Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 123-131

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.014

Genetic Diversity among Rice Yellow Stem Borer, *Scirpophaga incertulas* Revealed Using ISSR Markers

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Keywords

Genetic variability, ISSR, Yellow stem borer

Article Info

Accepted:
04 March 2018
Available Online:
10 April 2018

A B S T R A C T

Rice Stem borer is an important rice pest in India. In the present study, the genetic variability was analyzed among geographically isolated populations of rice yellow stem borer (YSB) from sixty locations of Odisha using ISSR marker technique. Fifty ISSR primers were screened and eleven ISSR primers were selected on the basis of clarity, usability, and reproducibility of their banding patterns. The 11 primers produced a total of 67 bright and discernible bands, all of which were polymorphic. Three unique bands were identified which will be useful for developing diagnostic markers. Genetic similarity among YSB populations varied from 0.02 to 0.1, indicating that wide genetic variation exists among YSB populations at molecular level. Most of the populations could be uniquely distinguished from each other and grouped into four major clusters at 20 % level of genetic similarity.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops which feed more than 3 billion people. It has been estimated that more than 200 million tons of rice are being lost annually due to insect pests (Khan *et al.,* 1991). Among different stem borers, yellow stem borer (YSB), *Scirpophaga incertulas* shares more than 70 percent of total stem borer population. YSB is distributed mainly in Southeast Asia, China, Afghanistan and India. Grain yield losses of 3 to 95% due to yellow stem borer (YSB) damage have been reported in India (Muralidharan and Pasalu, 2006). It attacks rice throughout its growth period from the seedling stage to maturity. At the vegetative stage, the larva can damage the tillers causing dead hearts, in which the young tiller and leaves of the tiller turn brown and die. During the reproductive stage, injury to tillers can destroy the panicles resulting in whiteheads. This specialist species, *S. incertulas* is the most destructive and dominant species of rice crop in Odisha, India. An estimate made at Central Rice Research Institute (CRRI), Cuttack suggests that everyone per cent increase in stem borers incidence at the vegetative phase registered a loss of 0.28 per cent in yield which at heading stage was shown 0.62 per cent. The potential losses of 40-50 per cent stems before harvest have been estimated due to yellow stem borer alone (Israel and Abraham, 1967). For effective pest management, understanding genetic diversity of insect pest is a

prerequisite. Development in molecular markers provides a great opportunity for quick and reliable estimation of genetic diversity.

Inter simple sequence repeats (ISSR) are one such marker system which has been extensively used for genetic analysis of plants and animals (Zeitkiewicz *et al.,* 1994; Reddy *et al.,* 1999b; Bornet *et al.,* 2002; Vijayan, 2006). Further, the technique of ISSR amplification is sensitive enough to differentiate closely related individuals (Zietkiewicz *et al.,* 1994) and assess the genetic diversity in germplasm (Wolfe *et al.,* 1998). ISSRs are presumably noncoding loci and are dispersed throughout the genome. In this study we describe the use of ISSR markers to evaluate the extent of genetic relationships and richness among 60 YSB populations of Odisha, India.

Materials and Methods

Insect materials

The female moths of yellow stemborers (YSB) were collected from sixty different locations in Odisha (Table 1) and were preserved in 95 per cent ethanol at 4^0C for genomic DNA isolation.

