Nilaparavata lugens (stal) genetic divergence analysis

Nilaparavata lugens (Stal.)

Nilaparvata lugens is generally called as brown plant hopper or Asian rice brown plant hopper (BPH). *N. lugens* comes under order: Hemiptera, Suborder: Auchennorrhyncha and Family: Delphacidae. The delphacid genus *Nilaparvata* consists of 18 species. *Nilaparvata* is a crucial genus economically, with one amongst its member *N. lugens* being serious insect pests of rice and are cosmopolitian particularly in tropical Asia wherever rice crops are continuously cultivated (Wilson and Claridge, 1991). These insects are found throughout the year in tropical regions. In temperate areas such as in middle China, Korea and Japan, however, they do not survive within the winter season, and migrate into these countries every year with the new rice season (Park, 1973; Kisimoto, 1976; Asahina and Tsruoka, 1986; Uhm *et al.* , 1988).

The species is mostly yellowish brown to dark brown (Fig. 1). The carina on vertex is faint and the median carina on the frons is distinct. In *Nilaparvata* genus the first tarsal segments bears several small spines. *N. lugens* is mostly seen on paddy fields *.* The key characters of the family are the presence of a movable spur at the base of the hind tarsi. To distinguish the major groups within the family mostly the structure of the spur is considered. In case of doubts the male genetalial characters are also used to confirm the identity of species. The male genitalia are distinctive with aedeagus slender and upturned. The parameres are distinctively shaped. In female inner margin of the valvifer VIII is rounded at the base (Wilson and Claridge, 1991).

Both nymphs and adults of BPH cause damage rice plants through intensive feeding on them. BPH conjointly transmits viruses such as rice ragged stunt (RRSV) and rice grassy stunt (RGSV) (Hibino, 1996). The foremost severe outbreak of the BPH in India occurred in Kerala state at the end of 1973 and early in 1974 (Koya, 1974; Nalinakumari and Mammen, 1975). It occurred in the ‘ Kole’ lands of Trichur district and Kuttanad area in Kottayam and Alleppy districts. In many several fields the damage was so great that growers abandoned the crop (Das *et al* ., 1972).

(d) adults on paddy plant

The partial coding sequence of mitochondrial COI gene of *N. lugens* was PCR amplified using the forward primer with DNA sequence 5′ TAAACTTCAGGGTGACCAAAAAATCA 3′ and the reverse primer with DNA sequence 5′ GGTCAACAAATCATAAAGATATTGG 3′. The PCR amplification of partial COI sequence of *N. lugens* isolated from Kerala, India yielded a product with 658 bp. The DNA sequence obtained, its conceptual translation product, nucleotide BLAST, peptide BLAST and Chromatogram are presented in figures (2-6). The sequence was deposited within the GenBank with a GenBank Accession No. KJ796483.

> *Nilaparvata lugens* cytochrome oxidase subunit I gene | Voucher SRCUNILA01| partial cds; mitochondrial| 658 bp| Accession No. KJ796483

> *Nilaparvata lugens* | Voucher SRCUNILA01| cytochrome oxidase subunit I, partial (mitochondrion) peptide| 219 AA.

Nilaparvata lugens voucher AIN18001AP1 Cytochrome oxidase subunit I (COX1) gene, partial cds; mitochondrial Sequence ID: gb| KC858992. 1| *Nilaparvata lugens* | Length: 658

Nilaparvata lugens cytochrome oxidase subunit I (mitochondrion)GenPeptGraphicsSequence ID: lcl| 82965| *Nilaparvata lugens* | Length: 219

The COI nucleotide sequence analysis revealed the composition of nucleotides in the COI gene of *N. lugens* isolated from Kerala (Table 2). The COI sequence of *N. lugens* showed bias to nucleotide AT, with following composition of nucleotides T= 27. 9%, C= 15. 9%, A= 35. 3% and G= 20. 9%. The COI nucleotide composition analysis showed the variation in composition of each nucleotide of *N. lugens* isolated from Kerala and geographically isolated populations like Karnataka (KC858992), China (AB572318, KC476395 and KC476394).

Alyses of Evolutionary Divergence were conducted using the Maximum Composite Likelihood model. The analysis concerned 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There have been a total of 258 positions in the final data set. Evolutionary analyses were conducted in MEGA6. The evolutionary divergence analysis of *N. lugens* depicts the degree of divergence in the Delphacidae family of different geographically isolated populations of *N. lugens* is shown in table (3).

From the data given in the table (3) it is clear that the *N. lugens* showed 0. 02% evolutionary divergence with *N. lugens* isolated from Karnataka and the

species from China. *Mongoliana serrata* species is the one which showed a maximum of 2. 41% of evolutionary divergence with *N. lugens* isolated from Kerala.

The phylogeny tree generated using NJ method showing the phylogenetic position of *N. lugens* isolated from Kerala is given in figure (7). Phylogenetically *N. lugens* (JN391181) and (AB572314) showed to be the closest relatives of *N. lugens* of Kerala. They were arranged in were aligned in single clad and divided into two sub branches separately.

Discussion

The sample were successfully sequenced using the forward and reverse primers to get forward and reverse sequences of 658 bp. The sequence identification with the NCBI BLAST tool disclosed that closest similarity of 99% is shown by *N. lugens* (GenBank accession No. KC858992) isolates from Karnataka. The above data with NCBI BLAST tool revealed that no 100% sequence similarity for COI gene are available in the data base. It can be interpreted that the resultant sequence obtained for the *N. lugens* Kerala is novel. The COI sequence obtained during this study showed variation with other species of identical family pointing its use as a DNA barcode to spot the species. The COI sequence could also be used for evolutionary studies and host insect relation studies of *N. lugens* . The most of the hemipteran species mitogenomes nucleotide compositions are significantly biased toward A and T. The mitochondrial genome of hemipteran species has considerable variation in base composition among different hemipteran species (Zhang *et al.* , 2013). The COI nucleotide sequence analysis of *N.*

lugens isolated from Kerala and other geographically isolated population also showed 63 to 65% AT content in the COI sequence which is varied from the total AT content of J strand which is 70 to 74. 42%. These results indicated that, in the hemiptera the AT content of COI gene will be less compared to the total AT content of J strand. In the nucleotide triplet code, there is strong compulsion in the nucleotide changes in second position of all codons and first position of many codons. Due to the degenerative character of the triplet code third position of many codons and first position of some codons is less constraint.

The variations in the strong constraint positions lead to the variations in the amino acids sequence. But the variations in the less constraint position will not affect (silent) the phenotype and these less constrained codon positions evolved at high rate (Nei, 1987; Irwin et al., 1991).

The genetic divergence analysis revealed the genetic difference within the species *N. lugens* and between the related species of order Hemiptera. The COI sequence of *N. lugens* showed considerable variations with the related species. Thus the COI sequence isolated during this study can be used as a barcode to spot this insect species. Within the evolution tree the *N. lugens* isolated from totally different geographical locations are formed from a common ancestor. This indicates that the *N. lugens* is a monophyletic species.