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Development of SSRs and its application in genetic diversity study of Indian population of *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae)

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Abstract
Pink stem borer (PSB) has become the major pest of cereals in India and other parts of the Asia. The wide geographic distribution and broad host range of PSB is likely to result in high genetic variability within the species. To understand this in better way we have identified six polymorphic SSRs out of 64 SSRs developed from 497 genomic DNA sequences available in NCBI database. These six SSRs were able to show the genetic difference among the *Sesamia inferens* population with respect to their host preference. The result of UPGMA dendrogram and Principal component analysis by using jaccards similarity coefficient data, different populations of *S. inferens* were clustered according to host. These results suggest a low level of inter-population gene flow in pairwise populations from sorghum, sugarcane, maize and rice fields in India. Such levels of differentiation among populations may indicate only a moderate dispersal capacity of *S. inferens*, even when no remarkable geographic barriers exist. For an effective management of this pest in the future, there is urgent need for a better understanding of the gene flow of sympatric *S. inferens* populations associated with different host plants within its distribution range.

Keywords: *Sesamia inferens*, microsatellites, pink stem borer

Introduction
The pink stem borer, *Sesamia inferens* is an important polyphagous pest of cereals [51, 8, 48, 21]. It is widely distributed in India, Ceylon, Pakistan, Myanmar, Thailand, Vietnam, Indonesia, Philippines, Taiwan, China and Japan [4, 24]. In India, it is reported as a major pest of maize, sugarcane, sorghum, wheat and rice in Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, Madhya Pradesh, Maharashtra, Orissa, West Bengal, Bihar, Assam, Uttar Pradesh, Delhi, Haryana, Uttarakhand, Chhattisgarh and Punjab which causes the huge economic losses [33].
The detection of genetic differentiation among and within insect populations will be major step for improvement of any pest management practice such as biotype ecology and evolution, biological control, resistance management and insect-plant interactions [20, 22, 10, 12, 1]. Though various researchers concentrated only on biological, ecological and management aspects of *S. inferens* [3, 31, 2, 27, 14], research on its genetic structure, phylogeography [46] and genetic diversity with DNA markers [9, 39] is very rare at global level and in India there is no much work on genetic diversity study *S. inferens* by using advanced DNA markers.
In genetic diversity and populations study among the DNA markers, microsatellites (SSR) used widely [7, 30, 39]. SSRs repeat motifs of 1–6 bp in length are hypervariable and widely spread in eukaryotic genomes [43, 32]. SSRs are reproducible, multiallelic, co-dominant, abundant, and cover the whole genome and have become markers of choice for diversity analysis and genome analysis [26, 50, 47, 15, 44].
The first set of SSRs by Tang [39] developed in *Sesamia inferens* through enriched cDNA libraries construction methods explained by Zane [49] and Xu [45]. The development of SSR markers requires a great deal of time, effort, and investment in the construction and screening of genomic libraries and sequencing of clones containing SSRs, primer development, and their validation [19]. However, a large number of expressed sequence tags (ESTs) and genomic and also mitochondrial DNA sequences [8] available in public databases provide an alternative method of microsatellite development. SSRs can be computationally mined from EST and genomic DNA databases [40].

With the advances in bioinformatics, it is possible to mine and analyze large-scale EST and genomic datasets efficiently and exhaustively in various organisms [11, 35]. The objectives of present study was the development of EST-SSRs from the available genomic DNA sequence database from the NCBI and validation of SSRs for the study of genetic diversity in *S. inferens* population collected from the various hosts and regions of India.

Material and Methods

Insect collection and DNA isolation

Collection of random samples of *S. inferens* was done in different period according to availability of infestation on four different hosts including maize, rice, sugarcane and sorghum and also from different location of India (Table 1) during the 2015-16 periods. About 20 larvae collected for each location with respect to host. The larval samples were collected at the rate of one larvae per individual plant from 20 different plant selected random within field. All collected larvae from each populations belonging to a particular location and host were separately preserved in 95% ethanol at -80ºC and used for DNA extraction. All larvae were thoroughly washed with formaldehyde and alcohol and the gut contents were removed to avoid contamination of any other DNA. The genomic DNA isolation involved use of an animal kit (QIAGEN cat# 69504) by standard procedures.

