

# Analysis of the nonstructural and structural polyprotein regions, and complete genome sequences of Israel acute paralysis viruses identified from honeybees (*Apis mellifera*) in Korea



Kondreddy Eswar Reddy<sup>a,b,\*\*,1</sup>, Jin Hyeong Noh<sup>a,1</sup>, Young-Ha Kim<sup>a</sup>, Mi Sun Yoo<sup>a</sup>,  
Huong Thi Thanh Doan<sup>a,c</sup>, Mummadireddy Ramya<sup>b</sup>, Suk-Chan Jung<sup>a</sup>,  
Dong Van Quyen<sup>c</sup>, Seung-Won Kang<sup>a,\*</sup>

<sup>a</sup> Parasitology and Insect Disease Research Laboratory, Animal and Plant Quarantine Agency, 480 Anyang, 6 dong, Anyang 420-480, South Korea

<sup>b</sup> Plant Molecular Biology Lab, Department of Botany, S. K. University, Anantapur, Andhrapradesh, India

<sup>c</sup> Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam

## ARTICLE INFO

### Article history:

Received 6 May 2013

Returned to author for revisions

31 May 2013

Accepted 10 June 2013

Available online 22 July 2013

### Keywords:

*Apis mellifera*

Honeybee

Israel acute paralysis virus

Phylogenetic tree

## ABSTRACT

Phylogenetic trees were constructed for 24 partial nucleotide sequences of the nonstructural polyprotein (ORF1) and structural polyprotein regions (ORF2) of Korean IAPV genotypes, as well as eight previously reported IAPV sequences from various countries. Most of the Korean genotypes formed a distinct cluster, separate from other country genotypes. To investigate this phenomenon in more detail, three complete IAPV genome sequences were identified from different regions in Korea, i.e., Korea1, Korea2, and Korea3. These sequences were aligned with eight previously reported complete genome sequences and various genome regions were compared. The Korean IAPVs were very similar to those from China and Israel, but highly diverged from USA and Australian genotypes. Interestingly, they showed greater variability than the USA and Australian genotypes in ORF1, but highly similar to the Australian genotype in the ORF2 region. Thus, genetic recombination may account for the spatial distance between the Korean IAPV genotypes and those from other countries.

© 2013 Elsevier Inc. All rights reserved.

## Introduction

The honeybee, *Apis mellifera*, is the world's most economically important insect pollinator and over one-third of the human diet is considered to depend on honeybee pollination (McGregor, 1976; Delaplane and Mayer, 2000). The market value of the food pollinated by honeybees exceeds \$US 212 billion worldwide (Gallai et al., 2009). However, the health and vigor of honeybees worldwide are compromised by various disease agents, including viruses, bacteria, fungi, protozoa, and parasitic mites (Bailey and Ball, 1991; Ellis and Munn, 2005). Viruses are probably the least well understood of these pathogenic agents, due to a lack of information about the fundamental dynamics of viral disease outbreaks.

Israel acute paralysis virus (IAPV) infections of bees are characterized by shivering wings, followed by progression to paralysis and death outside the hive. IAPV was first detected in Israel (Maori et al., 2007) but was later found to have a global distribution (Cox-Foster et al., 2007; Blanchard et al., 2008; Palacios et al., 2008). The presence of IAPV has been strongly correlated with a new syndrome in honeybees that has caused losses in the USA and Europe, which is known as colony collapse disorder (CCD) (Cox-Foster et al., 2007). IAPV has been characterized as a new member of the Dicistroviridae family and is closely related to the Kashmir bee virus (KBV) and Acute bee paralysis virus (ABPV), although it differs genetically and serologically (Maori et al., 2007).

IAPV is a positive-strand RNA virus that contains two long open reading frames (ORFs), which are separated by an intergenic region. The 5'-ORF encodes a nonstructural polyprotein (Maori et al., 2007) that contains several signature sequences for a helicase, protease, and RNA-dependent RNA polymerase. The structural polyprotein, which is located downstream of the nonstructural polyprotein, encodes two capsid proteins (Maori et al., 2007).

