TAXONOMIC STUDIES AND GENETIC VARIABILITY OF RICE PLANTHOPPERS IN ANDHRA PRADESH

By

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B.Sc. (Ag.)

THESIS SUBMITTED TO THE
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CERTIFICATE

Mr. **T. RAMA MOHAN SURI** has satisfactorily prosecuted the course of research and that the thesis entitled **"TAXONOMIC STUDIES AND GENETIC VARIABILITY OF RICE PLANTHOPPERS IN ANDHRA PRADESH"** submitted is the result of original research work and of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **"TAXONOMIC STUDIES AND GENETIC VARIABILITY OF RICE PLANTHOPPERS IN ANDHRA PRADESH"** submitted in partial fulfillment of the requirements for the degree of Master of Science in the major field of **ENTOMOLOGY** of the Acharya N.G. Ranga Agricultural University, Hyderabad is a record of the bonafide research work carried out by Mr. **T. RAMA MOHAN SURI** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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I, Mr. **T. RAMA MOHAN SURI** hereby declare that the thesis entitled "**TAXONOMIC STUDIES AND GENETIC VARIABILITY OF RICE PLANTHOPPERS IN ANDHRA PRADESH"** submitted to the Acharya N.G. Ranga Agricultural University for the degree of Master of Science in Agriculture in the major field of **Entomology** is the result of original research work done by me. I also declare that the thesis or any part there of has not been published earlier in any manner.

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ABSTRACT

Name of the Author	:	**T. RAMA MOHAN SURI**
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The planthoppers, both brown planthoppers and whitebacked planthoppers collected from rice fields of five different locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam were studied for taxonomic differences and for their genetic variability. The male genital structures of brown and black colour forms of brown planthoppers and whitebacked planthoppers were observed under Olympus Trinocular Research Microscope (400X). There are no variations in genital structures of brown and black forms and these are mere colour variants of brown planthopper, *Nilaparvata lugens* (Stal) and are not different species or subspecies in all the five locations. Similarly, there are no variations in

male genital structures of whitebacked planthopper, *Sogatella furcifera* (Horvath), collected from all the five locations.

After confirming the taxonomic study, the samples were subjected to further molecular analysis for their genomic DNA by using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique. But the DNA fingerprinting method showed very little differences among the populations of five locations and also for the brown and black forms of brown planthopper, which might be the genetic variations among these populations.

Hence, both taxonomic studies and molecular studies which were conducted have been proved that both brown and black forms of brown planthopper belongs to only one species namely *Nilaparvata lugens* (Stal) and the Whitebacked planthopper populations of five locations of Andhra Pradesh belong to only one species namely *Sogatella furcifera* (Horvath). Hence, there are no biotypes existing in both brown planthopper and whitebacked planthopper populations in five geographical regions of Andhra Pradesh.

ABBREVIATIONS

The following abbreviations have been used throughout this studies without definition.

AFLP = Amplified Fragment Length Polymorphism

Bp = base pair

BSA = Bulk segregation analysis

CTAB = N-Cetyl-N,N,N-trimethyl ammonium bromide

dNTP = Deoxy nucleotide triphosphate

EDTA = Ethylene Diamine Tetra Acetate

MAS = Marker aided selection

Min = Minute

µg = Micro gram

µM = Micro Mole

µl = Micro litre

mg = Milli gram

ng = Nano gram

PCR = Polymerase Chain Reaction (PCR)

RAPD = Random Amplified Polymorphic DNA (RAPD)

RFLP = Restriction Fragment Length Polymorphism

RILs = Recombinant Inbred Lines

STMS = Sequence Tagged Microsatillites

SSR = Simple Sequence Repeat

Taq = *Thermus aquaticus*

TBE = Tris Boric acid EDTA buffer

TE = Tris EDTA

UV = Ultra violet

Da = Dalton

DNA = Deoxyribonucleic acid

SDS = Sodium dodecyl sulfate

U = Unit

RNase = Ribonuclease

CHAPTER – I

INTRODUCTION

In India rice is cultivated round the year in one or the other part of the country, in diverse ecologies spread over 44 million hectares with a production of around 90 MT, representing the largest area and the second highest production in the world. This production almost tripled from 30.4 MT in 1966 to a record production of 93.3 MT, with an average productivity of 2.08 t/ha in 2001-02 (Mangala Rai, 2006)

Population of rice consumers continues to grow. It is estimated we will have to produce 38% more rice in 2030. This increased demand will have to be met from less land, with less water, less labor and fewer chemicals. This additional rice must be produced from rice varieties with higher yield potential and dense micronutrients with pest and disease resistant capacity.

Rice is grown under a wide range of agro climatic conditions. It is attacked by more than 100 insect species but relatively few cause significant economic losses. Rice is essentially a crop of hot humid environment conducive to the survival and proliferation of various insect pests. The rice brown planthopper (BPH), *Nilaparvata lugens* (Stal) and whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath) (Family: Delphacidae, Order : Hemiptera)are the

most devastating pests of rice in tropical & temperate Asia. Under favourable conditions, these insects cause severe damage by feeding on rice plant. The damage done is commonly called as "**Hopperburn**" and sometimes kills the entire crop at the time of flowering. At high population density of the insect pest, complete drying of the plant is observed.

It was recorded that at a population density of more than 400-500 nymphs or 200 adults per plant, BPH can cause complete loss of rice plants or crop loss may be 100%. Besides doing direct damage to the rice plant by causing physiological damage, it acts as vector of Grassy stunt virus and ragged stunt (Wilson and Claridge, 1991).

The recent losses to rice crop due to brown planthopper and grassy stunt (GS) disease in Asia amounted to about US $ 300 million. Twenty two species of planthoppers have been identified in China, Rao (2006) reported 23 species of delphacid planthoppers from south India, of which the genera *Nilaparvata* and *Sogatella* are economically important. The genus *Nilaparvata* consists of fifteen species and among these species, the brown planthopper is the only species causing economic damage to the rice plant (Mochida and Okada, 1979).

Nilaparvata lugens (Stal) incidence gradually increasing during last decade, damage became serious because rice cropping systems have changed from single to double or multiple crop system and from interplanting to successive planting leading to major outbreak of brown planthopper throughout Asia during 1972-

1975. Now a days this pest is not controlled by many insecticides which proved to be effective previously. This may be due to the development of resistance or may be due to development of new species or new biotypes.

The present studies are planned in order to confirm the species status of brown planthopper and whitebacked planthopper with the following objectives--

1. Collection of brown planthoppers (BPH) *Nilaparvata lugens* (Stal) and whitebacked planthopper (WBPH) *Sogatella furcifera*(Horvath) in different geographical areas like Bapatla, Nellore, Maruteru, Warangal and Srikakulam of Andhra Pradesh.

2. Study of variations in genetalia structures of these planthoppers collected from the above locations.

3. Study of genetic analysis for their genomic DNA by using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique.

CHAPTER – II

REVIEW OF LITERATURE

The planthoppers belong to the super family fulgoroidea of Auchenorrhynchous- Hemiptera. Melichar (1903) was the pioneer worker in the field of oriental leaf and planthoppers. Distant (1906 and 1916) worked on the taxonomy of Indian Delphacidae and described 15 genera including 34 species. Ishihara and Lowe (1969) reported brown planthopper (BPH) *Nilaparvata lugens* (Stal) and whitebacked planthopper (WBPH) *Sogatella furcifera*(Horvath) from different rice growing states in India. Mammem and Menon (1972) reported 29 new species of delphacids recorded for the first time in India. Mammem and Menon (1974) studied 44 species of delphacids and given a key for seperation of 25 genera of Indian delphacids.

In China 22 species of planthoppers on rice reported in the past 20 years, among these the commonest and most injurious planthoppers on rice are *Nilaparvata lugens* (Stal) and *Sogatella furcifera* (Horvath). Kalode (1983) reported the commonly occurring Auchenorrhynchan rice fauna from India including both *Nilaparvata lugens* (Stal) and *Sogatella furcifera* (Horvath). O'Brien and Wilson (1985) have given comprehensive account of planthopper systematics and their external morphology and an illustrated key to the 20 planthopper families.

2.1 Genus Nilaparvata Distant

Nilparvata lugens (Stal) has become a significant pest in south and south-east Asia, India, Japan, Sri Lanka, Philippines and several other countries in the early 1970s. The macropters potentially migrate and are responsible for colonizing the new fields. At the time of colonization the "Macropterous" forms in rice fields both under temperate (Kisimoto,1979) and tropical condition (Duck *et al.,*1979) settle down on rice plant and produce the next generation in which most of the females develop as Brachypterous (Nasu , 1967).

Okada (1977) reported 14 species under this genus of which he described seven species and provided a key for six species and one unknown species in the genus.

Mochida and Okada (1979) reported that there are 14 determined and two undetermined species as the members of the genus *Nilaparvata* so far in the world. Wilson and Claridge (1991) described this genus on the basis of small spines on the first tarsal segment of the hind leg and also provided the key for identification of males and females.

Seven planthopper species (Delphacid : Homoptera) associated with different rice ecosystems were reported for the first time from Andhra Pradesh. An

illustrated key is provided to identify 10 known planthoppers from Andhra Pradesh. (Lakshmi Narayana *et al*., 2005).

2.2 Genus Sogatella Fennah

In Japan, the grain loss caused by this pest has reached up to 90% in some fields (Suenaga, 1971). A study on the percent loss of paddy caused by the artificial infestation of the *Sogatella furcifera* (Horvath) was conducted by (Khatri *et al.,* 1983). They reported that the percent of grain loss varied from 11-39 when 15 insects/hill were released at varying stages of plant growth.

The pre-oviposition period ranges between 3-8 days. Eggs are laid in longitudinal rows within the leaf midribs. On an average, a female hopper laid 164 eggs in tests in India (Vaidya and Kalode, 1981) but in Japan, the total number of eggs laid per female ranged between 300-350 (Suenaga, 1963). Eggs are similar in size and shape to that of *Nilaparvata lugens* (Stal), but have a longer, more pointed, egg plug.

The eggs hatch in about 6 days. Nymphs are white to a strongly mottled dark grey or black and white in colour. They reach adulthood in 12-17 days passing through five instars. It may be that the ecological niche vacated by the *Nilaparvata lugens* (Stal) is gradually being occupied by *Sogatella furcifera* (Horvath) (Khan and Saxena, 1985)

Horvath (1899) first erected the species *furcifera* under *Delphax* on the basis of male specimens collected from Japan. Fennah (1963) subsequently changed the genus name *Delphax* to *Sogatella* and he also described 16 species in this genus and provided a key for seperation of these specimens. Asche and Wilson (1990) have redefined the genus *Sogatella* and related groups and provided a key to males of the fourteen included species. According to them the species of *Sogatella* are found throughout the subtropical and tropical regions of the world.

2.3 Biotypes of brown planthopper (BPH)

The term ‘Biotypes’ has been coined for the strains of many phytophaghus insect species which are morphologically indiscriminable but differ by survival and development on particular host or preference for feeding, oviposition or both.

Several biotypes of *Nilaparvata lugens* (Stal) are known to occur throughout the tropical Asia. Till now from Southeast Asia 1st and 2nd biotypes, from Philippines 3rd biotype and from India the 4th biotypes were reported. They are distinguishable by their differential virulence, honeydew productions and ability to survive on various rice varieties.

2.4 DNA markers

In recent years, DNA based molecular markers have been extensively used in many areas such as gene mapping and gene tagging, genome identification, analysis of genetic diversity and estimation of genetic relatedness (Krap and

Edwards, 1997; Mohan *et al.,* 1997a; Katiyar *et al.,*2000). DNA based methodologies have been found to be best alternatives to differentiate closely related organisms (Goodwin and Annis, 1991; Mohan *et al.,* 1997b). The use of DNA based markers allows efficient comparison because genetic differences are detectable at all stages of development of the organism unlike iso-enzymes which may show age dependent changes.

Several DNA marker techniques have been developed in recent years. They differ in principle, detection of polymorphism rate, application, cost and time requirement. All the DNA marker techniques aim at detection and exploitation of naturally occurring DNA polymorphism and are broadly placed in two categories.

1. Blot hybridization based techniques.

2. Polymerase Chain Reaction (PCR) based techniques.

Blot hybridization techniques used cloned or synthetic oligonucleotide probes to identify differences in the size of specific or number of fragments, following digestion of genomic DNA with restriction enzymes. Restriction Fragment Length Polymorphisms (RFLP), minisatellites or Variable Number Tandem Repeats (VNTRs) (Jeffreys *et al.,* 1985) etc are of such type of techniques.

