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ASSESSMENT OF GENETIC VARIABILITY IN RICE YELLOW STEM BORER POPULATIONS OF ODISHA USING RAPD MARKERS

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ABSTRACT

Yellow stem borer (YSB), *Scirpophaga incertulas* Walker is considered to be the most serious pest in irrigated and deep water rice in South-East Asia. It causes yield losses estimated at 15-20% and reaching up to 60% during severe out breaks. Application of insecticides for control of the pest possesses many problems. Ordinary ground applications are limited to the pre-flood period and spraying is not possible when water is deeper than 50 cm. The use of resistant varieties seems to be the best alternative. Host-plant resistance is likely to be more durable if it employs an array of resistance genes encoding diverse mechanisms of resistance using marker-assisted selection (MAS) breeding approach. Durability of resistance depends on the population structure and mobility of the insect; resistance is more likely to break down if the pest population is genetically diverse or if rates of mutation or migration are high. DNA fingerprinting of insect populations provides insight into genetic diversity of the insect populations, which help to develop varieties resistant to the pest. In the present study, we analyzed the genetic variability between geographically isolated populations of YSB from sixteen places of Orissa using RAPD marker technique. Ten arbitrary 10-mer oligonucleotide primers were used to amplify genomes of yellow stem borer populations. A total of 104 bands were amplified, of which 99(95.1%) were polymorphic. Thirty three unique bands were identified which will be useful for developing diagnostic markers. Genetic similarity among YSB populations varied from 0.24 to 0.651, with an average of 0.415 indicating that wide genetic variation exists between YSB populations at molecular level. All the populations could be uniquely distinguished from each other and grouped into three major clusters at 38% level of genetic similarity. Further study with host differentials can ascertain their biotype status.

KEYWORDS: Rice, Yellow Stem Borer (YSB), Genetic Variability, RAPD

ABBREVIATIONS

The following abbreviations have been used throughout in these studies without definition.

Table

Aflp	Amplified Fragment Length Polymorphism
bp	Base pair
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 liter
Mg	Milli gram

ng	**Nano gram**
PCR	**Polymerase Chain Reaction**
RAPD	**Randomly Amplified Polymorphic DNA**
STMS	**Sequence Tagged Microsatellites**
SSR	**Simple Sequence Repeat**
Taq	**Thermusaquaticus**
TBE	**Tris Boric acid EDTA buffer**
TE	**Tris EDTA**
UV	**Ultra violet**
YSB	**Yellow Stem Borer**
RFLP	**Restriction Fragment Length Polymorphism**
DAF	**DNA Amplification Fingerprinting**
EtBr	**Ethidium Bromide**
VNTRs	**Variable Number Tandem Repeats**
BPH	**Brown Plant Hopper**
Mm	**Milli molar**
Rp	**Resolving power**

INTRODUCTION

Rice is the predominant staple food for 15 countries in Asia and the Pacific, ten countries in Latin America and the Caribbean, one country in North Africa and seven countries in sub-Saharan Africa (FAO, 1999), providing 27 percent of dietary energy supply, 20 percent of dietary protein and 3 percent of dietary fat. Rice can contribute nutritionally significant amounts of thiamine, riboflavin, niacin and zinc to the diet, but smaller amounts of other micronutrients. In India alone 42.8 % of land is under irrigation & total productivity of rice is about 6.3 t/ha. It is grown on over 145 million ha in more than 110 countries and occupies almost one-fifth of the total world cropland under cereals. Demand for rice is increasing at a rate of 2% annually but the rate of growth of rice production has slowed by 1.2% .It has been projected that by the year 2020 AD, the global demand for rice would increase by 65% requiring production of 858 million tons of unmilled rice as compared to present production of 550 million tons. If this goal is to be met, it is necessary to use rice varieties with higher yield potential, durable resistance to diseases and insect pests and tolerance to abiotic stresses.If present trend continues, the demand for rice will exceed the population by the end of the century. The most challenging task before the research workers is how to sustain the yield in order to meet the demand of the growing population. One of the major constraints for stable higher production is the biotic stress.

The rice plant has been thesubject to attack by more than 100 species of insects; 20 of them can cause economic threat to the cultivation of rice. Together they infest all parts of the plant at all growth stages and a few transmit viral diseases leading to indirect damage. The major insect pests that cause significant yield losses are leafhoppers, plant hoppers, stem borers, gall midge and leaf folders. Rice stem borers occupy the major status as pest and cause considerable damage to the rice cultivation in almost in all the seasons throughout the rice growing stages.

The rice stem borers are worldwide in distribution (*Dale 1994*) and are considered as most important of all (*Pathak 1975, CIBA 1969*).Most stem borers belong to the families Pyralidae and Noctuidae, and the immature insects, the caterpillars, tunnel into the stems and feed on the soft tissues and cause the injury.Yellow Stem Borer (YSB),*Scirpophagaincertulas* Walker (Pyralidae, Lepidoptera) is a serious pest of rice. It feeds only on rice plants (Monophagous feeding), while other species of stem borer feed on barley, sorghum, maize, wheat, and grasses(Polyphagous feeding).It is, distributed primarily in the tropics and also occurs in the temperate areas where

