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

Soumya Bharati Babu[1,2], Govindharaj Guru-Pirasanna-Pandi[1,*], C. Parameswaran[1], Jayaraj Padhi[2], G. Basana-Gowda[1], M. Annamalai, Naveenkumar Patil[1], Chanchala Meher[1], S. Sabarinathan[1] and Prakash Chandra Rath[1]

[1]ICAR-National Rice Research Institute, Cuttack 753 006, India
[2]Odisha University of Agriculture and Technology, Bhubaneswar 751 003, India

Brown planthopper, *Nilaparvata lugens* (Stål) is one of the most destructive pests of rice in Southeast Asia. It expresses a differential reaction to resistant rice cultivars and various insecticide groups in different geographic locations. Therefore, genetic diversity among *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 analysed using with genomic simple sequence repeat (SSR) markers. Results revealed that the 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 a 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 remaining two clusters. Similarly, the North and West Indian populations shared the same compartment in principal coordinate analysis. This variation might be associated with the *N. lugens* migration due to wind movement of the southwest monsoon in two branches, viz. Arabian Sea branch and Bay of Bengal branch. The present study provides 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.

Keywords: Brown planthopper, genetic diversity, microsatellite markers, monsoon, rice.

RICE (*Oryza sativa* L.), is a staple crop and the 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/ha (ref. 1). To keep pace with the increasing world population and dietary demands, global food production must be increased by 70% by 2050 (ref. 2). However, rice production is constantly threatened by various insect-pest attacks that inflict 28% economic loss every year[3]. The brown planthopper, *Nilaparvata lugens* is a notorious pest of rice that causes 70–100% yield loss in the Asian subcontinents[4,5]. It destroys the rice crop by sucking the phloem sapthat leads to distinct hopper-burn symptoms, and indirectly damages the crop by transmitting viral infections such as grassy stunt and ragged stunt.

Chemical control and the use of resistant/tolerant varieties are the most efficient methods of pest management in different rice-growing areas[6,7]. Unfortunately, continuous and high doses of insecticides have led to insecticidal resistance and a resurgence in the *N. lugens* populations. Furthermore, monocropping and extensive 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 extensively 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 destructing[9]; this 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 (ref. 12). A varied virulent reaction to a group of insecticides has also been observed 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 varies in different rice-growing regions in India. For example, in Karnataka, Mandya and Soraba populations were found to be more sensitive to insecticides than the Gangavati and Kathalgere populations[17]. The target site insensitivity or resistance of *N. lugens* populations has

*For correspondence. (e-mail: Guru.G@icar.gov.in)

been attributed to mutation or upregulation of detoxifying enzyme genes[18]. To identify underlying mechanism of evolving resistance in different *N. lugens* populations, their genetic structure and diversity need to be thoroughly studied using 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 endorse a sound understanding of genetic variability, environmental adaptability and migration pattern of *N. lugens* to formulate rational control tactics. Hence, we made an effort 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 and single-nucleotide polymorphism markers have been used by entomologists worldwide[19]. Among 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 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 geographical areas of India using genomic markers.

Material and methods

Insect sampling location

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

DNA extraction and quantification

The genomic DNA from 22 *N. lugens* populations was isolated using cetyltrimethyl ammonium 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 1 h. Next, 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 was collected in fresh microtubes. For precipitation of the DNA, pre-chilled isopropanol (450 µl) was added and 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. DNA pellets were dried and resuspended in 100 µl tris-EDTA (ethylene diamine tetra acetic acid) buffer. The quantity and quality of the extracted DNA were estimated using NanoDropOne^{C} (Thermo Fisher Scientific, USA).

PCR amplification with SSR markers

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

Data analysis

The banding patterns were observed with a gel documentation system (BIORAD, USA). The 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 (F_{ST}) estimation and principal coordinate analysis (PCoA) of the 22 *N. lugens* populations were performed using GenAlEx

