAT
China       ATGCCGAATTATTCATACACCCCCACCATCGGGCGTACTTACGTGTACGACAATAAATAT
Thailand    ATGCCGAATTATTCATACACCCCCACCATCGGGCGTACTTACGTGTACGACAATAAATAT
            ************************************************************

India       TACAAAAAACTTGGGCTGTCTTATCAAAAACGCCAAGCGCAAGAAGCACCTAGTCGAACAT
China       TACAAAAAACTTGGGCTGTCTTATCAAAAACGCCAAGCGCAAGAAGCACCTAGTCGAACAT
Thailand    TACAAAAAACTTGGGCTGTCTTATCAAAAACGCCAAGCGCAAGAAGCACCTAGTCGAACAT
            ************************************************************

India       GAACAAGAGGAGAAGCAATGGGATCTTCTAGACAACTACATGGTTGCCGAAGATCCCTTT
China       GAACAAGAGGAGAAGCAATGGGATCTTCTAGACAACTACATGGTTGCCGAAGATCCCTTT
Thailand    GAACAAGAGGAGAAGCAATGGGATCTTCTAGACAACTACATGGTTGCCGAAGATCCCTTT
            ************************************************************

India       TTAGGACCGGGCAAAAACCAAAAACTTACCCTTTTTAAAGAAATTCGCAGTGTGAAACCC
China       TTAGGACCGGGCAAAAACCAAAAACTTACCCTTTTTAAAGAAATTCGCAGTGTGAAACCC
Thailand    TTAGGACCGGGCAAAAACCAAAAACTTACCCTTTTTAAAGAAATTCGCAGTGTGAAACCC
            ************************************************************

India       GATACCATGAAGTTAATCGTCAACTGGAGCGGCAAAGAGTTTTTGCGTGAAACTTGGACC
China       GATACCATGAAGTTAATCGTCAACTGGAGCGGCAAAGAGTTTTTGCGTGAAACTTGGACC
Thailand    GATACCATGAAGTTAATCGTCAACTGGAGCGGCAAAGAGTTTTTGCGTGAAACTTGGACC
            ************************************************************

India       CGTTTTGTTGAGGACAGCTTCCCCATTGTAAACGACCAAGAGGTGATGGACGTGTACCTC
China       CGTTTTGTTGAGGACAGCTTCCCCATTGTAAACGACCAAGAGGTGATGGACGTGTACCTC
Thailand    CGTTTTGTTGAGGACAGCTTCCCCATTGTAAACGACCAAGAGGTGATGGACGTGTACCTC
            ************************************************************

India       GTCGCCAACCTCAAACCCACACGCCCCAACAGGTGCTACAAGTTCCTCGCTCAACACGCT
China       GTCGCCAACCTCAAACCCACACGCCCCAACAGGTGCTACAAGTTCCTCGCTCAACACGCT
Thailand    GTCGCCAACCTCAAACCCACACGCCCCAACAGGTGCTACAAGTTCCTCGCTCAACACGCT
            ************************************************************

India       CTTAGGTGGGAAGAAGACTACGTGCCCCACGAAGTAATCAGAATTGTGGAGCCATCCTAC
China       CTTAGGTGGGAAGAAGACTACGTGCCCCACGAAGTAATCAGAATTGTGGAGCCATCCTAC
Thailand    CTTAGGTGGGAAGAAGACTACGTGCCCCACGAAGTAATCAGAATTGTGGAGCCATCCTAC
            ************************************************************

India       GTGGGCATGAACAACGAATACAGAATTAGTCTGGCTAAAAAGGGCGGCGGCTGCCCAATC
China       GTGGGCATGAACAACGGATACAGAATTAGTCTGGCTAAAAAGGGCGGCGGCTGCCCAATC
Thailand    GTGGGCATGAACAACGAATACAGAATTAGTCTGGCTAAAAAGGGCGGCGGCTGCCCAATC
            ***************-********************************************