Table 1: Populations of *S. inferens* from different geographical locations in India

S. No.	Collection site	State	Host	Time	Co-ordinates
1	Hyderabad	Andhra Pradesh	Rice	September 2014	17º 36'N, 78º 47'E
2	Killikulam	Tamil Nadu	Rice	March 2015	8 º 35'N, 77 º 66'E
3	Pattambi	Kerala	Rice	February 2015	10º 82'N, 76º 20'E
4	Karnal	Haryana	Sugarcane	January 2015	29º 69'N, 76º 98'E
5	Kannur	Kerala	Sugarcane	April 2015	11º 86'N, 75º 35'E
6	Gulbarga	Karnataka	Sorghum	December 2014	17º 33'N, 76º 83'E
7	Ludhiana	Punjab	Maize	January 2015	30° 90'N,75° 80'E
8	Bagalkot	Karnataka	Maize	October 2014	16º 18'N, 75º 69'E
9	Hyderabad	Andhra Pradesh	Maize	November 2014	17º 36'N, 78º 47'E
10	Bangalore	Karnataka	Maize	August 2014	12º 96'N, 77º 56'E
11	Shimoga	Karnataka	Maize	October 2014	13º 93'N, 75º 56'E
12	Koppala	Karnataka	Maize	October 2014	15º 35'N, 76º 15'E
13	Almora	Uttarakhand	Maize	December 2014	29º 59'N, 79º 65'E
14	Gulbarga	Karnataka	Maize	November 2014	17º 33'N, 76º 83'E
15	Delhi	Delhi	Maize	January 2015	28º 61'N, 77º 23'E
16	Solapur	Maharashtra	Maize	October 2014	17º 68'N, 75º 92'E
17	Raichur	Karnataka	Maize	February 2015	16º 20'N, 77º 37'E
18	Kolar	Karnataka	Maize	February 2015	13º 13'N, 78º 13'E
19	Coimbatore	Tamil Nadu	Maize	March 2015	11º 01'N, 76º 97'E
20	Ambikapur	Chhattisgarh	Maize	January 2015	23º 12'N, 83º 20'E

Microsatellite development

A set of genomic DNA sequence inserts of the genus *Sesamia* (not published) available in the NCBI database (http://www.ncbi.nlm.nih.gov/nucleotide) as on 10th October, 2013 were retrieved. The repetitive elements and other interspersed repeats were masked by the software EGassembler webserver [25]. The software automatically screens and cleans for various contaminants in the retrieved sequences. The server clustered and assembled the sequences into contigs using CAP3 [13] with the criterion of 80% overlap identity between one end of a default read to another end. In considering the efficiency of the primer design and polymerase chain reaction (PCR), DNA sequences of less than 100 bp were not included in the analysis. The identification of the microsatellites primer pairs was designed using WebSat software [23], with the following repeat threshold settings: 15 repeats for mono-, 8 for di-, 4 for tri-, 3 repeats for tetra-, 2 repeats for penta and hexa-nucleotide SSRs.

PCR conditions

PCR reactions involved use of 200 ng genomic DNA, 0.20 µM mixed forward and reverse primers, 1X Buffer (10 mM de Tris-HCl, pH 8.2, 50 mMKCl, Triton 0.1%, BSA 1mg/ml), 1.5 mM MgCl2, 0.2 mMdNTPs, 1 U Taq polymerase and lastly sterile distilled water in a 10-µL reaction volume. Amplification involved a GeneAmp PCR 9700 System thermal cycler (Applied Biosystems Inc.) programmed to 94 °C for 5 min, followed by 30 cycles of 94 °C for 30s, each primer standard annealing temperature (Table 2) for 40s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min and stored the samples at 4 °C still electrophoresis.

Electrophoresis of PCR products

PCR products were analyzed by electrophoresis in 3.5% SFR agarose gel electrophoresis run at 100 V/cm for 1.5 h in 0.5x TBE buffer. The banding pattern was visualized using the ethidium bromide (1x) staining method. The stained, products were visualised and photographed with gel documentation system (UVPRO, UK). The samples were analyzed twice for all primers to test the reproducibility of bands.

Scoring of bands and statistical analysis

Based on log molecular weight of the co-migrating 100 bp DNA marker (Fermentas Inc., www.fermentas.com) and their migration distances scatter plots were established and trend lines with best fit was fitted. Based on the mathematical expression of the trend lines the molecular weight of the fragment corresponding to their migration distances was calculated. The individual DNA bands were scored as present or absent (1/0) in the amplification profile of each sample. Only clear bands with good resolution were scored. Binary data were used to obtain cluster analysis of the similarity matrices following Unweighted Pair Group with Arithmetic Averages, UPGMA [37] using SAHN and Principal component analysis (PCA) using Eigen vectors and Eigen values function

of NTSYS-pc version 2.1 program [34]. The genetic similarity was determined using the jaccards similarity coefficient. The percentage of polymorphism was calculated as the proportion of the polymorphic markers to the total number of markers. The polymorphism information content value was also determined [36]. According to Nei's [28] analysis of molecular variation (AMOVA) was calculated in GenAlEx (Genetic Analysis in Excel) v. 6.5 package [29].