Insect DNA isolation

Female moths of yellow stem borers were used for isolation of genomic DNA (Anonymous, 1993). Single adult female was soaked in 50 μl of extraction buffer for 10 minutes and then homogenized in 1.5 ml of eppendorf tube with sterilized polypropylene pestle gently and thoroughly and washed with 350 μl of extraction buffer. Ten microliter of proteinase-K (10 mg/ml) was added, mixed well and incubated at 37ºC for 1hr followed by addition of 400μl of equilibrated phenol. The samples were centrifuged at 12000 rpm for 10 minutes. The upper layer was carefully transferred to another tube and an equal volume containing 200μl of phenol and 200μl of chloroform: isoamyl alcohol (24:1) was added, mixed well and the solution was centrifuged at 12000 rpm for 10minutes. Then the supernatant was transferred to a new tube and ten microliter of RNAase (10 mg/ml)was added and incubated for 1 hr at 37ºC followed by addition of 400μl chloroform: isoamyl alcohol(24:1) mixed by brief vortexing and centrifuged at 12000 rpm at 40ºC for 10 minutes. The upper aqueous layer was carefully transferred to another Eppendorf tube and to it 10μl of NaCl (5M) and twice the volume of ice cold absolute alcohol was added. An overnight incubation was carried out at –20 ºC followed by centrifugation at 12,000 rpm for 10 minutes. The DNA pellet was washed with 100μl of 70 per cent ethanol, dried at room temperature and dissolved in 50 μl of TE buffer. The quality and quantity of DNA were estimated electrophoretically using λ DNA as standard, and stored at –20 ºC for further analysis.

PCR amplification and agarose gel electrophoresis

A total of 50 ISSR primers developed by the University of British Columbia Biotechnology Laboratory (www.biotech.ubc.ca) were initially screened, and the 11 primers that produced bright, clear, and reproducible fragments were utilized for further study (Table 2). Each 20µl of PCR reaction mixture Consisted of 25ng of genomic DNA, 1X PCR buffer {75 mMTris-HCl (pH 9.0), 50mMKCl, 20 mM $(NH_4)_2SO_4$}, 200µM dNTP mix (MBI Fermantas, Lithuania, USA), 5 pmol of primers, 2 mM of $MgCl_2$ and 1U of *Taq*DNA polymerase (Biotools, Spain).

Amplification was carried out in a thermal cycler (Perkin Elmer, Cetus, USA),with initial denaturation temperature of 93^0C for 2 min followed by 44 cycles of 93^0C for 1 min, 36^0C

for 1 min and 72^{0}C for 2 min and final extension at 72^{0}C for 5 minute. The amplified products were size fractionated on a 1.5 per cent agarose gel containing ethidium bromide. The gel was visualized under UV using Gel Imaging System (Fluor Chem TM 5500, Alpha Innotech, USA). PCR amplification with each primer was carried in two replicators.

Data scoring and statistical analysis

Since ISSR markers were dominantly inherited, each band was assumed to represent the phenotype at a single biallelic locus (Williams *et al.,* 1990). Only bright and discernible fragments across all the population samples were included in the statistical analysis.

ISSR bands were scored as present (1) or absent (0) in agarose gels and entered into a binary matrix representing the ISSR profile of each population. Initially, the potential of ISSR markers for estimating genetic variability of YSB were examined by measuring the marker informativeness through the counting of bands. The bands were counted as: total number of amplified bands (TB); number of polymorphic bands (PB), i.e. bands that were not amplified in all population; number of unique bands (UB), i.e. bands amplified in single population.

To analyze the suitability of ISSR markers to evaluate genetic profiles of YSB, the performance of the markers was measured using four parameters: polymorphic information content (PIC), heterozygosity (H), marker index (MI) and resolving power (RP).

Polymorphic information content (PIC) and Heterozygosity (H)

The PIC value as well as the H value for each locus was calculated using the polymorphism information calculator i.e. PICcalc: an online program to calculate polymorphic information content for molecular genetic studies developed by Nagy *et al.,* 2012 (http://w3. georgikon.hu/pic/english/default.aspx).

Marker Index (MI)

The marker index (MI) was calculated as described by Smith *et al.,* (1997) and Lu¨ bberstedt *et al.,* 2000: Marker indices were calculated as the product of PIC and the number of polymorphic bands per assay unit (POL)

MI= POLxPIC

Resolving Power (RP)

The resolving power (RP) of each primer was calculated according to Prevost and Wilkinson 1999: (Rp) = Σ Ib, where Ib represents the informative fragments. The Ib can be represented on a scale of 0-1 by the following formula:

1- {2x(0.5- Pi)}where p is the proportion of populations containing the band.