India       ATGAACATCCACAGCGAGTACACCAACTCGTTCGAGTCGTTTGTGAACCGCGTCATATGG
China       ATGAACATCCACAGCGAGTACACCAACTCGTTCGAGTCGTTTGTGAACCGCGTCATATGG
Thailand    ATGAACATCCACAGCGAGTACACCAACTCGTTCGAGTCGTTTGTGAACCGCGTCATATGG
            ************************************************************

India       GAGAACTTCTACAAACCCATCGTTTACATCGGCACAGACTCTGCCGAAGAAGAGGAAATC
China       GAGAACTTCTACAAACCCATCGTTTACATCGGCACAGACTCTGCCGAAGAAGAGGAAATC
Thailand    GAGAACTTCTACAAACCCATCGTTTACATCGGCACAGACTCTGCCGAAGAAGAGGAAATC
            ************************************************************

India       CTAATTGAGGTTTCTCTCGTTTTCAAAATAAAGGAGTTTGCACCAGACGCGCCTCTGTTC
China       CTAATTGAGGTTTCTCTCGTTTTCAAAATAAAGGAGTTTGCACCAGACGCGCCTCTGTTC
Thailand    CTAATTGAGGTTTCTCTCGTTTTCAAAATAAAGGAGTTTGCACCAGACGCGCCTCTGTTC
            ************************************************************

India       ACTGGTCCGGCGTATTAA
China       ACTGGTCCGGCGTATTAA
Thailand    ACTGGTCCGGCGTATTAA
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Fig. 5: Clustal W multiple sequence alignment of *Bombyx mori* nucleopolyhedrovirus (NPV) Polyhedrin Gene sequence of Indian isolate of nucleopolyhedrovirus (NPV) Polyhedrin Gene with sequences of nucleopolyhedrovirus (NPV) Polyhedrin Gene isolates of China and Thailand.

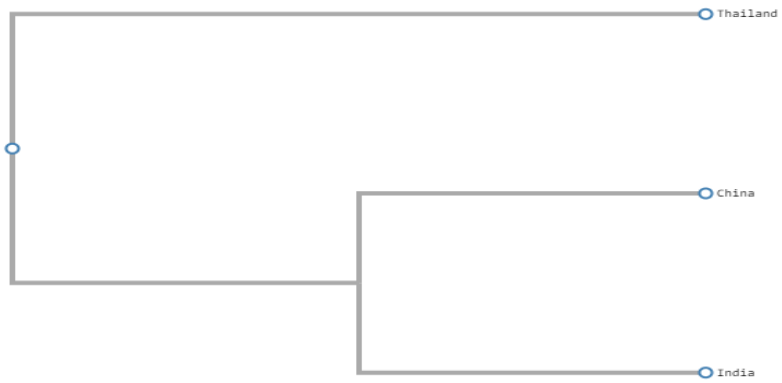

Fig. 6: Phylogenetic analysis *Bombyx mori* nucleopolyhedrovirus (NPV).

In silkworm, *B. mori* the most common mode of entry of NPV is per oral suspension as in the case of other lepidopteran insects. Individual feeding of a piece of leaf coated with 10 µl suspension of $2x10^5$ OBs/ml ensured the ingestion of a dosage of about 2000 OBs/larva which was sufficient to initiate the infection since about 50% mortality occurred prior to eclosion of adult moths. Usually a successful infection depends on the ingestion of sufficient virus to initiate replication in the host. The virus uptake is controlled by larval behavior, feeding rate, and the concentration and distribution of the viral dosage (Hunter *et al.,* 1984). In this study, the naturally infected fifth instar larvae were collected from Athiyanur village. Method of experimental silkworm infection by Hunter *et al.* (1984) was followed. The infected 5th instar larvae become elevated intersegmental membrane, swelled body and white turbid haemolymh containing a large number of viral polyhedral occlusion bodies (OBs) were observed. The cadaver was white at the time of death and became dark within 3-5 h as they decomposed. Rashmi *et al.* (2016) have morpho-logically identified the 5th instar larvae of infected silkworm and detected the early infection of Grasserie virus (BmNPV) polyhedrin gene (polh) in silkworm *Bombyx mori* by PCR technique.