Results

Identification and designing of SSRs

A total of 497 genomic DNA sequences (not published) belonging to *Sesamia* genus with different species (*S. calamistis, S. nonagrioides, S. inferens, S. poephaga, S. epunctifera, S. penniseti, S. veronica, S. fermata, S. pennipuncta and Sesamia sp*.) were obtained from NCBI and used for further computations and analysis, since no ESTs database was available as on 10th October, 2013. After screening for the repetitive elements and other interspersed repeats, only 109 (21.93%) sequences (not published) were used as contigs for the finding and designing primers for flanking repeats. Total 64 SSRs (58.71%) were designed by WebSat software (not published) and out of 64 randomly 47 (not published) were synthesized through Bioserve (Bioserve Biotechnologies, Hyderabad), and used to asses genetic diversity of 20 population *S. inferens* collected from different region and hosts (Table 1).

Table 2: Polymorphic SSR markers utilized in the study, no. of markers generated for populations collected from different host and PIC values

Locus	Accession No.	Motifs	Primers	Ta (°C)	Allele size range (bp)	N_A	Polymorphism (%)	PIC
SI-SSR3	JF274118.1	(CATTTA)2	F-AGTAACCCCAGTACATATTCAACCT	53	169-371	6	100	0.69
			R-CGGGCTCCAATTCAAGTTA					
SI- SSR17	HQ588158.1	(AAACT)2	F-GGAAAATGTTAAGTACGCCAGC	53	122-228	7	100	0.66
			R-AGAACCTCTCAAATTAGCCGTG					
SI- SSR 21	EU979119.1	(TTCTT)2	F-AATAGGACCACCGCTATGAAAA	51	127-215	7	100	0.64
			R-AGTAATGGTGCGAATTTGTGTG					
SI- SSR 25	HQ636625.1	(GCAACC)2	F-AACAACAACGTGAGCTACCAGA	53	121-240	3	100	0.79
			R-GTGTGTTTTCAGAGATGTCCCA					
SI- SSR 34	AY587143.1	(TCTT)3	F-ACTGCTGCTCATTGTATGTTGG	52	165-260	2	50	0.42
			R-TTTATTGAAGATGACACGGCTG					
SI- SSR 36	AY587146.1	(ACAAT)2	F-GTCTACATCGTGGGTCAGATCA	55	109-192	3	75	0.51
			R-ACGTAAGTATCACCCCATCCTG					
SI- SSR 37	EU825763.1	(GATGT)2	F-CACGTACATAATTGCATTCGCT	52	106-209	7	100	0.81
			R-TCAGTAGACGAAGCTCGAAACA					

(F = forward; R = reverse; Ta = annealing temperature; *N*A = number of alleles per locus; PIC = polymorphic information content)

Characterization of *S. inferens* by SSRs

The genetic variability of 20 populations of *S. inferens* was investigated by PCR analysis of DNA using 47 SSRs, from that only 6 SSRs produced scorable polymorphic markers in each DNA sample and remaining 41 SSRs was found to produce a single monomorphic band in DNA of all population samples (Table 2). A total of 35 markers with size range from 106-371 bp from 6 SSRs were available for analysis across the different populations. The highest numbers of 7 markers were produced by the primers SI SSR17, SI SSR21 and SI SSR37, followed by 6 markers by SI SSR3 with high degree of polymorphism 50-100%. The primer SI SSR37 was found to be highly informative to differentiate the host associated populations with a polymorphism information content value of 0.81 (Table 2). The calculation of the jaccards coefficient pair wise similarity value matrices was based on the presence or absence of SSR bands. The coefficient values ranged from 0.37-0.94 (Table 3). The *S. inferens* populations occurring on rice from Killikulam and Pattambi were found to be very closely related with a coefficient of 0.94, while the population occurring on rice from Pattambi and maize from Raichur was found to differ widely with a coefficient value of 0.37. The population on sorghum was found to be distantly related to the other hosts with lower similarity coefficients.