Similarity coefficient

Data were analysed to obtain Jaccard (1908) coefficients among the populations by using NTSYS-pc version 2.11W (Rohlf 1997). The SIMQUAL program was used to calculate the Jaccard's coefficients.

A common estimator of genetic identity and was calculated as follows: Jaccard's coefficient = *N*AB/(*N*AB + *N*A + *N*B), where *N*AB is the number of bands shared by samples, *N*A represents amplified fragments in sample A, and *N*B represents fragments in sample B. Similarity matrices based on these indices were calculated.

The similarity co-efficients were then used to construct a dendrogram using the UPGMA (Unweighted Pair-Group Method with Arithmetical average) cluster analysis (Sneath and Sokal, 1973). All procedures were computed with the computer package NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, Biostatistics, New York, USA, software version 2.02j package) (Rohlf, 1993).

Results and Discussion

DNA isolation and quantification

The genomic DNA was successfully isolated from yellow stem borer moths following modified genomic DNA isolation method in our studies. Purification and quantification of genomic DNA isolated from each individual moth was done and fractionated on agarose gel (0.8%). The DNA bands of all individuals were intact and did not show any smearing. This reflects good quality DNA preferable for ISSR analysis. The concentration of genomic DNA ranges from 25 ng/µl to 50 ng/µl.

ISSR band profile

Fifty ISSR primers were screened and Eleven ISSR primers were selected on the basis of clarity, usability, and reproducibility of their banding patterns; the data are shown in (Table 2). The 11 primers produced a total of 67 bright and discernible bands, all of which were polymorphic. The number of bands produced by individual primers was in the range of 4-8 (Table 2). The size of the polymorphic bands ranged from 300bp to1600bp. The representative banding patterns are shown in Figure 1 and 2. The primers differed greatly in their potential usability as indicated by the number of scorable amplified bands, e.g. the primer UBC808 and UBC826 produced as many as 8 bands each, while primersUBC812 and UBC873 amplified only 4 bands. Highest number of common bands i.e. 7 bands each was amplified by four primers UBC (AC) 8TC, UBC 811, UBC 856 and UBC 880. Whereas, UBC 864 and UBC 825 amplified six bands each while primer UBC (AC) 8AT amplified five bands. Unique bands of 300bp (Godiapokhari YSB population), 900bp (Sanagaon YSB population) and 1400bp (Sambalpur YSB population) were amplified by the ISSR primers UBC812, UBC811 and UBC812.

ISSR marker performance

The polymorphic information content varied from 0.264(UBC825) to 0.368 (UBC873) The highest heterozygosity value (0.487) was obtained with ISSR primer UBC873 and the lowest heterozygosity value (0.255) was observed in ISSR primer UBC856. Whereas the marker index ranged from 1.264 (UBC812) to 2.359 (UBC(AC)8TC). The resolving power (RP) is a parameter that indicates the discriminatory potential of the primers chosen. The highest RP value was observed with the ISSR primer UBC864 (3.778) and the lowest with the ISSR primer UBC856 (1.750) (Table 3).

Dendrogram analysis

UPGMA clustering method was used to construct the genetic distance matrix. The values of genetic distance ranged from 0.00 to 0.1, suggesting a great genetic base.

At 20% level of genetic similarity the sixty YSB populations were grouped into four main clusters. Cluster I consists of 4 YSB populations (Chatia, Diaspatna, Gopinathpur and Sesagaon). Cluster II comprises a maximum of 52 YSB populations. Cluster III has a single YSB population from Balarampur and Cluster IV comprises of three YSB populations i.e. Salepur, Naugada and Soro respectively.

