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Genetic variability of populations of *Nilaparvata lugens* stal (Delphacidae: Hemiptera) as revealed by random amplified polymorphic DNA

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ABSTRACT

The Brown Plant Hoppers (BPHs) is one of the most serious insect pests in Asia where rice is widely planted. It is a typical vascular feeder and considered one of the most serious pests of all rice herbivores. It causes reduction in leaf area, photosynthetic rate, nitrogen concentrations of leaf and stem, chlorophyll contents and organic dry weight. It also causes high infestation ratios, reduction in egg numbers and survival ratios of eggs and nymphs on susceptible rice plants. Besides it also transmits several viral diseases that cause crop damage .However, BPH has dramatically emerged as a major pest causing massive losses in tropical rice production under the "Green Revolution" with high yielding IR varieties. In 2005, India reported a loss of 2.7 million tons of rice due to direct damage. About twelve populations of N.lugens were collected from different localities of Tamilnadu from the rice field and were subjected to RAPD analysis using twenty random decameric primers. Out of the twenty RAPD primers tested, three primers have produced clear, consistent and reproducible RAPD bands for all the populations. UPGMA based dendrogram analysis grouped all the populations of N.lugens into many clusters based on the genetic similarity coefficient.

KEYWORDS: Brown Plant Hopper, N.lugens, Green revolution, RAPD, UPGMA, Dendrogram, Genetic Similarity

INTRODUCTION

Rice is one of the agronomically and nutritionally important cereal crops and it is the principal staple food in developing countries. Many diseases and insects harass the rice plant, among these insect pests, plant hoppers, stem borers and gall midges are the most serious pests of rice (Joshi *et al.,* 2000). The Brown Plant Hopper (BPH) is a typical vascular feeder and considered one of the most serious pests of all rice herbivores. BPHs cause reductions in leaf area, photosynthetic rate, leaf and stem nitrogen concentrations, chlorophyll contents and organic dry weight. It also causes high infestation ratios, egg numbers and survival ratios of eggs and nymphs on susceptible rice plants (Cagampang *et al.,* 1974). In the field, feeding by large numbers of BPH usually causes ''hopperburn'' i.e. drying of the leaves and wilting of the tillers, resulting in the dramatic reductions in the yield of susceptible rice varieties (Sogawa, 1982). Kazushige *et al.* (2003) reported that prior to 1970, these plant hoppers were not regarded as economic pests of rice in the traditional low input sustainable paddy ecosystems. However, BPH has dramatically emerged as a major pest causing massive losses in tropical rice production under the "Green Revolution" with high yielding IR varieties. The BPH also transmits ''grassy stunt'' and ''ragged'' diseases as a virus vector (Rivera *et al.,* 1966). In China, BPH caused only occasional damage in the

Southern rice growing areas before the 1960s, whereas widespread outbreaks occurred frequently in the 1990s in the rice-producing areas of Southern and Central China. In addition to being a sucking insect that causes direct damage to the crop, it also transmits several viral diseases that cause additional damage to the crop (Huang *et al*. 2001). To date, genetic variations among populations of *N.lugens* have been examined using allozyme electrophoresis and using nuclear and mitochondrial DNA. Demayo *et al.* (1990) used starch gels to examine variation among populations in the Philippines and found little population structure.

More recently, Hoshizaki (1994) has used isoelectric focusing to investigate a range of laboratory populations from Asia (including Sri Lanka, Java, Malaysia, Philippines, Taiwan and Ishigaki Island, but not China nor Vietnam) and several Japanese populations from the field (Kyushu and Honshu Islands). By virtue of its simple structure, maternal inheritance and relatively rapid evolutionary rates, mitochondrial DNA has become a widely used marker for understanding population structure and phylogenetic relationships of insects (Simon *et al.*, 1994; Roderick, 1996). Genomic DNA fingerprinting using random amplification of polymorphic DNA (RAPD) has been found to be useful in differentiating very closely related bacteria and this method has been used for identification of most of the insects and microbes including genetic analyses (Kosier *et al*., 1993; Kay *et al*., 1994). The present investigation was carried out to establish genetic pattern and genetic groups of different isolates of *N. lugens* collected from the rice field using the technique of RAPD-PCR technique.

