Journal of Scientific Research and Studies Vol. 5(3), pp. 58-64, March, 2018
ISSN 2375-8791

http://www.modernrespub.org/jsrs/index.htm

Full Length Research Paper

Population structure of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) based on PCR analysis in West Sumatra, Indonesia

Enie Tauruslina A[1]*, Trizelia[2], Yaherwandi[2] and Hasmiandy Hamid[2]

[1]Department of Agriculture, Food and Horticulture Plant Protection Office, West Sumatera, Indonesia.
[2]Department of Plant Protection, Faculty of Agriculture, University of Andalas, Padang, West Sumatra, Indonesia.

*Corresponding author. E-mail: etauruslina@yahoo.com

Accepted 14 March, 2018

This research was conducted to determine the population structure of brown planthopper (*Nilaparvata lugens*) in the Province of West Sumatra, Indonesia. *N. lugens* sample location was selected from fourteen districts for determining the sample collection of the population structure. The localities were Tanjung Mutiara, IV Nagari, Lubuk Basung, Kinali, Lengayang, Koto VII, X Koto Singkarak, Rambatan, Sitiung, Koto Baru, Nanggalo, Kuranji, Talawi and Sungai Geringging. Population genetic variability was performed using Polymerase Chain Reaction (PCR) and DNA analysis using SSRNLS24, SSRBM1262 and SSRBPH06 primers. The results of genetic characterization based on PCR revealed a clear population structure. Population structure of *N. lugens* is diversed based on PCR SSR analysis. Amplification of *N. lugens* gene shows that sample 1 to 14 of DNA bands was a consistent pattern of DNA bands. The dendrogram population of *N. lugens* was eight samples, with genetic similarity at > 0.50.

Key words: Rice, marka SSR, phylogenetic, *Nilaparvata lugens.*

INTRODUCTION

Brown planthopper (*Nilaparvata lugens* Stal.) was an old village. It was a problem in rice cultivation in Indonesia. The pest was included in the order Homoptera, sub order Auchenorrhyncha, infra order Fulgoromorpha, family Delphacidae, genus Nilaparvata and species *Nilaparvata lugens* (Baehaki, 2012). The pest was widespread in the Palaeartik region (China, Jepang and Korea), Oriental (Bangladesh, Kamboja, India, Indonesia, Malaysia, Serawak, Taiwan, Muangthai,Vietnam and Filipina) and Australian (Australia, Fiji Islands, Kaledonia, Solomon Islands and New Gunea). Based on the data shown, brown planthopper has become the global pest (Catindig et al., 2009). The pest can fly far from one country to another. The migratory of *N. lugens* was higher in subtropical areas and temperate regions than tropica regions. The macroptera of *N. lugens* have a preoviposition period longer and hunger in the temperate and subtropical regions than in the tropical regions. The duration of preoviposition hunger was thought to be useful for long distance migration before reproduction. These findings support the hypothetical migration system of brown planthopper in East Asia, which have maintained long distance migration capabilities throughout the year (Wada et al., 2007, 2009). In West Sumatra, *N. Lugens* attacked for 5 years (2013-2017), which was 2,513.10 ha (BPTPH, 2017). Based on the data, population structure research is required. The research of *N. lugens* population structure in West Sumatra was never done. The research purpose was to know the *N. lugens* population structure. The research used SSR moleculer markers. SSR moleculer markers include PCR-based marker (Bardakoi, 2001). SSR PCR has to ability to detect high polymorphisms, detect potential geographically separated population variations and individual variations in population (Luque et al., 2002; Vijayan et al., 2006).

Table 1. Study areas.