PCR based techniques use either random or specific primer(s) to amplify random or specific DNA fragments from genome. They are easily amenable for automation, simple to perform and can be used to assay a large number of individuals. These include Randomly Amplified Polymorphic DNA (RAPD) (Welsh and Mc Clelland , 1990; Williams *et al .,*1990), Sequence Characterized Amplified Region (SCAR) (Zebeau and Voc, 1993) and Inter Simple Repeat (ISSR) polymorphism .

DNA markers are finding varied application in wide variety of species from animal to plant in genetic studies, breeding programs, both basic and applied research, and legal purposes. They have been utilized for gene and genome mapping, genome studies, fingerprinting for identification and assessment of genetic variability of breeding lines, management of genetic resources, prediction of hybrid performance, management of alien gene introgression, improvement of various breeding methodologies, population and ecological studies, monitoring of gene(s) in genetic engineering or gene therapy experiment, identification of somatic hybrids, map based cloning of desired genes etc.

2.5 Randomly Amplified Polymorphic DNA (RAPD)

RAPD is a Polymerase Chain Reaction (PCR) based DNA marker technique, which uses a single arbitrary short oligonucleotide primer (9-10bp) instead of a pair of specific primers (Welsh and Mc Clelland, 1990; Williams *et al.,* 1990). The primer binds randomly in target genome and amplification takes

place when the given DNA sequence (complementary to that of the primer) present in the genome to the opposite DNA strands in opposite orientation, within a reasonable amplification. For the most plant species a single primer (random sequence with at least 50% G and C and lacking inverted repeats) are predicted to generate, on a gel matrix by electrophoresis and visualized in the presence of ethidium bromide or silver staining. Polymorphism result mainly due to changes in primer binding site or form changes which after the distance of adjacent primer binding site beyond amplified products and hence RAPDs are dominant markers. Presence of particular band is dominant and absent is taken as recessive.

The RAPD technique is simple, cost effective, requires no radioactivity and is well suited to analyze a large number of samples. The procedure requires very small amounts of DNA, user universal primer and does not require cloning or prior knowledge.

2.6 DNA fingerprinting

DNA fingerprinting is a well known technique for establishing authenticity. Apart from its forensic applications, it has also evolved as a simple, yet elegant technique in solving paternity disputes, confirming quality parameters in plant biotech industries, characterizing crop varieties, micro organisms, protecting breeders right and other applications in plant sciences. DNA fingerprinting has been recently used by many workers to evaluate the genetic variability or diversity

at molecular levels. PCR based DNA markers; especially STMS facilitate an unbiased assay of genetic diversity.

Insect DNA fingerprinting reveals biodiversity of insect at all levels from fields of different regions, improves development strategies. Genetic markers have been used extensively to study insect population structure, biotypes, species and monitor the spread of insecticide resistance (Hoy, 1994). More recently, a diversity of DNA techniques have been applied, including Randomly Amplified Polymorphic DNA (RAPD) (Welsh and Mc Clelland, 1990; Williams *et al.,* 1990). Restriction Fragment Length Polymorphism (RFLP), minisatellites or Variable Number Tandem Repeats (VNTRs) (Jeffreys *et al.,* 1985) etc, are some such type of techniques. A frequent objective of research on host plant resistance to insect has been establish the development of genetic markers to distinguish the biotypes collected from different regions or adapted to different crop resistance genes (Black *et al.,* 1992). In case of BPH with different biotypes occurring in different regions, DNA fingerprinting is useful in characterizing population diversity, guiding breeding programs and improving strategies for the varietal deployment.

Mun et *al.* (1999) examined the species of delphacid planthoppers, *Nilaparvata lugens* (Stal) and *Sogatella furcifera* (Horvath). An 850 base pair region of mitochondrial DNA Cytochrome oxidase-I (Co-I) was sequenced from a total of 71 individuals collected from 11 localities in seven countries: Korea,

Philippines, China, Bangladesh, Malaysia, Vietnam and Thailand. Populations from China did share halotypes with Korea, which was consistent with the hypothesis that China was the source for yearly immigration in to Korea. Some scientists used starch gels to examine variation among populations of *Nilaparvata lugens* (stal) genetically in the Philippines and found little population structure.

Jones *et a*l. (1996) inferred molecular phylogenies of *Nilaparvata lugens* (Stal) and related species using amplification and restriction site analysis of both mitochondrial DNA and nuclear DNA. In this study, the mitochondrial DNA (16srNA, CoI, CoII) showed some, but limited, variability between populations of *Nilaparvata lugens* (stal) and related species. The limited variation observed in the nuclear DNA (ITSI) strongly support a division between the *Nilaparvata lugens* (Stal) populations in the Asia and Australia, regardless of host plant associations.

Shufran and Whalon (1995) used Random Amplified Polymorphic DNA (RAPD) amplified by the Polymerase Chain Reaction (PCR) to estimate the relatedness of three biotypes of *Nilaparvata lugens* (Stal) from the Philippines. Each individual brown planthopper had a unique set of RAPD bands, but no bands were diagnostic for any one biotype. Brown planthopper types 1, 2 and 3 were genetically homogeneous. This supports the results of other studies which conclude that brown planthopper biotypes are not sub-specific categories, but merely represent individual variation for virulence to rice cultivars.

Black *et al.* (1992) differentiated the insect populations of aphid biotypes which are reproductively isolated, by the help of genetic fingerprints of brown planthopper developed by using Random Amplified Polymorphic DNA – Polymerase Chain Reaction (RAPD) - (PCR). Saxena *et al*. (1991) found minor differences in allele frequencies of 20 enzymatic loci among biotypes 1,2 and 3. However, they interpreted this to mean that brown planthopper biotypes were undergoing speciation. This is done by isozyme results.

Den Hollander (1989) also found slight differences in frequencies of RAPD bands among the same biotypes, but it is more likely that differences in frequencies of isozymes and RAPD may be due to drift effects associated with greenhouse culturing for many years. Khan and Saxena (1990) showed that biotypes 2 and 3 can be present at low frequencies in biotype 1 populations maintained on TN1 therefore; it is possible that some of the homogeneity between biotypes 1 and 2, and between biotypes 1 and 3 may be due to this phenomenon. However, this does not explain the homogeneity between biotypes 2 and 3.

Pathak and Heinrichs (1982) reported that virulence characteristics of one biotype can be changed to that of another biotype by simply rearing populations on a different cultivar in as few as eight generations. Furthermore, individual brown planthoppers cannot be readily classified to biotype using morphology. Clap *et al.* (2000) Figuerao *et al.* (1999) reported that only a small amount of DNA is required for Polymerase Chain Reaction (PCR).

Naito (1982), Saxena and Barrion (1983b) and Tyagi (1983) reported that cytological studies in recent times are being considered as one of the routes to the biotypic characterization of insect species of one of the important pests on rice like Brown planthopper, *Nilaparvata lugens* (Stal) which exists in the form of many biotypic populations.

Claridge (1979), Saxena and Barrion (1983a), Saitoh *et al.* (1970) and Liquido (1978) reported with the help of chromosomal studies that the total number of chromosomes was the same as that of its counterpart in the Philippines and the chromosome arrived in these experiments is of $2n = 29$ for brown planthopper. But the subsequent findings including the recent investigations clarified the previous revealation and expressed the number as 30.

CHAPTER - III

MATERIALS AND METHODS

The present investigations were undertaken on taxonomic studies and genetic variability of rice planthoppers of five different locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam. The taxonomic studies were conducted at Agricultural College, Bapatla, Guntur district and the genomic DNA fingerprinting work was conducted at Cuttack (Orissa) with the colloboration of Central Rice Research Institute (CRRI) during 2006-2007. The details of the materials used and the methods employed during the course of investigation are given in this chapter.

3. 1 SOURCE OF MATERIAL STUDIED

Planthopper collections were made in different rice ecosystems in the following five locations of Andhra Pradesh.

1. NELLORE
2. BAPATLA
3. WARANGAL
4. MARUTERU
5. SRIKAKULAM

3. 2 METHODS OF STUDY

3.2.1 Collection, Killing, Drying and Preservation of the Specimens

Both brown planthoppers, *Nilaparvata lugens* (Stal) and whitebacked planthoppers, *Sogatella furcifera* (Horvath) were collected from different fields of five different geographical locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam by sweep-net method and trapped in aspirator. They were killed with cotton swab wetted with a few drops of ethyl acetate and care was taken to use little quantity of it for killing the insects as excess of it causes curling and twisting of the wings. The killed specimens were dried in hot air oven at 45-50^{0}C for about 2 to 3 hours. The dried specimens were preserved in glass vials and labelled. A paper label indicating collection data written with pencil was placed in each vial. A narrow strip of filter paper with its tip dipped in formaldehyde was placed in each vial, held hanging with the cork not touching the specimens which prevents fungal infection during long storage.

3.2.2 Processing of Material for Study

A. Mounting of specimens

The specimens were mounted on a triangular thick white paper points on the right side of the thorax by using the gum prepared by diluting quick fix in amyl acetate. This facilitates the examination of head, wings, legs and abdomen from all

desired angles and also useful for easy detaching of the abdomen for the study of male genitalia. The label with information regarding host plant, locality, date of collection and name of the collector was transfixed to each specimen.

B. Preparation of male genitalia

To study the male genitalia, the specimen was firmly supported on a cork piece on its back and the abdomen was removed from the thorax with the help of sharp needle by pressing down at the junction of the thorax and abdomen. The detached abdomen was transferred into a cavity dish containing few millilitres of 10 per cent potassium hydroxide and kept for over night at room temperature. Abdomen was then transferred to another small petri dish containing water. The digested soft tissues or internal body contents were pressed out by means of a pair of fine needles or forceps. Then the abdomen is rinsed twice or thrice in water to remove the potassium hydroxide remnants.

The abdomen was then transferred to a glycerin drop on the glass slide. The treatment rendered the entire abdomen completely transparent which was sufficient in many cases to permit the study of genitalia. The male genitalia was dissected out under Binocular Stereo Zoom Microscope following the technique for detailed examination and for illustrations. After the identification and illustrations, the dissected parts of genitalia were placed inside the abdomen, which was finally stored in a micro vial in a drop of glycerin. The vial was

stoppered with a cork and transfixed to the same pin, which was holding the rest of the specimen.

3.2.3 Illustrations

For making illustrations, the genital parts were kept in place by placing a very small quantity of fevi-stick gum at the bottom of the cavity slide before placing a glycerin drop in it. The dissected genital parts were gently pressed after arranging them in required orientation for drawing illustrations. The dissected male genital structures were further studied in detail with Olympus Trinocular Research Microscope and illustrations were made with the same microscope using drawing apparatus. All the drawings were made with pencil and inked later.

3.3 GENOMIC DNA-FINGERPRINTING

3.3.1 Insect materials stored for genomic study

Both brown planthoppers *Nilaparvata lugens* (Stal) and whitebacked planthoppers *Sogatella furcifera* (Horvath) were collected from different fields of five different geographical locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam by sweep-net method and trapped in aspirator. The collected insects were preserved in 95% ethanol at 4˚C for genomic DNA isolation.

3.3.2 Isolation of genomic DNA

The given steps were followed to isolate genomic DNA from females of planthoppers of rice collected from five locations of Andhra Pradesh.

1. Soak single adult female in 50µl of extraction buffer for 10 minutes.

2. Homogenize the insect in 1.5ml of eppendorf tube with sterilized polypropylene pestle gently and thoroughly.

3. Add once again 350 µl of extraction buffer slowly by rinsing the pestle.

4. Add 10 µl of 10mg/ml proteinase-k and incubate at 37˚C for 1 hr.

5. Add 400 µl of equilibrated phenol to the tube invert 30 times and spin at 14.000 rpm at 25˚C for 10 minutes.

6. Then transfer the supernatant to a new tube with a pipette avoiding protein and debris layer.

7. Add 10 µl of 10 mg/ml RNAase and incubate for 1 hr at 37˚C.

8. Then add 200 µl phenol /and 200 µl chloroform-isoamyl alcohol(24:1), invert 30 times and spin at 14,000 rpm at 10˚C for 10minutes and transfer the supernatant to a new tube with plastic pipette

9. Add 400 µl of chloroform-isoamyl alcohol(24:1), invert 30 times and spin at 14,000 rpm at 10˚C for 10minutes

10. Collect the supernatant in to a fresh tube

11. Add 10 µl of 5M NaCl and double the volume of contents present in the tube(2X) ice-cold absolute ethanol was added.

12. Mix gently and leave at -20˚C for 2 hr to over night.

13. Spin at 14,000 rpm at 4˚C for 10 min.

14. Remove the supernatant with pipette taking care not to dislocate the DNA pellet.

15. Add 200 µl of 70% ethanol to the DNA pallet. Leave for 10 minutes, spin at 14000 rpm at 4˚C for 10 minutes and remove the supernatant.