MATERIALS AND METHODS

The BPHs *(N.lugens)* insect pest species were collected from twelve different localities of southern parts of Tamil Nadu, India (Table 1) in rice plant (*Oryza sativa*) ecosystem with the use of sweeping net and hand picking method. The collected insects were transformed into a new sterilized specimen bottle for DNA isolation. DNA was extracted using phenol-chloroform-isoamyl alcohol method (Maniatis *et al*., 1982) with some modifications. 20ng of DNA was dissolved in 20µl PCR reaction buffer containing 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.2 mM dNTPs, 20 pM of primer and 0.5 U of DNA polymerase. Twenty primers (RAPD Kit A1 to RAPD Kit A20) obtained from Integrated DNA Technologies (IDT), USA were used for RAPD-PCR studies. PCR technique was conducted according to the methods of Williams *et al*. (1990) with initial heat step (94ºC for 5 minutes), 40 cycles of denaturation (94ºC for 1 minute), annealing (36ºC for 1 minute) and extension (72ºC for 2 minutes) and a finial extension step (72ºC for 7 minutes).

Primer Code	Sequence
RAPD Kit A1	5′ CAGGCCCTTC 3′
RAPD Kit A2	5′ TGCCGAGCTG 3′
RAPD Kit A3	5′ AGTCAGCCAC 3′
RAPD Kit A4	5′ AATCGGGCTG 3′
RAPD Kit A5	5′ AGGGGTCTTG 3′
RAPD Kit A6	5′ GGTCCCTGAC 3′
RAPD Kit A7	5′ GAAACGGGTG 3′
RAPD Kit A8	5′ GTGACGTAGG 3′
RAPD Kit A9	5′ GGGTAACGCC 3′
RAPD Kit A10	5′ GTGATCGCAG 3′
RAPD Kit A11	5′ CAATCGCCGT 3′
RAPD Kit A12	5′ TCGGCGATAG 3′
RAPD Kit A13	5′ CAGCACCCAC 3′
RAPD Kit A14	5′ TCTGTGCTGG 3′
RAPD Kit A15	5′ TTCCGAACCC 3′
RAPD Kit A16	5′ AGCCAGCGAA 3′
RAPD Kit A17	5′ GACCGCTTGT 3′
RAPD Kit A18	5′ AGGTGACCGT 3′
RAPD Kit A19	5′ CAAACGTCGG 3′
RAPD Kit A20	5′ GTTGCGATCC 3′

Sequence of the Primers

Amplification was performed using a programmable thermal Cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers [λ DNA digested with EcoRI and HindIII (Bangalore GeNei, India)] were

Sl. No.	Collection Place	Collection Method	Code	Collection Date	Ecosystem
1.	Madurai	Sweeping Net	I	***17.07.2010***	***Rice Field***
2.	Sivagangai	Sweeping Net	II	***26.06.2010***	Rice Field
3.	Virudhunagar	Sweeping Net	III	***13.06.2010***	Rice Field
4.	Ramanathapuram	Sweeping Net	IV	***12.06.2010***	Rice Field
5.	Theni	***Sweeping Net***	V	***22.05.2010***	Rice Field
6.	Dindigul	Sweeping Net	VI	***23.05.2010***	Rice Field
7.	Pudukkottai	Sweeping Net	VII	***29.05.2010***	Rice Field
8.	Thanjavur	Sweeping Net	VIII	*29.05.2010*	Rice Field
9.	Trichy	Sweeping Net	IX	30.05.2010	Rice Field
10.	Coimbatore	Sweeping Net	X	15.05.2010	Rice Field
11.	Salem	Sweeping Net	XI	08.05.2010	Rice Field
12.	Erode	Sweeping Net	XII	09.05.2010	Rice Field

Table 1: Details of collection of *N.lugens* from various parts of Tamilnadu, India

loaded onto a 1.6% Tris-Borate-EDTA agarose gel (Sambrook *et al*., 1989) and run for 4hours at 50V. The gels were stained with ethidium bromide (0.5 µg) and photographed using DP-001FDC Photo Documentation System (Vilber Lourmat, France). The RAPD products were analysed using the Bioprofile 1D software (Vilber Lourmat, France). Cluster analysis was performed and dendrogram was plotted based on pair wise genetic distance estimated using the unweighted pair group method with arithmetic mean (UPGMA) based on Nei (1978).

