Genetic analysis of brown planthopper, *Nilaparvata lugens* (Stål.) (Hemiptera: Delphacidae) based on microsatellite markers

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Abstract

Brown planthopper, *Nilaparvata lugens* (Stål.) is one of the most ruinous pest of rice in south-east Asia. This insect tends to express a differential reaction to resistant rice cultivars and various insecticide groups in different geographic locations. Therefore, genetic diversity among the *N. lugens* populations must be understood for their effective management. Hence, in the present study, the genetic structure and diversity of 22 *N. lugens* populations collected from 22 hotspot regions of India were studied with genomic simple sequence repeats markers. Results revealed that mean genetic diversity was 0.399 and polymorphic information content was 0.337 in the 30 selected SSR markers. Further, molecular variance revealed only a 2% variation among the populations and 98% within the population. In cluster and population structure analysis, all 22 populations were sub-grouped into three groups.Interestingly, the North and West Indian populations showed high genetic similarity and assembled into one cluster in cluster analysis. The East and South Indian populations were evenly segregated into the rest two clusters. Similarly, the North and West Indian populations were again deemed to be occupied the same compartment in Principal coordinate

analysis. This variation might be associated with the *N. lugens* migration due to wind movement of the south-west monsoon in two branches *viz.* Arabian Sea branch and Bay of Bengal branch. The present study provides the molecular evidence for genetic variation among different populations of *N. lugens* in India. The information could be helpful to devise an efficient management strategy against this pest in different rice ecosystems.

Key words: Brown planthopper, populations, genetic diversity, monsoon, India

1. Introduction

Rice (*Oryza sativa* L.), is the foremost imperative staple crop and a major source of nutrition for 90% of the Asian population. It is produced in more than 100 countries worldwide, but India and China together contribute more than 50% of the rice production. India's total rice production is about 117.47 million tonnes, with a productivity of 2.6 tonnes per ha[1]. To keep pace with the increasing world population and dietary demands, global food production is needed to be increased by 70% by 2050[2]. However, rice production is constantly threatened by various insect-pest attacks that inflict 28% economic losses every year[3]. The brown planthopper, *Nilaparvata lugens* is a notorious pest of rice that causes 70-100% yield losses in the Asian subcontinents[4-5]. They destroy the crop by sucking the phloem sap that leads to distinct hopper-burn symptoms and indirectly damage the crop by transmitting viral infections such as grassy stunt and ragged stunt.

The chemical control and use of resistant/tolerant varieties are the most efficient methods of pest management in different rice growing areas[6-7]. But unfortunately, continuous and high doses of insecticide lead to insecticidal resistance and a resurgence in the *N. lugens* population. Furthermore, monocropping and greater use of nitrogenous fertilizers have been linked to *N. lugens* outbreaks becoming more common and intense[8]. In the last few decades, *N. lugens* has been hugely attacking rice and the fact that its outbreaks occur every year in some regions in India suggests the development of new virulent populations that can easily overcome the resistant traits in rice. So far, four *N. lugens* biotypes have been identified in rice ecologies worldwide, of which biotype-4 is the most damaging one[9], which is predominantly found in India[10-11]. It is well documented that many rice varieties/resistant sources previously resistant to this pest have become susceptible to biotype-4[12]. Apart from this, a varied virulent reaction to a group of insecticides has been seen in the *N. lugens* populations from different geographical locations[13-14] due to differential gene activation[15-16].

Likewise, *N. lugens* susceptibility to insecticides also varied in different rice-growing regions in India, like in Karnataka state, Mandya and Soraba populations were found to be more sensitive to insecticides than the Gangavati and Kathalgere populations[17]. The *N. lugens* population's target site insensitivity or resistance has been attributed to mutation or upregulation of detoxifying enzyme genes[18]. To know the underlying mechanism of evolving resistance in different *N. lugens* population, their genetic structure and diversity need to be thoroughly investigated with different populations from India.

Moreover, due to a dearth of comprehensive knowledge and studies on population structure, gene flow, and genetic diversity within a biotype of *N. lugens* and ineffective coordination of pest management strategies for different populations, *N. lugens* populations have not been suppressed successfully. All these factors urge for a sound understanding of genetic variability, environmental adaptability, and the migration pattern of *N. lugens* to formulate rational control tactics. Hence, an attempt was made to evaluate the population structure and genetic diversity of *N. lugens* in India.

To retrieve the genomic basis of insect diversity and gene pool, different markers like microsatellite markers, mitochondrial DNA, single nucleotide polymorphism markers have been used by entomologists worldwide[19]. Amongst them, microsatellites have been exploited as popular markers in entomological research because of excessive abundance and noticeable variability in their genome[20]. Earlier studies have proven that in addition to population genetics, microsatellite markers are also helpful in resolving the phylogenetic problems of closely related species[21-22]. Hence, this study was designed to explore the genetic structure and diversity of *N. lugens* from distinct geographic areas of India using genomic markers.

2. Material and methods

2.1. Insect sampling location

Twenty-two *N. lugens* populations were sampled from various hotspot regions representing different agroclimatic zones of India during three consecutive years 2020-22 (Figure 1; Supplementary Table 1). Adults of *N. lugens* were collected using an aspiratoreach year that was preserved with 95% ethanol and stored at -20°C.

