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Characterization of Genetic Diversity of Rice Genotypes Showing Resistance to Rice Leaf Folder, *Cnaphalocrocis medinalis* Using SSR Markers

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ABSTRACT

Keywords

Rice leaffolder, *Cnaphalocrocis medinalis*, SSR Markers, Resistance.

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Twenty rice genotypes which recorded resistant and moderately susceptible reaction against rice leaf folder were characterized by using SSR markers. Out of eleven primers of SSR markers tested, seven primers were found to be polymorphic in nature. A total of 123 amplified bands were seen with 78 % polymorphism with the primers RM 3691, RM 600, RM 14 and RM 228. And it was also observed that all the bands were in the range of 100 bp to 1000bp. A clear variation in the banding pattern was observed among the resistant and susceptible genotypes with the primer RM 3691. The Jaccard's similarity values ranged from 0-89 % indicating the presence of wide range of genetic diversity. The dendrogram showed variable degree of relationship among the genotypes. The resistant and moderately susceptible genotypes were grouped into two different clusters indicating that they were significantly different at molecular level also. In cluster-I, INRC-3021 and W1263 were very closely related and in cluster-II, NLR 145 and Sri Satya were closely related with the similarity index of 86 and 89 %, respectively.

Introduction

Rice (*Oryza sativa* L.) is one of the most significant cereals and is the staple food for more than 2 billion people, mostly in developing countries. India leads the world in rice with 44.06 million hectares with a production of 105.32 million tonnes, but productivity is only 75 % of the world average of 4.02 tonnes ha⁻¹ (Directorate of Rice Development, 2014). Insect pests are the major constraints in enhancing rice productivity which cause 20-30 % loss, besides diseases and weeds (Behura *et al.*, 2011). Nearly 300 spp. of insect pests are attacking the paddy crop at various stages and

among them only 23 species cause notable damage (Pasalu and Katti, 2006).

The Rice leaffolder, *Cnaphalocrocis medinalis* earlier considered as a minor pest has gained the status of major pest with the wide spread of high yielding rice varieties, changes in the cultural practices, multiple cropping patterns, reduced genetic variability of high yielding rice varieties and application of high levels of nitrogenous fertilizers (Nanda *et al.*, 2000). For managing the pest, host plant resistance is one of the components in Integrated Pest Management (IPM) in

which identification of phenotypic, biochemical and molecular markers are included. Among the different classes of molecular markers, SSR markers are often chosen as the preferred markers for a variety of applications in breeding because of their multi-allelic nature, co-dominant inheritance, relative abundance and extensive genome coverage (Gupta and Varshney, 2000). It is essential to know the genetic diversity at molecular level for developing a resistant variety among the available genotypes. Keeping these points in view, present study was undertaken with the objective of estimating genetic diversity in rice genotypes, which showed resistance to rice leaf folder in field level, using SSR markers.

Materials and Methods

Field screening of rice genotypes against Rice leaf folder

Fifty rice genotypes collected from different locations were raised under natural field conditions at ARS, Nellore during *kharif* 2009 and 2010. Two rows of 25 hills in each row were transplanted for each entry with two replications. TN1 was used as susceptible check. All the recommended agronomic practices except leaf folder protection measures were adopted during the experiment. Observations on leaf folder damage were recorded on five randomly selected plants in each genotype at 25, 40 and 70 DAT. Per cent damage was calculated by using the following formula.

$$\% \text{ Damage} = \frac{\text{Number of damaged leaves per hill}}{\text{Total number of leaves per hill}} \times 100$$

Based on the mean % damage during two seasons, Rice genotypes were categorized into Highly Resistant (0 %), Resistant (1-15 %), Moderately Resistant (16-30 %), Moderately susceptible (31- 50 %), Susceptible (51-75 %) and Highly Susceptible (>75%) by following

IRRI Standard Evaluation System (SES) for rice, 1980. A total of twenty rice genotypes which exhibited resistant and moderately susceptible reaction against rice leaf folder were characterized using SSR markers.

DNA extraction and SSR marker analysis

The total DNA of each rice genotypes was extracted from leaves using the modified CTAB method (Murray and Thomson, 1980). Then the quality and quantity of DNA was verified by spectrophotometrically and also by using Nano drop procedure.