Study areas (Regency / Districts)	Altitude (meter upper of the sea)	Geographic position
Agam Tanjung Mutiara	1 – 2	00°03' North Latitude 100°22' East Longitude
Agam IV Nagari	0 – 500	00°01'34"– 00°28'43" South Latitude 99°46'39" – 100°32'50" East Longitude
Agam Lubuk Basung	0 – 500	00°01'34"– 00°28'43" South Latitude 99°46'39" – 100°32'50" East Longitude
Pasaman Barat Kinali	913	00°33'– 00°11' South Latitude 99°10' – 100°04' East Longitude
Pesisir Selatan Lengayang	0 – 1000	0°59'– 2°28'6' South Latitude 100°19' – 101°18' East Longitude
Sijunjung Koto VII	120 – 635	0°18'43" – 1°41'46" South Latitude 100°46'50" – 101°53'50" East Longitude
Solok X Koto Singkarak	400	00°36'25" – 00°49'13" South Latitude 100°27'05" – 100°38'46" East Longitude
Tanah Datar Rambatan	400 – 1000	00°17" – 00°39" South Latitude 100°19" – 100°51" East Longitude
Dharmasraya Sitiung	115	00°47'07" – 00°41'56" South Latitude 101°09'21" – 101°54'27" East Longitude
Dharmasraya Koto Baru	100 – 1500	00°47'07" – 00°41'56" South Latitude 101°09'21" – 101°54'27" East Longitude
Padang City Nanggalo	0 – > 1000	00°44"00" – 01°08'35" South Latitude 100°05'05" – 100°34'09" East Longitude
Padang City Kuranji	0 – > 1000	00°44"00" – 01°08'35" South Latitude 100°05'05" – 100°34'09" East Longitude
Sawahlunto City Talawi	250 – 650	0°34' – 0°46' South Latitude 100°41' – 100°49' East Longitude
Padang Pariaman Sungai Geringging	0 – 10	0°11' – 0°49' South Latitude 98°36' – 100°28' East Longitude

MATERIALS AND METHODS

Study area

The research was conducted in fourteen districts of West Sumatra. The localities were attacked by *N. lugens*. It was Tanjung Mutiara, IV Nagari, Lubuk Basung (Agam Regency), Kinali (Pasaman Regency), Lengayang (Pesisir Selatan Regency), Koto VII (SijunjungRegency), X Koto Singkarak (Solok Regency), Rambatan (Tanah Datar Regency), Sitiung, Koto Baru (Dharmasraya Regency), Nanggalo, Kuranji (Padang City), Talawi (Sawahlunto City) and Sungai Geringging (Padang Pariaman Regency). The study areas are shown in Table 1.

Sample collection

The sample collection was imago *N. lugens*; it was obtained from an attacked location.

DNA Extraction

The DNA extracted from imago of *N. lugens* (only head and thorax) contained 60 µl of extracted buffer and 2-3 iron balls (gotri) in a 1.5 ml micro tube. The tubes were used with a *tissue lyser* at a speed of 25x /s as much as 2 times, for 1 min. The tubes were incubated in a water bath at 65°C for 40 min and mixed with 10 µl of KaAc 5 M, *vortex* and the water phase was transferred to a fresh tube for 40 min. The tubes were spinned on 14.000 rpm for 20 min. The new tubes were prepared with 1.5 ml and cold absolute ethanol of 70% (–20°C). The supernatant was discarded in the tubes with 1.5 ml and mixed with 100 µl of cold absolute ethanol at room temperature for 10 min. The tubes were spinned for 20 min, then ethanol was poured with a pipette. DNA pellets were washed with 100 µl cold ethanol at 70% and spinned for 10 min. Ethanol was removed and DNA pellets were dried, then DNA was resuspended in 30 µl TE (Tris EDTA pH 8.0) and mixed with 5 µl RNAse A.

Table 2. The type of primers used for detection polymorphism of *N. lugens* sample.