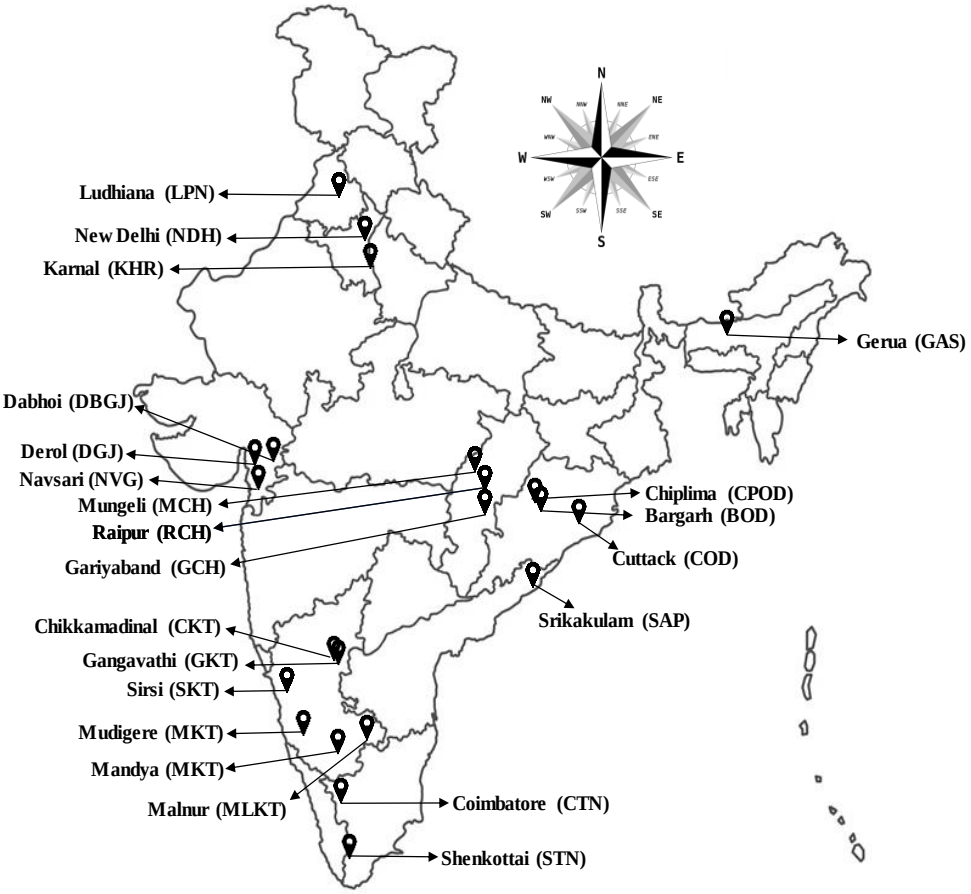


Figure 1. *Nilaparvata lugens* collection sites from different rice cultivating regions in India

2.2. DNA extraction and quantification

The genomic DNA from 22 *N. lugens* populations was isolated using cetyltrimethylammonium bromide (CTAB) method[10]. In brief, the preserved sample of *N. lugens* was pulverized in 500 µl of CTAB solution (2% w/v) using a sterile micro-pestle and then incubated in a water bath at 65°C for 1h. Then, 24: 1: 1 v/v ratio of phenyl: chloroform: isoamyl alcohol (PCI) was added @ 500 µl/sample and after centrifugation at 12,000 rpm for 10 min, the aqueous phase is collected in fresh microtubes. For precipitation of DNA, pre-chilled isopropanol (450 µl) was added and was stored at –20°C for 15–30 min and then centrifuged at 12,000 rpm for 15 min at 4°C. The resultant DNA pellets were centrifuged with 70% ethanol at 12,000 rpm for 10 min at 4°C with 70% ethanol. DNA pellets were dried and re-suspended in 100 µl Tris-EDTA (Ethylene Diamine Tetra Acetic Acid) buffer. The quantity and quality of extracted DNA were estimated by using NanoDrop One^{C} (Thermo Fisher Scientific, USA).

2.3 PCR amplification with SSR markers

Initially, sixty-three SSR primers were used for the present study. After the screening, 30 SSR primers with clear allelic bands were used for data analysis (Supplementary Table 2). The thermal cycle for PCR comprised of an initial denaturation at 95°C/3 min, followed by 34 cycles of denaturation at 94°C/30 sec, annealing temperature (Supplementary Table 2) for 30 sec, and extension at 72°C/1 min, and a final extension at 72°C/10 min. Later, the resultant PCR product was visualized using TAE buffer (40 mM Tris-acetate, 1 mM EDTA) in 2% (w/v) agarose gel. The study repeated three years from 2020 to 2022 to confirm the results.

2.4 Data analysis

The banding patterns were observed with a gel documentation system (BIORAD, USA). Those SSR bands were analysed, and scoring was given (1 for presence; 0 for absence; 9 for not amplified, missing value). POWER MARKER Ver 3.25 was used to estimate polymorphic information content (PIC), major allele frequency, number of alleles per locus, and genetic diversity of the selected markers[23]. Analysis of molecular variance (AMOVA), Fixation index (Fst) and principal coordinate analysis (PCoA) of the 22 *N. lugens* populations was performed by GenAlEx ver.6.502[24]. The Mantel test achieved genetic isolation by distance[25]. STRUCTURE version 2.3.4 software was used for genetic structure and admixture detection in the 22 *N. lugens* populations. Similarly, Structure Harvester Ver.0.6.193 software calculated K-value by ΔK method[26-27]. The unweighted neighbor-joining (NJ) tree was constructed with 30 molecular markers using DARwin version 6.0.21 for genetic diversity and phylogenetic analysis.