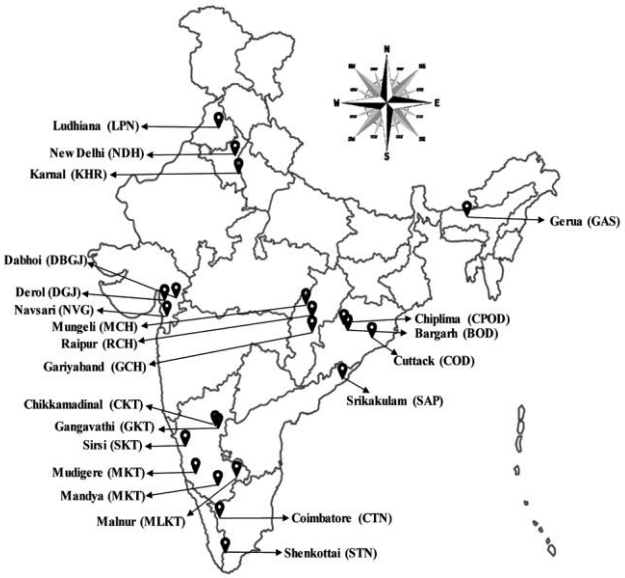

Figure 1. *Nilaparvata lugens* collection sites from different rice-cultivating regions of India.

Table 1. Genetic diversity parameters and amplification pattern of 30 simple sequence repeats (SSRs) markers in different *Nilaparvata lugens* populations

Markers	Major allele frequency	No. of alleles	Gene diversity	Heterozygosity	Polymorphic information content
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

ver. 6.502 (ref. 24). The Mantel test achieved genetic isolation by distance[25]. STRUCTURE ver. 2.3.4 was used for genetic structure and admixture detection in the 22 *N. lugens* populations. Similarly, STRUCTURE HARVESTER ver. 0.6.193 was used to calculate *K*-value employing the Δ*K* method[26,27]. The unweighted neighbor-joining (NJ) tree was constructed with 30 molecular markers using DARwin ver. 6.0.21 for genetic diversity and phylogenetic analysis.

Results and discussion

Genomic analysis

In the present study, 30 SSR markers were selected for genotypic diversity analysis of 22 *N. lugens* populations based on polymorphism and allelic banding patterns (Table 1). A total of 69 alleles were detected, of which 89.85% (62 bands) was polymorphic, resulting from 23 SSR markers. Alleles per locus ranged from 1 to 5 with a mean of 2.533. The amplicon size varied from 100 to 300 bp in primers NLGS1360 and NLGS1627 respectively. The heterozygosity was found to be 0.205, and varied from 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. The mean value of the major allele frequency for all 30 markers was 0.683. The mean genetic diversity of all the 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. The average PIC was 0.337 and varied from 0.208 to 0.671 (Table 1).

AMOVA and coordinates analysis

AMOVA was used to analyse the distance matrix of inter- and intra-*N. lugens* populations collected from four ecological zones (North, East, West and South) of India. The result revealed that the intra-population variation was only 2%, whereas it was 98% within the populations (Table 2 and Figure 2 *a*). 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 were recorded between the North and West Indian populations, and East and South

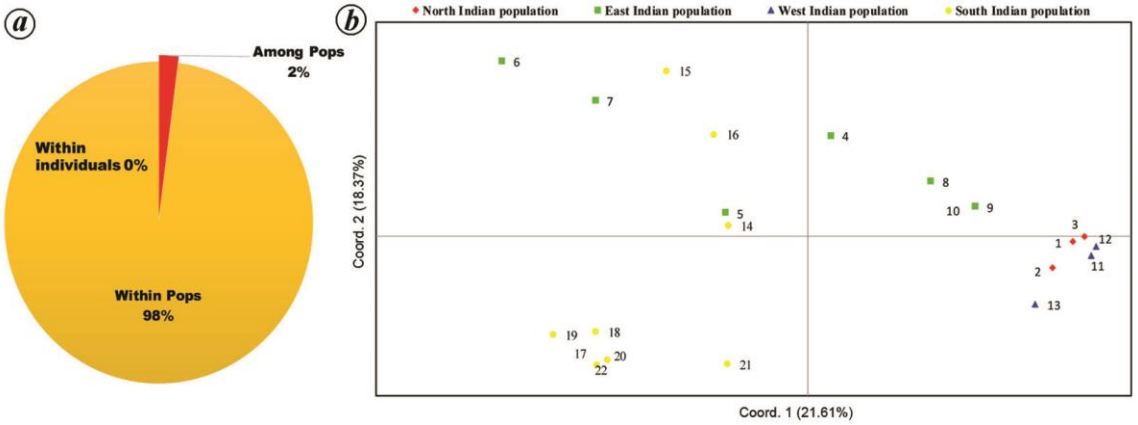

Figure 2. ***a***, Analysis of molecular variance. ***b***, Principal coordinate analysis of 22 *N. lugens* populations linked to 30 simple sequence repeats (SSR) markers.