16. Add finally with 200 µl 100% ethanol, spins at 14000 rpm at 4˚C for 10 minutes and remove the supernatant taking care of DNA pellet.

17. Dry the pellet under vacuum for 30 min.

18. Add 50µl of TE (Tris HCl & EDTA) to each pellet and leave at 4˚C overnight.

19. Store DNA samples at -20˚C till further analysis.

3.3.3 Measurement of DNA concentration and quality checking

A. Agarose gel electrophoresis

In order to know intactness of genomic DNA, presence of proteins and (or) RNA contaminates, an aliquot 4µl of each sample was subjected to agarose gel (0.8%) electrophoresis for about 2 hours along with 500 ng of molecular weight marker (Lambada / ECoRI). The gel was stained with ethidium bromide (0.5 µg/ml), viewed under UV Tran-illuminator and photographed immediately for further interpretation using Gel-Doc system (Bio Rad). The quantity of genomic DNA was determined by comparing with molecular weight markers.

B. RAPD primers and RAPD Reactions

Eight arbitrary 10 oligonucleotide primers were used for DNA amplification. These primers were from commercially available RAPD primer kits (Operon Technologies, Almeda California, USA). Each primer had a GC content of 60% (or) higher.

C. Polymerase Chain Reaction (PCR)

The PCR amplification was performed in a 20 µl reaction mixture volume containing 24 ng of DNA, 1X buffer, 200 mM dNTP, 20 ng of primer, 2 mM of

magnesium chloride and 1U of *Taq* (*Thermus aquaticus*) DNA polymerase enzyme. A single primer was included in each PCR reaction.

3.3.4 Preparation of reaction mixture

The reaction (master mix) was prepared as follows:

Reagent	**Final Concentration**	**Volume in µl**
Sterile de-ionized water	-	8.5 × 20 = 170.0
10× PCR buffer	IX	2.0 × 20 = 40.0
10 mM dNTP	200µM	0.4 × 20 =8.0
25 mM magnesium chloride	2mM	1.6 ×20 =32.0
Primer	20ng	1.3 ×20 =26.0
Taq DNA polymerase	1 U	0.2 ×20 =4.0
	Total	14.0 ×20 =280.0

The contents were mixed well, 14 µl was distributed to each tube.

Six µl of DNA (24ng) sample was added to each tube, mixed well and briefly centrifuged to collect drops from wall of tube. The amplification was carried out in Thermo Hybaid Thermal Cycler for 45 cycles under following PCR conditions

Thermal cycling

Step	**Temperature (^{0}C)**	**Duration (min)**	**Cycle**
Initial denaturation	93	2	1
Denaturation	93	1	45
Annealing	36	1	
Extension	72	2	
Final extension	72	5	1

3.3.5 Gel Electrophoresis and detection of amplified products:

Four micro liter of loading buffer was added to amplified PCR products, mixed well, centrifuged briefly to collect drops from wall of tube. Twelve micro liters of amplified products of each sample was loaded on 1.5% agarose gel in 1X TBE (Tris, Borate & EDTA) buffer to separate the amplified fragments. The electrophoresis was done for about 3 hours at 6o volts. The molecular weight

marker (100 bp ladder plus) was used to compare the molecular weights of amplified products. After electrophoresis the gel was stained with ethidium bromide for 20 min. The gel was visualized under UV Tran-illumination and was photographed using Gel-Doc system (Bio-Rad). DNA fragment sizing and matching was done by scoring photographs directly. Individual bands within lanes were assigned to a particular molecular weight comparing with the DNA molecular weight marker. Total number of bands within each lane and number of polymorphic bands were noted.

3.3.6 Analysis of DNA Fingerprint pattern

The RAPD amplified bands were scored as present (1) or absent (0) for each primer population combination. The data entry was done into a binary data matrix as discrete variable. Jaccards's Coefficient of similarity was measured and a dendrogram was generated based on similarity coefficients by using Unweighted Pair Group Method with Arithmetic Average (UPGMA). Most informative primers were selected based on the extent of polymorphism. The average similarity index for all pair wise comparisons (Xd) were calculated and used to estimate the probability of DNA fingerprints of two populations being identical by chance employing the formula (Xd)n where Xd = average similarity index and n=the average number of amplified products per population.

CHAPTER - IV

RESULTS

The taxonomic studies and genetic variability of rice planthoppers in Andhra Pradesh were undertaken. The planthoppers viz., brown planthopper *Nilaparvata lugens* (Stal) and whitebacked planthopper *Sogatella furcifera* (Horvath) collected from Nellore, Bapatla, Warangal, Maruteru and Srikakulam were brought to the laboratory, processed and taxonomic characters were studied. Some populations of all the samples stored in 95% ethanol and preserved at 4°C were taken to Central Rice Research Institute (CRRI), Cuttack for molecular analysis.

Now a days this pest is controlled on rice with many insecticides which proved to be effective previously. This may be due to the development of insecticidal resistance by this pest or may be due to the development of new species or new biotypes. Hence the present studies were planned on taxonomy and genetic variability of rice planthoppers and the results obtained are presented in this chapter.

4.1 COLLECTION OF PLANTHOPPERS FROM DIFFERENT GEOGRAPHICAL AREAS OF ANDHRA PRADESH

Both brown planthoppers , *Nilaparvata lugens* (Stal) and whitebacked planthopper *Sogatella furcifera* (Horvath) were collected from five locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam. The

collected insects were dried and carefully separated as brown planthopper and whitebacked planthopper. Among brown planthoppers, two forms were found which are phenotypically different in their colouration and they are brown and black forms.

4.2 STUDY OF GENITAL STRUCTURES

The male genetalia of brown planthopper of both the colour forms and whitebacked planthopper were dissected to study the structural variation of Genital style, Anal tube and Aedeagus from all the insects of five locations. All these structures were observed under Olympus Trinocular Research Microscope (400X) and line diagrams of these structures were drawn with the help of Drawing Attachment (Fig:1-5) & (Plate-1). It was observed that there are no variations in the genetalia structures of brown and black forms and hence these two forms are mere colour variants and are not different species or subspecies in all the five locations. Hence, it was concluded that these belong to a single species as *Nilaparvata lugens* (Stal). This colour variation is more pronounced in nymphs and less pronounced in adults. With in the nymphs also only later instars show remarkable variations in colour.

The genital structures of whitebacked planthopper from all the five locations viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam were also observed under the same microscope and drawn the line diagrams (Fig:6) & (Plate-2). There are no variations in the male genital structures in all the specimens

collected from all the five locations. Hence, they are one and the same species, *Sogatella furcifera* (Horvath).

4.3 Study of genetic analysis for their genomic DNA by using random amplified polymorphic DNA – polymerase chain reaction (RAPD – PCR) technique at CRRI, Cuttack.

Modified genomic DNA isolation method was successfully used to isolate genomic DNA from the adult females of both brown planthoppers and whitebacked planthoppers. The Agarose gel electrophoresis showed high molecular weight with high purity genomic DNA (Plate : 3-5)

RAPD (Random Amplified Polymorphic DNA) markers are short amplified DNA fragments synthesized by *Taq* DNA polymerase from 8 to 10 base-pair oligomers of arbitrary sequence (Welsh and Mc Clelland, 1990; Williams *et al.,*1990) that may reveal single-base changes in the primer-target site as well as larger deletions or insertions in DNA samples. These differences are observed as changes in the presence or absence of DNA fragments of a particular molecular weight in an amplification profile. RAPD markers have been applied to taxonomic comparisons of many species.

If a population is considered as a group of individuals that has been selected for expression of specific traits in a background of otherwise randomly distributed genetic variation, then a bulked sample from 5 individuals may

similarly be expected to be over-represented with respect to markers linked to these traits.

Comparison of RAPD profiles from bulked DNA samples of different locations of Andhra Pradesh may therefore, reveal markers that distinguish between them.

Fifteen arbitrary 10-mer oligonucleotide primers were used in the RAPD analysis. The amplification pattern of RAPD primers was summerised in 0, 1 format (Table-1). Among these only five primers successfully amplified and a total of 34 reproducible bands, of which 22 are polymorphic. The number of bands per primer ranged from two (OPN-15) to twelve (OPN-16). The size of the amplified product varied from 300bp to 2800bp. Maximum number of polymorphic bands (i.e. 12) were obtained with the primer OPN-16, while the others viz.OPN-18, OPN-19, OPN-20 showed very little polymorphism, but OPN-15 failed to show polymorphism (Table-2 & 3).

Similarity index of pair-wise comparison estimated on the basis of 5 primers ranged from 0.92 to 0.46 with an average similarity index of 0.66 (Table-4).

A dendrogram was constructed based on the similarity matrix data and Jaccard's coefficient by applying Unweighted Pair Group Method with Arithmetic Average computer program version 1.8 (Fig-7). Each major group consists of

three sub groups. First major group consists of all five bulked samples of brown BPH of five different locations, which has three sub-groups i.e. first sub group includes the populations of Nellore and Bapatla. Second subgroup includes Warangal and Maruteru and Third sub group includes Srikakulam alone (Fig-7)

Second major group includes all five samples of black BPH, which contains three sub groups at which first sub group includes Nellore and Bapatla, second sub group includes Warangal and Maruteru and third sub group includes Srikakulam (Fig-7). These little variations are genetic variations within the population. Hence, there is no difference between brown and black colour forms of brown planthopper in all the five locations which indicates these are mere colour forms.

Third major group includes all five samples of whitebacked planthopper, with three sub groups at which first sub group includes Nellore and Bapatla, this little variations are within the population, second sub group includes Warangal and third sub group includes Maruteru and Srikakulam (Fig-7). Hence, there is no difference between populations of whitebacked planthoppers collected from five locations, which indicates the same species. Based on these results, it was concluded that there are no biotypes existing in both brown planthopper and whitebacked planthopper populations in five geographical regions of Andhra Pradesh.

CHAPTER - V

DISCUSSION

Brown planthopper**,** *Nilaparvata lugens* (Stal) has become a significant pest in south and south-east Asia, India, Japan, Sri Lanka, Philippines and several other countries in the early 1970s. It is a phloem feeding insect, commonly found in rain fed and irrigated wetland environments during the reproductive stage of rice plant. It infects all growth stages but the most susceptible stages are early tillering to flowering or during first 30 days after seedling until the reproductive stage. The removal of plant sap and blockage of vessels by the feeding tube sheath cause the tillers to dry and turn brown. This condition is called "**hopperburn**" (Chen and Cheng, 1979).

5.1 STUDY OF GENITAL STRUCTURES

The male genital structures of both colour forms of brown planthopper and whitebacked planthoppers were observed under Olympus Trinocular Research Microscope (400X) and line diagrams of these structures were drawn with the help of Drawing Attachment (Fig: 1-5). It was observed that there were no variations in the genital structures of brown and black forms and these forms are mere colour variants and are not different species or subspecies in all the five locations. Hence, it was concluded that these belong to a single species as *Nilaparvata lugens* (Stal).

The genital structures of whitebacked planthopper for all the five locations viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam were also observed under the same microscope and drawn the line diagrams (Fig.6). There are no variations in the male genital structure in all the specimens collected from all the five locations.

The published data is not available regarding the comparision of genital structures of brown planthopper and whitebacked planthopper in different locations of Andhra Pradesh. However, Rao Rama Subba (2006) reported that there are no variations in genital structures of both brown and black forms of brown planthopper, representing only one species i.e., *Nilaparvata lugens* (Stal) and these results are in accordance with the present studies. In order to confirm the biotype status, the molecular analysis of these samples was undertaken.

5.2 STUDY OF GENETIC ANALYSIS AT GENOMIC DNA - LEVEL BY RAPD – PCR TECHNIQUE

A frequent objective of research on host plant resistance to insect has been development of genetic markers to distinguish the biotypes collected from different regions or adapted to different crop resistance genes (Black *et al*., 1992). RAPD profiling was found efficient enough to reveal usable level of DNA polymorphism among insect populations.

The main purpose of present study is to analyse genetic variability of intra-population of brown planthoppers at molecular level. PCR-based molecular marker techniques, especially Random Amplified Polymorphic DNA (RAPD) have been used for analysis of genetic variability between female populations of brown planthopper.