Primers	Temperature Annealing	Primer Sekuens 5' – 3'
SSRNLES24	55°C, 30 seconds	primer *forward* SSR (F5 – AGCTCCAATGACAGGTG-3') *reverse* SSR (R5-CCTTTCTTTCAAACAAGGTT AACAA-3')
SSRBM1262	55°C, 30 seconds	primer *forward* SSR (F5 – AGAATCATTGATGAGTCAAGC-3') *reverse*SSR (R5-TCACACACA CTCTCTCACAAA-3')
SSRBPH06	55°C, 30 seconds	primer *forward* SSR (F5 – AAAACATTCCACATTAGCCT-3') *Reverse*SSR (R5-CCTTTTGATTGTATA GGAGC-3')

Table 3. The reaction composition of PCR

Component	Volume
Tag	1 U
dNTPs	0.2 mM
$MgCl_2$	2.5 mM
Primer F+R	0.3 – 0.5 µM
DNA template	10 – 20 ng
PCR buffers	1 x
Total	10 µl

Table 4. The profil of PCR SSR.

Stage	Temperature (°C)	Duration	Cycle
Pre-denaturation	94	5 min	Stages 2 to 4 repeated 40 times
Denaturation	94	30 seconds	
Patching	55	30 seconds	
Extension	72	60 seconds	
Final extension	72	10 min	

The tube was shaken for 10 min and incubated at 37°C for 1 h. The concentrate of DNA was measured with spectrophotometer at an absorbance of 260 and 280 nm. DNA was concidered pure if the OD260/OD280 ratio ranged from 1.6–1.9. The DNA extracted was based on Gene JET^{TM} *Genomic DNA Purification Kit*®Fermentas (Latief et al., 2012).

PCR Amplification of *N. lugens* DNA

The amplification of DNA from *N. lugens* DreamTag *Master Mix* Fermentas was used with SSR primers. The SSR primers shown in Table 2. The choice of primers was based on the optimzation result, a higher polymorphism and a clearly amplified band. The DNA of bands amplification was converted to binary data, based on score. DNA bands were valued at 1 if the DNA bands were presented, and valued at 0 if the DNA bands were not.

The reaction composition was based on the PCR process referred to with protocol kit PCR ®Fermentas (Jing et al., 2011) and is detailed as shown in Table 3. The profile of PCR SSR is shown in Table 4.

The process of PCR was as follows: *pre-denaturation* at 94°C for 5 min with 40 cycles (*denaturation* at 94°C for 30 s; *annealing* at 55°C for 30 s; *extension* at 72°C for 60 s and *final elongation* at 72°C for 10 min). Denaturation, annealing and extention were 40 cycles.

Population structure

The method of analysis was a cluster analysis on *N.*

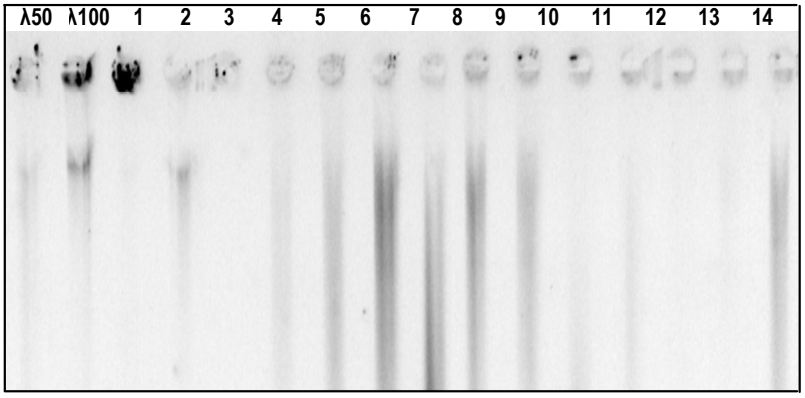

Figure 1. Electroferogram of genomic *N. lugens* DNA used fourteen samples. Holes λ 50, λ100, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14, respectively are λ50 = 50 ng standard λ, λ100 = 100 ng standard DNA λ, 1 = Tanjung Mutiara, 2 = IV Nagari, 3 = Lubuk Basung, 4 = Kinali, 5 = Lengayang, 6 = Koto VII, 7 = X Koto Singkarak, 8 = Rambatan, 9 = Sitiung, 10 = Koto Baru, 11 = Nanggalo, 12 = Kuranji, 13 = Talawi,14 = Sungai Geringging