Table.1 Names of YSB populations along with their latitude and longitude

SL NO	PLACE	LATITUDE	LONGITUDE	SL NO	PLACE	LATITUDE	LONGITUDE
1.	Chatia	20.610352	86.060715	31	Khuntuni	20.565808	85.724355
2.	Gandadhara	19.925586	84.741669	32	Balarampur	20.674389	85.491690
3.	Sonepur	20.848014	83.895004	33	Chandola	20.217775	86.204840
4.	Bhola	20.352135	85.723525	34	Mendhasala	20.267884	85.698696
5.	Pipili	20.111801	85.835287	35	Dhirapur	20.035385	86.010180
6.	Dolanagar	20.525678	86.261571	36	Nandighora	20.481750	86.264050
7.	Jijinipur	20.163763	86.055113	37	Sambalpur	21.466222	83.975164
8.	Maniabandha	20.440384	85.433271	38	Begonia	20.121211	85.272819
9.	Thengapada	21.021260	84.005319	39	Barahdebta	21.553116	86.440930
10.	Aruha	21.315110	86.756100	40	Kundeisara	21.122566	84.482847
11.	Simulia	20.779435	86.750170	41	Manmunda	20.493593	83.551260
12.	Odagoan	20.015931	84.986955	42	Dardera	20.123012	85.122087
13.	Diaspatna	20.045606	85.501116	43	Parabil	21.147450	85.157605
14.	Srikantpur	20.002488	85.541687	44	Narayanpur	18.958926	84.246597
15.	Hindol	20.610201	85.201073	45	Jagannathpur	20.241672	86.348449
16.	Danagohiri	20.145538	85.544973	46	Bhootmundei	20.192750	86.344038
17.	Dadha	20.404350	85.833990	47	Chandaka	20.368331	85.764742
18.	Kuruda	18.935649	84.220799	48	G.Rambha	19.516285	85.089949
19.	Teismile	20.202066	86.042890	49	Redhakhol	21.068382	84.335271
20.	Chianpur	21.448922	87.026396	50	Salepur	20.481199	86.117729
21.	Nischintkoili	20.479101	86.184712	51	Sanagaon	20.508985	84.960090
22.	Balipatna	20.198231	85.958554	52	Nuagada Ghati	20.251188	85.202436
23.	Shrikhandapur	19.500714	85.465942	53	Soro	21.163581	86.431763
24.	Akhuapada	20.926156	86.288854	54	Sesagaon	20.291834	85.324031
25.	Ranjisura	21.222878	86.540188	55	Lalitgiri	20.591630	86.264108
26.	Vellipadia	21.430947	87.043491	56	CRRI	20.454577	85.931646
27.	Gopinathpur	20.383672	85.372397	57	Kendrapara	20.500000	86.416700
28.	Godiapokhari	20.166605	85.856464	58	Mangarajpur	20.767743	86.222202
29.	Indupur	20.613815	86.406746	59	Mallipura	20.290110	86.520845
30.	Mahanga	20.559876	86.193085	60	Nalanga	20.038985	85.884440

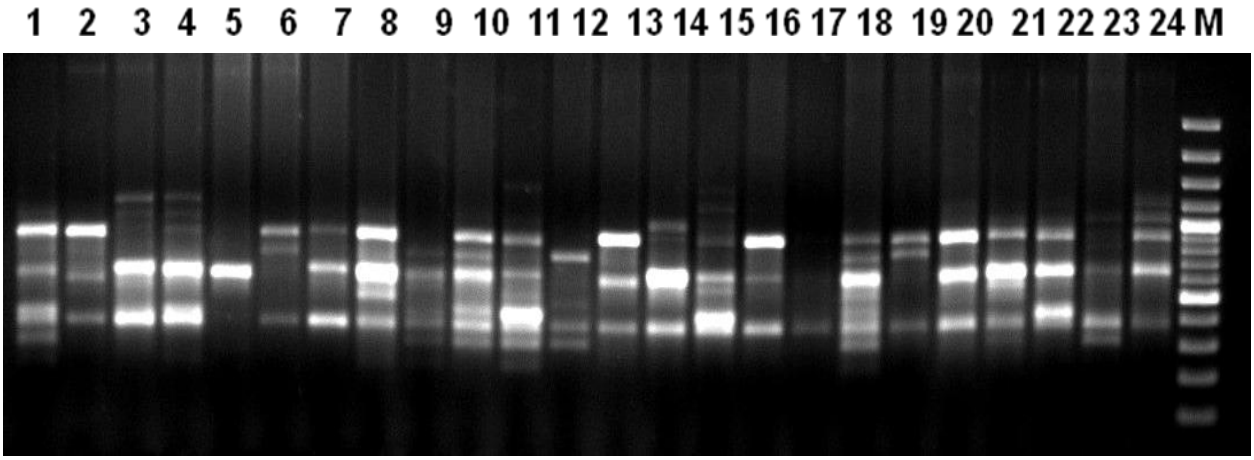
Table.2 Amplification pattern of ISSR primers along with their sequences