Shufran and Whalon (1995) used Random Amplified Polymorphic DNA (RAPD) amplified by the Polymerase Chain Reaction (PCR) to estimate the relatedness of three biotypes of *Nilaparvata lugens* (Stal), from the Philippines. Each individual brown planthopper had a unique set of RAPD bands, but no bands were diagnostic for any one biotype. Brown planthopper types 1, 2 and 3 were genetically homogeneous. This supports the results of other studies which conclude that brown planthopper biotypes are not sub-specific categories, but merely represent individual variation for virulence to rice cultivars.

In the present study, modified method of DNA isolation was found suitable for extraction of good quality and high molecular weight genomic DNA from adult female. RAPD technique was found efficient enough to reveal usable level of DNA polymorphism among adult females.

Jones *et a*l. (1996) inferred molecular phylogenies of *Nilaparvata lugens* (Stal) and related species using amplification and restriction site analysis of both mitochondrial DNA and nuclear DNA. In this study, the mitochondrial DNA

(16srNA, CoI, CoII) showed some, but limited, variability between populations of *Nilaparvata lugens* (stal) and related species.

In the present investigations a dendrogram was constructed based on the similarity matrix data and Jaccard's Coefficient by applying Unweighted Pair Group Method with Arithmetic Average computer program version 1.8. Each major group consists of three sub groups(Fig-7).

First major group consists of all five bulked samples of brown BPH of five different locations, which has three sub-groups i.e. first subgroup includes the populations of Nellore and Bapatla. Second subgroup includes Warangal and Maruteru and Third sub group includes Srikakulam alone (Fig-7)

Second major group includes all five samples of black BPH, which contains three sub groups at which first sub group includes Nellore and Bapatla, Second subgroup includes Warangal and Maruteru and third sub group includes Srikakulam. These results indicate that these brown and black forms of brown planthopper are mere colour variants due to genetic variation within the population.

Third major group includes all five samples of whitebacked planthopper, with three subgroups at which first subgroup includes Nellore and Bapatla, second subgroup includes Warangal and third subgroup includes Maruteru and Srikakulam.

Mayank Sharma (2004), also analysed different samples of brown planthopper populations using RAPD-PCR technique and provided dendrogram showing some differences between the samples.

The present study confirms the species status of brown planthopper, *Nilaparvata lugens* (Stal) and whitebacked planthopper, *Sogatella furcifera* (Horvath). The variations found in both genital structures and DNA finger printing studies are mere genetic variations within the populations of five locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam, but they are not the biotypes. The color variations within the population of brown planthopper are mere colour variants which may be due to genetic variations within the population.

CHAPTER –VI

SUMMARY

The planthoppers both brown planthoppers and whitebacked planthoppers were collected from rice fields of five locations of Andhra Pradesh viz., Nellore, Bapatla, Warangal, Maruteru and Srikakulam. The collected planthoppers were separated accordingly and some populations stored in 95% ethanol at 4°C for molecular analysis, which was done at CRRI, Cuttack. The remaining population was dried and kept for genital studies.

The taxonomic study revealed that the genital structures namely, genital style, anal tube and aedeagus of all the populations are almost similar . So the brown and black forms of brown planthopper are considered as only the colour variants of the species *Nilaparvata lugens* (Stal). The populations of whitebacked planthoppers of five locations are one and the same belonging to the species *Sogatella furcifera* (Horvath). For further examination the DNA fingerprinting analysis was also done at CRRI, Cuttack.

The genital structures were observed under Research Microscope to find the variations among the planthopper populations belonging to five different locations of Andhra Pradesh, revealed that there are very minor variations. These structural variations are not enough to identify them as new species or subspecies.

The similarity index values also showed very less difference among these populations. The dendrogram drawn with the help of Jaccard's Coefficient and UPGMA (Unweighted Pair-Group Method Arithmetic Average) analysis also showed little differences among the populations.

Hence, the brown and black forms of brown planthopper are considered as mere colour variants of one and the only species *Nilaparvata lugens* (Stal) and coming to location differences of both brown planthoppers and whitebacked planthoppers are very minute which cannot be considered for biotypic status.

REFERENCES

Asche M and Wilson M R 1990 The delphacid genus *Sogatella* and related groups: a revision with special reference to rice-associated species (Homoptera : Fulgoroidea) Systematic Entomology 15: 1-42.

Black W C IV, Du Teau, N M , Putera, G J and Nechols J R 1992 Use of random amplified polymorphic DNA Polymerase chain reaction (RAPD-PCR) to detect DNA polymorphism in aphids. *Bulletin of Entomological Research* 82:151-159.

Chen C N and Cheng C 1979 Ecological physiology of rice plants attacked by brown planthopper. In proceeding of the Roc- Japan symposium on rice productivity, institute of botany, *Academia Sinicia* pp 135-146.

Clapp J P, Van Der Stoel C D and Van Der Putten W H 2000 Rapid identification of cyst (Heterodera sp., Globodera sp.) and root-knot (Meliodogyne sp.) nematodes on the basis of ITS2 sequence variation detected by polymerase chain reaction (PCR)- Single-Strand Conformational Polymorphism (SSCP) in cultures and field samples. Molecular Ecology 9:1223-1232.

Claridge M F 1979 Biotaxonomic studies on leafhopper pests for rice. ODA Research Scheme R3282 Second Annual Report 1978/79; Cardiff, Wales, U. K. 79.

Den Hollander J 1989 Electrophoretic studies on planthoppers and leafhoppers of agricultural importance. In eletrophoretic studies on agricultural pests. Systematic Association. Special Volume.39 (Edited by Loxdale H D and Den Hollander J) 297-315. Clarendon press, Oxford.

Distant W L 1906 Rhyncota : Homoptera In, the fauna of British India, including Ceylon and Burma C T Binghan (ed.) published under the authority of the secretary of state for India in Council 3: 1-151.

Distant W L 1916 The fauna of British India, including Ceylon and Burma, Rhyncota vol VI Today and Tomorrow's printers and publishers 24 B/5, Karol Bagh, New Delhi-5 pp: 248.

Duck V A , Mishra B C, Alam A and Chen C N 1979 Ecology of the brown hopper in the tropics. In brown plant hopper : Threats to Rice Production in Asian International Rice Research Institute, Philippines, pp 61-98.

Fennah R G 1963b The delphacid species complex known as *Sogatella furcifera* (horvath) (Homoptera : Fulgoroidea) Bulletin of Entomological Research 54: 45-79.

Figuerao C , Simon J C, Le Gallic J F and Niemeyer H M 1999 Molecular markers to differentiate two morphologically-close species of the genus Sitobion. Entomologia Experimentalis Applicata 92: 217-225.

Goodwin P H and Annis S L 1991 Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplify polymorphic DNA assay. *Applications of Environmental Microbiology* 57: 2482-2486.

Horvath G 1899 Hemepteres de File de Yesso (Japan) Termeszetatrajzi Fiizetek Budapest 22: 365-379.

Hoy M A 1994 Insect molecular genetics: An introduction to principle and Application, San Diego: Academic Press.

Ishihara J and Lowe A 1969 The leafhoppers of rice fields in India All India Co-ordinated rice improvement project Report. Rajendra nagar, Hyderabad-30, Andhra Pradesh, India 1-14.

Jeffery A J, Wilson V and Thein S L 1985 Hypervariable 'minisatellites' region in human DNA. *Nature* 314:67-73.

Jones P L, Gacesa P and Butlin R K 1996 Systematics of *Nilaparvata lugens* (Stal) and related species using nuclear and mitochondrial DNA. 133-148 in Symondson W O C and Liddell J E (ed.). The ecology of agricultural pests. London, Chapmen and Hall.

Kalode M B 1983 Leafhoppers and planthoppers of rice in India in proceedings of the 1st International workshop on Bio-taxonomy, classification and Biology of leafhoppers and planthoppers (Auchenorrchyncha) of Economic importance. Knight W J, Pant N C, Robertson T S and Wilson M

R (EDS.) 500 PP London, 4-7 October 1982, Commonwealth Institute of Entomology, 56 Queen's Gate, London 225-245.

Karp A and Edward K J 1997 DNA markers; a global overview. In: Caetano Anolles G and Gressshoff P M (eds.) DNA markers : protocols applications and overview (PP 1-13). Wiley-V C H. New York.

Katiyar S K, Channdel G, Tan Y, Zhang Y, Huang B, Nugaliyadda L, Fernando K, Bentur J S, Inthavong S, Constantino S and Bennet J 2000 Biodiversity of Asian Rice gall midge *Orseolia oryzae* (Wood Mason) from five countries examined by AFLP analysis. *Genome* 43: 322-332.

Khan Z R and Saxena R C 1985 A selected bibliography of the *Sogatella furcifera* (Horvath) (Homoptera : Delphacidae). *Insect Science Application* 6: 115-134.

Khan Z R and Saxena R C 1990 Purification of biotype 1 population of *Nilaparvata lugens* (Stal). Insect Science Applications 11: 55-62.

Khatri A K, Gangrade G A and Rathore V S 1983 Studies on the crop losses in rice caused by the *Sogatella furcifera* (Horvath) and the economic injury in India. *Tropical Pest Management* 29:220-223.

Kisimoto R 1979 Bionomics, forecasting of outbreaks and injury caused by Rice planthopper. *In The Rice Brown Planthopper*. Food and fertilizer technology center for Asia and pacific region, Taipei, Taiwan, pp.7-41.

Lakshmi Narayana N, Rama Subba Rao V and Rajasekhar P 2005 New Records of Planthoppers (Delphacidae : Homoptera) Associated with Rice Ecosystems from Andhra Pradesh. The Andhra Agriculture Journal, 52 (3&4): 488-495.

Liquido N J 1978 Morphology, Cytology and Breeding behaviour of *Nilaparvata lugens* (Stal) (Delphacidae:Homoptera) M. S Thesis, University of Philippines, Los Banon, Philippines (abstract).

Mammen K V and Menon M G R 1972 New records of Delphacids from India Entomologists Newsletter 2: 7-9.

Mammen K V and Menon M G R 1974 Taxonomic key for identification of Indian Delphacidae. Agriultural Research Journal of Kerala 12(2): 146-150.

Mangala Rai 2006 Rice culture in agriculture: An Indian Perspective. Abstracts of Plenary Session of International Rice Congress.

Mayank Sharma 2004 Molecular Analysis of Brown Planthopper Populations, Guru Ghasidas University, Bilaspur, Chhatisgarh, M. Sc. Thesis in Biotechnology

Melichar L 1903 Hom Fauna Von Ceylon, Verleg Von Felix L. demes Berlin pp. 219.

Mochida O and Okada T 1979 Taxonomy and Biology of, *Nilaparvata lugens* (stal) (Homoptera: Delphacidae) In, Brown plant hoppers: Threat to rice production Asia. IRRI Los banos, Philippines, PP 1-9.

Mohan M, Nair S, Bhagwat A, Krishna T G, Yano M, Bhatia C R and Sasaki T (1997a). Genome mapping, molecular markers and markers-assisted selection in plants. *Molecular Breeding* 3 : 87-103.

Mohan M, Satyanarayan P V, Kumar A, Srivastava M N and Nair S (1997b) Molecular mapping of resistance specific PCR based marker linked to a midge resistance gene (Gm4t), in rice. *Theory of Applied Genetics* 95:777-782.

Mun J H, Song Y H, Heong K L and Rodgick G K 1999 Genetic variation among Asian populations of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcitera* (Hemiptera : Delphacidae) : Mitochondrial DNA sequences, Bulletin of Entomological Research 89: 245-253.

Naito T 1982 Chromosome number differentiation in sawflies and its systematic implication (Hymenoptera:Tenthredinidae). Kontyu 50(4):569-587.

Nasu S 1967 Taxonomy, Distribution, Host range, life cycle and control of rice leafhopper. In: Major insect pest of rice .Proceeding of a Symposium in the International Rice Research Institute, Philippines, September. 1964. Baltimore, Md. (USA): johns Hopkins Press. Pp 493-523.

O'Brien L B and Wilson S W 1985 Planthopper systematics and external morphology In, Nault L R and Rodriguez J G (Eds.) The leafhoppers and planthoppers John Wiley and Sons, New York pp. 61-102.

Okada T 1977 Taxonomic characters for identification of Rice Brown planthopper *Nilaparvata lugens* (stal) and its related species in the Asian and Pacific region. The rice brown planthopper. Food and Fertilizer Technology Centre for the Asian and Pacific region 1-26.

Pathak P K and Heinrichs E A 1982 Selection of biotype populations 2 and 3 of *Nilaparvata lugens* (Stal) by exposure to resistant rice varietes. Environmental Entomology 11: 85-90.