RESULTS AND DISCUSSION

DNA isolated from twelve different isolates of *N.lugens* was characterized through RAPD using twenty decameric random primers. Out of these

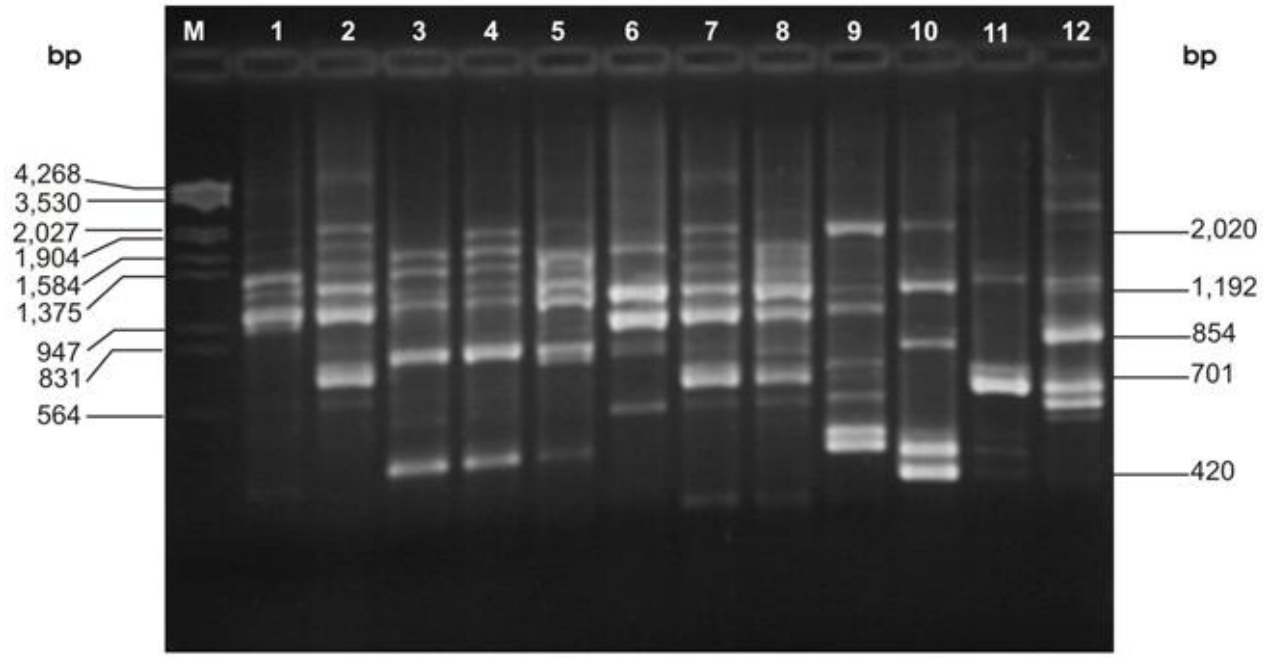

Figure.1a. Random Amplified Polymorphs DNA of Different isolates of *N. lugens* generated by the primer RAPD Kit A3.