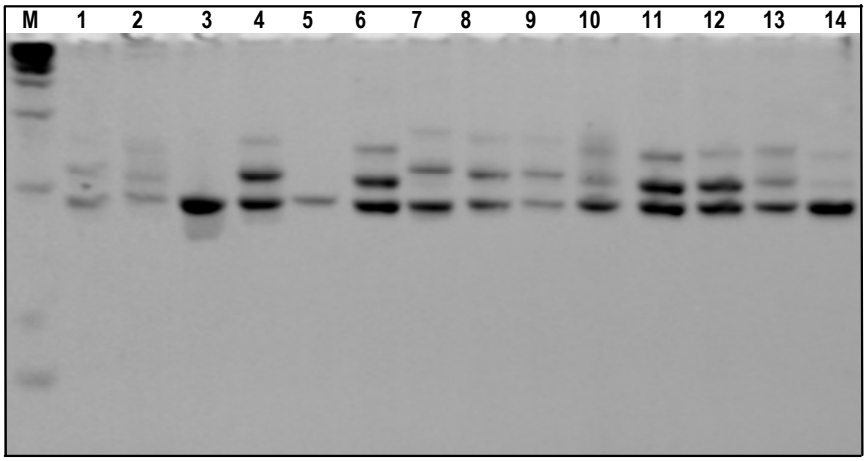

Figure 2. Electroferogram of acrylamide gel 8% used SSRNLES24 primer. Holes M, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 dan 14, respectively are Marker 100 bp DNA Ladder, 1 = Tanjung Mutiara, 2 = IV Nagari, 3 = Lubuk Basung, 4 = Kinali, 5 = Lengayang, 6 = Koto VII, 7 = X Koto Singkarak, 8 = Rambatan, 9 = Sitiung, 10 = Koto Baru, 11 = Nanggalo, 12 = Kuranji, 13 = Talawi,14 = Sungai Geringging

lugens group. It was based on similarity and dendrogram. The kinship relationship population of *N. Lugens* was known. The phylogenetic analysis of *N. lugens* DNA was SSR primers molecular markers. The phylogenetic analysis was done with Clustering UPGMA NTSys 2.1 method (Rohlf, 2000).

RESULTS

DNA Extraction

DNA extraction was DNA electrophoresis. There were fourteen samples of *N. lugens* imago. The DNA extracted was an electropherogram as shown in Figure 1.

PCR Amplification

PCR amplification was primers of SSRNLES24, SSRBM1262 and SSRBPH06. The PCR amplification was an electropherogram as shown in Figures 2, 3 and 4.