temperature remains above 10ºC and annual rainfall is more than 1,000 mm. It is the predominant species in Bangladesh, India, Malaysia, Pakistan, the Philippines, Sri Lanka, Thailand, Vietnam, and parts of Indonesia. It is abundant both on lowland rice and upland rice and attack young plants even in the nursery (*Litsinger et al., 1987*). It requires high moisture (90-100%) and is a notorious pest of deepwater rice. It is because the deep water rice has longer stem due to which the larvae moves upwards as it is negatively geotropic in nature. Stem borer damage becomes evident as : (a) **dead heart** , in which the death of the central shoot occurs that leads to development of transparent patches and eventually it dries up and the central leaf whorl does not unfold and this occurs in the vegetative stage of the plant; and (b) **white ear head**, in which the larvae enters the tillers during grain filling that results in whole panicle turning chaffy & whitish in colour and this occurs in the reproductive stage of the plant. Significant losses are also inflicted by larvae that feed within the stem without severing the growing plant parts at the base. Such damage results in reduced plant vigor, fewer tillers, and many unfilled spikelets.

Resistance is a relative attribute and is measurable only in relation to susceptibility. Resistant populations are sought as the major tactic in pest management. Insect resistance is a relative property & can be defined only in comparison to other more susceptible varieties; it can be defined as “those heritable characteristics possessed by the plant, which influence, the ultimate degree of damage done by insects.”Varieties resistant to one species of stem borer are not necessarily resistant to other species. Differences in resistance to stem borer among varieties are only quantitative. All shades of resistance from moderate to high are seen in rice genotypes, however, very high level of resistance against stem borer was not noticed in any of the genotypes. Even varieties classified as resistant suffer some damage under heavy insect populations. However, several wild rice species have high levels of resistance to stem borers. Genetic studies have shown that the resistance is polygenic in nature. Insect resistance mainly involves some morphological or some non-specialized biochemical / physiological factors of the host plants. Overcoming of such mechanisms of resistance would require a change in the habit or the physiology of the insect pest, which is expected to be governed by more than one gene. The incorporation of insect resistance into modern populations is a major objective of most rice breeding programs in Asia. The selection of superior genotypes by conventional plant breeding is time consuming and often dependent upon environmental conditions. As a result, plant breeders are interested in improved techniques that will make the selection of better varieties more reliable.

Traditionally used morphological and biochemical markers have not been found to be discriminative enough, warranting more precise techniques. Further, these markers are not reliable because many characters of interest have low heritability and are genetically complex. Several DNA marker techniques are now available. They are more reliable, and remain unaffected across different growth stages, seasons, locations and agronomic practices. In fact, the restriction fragment length polymorphism (RFLP) approach has been used successfully to identify genetic markers in plants, including rice.However, the RFLP technique needs specific probes for the target DNA sequences, and use of radioactive elements makes it more costly and tedious. The development of Polymerase Chain Reaction (PCR) based techniques has offered a good alternative to the RFLP analysis. The PCR based randomly amplified polymorphic DNA (RAPD), microsatellite and amplified fragment length polymorphism (AFLP) approach requires less DNA, and is technically simple, quicker and cheaper compared to the RFLP. These markers have been recently utilised for many purposes including genome mapping, gene tagging, estimation of genetic diversity, variety differentiation, resolution of uncertain

parentage and purity testing. RAPD analysis is more convenient, simple and requires little amount of genomic DNA, making it suitable for analysis single insect at molecular level.Among the various techniques available, RAPD analysis is potentially simple, rapid, reliable DNA fingerprinting method, making it suitable for analysis single insect at molecular level.

A wide range of research works have been done on rice applying molecular techniques but a little work has been done on the study of genetic variability among different populations of yellow stem borer of Odisha at DNA level, in spite of it being a major rice pest. Therefore, the present investigation was carried out in order to assess the genetic variability exists among morphologically indistinguishable populations of YSB from various hotspot locations of Odisha, where high-intensity borer infestation is favoured by multiple rice cropping, cultural practices, and climatic conditions using DNA fingerprinting techniques.

MATERIALS AND METHODS

Insect Materials

The female moths of yellow stem borers (YSB) were collected from sixteen different locations in Odisha. (Table 1)

The adult females were preserved in 95% ethanol at 4^0C for genomic DNA isolation.

Isolation of Genomic DNA

Modified method was used to isolate genomic DNA from female moths of yellow stem borers as follows:

- Soak single adult females in 50 μl of extraction buffer (see appendix) for 10 minutes.
- Homogenize the females in 1.5ml of eppendorf tube with sterilized
- Polypropylene pestle gently and thoroughly.
- Add once again 350 μl of extraction buffer slowly by rinsing the pestle.
- Add 10 μl of 10 mg/ml Proteinase-K and incubate at 37^0C for 1 hr.
- Add 400 ml of equilibrated phenol to the tube invert 30 times and 14,000 rpmat 25^0C for 10 minutes.
- Then transfer the supernatant to a new tube with a pipette avoiding protein and debris layer.
- Add 10 μl of 10 mg/ml RNase and incubate for 1 hr at 37^0C.
- Then add 400 ml phenol / chloroform- isoamyl alcohol, invert 30 times and spin at 14,000 rpm at 4^0C for 5 minutes and transfer the supernatant to a new tube with plastic pipette and add 9 µl of 5M NaCl.
- Add 2X volume of ice-cold absolute ethanol.
- Mix gently and leave at -20^0C for 2 hr (or) over night at -20^0c.
- Spin at 14,000 rpm at 4^0C for l0 min.
- Remove the supernatant with pipette taking care not to dislocate the DNA pellet.

