GENETIC VARIATION OF WILD RICE IN VIETNAM

Nguyen Thi Lang*[1], Trinh Thi Luy[1], Pham Thi Thu Ha[1],
Tran Bao Toan[1], Bui Chi Buu[2] ,Yoshimichi Fukuta[3]

Abstract

The study aimed at identifying rice gene pools carrying blast resistant genes via phenotyping and genotyping for wild rice in Vietnam. The experiments were carried out at CLRRI (Cuu Long Delta Rice Research Institute) laboratories, greenhouses and experimental fields, Thoi Lai, Can Tho, from 2011 October to 2015 October. Major research activities consisted of evaluating the genetic diversity of wild rice accessions. A total of 101 accessions of some wild rice species in the genebank of Cuu Long Rice Research Institute, Vietnam were used to clarify the diversity using SSR markers. Molecular diversity analysis using 38 polymorphic SSR markers revealed among the 101 accessions. The 101 Vietnam accessions generated 2 clusters at 0.76 similarity coefficient. Some with the same variety names were grouped into different clusters. Genotyping blast resistance among wild rice via molecular markers as SSRs (simple sequence repeats) showed the results of a total of 101 wild rice accessions were classified into two cluster groups A , and B based on the polymorphism data for 120 alleles of 38 SSR markers. Te Tep varieties including *O. punctala-1* , *O. latifolia-1, O. nivara-1* were categorized into group B. The other two groups A (98 accessions) as an LTH -type control was also categorized into group A. Phenotyping blast resistance among wild rice for blast isolates collected from the Mekong Delta. Against 13 standard differential blast isolates which were selected based on the reactions to monogenic lines for targeting 101 resistance genes as the differential accessions, these resistant of rice accessions were investigated. Based on the cluster analysis, these accessions were classified into three groups, A, B and C.

Keywords: Blast, genetic diversity, SSR markers, wild rice

INTRODUCTION

Recent advances in molecular biology, principally the development of the polymerase chain reaction (PCR) for amplifying DNA, DNA sequencing and data analysis have resulted in powerful techniques which can be used for the screening, characterization and evaluation of genetic diversity. With molecular marker techniques, powerful tools have been developed so that genetic resources can be accurately assessed and characterized. Several types of molecular markers are available for evaluating the extent of genetic variation in rice (Ni *et al.*, 2002). These include restriction fragment length polymorphism (RFLP) (Botstein *et al.*, 1980), random amplified polymorphic DNA (RAPD) amplified fragment length polymorphism (AFLP)), and microsatellites or simple sequence repeats (SSR) (Mc. Couch, 1988, Temnykh *et al.*, 2000 and Lang *et al.*, 2009). Development of host plant resistance is the most effective means of disease management. As many as genes conferring resistance some genes for resistance to blast have been identified to various races of the pathogen have been identified and utilized in rice breeding programs. To evaluate the genetic backgrounds and introgression of resistance genes from donor varieties, the graphical genotypes of the population were constructed using 38 simple sequence repeats. However, large-scale and long-term cultivation of varieties carrying a single gene for resistance resulted in a significant shift in pathogen race frequency with the consequent breakdown of resistance in these cultivars. This report the gene for blast resistance, genes identified wild rice.

Characterization and evaluation of diversity among traditional varieties will provide plant breeders information necessary in the identification of initial materials for hybridization to produce varieties with improved productivity and quality.

The objectives of the study are: To evaluate genetic diversity of the wild rice accessions in the genebank of Cuu Long Rice Research Institute, Vietnam using morphological characters and microsatellite markers.

Genetic diversity of Vietnam wild rice species using SSR markers.

MATERIALS AND METHODS

Plant materials

A total of 102 accessions of several wild rice species included and 2 checked varieties such as Te Tep and LHT rice (data are not shown).