Table 3: Similarity coefficient matrices for *S. inferens* populations collected from different host species using SSR markers

	SI 1	SI 2	SI 3	SI 4	SI 5	SI 6	SI 7	SI 8	SI 9	SI 10	SI 11	SI 12	SI 13	SI 14	SI 15	SI 16	SI 17	SI 18	SI 19	SI 20
SI 1	1																			
SI 2	0.94	1																		
SI 3	0.88	0.94	1																	
SI 4	0.42	0.42	0.42	1																
SI 5	0.48	0.48	0.48	0.94	1															
SI 6	0.54	0.54	0.54	0.42	0.42	1														
SI 7	0.62	0.62	0.57	0.51	0.57	0.62	1													
SI 8	0.54	0.54	0.48	0.60	0.60	0.48	0.80	1												
SI 9	0.68	0.68	0.68	0.45	0.51	0.57	0.82	0.74	1											
SI 10	0.60	0.60	0.54	0.48	0.54	0.54	0.91	0.77	0.8	1										
SI 11	0.48	0.54	0.48	0.54	0.54	0.48	0.80	0.82	0.74	0.82	1									
SI 12	0.71	0.71	0.65	0.48	0.54	0.48	0.80	0.82	0.80	0.77	0.77	1								
SI 13	0.57	0.57	0.51	0.51	0.57	0.51	0.77	0.80	0.71	0.74	0.74	0.85	1							
SI 14	0.51	0.57	0.51	0.45	0.51	0.57	0.82	0.80	0.77	0.85	0.91	0.80	0.82	1						
SI 15	0.54	0.60	0.54	0.48	0.54	0.48	0.74	0.71	0.74	0.71	0.65	0.65	0.74	0.68	1					
SI 16	0.60	0.65	0.60	0.54	0.60	0.48	0.80	0.77	0.68	0.77	0.71	0.77	0.74	0.74	0.77	1				
SI 17	0.42	0.42	0.37	0.54	0.54	0.48	0.74	0.82	0.62	0.71	0.71	0.71	0.85	0.74	0.77	0.77	1			
SI 18	0.62	0.62	0.57	0.57	0.62	0.51	0.77	0.85	0.71	0.80	0.68	0.80	0.82	0.71	0.74	0.80	0.74	1		

SI 19	0.57	0.57	0.51	0.62	0.68	0.51	0.88	0.85	0.71	0.85	0.74	0.80	0.82	0.77	0.80	0.91	0.85	0.88	1	
SI 20	0.60	0.60	0.60	0.54	0.60	0.60	0.80	0.77	0.68	0.77	0.65	0.77	0.74	0.68	0.65	0.82	0.77	0.80	0.85	1

[SI- *Sesamia inferens*, SI 1-Hyderabad (rice), SI 2-Kilikillam (Rice), SI 3-Pattambi (Rice), SI 4-Karnal (Sugarcane), SI 5-Kannur (Sugarcane),SI 6-Gulberga (Sorghum),SI 7-Ludhiana(Maize), SI 8-Bagalkot(Maize), SI 9-Hyd(Maize), SI 10-Bangalore(Maize), SI 11-Shimoga(Maize), SI 12-Koppal (Maize), SI 13-Almora (Maize), SI 14-Gulb (Maize), SI 15-Delhi (Maize), SI 16-Solapur(Maize), SI 17-Raichur (Maize), SI 18-Kolar (Maize), SI 19-Coimbatore (Maize), SI 20-Ambikapur (Maize)].

In present study AMOVA result shows approximately, 74% of molecular variability was among populations and 26% could be found within population groups (Table 4).

Table 4: Statistical analyses of molecular variance among populations and within populations of *S. inferens*

Source	df	Sum of square	Mean sum of square	Estimated variance	% total variance
Among Population	3	15.952	5.317	1.337	74
Within Population	17	8.143	0.479	0.479	26
Total	20	24.095		1.816	100

Signifcant levels are based on 1000 permutations; df: degree of freedom, $p \leq 0.001$.

The jaccards coefficient matrices were then utilized to cluster the data using the un-weighted pair-group method analysis of [37]. The dendrogram (Fig. 1) revealed the existence of four principle clusters and a single sub-cluster. The population occurring on sorghum stood out in a single cluster (A), while the population occurring on sugarcane from different location grouped together in cluster B, the population on maize from different regions grouped together in cluster C and finally the population of rice from three location grouped together in cluster D. In cluster C, population of maize sub clustered in to four clusters, but all these clusters was not separated according to the geographical location or states. Data of the similarity matrices used for principal component analysis (PCA), PCA shows the same similar pattern of grouping of *S. inferens* populations according to the hosts (Fig. 2), the PCA plot was in agreement with UPGMA dendrogram.