- Add 200 μl of 70% ethanol to the DNA pellet. Leave for 5 minutes, spin at 14000rpm at 4^0C for 5 minutes and remove the supernatant.
- Add finally with 200 μl 100% ethanol, spins at 14000 rpm at 4^0C for 5 minutes and remove the supernatant taking care of DNA pellet.
- Dry the pellet under vacuum for 30 min.
- Add 50 μl of TE to each pellet and leave at 4^0C overnight.
- Store DNA samples at -20^0C till further analysis.

MEASUREMENT OF DNA CONCENTRATION AND QUALITY CHECKING- WHERE DNA BANDS PRESENT & PRO,RNA

Agarose Gel Electrophoresis

In order to know intactness of genomic DNA, presence of proteins and (or) RNA contaminants, an aliquot 4 µl of each sample was subjected to agarose gel (0.8%) electrophoresis for about 2 hours along with 500 ng of molecular weight marker (Lambda/*ECoRI*). The gel was photographed immediately for further interpretation using Gel-Doc system (Alpha-Innotech). The quantity of genomic DNA was determined by comparing with molecular weight markers.

RAPD Primers and RAPD Reactions

Ten random decamer oligonucleotide primers were used for amplification from genomes of YSB. These primers were from commercially available RAPD primer kits (Operon Technologies, Almeda California, USA). Each primer had a GC content of 60% (or) higher..(Fig. List.2)

Polymerase Chain Reaction (Pcr)

The PCR amplification was performed in a 20 µl reaction mixture volume containing 15 ng of DNA, 1X buffer, 200 mMdNTP, 20 ng of primer, 2 mM of magnesium chloride and 1U of *Taq(Thermusaquaticus*) DNA

polymerase enzyme. A single primer was included in each PCR reaction.

Preparation of Reaction Mixture

The reaction (master mix) was prepared as follows :

Table

Reagent	Final Concentration	Volume in µL
Sterile de-ionized water	**-**	**8.5 x 20 = 190.0**
10x PCR buffer	**IX**	**2.0 x 20 = 40.0**
10 mMdNTP	**200 µM of each**	**0.4 x 20 = 8.0**
25 mM magnesium chloride	**2Mm**	**1.6 x 20 = 32.0**
Primer	**20 ng**	**1.3 x 20 = 26.0**
***Taq* DNA Polymerase**	**1 U**	**0.2 x 20 = 4.0**
	Total	**14.0 X 20 = 280.0**

The reaction mixture was mixed well; 14 µl was distributed to each tube. Two µl of DNA (15 ng) sample was added to each tube, mixed well and briefly centrifuged to collect drops from wall of tube. The amplification was carried out in Lark Thermal Cycler (Model L196G) for 45 cycles under following PCR conditions:

Thermal Cycling

Table

Step	Temperature (0C)	Duration (Min)	Cycle
Initial denaturation	93	2	1
Denaturation	93	1	45
Annealing	36	1	
Extension	72	2	
Final extension	72	5	1

Gel Electrophoresis and Detection of Amplified Products

Four micro liter of loading buffer was added to the amplified PCR products, mixed well, centrifuged briefly to collect drops from wall of tube. Twelve microliter of amplified products of each sample was loaded on 1.5% agarose gel in 1X TBE buffer to separate the amplified fragments. The electrophoresis was done for about 3 hours at 60 volts. The molecular weight marker (100 bp ladder plus) was used to compare the molecular weights of amplified products. Gel-Documentation was carried out using the system FluorChem5500 (Alpha-Innotech).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.

Statistical Analysis of DNA Fingerprint Pattern

The RAPD amplified bands were scored as present (1) absent (0) for each primer population combination. The data entry was done into a binary data matrix as discrete variable and subjected to various statistical analysis to draw definite conclusion.

Percent of Polymorphic Loci

A locus can be defined as polymorphism when frequency of marker (allele) is less than 1.0.

$$\frac{\text{Number of polymorphic bands} \times 100}{\text{Total number of bands compared}}$$

Percent of polymorphic loci =

Primer Efficiency

Primer resolving power was used to identify the primer that distinguishes the cultivars most efficiently. Resolving power is calculated to identify the best informative primers.

Resolving Power of the primer (Rp)

$Rp = \sum Ib$

Ib = 1- {2X(0.5 - Pi)}

Where,

Ib = Sum of bands in formativeness

pi= It is the proportion of the n genotypes/populations containing the band.

Similarity Coefficient

For similarity coefficient selected population/genotype was compared with rest of populations/genotypes. Greater the value of coefficient, compared populations/genotypes will be more similar to selected population. The value of coefficient also signifiesabout the extent of similarity between two genotypes.

Jaccard's similarity =

n_{xy}= Number of bands common in sample a and b

n_1= Total number of bands present in all the sample.

n_2= Number of bands not present in sample a and b but found in

other samples.

Jaccard's co-efficient of similarity was obtained using NTSYS-PC

(Version 2.01).

The average similarity index for all pair wise comparisons (X_D) were calculated and used to estimate the probability of DNA fingerprints of two populations being identical by chance.

Employingformula $(X_D)^n$, where, X_D = average similarity index and n = average no. of amplified products per population.