Despite the worldwide distribution and frequency of honeybee virus strains, very few studies have focused on the genetic diversity of these viruses. Direct sequencing of amplicons and phylogenetic analyses of sequences can provide insights into the

\* Corresponding author. Fax: +82314671828.

\*\* Corresponding author at: Parasitology and Insect Disease Research Laboratory, Animal and Plant Quarantine Agency, 480 Anyang, 6 dong, Anyang 420-480, South Korea

E-mail addresses: [eswar@mail.hallym.ac.kr](mailto:eswar@mail.hallym.ac.kr),  
[eswar4uk@gmail.com](mailto:eswar4uk@gmail.com) (K.E. Reddy),  
[kangsw777@korea.kr](mailto:kangsw777@korea.kr) (S.-W. Kang).

<sup>1</sup> Co-first authors (contributed equally).

genetic relationships among different virus strains. This approach may even allow the prediction of virus taxonomies, which has already been achieved in many cases (Berenyi et al., 2007; Tapaszti et al., 2009; Reddy et al., 2013).

To the best of our knowledge, very few studies have focused on IAPV infection in Korea, so the present study was to be the first to analyze and report the complete nucleotide sequences of three IAPV genomes from South Korea and compared them with the nucleotide sequences of IAPV genotypes from different countries. The phylogenetic relatedness of the partial nucleotide sequences of the IAPV nonstructural polyprotein region (ORF1) and the structural polyprotein region (ORF2) were also analyzed in strains from different regions of South Korea to assess the genetic relationships among IAPV strains with various geographic origins.

## Results

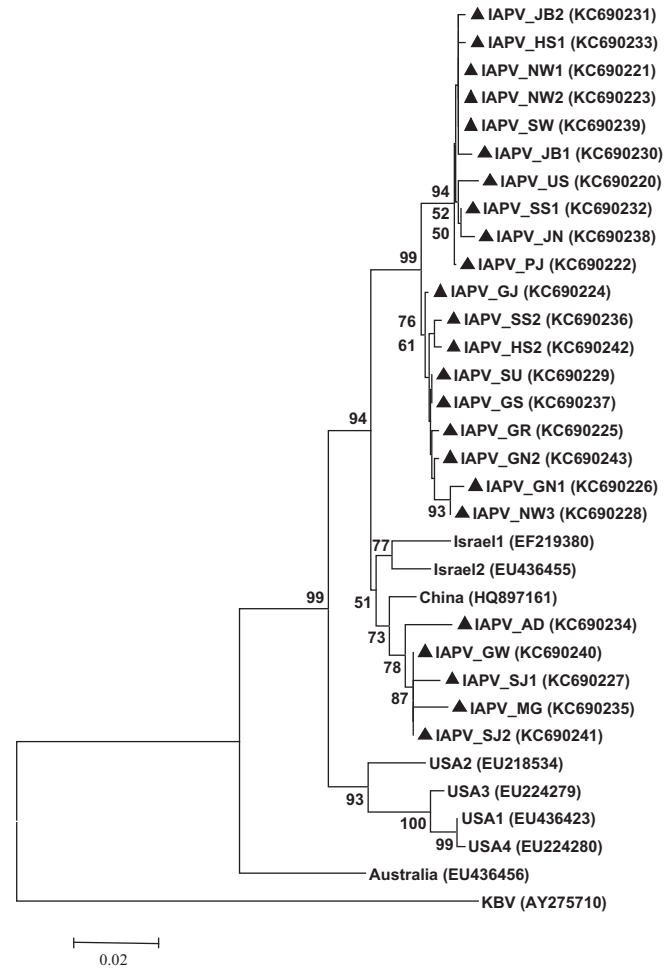
### Detection of the IAPV samples

Initially, two primer pairs were used to screen for the presence of IAPV in 89 colonies of *A. mellifera* samples, finally 24 infected samples were identified in the present study.