3. Results and Discussion

3.1 Genomic analysis

In the present study, 30 SSR markers were selected for the genotypic diversity of 22 *N. lugens* populations based on the polymorphism and allelic banding patterns (Table 1). A total of 69 alleles were detected, of which 89.85% (62 bands) were polymorphic, resulting from 23 SSR markers. Alleles per locus ranged from 1-5 with a mean of 2.533. The amplicon size varied from 100bp to 300bp in primers NLGS1360 and NLGS1627, respectively. Observed heterozygosity was found to be 0.205 with a range of 0.136 (NLGS1807 and NLGS3014) to 1.000 (NLGS1871). For 23 polymorphic SSR markers, the major allele frequency varied from 0.386 to 0.864. Further, the mean value of major allele frequency for all 30 markers was 0.683. The mean genetic diversity of all 30 markers amplified in 22 *N. lugens* populations was 0.399, of which a maximum of 0.710 and minimum of 0.236 was

found in the NLGS246 and NLGS1420 markers, respectively. Besides, the average polymorphic information content (PIC) was 0.337 and varied from 0.208 to 0.671 (Table. 1).

Table 1. Genetic diversity parameters and amplification pattern of 30 SSR markers in different *N. lugens* populations

Markers	Major Allele Frequency	No. of allele	Gene Diversity	Heterozygosity	PIC
NLGS751	0.523	2	0.499	0.045	0.374
NLGS1405	0.818	3	0.310	0.273	0.282
NLGS1915	0.682	3	0.459	0.000	0.387
NLGS1492	0.386	3	0.650	0.318	0.574
NLGS1871	0.500	2	0.500	1.000	0.375
BM1372	0.773	2	0.351	0.000	0.290
BM1378	0.568	5	0.593	0.545	0.537
BM1432	0.545	3	0.549	0.045	0.456
BM1443	0.773	3	0.376	0.273	0.344
BM1446	0.682	2	0.434	0.455	0.340
BM1464	0.477	3	0.542	0.955	0.437
BM1471	0.818	4	0.317	0.182	0.299
NLGS264	0.455	5	0.710	0.364	0.671
NLGS2996	0.545	4	0.616	0.364	0.560
NLGS775	1.000	1	0.000	0.000	0.000
NLGS1627	0.500	3	0.616	0.182	0.542
NLGS873	0.659	2	0.449	0.409	0.348
NLGS1360	0.727	2	0.397	0.000	0.318
NLGS825	1.000	1	0.000	0.000	0.000
NLGS2379	0.545	2	0.496	0.000	0.373
NLGS2735	0.500	2	0.500	0.000	0.375
NLGS1016	0.500	3	0.574	0.273	0.484
NLGS1807	0.841	3	0.278	0.136	0.257
NLGS1420	0.864	2	0.236	0.000	0.208
NLGS2289	1.000	1	0.000	0.000	0.000
NLGS2282	0.773	2	0.351	0.000	0.290
NLGS3014	0.614	3	0.505	0.136	0.416
NLGS2828	0.409	3	0.657	0.182	0.583
NLGS1487	1.000	1	0.000	0.000	0.000
NLGS271	1.000	1	0.000	0.000	0.000
Mean	0.683	2.533	0.399	0.205	0.337

3.2 AMOVA and coordinates analysis

The molecular variance (AMOVA) was evaluated to analyse the distance matrix of inter and intra *N. lugens* population collected from India's four ecological zones (North, East,

West and South zones). The result revealed that the intra-population variation was only 2% where as it was 98% within the populations (Table 2; Figure 2a). The highest pairwise F_{ST} value of 0.723 was recorded between the North Indian and East Indian populations showing more significant genetic variance. The lowest value of 0.156 was recorded between the South Indian and West Indian populations. Similarly, pairwise F_{ST} values of 0.388 and 0.382 between the North and West Indian & Wast and South Indian population denoting low and moderate genetic variability, respectively (Table 3). The principal coordinate analysis (PCoA) result further confirmed genetic similarity among the North and West Indian populations occupying separate compartments than the rest (Figure 2b). The first and second compartments *viz.* PC1 and PC2 presented a percent variance of 21.61% and 18.37%, respectively (Supplementary Table 3). The Mantel test did not support a significant association (r^2 = 0.0287; p = 0.124) between genetic variation based on geographic distance (Figure 3), suggesting no genetic isolation-by-distance (IBD) effect was present in the 22 populations.

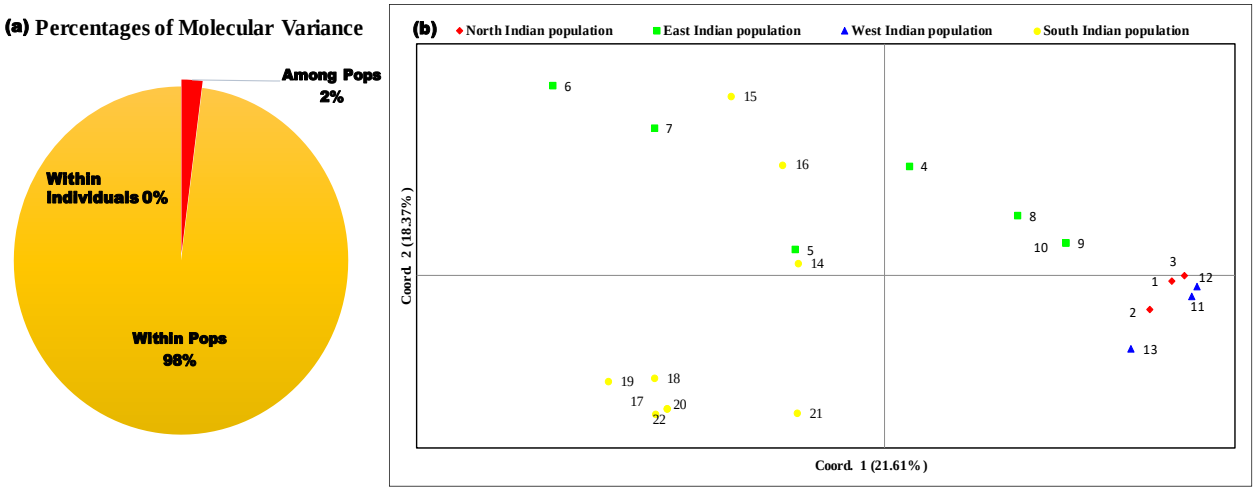


Figure 2. (a) Analysis of molecular variance, **(b)** Principal Coordinate Analysis of 22 *N. lugens* population linked to 30 markers.