Rao Rama Subba V 2006 Biosystematics of planthopper fauna (Homoptera : Fulgoridea : Delphacidae) of Economic importance in Andhra Pradesh. Final Report of ICAR Adhoc Research Project submitted to Indian Council of Agricultural Research, Krishi Bhavan, New Delhi.

Saitoh K, Kudoh K and Mochida O 1970 A study of the male meiotic chromosomes in two homopteran species of economic importance, Science. Hirosaki University 17: 32-37.

Saxena R C and Barrion A 1983a Chromosomal polymorphism among the biotype populations of the brown planthopper, *Nilaparvata lugens* (Stal)

(Homoptera:Delphacidae). Of 14th Annual Conference of the Pest Control Council of the Philippines, Manali, May 5-98, 1983, 10(abstract).

Saxena R C and Barrion A A 1983b Biotype of the brown planthopper, *Nilaparvata lugens* (Stal). Korean Journal of Plant Protection 22(2):52-66.

Saxena R C, Demayo C G and Barrion A 1991 Allozyme variation among biotypes of *Nilaparvata lugens* (Stal) in Philippines. Biochemical Genetics 29:115-123.

Shufran K A and Whalon M E 1995 Genetic analysis of brown planthopper biotypes using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). Insect Science Applications. 16 : 27-33.

Suenaga H 1963 Analytical studies on the ecology of the two species of planthoppers, the *Sogatella furcifera* (Horvath) and *Nilaparvata lugens* (Stal) with special reference to their outbreaks (in Japan). *Bulletin of Kyushu Agricultural Exp. Stn*. 3: 1-5.

Suenaga H 1971 Method 80. In Chirappa journal (ed.) Crop loss assessment methods. FAO Manual of the evaluation and prevention of losses by pests, diseases and weeds. Common Wealth Agriculture. Bureaux, Farnham Royal, Slouth, U. K., by arrangement with FAO of the United Nations.

Tyagi B K 1983 Cytogenetics, cytotaxonomy and cytophylogeny of dragonflies (Odonata).Proceedings of the First All India Symposium on Physiology of Insect Reproduction, Nagapur 6 (abstract).

Vaidya G R and Kalode M B 1981. Studies on biology and varietal resistance to *Sogatella furcifera* (Horvath) in rice. *Indian Journal of Plant Protection* 9: 3-12.

Welsh J and Mc Clelland M 1990 Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18: 7213-7218.

William J G K, Kubelik A R, Livak K J and Tingey S V 1990 DNA polymorphism's amplified by arbitrary primers are useful as genetic markers. *Nuclei Acids Research* 18: 6531-6535.

Wilson M R and Claridge M F 1991 Hand book for the identification of leafhoppers and planthoppers of rice. C.A.B International, Wallingford Oxon OX10 8DE, UK. pp: 142.

Zabeau M and Vos P 1993 Selective restriction fragment amplification : a general method for DNA finger printing. *European Patent Application*. Publication number EP 0534858 A1. Mochida O 1978 BPH "Hamma Wereng" problems on rice in Indonesia. CRIA-IRRI, Sukamandi, Indonesia pp.76.

APPENDIX

Reagents and solutions

A: 1.0 M Tris- HCl (pH 8.0)

Trizma base (Mw=121.14) =12.11g

Dissolve in sterile de-oinised water, adjust pH to 8.0 with cone. HCl, make up volume to 100 ml with de-ionised water and autoclave at 15 Psi for 15 min.

B: 0.25 M EDTA (pH 8.0)

EDTA (disosium salt; Mw=372.3)=9.31g

Dissolve, adjust pH to 8.0 with 5 N NaO11, make up volume to 100 ml with de-   ionised water and autoclave at 15 Psi for 15 min.

C: 5.0M NaCl

NaCl

Dissolve, make up volume to 100 ml with de-ionized water and autoclave at 15 Psi for   15 min

D: Extraction buffer

1M Tris-HCl (pH 8.0) = 10 ml

0.25 M EDTA (pH 8.0) =8 ml

5 M NaCl = 28 ml

1.5% CTAB (w/v) =1.5gm

Dissolve, make up to 100 ml with de-ionized water and autoclave at 15 Psi for 15 min.

E: 10% working CTAB

10%CTAB = 10 gm

5 M NaCl =14 ml

Dissolve in water, make up to 100 ml and autoclave at 15 Psi for min.

F: 3M NaOAC (pH 6.8)

Sodium Acetate =40.83 gm

Dissolve, adjust pH to 6.8 with glacial acetic acid, make up volume to 100 ml with de-ionized water and autoclave at 15 Psi for 15 min.

G: Chloroform: iso-amyl alcohol Mixture(24:1)

Cloroform =96ml

Iso-amyl alcohol =4ml

H: Iso-propanol (cold)

Filter sterilizes and store at 4°C

I: 70% Ethanol (100ml)

Absolute alcohol =70ml

Double distilled water =30ml

J: R Nase stock

1 M Tris-HCl (pH 8.0) =100µl

5 M NaCl =300µl

R Nase =10 mg (Sigma)

Adjust volume to 1 ml with de-ionized water, boil for 15 minutes and allow to cool slowly and store at -20°C

K: TE (10:1)

1 M Tris-HCl (pH 8.0) =1 ml

0.25 M EDTA (pH 8.0) =0.4ml

Dissolve; make up volume to 100 ml with de-ionized water and autoclave at 15 Psi for 15 min.

L: 10X TBE (pH 8.0)

Trizma base	=108 gm
Boric acid	=55 gm
EDTA	=9.3 gm

Dissolve and make up volume to 1000 ml with double distilled water.

INSTRUMENT USED FOR EXPERIMENT

1. BIOTECH agarose gel electrophoresis system.
2. LAB-LINE thermal rocker.
3. Gel-Doc system (Bio Rad).
4. Refrigerated micro centrifuge (HERAEUS Biofuge 17RS).
5. Water bath incubator (JULABA).
6. Microwave oven (KELVINATOR).
7. Laminar flow (KLENZAIDS).
8. LAB-LINE orbit environ shaker incubator.
9. LAB-LINE- 20° C freezer.
10. GODREJ refrigerator.
11. PRECISA weight marker.
12. REMI refrigerated centrifuge.

13. SYSTRONIC digital pH meter.

14. WISWD 65°C water bath incubator.

15. ZIEGRA ice-maker.

16. LABCONCO deionizer.

17. YORCO vertical autoclave.

18.PERKIN ELMER DNA Thermal Cycler

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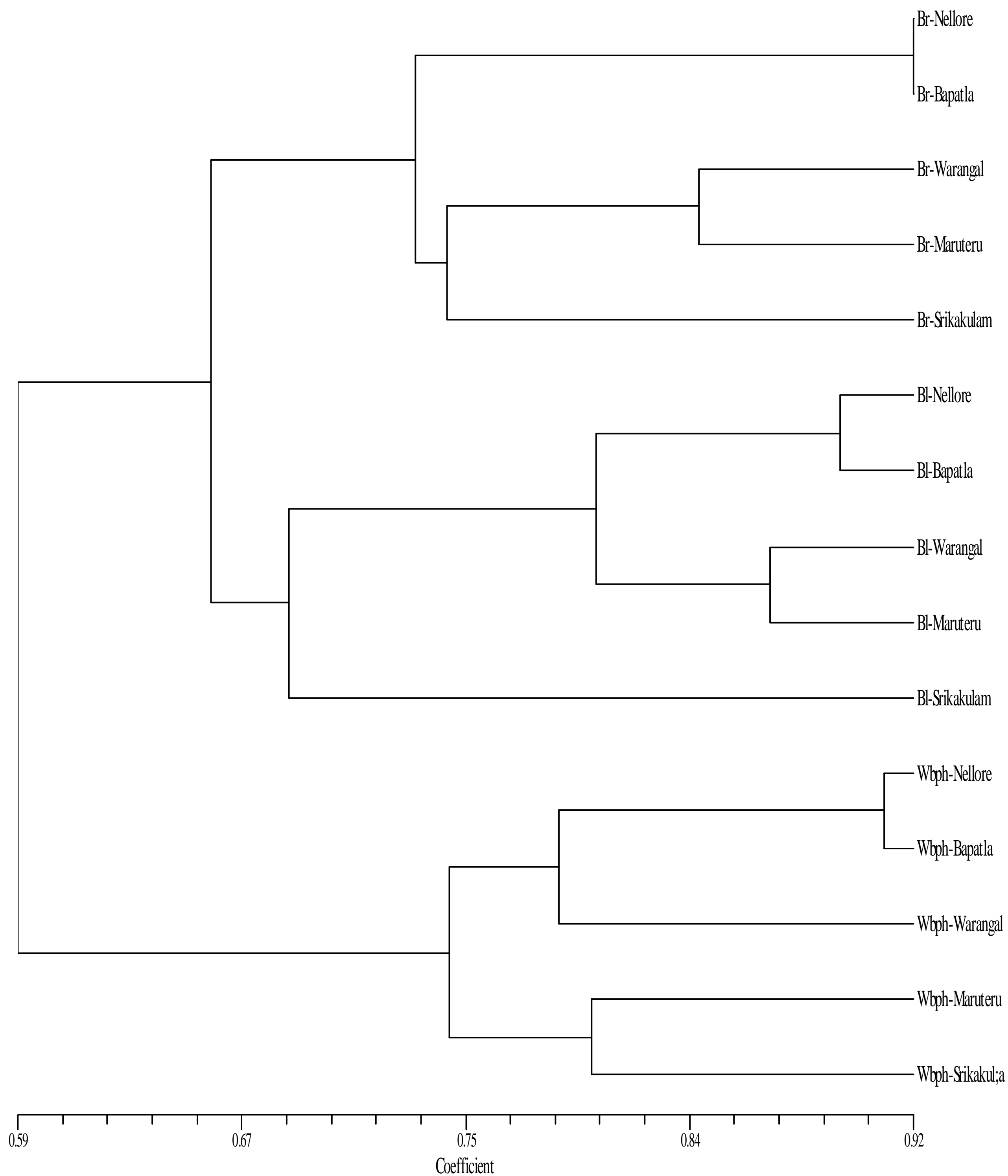


Fig-7. Dendrogram generated by Jaccard's Co-efficient using UPGMA (Unweighted Pair Group Mean with Arithmetic Average) analysis computed from pairwise comparisons of RAPD bands of brown & black forms of brown planthopper and whitebacked planthoppers of rice of five locations of Andhra Pradesh.

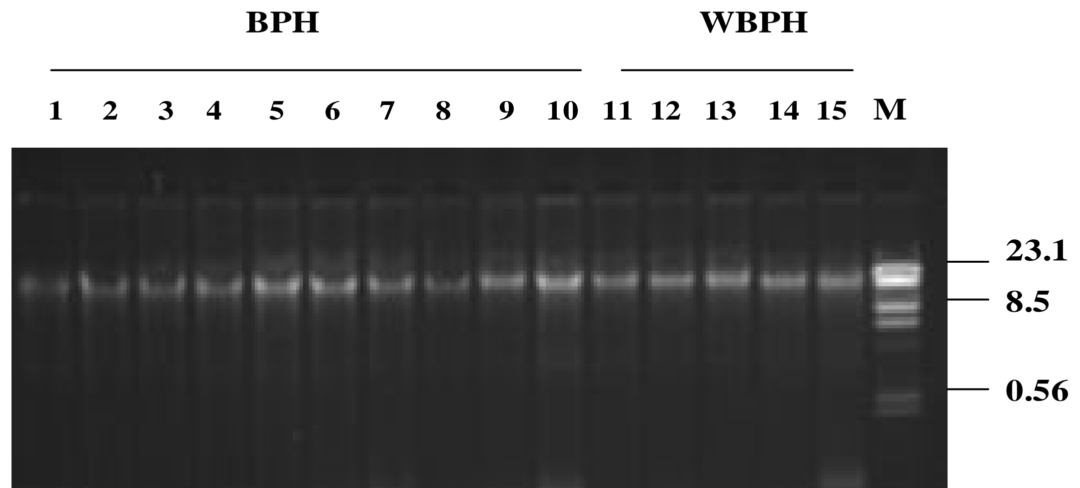


PLATE:3 Genomic DNA of bulk females of BPH

- M= Molecular weight marker (Lambda/*HindIII*)
- Numbers on top of the lanes correspond to individuals of BPH
- Numbers on the right margin represent molecular weight markers in kb
- 1,2,3,4,5– Brown forms of brown plant hopper.
- 6,7,8,9,10—Black forms of brown plant hopper.
- 11,12,13,14,15—White backed plant hopper.