Table 2. AMOVA among and within populations

Source	d*f*	Sum of square	Mean square	Estimated variance	Percentage of variation
Among populations	2	678,005.444	339,002.722	6,298.787	2
Within populations	15	4,565,390.889	304,359.393	304,359.393	98
Within individuals	18	0.000	0.000	0.000	0
Total	35	5,243,396.333	–	310,658.180	100

Table 3. Pairwise F_{ST} estimates among three populations of *N. lugens* (allele data)

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

Indian populations denoting low and moderate genetic variability respectively (Table 3). The PCoA result further confirmed genetic similarity between the North and West Indian populations, which occupied separate compartments compared to the rest (Figure 2 *b*). The first and second compartments, viz. PC1 and PC2 presented a 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 on geographic distance (Figure 3), suggesting that no genetic isolation-by-distance (IBD) effect was present in the 22 populations.

Cluster and population structure analysis

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

Similarly, population structure analysis revealed three sub-groups of the 22 *N. lugens* populations based on a threshold value; >70% with a highest value of 7.522 for ΔK at $K = 3$ (Figure 4 *b* and *c*; Supplementary Table 4). Further, sub-population II was the largest, including all Karnataka and Andhra Pradesh populations along with two East and West Indian populations. Besides, sub-population I (SPI) consisted of all three North India populations, followed by sub-population III which included all Odisha and Tamil Nadu populations along with the Gariyaband 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).

N. lugens is a major insect pest in South and Southeast Asia, that causes 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

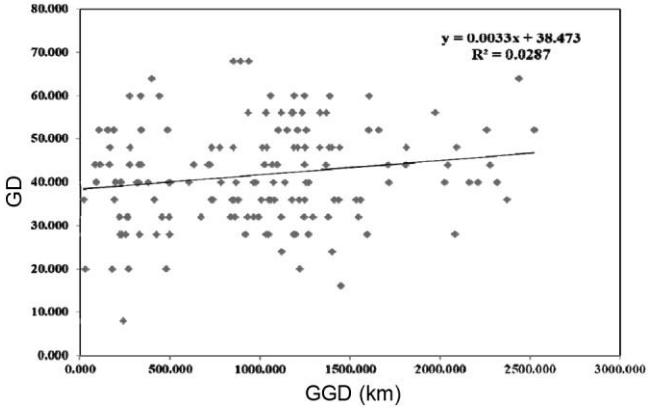

Figure 3. Mantel test showing regression of genetic distance (GD) of 22 *N. lugens* populations against geographical distance (GGD).