Cluster Analysis

Cluster analysis is used to generate dendrogram which shows the genetic relationships among genotypes/populations under study. A dendrogram based on Jaccard's coefficient genetic similarity was generated by using Unweighted Pair group Method with ArithmeticMean(UPGMA). The computer package NTSYS-PC was used for cluster analysis (Version 2.01).

The results obtained in the present study are presented below.

DNA Isolation and Quantification

The genomic DNA isolation method was successfully used to isolate genomic DNA from yellow stem borer moths in our studies. Purification and quantification of genomic DNA isolated from each individual moth was done and fractionated on agarose gel (0.8%)(Figure No.7). The DNA bands of all individuals were intact and did not show any smearing. This reflects good quality DNA preferable for RAPD analysis. RNase treatment was done and there was no RNA left in the genomic DNA of any of the samples. The concentration of genomic DNA ranges from 12 ng/µl to 40

ng/µl.

RAPD Amplification

PCR amplification of DNA isolated from 16 YSB populations was done following the same protocol for all the ten random primers. PCR products run in agarose gel were scored manually. Each band produced by PCR amplification was examined by comparing with DNA size marker (100 bp ladder plus).The amplifications were carried out twice to check for reproducibility. Occasionally, the intensity of some bands was reduced orincreased slightly, but the total number of bands obtained with a primer remained the same.

Band Diversity

Ten random 10-mer oligonucleotide primers were used in RAPD analysis. The amplification pattern of RAPD primers was summarized in Table 3. All the ten random primers successfully amplified a total of 104 reproducible bands, of which 99 (95.1%) are polymorphic. The number of bands per primer ranged from six (OPH12) to thirteen (OPA13) with an average of 10.4 bands per primers. The size of the amplified product varied from 250bp to 1990bp. All the ten primers revealed polymorphism between individuals with an average of 10.4 bands per primer. Out of the 99 polymorphic bands, maximum numbers of polymorphic bands (i.e.12) were obtained with the primers, A04, OPC05 and OPA13 (Fig. No.9& 13).The primer, OPM05 amplified the second highest number of polymorphic bands (i.e., 11). Five primers, AO3, OPA13, OPM05, OPN04 and OPN13 amplified monomorphic bands. Thirty three unique bands were identified. The highest numbers of unique bands were obtained with the primer OPM05.The primers A03, A04, OPA13 and OPH12 amplified three, six, two and one unique bands, respectively. Amplification pattern with primers (AO3, OPA13,OPM05,OPN04 and OPN13)has been shown in Fig(8,10,11,12).Resolving power of random primers varied from 3.250 (OPH12) to 11.750 (OPA13), indicating that all the primers are efficient in differentiating populations of YSB.

Genetic Variation Among YSB Populations

Genetic similarity (Jaccard's coefficient of similarity) among YSB populations varied from 0.24 to 0.651 with an average of 0.415, indicating that wide genetic variation exists between YSB populations. Ranjisura showed lowest genetic similarity with Daispatna YSB population while Jijinipur showed highest genetic similarity with Teismile YSB population.

Cluster Analysis

Cluster analysis using UPGMA, dendrogram classified all the 16 rice YSB populations into two major groups, I & II. Group I is classified into 3 sub-clusters IA,IB,IC and group II into 2 sub-clusters IIA, IIB (Table-4).

All the individual populations of YSB included in the study could be distinguished precisely from each in pair wise comparison over all the ten RAPD primers. All the 10 primers provided high resolution power enabling nearly2x 10^{-13} populations to be precisely identified. (table-5)

RESULTS AND DISCUSSIONS

The yellow stem borer, *Scirpophaga incertulas* is a serious pest of rice and is common in Southeast and East Asia, China, the Indian continent, and Afghanistan. It attacks rice throughout its growth period. They infest plants from the seedling stage to maturity. Although worldwide in distribution, rice stem borers are particularly destructive in Asia, Middle East and Mediterranean regions. Although stem borer damage becomes evident only as dead heart and whitehead,

significant losses are also inflicted by larvae that feed within the stem without severing the growing plant parts at the base. Such damage results in reduced plant vigor, fewer tillers, and many unfilled spikelets.

The use of resistant varieties seems to be the best alternative. Host-plant resistance is likely to be more durable if it employs an array of resistance genes encoding diverse mechanisms of resistance using marker-assisted selection (MAS) breeding approach. Durability of resistance depends on the population structure and mobility of the insect; resistance is more likely to break down if the pest population is genetically diverse or if rates of mutation or migration are high. DNA fingerprinting of insect populations provides insight in to genetic diversity of the insect populations, which help to develop varieties resistant to the pest.

The main purpose of present study is to assess the genetic variability amongpopulations of YSB atmolecular level. PCR-based molecular marker techniques, especially Randomly Amplified Polymorphic DNA

(RAPD) have been used for analysis of genetic variability between populations of yellow stem borers (*Kumar et al., 2001*).RAPD-PCR has found wide applications in agricultural entomology for estimatinggenetic relationships in several insect species. For example, it has been used

to detect the geographic origin of insect pests (*Armstrong and Wratten, 1996; Gafnyet al., 1996*).

In the present study, modified method of DNA isolation was found to be 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.