Table 2. AMOVA among and within population

Source	df	Sum of square	Mean square	Estimated Variance	Percentage of variation
Among Population	2	678005.444	339002.722	6298.787	2%
Within Population	15	4565390.889	304359.393	304359.393	98%
Within individual	18	0.000	0.000	0.000	0%
Total	35	5243396.333	-	310658.180	100%

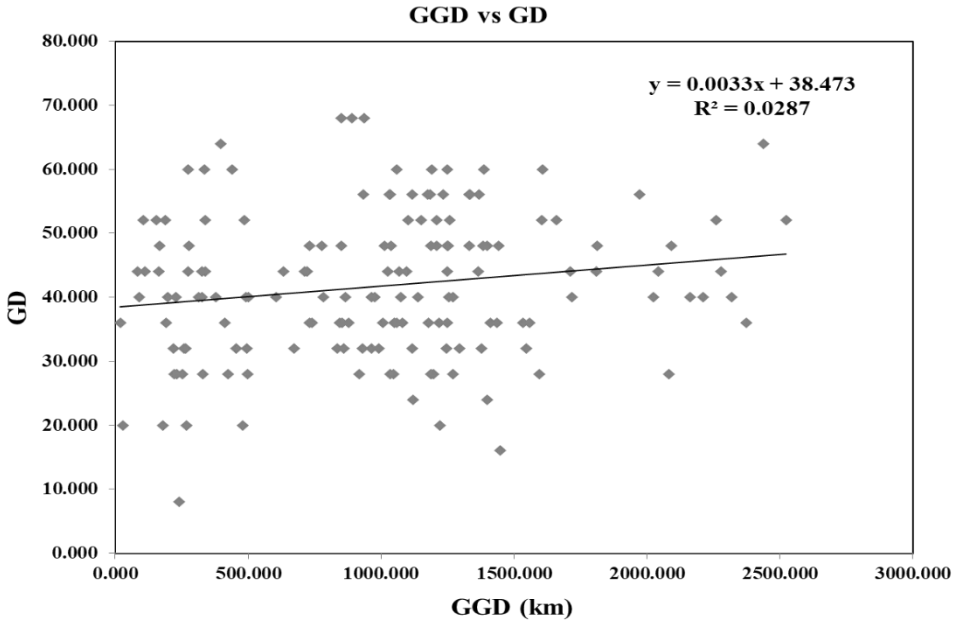


Figure 3. Mantel test showing regression of genetic distance (GD) of 22 *N. lugens* populations against geographical distances (GGD).

Table 3. Pair-wise FST estimates among three populations *N. lugens* (Allele Data)

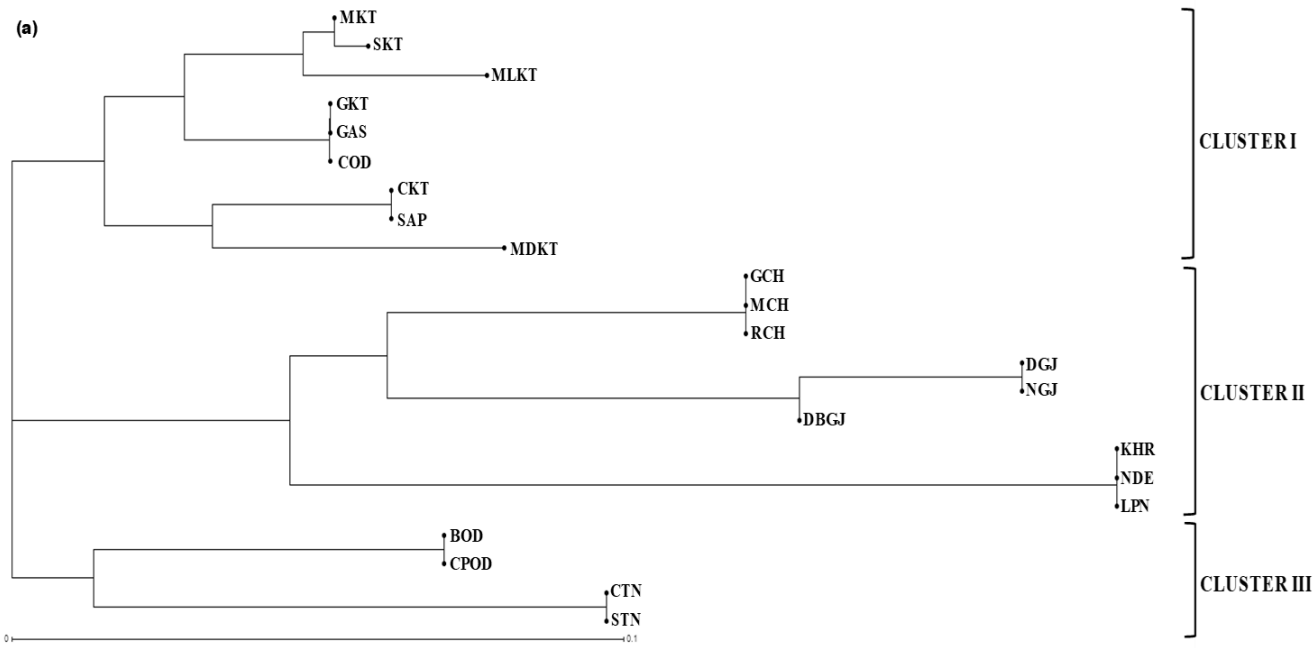
	North Indian population	West Indian population	East Indian population	South Indian population
North Indian population	0.000			
West Indian population	0.388	0.000		
East Indian population	0.723	0.353	0.000	
South Indian population	0.374	0.156	0.382	0.000