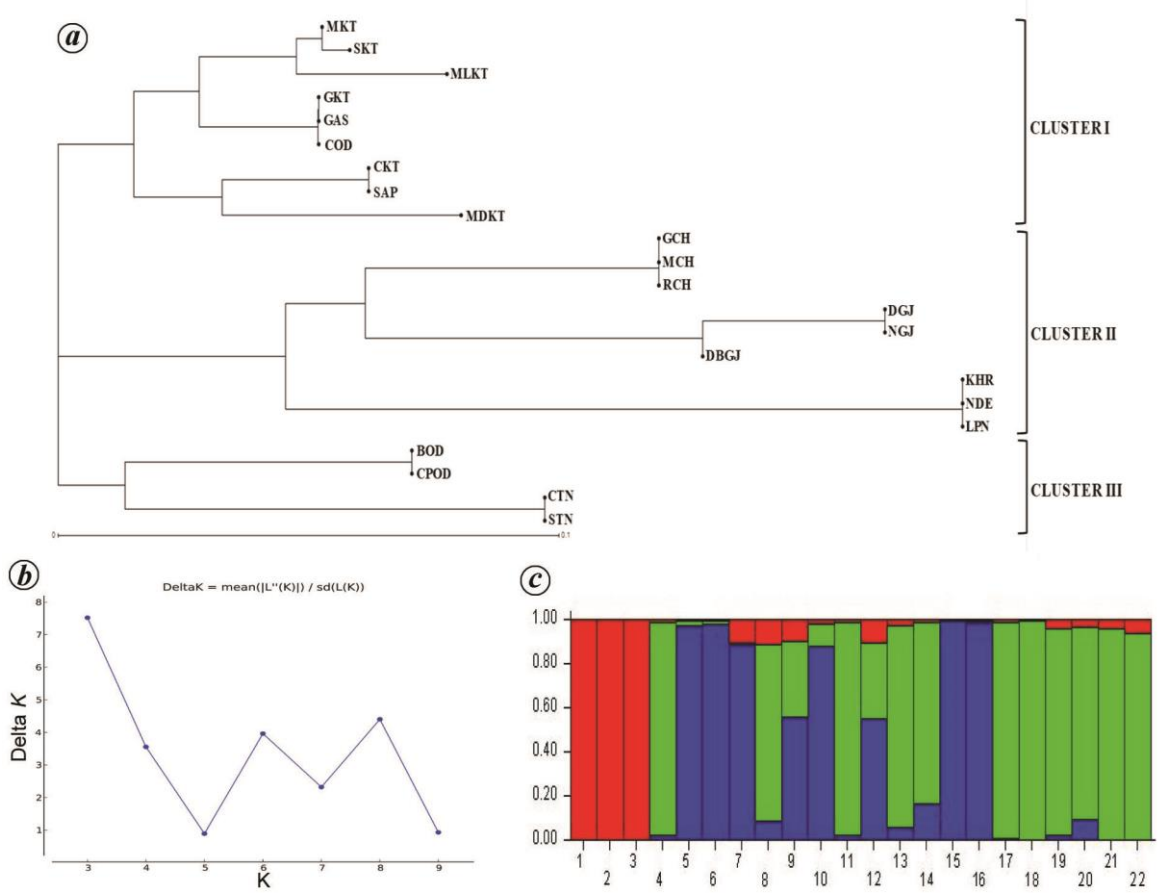

Figure 4. ***a***, Unrooted Neighbor–Joining (NJ) tree representing three clusters. ***b***, Delta-*K* value. ***c***, Population structure showing bar plot partitioning the entire population into three subgroups based on coloured segments for Δ*K* at *K* = 3 of 22 *N. lugens* populations linked to 30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

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 the North, South, East and West agro-ecological zones, was examined with 30 SSR markers.

The SSR markers have been widely used for examining bio-geographical relationships and genetic diversity of

intra- and inter-populations[19,30]. In the present study, 30 SSR markers displayed a 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 the present study, the PIC value represents a medium level of polymorphism with 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 the Indian populations because of their migration habit[11,33,34].

Cluster analysis revealed genetic relationships between different *N. lugens* populations, which were classified into three groups. Among the three major clusters in cluster analysis, the North Indian and West Indian populations were grouped with the Chhattisgarh population in cluster II, revealing genetic relatedness among them, whereas the East Indian and South Indian populations were evenly distributed in clusters I and III. From this result, the gene flow pattern can be correlated with the yearly wind current in the country, as the population genetic structure changes due to random genetic drift, dispersion and migration[35,36]. Being a soft flyer, *N. lugens* generally depends on prevailing wind current and direction for its 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 largely influenced by the southwest (SW) monsoon, which is a key component of yearly rainfall in the country[37,39]. The SW monsoon arrives during June–July in two branches. The Arabian Sea branch flows along the coastal region of the Western Ghats towards western and northwestern India. This could explain the genetic similarity observed in the present study between the West Indian 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 the South Indian and East Indian populations in the present study. Homogeneity in *N. lugens* populations has also been reported in earlier studies, with a majority of them sampled from East and South India[34]. The ΔK method in population structure analysis identified three potential structures at $K = 3$ that represent some genetic diversity in the population. PCoA successfully distinguished the North Indian and West Indian populations from the others. Moreover, variation in the North Indian and South Indian populations suggests modifying the pest management strategies to target gene-specific traits in Indian *N. lugens* that could be effective for both the North Indian and South Indian populations.

The Mantel test for IBD analysis showed a non-significant correlation between genetic and geographical distances of 22 *N. lugens* populations, suggesting unrestricted gene flow among them. Similar to our results, IBD analysis results 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* populations revealed a homogenous population, with only 2% variation between the populations and 98% variation within the populations. Previous studies has also reported low genetic differentiation (2.84%) among the Indian *N. lugens* populations[34,42].

Conclusion

In this study, we have analysed the population structure and gene migration among 22 *N. lugens* populations in India using a genomic SSR marker. The findings revealed the presence of two genetic groups, North and West Indian, East and South Indian, as well as genetic homogeneity within the groups. From this, it can be speculated that genetic diversity in the populations is due to their two-way migration strengthened by the Arabian Sea branch and Bay of Bengal branch of the SW monsoon in India, which may be a major factor for sub-grouping. These results could be useful in designing different pest management strategies based on geographical locations to target specific genetic traits shared by different *N. lugens* populations in India.