All the ten random primers successfully amplified a total of 104 reproducible bands, of which 99 (95.1%) are polymorphic. The size of bands varied from 250bp to 1990bp.Thirty three unique bands were identified which could be developed into diagnostic markers to identify particular population of YSB. Similar to our study, *Kumar et al(2001)* used RAPD markers to assess the genetic diversity of 28 YSB populations collected from different hotspots of India. They have used 32 RAPD primers which amplified a total of 354 scorable bands with 11.06 bands per primer. The molecular weight range of bands varied from 0.5–2.5 kbp.

Genetic similarity (Jaccard's coefficient of similarity) among YSB populations varied from 0.24 to 0.651 with an average of 0.415, indicating that wide genetic variation exists between YSB populations. Ranjisura showed lowest genetic similarity with Daispatna YSB population while Jijinipur showed highest genetic similarity with

TeismileYSB population.Cluster analysis based on UPGMA, dendrogram grouped all the 16 populations of YSB into two different major groups corresponding to their geographical location (Fig.14). First major group consists of three subgroups, the first sub group consists of two populations and the second and third sub group consists of three populations each. The second major group consists of two subgroups the first sub group consist of six populations and the second sub group consist of two populations.

According to the study ofKumar et al.(2001) using RAPD-PCR analysis,genetic distance (GD)among 26 YSB, *Scirpophaga incertulas* populations varied from 6 to 48%. The importantconclusion drawn from the above analysis is that, with the exception of the pestpopulation from Pattambi, genetic distance is observed to be independent of geographic distance, suggesting that gene flow between populations is independentof geographic distance and appears to be

unrestricted.

In the case of *S. incertulas*, since host tolerance is polygenic in nature (*Khush, 1977*), environmental factors such as temperature,humidity, and hours of sunshine, as well as the genetic structure of the populationin any particular region, would play an important role in host tolerance. In thiscase, if the population structure had contributed significantly to the observed differencesin levels of pest incidence on the rice variety grown in different regions, we would expect to find RAPD markers specific to the different pest populationsand clustering based on geographical distribution.

In a similar study on brown planthopper (BPH) biotypes using RAPD markers,*Shufran and Whelon (1995*) have shown that though the three biotypes (1, 2,and 3) cannot be classified into sub-specific categories based on morphology,isozymes, or RAPD-PCR, they differ in their virulence characteristics. Accordingto these authors, BPH biotypes vary in their ability to injure resistant rice cultivarswith different resistant genes. In the case of the brown planthopper, resistance isgoverned by a single major gene.

In rice, no complete source of resistance to *S. incertulas* has been identified inspite of screening all the germplasm at the IRRI, Philippines (*Chaudharyet al., 1984; Khush, 1977*). Hosttolerance to *S. incertulas* is polygenic in nature (*Khush, 1977, 1992*) and there are very few reports of the number of genes contributing to host tolerance or of molecular markers linked to resistance. Hence, to determine if the different populations exhibit variation in their ability to injure the host plants, it would be necessary to study in detail the various factors contributing to pest infestation. These factors include, the composition of insect gut enzymes such as proteolytic enzymes (e.g.,trypsin, chymotrypsin), amylases, xylanolytic, cellulolytic, and other related enzymes that help the larvae break down or digest the stem components (*Applebaum, 1985)* and the composition of the host genotype, i.e., different types of naturally occurring phytochemicals present in the host such as lectins, waxes, phenolics, amino acids, sugars, silicates, amylase inhibitors, proteinase inhibitors, etc., which are known to confer resistance to plants against herbivorous insects (*Broadway, 1996*).

CONCLUSIONS

The results obtained in the present study are summarized briefly below.

Ten random 10-mer oligonucleotide primers were used in RAPD analysis. All the ten random primers successfully amplified a total of 104 reproducible bands, of which 99 (95.1%) are polymorphic. The number of bands per primer ranged from six (OPH12) to thirteen (OPA13) with an average of 10.4 bands per primers. The size of the amplified product varied from 250bp to 1990bp. All the ten primers revealed polymorphism between individuals with an average of 10.4 bands per primer. Out of the 99 polymorphic bands, maximum numbers of polymorphic bands (i.e.12) were obtained with the primers, A04 & OPA13.The primer, OPM05 amplified the second highest number of polymorphic bands (i.e., 11). Five primers, OPA3, OPA13 ,OPM05,OPN4 and OPN13 amplified monomorphic bands. Thirty three Unique bands were identified which would be useful for developing diagnostic markers.

Genetic similarity (Jaccard's coefficient of similarity) among YSB populations varied from 0.24 to 0.651 with an average of 0.415, indicating that wide genetic variation exists between YSB populations. Ranjisura showed lowest genetic similarity with Daispatna YSB population while Jijinipur showed highest genetic similarity with Teismile YSB population Cluster analysis grouped all the 16 YSB populations into two different major groups corresponding to their geographical location. First major Sub-group consists of two populations (i.e. Ranjisura ,Srikantpur). The second major sub-group

consists 3

populations(Akhuapada, Vellipadia,Sisua) and the third major subgroup also consists of 3 populations(Aruha, Sukarpada , Nalanga). The second major group consists of two sub-groups. The first sub-group consist of six populations (i.e., Daispatna, Jaganathpur,Teismile, Jijinipur,Dolanagar, Kishannagar) while the second sub-group consist of two populations (i.e. Chandaka, Jamujhadi).

All the individual populations of YSB included in the study could be distinguished precisely.