3.3 Cluster and Population structure analysis

The clustering framework based on Neighbor-joining (NJ) tree categorised the 22 *N. lugens* populations into three major clusters (Figure 4a). Both cluster I and II consists of nine *N. lugens* populations each. All North Indian (Ludhiana, Punjab; New Delhi, Delhi; and Karnal, Haryana) and West Indian (Navsari, Gujarat; Derol, Gujarat; and Dabhoi, Gujarat) populations were grouped into cluster II along with Chhattisgarh population. The East (Gerua, Assam; Cuttack, Odisha; Chiplima, Odisha; Bargarh, Odisha; Raipur, Chhattisgarh; Mungeli, Chhattisgarh; and Gariaband, Chhattisgarh) and South Indian (Srikakulam, Andhra Pradesh; Shenkottai, Tamil Nadu; Coimbatore, Tamil Nadu; Mandya, Karnataka; Sirsi, Karnataka; Mudigere, Karnataka; Malur. Karnataka; Chikkamadinal, Karnataka; and Gangavathi, Karnataka) populations were randomly distributed in cluster I and III. Moreover, the majority of South Indian populations (Karnataka and Andhra Pradesh populations) were

grouped in cluster-I along with three east Indian populations (Gerua, Assam; and Cuttack, Odisha) and the rest were grouped in cluster-III (Figure 4a).

Similarly, the population structure analysis again displayed three sub-groups of the studied 22 *N. lugens* populations based on a threshold value; >70% with a highest value of 7.522 for ΔK at K = 3 (Figure. 4b & 4c) (Supplementary Table 4). Further, sub-population II (SPII) was the largest subpopulation which entails all Karnataka (KT) and Andhra Pradesh (AP) populations along with two east and west Indian populations. Besides, sub-population I (SPI) consists of all three populations of North India followed by Sub-population III (SPIII), which includes all Odisha and Tamil Nadu populations along with Gariyaband; Chhattisgarh population. The analysis also showed two populations (Mungeli and Derol) from Chhattisgarh were found to be admixture. The net nucleotide distance of three sub-populations was calculated (Supplementary Table 5) and the expected heterozygosity with alpha value ($\alpha = 0.060$) was found between the sub-populations (Supplementary Table 6).



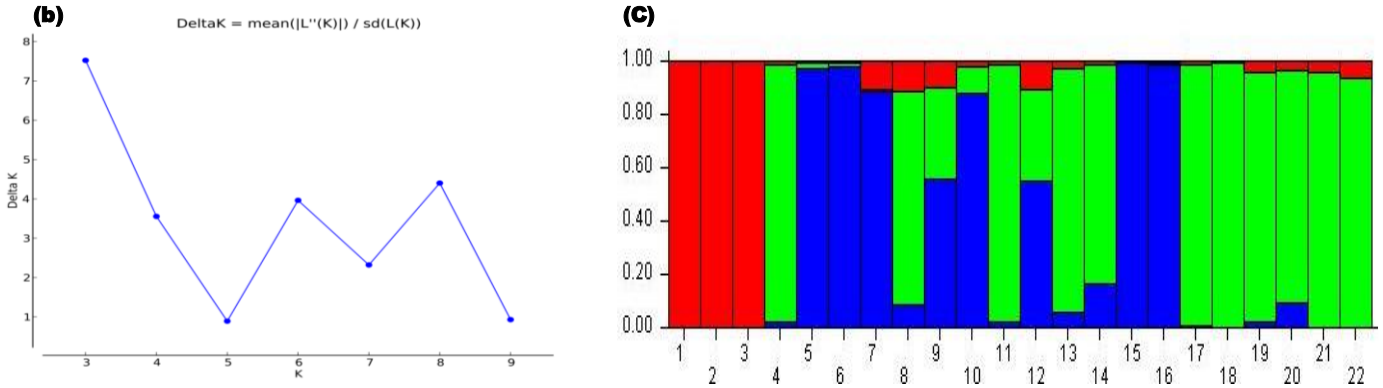


Figure 4. (a) Unrooted Neighbor-Joining (NJ) tree representing three clusters; (b) Delta-K value; (c) population structure showing bar plot partitioned the entire population into three subgroups based on coloured segments for ΔK at K = 3 of 22 *N. lugens* population linked to 30 markers.

N. lugens is the major insect pest in South and south-east Asia, causing an annual loss of 70-100% every year in rice. This pest continuously evolves with new resistance traits to many insecticidal groups and well-known resistant rice cultivars[6-7,28] with varied resistance reactions in different locations[13-14,17]. The varietal screening trial under the All India Coordinated Rice Improvement Project (AICRIP) revealed a speckled response in *N. lugens* populations from various locations to known gene differential rice varieties claimed some level of genetic diversity in the country[6, 29]. To unveil the differential resistance reaction, it is essential to identify the genetic diversity in various geographic populations. In this study, genetic variation among 22 *N. lugens* populations sampled from 22 hotspot regions of India which encompass north, south, east and west agroecological zones, was examined with 30 SSR DNA markers.