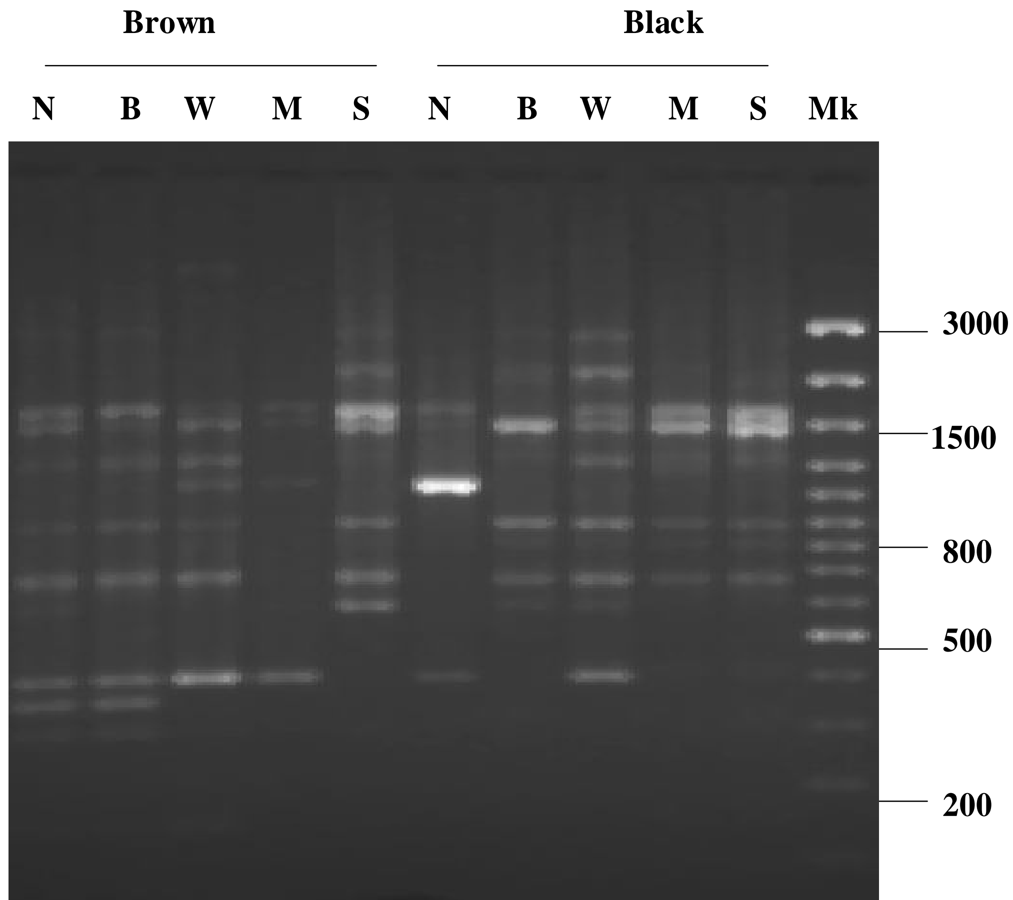


PLATE - 4: RAPD profiles of amplified bulk DNA of (brown & black) brown plant hopper of five locations obtained with primer,OPN-16

- **N=Nellore,**
- **B=Bapatla,**
- **W=Warangal,**
- **M=Maruteru,**
- **S=Srikakulum**

- **Mk = Molecular weight marker (100bp DNA ladder plus)**
- **Numbers on the right margin represent molecular weight markers in base pair**

White backed planthoppers

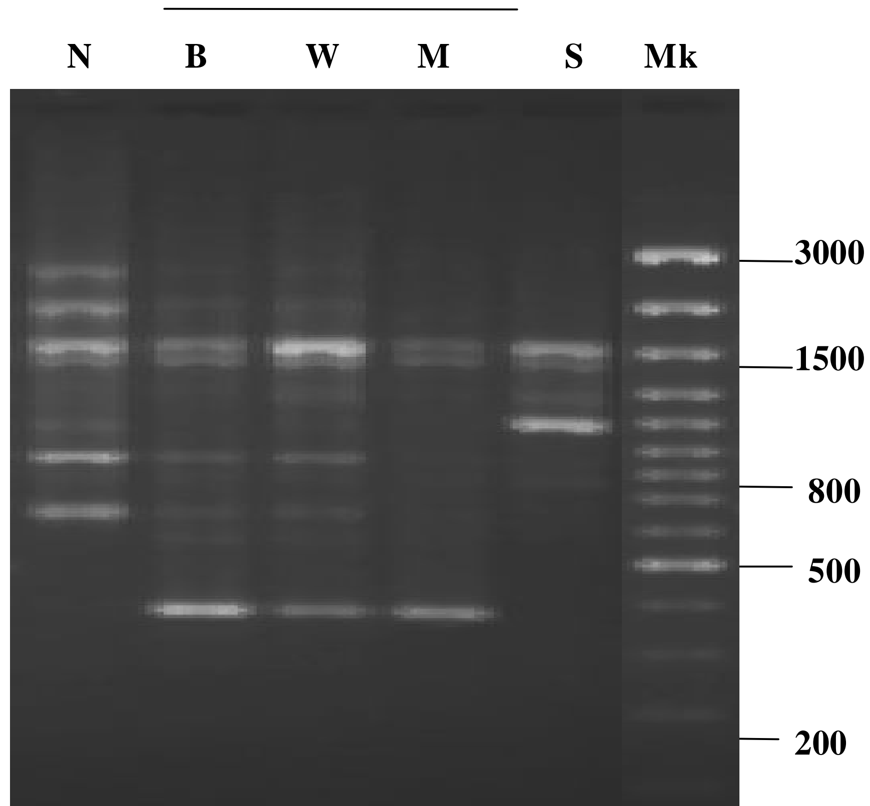


PLATE - 5: RAPD profiles of amplified bulk DNA of White backed planthoppers of five locations obtained with primer,OPN-16.

- **N=Nellore**
- **B=Bapatla**
- **W=Warangal**
- **M=Maruteru**
- **S=Srikakulum**

- **Mk = Molecular weight marker (100bp DNA ladder plus),**
- **Numbers on the right margin represent molecular weight markers in base pair**

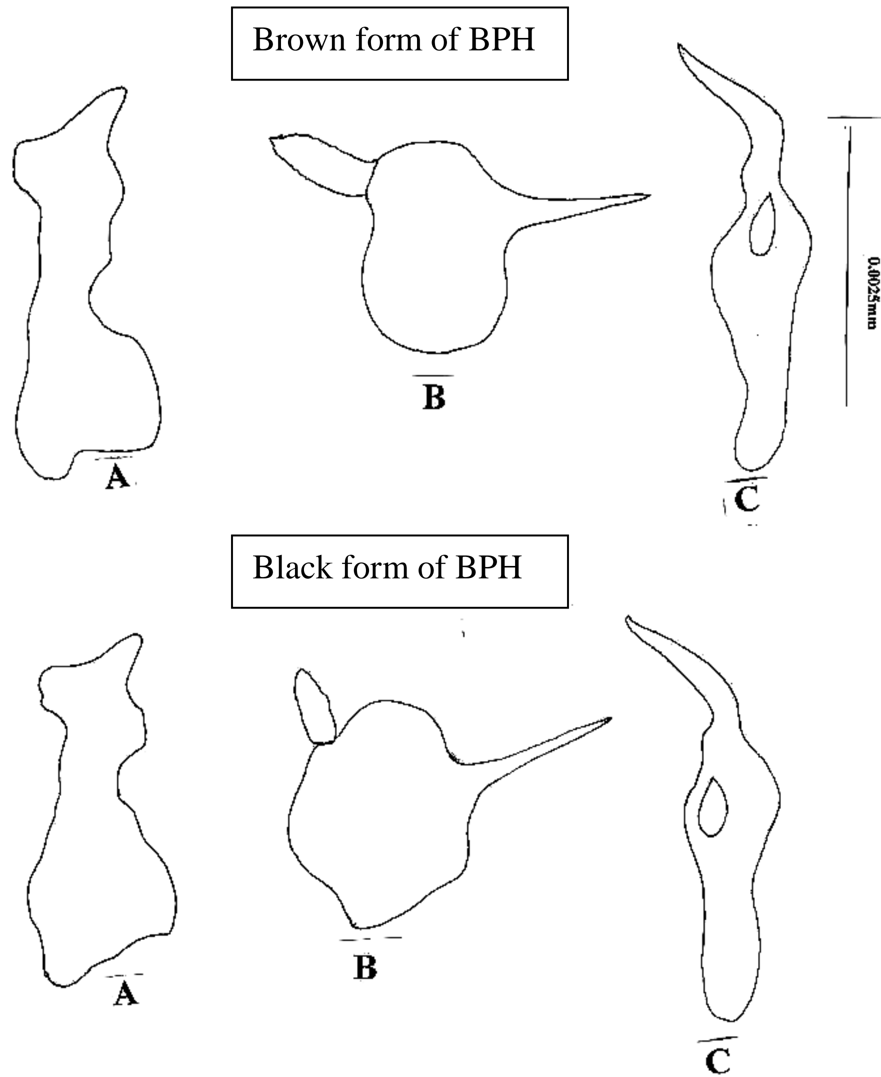


Fig-5. Genital structures of brown and black forms of brown plant hopper, *Nilaparvata lugens* (Stal), from Srikakulam region.

A – Genital style
B – Anal tube
C – Aedeagus

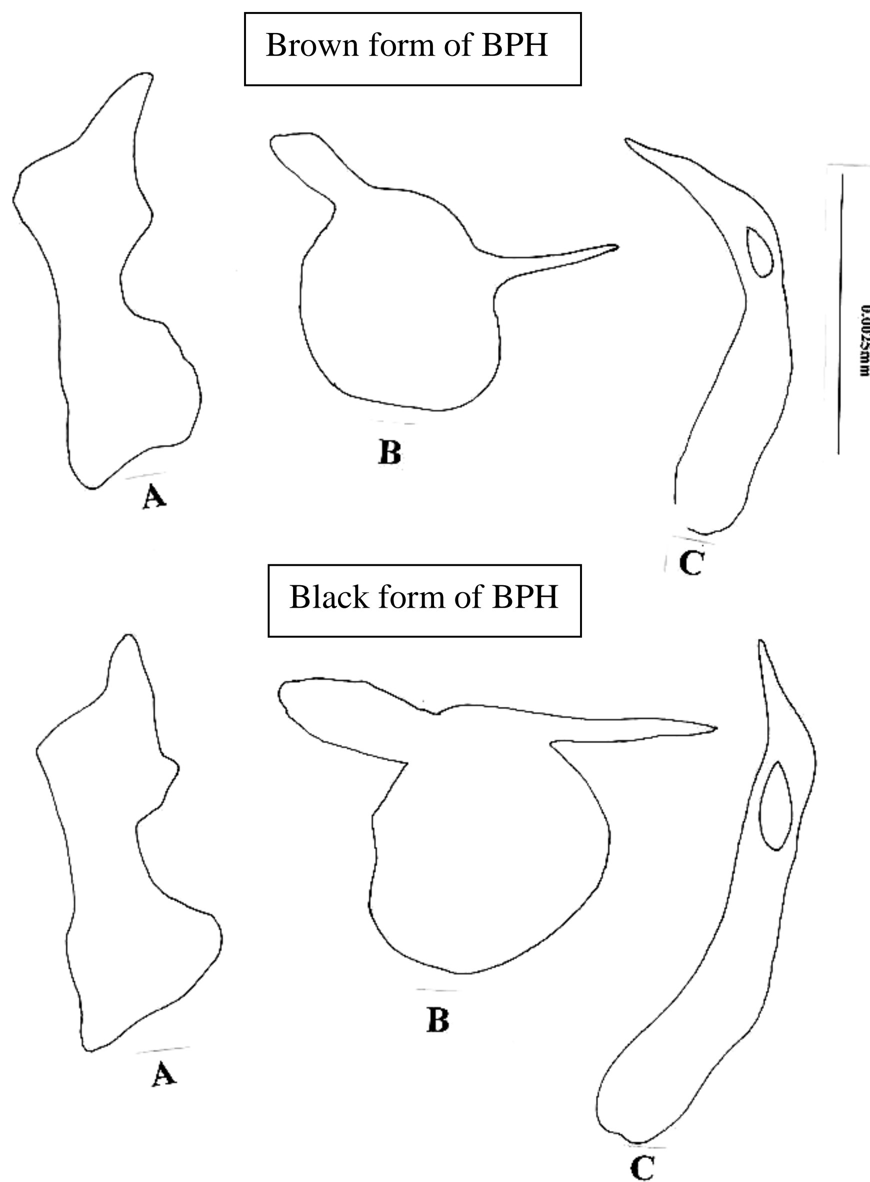


Fig-1. Genital structures of brown and black forms of brown plant hopper, *Nilaparvata lugens* (Stal), from Nellore region.