Population structure

The genetic similarity matrix of *N. lugens* DNA of the

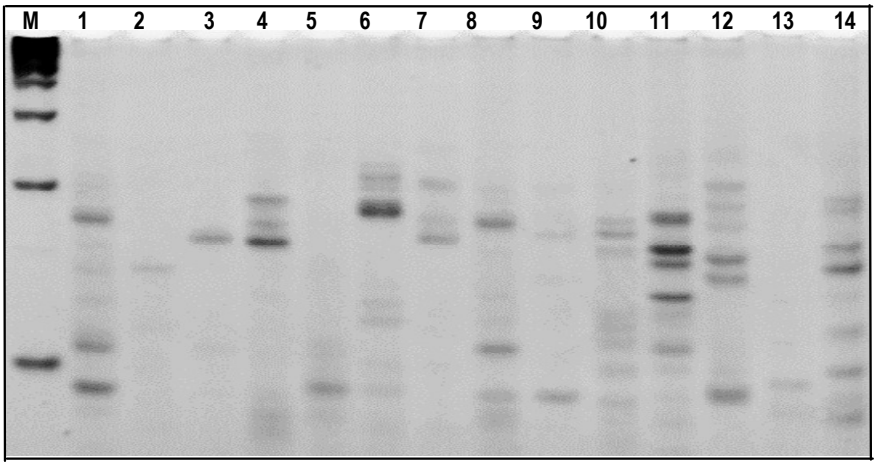

Figure 3. Electroferogram of acrylamide gel 8% used SSRBM1262 primer. Holes M, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 dan 14 respectively are Marker 100 bp DNA Ladder, 1 = Tanjung Mutiara, 2 = IV Nagari, 3 = Lubuk Basung, 4 = Kinali, 5 = Lengayang, 6 = Koto VII, 7 = X Koto Singkarak, 8 = Rambatan, 9 = Sitiung, 10 = Koto Baru, 11 = Nanggalo, 12 = Kuranji, 13 = Talawi,14 = Sungai Geringging

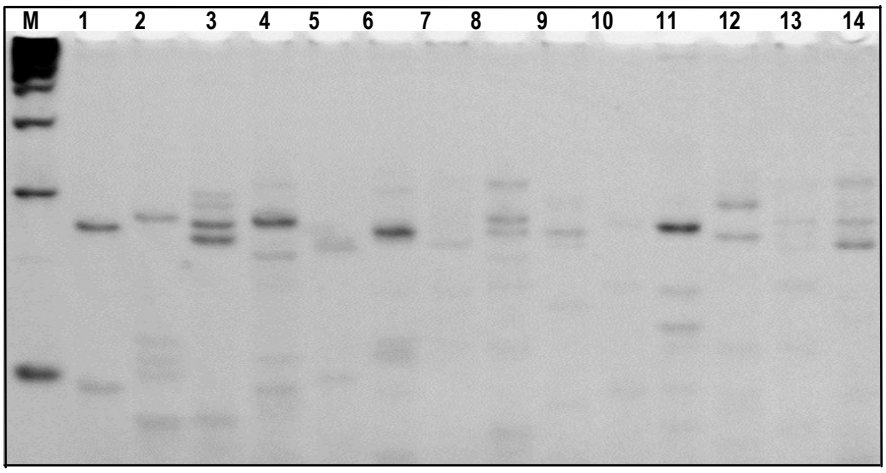

Figure 4. Electroferogram of acrylamide gel 8% used SSRBPH06 primer. Holes M, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 dan 14 respectively areMarker 100 bp DNA Ladder, 1 = Tanjung Mutiara, 2 = IV Nagari, 3 = Lubuk Basung, 4 = Kinali, 5 = Lengayang, 6 = Koto VII, 7 = X Koto Singkarak, 8 = Rambatan, 9 = Sitiung, 10 = Koto Baru, 11 = Nanggalo, 12 = Kuranji, 13 = Talawi,14 = Sungai Geringging

genetic similarity dendrograms was estimated. It was a method of arithmetic averages (UPGMA). The cophenetic correlation was calculated to check the clustering consistency (Manly, 1997). All samples were performed with software NTSYS-pc version 2.1 (Rohlf, 2000). The genetic similarity dendrogram is shown in Figure 5.

DISCUSSION

In Figures 2, 3 and 4, analysis of the PCR amplification showed that fourteen samples of imago *N. Lugens* and DNA bands have clear patterns. The primers of SSRNLES24, SSRBM1262 and SSRBPH06 were gene amplification on forward and reverse capacity. All samples (sample 1 to 14) of DNA bands showed that the pattern of DNA bands are consistent. According to Reddy et al. (1999), molecular marker was a simple method with the ability to detect a high polimorphism, reliable and sensitive to distinguish individuals who have a closer relationship. The formed DNA bands were considered as a single character representing a DNA locus. The same DNA bands were considered a single locus (Agisimanto et al., 2007).