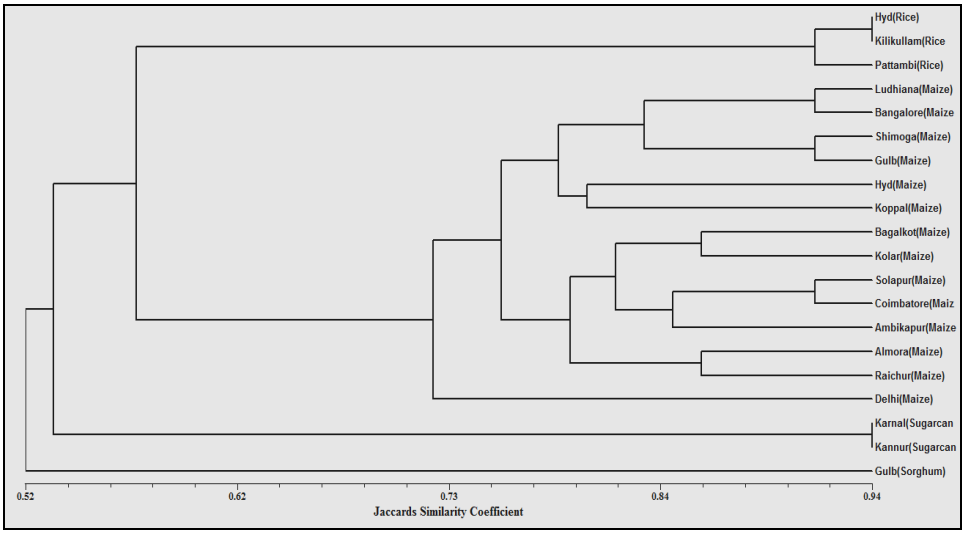

Fig 1: Dendrogram from jacards coefficient matrices of pairwise similarity in SSR analysis between *S. inferns* populations using the UPGMA.

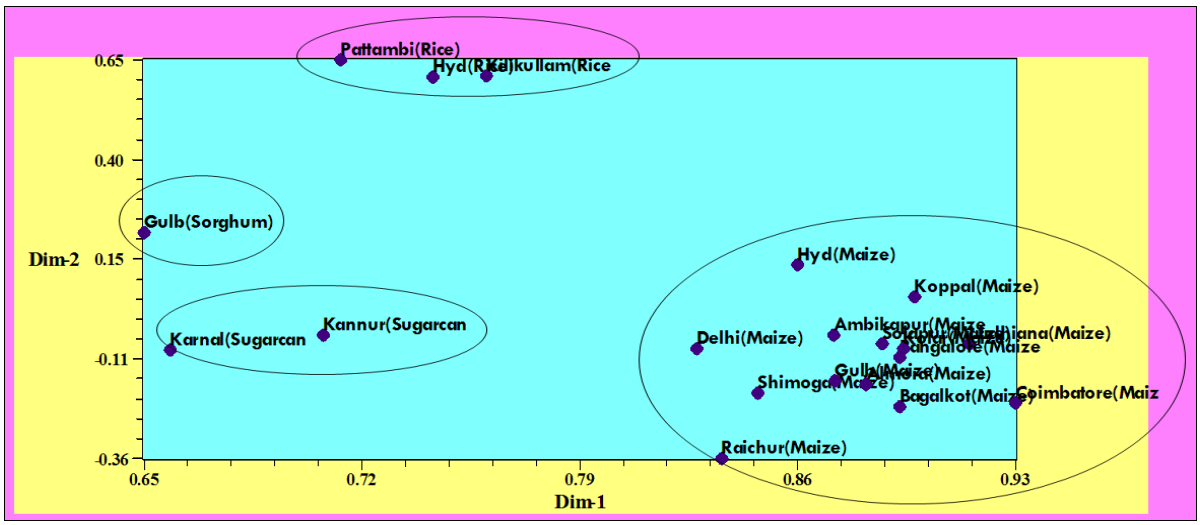

Fig 2: Associations among *S. inferens* populations obtained from a principal component analyses, using the jaccards similarity coefficients of 35 SSRs marker