Sl. No	Primer Name	Primer sequence(5'-3')	Tm (°C)	Band size range (bp)	No. of scored bands	No of unique bands	Polymorphic Bands (%)
1	UBC (AC)8TC	ACACACACACACACACTC	53.7	400-1000	7	0	7(100%)
2	UBC808(AG)8C	AGAGAGAGAGAGAGAGC	52.8	300-900	8	0	8(100%)
3	UBC811(GA)8C	GAGAGAGAGAGAGAGAC	52.8	300-900	7	1	7(100%)
4	UBC812(GA)8A	GAGAGAGAGAGAGAGAA	50.4	300-700	4	1	4(100%)
5	UBC825(AC)8T	ACACACACACACACACT	50.4	500-1000	6	0	6(100%)
6	UBC826(AC)8C	ACACACACACACACACC	52.8	400-1600	8	0	8(100%)
7	UBC856(AC)8CTA	ACACACACACACACACCTA	54.5	600-1450	7	0	7(100%)
8	UBC 864	ATGATGATGATGATGATG	46.9	500-1000	6	0	6(100%)
9	UBC 873	GACAGACAGACAGACA	49.2	400-800	4	0	4(100%)
10	UBC 880	GGAGAGGAGAGGAGA	50.6	300-1400	7	1	7(100%)
11	UBC (AC)8AT	ACACACACACACACACAT	51.4	400-800	5	0	5(100%)

Table.3 ISSR marker performance

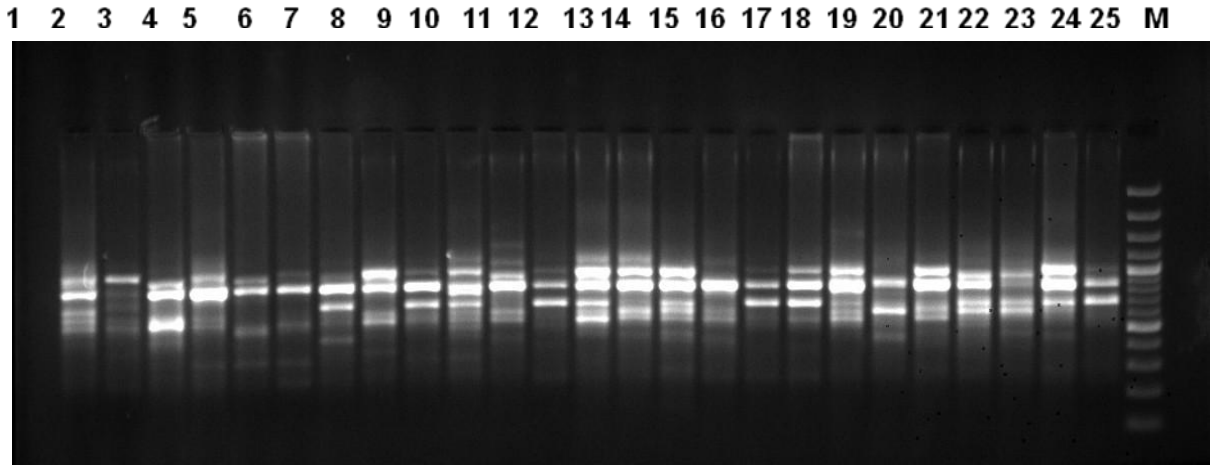
Sl. No.	Primer Name	Primer Resolving power (RP)	Heterozygosity (H)	Polymorphic information content (PIC)	Marker index (MI)
1	UBC (AC)8TC	3.639	0.429	0.337	2.359
2	UBC 808(AG)8C	3.250	0.386	0.311	2.488
3	UBC 811(GA)8C	2.806	0.365	0.298	2.086
4	UBC 812(GA)8A	1.806	0.394	0.316	1.264
5	UBC 825(AC)8T	1.944	0.313	0.264	1.584
6	UBC 826(AC)8C	3.139	0.355	0.292	2.336
7	UBC856(AC)8CTA	1.750	0.255	0.222	1.554
8	UBC 864	3.778	0.470	0.359	2.154
9	UBC 873	2.806	0.487	0.368	1.472
10	UBC 880	2.556	0.342	0.283	1.981
11	UBC (AC)8AT	3.417	0.483	0.366	1.83