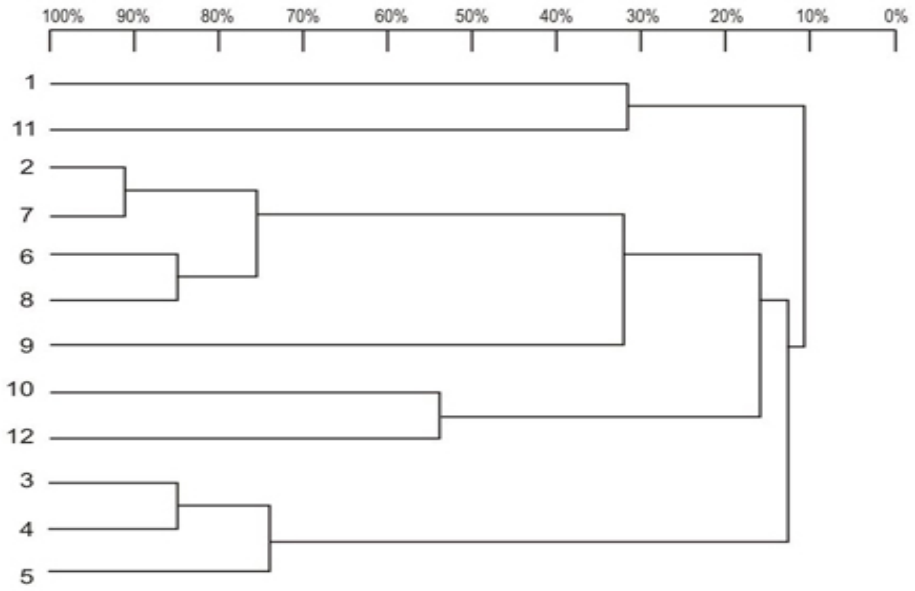

Figure. 1b. Dendrogram with homology coefficient % UPGMA N.lugens generated by the primer RAPD Kit A3.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.00											
2	0.20	1.00										
3	0.13	0.18	1.00									
4	0.13	0.33	0.89	1.00								
5	0.13	0.12	0.75	0.67	1.00							
6	0.29	0.73	0.15	0.15	0.15	1.00						
7	0.22	0.92	0.18	0.18	0.18	0.80	1.00					
8	0.25	0.83	0.18	0.18	0.18	0.89	0.91	1.00				
9	0.13	0.33	0.22	0.40	0.44	0.22	0.36	0.20	1.00			
10	0.13	0.20	0.18	0.25	0.29	0.13	0.22	0.13	0.25	1.00		
11	0.33	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	1.00	
12	0.13	0.18	0.18	0.18	0.25	0.25	0.20	0.22	0.22	0.57	0.25	1.00

Figure.1c. Similarity index based on RAPD profiles of N.lugens generated by the primer RAPD Kit A3.

twenty primers tested, three primers namely RAPD KitA03 ($^{5'}$ AGTCAGCCAC $^{3'}$), KitA07 ($^{5'}$ GAAACGGGTG $^{3'}$) and KitA09 ($^{5'}$ GGGTAACGCC $^{3'}$) alone produced reliable and reproducible RAPD fingerprints for the *N.lugens* (Figures - 1a, 1b & 1c). These three primers amplified to a total of 157 scorable bands in the molecular weight range of approximately 250bp to 3000bp.