Discussion

In nature, polyphagous pests tend to be mono or oligophagic at the micro ecological level and their populations could be made up of individuals that are predominantly monophagous [17]. The selective use among diverse resources may lead to the evolution of ecological specialization and adaptation [5, 18]. The versatility of *S. inferens* may be due to the presence of a strong genetic variability governing the behavior [39, 9] making it a serious pest on several cereals. In this regard a better understanding of the genetic differences of polyphagous pest like *S. inferens* can be very useful to understand the structure and population dynamics, their behavior and response to various selection pressures. The wide geographic distribution and broad host range of *S. inferens* is likely to result in high genetic variability within the species [39]. In *S. inferens* information on the existence of molecular markers was nil due to it was minor pest, since now its gaining importance as major pest and need to develop the management strategies. A few studies have demonstrated recently development of SSRs and study the genetic variability of *S. inferens* by Tang [39] from China and also diversity study by RAPD markers in Iran [9]. However, none of the studies reported the development of SSRs and study the genetic variability of *S. inferens* pest population of India. This is the first study to develop SSRs sets from genomic DNA sequences from the NCBI database and use them for diversity analysis in India.

In present study AMOVA result shows approximately, 74% of molecular variability was among populations and 26% could be found within population groups (Table 4). Significant genetic variation among *S. inferens* populations was also reported in *S. inferens* from cultivated rice fields in Yangzhou and Guiyang populations from China by using SSRs [39]. In relative species of *Sesamia nonagrioides* significant variation has been found within and among population collected from different host cultivated in Iran by Cheraghali [9] and European populations collected from maize [10, 22].

Genetic differences may evolve if the physiological adaptation of an insect to a certain host plant causes a decrease in performance on the alternative host [41, 6 42]. For example, Jindal [16] detected a significant host-plant effect on genetic differentiation of *S. inferens* populations from India, Korea and China using DNA barcode CO I gene amplification and also in the different host population of *S. nonagrioides* from Iran by [9]. In our study, populations of first cluster in the UPGMA tree were collected on sorghum, the second cluster included two populations from sugarcane, third cluster included fourteen populations from maize and fourth cluster included three populations of rice (Fig. 1). It seems that the clustering reflects the diversity as related to host plants rather than isolation by geographical distances. Since, moth of *S. inferens* had the strongest flight capability and the flight distance of female and male moths were over 32 and 50 km, respectively [38].

Conclusion

However, it is difficult to conclude to what extent different host plants can affect our results regardless of geographic distances. Number of SSRs used was small in number and size of the population for different host should be included more number to understand the exact gene flow and diversity in *S. inferens* with different host. In order to shed light on the evolutionary history of *S. inferens* in India, there is an urgent need for a better understanding of the gene flow of sympatric *S. inferens* populations associated with different major host plants, including wild grasses. Such studies should be done along the distribution range of this species in India by developing the more number of species specific SSRs and SNPs markers through recent advanced technology, since SSRs from database limited to only some extend. We conclude that the microsatellite markers described herein will be useful in studying population genetics within *S. inferens*. Furthermore, some of the microsatellites and SNPs have to develop to understand the wide geographically distributed and wide host range species, *S. inferens*. It will help to develop the effective management strategies to control its economic loss to different crop hosts.

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References

1. Agunbiade TA, Coates BS, Datinon B, Djouaka R, Sun W, Tam M *et al*. Genetic Differentiation among *Maruca vitrata* F. (Lepidoptera: Crambidae) Populations on Cultivated Cowpea and Wild Host Plants: Implications for Insect Resistance Management and Biological Control Strategies. PLoS ONE. 2014; 9(3):92072.
2. Anil KA. Digestive enzymes of sugarcane pink stem borer Sesamia inferens (Walker), Lepidoptera. Journal of Research on Lepidoptera. 1976; 15(2):153-162.
3. Areekul S, Chamchanya. Effects of humidity, temperature, and light on the growth and development of *Sesamia inferens* (Walker). Kasetsart J. 1973; 7:65-75.
4. Azuma S. Biological studies on the sugar cane insect pests in Okinawa, with special reference to the change of their composition and infestation in relation to the introduction of new commercial sugar cane varieties. Bull. Coll. Agric. Univ. Ryukyu. 1977; 24:1-158.
5. Berenbaum M. Introduction to the symposium: on the evolution of specialization. American Naturalist. 1996; 148:78-83.
6. Busato GR, Grutzmacher AD, Garcia MS, Giolo FP, Martin AF. Consumo e utilização de alimentopor *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) originária de different esregioes do Rio Grande do Sul, nasculturas do milho earrozirrigado. Neotropical Entomology. 2002; 31:525-529.
7. Carleton KL, Streelman JT, Lee BY, Garnhart N, Kidd M, Kocher TD. Rapid isolation of CA microsatellites from the tilapia genome. Anim. Genet. 2002; 33:140-144.
8. Chai HN, Du YZ. The complete mitochondrial genome of the Pink Stem Borer, *Sesamia inferens*, in comparison with four other noctuid moths. Int. J Mol. Sci. 2012; 13:10236-10256.
9. Cheraghali Z, Esfandiari M, Mossadegh MS, Memari HR. Genetic diversity of populations of the stem borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in southern and southwestern Iran, using RAPD-PCR. J Zool. 2015; 11:70-75.
10. De La Poza M, Farinos PG, Berioz B, Ortego F, Hernanadez-Crespo P, Castanera P. Genetic structure of *Sesamia nonagrioides* (Lefèbvre) populations in the