PCR was performed in 25 µl of reaction mixture using 2.5µl of 10X PCR reaction buffer, 2.5µl of 25 mM MgCl₂, 0.5µl of 10 mM dNTPs, 2 pmol of each forward and reverse primer, 0.2µl of 3U/µl of Taq DNA polymerase (Genei, Bangalore), 100-200ng of genomic DNA and 18.9µl sterile distilled water. The amplification was performed in a PCR machine (Eppendorf Programmable thermal controller, Hamburg, Germany). PCR amplifications profile was with initial denaturation of DNA at 94°C for 5min followed by 35 cycles of amplification at 94°C for 1 min with annealing temperatures of 54-60°C for 1min, extension step at 72°C for 2min and final extension was made at 72°C for 5min. Then the PCR product was finally kept at 4°C for electrophoresis. The annealing temperatures were optimized individually for each primer (Table 2). Before loading PCR Products in vertical gels, amplification was checked on 2.0% agarose gel. Based on agarose gel electrophoresis of amplification product, the separation of DNA fragments was carried out using 9% non-denaturing polyacrylamide gel electrophoresis (PAGE).

After PCR amplification, 3µl of bromo phenol blue dye was added to 25µl reaction mixture. Then 25µl of this reaction mixture was loaded by using eppendorf micropipette on each lane

of 13-track gel of 9% non-denaturing PAGE and as the base pair marker, 50kb DNA ladder was loaded on one corner of the gel. Composition of 30ml of 9% PAGE gel was 16.5ml of distilled water, 4.5ml of 10X TBE, 9ml of acrylamide-methylbisacrylamide 29:2:0:8 (V/V), 40µl of TEMED and 180µl of 10% APS (ammonium per sulphate). Electrophoresis was done at 100 volts for 3-4 hours in 1X TBE running buffer. PCR products were visualized by using silver staining protocol (Kolodny, 1984).

The banding pattern was observed under white lamp trans-illuminator using gel documentation system (Alpha Innotech, USA) in an auto exposure mode and the gel picture was documented. Clearly resolved, unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicates the presence and absence of bands in each variety respectively. The data matrix thus generated was used to calculate Jaccard's similarity coefficient for each pair-wise comparison. The coefficients were calculated *Insilico* following Jaccard (1908), using the following formula.

$$\text{Similarity coefficient} = \frac{a}{n}$$

where,

a = Number of matching bands for each pair of comparisons

n = Total number of bands observed in two samples.

The similarity co-efficients were subjected to Unweighted Pair-Group Method of Arithmetical averages (UPGMA) cluster analysis to for grouping the genotypes based on their overall similarities. Statistical Package for Social Sciences (SPSS) package was used for the cluster analysis and subsequent dendrogram preparation.

Results and Discussion

Field evaluation of rice genotypes against rice leaf folder

The mean percent leaf damage (*kharif* 2009 and 2010) due to leaf folder in fifty rice genotypes ranged from 11.4 to 37.4. The lowest damage was recorded in Jhitpiti (11.4%) and highest damage was observed in NLR-145 (37.4%). Based on overall leaf folder percent damage during two years, the genotypes were categorized into various groups according to Standard Evaluation System for Rice given by IRRI, 1980. Out of 50 genotypes of rice, ten genotypes were found resistant, thirty were moderately resistant and ten genotypes showed moderately susceptible reaction against leaf folder. A total of twenty rice genotypes which exhibited extreme level resistant and moderately susceptible reaction against rice leaf folder were evaluated using SSR markers (Table 1).

Characterization through SSR markers

Primer selection and survey

In the amplification of genomic DNA of the twenty selected rice genotypes using 11 primers of SSR markers (Table 3), ten primers given reproducible and scorable bands with high percentage of polymorphism. PCR amplification with these primers was done twice before scoring for presence or absence of bands.

Banding pattern

With PAGE gel analysis among 10 primers, seven primers were found to be polymorphic. A total of 123 amplified bands were seen with 70.8 % polymorphism. The number of amplified fragments ranged from 2 to 33 with an average of 8.72 per primer (Table 3).

Herrera *et al.*, (1999) used a set of 48 microsatellites to assess the genetic diversity of 11 Venezuelan rice cultivars and a total of 203 alleles were detected and the number of alleles per marker ranged from 2 to 9, with an average of 4.23.

The maximum polymorphism in PCR reaction was observed with the primers RM 3691, RM 600, RM 14 and RM 228 and these showed

100 % polymorphism as all the bands were polymorphic with size ranging from 100 bp to 1000 bp. The primers RM-495, RM 5380 and RM -6867 showed 0 % polymorphism as all were monomorphic. Selvaraju *et al.*, (2007) reported that the primers RM 495, RM 600, RM 14 and RM 3691 were significantly associated and alleles amplified with rice leaf folder resistance.