[1] Cuu Long Delta Rice Research Institute (CLRRI)
[2] Institute of Agricultural Sciences for Southern Vietnam (IAS)
[3] Japan International Research Center for Agricultural Sciences (JIRCAS)
* Corresponding author: ntlang@hcm.vnn.vn

A total of 102 Vietnam wild rice accessions and 28 IRRI varieties included were characterized their blast resistance to 13 selected differential blast isolates from Vietnam race 1 (Can Tho), race 2 (Vinh Long), race 3 (Tien Giang), race 4 (An Giang), race 5 Ben Tre), race 6 (Dong Thap), race 7 (Long An), race 8 (Bac Lieu), race 9 (Soc Trang), race 10 (Tra Vinh), race 11 (Hau Giang), race 12 (Kien Giang) and race 13 (Ca Mau).

Inoculation and evaluation of rice resistance were performed following the previous method described by IRRI SES (1996); Hayashi and Fukuta (2009).

Molecular- Based Characterization and Analysis Using SSR

DNA extraction

The ninety accessions were grown in pots. Maximum protection was employed to ensure healthy and disease free-growth of seedlings. Leaves were collected 2-3 weeks after planting for extraction of DNA.

Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents and equipment were followed according to Sambrook *et al.,* (1989). Molecular work was conducted at the Genetics and Plant Breeding Department of Cuu Long Rice Research Institute, Cantho, Vietnam and Genome Mapping Laboratory (GML) of the International Rice Research Institute (IRRI), Philippines.

DNA suitable for PCR analysis was prepared using a simplified procedure according to Mc Couch *et al.,* (1988). A piece of young rice leaf (2 cm) was collected and placed in labeled 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a Spot Test Plate (Thomas Scientific) after adding 400 µl of extraction buffer. Grinding was done until the buffer turned green, an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of extraction buffer was added into the well by pipetting. Around 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air- dried and resuspended in 50 µl of TE buffer (Lang, 2002).

DNA quality checks used 1% agarose by melting 3g agarose in 300ml TAE buffer. The mixture was heated in microwave for 5 - 6 minutes and then cooled to around 55 - 60^{0}C. This was then poured on prepared electrophoresis box with combs. Gels were ready and combs removed after about 45 minutes. Seven microliliters of DNA sample plus 3µl loading buffer (Tris 1M pH = 8.0, glycerol, EDTA 0.5M pH = 8.0, xylene cyanol 0.2%, bromphenol blue 0.2% and distilled water) was run at 70-80v, 60 mA for 45 minutes or until loading buffer dye moved far from the wells. Gel was then taken out and stained with ethidium bromide after which was visualized under UV light.

Microsatellite Analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, band detection and scoring.

PCR assay

Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 312 microsatellite primer pairs currently available for rice (Temnykh *et al.,* 2000). The PCR reaction was as follows:

Reactions were overlaid with mineral oil and processed in a Programmable Thermal Controller programmed for 35 cycles of 1 min at 94^{0}C, 1 min at 55^{0}C and 2 min at 72^{0}C, with a final extension at 75^{0}C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product which was then denatured at 94^{0}C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel.

Band detection and scoring

Plates were separated using a plastic wedge and removed from the tank. The acrylamide gel was soaked in ethidium bromide staining solution for 15 to 20 minutes. Bands in the ethidium bromide-stained gels were detected and photographed under UV light. Allelic bands were scored as 1 or 0 for presence or absence, respectively. Data were entered directly into an Excel spreadsheet.

Data Analysis

Analysis of variance

The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

Pair–wise comparisons of the lines based on the presence or absence of unique and shared polymorphic products were used to calculate genetic similarity coefficients. Similarity coefficients were calculated using Nei and Li distance measure (Nei and Li, 1979) in the NTSYS-PC Numerical Taxonomy and Multivariate Analysis System (Rohlf, 1990). The lines

were clustered based on similarity coefficients using the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm.