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Table 1: List of Rice Yellow Stem Borer Populations Used in DNA Fingerprinting

Sl. No.	Place
1	Ranjisura
2	Shrikantpur
3	Akhuapada
4	Aruha
5	Daispatna
6	Vellipadia
7	Sisua
8	Sukarpada
9	Dolanagar
10	Jaganathpur
11	Theismile
12	Chandaka
13	Jijinipur
14	Kishannagar
15	Jamujhadi
16	Nalanga

Table 2: Details of Random Primers Used, Indicating Their Sequence (5'-3') And GC Content

Sl. No.	Name of Random (RAPD) Primer	Sequence(5' -3')	No of Bp	GC Content (%)
1	A03	AGTCAGCCAC	10	60
2	A04	AATCGGGCTG	10	60
3	OPA13	CAGCACCCAC	10	70
4	OPH12	ACGCGCATGT	10	60
5	OPM-05	GGGAACGTGT	10	60
6	OPC-05	GATGACCGCC	10	70
7	OPH-04	GGAAGTCGCC	10	70
8	OPN-04	GACCGACCCA	10	70
9	OPN-13	AGCGTCACTC	10	60
10	OPQ-12	AGTAGGGCAC	10	60

Table 3: Amplification Pattern of RAPD Primers Used in DNA Fingerprinting of Rice Yellow Stem Borer

Sl. No.	RAPD Primer	Band Size of Range(Bp)	Total No. of Bands	No. of Polymorphic Bands	No. of Monomorphic Bands	No of Unique Bands	R_P
1	A03	350-1500	8	7	1	3	7.875
2	A04	290-1990	12	12	0	6	5.125
3	OPA13	395-1550	13	12	1	2	11.750
4	OPH12	600-1250	6	6	0	1	3.250

5	OPM05	450-1300	12	11	1	8	4.000
6	OPC05	395-1200	15	15	0	3	10.875
7	OPH04	590-1990	11	11	0	4	8.250
8	OPN04	300-1020	9	8	1	0	6.875
9	OPN13	400-1400	9	8	1	3	6.625
10	OPQ12	250-1200	9	9	0	3	6.625
		TOTAL	**104**	**99**	**5**	**33**	**71.25**
		AVERAGE	**10.4**	**9.9**	**0.5**	**3.3**	**7.125**
			%	**95.1**	**4.8**	**31.7**	

Table 4: Clustering Pattern of Rice Yellow Stem Borer Populations

Cluster & Sub Cluster		No. of Population(S)	Name of Population(S)
I	**IA**	**2**	Ranjisura, Srikantpur
	IB	**3**	Akhuapada, Vellipadia, Sisua
	1C	**3**	Aruha,Sukarpada,Nalanga
II	**IIA**	**6**	Daispatna, Jaganathpur,Teismile, Jijinipur,Dolanagar, Kishannagar
	IIB	**2**	Chandaka,Jamujhadi

Table 5: Analysis of DNA Fingerprinting Using RAPD Primers

Attributes	Ten Primers
Average bands for each Population	33.25
Average similarity index(X_D)	0.415
Probability of identical match by chance$(X_D)^n$	$2x10^{-13}$

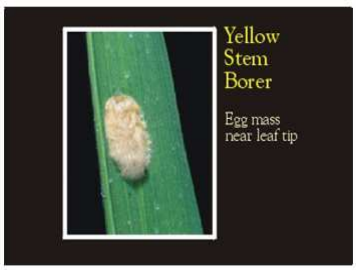
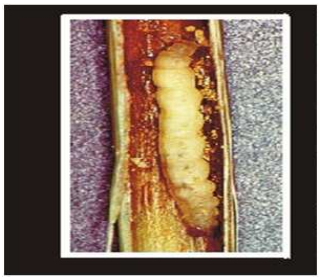


Figure 1



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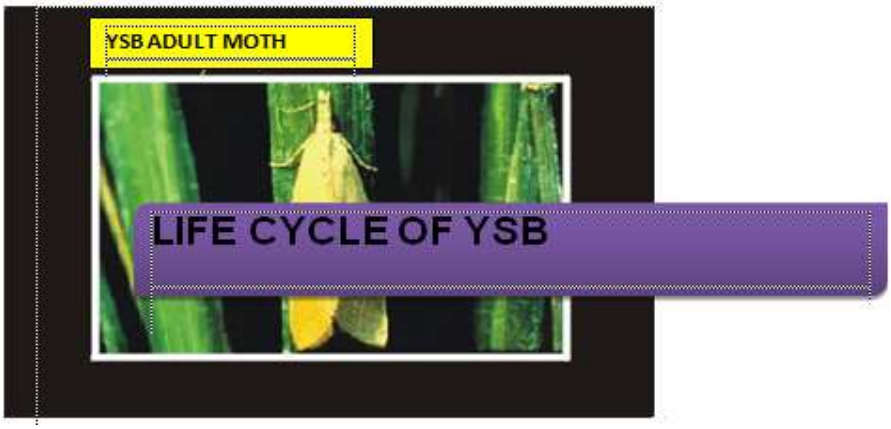

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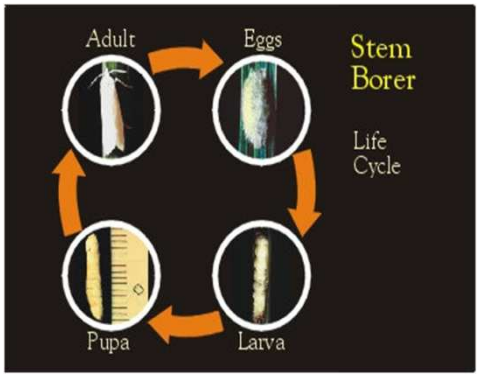