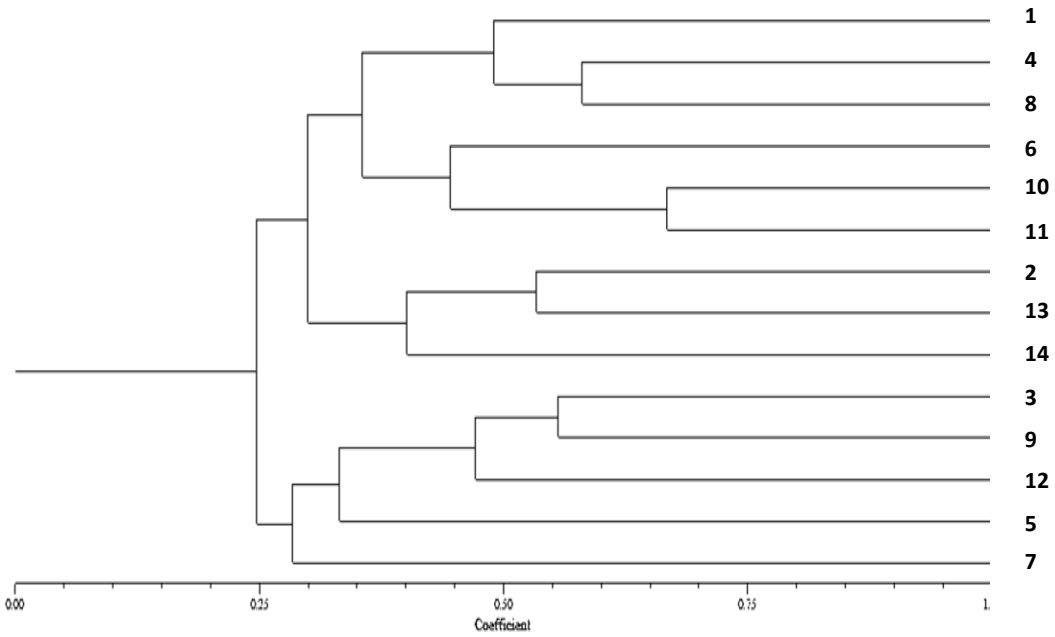

Figure 5. Dendrogram grouping used fourteen samples imago *N. lugens* on genetic similarity in West Sumatra Province. 1 = Tanjung Mutiara, 2 = IV Nagari, 3 = Lubuk Basung, 4 = Kinali,5 = Lengayang, 6 = Koto VII, 7 = X Koto Singkarak, 8 = Rambatan, 9 = Sitiung, 10 = Koto Baru, 11 = Nanggalo, 12 = Kuranji, 13 = Talawi,14 = Sungai Geringging

Figure 5 shows that there is a significant kinship relationship with the value of the coefficient of similarity between 0 and 1. The smaller the value of similarity (close to 0), the farther away is the kinship relationship; and the larger the value of similarity (close to 1), the closer the kinship relationship. The based clustering analysis shows that the kinship was very close to similarity value > 0.50, and the kinship was slightly different with similarity value < 0.50 (Harti, 1988; Lynch and Milligan, 1994). The dendrogram shows that in the group based on genetic similarity, there were some populations that showeda population cluster with population of other locations. The clustering population of *N. lugens* was based on genetic similarity at 50%. The highest genetic similarities were Koto Baru and Nanggalo (0.68), Kinali and Rambatan were 0.63, Lubuk Basung and Sitiung were 0.61, and then IV Nagari and Talawi were 0.59. It was justifying on a high genetic similarity among them. The similarity was lowest (< 0.50) in six samples of *N. lugens* location. According to Ghislain et al. (2006), SSR markers detect highly repetitive regions in the genome that can be derived from untranslated regions and Lintrons (Ghislain et al., 2006).

Genetic similarity is affected by some factor in population structure, such as insect behaviour, agroecosystem conditions and abiotic factors that affect the distribution of insects (Roderick, 1996; Slatkin, 1994). According to Gullan and Cranston (2000), the clustering patterns formed tend to be based on geographical variation and altitude.

Conclusion

The research was used for phylogenetic analysis of *N. lugens* DNA based on analysis of SSR primers molecular markers. The primers were gene amplification in forward and reverse capacity. All samples (sample 1 to 14) of DNA bands showed that DNA pattern bands are consistent. The clustering population of *N. lugens* genetic similarity was eight samples, based on > 50%. The results obtained became the next basic research.