In this study, we have optimized the polymerase chain reaction for amplification of a target DNA gene sequence from baculoviruses. Viral DNA obtained from infected 5th instar larvae were good sources of DNA for PCR amplification. The polyhedron gene, which has about 80% sequence homology among NPVs (Rohrmann, 1986), proved to be a suitable gene for the development of a generic amplification technique. The polyhedrin gene also contains less conserved regions from which specific primers have been designed. The present study performed the PCR amplification and sequencing of BmNPV polyhedrin gene from natural and experimental infected fifth instar larvae of silkworm. Both naturally and experimentally infected BmNPV silkworm yielded the amplification product of ~738 bp. We also performed agarose gel electrophoresis analysis of PCR amplified DNA gene fragment from different organs of natural and experimental infected *B. mori*larvae with target DNA marker. Khurad *et al.* (2004) have reported the PCR amplification of 473 bp gene of BmNPV isolated from infected silkworm larvae.

Conclusion

We have developed a PCR procedure with suitable forward and reverse primers capable of amplifying ~738 bp from infection of Grasserie virus (BmNPV) polyhedrin gene (polh) in silkworm *Bombyx mori*. DNA amplification coupled with restriction enzyme analysis of the PCR products proved to be a specific and powerful technique. The technique will also be helpful to promote the antiviral product against BmNPV.

References

Acharya A, Sriram S, Sehrawat S, Rahman M, Sehgal D and Gopinathan K. (2002) *Bombyx mori* nucleopolyhedrovirus: Molecular biology and biotechnological applications for large-scale synthesis of recombinant proteins. Curr Sci. 83: 455-465.

Alletti GG, Sauer AJ, Weihrauch B, Fritsch E, Undorf-Spahn K, Wennmann JT and Jehle JA. (2017) Using next generation sequencing to identify and quantify the genetic composition of resistance-breaking commercial isolates of *Cydia pomonella* granulovirus. Viruses 9: 250.

Ardisson-Araújo DMP, Melo FL, Andrade MDS, Brancalhão RMC, Báo SN and Ribeiro BM. (2014) Cyomplete genome sequence of the first non-Asian isolate of *Bombyx mori* nucleopolyhedrovirus. Virus Genes 49: 477-484.

Attathom T, Attathom S, Kumpratueang S and Audtho M. (1994) Early detection of Grasserie disease of silkworm, *Bombyx mori* by DNA probe. In Proc 32nd Kasetsart University Anual Conference: Plant Science, Kasetsart University, Bangkok, Thailand, pp. 257-271.

Bao YY, Tang XD, Lv ZY, Wang XY, Tian CH, Xu YP and Zhang CX. (2009) Gene expression profiling of resistant and susceptible *Bombyx mori* strains reveals nucleopolyhedrovirus-associated variations in host gene transcript levels. Genomics 94: 138-145.

Chitra C, Karanth NGK and Vasantharajan VN. (1975) Diseases of mulberry silkworm, *Bombyx mori*. J Sci Ind Res. 34: 386-401.

Gani M, Chouhan S, Babulal, Gupta RK, Khan G, Bharath Kumar N, Saini P and Ghosh MK. (2018a) *Bombyx mori* nucleopolyhedrovirus (BmNPV): Its impact on silkworm rearing and management strategies. J Biol Control 31: 189-193.

Gani M, Josepha AVM, Satish L, Chouhan S, Gadwala M, Ahmad MN and Sivaprasad V. (2018b) Prevalence and molecular characterization of viruses causing diseases in *Bombyx mori* L. (Lepidoptera: Bombycidae) from different climatic regions of India. J Biol Control 32: 252-256.