Table.1 The selected rice genotypes (20) which were resistant and moderately susceptible to Rice leaf folder

Resistant Genotypes				Moderately Susceptible Genotypes			
S. No	Genotype Name	% Leaf Damage	Damage Rating	S.No	Genotype Name	% Leaf Damage	Damage Rating
1	NLR 3010	14.11	1	11	NLR 20127	32.06	5
2	NLR 40059	14.21	1	12	NLR 20128	32.63	5
3	RP-2068-18-3-5	12.02	1	13	NLR 33636-4	36.69	5
4	Jhitpiti	11.40	1	14	NLR 33671-1	36.79	5
5	INRC-202	13.15	1	15	NLR 33671-7	32.17	5
6	INRC-1997	14.30	1	16	RP-4688-53-2-1255	36.02	5
7	INRC-3021	13.37	1	17	TN 1	37.16	5
8	W- 1263	14.43	1	18	IR -36	33.36	5
9	LF 293	14.39	1	19	NLR 145	37.39	5
10	LF 333	13.24	1	20	Sri satya	31.95	5

Table.2 List of SSR primers selected for DNA amplification

S.No	Primer Name	Forward primer	Reverse primer	Annealing temp (°C)
1	RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	58
2	RM600	AAACGTGTGTTAGCCTGTTAGG	CATATGCTAGTGGTGCTAGCG	58
3	RM576	GGACGGCGAGTTCATAAATAG	CTTGATGGGATAAAAGCATCAG	54
4	RM3412	AAAGCAGGTTTTCTCCTCC	CCCATGTGCAATGTGTCTTC	54
5	RM14	CCGAGGAGAGGAGTTCGAC	GTGCCAATTCCTCGAAAAA	58
6	RM5100	AAAAGAGTCTCTTCTCTCT	AGGACACATACTGTGTATAA	54
7	RM5405	CACTCTCACACTCACCAGCG	GTCGTCTCGCTCTCATCTCC	60
8	RM5380	CCACCCTGTTTCATCTCGC	ATGGCGATACCACCACTCTC	60
9	RM3691	GCTGATGGTCAAAGATCAGG	ATGTGTCTGCTGGCACAGAG	58
10	RM6867	AGAGAGCACAAATCGGAGTCG	GCAGCAGCAACAAGATGTTC	58
11	RM228	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	58

Table.4 Jaccard's similarity coefficient of twenty selected genotypes of rice based on polymorphism obtained with 10 primers

	NLR 3010	NLR 4005 9	RP- 206 8- 18- 3-5	Jhitp iti	INR C- 202	INR C- 1997	INR C- 3021	W- 1263	LF 293	LF 333	NLR 2012 7	NLR 2012 8	NLR 3363 6-4	NLR 33671 -1	NLR 33671- 7	RP- 4688- 53-2- 1255	TN 1	IR- 36	NLR 145	Sri Sathya
NLR 3010	1.00																			
NLR 40059	0.20	1.00																		
RP-2068- 18-3-5	0.09	0.07	1.00																	
Jhitpiti	0.13	0.22	0.22	1.00																
INRC-202	0.12	0.21	0.15	0.32	1.00															
INRC-1997	0.06	0.10	0.22	0.12	0.32	1.00														
INRC-3021	0.06	0.11	0.31	0.13	0.27	0.69	1.00													
W -1263	0.06	0.11	0.31	0.17	0.22	0.59	0.86	1.00												
LF 293	0.07	0.06	0.46	0.14	0.18	0.25	0.41	0.41	1.00											
LF 333	0.07	0.06	0.36	0.14	0.13	0.25	0.33	0.33	0.47	1.00										
NLR 20127	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	1.00									
NLR 20128	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.31	1.00								
NLR 33636-4	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.05	0.41	0.40	1.00							
NLR 33671-1	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.04	0.47	0.38	0.67	1.00						
NLR 33671-7	0.00	0.05	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.04	0.29	0.26	0.50	0.47	1.00					
RP-4688- 53-2-1255	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.04	0.27	0.19	0.27	0.45	0.41	1.00				
TN 1	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.13	0.10	0.18	0.23	0.21	0.36	1.00			
IR-36	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.18	0.15	0.18	0.23	0.16	0.36	0.33	1.00		
NLR 145	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.06	0.05	0.05	0.05	0.09	0.29	0.38	1.00	
Sri Sathya	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.06	0.05	0.05	0.04	0.09	0.35	0.35	0.89	1.00

Table.3 Allelic variation of SSR primers used for DNA amplification of selected rice genotypes for leaf folder resistance

