Phylogenetic Relationship of DpNPV (Diaphania pulverulentalis Nuclear Polyhedrosis Virus) with Other Lepidopteran NPVs based on Polyhedrin Gene

P. Priyadharshini1*, K.C. Narayanaswamy2 and C.A. Mahalingam3
1Department of Agrl.Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu
2Department of Sericulture, University of Agricultural Sciences, Bangalore-560 065, Karnataka
3Department of Sericulture, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu

The mulberry leaf-roller, Diaphania pulverulentalis (Hampson) is a major defoliator pest of mulberry in sericultural tracts of Karnataka, India. A nuclear polyhedrosis virus isolated from D. pulverulentalis larvae was analysed via its polyhedrin gene, which was amplified from DpNPV DNA in polymerase chain reaction using degenerate set of primers and the sequence was analysed phylogenetically with other polyhedrin genes. The analysis revealed that DpNPV was closely related to Galleria mellonella NPV, Plutella maculipennis NPV and Autographa californica NPV and distinct from Bombyx mori NPV.

Keywords: Diaphania pulverulentalis, nuclear polyhedrosis virus, polyhedrin gene, phylogenetic analysis.

Baculoviruses (Baculoviridae) are a diverse group of insect viruses, with good potential as biological insecticides (Moscardi, 1999) mainly used to control insect pest populations due to their high species-specificity, efficiency against certain pests and safety (Blissard and Rohrmann, 1990). Traditional approaches for classification of organisms using morphological features or molecular sequences such as ribosomal DNA (Woese et al., 1990) do not easily transfer to viruses. For lack of fossil records for viruses, their streamlined gene complements and the plasticity of their genomes compared to cellular organisms. Never theless, viral gene sequences useful for reconstructing phylogenies have been identified. DNA polyhedrin gene homologs have been used to infer phylogenetic relationships among DNA viruses, but other genes such as DNA polymerase, egt, gp41, lef2 and gp37 have also been used (Chen et al., 1999; Jin et al., 1999; Kang et al., 1998).

Productivity in sericulture is mainly based on the quality and yield of mulberry leaves, which are the only source of food for rearing of silkworms. The occurrence of NPV on mulberry leaf-roller, Diaphania pulverulentalis, a major pest of mulberry in South India has been reported (Rajadurai et al., 1999). In the present study, a polyhedrin gene was sequenced from DpNPV (Diaphania pulverulentalis nuclear polyhedrosis virus) and a phylogenetic tree based on polyhedrin gene sequence was constructed and the phylogenetic position of DpNPV was resolved.

Materials and Methods

The DpNPV polyhedrin gene (Diaphania pulverulentalis nuclear polyhedrosis virus) was amplified in PCR reactions using degenerate set of primers for polyhedrin. The forward and reverse primers used for PCR were GTTAAA CCGGACACC ATGAAGC and AACTTCTACAA GCCCATCGTTTAC. Reaction products were cloned into pGEM-T easy plasmids (promega) and automatically sequenced (Bangalore Genei, Bangalore). The sequence obtained was deposited in GenBank under the accession number of FJ 901339.

In order to assess the relationship of DpNPV polyhedrin with other lepidopteran polyhedrin NPVs, phylogenetic analysis of baculovirus occlusion matrix proteins was carried out. Multiple sequence comparison and phylogenetic inferences were performed with CLUSTAL W package and trees were constructed using Neighbourhood method. For this analysis, lepidopteran NPVs and those with the highest amino acid identity with DpNPV polyhedrin genes were used. The DpNPV polyhedrin gene sequence was compared with other polyhedrin genes and granulin gene sequences such as Bombyx mori NPV (DQ231336), Galleria mellonella MNPV (AY706696), Lymamtria monacha NPV (AY706702), Lymamtria xylina NPV (AY706703), Malacosoma neustria NPV(AY706708), Mamestra brassicace NPV (AY706705), Peridroma marginalosa NPV (AY706710), Actias selene NPV (AY706680), Agrotis segetum NPV(AY706683), Anagraphe falcifera NPV(AY706686), Antheraea pernyi NPV (AY706687), Autographa biloba NPV(AY737724), AY706681 Autographa californica NPV (AY706681), Buzura suppressaria NPV (DQ231342), Plutella maculipennis NPV (AY706713), Samia cythia NPV (AY706711), Spodoptera litura NPV (AY706715), Spodoptera littoralis NPV (AY706717), Agrotis segetum granulovirus (AY706661), Choristoneura...
murinana granulovirus (AY706663), Cydia pomonella granulovirus (AY706667), Hyphantria cunea granulovirus (AY706669), Pieris brassicae granulovirus (DQ235253), Pieris rapae granulovirus (AY706673) and Spodoptera frugiperda granulovirus (AY706677) downloaded from GenBank.