SSR markers were widely used for examining bio-geographical relationships and genetic diversity of intra and inter-populations[19, 30]. In our analysis, 30 markers displayed the mean PIC value of 0.337, allele per locus of 2.533, and major allele frequency of 0.683 among all populations. Earlier researchers reported similar results in genetic diversity analysis of *N. lugens* biotypes, with the average allele per locus ranging from 2.3±0.1 (biotype 1) to 4.5±0.2 (biotype Y)[31]. In our study, PIC value represents a medium level of polymorphism with the least heterozygosity in the selected populations[32]. Many earlier studies on *N. lugens* confirmed genetic homogeneity due to a great degree of gene flow in Indian populations due to its migration habit[11, 33-34].

Cluster analyses revealed genetic relationships between different *N. lugens* populations, which were classified into three groups. Among the three major clusters in cluster analysis, north, and west Indian populations were assembled with the Chhattisgarh population in cluster II, revealing genetic relatedness among them, whereas east and south Indian populations were evenly distributed in cluster I and cluster III. From this result, the gene flow pattern can be correlated with the country's yearly wind current, as a population genetic structure changes due to random genetic drift, dispersion and migration[35-36]. Being a soft flyer, *N. lugens* generally depend on prevailing wind current and direction for their migration[37]. Polymorphic markers like SSR are suitable for accessing the migratory behaviour of a species as they are co-dominant and show greater diversity per locus than other conventional methods[36]. Similarly, microsatellite markers were used to study the genetic structure and migration in migratory insects like *N. lugens*[36]; *Sitobion avenae* (Fabricius)[35] and *Mythimna separata* (Walker)[38].

The migration of *N. lugens* in India is heavily influenced by the southwest monsoon, which is a key component of the country's yearly rainfall[37, 39]. South-west monsoon arrives during June-July in two branches. The Arabian Sea branch flows along the coastal side of Western Ghats mountain towards western and north-western India. This could explain the genetic similarity observed in our study between West and North Indian populations. In contrast, the Bay of Bengal branch is the main cause of rainfall in southeast and north-east India, which explains the even gene flow among south and east Indian populations in the present study. Homogeneity in *N. lugens* populations was also reported by earlier researchers, where a majority of them were sampled from east and south India[34]. While the ΔK method in population structure analysis again identified three potential structures at $K = 3$ that represent some genetic diversity in the population. Again, the Principal coordinate analysis (PCoA) successfully distinguished between the north and west Indian populations from others. Moreover, variation exit in the North and South Indian populations suggests modifying the pest management strategies to target the gene-specific traits in Indian *N. lugens* that could be effective for both North and South Indian populations.

Mantel test for IBD analysis showed a nonsignificant correlation between genetic and geographic distances of 22 *N. lugens* populations suggesting unrestricted gene flow among them. Similar to our results, IBD analysis result were found to be non-significant in different migratory insects like *Apis gossypii* (Glover) [40] and plant hoppers[34, 41]. AMOVA of 22 *N. lugens* population revealed a homogenous population with only 2% variation between the

populations and 98% variation within the population. Previous worker also reported low genetic differentiation (2.84%) among the Indian *N. lugens* populations[34, 42].

5. Conclusion

In this study, we have analysed the population structure and gene migration among the twenty-two *N. lugens* geographical populations in India using a genomic SSR marker. The findings revealed the presence of two genetic groups, North and West India & East and South India, as well as genetic homogeneity within the groupsFrom this, it can be speculated that genetic diversity in the population is due to their two-way migration strengthening by the Arabian Sea branch and Bay of Bengal branch of southwest monsoon in India, which may be a major factor for sub-grouping. Our results could be useful in designing different pest management strategies based on geographical locations to target specific genetic traits shared by different *N. lugens* population of India.

References

1. Pathak, H., Tripathi, R., Jambhulkar, N. N., Bisen, J. P., Panda, B. B., Eco-regional Rice Farming for Enhancing Productivity, Profitability and Sustainability. NRRI Research Bulletin No. 22. ICAR-National Rice Research Institute, Cuttack 753006, Odisha, India, 2020; pp. 28.
2. Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., Mortensen, D. A., Agriculture in 2050: Recalibrating targets for sustainable intensification. *Bioscience*, 2017, **67**(4), 386-391. https://doi.org/10.1093/biosci/bix010.
3. Mondal, D., Ghosh, A., Roy, D., Kumar, A., Shamurailatpam, D., Bera, R., Ghosh, S., Bandopadhyay, P., Majumder, A., Yield loss assessment of rice (*Oryza Sativa* L.) due to different biotic stresses under system of rice intensification (SRI). *J. Entomol. Zool. Stud.* 2017, **5**(4), 1974-1980.
4. Pandi, G. G. P., Chandar, S., Pal, M., Soumia, P. S., Impact of elevated CO2 on *Oryzasativa* phenology and brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) population. *Curr. Sci*. 2018, **114**(8), 1767-1777. https://doi.org/10.18520/cs/v114/i08/1767-1777
5. Jena, M., Pandi, G. G. P., Adak, T., Rath, P. C., Gowda, G. B., Patil, N. B., Golive, P., Mahapatra, S. D., Paradigm shift of insect pests in rice ecosystem and their management strategy. *Oryza*, 2018, 55, 82-89.