Figure 4

Figure 5: Dead Heart

Figure 6: White Ear Head

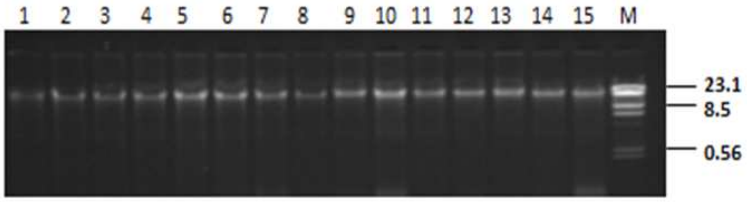
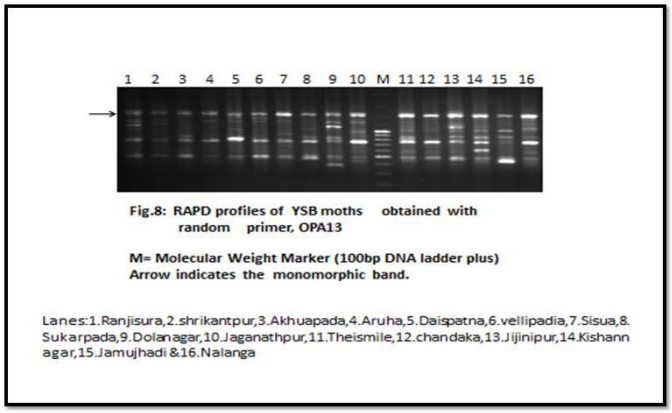


Figure 7:Genomic DNA of Moths of Yellow Stem Borer

M=Molecular Weight Marker(Lambda/Hindlll)

Numbers on the Margin Represent Molecular

Weight Markers in Kb.



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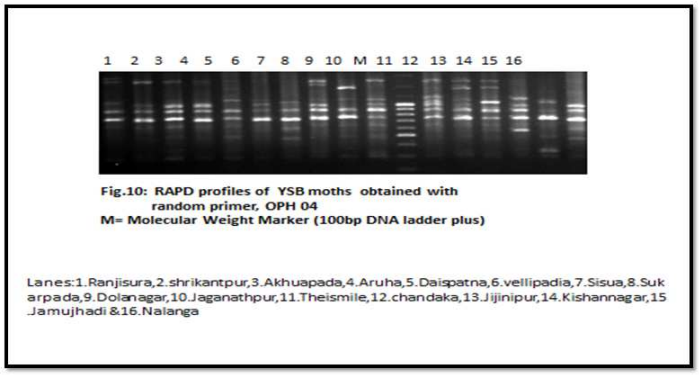

Figure 9

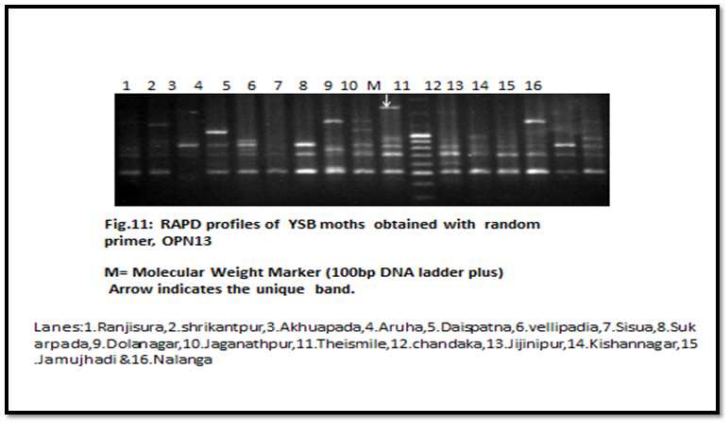

Figure 10

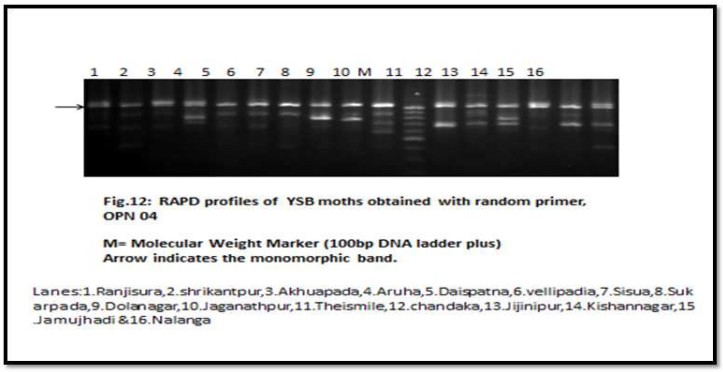

Fig.12: RAPD profiles of YSB moths obtained with random primer, OPN 04

M= Molecular Weight Marker (100bp DNA ladder plus)
Arrow indicates the monomorphic band.