Fig.1 Amplification pattern with ISSR primer UBC 808



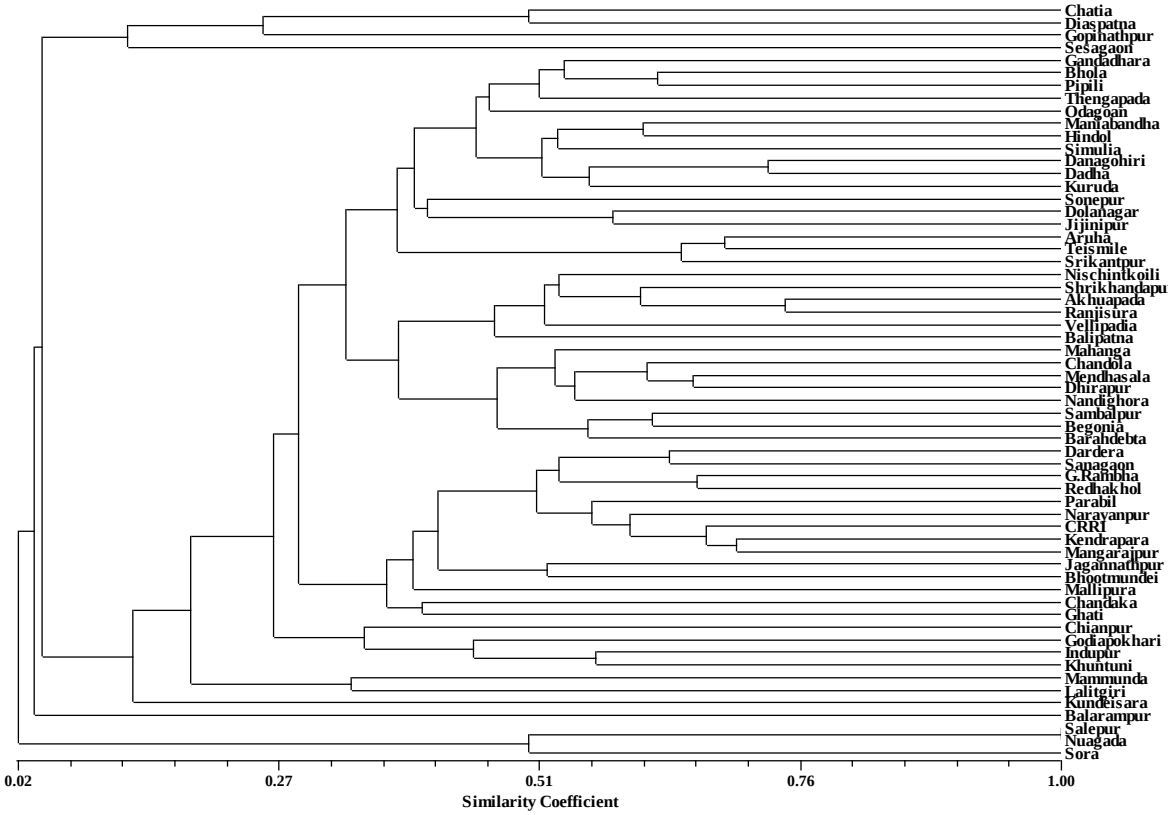
Numbers above the lanes indicate the YSB populations
M= 100Base pair DNA Ladder