1. Pathak, H., Tripathi, R., Jambhulkar, N. N., Bisen, J. P. and Panda, B. B., Eco-regional rice farming for enhancing productivity, profitability and sustainability, NRRI Research Bulletin No. 22, ICAR-National Rice Research Institute, Cuttack, 2020, p. 28.
2. Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W. and 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. *et al*., 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. and Soumia, P. S., Impact of elevated CO_2 on *Oryza sativa* phenology and brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) population. *Curr. Sci*., 2018, **114**(8), 1767–1777.
5. Jena, M. *et al*., Paradigm shift of insect pests in rice ecosystem and their management strategy. *Oryza*, 2018, **55**, 82–89.
6. Anant, A. *et al.*, Evaluation of brown plant hopper *Nilaparvata lugens* (Stal) resistance. *Indian J. Entomol*., 2021, **83**(2), 223–225.
7. Horgan, F. G., Penalver, C. A., Arida, A., Ferrater, J. B. and 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. *et al.*, 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. and 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. *et al.*, 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. *et al.*, 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. *et al.*, Genetic dissection and identification of candidate genes for brown planthopper, *Nilaparvata lugens* (Delphacidae: Hemiptera) resistance in 'farmers' varieties of rice in Odisha. *Crop Prot*., 2021, **144**, 105600.
13. Mohan, U., Lakshmi, V. J., Sharma, S., Katti, G. R., Chirutkar, P. M. and Krishnaiah, N. V., Monitoring of insecticide resistance in rice brown planthopper *Nilaparvata lugens* (Stål) in Nalgonda District of Telangana state, 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. and 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 Manage. Sci*., 2016, **72**, 140–149.
16. Wu, S. F. *et al*., 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. and 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. and Han, Z., Biochemical mechanisms of imidacloprid resistance in Nilaparvatalugens: over-expression of cytochrome P450 *CYP6AY1*. *Insect Biochem. Mol. Biol*., 2013, **43**, 1021–1027.
19. Vieira, M. L., Santini, L., Diniz, A. L. and 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. and 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. and Safinaz, E., New microsatellite and CAPS-microsatellite markers for clarifying taxonomic and phylogenetic relationships within *Origanum* L., *Mol. Breed*., 2014, **34**, 643–654.
22. Hashem, M. H. *et al.*, Genetic divergence and phylogenetic relationship of the rabbitfish *Siganusrivulatus* 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. and Muse, S. V., Power marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, 2005, **21**, 2128–2129.
24. Peakall, R. and 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 HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour*., 2012, **4**, 359–361.
27. Evanno, G., Regnaut, S. and 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. and 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 Plant Pathology). ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, 2018, vol. 2, pp. 2–3.
30. Duan, X. L., Peng, X., Qiao, X. F. and 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. and 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. and 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, S. and 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. and 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. and Sunnucks, P., Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Mol. Ecol.*, 2003, **12**, 21–34.
36. Liu, J. N., Guia, F. R. and Lib, Z. Y., Genetic diversity of the planthopper, *Sogatella furcifera* in the Greater Mekong Subregion detected by inter-simple sequence repeats (ISSR) markers. *J. Insect Sci.*, 2010, **10**(52), 1–19.
37. Yang, S. J., Bao, Y. X., Chen, C., Lu, M. H., Liu, W. C. and 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. and Wu, J. X., Microsatellite-based analysis of genetic structure and gene flow of *Mythimna separate* (Walker) (Lepidoptera: Noctuidae) in China. *Ecol. Evol*., 2019, **9**(23), 13426–13437; doi:10.1002/ece3.5799. PMID: 31871655; PMCID: PMC6912921.
39. Otuka, A., Dudhia, J., Watanabe, T. and 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. *Agric. Entomol*., 2005, **7**(1), 1–9.
40. Nam, H. Y., Park, Y. and 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. and Rauf, A., The genetic structure of *Nilaparvata lugens* (Stal) in Java populations. *Hayati. J. Biosci*., 2020, **27**(4), 333–334.
42. Babu, S. B. *et al.*, Genetic analysis of brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) based on DNA markers. Research Square preprint, posted on 30 January 2023; https://doi.org/10.21203/rs.3.rs-2508969/v1.

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