S.No.	Primer Name	Total No. of alleles	No. of polymorphic alleles	Amplification size (bp)	% Polymorphism
1	RM495	8	0	190	0
2	RM600	18	18	285	100
3	RM576	6	6	Non distinct	100
4	RM3412	0	0	Non distinct	0
5	RM14	14	14	200	100
6	RM5100	10	10	370	100
7	RM5405	12	2	250	16.7
8	RM5380	3	0	Non distinct	0
9	RM3691	33	33	250	100
10	RM6867	6	0	200	0
11	RM228	13	13	115	100
	Total	123	96		78.05
	Average		8.72		

Fig.1 Gel profile showing the amplification of SSR primer RM 3691 with 20 selected rice genotypes

Fig.2 Gel profile showing the amplification of SSR primer RM 14 with 20 selected rice genotypes

Note: The numeric number above the lane indicates the genotype number as in Table 1 and M indicates the Marker - 50 kb

Fig.3 Dendrogram depicting variation among rice genotypes based on SSR analysis

A clear variation in the banding pattern was observed among resistant and moderately susceptible genotypes with the primer RM 3691. In majority of genotypes, a maximum number of 33 amplified bands at 100 bp and at 250 bp size were present (Fig. 1). In the profile of RM 14, a clear variation in banding pattern was observed with 14 scorable polymorphic bands present at 200 bp and 450 bp size (Fig. 2). In the profile of remaining primers there was a clear variation among different genotypes. The similarity index values ranged from 0 to 89 % indicating the presence of wide range of genetic diversity at molecular level among the genotypes, tested for leaf folder resistance (Table 3).

Cluster analysis

Similarity coefficients were subjected to Unweighted Pair Group Method on Arithmetic average (UPGMA) using paired matrix values and are presented in figure 3. The dendrogram exhibited variable degree of relationship among the genotypes studied and indicated two major clusters. The ten resistance genotypes and other ten moderately susceptible genotypes to rice leaf folder were grouped into two different clusters indicating that they are significantly different at molecular level and among cluster-I, INRC-3021 and W1263 were very closely related and in cluster-II, NLR 145 and Sri

Satya were closely related with the similarity index of 86 and 89 %, respectively.

In conclusion, results revealed that 70.8 percent polymorphism existed among twenty genotypes using SSR markers indicated the presence of wide range of genetic diversity at molecular level. Dendrogram clearly distinguished the resistant and moderately susceptible genotypes for rice leaf folder, *Cnaphalocrocis medinalis* by clearly forming into two different clusters indicating that they are significantly different at molecular level and were correlated with the field performance.

In future, Correlation studies may be conducted among different genotypes with the data of parentage for drawing more conclusions.

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References

- Behura, N., Sen, P., Kar, M.K. 2011. Introgression of yellow stem borer (*Scirpophaga incertulus*) resistance gene into cultivated rice (*Oryza* sp.) from wild spp. *Indian J. Agri. Sci.*, 81: 359-362.
- Directorate of Rice Development. 2014. Status paper on Rice. 151. <http://drd.dacnet.nic.in/>
- Gupta, P.K. and Varshney, R.K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, 113: 163-185
- Herrera, T.G., Duque, D.P., Almeida, I.P., Nunez, G.T., Pieters, A.J., Martinez, C.P and Tohme, J.M. 1999. Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electronic J. Biotechnol.*, 11(5): 1-6
- International Rice Research Institute. Standard Evaluation System for Rice. 1980. III edition. IRRI, Los Banos, Philippines, 123.
- Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bulletin de la Société vaudoise des sciences naturelles*, 44: 223-270.
- Kolodny, G.M. 1984. An improved method for increasing the resolution and sensitivity of silver staining of nucleic acid bands in polyacrylamide gels. *Anal. Biochem.*, 138: 66-67.
- Murray, M. and Thompson, W. 1980. The isolation of high weight plant DNA. *Nucleic Acids Res.*, 8: 4321-4325.
- Nanda, U.K., Mahapatra, G.K., Sahoo, A and Mahapatra, S.C. 2000. Rice Leaf folder: Integrated neem derivatives in its management. *Pestol.*, 24(7): 31-34.
- Pasalu, I.C., Katti, G., 2006. Advances in eco friendly approaches in rice IPM. *J. Rice Res.*, 1(1): 83-90.
- Selvaraju, K., Shanmugasundaram, P., Mohankumar, S., Asaithambi, M and Balasaraswathi, R. 2007. Detection of quantitative trait locus for leaf folder resistance in rice on linkage group 1 based on damage score and flag leaf width. *Euphytica*, 157: 35-43.

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