6. Anant, A., Govindharaj, G. P. P., Gajendra, C., Gowda, G. B., Patil, N., Annamalai, M., Adak, T., Rath, P., Jena, M., Evaluation of Brown plant hopper *Nilaparvata lugens* (stal.) resistance. *Indian J. Entomol*., 2021a, **83**(2), 223– 225.
7. Horgan, F. G., Penalver, C. A., Arida, A., Ferrater, J. B., Bernal, C. C., Adaptation by the Brown planthopper to resistant rice: a test of female-derived virulence and the role of yeast-like symbionts. *Insects*., 2021, 12, 908. https://doi.org/10.3390/ insects12100908
8. Hu, G., Lu, F., Zhai, B. P., Lu, M. H., Liu, W. C., Zhu, F., Wu, X. W., Chen, G. H., Zhang, X. X., Outbreaks of the Brown Planthopper *Nilaparvata lugens* (Stål) in the Yangtze River Delta: Immigration or Local Reproduction?. *PLOS ONE.*, 2014, **9**(2), e88973. https://doi.org/10.1371/journal.pone.0088973.
9. Muduli, L., Pradhan, S. K., Mishra, A., Bastia, D. N., Samal, K. C., Agrawal, P. K., Dash, M., Understanding Brown Planthopper Resistance in Rice: Genetics, Biochemical and Molecular Breeding Approaches. *Rice Sci.*, 2021, **28**(6), 532-546. https://doi.org/10.1016/j.rsci.2021.05.013.
10. Babu, S. B., Pandi, G. G. P., Parameswaran, C., Anant, A. K., Padhi, J., Bansal, R., Priyadarsini, S., Patra, B. C., Gowda, G. B., Annamalai, M., Patil, N., Rath, P. C., Genomic analysis and finding of candidate genes for *Nilaparvata lugens* (stål) resistance in Indian pigmented and other indigenous rice genotypes. *Crop Prot*., 2022, 156, 105959. https://doi.org/10.1016/j.cropro.2022.105959.
11. Pandi, G. G. P., Anant, A. K., Choudhary, J. S., Babu, S. B., Gowda, B. G., Annamalai, M., Patil, N., Adak, T., Panneerselvam, P., Rath, P. C., Molecular diversity of *Nilaparvata lugens* (Stål.) (Hemiptera: Delphacidae) from India based on internal transcribed spacer 1 (ITS1) gene. *Curr. Sci*., 2022, **122**(12), 1392-1400.
12. Anant, A. K., Pandi, G. G. P., Jena, M., Chandrakar, G., Chidambaranathan, P., Raghu, S., Gowda, G. B., Annamalai, M., Patil, N., Adak, T., Ramasamy, N., Rath, P. C., Genetic dissection and identification of candidate genes for brown planthopper, *Nilaparvata lugens* (Delphacidae: Hemiptera) resistance in 'farmers' varieties of rice in Odisha. *Crop Prot.*, 2021b, 144, 105600.
13. Mohan, U., Lakshmi, V. J., Sharma, S., Katti, G. R., Chirutkar, P. M., Krishnaiah, N. V., Monitoring of insecticide resistance in Rice brown planthopper *Nilaparvata lugens* (Stål) in Nalgonda District of Telangana State, India. *India Ann Plant Prot Sci.*, 2019, **27**(2), 172-176.

14. Deosi, K. K. and Suri, K. S. Status of insecticide resistance in rice brown planthopper (*Nilaparvata lugens*) in Punjab. *Indian J. Agric. Sci*., 2022, **92**(2), 203-207. https://doi.org/10.56093/ijasv92i2.122216
15. Garrood, W. T., Zimmer, C. T., Gorman, K. J., Nauen, R., Bass, C., Davies, T. G., Field-evolved resistance to imidacloprid and ethiprole in populations of brown planthopper *Nilaparvata lugens* collected from across South and East Asia. *Pest Manag Sci*., 2016, 72, 140–149.
16. Wu, S. F., Zeng, B., Zheng, C., Mu, X. C., Zhang, Y., Hu, J., Zhang, S., Gao, C. F., Shen, J. L., The evolution of insecticide resistance in the brown planthopper (*Nilaparvata lugens* Stål) of China in the period 2012–2016. *Sci Rep*., 2018, 8, 4586. https://doi.org/10.1038/s41598-018-22906-5.
17. Basanth, Y. S., Sannaveerappanavar, V. T., Gowda, D. K. S., Susceptibility of Different Populations of *Nilaparvata lugens* from Major Rice Growing Areas of Karnataka, India to Different Groups of Insecticides. *Rice Sci*., 2013, **20**(5), 371-378.
18. Ding, Z., Wen, Y., Yang, B., Zhang, Y., Liu, S., Liu, Z., Han, Z., Biochemical mechanisms of imidacloprid resistance in *Nilaparvata lugens*: Over-expression of cytochrome P450 *CYP6AY1*. *Insect Biochem Mol Biol.*, 2013, 43, 1021-1027.
19. Vieira, M. L., Santini, L., Diniz, A. L., Munhoz, C., Microsatellite markers: what they mean and why they are so useful. *Genet Mol Biol*., 2016, **39**(3), 312-328. https://doi.org/10.1590/1678-4685-GMB-2016-0027
20. Benali, S., Mohamed, B., Eddine, H. J., Neema, C., Advances of molecular markers application in plant pathology research. *Eur J Sci Res*., 2011, 50, 110-123.
21. Ince, A. G., Mehmet, K., Safinaz, E., New microsatellite and CAPS-microsatellite markers for clarifying taxonomic and phylogenetic relationships within Origanum L., *Mol Breeding*., 2014, 34, 643-654.
22. Hashem, M. H., Dalal, S. A., Areej, S. J., Nader, R., Abdelsalam, R. M., Abd, E. A., Ahmed, E. K., Amani, S. A., Abdallah, T. M., Ahmed, S. A., Manal, E. S., Hesham, A. H., Genetic divergence and phylogenetic relationship of the rabbitfish *Siganus rivulatus* inferred from microsatellite and mitochondrial markers. *J King Saud Univ Sci.*, 2022, **34**(4), 101943. https://doi.org/10.1016/j.jksus.2022.101943.
23. Liu, K., Muse, S. V., Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*., 2005, 21, 2128-2129.
24. Peakall, R., Smouse, P. E., Genalex6.5: genetic analysis in excel population genetic software for teaching and research an update. *Bioinformatics.*, 2012, 28, 2537-2539.