RESULTS AND DISCUSSION

Molecular Diversity Analysis Using

Polymorphism of microsatellite markers

All of the primer pairs used in this study generated polymorphic bands among the genotypes. A total of 120 loci were assigned to the 38 microsatellite primer pairs. As previously reported, RM4 and RM20 each detected two loci. A total of 120 alleles were detected among the 101 rice genotypes with an average of 3.1 alleles per locus (Table 1). The number of alleles per locus ranged from 2 to 6 (in RM 3164). The total alleles identified in the 101 genotypes were classified into two categories:

A dendrogram based on cluster analysis using an unweighted pair group method (UPGMA) with the module of SAHN in the NTSYS-pc package. The cluster analysis showed significant genetic variation among upland rice varieties studied and the genetic distance ranged from 0 to 0.76 (Figure 1). With genetic distance of 0.76, the cluster revealed two major groups, A, B, in the wild rice accessions. The first group, A, is contained 98 accessions.

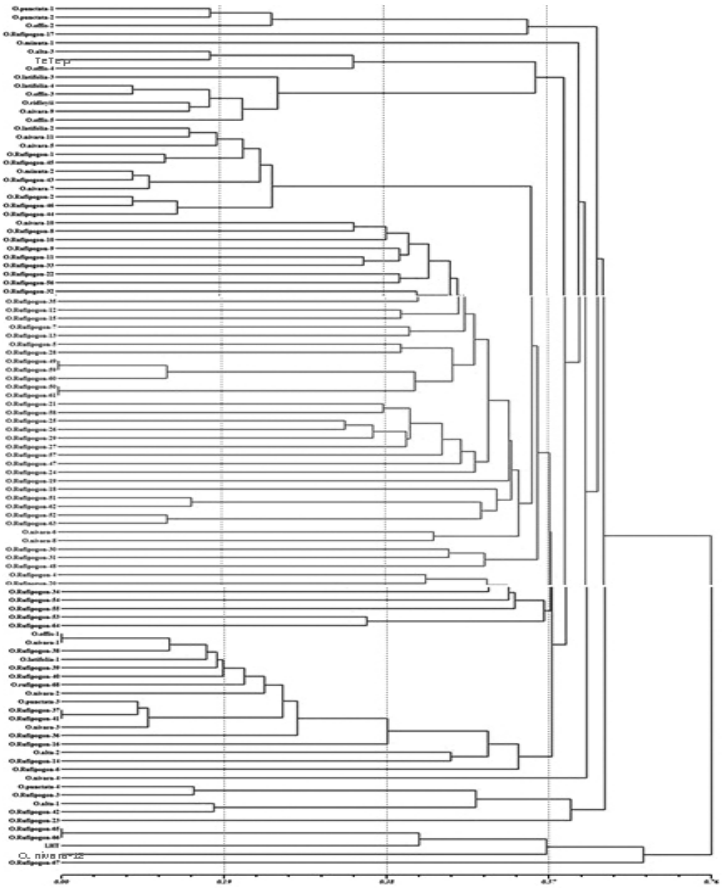

Figure 1. Classification of rice accessions based on genetic distance calculated from 38 microsatellite markers of 101 wild rice accessions

The first sub group A contained the largest cluster contained 98 rice accessions. The second group, B, is small group accession cluster contained 4 accessions. Most of this group contained lowland rice accessions. The A group is divided into five sub groups. The first sub group, A1, contained 4 rice varieties consisting 26 upland rice. The fist sub group, A1, included 4 rice, A 3 is big sub group, A 4 contained only 1 accession: *O. nivara*-4 and A 5 contained only 1 accession: *O.punctata -4*. The second group contained 5 accession such as: 3 *O. rufipogon (- 66, 65 - 67), LHT, O. nivara* -27. The low PIC values were observed for the primers of RM1089 (0.077) on chromosome 5, RM1134 on chromosome 7 (0.147), RM8203 on chromosome 3 (0.241). The PIC values of the remaining microsatellite loci were all above 0.50 to 0.728.