A – Genital style
B – Anal tube
C – Aedeagus

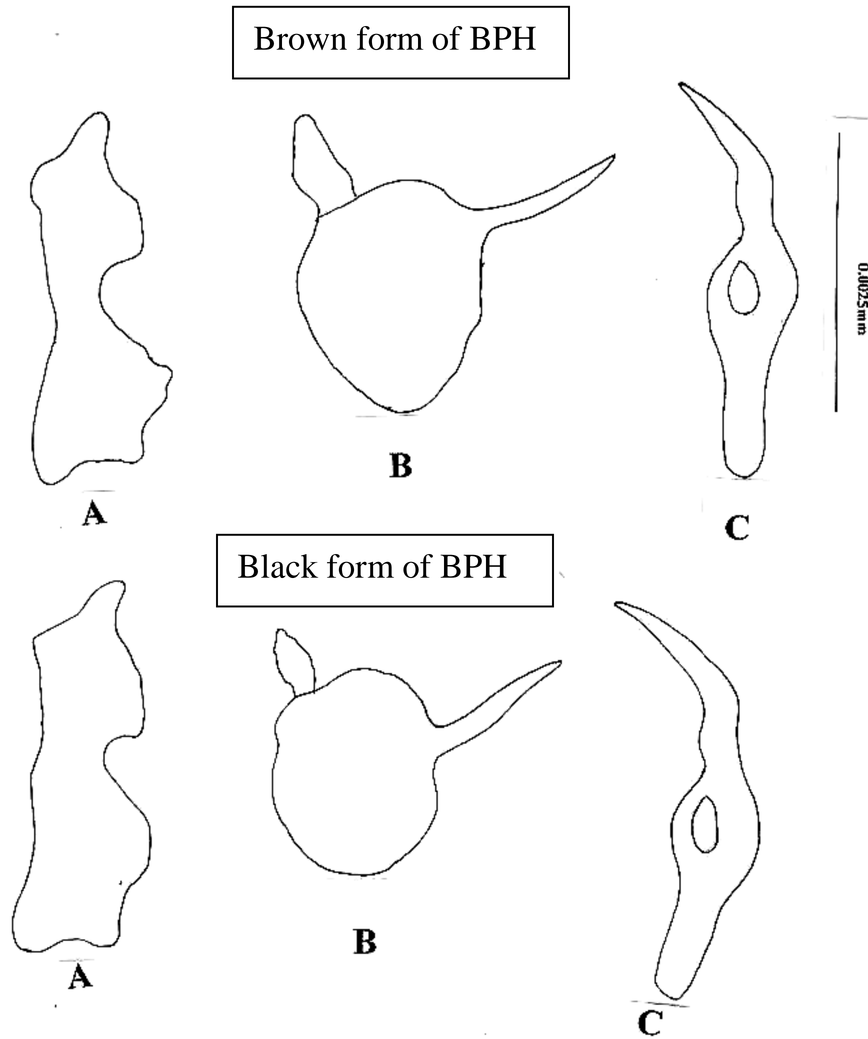


Fig-2. Genital structures of brown and black forms of brown plant hopper, *Nilaparvata lugens* (Stal), from Bapatla region.

A – Genital style
B – Anal tube
C – Aedeagus

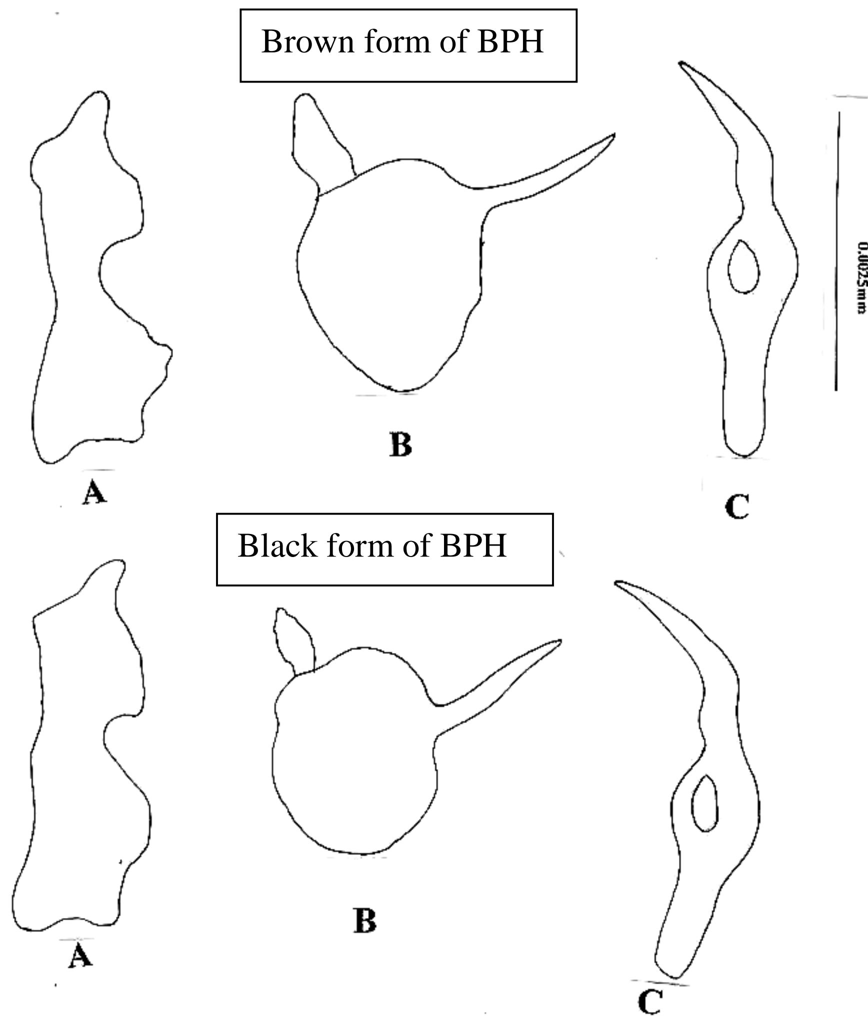


Fig-4. Genital structures of brown and black forms of brown plant hopper, *Nilaparvata lugens* (Stal), from Maruteru region.

A – Genital style
B – Anal tube
C – Aedeagus

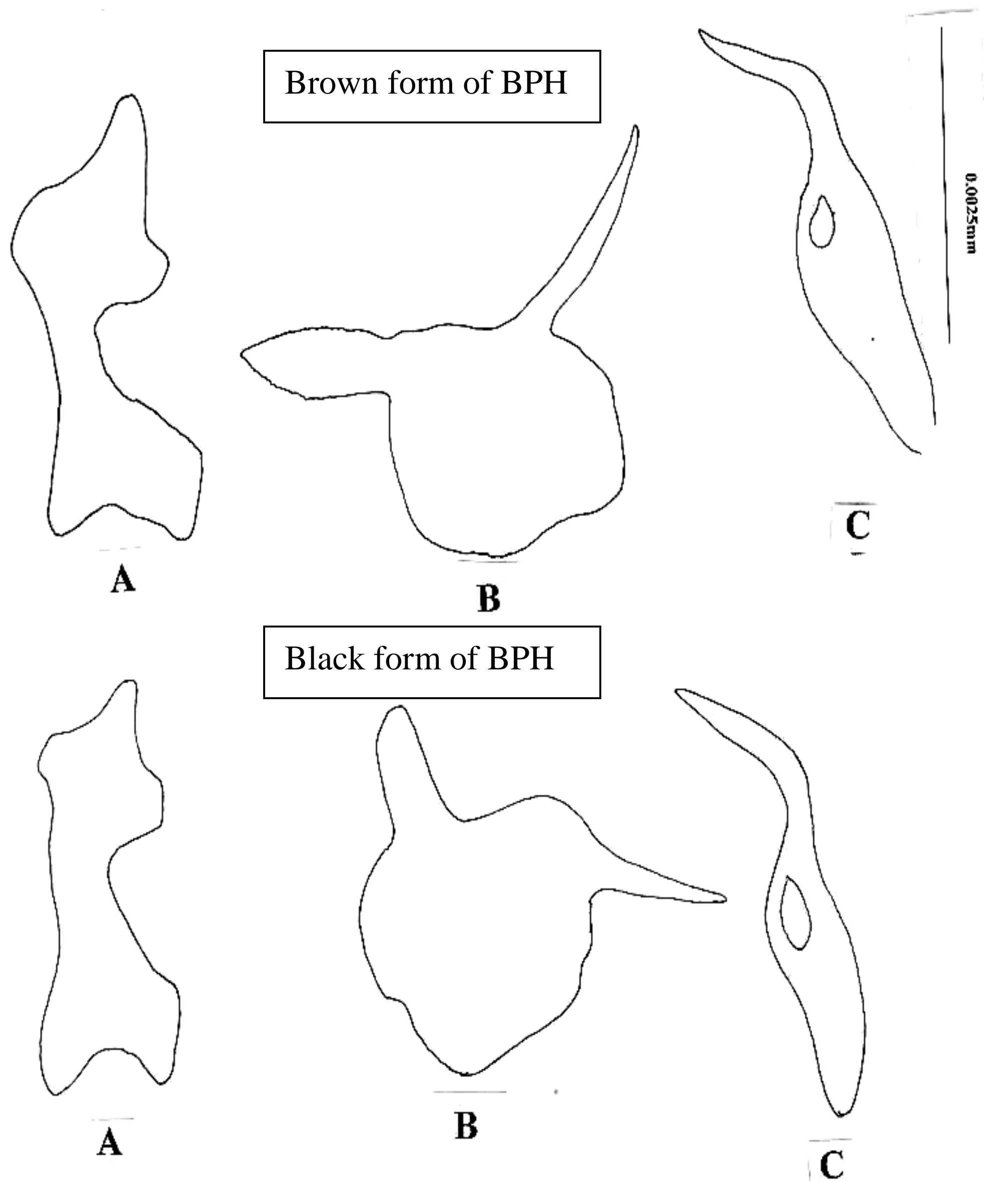


Fig-3. Genital structures of brown and black forms of brown plant hopper, *Nilaparvata lugens* (Stal), from Warangal region.

A – Genital style
B – Anal tube
C – Aedea

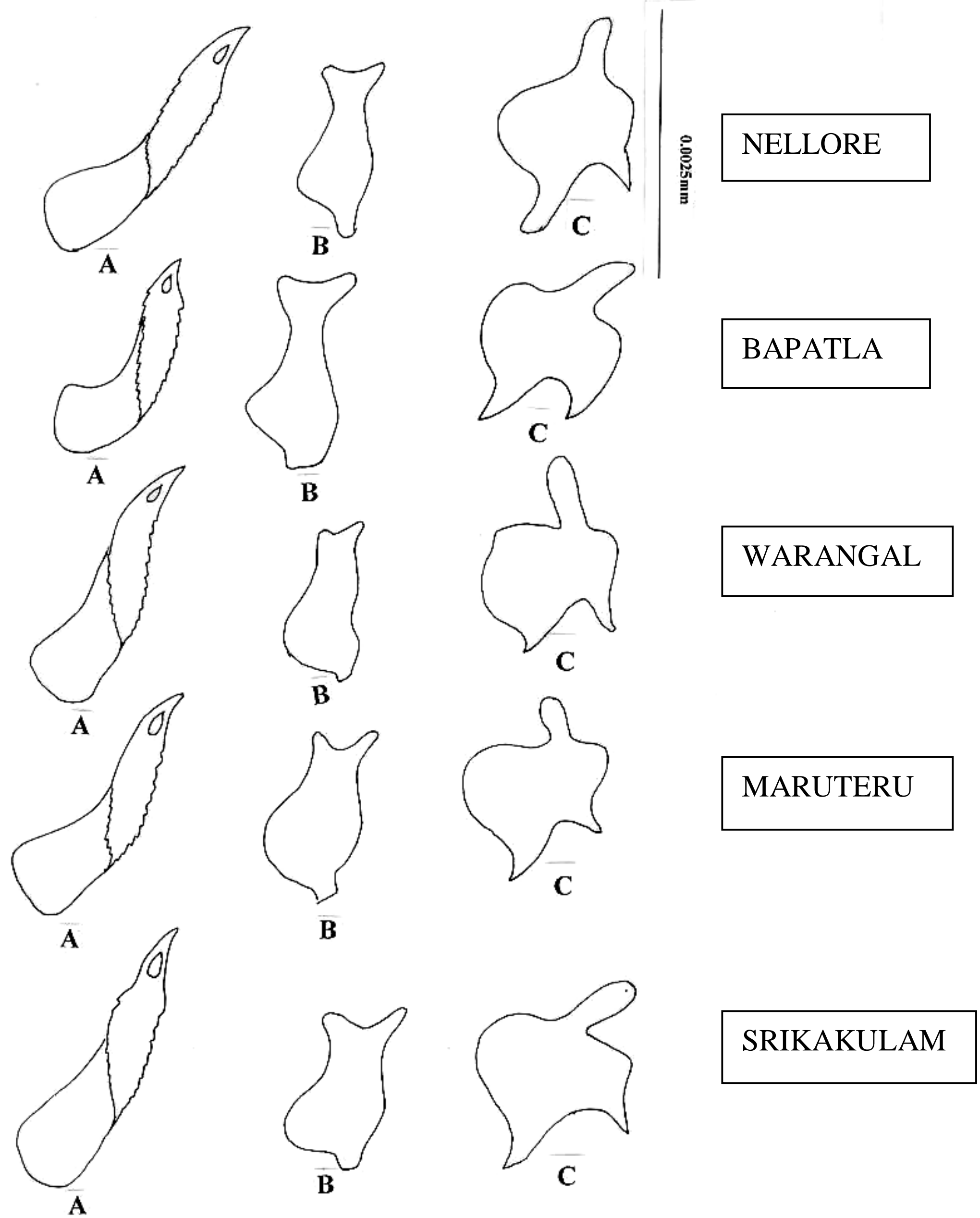


Fig-6. Genital structures of Whitebacked planthopper, *Sogatella furcifera* (Horvath), from different regions of Andhra Pradesh.