25. Mantel, N., The detection of disease clustering and a generalized regression approach. *Cancer Res*., 1967, 27, 209-220.
26. Earl, D. A., Structure HARVEASTER: a website and program for visualizing structure output and implementing ant Evanno method. *Conserv Genet Resour*., 2012, 4, 359–361.
27. Evanno, G., Regnaut, S., Goudet, J., Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol., 2005, 14, 2611–2620.
28. Jena, K. K., Kim, S., Current status of brown planthopper (*N. lugens*) resistant and genetics. *Rice*., 2010, 3, 161-171.
29. AICRIP, All-India Coordinated Rice Improvement Programme progress report entomology and plant pathology. ICAR-Indian Institute of Rice Research. 2018, 2, 19-20.
30. Duan, X. L., Peng X, Qiao X F, Chen M H, Life cycle and population genetics of bird cherry-oat aphids *Rhopalosiphum padi* (L.) (Hemiptera: aphididae) in China: an important pest on wheat crops. *J Pest Sci.,* 2017, 90, 103–116.
31. Jing, S., Liu, B., Peng, L., Peng, X., Zhu, L., Fu, Q., He, G., Development and use of EST-SSR markers for assessing genetic diversity in the brown planthopper (*Nilaparvata lugens* Stål). *Bull Entomol Res*., 2012, 102, 113–122.
32. Serrote, C. M. L., Reiniger, L. R. S., Silva, K. B., Rabaiolli, S. M. D. S., Stefanel, C. M., Determining the Polymorphism Information Content of a molecular marker. *Gene*., 2020, 726, 144175. https://doi.org/10.1016/j.gene.2019.144175
33. Srinivasa, N., Chander, S., Twinkle, Chandel, R. K., Genetic homogeneity in brown planthopper, *Nilaparvata lugens* (Stål) as revealed from mitochondrial cytochrome oxidase I. *Curr Sci*., 2020, **119**(6), 1045-1050.
34. Tyagi, S., Narayana, S., Singh, R. N., Srivastava, C. P., Twinkle, S., Das, S. K., Jeer, M., Migratory behaviour of Brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), in India as inferred from genetic diversity and reverse trajectory analysis. *3 Biotech.*, 2022, 12, 266.https://doi.org/10.1007/s13205-022-03337-6.
35. Llewellyn, K. S., Loxdale, H. D., Harrington, R., Brookes, C. P., Clark, S. J., Sunnucks, P., Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Molecular Ecology*., 2003, 12, 21–34.
36. Liu, J. N., Guia, F. R., Lib, Z. Y., Genetic diversity of the planthopper, *Sogatella furcifera* in the Greater Mekong Subregion detected by inter-simple sequence repeats (ISSR) markers. *Journal of Insect Science*., 2010, **10**(52).

37. Yang, S. J., Bao, Y. X., Chen, C., Lu, M. H., Liu, W. C., Hong, S. J., Effect of the Asian monsoon on the northward migration of the brown planthopper to northern South China. *Ecosphere.*, 2022, **13**(10), e4217.
38. Li, M. M., Li, B. L., Jiang, S. X., Zhao, Y. W., Xu, X. L., Wu, J. X., Microsatellite-based analysis of genetic structure and gene flow of *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) in China. *Ecology and Evolution*., 2019, **9**(23), 13426-13437. doi: 10.1002/ece3.5799. PMID: 31871655; PMCID: PMC6912921
39. Otuka, A., Dudhia, J., Watanabe, T., Furuno, A., A new trajectory analysis method for migratory planthoppers, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) and *Nilaparvata lugens* (Stål), using an advanced weather forecast model. *Agricu Entomol*., 2005, **7**(1), 1-9.
40. Nam, H. Y., Park, Y., Lee, J. H., Population Genetic Structure of *Aphis gossypii* Glover (Hemiptera: Aphididae) in Korea. *Insects*., 2019, **10**(10), 319.doi: 10.3390/insects10100319. PMID: 31561591; PMCID: PMC6835795
41. Winnie, R. M., Rafudin, R., Widiarta, I. N., Rauf, A., The Genetic Structure of *Nilaparvata lugens* (Stal.) in Java Populations. *Hayati J of Biosci*. 2020, **27**(4),333-334.
42. Babu, S. B., Pandi, G. G. P., Parameswaran, C., Padhi, J., Patra, B. C., Gowda, G. B., Annamalai, M., Patil, N., Meher, C., Sabarinathan, S., Rath, P. C., Genetic analysis of brown planthopper, Nilaparvata lugens (Stål.) (Hemiptera: Delphacidae) based on DNA Markers. Research Square Pre-print . posted 30 Jan, 2023. https://doi.org/10.21203/rs.3.rs-2508969/v1.