### Phylogenetic analysis of IAPV nonstructural polyprotein region and structural polyprotein region genotypes

Phylogenetic trees were constructed for the Korean IAPV partial nonstructural polyprotein regions and structural polyprotein regions, which were compared with previously reported IAPV sequences from various countries. The partial nonstructural polyprotein region shared 95–99% sequence identity among Korean isolates, while the Korean genotypes shared 91–98% similarity with genotypes from other countries. The structural polyprotein region shared 93–99% identity among the Korean genotypes and 89–98% homology with genotypes from other countries. The Korean genotypes were highly conserved and they formed geographically separated sub-clusters, which were distinct from genotypes from other countries. Most of the genotypes were separated from those identified in the USA and Australia. However, a small number were closely related to China, Israel1, Israel2, and Australia. Compared with the structural polyprotein region, the nonstructural polyprotein region exhibited greater diversity compared with the genotypes from other countries.

The phylogenetic tree based on the partial nonstructural polyprotein region sequences had a very different topology where the IAPV genotypes were separated completely from the KBV genome sequence, which was used as an external node (Fig. 1). As shown in the tree, the Australian (91–93%) and USA genotypes (93–94%) were clearly segregated from Korean genotypes, which formed separate branches. The isolates investigated were highly conserved and they formed three sub-clusters. However, IAPV\_AD, IAPV\_GW, IAPV\_MG, IAPV\_SJ1, and IAPV\_SJ2 Korean genotypes were separated and shared greater similarity with the China, Israel1, and Israel2 genotypes (96–98%), compared with the remaining Korean genotypes, and together they formed a separate sub-cluster (Fig. 1). In the tree based on the partial structural polyprotein region, all of the IAPV genotypes were separated as a main branch from the KBV genotype, which was used as an external node (Fig. 2). The second main branch divided into two main groups. One main group contained the investigated Korean genotypes (except for IAPV\_AD and IAPV\_MG) with 94–99% similarity and it was also sub-divided into small sub-clusters. The second main group contained genotypes from different countries and the Korean IAPV\_AD and IAPV\_MG genotypes. In this group, the USA strains formed a separate sub-cluster. Interestingly, the IAPV\_AD and IAPV\_MG Korean genotypes were more similar (97–98%) to those



**Fig. 1.** Phylogenetic tree showing IAPV genotypes from Korea and other countries. The nucleotide sequences of the partial nonstructural polyprotein regions of 24 *A. mellifera* IAPV genotypes from different Korean regions (the final two capital letters of each sample name indicate their place of origin) and eight *A. mellifera* IAPV genotypes from other countries were aligned using CLUSTALW. The phylogenetic tree was constructed with MEGA5 using the neighbor-joining method. The accession number of each viral sequence is indicated in parentheses.

from Australia (92% for the ORF1 region) than the remaining Korean genotypes.

### Phylogenetic analysis of complete IAPV genome sequences

A phylogenetic analysis was conducted using the complete Korea1, Korea2, and Korea3 IAPV genome sequences, and the results were compared with previously reported complete IAPV genome sequences (Fig. 3). As shown in Fig. 3, the tree diverged to form two main branches. All of the IAPV genotypes formed the first main branch, while the KBV genome was highly diverged (76–77%) and formed a second main branch. The IAPV genotypes in the first main branch split into two sub-branches. One sub-branch contained the Australian genotype while the second sub-branch split into two groups, one of which contained all of the USA genotypes and the other contained the Korea1, Korea2, Korea3, China, Israel1, and Israel2 genotypes. Thus, three Korean genotypes and the Chinese genotype sub-clustered with the Israeli genotypes.

### Comparison of the variability between different IAPV genomic regions

Three complete Korean IAPV genome sequences were identified from different regions in Korea and they were aligned with

Download English Version:

<https://daneshyari.com/en/article/6140867>

Download Persian Version:

<https://daneshyari.com/article/6140867>

[Daneshyari.com](https://daneshyari.com)