A – Aedeagus
B – Genital style
C – Anal tube

TABLE - 1

Band scoring of five primers to different populations

Primer	Band size	N	B	W	M	S	n	b	w	m	s	W1	W2	W3	W4	W5
OPN-16	**300**	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	400	1	1	1	1	0	1	0	1	0	1	1	1	1	0	0
	600	0	0	0	0	1	1	1	0	0	0	1	1	1	0	1
	680	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
	800	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0
	900	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
	1100	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0
	1200	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0
	1500	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1
	1700	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2000	0	0	0	0	1	1	1	0	0	0	1	1	0	1	0
	2800	1	1	0	0	1	1	0	1	1	0	0	0	0	0	0
OPN-18	**350**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	450	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	800	1	1	0	1	1	1	1	1	1	0	0	0	1	0	0
	900	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
	1000	0	0	0	1	0	1	1	1	1	1	1	0	0	0	0
	1300	1	1	1	1	0	1	0	1	1	1	0	0	1	0	0
OPN-19	**450**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	600	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	700	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	900	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	950	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	1000	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1200	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
	1600	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0
OPN-20	**550**	1	1	1	1	1	0	0	0	0	0	0	1	1	0	1
	1150	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1400	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0
	1750	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
OPN-15	**500**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	850	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

TABLE-2

Primers, their sequences and band patterns

Primer	Nuecleotide Sequence	No.of bands scored	No.of poly. bands	Size range of scored fragments
OPN-20	GGTGCTCCGT	4	3	300-2800
OPN-16	AAGCGACCTG	12	11	350-1600
OPN-18	GGTGAGGTCA	7	5	450-1600
OPN-19	GTCCGTACTG	9	3	550-1750
OPN-15	TCGCCGGTTC	2	0	500-850
OPN-3	GGTACTCCCC	-	-	-
OPH-15	AATGGCGCAG	-	-	-
OPH-2	TCGGACGTGA	-	-	-
OPH-16	TCTCAGCTGG	-	-	-
OPJ-1	CCCGGCATAA	-	-	-
OPH-10	CCTACGTCAG	-	-	-
OPG-5	CTGAGACGGA	-	-	-
OPG-10	AGGGCCGTCT	-	-	-
OPR-5	GACCTAGTGG	-	-	-
OPR-15	GGACAACGAG	-	-	-
	Total-	34	22	

TABLE - 3

Number of band fragments obtained by five primers to different populations of Planthoppers

Individual	OPN-15	OPN-16	OPN-18	OPN-19	OPN-20	Total
1	2	7	5	7	3	24
2	2	6	5	7	4	24
3	2	7	4	7	3	23
4	2	5	6	7	3	23
5	2	9	4	7	3	25
6	2	9	7	8	2	28
7	2	7	6	8	2	25
8	2	9	7	9	2	29
9	2	6	7	9	3	27
10	2	2	6	8	2	20
11	2	7	3	7	2	21
12	2	7	2	7	3	21
13	2	6	4	7	2	21
14	2	5	3	7	1	18
15	2	5	3	6	2	18

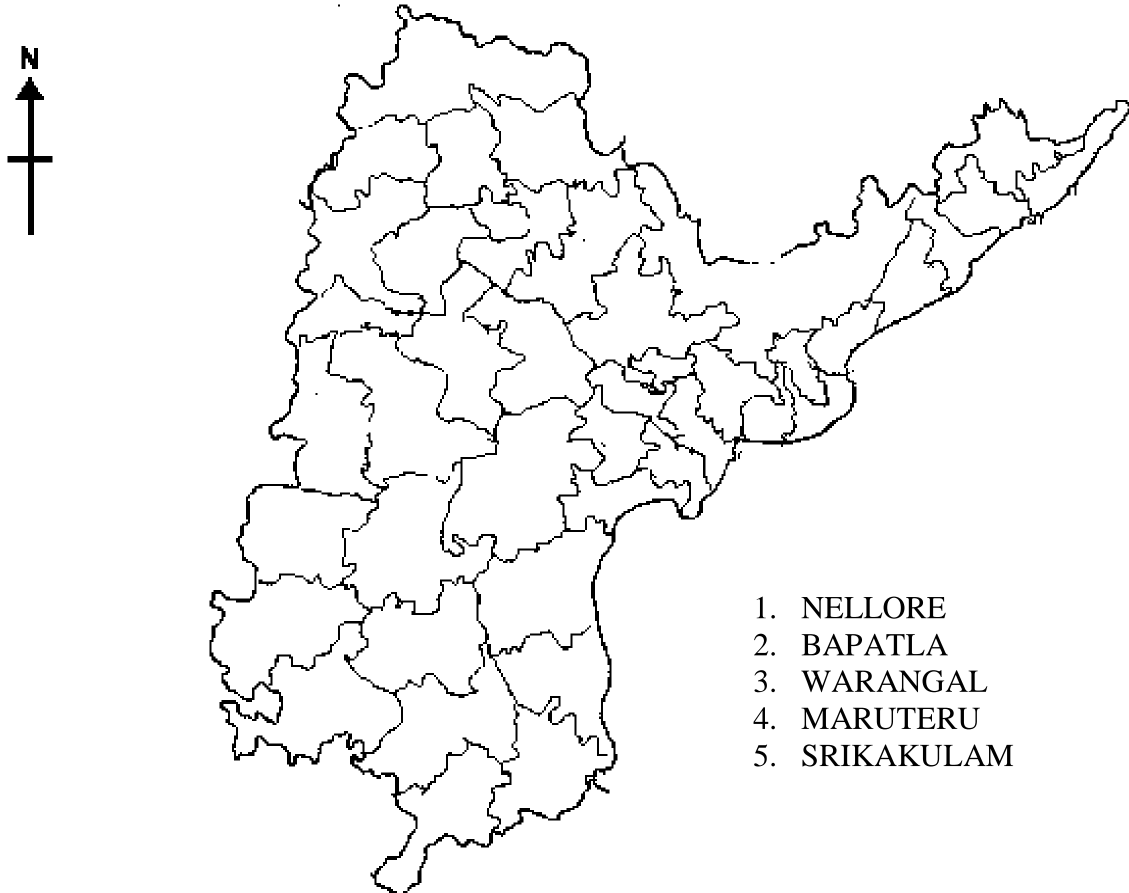
	Brown BPH	Black BPH	WBPH
NELLORE -	1	6	11
BAPATLA -	2	7	12
WARANGAL -	3	8	13
MARUTERU -	4	9	14
SRIKAKULAM-	5	10	15

TABLE - 4

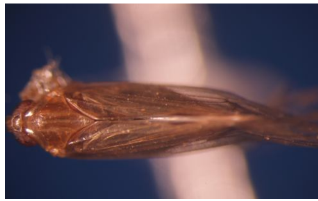
Similarity matrix for Jaccard's Coefficient for Plant hopper populations based on 34 bands obtained with RAPD primers

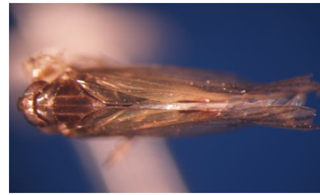
	Br-Nellore	Br-Bapatla	Br-Warangal	Br-Maruteru	Br-Srikakulam	Bl-Nellore	Bl-Bapatla	Bl-Warangal	Bl-Maruteru	Bl-Srikakulam	Wbph-Nellore	Wbph-Bapatla	Wbph-Warangal	Wbph-Maruteru	Wbph-Srikakul;am
Br-Nellore	1														
Br-Bapatla	**0.92**	1													
Br-Warangal	0.81	0.74	1												
Br-Maruteru	0.74	0.67	0.84	1											
Br-Srikakulam	0.75	0.68	0.78	0.71	1										
Bl-Nellore	0.73	0.68	0.65	0.65	0.71	1									
Bl-Bapatla	0.63	0.58	0.61	0.61	0.72	0.89	1								
Bl-Warangal	0.71	0.66	0.73	0.73	0.69	0.84	0.74	1							
Bl-Maruteru	0.71	0.71	0.61	0.61	0.62	0.83	0.79	0.87	1						
Bl-Srikakulam	0.63	0.63	0.65	0.72	0.51	0.71	0.67	0.69	0.68	1					
Wbph-Nellore	0.55	0.55	0.57	0.52	0.59	0.69	0.71	0.56	0.61	0.57	1				
Wbph-Bapatla	0.61	0.61	0.63	0.52	0.64	0.63	0.64	0.52	0.55	0.52	0.91	1			
Wbph-Warangal	0.73	0.67	0.69	0.63	0.64	0.69	0.64	0.61	0.61	0.57	0.75	0.83	1		
Wbph-Maruteru	0.56	0.51	0.57	0.46	0.59	0.58	0.65	0.52	0.55	0.52	0.77	0.77	0.69	1	
Wbph-Srikakul;am	0.62	0.56	0.64	0.52	0.65	0.53	0.59	0.47	0.51	**0.46**	0.69	0.77	0.77	0.81	1

ANDHRA PRADESH



❖ AP Map showing the locations of Plant hopper collections.

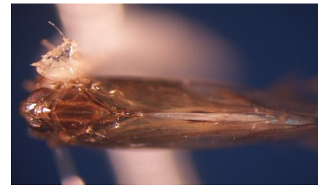

NELLORE


NELLORE

BAPATLA

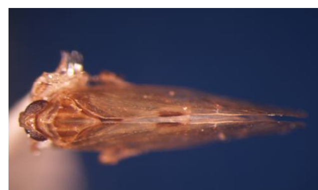
BAPATLA

WARANGAL


WARANGAL

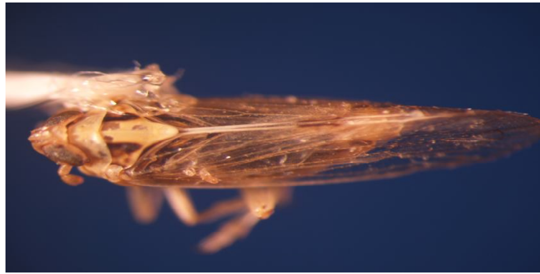
MARUTERU

MARUTERU


SRIKAKULAM

SRIKAKULAM

Plate: 1 Brown and black forms of brown planthopper, *Nilaparvata lugens* (Stal) of different locations of Andhra Pradesh.

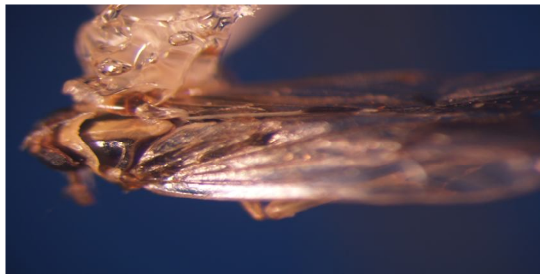


NELLORE

BAPATLA

WARANGAL

MARUTERU



SRIKAKULAM

Plate: 2 Whitebacked planthoppers, *Sogatella furcifera* (Horvath) of different locations of Andhra Pradesh.