Fig.2 Amplification pattern with ISSR primer UBC 864



Numbers above the lanes indicate the YSB populations

Fig.3 Dendrogram showing genetic relationship among YSB populations based on loci amplified by ISSR primer



The second major cluster II comprising of 52YSB populations is further sub-divided into four Sub clusters i.e., A, B, C and D. Sub cluster A consists of 31YSB populations (Gandadhara, Sonepur, Bhola, Pipili, Dolanagar, Jijinipur, Maniabandha, Thengapada, Aruha, Simulia, Odagoan, Srikantpur, Hindol, Danagohiri, Dadha, Kuruda, Teismile, Nischintkoili, Shrikhandapur, Akhuapada, Ranjisura, Vellipadia, Balipatna, Mahanga, Chandola, Mendhasala, Dhirapur, Nandighora, Sambalpur, Begonia and Barahdebta). The subcluster B comprises of 14YSB populations (Dardera, Sanagaon, G.Rambha, Redhakhol, Parabil, Narayanpur, NRRI, Kendrapara, Mangarajpur, Bhootmundei, Jagannathpur, Mallipura, Chandaka and Ghati) and subcluster C and D consists of four (Chianpur, Godiapokhari, Indupur, khuntuni) and three (Manmunda, Lalitgiri, Kundeisara) YSB populations respectively (Fig. 3).

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. ISSR technique was found efficient enough to reveal usable level of DNA polymorphism among adult females.

As ISSR-PCR DNA fingerprinting method targets random SSR or microsatellites, it has been used extensively to uncovering species level divergences and also a good tool for distinguishing geographic populations (Zietkiewicz *et al.*, 1994). In present study, among 50 ISSR primers screened only eleven ISSR primers were found polymorphic. All primers successfully amplified a total of 67

reproducible polymorphic bands. The size of bands varied from 300bp to 1600bp. Mohammad Javad Ardeh (2013) studied the utility of ISSR-primers to make difference among populations of parasitoid, *Eretmocerus mundus* mercet (hymenoptera: aphelinidae). From 60 ISSR primers, checked, 64 bands were obtained from 16 primers. The bands size ranged from above 200 to 1000 bp for different primers. The rate of divergence among the bands was strong enough to make a clear cut difference among the studied specimens. Three unique loci were identified which could be developed into diagnostic markers to identify particular population of YSB. Genetic similarity among YSB populations ranged from 0.00 to 0.1, revealing wide genetic variation among YSB populations. Cluster analysis based on UPGMA, dendrogram grouped all the 60 populations of YSB into four different major groups corresponding to their geographical location. Velu *et al.,* (2008) used ISSR markers to determine genetic relationships among mutant silkworm strains of *Bombyx mori*. The dendrogram produced using the unweighted pair group method with arithmetic means (UPGMA) and cluster analysis made using Nei's genetic distance resulted in the formation of one major group containing 6 groups separated 20 mutant silkworm strains. Similar to our study, Kumar *et al.,* (2001) used ISSR markers to assess the genetic diversity of 28 YSB populations collected from different hotspots of India. They used 9 ISSR primers which gave rise to 79 amplification products of which 67 were polymorphic. A dendrogram constructed from this data indicates that there is no geographical bias to the clustering and that gene flow between populations appears to be relatively unrestricted. Liu *et al.,* (2010) employed inter-simple sequence repeat (ISSR) markers to investigate the genetic diversity and differentiation of 47 populations of white backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae) sampled from 14 prefectures of the Greater Mekong Subregion. A total of 14 selected primers yielded 121 bright and discernible bands, with an average of 8.6 bands per primer. Neighbor-Joining cluster analysis of the 47 populations showed two major clusters, one consisting of mostly southwestern Yunnan Province and Myanmar populations; and the other one consisting of southeastern and central of Yunnan Province plus Vietnam and Laos populations. NIE *et al.,* (2012) used eight different inter simple sequence repeat (ISSR) markers as tools to investigate genetic variability and population differentiation in the Chinese alligator, *Alligator sinensis.* Eleven polymorphic bands (17.2%) out of a total of 64 were generated from 110 individuals in three populations. Analysis of molecular variation showed that most of the genetic variation (98.0%) occurred within the populations.