Gani M, Senger S, Lokanath S, Saini P, Bali K, Gupta R, Sivaprasad V, Johannes AJ and Jörg TW. (2021) Patterns in genotype composition of Indian isolates of the *Bombyx mori* Nucleopolyhedrovirus and *Bombyx mori* Bidensovirus. Viruses 13: 901.

Goldsmith MR, Shimada T and Abe H. (2005) The genetics and genomics of the silkworm, *Bombyx mori*. Annu Rev Entomol. 50: 71-100.

Hamamura Y. (1959) Food selection by silkworm larvæ. Nature 183: 1746-1747.

Herniou EA, Luque T, Chen X, Vlak JM, Winstanley D, Cory JS and O'Reilly DR. (2001) Use of whole genome sequence data to infer Baculovirus phylogeny. J Virol. 75: 8117- 8126.

Hunter FR, Crook NE and Entwistle PF. (1984) Viruses as pathogens for the control of insects. In: Microbiological Methods for Environmental Biotechnology, (eds.) Grainger J.M. and Lynch J.M., Academic Press, New York, pp. 323–347.

Ke P, Walter M, Bärbel G, Vibor L, Yant SR, Luong T, Tomas C, Barbara M and Hans-Georg K. (2014) Quantitative microscopy of functional HIV post-entry complexes reveals association of replication with the viral capsid. Elife 3: e04114.

Khurad AM, Mahulikar A, Rathod MK, Rai MM, Kanginakudru S and Nagaraju J. (2004) Vertical transmission of nucleopolyhedrovirus in the silkworm, *Bombyx mori* L. J Invertebr Pathol. 87: 8-15.

Krishnaswami S, Narasimhanna MN, Suryanarayan SK and Kumararaj S. (1973) Manual of Sericulture. Vol. 2. Silkworm Rearing. FAO, Rome.

Kumar P, Baig M, Sengupta K and Govindaiah (1990) Handbook on Pest and Disease Control of Mulberry and Silkworm. https://digitallibrary.un.org/record/167892?ln=en.

Mallika K. (2006) Potential application of PCR based method for early detection of Grasserie disease of silkworm, *Bombyx mori*. Thesis, Graduate School, Kasetsart University.

McCook ART, Fernández BC and Ruiz A. (2021) Description of the main aspects influencing *Bombyx mori* L. (Lepidoptera: Bombycidae) rearing. Cuba J Agric Sci. 5: 1-13.

Meng L, Li H, Bao M and Sun P. (2017) Metabolic pathway for a new strain *Pseudomonas synxantha* LSH-70: From chemotaxis to uptake of n-hexadecane. Sci Rep. 7: 39068.

Nagaraju J and Goldsmith MR. (2002) Silkworm genomics-progress and prospects. Curr Sci. 83(4): 415-425.

Nataraju B, Datta RK, Baig M, Balavenkatasubbaiah M, Samson MV and Sivaprasad V. (1998) Studies on the prevalence of nuclear polyhedrosis in sericultural areas of Karanataka. Ind J Seric. 37: 154-158.

Nataraju B, Sivaprasad V, Datta RK, Gupta SK and Shamim M. (1994) Colloidal textile dye-based dipstick immunoassay for the detection of nuclear polyhedrosis virus (BmNPV) of silkworm, *Bombyx mori* L. J Invertebr Pathol. 63: 135-139.

Rashmi PJ and Raja IA. (2016) Polymerase chain reaction based detection of Grasserie virus, BmNPV in silkworm, *Bombyx mori*. Biosci Biotech Res Comm. 9(3): 471-474.

Ravikumar G, Urs SR, Prakash NV, Rao C and Vardhana K. (2011) Development of a multiplex polymerase chain reaction for the simultaneous detection of microsporidians, nucleopolyhedrovirus, and densovirus infecting silkworms. J Invertebr Pathol. 107: 193-197.

Reddy BK and Rao JV. (2009) Seasonal occurrence and control of silkworm diseases, grasserie, flacherie and muscardine and insect pest, uzi fly in Andhra Pradesh, India. Int J Ind Entomol. 18: 57-61.