The primer KitA03 amplified a total of 60

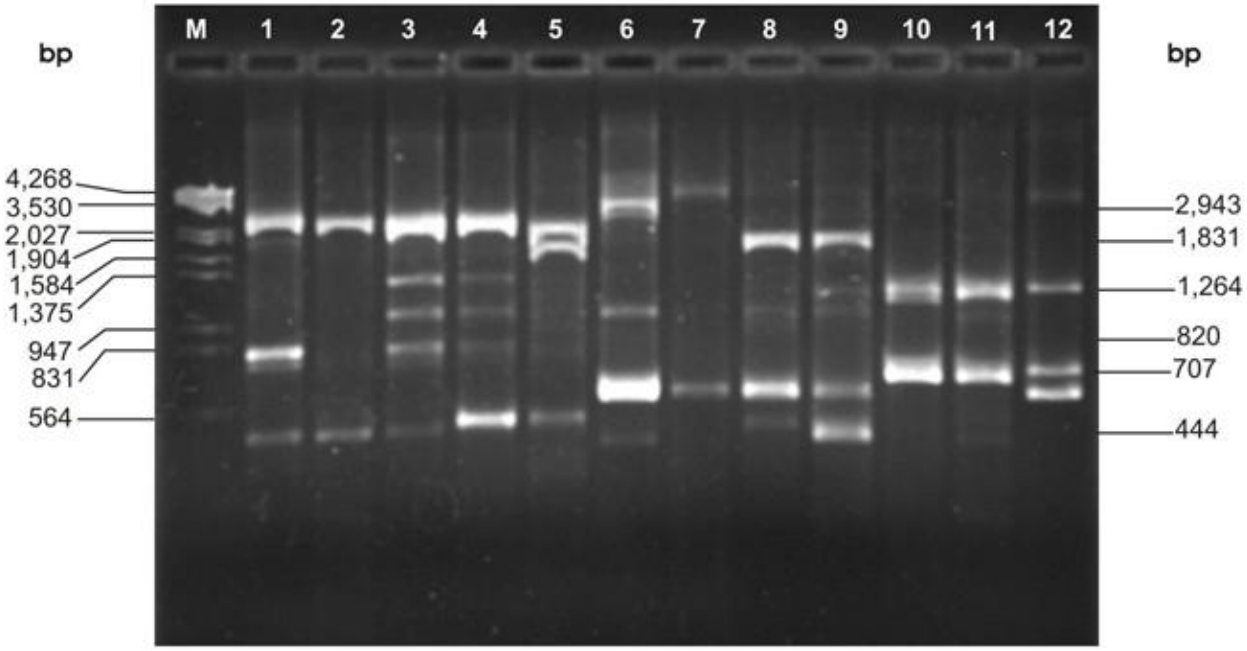

Figure. 2a. Random Amplified Polymorphs DNA of Different isolates of N.lugens generated by the primer RAPD Kit A7.