In conclusion, the ISSR markers gave complete, very reliable, reproducible and highly polymorphic fingerprints among populations of yellow stem borers. The present study reveals that PCR based fingerprinting techniques (ISSR) are informative for estimating the extent of genetic diversity as well as determining the pattern of genetic relationships among yellow stem borer populations from different regions of Odisha, India. In future, more number of ISSR markers should be used to study genetic relationships among more species and generas so that the most abundant SSR motifs can be identified and developed into microsatellite markers

Acknowledgement

We gratefully acknowledge the help and financial support provided by the Director, Central Rice Research Institute, Cuttack, India.

References

Anonymous 1993. Isolation of high molecular weight DNA from a single insect. Workshop on molecular biology of malaria vectors, Madras, India.

Bandong, J.P., and J.A. Litsenger. 2005. Rice crop stage susceptibility to the rice yellow stemborer *Scirpophagain certulas* (Walker). Int. J. Pest Manage. 51(1): 37–43.

Bornet, B., F. Goraguer, G. Joly & M. Branchard, 2002. Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). Genome 45(3): 481–484.

Israel, P. and Padmanabhan, S.Y. 1976. Biology and Control of Stem Borers of Rice in India Final Technical Report. CRRI, Cuttack, India.

Khan, Z.R., Litsinger, J.A., Barrion, A.T., Villanueva, F.F.D., Fernandez, N.J., Taylo, L.D., 1991. World Bibliography of Rice Stem Borers, vol. 415. Center of Insect Physiology and Ecology, International Rice Research Institute, Los Banos, Philippines, pp. 1794–1990.

Li, M.W., C.X. Hou, X.X. Miao, A.Y. Xu and Y.P. Huang, 2007. Analyzing genetic relationship in *Bombyx mori* using inter simple sequence repeat amplification. J. Economic Entomol., 100: 202-208.

Lv, J., L.T. Wilson, and M.T. Longnecker. 2008. Tolerance and compensatory response of rice to sugarcane borer injury". Environ. Entomol. 37: 796-807.

Muralidharan, K., and I.C. Pasalu. 2006. Assessments of crop losses in rice ecosystem due to stem borer damage. Crop Prot. 25: 409-417.

Nagy S, Poczai. P, Cernak I, Gorji AM, Hegedus G, Taller J. 2012. PIC calc: An Online Program to Calculate Polymorphic Information Content for Molecular Genetic Studies Biochem Genet 50:670–672.

Prevost A, Wilkinson MJ. 1999. A new system of comparing PCR Primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and applied Genetics*, 98(1): 107-112.

Reddy KD, Abraham EG, Nagaraju J. 1999b. Microsatellites of the silkworm, *Bombyx mori*: abundance, polymorphism and strain characterization. *Genome* 42: 1057-1065.

Rohlf FJ (1993) NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System), version 2.11. Exeter Software, Setauket.

Sneath, P.H.A., Sokal, R.R. 1973. Numerical taxonomy. W.H. Freeman and Company, San Francisco. 147–157.

Vijayan K, Anuradha HJ, Nair CV, Pradeep AR, Awasthi AK, *et al.*, (2006) Genetic diversity and differentiation among populations of the Indian eri silkworm, Samia cynthia ricini, revealed by ISSR markers. J Insect Sci 6: 1–11.

Wolfe, A.D., Q-Y. Xiang & S.R. Kephart, 1998. Diploid hybrid speciation in *Penstemon* (Scrophulariaceae). Proc Natl Acad Sci USA 95: 5112–5115.

Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR) – anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

How to cite this article:

Rudraksh Shovan Panda, Lambodar Behera and Sarat Chandra Sahu. 2018. Genetic Diversity among Rice Yellow Stem Borer, *Scirpophaga incertulas* Revealed Using ISSR Markers. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 123-131. doi: https://doi.org/10.20546/ijcmas.2018.704.014