Mediterranean area. Environmental Entomology. 2008; 37(5):1354-1360.
11. Ewing RM, Kahla AB, Poirot O, Lopez F, Audic S, Claverie JM. Large-scale statistical analyses of rice ESTs reveal correlated patterns of gene expression. Genome Res. 1999; 9:950-959.
12. Hoy M. Insect Molecular Genetics. An Introduction to Principles and Applications. 3rd Edition. Academic Press, San Diego, 2013.
13. Huang X, Madan A. CAP3: A DNA sequence assembly program. Genome Res. 1999; 9:868-877.
14. Huang CH, Yao HW, Ye GY, Cheng JA. Effects of sublethal dose of Fipronil on detoxifying enzymes in the larvae of *Chilo suppressalis* and *Sesamia inferens*. Chin. J Rice Sci. 2006; 20:447-450.
15. Jiao Y, Jia HM, Li XW, Chai ML, Jia HJ, Chen Z *et al*. Development of simple sequence repeat (SSR) markers from a genome survey of Chinese bayberry (*Myrica rubra*). BMC Genomics. 2012; 13:201-216.
16. Jindal V, Thakur A, Banta G, Singh M. Cataloguing genetic variations in *Sesamia inferens* populations infesting rice using DNA barcoding. In Genome. 2015; 58(5):233-233.
17. Karowe DN. Facultative monophagy as a consequence of feeding experience: behavioural and physiological specialization in *Colias philodice* larvae (Lepidoptera: Pieridae). Oecologia. 1989; 78:106-111.
18. Kawecki TJ. Sympatric speciation via habitat specialization driven by deleterious mutations. Evolution. 1997; 51:1751-1763.
19. Kayesh E, Zhang YY, Liu GS, Bilkish N, Sun X, Leng XP *et al*. Development of highly polymorphic EST-SSR markers and segregation in F_1 hybrid population of *Vitis vinifera* L. Genetics and Molecular Research. 2013; 12(3):3871-3878.
20. Kourti A. Mitochondrial DNA restriction map and cytochrome oxidase subunits I and II sequence divergence of corn stalk borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae). Biochemical Genetics. 2006; 44:321-331.
21. Mahesh P, Chandran K, Srikanth J, Nisha M, Manjunatha T. Natural Incidence of Sesamia inferens Walker, in Sugarcane Germplasm, Sugar Tech. 2013; 15(4):384-389.
22. Margaritopoulos JT, Gotosopoulos B, Mamuris Z, Skouras PJ, Voudouris KC, Bacandritsos N *et al*. Genetic variation among Mediterranean populations of *Sesamia nonagrioides* as revealed by RFLP mtDNA analysis. Bulletin of Entomological Research. 2007; 97:299-308.
23. Martins WS, Lucas DCS, De Souza Neves, Bertioli DJ. WebSat-A web software for microsatellite marker development. Bioinformation. 2009; 3:282. doi: 10.6026/97320630003282.
24. Mia AM, Iwahashi O, Seasonal changes in infestation level of sugarcane by the pink borer, *Sesamia inferens* (Lepidoptera: Noctuidae), in relation to a parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae), on Okinawa Island. Appl. Entomol. Zool. 1999; 34:429-434.
25. Masoudi-Nejad A, Koichiro T, Shuichi K, Yuki M, Masanori S, Masumi I *et al.* online bioinformatics service for large-scale processing, clustering and assembling ESTs and genomic DNA fragments. Nucleic Acids Res. 2006; 34:459-462.
26. Morand ME, Brachet S, Rossignol P, Dufour J, Frascaria-Lacoste N. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. Mol Ecol. 2002; 11:377-385.
27. Nagayama A, Arakaki N, Kishita M, Yamada Y. Emergence and mating behavior of the pink borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 2004; 39:625-629.
28. Nei M. Genetic distance between populations. The American Naturalist. 1972; 106:283-292.
29. Peakall Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 2012; 28:2537-2539.
30. Peterlunger E, Di G, Gaspero G, Cipriani P, Sivilotti L, Zulini M *et al*. Breeding strategy for the introgression of disease resistance genes into European Grapevine, 2002, 665-670. In VIII International Conference on Grape Genetics and Breeding, Kecskemet, Hungary. (http://www.actahort.org/books/603/index.htm; http://www.actahort.org/ books/603/603_91.htm).
31. Qureshi ZA, Anwar M, Ashraf M, Chatha NU, Arif MD. Rearing, biology and sterilization of the pink rice borer, *Sesamia inferens* Walker. Trop. Agric. Res. Ser. 1975; 5:75-79.
32. Rallo P, Dorado G, Martin A. Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). Theor Appl Genet. 2000; 101:984-989.
33. Reddy ML, Babu TR, Reddy DDR, Sreeramulu M. Determination of economic injury and threshold levels for pink borer *Sesamia inferens* (Walker) in maize, *Zea mays* L. International Pest Control. 2003; 45:260-263.
34. Rohlf FJ. NTSYS-PC: Numerical taxonomy and multivariate analysis system version *2.0*. Department of Ecology and Evolution. State University of New York, 1998.
35. Ronning C, Stegalkina S, Ascenzi R. Comparative analyses of potato expressed sequence tag libraries. Plant Physiol. 2003; 131:419-429.
36. Smith JSC, Chin ECL, Shu H, Smoth OS, Wall SJ, Senior ML *et al*. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparisons with data from RFLP and pedigree. Theoretical Applied Genetics. 1997; 95:163-170.
37. Sneath PHA, Sokal RR. *Numerical taxonomy*, San Francisco: W.H. Freeman and Company, 1973, 147-157.
38. Sun JZ, Zhang JX, Shen XS. The flight capabilities of rice stem borer moths *Tryporyza incertulas*, Chilo suppressalis and Sesamia inferens. Acta Entomologica Sinica. 1993; 36(3):315-322.
39. Tang XT, Xu J, Sun M, Xie FF, Du YZ. First Microsatellites from *Sesamia inferens* (Lepidoptera: Noctuidae). Annals of the Entomological Society of America. 2014; 107(4):866-871.
40. Varshney RK, Graner A, Sorrells ME Genic microsatellite markers in plants: features and applications. Trends Biotechnol. 2005; 23:48-55.
41. Via S. The genetic structure of the of the host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. Evolution. 1991; 45:827-852.
42. Via S, Conte G, Mason FC, Mills K. Localizing FST outliers on a QTL map reveals evidence for large genomic regions of reduced gene exchange during speciation with gene flow. Molecular Ecology. 2012; 21:5546-5560.
43. Vosman B, Arens P. Molecular characterization of