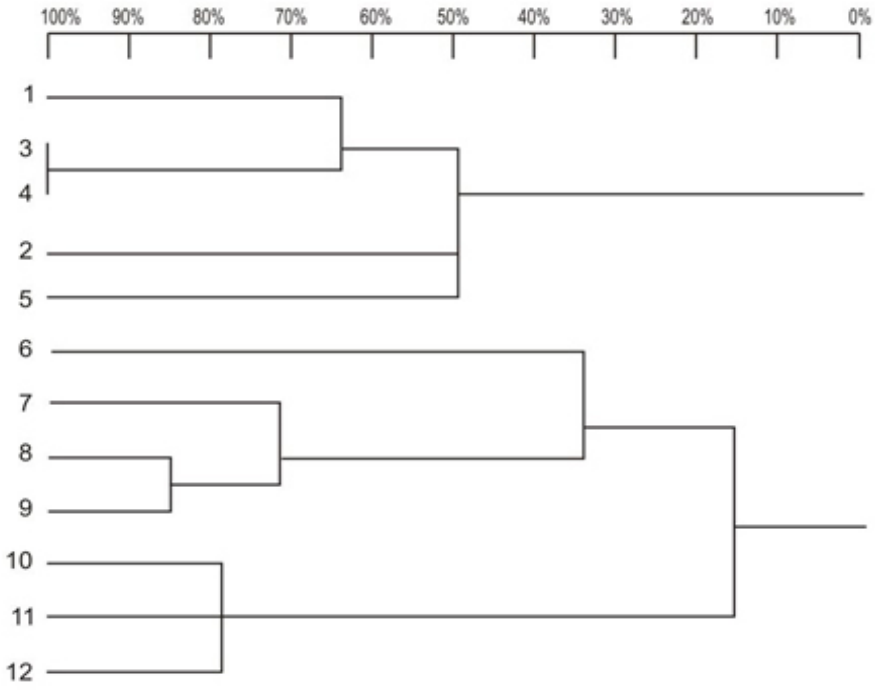

Figure. 2b. Dendrogram with homology coefficient % UPGMA N.lugens generated by the primer RAPD Kit A7.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.00											
2	0.50	1.00										
3	0.67	0.33	1.00									
4	0.67	0.33	1.00	1.00								
5	0.50	0.50	0.33	0.33	1.00							
6	0.00	0.00	0.29	0.29	0.00	1.00						
7	0.00	0.00	0.33	0.33	0.00	0.40	1.00					
8	0.00	0.00	0.29	0.29	0.40	0.33	0.80	1.00				
9	0.00	0.00	0.25	0.25	0.33	0.29	0.67	0.86	1.00			
10	0.00	0.00	0.00	0.00	0.00	0.19	0.19	0.19	0.33	1.00		
11	0.00	0.00	0.00	0.00	0.00	0.19	0.19	0.19	0.29	0.80	1.00	
12	0.00	0.00	0.00	0.00	0.00	0.19	0.19	0.19	0.29	0.80	0.67	1.00

Figure. 2c. Similarity index based on RAPD profiles of N.lugens generated by the primer RAPD Kit A7.

scorable bands and the size of the amplified products varied from 420bp to 2020bp (Figure 1a). The amplification patterns showed a maximum of seven bands in the isolates IV and IX (Figure - 1a: lane 4 & lane 9) and a minimum of three bands in isolate I (Figures - 1a: lane 1) for the primer Kit A03. The dendrogram exhibited four major clusters, comprised isolates of *N.lugens* I and XI in first cluster, isolates of II, VI, VII, VIII and IX in second cluster, isolates of X and XII in third cluster and isolates of III, IV and V in fourth cluster (Figure - 1b). The analysis of similarity index revealed that a maximum of 92% relatedness between II and VII isolates of *N.lugens* and a minimum of 13% of genetic similarity was observed between the isolate I with that of all the other isolates (Figure – 1c).

The primer KitA7 produced 42 scorable fragments with the molecular weight ranges from 444bp to 2,943bp (Figure - 2a). A maximum of five bands in isolates III and IV and a minimum of three bands in isolates II, VII and X were generated by Kit A7 primer. The dendrogram constructed from the RAPD profile of KitA7 revealed two distinct and separate clusters (Figure – 2b). One of the cluster grouped isolate I to V and the other grouped isolate VI to XII. The genetic similarity of 100% was recorded among the isolates of III and IV (Figure.2c).

The RAPD pattern produced by the primer KitA09 yielded 55 scorable fragments for twelve isolates of *N.lugens*. The amplified fragments were ranged from 264bp to 1,990bp (Figure - 3a). A maximum of seven bands in isolate IV and a minimum of two bands in isolate I were generated by Kit A09 primer. The dendrogram constructed from the RAPD profile of Kit09 exhibited two distinct and separate clusters (Fig. 3b). There are two cluster groups of isolates were obtained such as I to IV and V to XII. The highest genetic similarity of 100% was recorded among the isolates of V and IX and the least of 18% was recorded among isolates IV and VI (Figure – 3c).

Among these three primers (Kit A03, Kit A07 and Kit A09), Kit A03 produced the most polymorphic amplification patterns that could distinguish almost all the isolates from each other. The RAPD profile of different isolates of *N.lugens* generated by Kit A3 primer revealed a great degree of genetic polymorphism among the isolates.

The brown plant hopper (*Nilaparvata lugens*) is an enormously successful secondary insect pest that arose from the Green Revolution. It has been able to rapidly adapt to all resistant rice varieties and an array of pesticide chemistries (Liu *et al.,* 2005; Yang *et al.,* 2005 and Yin *et al.,* 2008). Rice entomologists and breeders have frequently observed that rice varieties may be resistant to *Nilaparvata lugens* in one geographic region but susceptible in another and rice varieties that were previously resistant may become susceptible over time. Plant hopper adaptation to new rice varieties has been demonstrated by increases in survival, body weight, honeydew production and reproduction (Claridge and den Hollander 1980, Pathak and Heinrichs 1982, Denno *et al.,* 1989).