Results and Discussion

The application of PCR combined with molecular phylogeny provides an excellent method for fast and reliable baculovirus identification and needs only tiny traces of a sample even if the virus cannot be recovered (Lange et al., 2004). The degenerate oligonucleotides used in the present study remained unchanged in their gene specific binding region. The partial sequence of DpNPV polyhedrin gene was determined and its length was 396 bp representing 63 per cent of the complete polyhedrin open reading frame. Comparison of the multiple sequences revealed that there was high degree of DNA sequence similarity between DpNPV with AcNPV, GmNPV, PmNPV and AbNPV and the base pair substitution took place at particular nucleotide through the entire length of the 396 bp. Even though, these NPVs showed variability with other NPVs and GVs, bases starting from 58 to 122 Table 1. Per cent identity of DpNPV with other NPV polyhedrin genes

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<th>Per cent Identity</th>
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The results also showed that the NPVs used in this analysis were clustered along at least four branches named in this study as group I, II (II-A, II-B, II-C), III (III-A, III-B ) and IV. The granuloviruses are placed as a sister group to the lepidopteran NPV. Within the NPV group, Group IV was used as an outgroup based on its polyhedrin gene sequence divergence from the remaining polyhedrins. Granuloviruses are used as an outgroup V which included Galleria mellonella GV, Spodoptera frugiperda GV, Agrotis segetum GV, Choristoneura murinana GV, Pieris brassicae GV, Pieris rapae GV and Hyphantria cunea GV which are highly different from D. pulverulentalis NPV (Fig. 1). The present study clearly indicated that D. pulverulentalis NPV was closely related to Galleria mellonella NPV, Plutella maculipennis NPV and Autographa california NPV and Autographa biloba NPV which showed 100 per cent identity (Table 1). The phylogenetic tree in Fig.1 implied that G. mellonella NPV, D. pulverulentalis NPV, P. maculipennis NPV, A. california NPV shared a common ancestral distinct from the other groups. The D. pulverulentalis NPV also clustered with Mamestra brassicae NPV (90.9%) and Buzura suppressaria NPV (90.9%) followed by Agrotis segetum NPV (89.4%), Peridroma margaritosa NPV (89.4%), Spodoptera litura NPV (88.6 %) and Spodoptera littoralis NPV (88.6%). The D. pulverulentalis NPV also showed identity with Malacosoma neustria NPV (85.6%) and B. mori (85.6%). D. pulverulentalis NPV was distantly related to Lymantria monacha NPV and Lymantria xylina NPV which showed identity of 84.0 and 82.4 per cent. The analysis also indicated that the same genus had close similarity which is confirmed with Autographa californica NPV and Autographa biloba.

Table 1. Per cent identity of DpNPV with other NPV polyhedrin genes
NPV, *Lymantria monacha* NPV and *Lymantria xy lina* NPV, *Spodoptera litura* NPV and *Spodoptera littoralis* NPV which corresponds closely to the host species separation within the host genus. This relationship apparently reflects a tight co-evolution between the viruses and their hosts. The GV s showed least similarity with NPVs ranging from 51.5 to 56.8 percent. The information obtained from the comparison of the phylogenies of the associated species is informative on the implied evolutionary interchange patterns (Brooks and McLennan, 1991). The actual interaction of viruses and hosts happened mainly at the cellular and molecular levels and therefore, the utilization of molecular data for virus phylogeny is appropriate. The increasing amount of molecular sequence data available from DNA and RNA viruses are being used in comparative studies which allow phylogenetic inferences and better understanding

![Phylogenetic tree analysis of DpNPV with other polyhedrin genes and Granuloviruses](image)

**Figure 1.** Phylogenetic tree analysis of DpNPV with other polyhedrin genes and Granuloviruses


It was concluded that the phylogenetic analysis based on polyhedrin gene sequences of DpNPV was closely related to *Galleria mellonella* NPV, *Plutella maculipennis* NPV and *Autographa californica* NPV and *Autographa biloba* NPV and distantly related to *Bombyx mori*. This molecular and biological information of DpNPV elucidated in the present study could be useful in understanding the relationships within viruses and to promote the use of baculoviruses in insect pest control strategies.

**References**


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