GATA/ GACA microsatellite repeats in tomato. Genome. 1997; 40:25-33.
44. Wijarat P, Keeratinijakal V, Toojinda T, Vanavichit A, Tragoonrung S. Genetic evaluation of *Andrographis paniculata* (Burm. f.) Nees revealed by SSR, AFLP and RAPD markers. J Med Plants Res. 2012; 6:2777-2788.
45. Xu H, Yang L, Xu P, Tao Y, Ma Z. cTrans: generating polypeptide databases from cDNA sequences. Proteomics. 2007; 7(2):177-179.
46. Yao YH, Du YZ, Zheng FS, Wang LP. The variation of mtDNA COII sequences in9 geo-populations of rice stem borer, *Sesamia inferens.* J Environ. Entomol. 2008; 30:39 43.
47. Yi G, Lee JM, Lee S, Choi D, Kim BD. Exploitation of pepper EST-SSRs and an SSR based linkage map. Theor Appl Genet. 2006; 114:113-130.
48. Zahra C, Mehdi E, Mohammad SM, Hamid RM. Genetic diversity of populations of the stem borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in southern and southwestern Iran, using RAPD-PCR. North-western journal of zoology. 2015; 11(1):70-75.
49. Zane L, Bargelloni L, Patarnello T. Strategies for microsatellite isolation: a review. Mol. Ecol. 2002; 11:1-16.
50. Zeid M, Schon C, Link W. Genetic diversity in recent elite faba bean lines using AFLP markers. Theor Appl Genet. 2003; 107:1304-1314.
51. Zhu HF, Fang CL, Wang LY. Economic insect fauna of China, Fasc. 7 Lepidoptera: Noctuidae (III). Science Press, Beijing, China, 1963.