The application of multilocus genotyping to the study of agricultural insect populations offers new opportunities to examine the origin, migration and amount of differentiation of plant hopper populations. It also offers a common tool that can be shared by rice entomologists to determine the degree of relatedness between regional plant hopper populations. These new techniques enable us to (1) identify the ancestral source of immigrants, (2) determine the number of migrants between geographic regions or host plants, (3) determine the amount of genetic diversity, and (4) determine the degree of differentiation between geographic populations. Coupled with the whole genome microarrays available for *N. lugens* and rice, a wealth of genomic resources are now available for the study of *N. lugens* and rice interactions (Chen *et al.,* 2009).

Molecular genetics tools have proven successful at detecting genetic variability. RAPD-PCR is a genetic technique that has been used successfully to identify genetic markers that characterize species in several insect orders, including Diptera, Homoptera and Hymenoptera (Black *et*

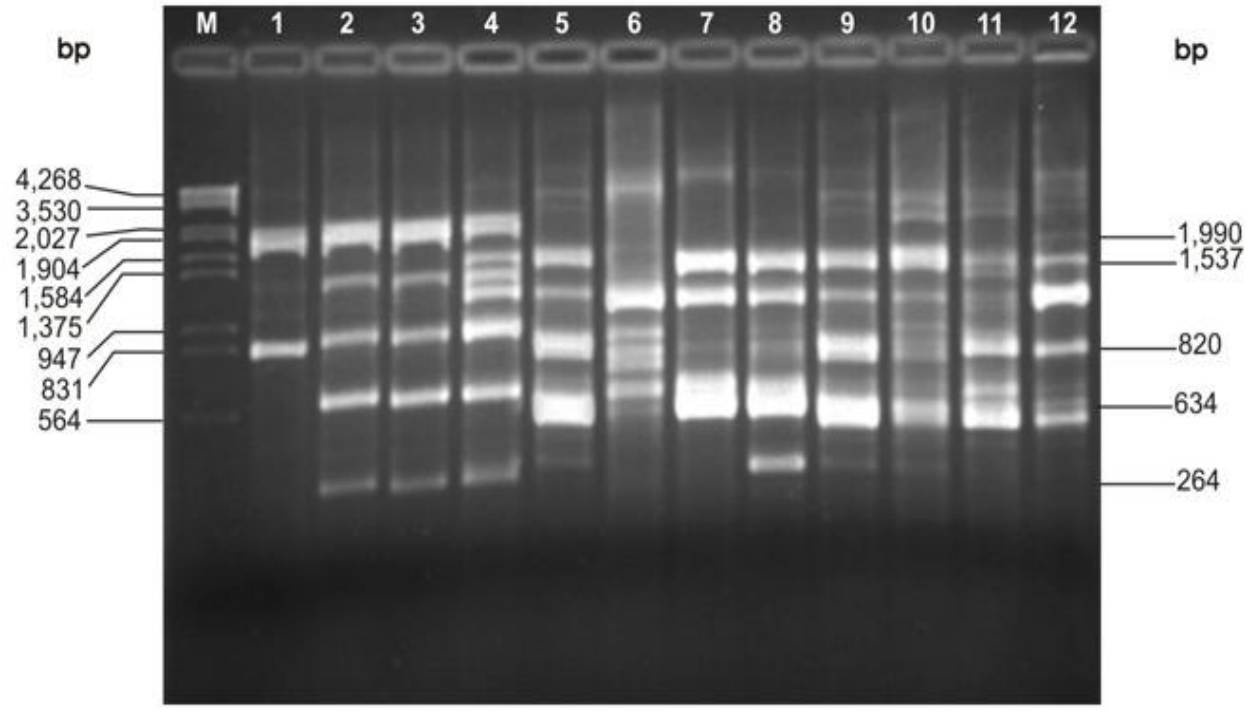

Figure. 3a. Random Amplified Polymorphs DNA of Different isolates of N.lugens generated by the primer RAPD Kit A9.

al., 1992; Kambhampati *et al.*, 1992; Haymer and McInnis 1994). Additionally, it has been useful in identifying the geographic origin of some introduced insect species (Mendel *et al.*, 1994). Puterka *et al.* (1993) have used RAPD-PCR to characterize phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diauraphis noxia* (Mordvilko). Dowdy and McGaughey (1996) also used RAPD-PCR to differentiate six populations of