Lanes:1.Ranjisura,2.shrikantpur,3.Akhuapada,4.Aruha,5.Daispatna,6.vellipadia,7.Sisua,8.Sukarpada,9.Dolanagar,10.Jaganathpur,11.Theismile,12.chandaka,13.Jijinipur,14.Kishannagar,15.Jamujhadi &16.Nalanga

Figure 11

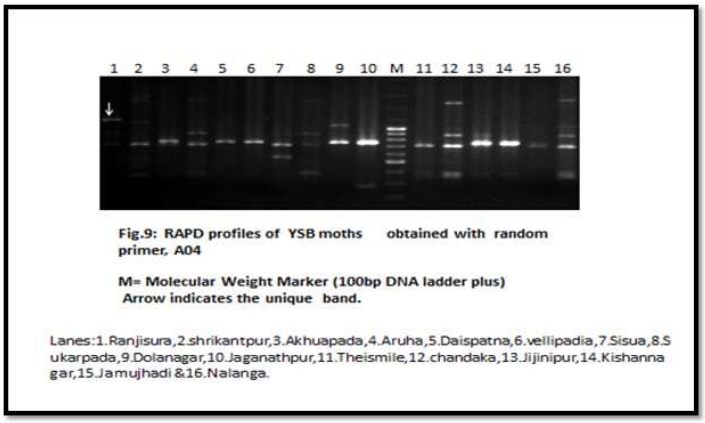

Fig.9: RAPD profiles of YSB moths obtained with random primer, A04

M= Molecular Weight Marker (100bp DNA ladder plus)
Arrow indicates the unique band.

Lanes:1.Ranjisura,2.shrikantpur,3.Akhuapada,4.Aruha,5.Daispatna,6.vellipadia,7.Sisua,8.Sukarpada,9.Dolanagar,10.Jaganathpur,11.Theismile,12.chandaka,13.Jijinipur,14.Kishannagar,15.Jamujhadi &16.Nalanga.

Figure 12

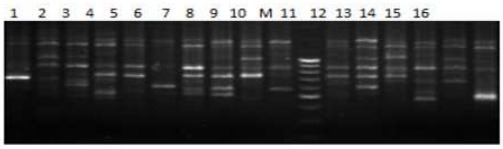

Fig.13:RAPD profiles of YSB moths obtained with random primer, OPC 05

M= Molecular Weight Marker (100bp DNA ladder plus)

Lanes:1.Ranjisura,2.shrikantpur,3.Akhuapada,4.Aruha,5.Daispatna,6.vellipadia,7.Sisua,8.Sukarpada,9.Dolanagar,10.Jaganathpur,11.Theismile,12.chandaka,13.Jijinipur,14.Kishannagar,15.Jamujhadi &16.Nalanga

Figure 13

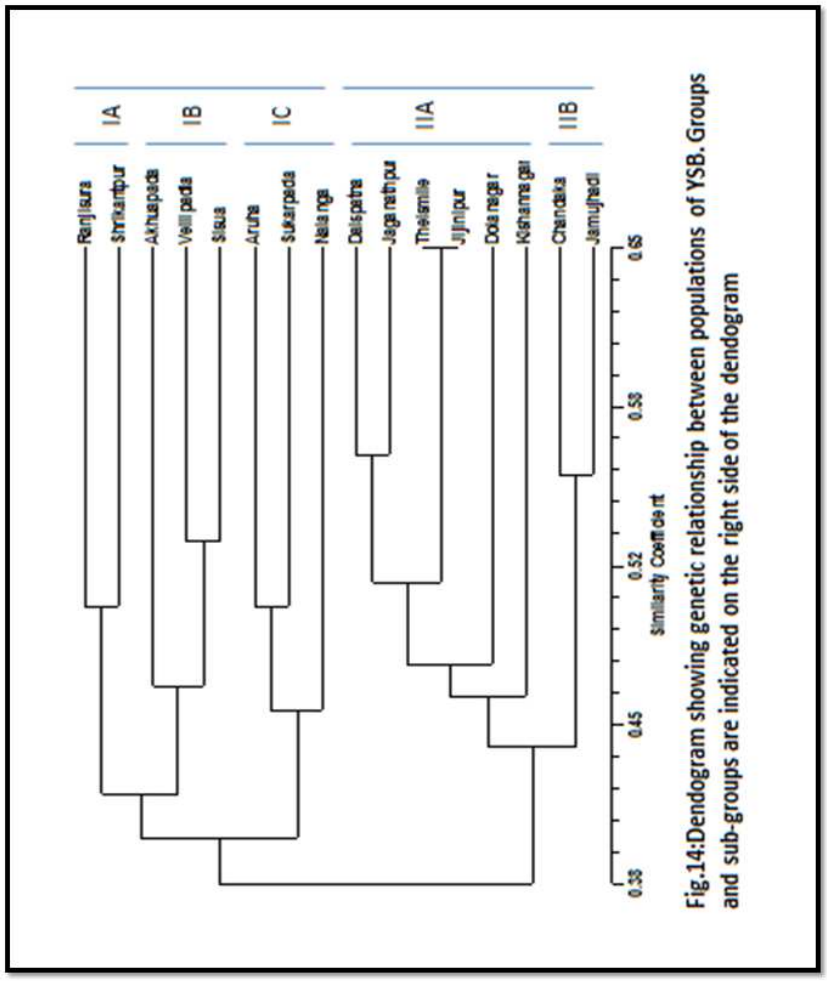

Fig.14:Dendogram showing genetic relationship between populations of YSB. Groups and sub-groups are indicated on the right side of the dendogram

Figure 14

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