REFERENCES

Agisimanto D, Supriyanto A (2007). Genetic diversity of Pamelo in Indonesia based on random primers of DNA amplified polymorphic. J. Hort. 17(1):17.

Baehaki SE (2012). Development of brown planthopper biotype in rice plants. Plant Food Sci. 7(1):8-17.

Bardakoi F (2001). Random amplified polymorphic DNA (RAPD) markers. Turk. J. Biol. 25:185-196.

BPTPH (2007). Laporan Tahunan. Balai Perlindungan Tanaman Pangan dan Hortikultura, Sumatera Barat. p. 450.

Catindig JLA, Arida GS, Baehaki SE, Bentur JS, Cuong LQ, Norowi M, Rattanakarn W, Sriratanasak W, Xia J, Lu Z (2009). Situation of plant hoppers in Asia. In K..Heong and B Hardy,Proc. Planthopper-new threat to the sustainabilityon intensive rice production system in Asia. International Rice Research Institute, Los Banos,Philippines. p.191-220.

Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM (2006). Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. Theor. Appl. Genet. 113:1515–1527.

Gullan PJ, Cranston PS (2000). The insect: an outline of entomology Malde: Blackwell Science. p. 155-156

Harti DL (1988). Population genetics: A primer. Academic Press, New York.
Jing S, Liu B, Peng L, Peng X, Zhu L, Gu Q, He G (2011). Bulletin of Entomological Research p. 1-10.DOI:1017/S0007485311000435.
Latief MA, Rafii MY, Mazid MS, Ali ME, Ahmed F, Omar MY, Tan SG (2012). Genetic dissection sympatric populations of brown planthopper, *Nilaparvata lugens* (Stal), using DALP-PCR molecular markers. The Scientific World Journal, Article ID 586831.
Luque C, Legal L, Staudter H, Gers C, Wink M (2002). Brief report: ISSR as genetic markers in Noctuids (Lepidoptera). Hereditas. 136:251-253.
Lynch M, Milligan BG (1994). Analysis of population genetic structure wih RAPD markers. Mol. Ecol. 3:91-99
Manly BFJ (1997). Randomization, bootstrap and Monte Carlomethods in biology. Chapman & Hall, London, p. 281.
Reddy KD, Nagaraju J, Abraham EG (1999). Genetic characterization of the silkworm Bombyx moriby simple sequence repeat (SSR) anchored PCR. Heredity 83:681-687.
Roderick GK (1996). Goegraphic structure on insect population: gene flow, phylogeography, and their uses. Ann. Rev. Ent. 41:325-352.
Rohlf FJ (2000). Program NTSYS-pc:numerical taxonomy and multivariate analysis system: version 2.1. Exeter Software, NewYork, p. 83.
Slatkin M (1994). Gene flow and population structure. In Real. LA (Ed). Ecological genetics. Princeton University Press.Princeton. New York. pp. 3-17.
Vijayan K, Anuradha HJ, Nair CV, Pradeep AR, Awasthi AK, Saratchandra B, Rahman SAS, Singh KC, Chakraborti R, Raje SU (2006). Genetic diversity and differentiation among population of the indian Eri Silkworm, Samia cynthia ricini, revealed by ISSR markers. J. Ins. Sci. 6(30):1-11.
Wada T, Ito K, Takahashi A, Tang J (2007). Variation of pre-ovipositional period in the brown planthopper, N*ilaparvata lugens*, collected in tropical, subtropica land temperate Asia. J. Appl. Entomol. 131(9-10):698-703.
Wada T, Ito K, Takahashi A, Tang J (2009). Starvation tolerance of macroptera brown planthopper, *Nilaparvata lugens* from temperate, subtropical and tropical populations in East and South-East Asia. Entomologia Experimentalis et Applicata 130:73-80.