Rohrmann GF. (1986) Polyhedrin structure. J Gen Virol. 67: 149991513.

Sajjan Dayanand B and Hinchigeri Shivayogeppa B. (2015) Structural organization of Baculovirus occlusion bodies and protective role of multilayered polyhedron envelope protein. Food Environ Virol. 7(4): 309-416.

Sambrook J and Russel DW. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA.

Samson MV, Baig M, Sharma SD, Balavenkatasubbaiah M, Sashidharan TO and Jolly MS. (1990) Survey on the relative incidence of silkworm diseases in Karnataka, India. Ind J Seric. 29: 248-254.

Shivaprakasam N and Rabindra RJ. (1995) Incidence of grasserie in silkworm, *Bombyx mori* L. in selected districts of Tamil Nadu. Ind J Seric. 34: 100-104.

Vanapruk P, Attathom T, Sanbatsiri K and Attathom S. (1992) Comparison of methods for the detection of nuclear polyhedrosis virus in silkworm, *Bombyx mori* Linn. In: Proc 30th Kasetsart University Annual Conference: Plant Science, Kasetsart University, Bangkok, Thailand, pp. 237-243.

Vasantharaj D and Ramamurthy VV. (2015) Elements of Economic Entomology, 8th edition, Brillion Publishing, New Delhi.

Vidya MJ. (1960) Indian sericulture problems of modernization. Publicity serial (Popular brochures). 1. Central Silk Board, Bombay, India, 330, 64.

Xia Q, Zhou Z, Lu C, Cheng D, Dai F, Li B, Zhao P, Zha X, Cheng T, Chai C, Pan G, Xu J, Liu C, Lin Y, Qian J, Hou Y, Wu Z, Li G, Pan M, Li C, Shen Y, Lan X, Yuan L, Li T, Xu H, Yang G, Wan Y, Zhu Y, Yu M, Shen W, Wu D, Xiang Z, Yu J, Wang J, Li R, Shi J, Li H, Li G, Su J, Wang X, Li G, Zhang Z, Wu Q, Li J, Zhang Q, Wei N, Xu J, Sun H, Dong L, Liu D, Zhao S. Zhao X, Meng Q, Lan F, Huang X, Li Y, Fang L, Li C, Li D, Sun Y, Zhang Z, Yang Z, Huang Y, Xi Y. Qi Q, He D, Huang H, Zhang X, Wang Z, Li W, Cao Y, Yu Y, Yu H, Li J, Ye J, Chen H, Zhou Y, Liu B, Wang J, Ye J, Ji H, Li S, Ni P, Zhang J, Zhang Y, Zheng H, Mao B, Wang W, Ye C, Li S, Wang J, Wong GK and Yang H. (2004) Biology Analysis, A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). Science 306: 1937-1940.

Xia Q, Li S and Feng Q. (2014) Advances in silkworm studies accelerated by the genome sequencing of *Bombyx mori*. Annu Rev Entomol. 59: 513-536.

Zhang ZJ, Zhang SS, Niu BL, Ji DF, Liu XJ, Li MW, Bai H, Palli SR, Wang CZ and Tan AJ. (2019) A determining factor for insect feeding preference in the silkworm, *Bombyx mori*. PLoS Biol. 17: 3000162.

Zhou Y, Chen K, Yao Q, Shen H, Liang G, Li X, Wang N and Yijia Li. (2010) Characterization of a late expression gene of *Bombyx mori* nucleopolyhedrovirus. Z Naturforsch. 65: 508-518.

Zhou Z, Yang H, Chen M, Lou C, Zhang Y, Chen K, Wang Y, Yu M, Yu F, Li J and Zhong B. (2008) Comparative proteomic analysis between the domesticated silkworm (*Bombyx mori*) reared on fresh mulberry leaves and on artificial diet. J Proteome Res. 7: 5103-5111.