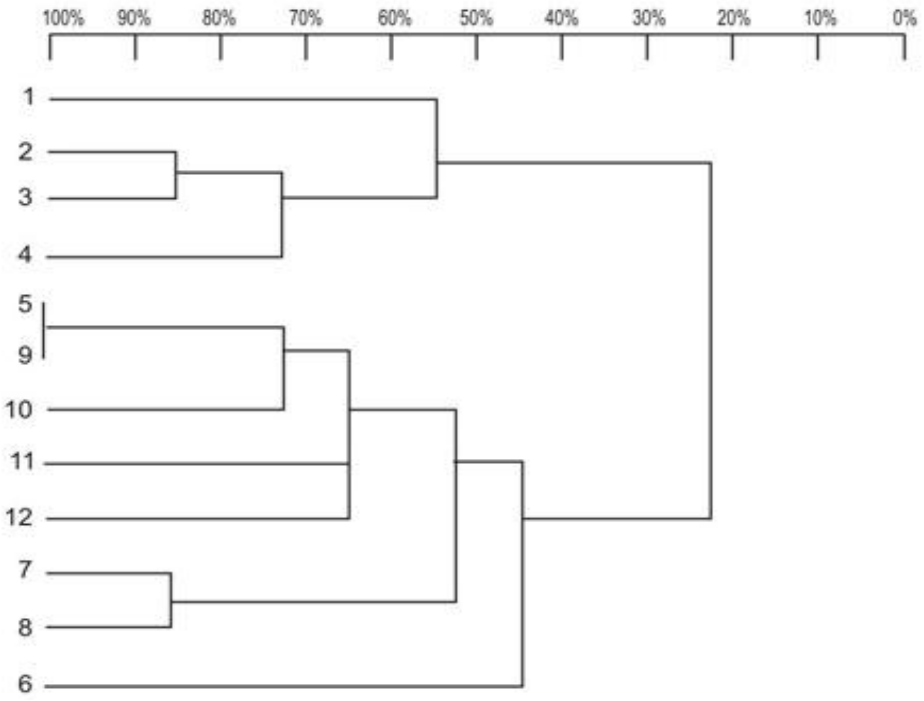

Figure. 3b. Dendrogram with homology coefficient % UPGMA N.lugens generated by the primer RAPD Kit A9.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.00											
2	0.57	1.00										
3	0.67	0.89	1.00									
4	0.50	0.73	0.80	1.00								
5	0.33	0.22	0.25	0.40	1.00							
6	0.29	0.20	0.22	0.18	0.44	1.00						
7	0.22	0.22	0.22	0.22	0.57	0.25	1.00					
8	0.22	0.22	0.22	0.20	0.50	0.22	0.86	1.00				
9	0.33	0.22	0.25	0.40	1.00	0.44	0.57	0.50	1.00			
10	0.29	0.20	0.22	0.36	0.89	0.40	0.50	0.44	0.89	1.00		
11	0.33	0.22	0.25	0.40	0.75	0.44	0.29	0.25	0.75	0.67	1.00	
12	0.33	0.22	0.25	0.60	0.75	0.22	0.29	0.25	0.75	0.67	0.75	1.00

Figure. 3c. Similarity index based on RAPD profiles of N.lugens generated by the primer RAPD Kit A9.

Indianmeal moth *Plodia interpuntella* (Hubner) and examined the genetic similarity within and among populations. Pornkulwat *et al.* (1998) and Srinivas *et al.* (2012) and Hassan (2013) used RAPD markers that were able to distinguish multivoltine from univoltine and bivoltine ecotypes. Saldanha (2000) has used RAPD-PCR to distinguish between local populations of *O. nubilalis* and found a large genetic group consisting of univoltine, bivoltine, and multivoltine ecotypes in Nebraska. The present study revealed that varied genotypic patterns and genetic groups of *N. lugens* associated with the rice. Further, genetic characterization would give concrete information on their association with the host plants based on its genotypic pattern.

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