Table 1: The PIC and number of alleles of 38 microsatellite markers

No.	Primer	Chro-mosome location	No. of allele	Map position (cM)	PIC	No.	Primer	Chro-mosome location	No. of allele	Map position (cM)	PIC
1	RM495	1	3	0 .3	0 .573	20	RM510	6	2	11 .5	0 .330
2	RM3604	1	4	26 .8	0 .685	21	RM276	6	3	33 .5	0 .487
3	RM1	1	4	29 .7	0 .712	22	RM162	6	3	108 .3	0 .642
4	RM8111	1	3	30 .8	0 .500	23	RM3138	6	3	110 .6	0 .622
5	RM259	1	5	38 .8	0 .728	24	RM1134	7	2	25 .4	0 .147
6	RM3865	2	3	21 .1	0 .508	25	RM11	7	3	67	0 .525
7	RM1347	2	2	26 .6	0 .452	26	RM408	8	3	0 .5	0 .603
8	RM3874	2	3	94 .3	0 .645	27	RM152	8	3	3 .3	0 .503
9	RM6959	3	3	65 .4	0 .561	28	RM7048	9	4	62 .4	0 .730
10	RM8208	3	2	89	0 .486	29	RM3164	9	6	72 .1	0 .800
11	RM168	3	3	122 .8	0 .607	30	RM258	10	4	48 .8	0 .655
12	RM8203	3	2	140 .1	0 .241	31	RM171	10	4	55 .59	0 .662
13	RM3317A	4	3	25 .4	0 .540	32	RM271	10	5	59 .4	0 .724
14	RM5586	4	2	56 .1	0 .298	33	RM552	11	4	40 .6	0 .727
15	RM3524	4	2	68 .3	0 .278	34	RM21	11	5	85 .7	0 .789
16	RM267	5	4	25	0 .703	35	RM247	12	5	26 .7	0 .743
17	RM413	5	3	26 .7	0 .575	36	RM7619	12	4	38 .1	0 .715
18	RM1089	5	2	37 .2	0 .077	37	RM7376	12	4	89 .5	0 .541
19	RM508	6	3	2 .3	0 .588	38	RM17	12	4	107 .4	0 .613

Table 2. Mean number of alleles based on microsatellite markers on different rice chromosomes

Group	Sub group	Mean of allele No. per SSR markers												*Mean*
		Chromosome												
		1	2	3	4	5	6	7	8	9	10	11	12	
A	1	2.80	1.58	1.13	0.08	0.33	0.50	0.88	0.25	1.50	1.33	1.75	1.50	*1.14*
	2	2.20	2.33	1.25	1.00	2.00	1.00	0.00	0.50	2.50	2.33	2.00	2.25	*1.61*
	3	1.33	1.30	1.11	0.85	1.74	0.65	0.58	0.52	1.68	1.65	1.95	1.69	*1.25*
	4	2.00	1.33	1.00	1.33	1.67	1.20	1.00	1.00	2.00	2.33	3.50	1.75	*1.68*
	5	1.44	1.27	1.15	0.53	2.07	1.76	0.90	1.70	2.50	2.00	1.90	1.25	*1.54*
	Mean	*1.95*	*1.56*	*1.13*	*0.76*	*1.56*	*1.02*	*0.67*	*0.79*	*2.04*	*1.93*	*2.22*	*1.69*	*1.44*
B		3.00	2.33	1.90	1.67	2.47	2.28	2.00	2.30	4.40	3.27	3.60	3.60	*2.74*
	Mean	*3.00*	*2.33*	*1.90*	*1.67*	*2.47*	*2.28*	*2.00*	*2.30*	*4.40*	*3.27*	*3.60*	*3.60*	*2.74*
Mean		*2.48*	*1.95*	*1.51*	*1.21*	*2.02*	*1.65*	*1.34*	*1.55*	*3.22*	*2.60*	*2.91*	*2.64*	*2.09*

Mean of allele number per locus and each chromosome reveals much lower in the improved varieties than landrace varieties (Table 2). The mean of allele number per locus group A is 1.44 .The mean number of alleles per locus observed in group B is 2.74 similar with total two group is 2.09.

Blast resistance of Vietnam wild rice species

Breeding efforts are aimed to introduce genes for blast resistance into desirable genetic background. Breeding of resistant varieties has been considered to be economical and effective for the control of rice blast disease although durable resistant varieties still rare. Rice resistance to blast disease has been explained by the gene to gene for gene theory between the resistance gene in the host and the avirulence gene in *P. grisea* (Kiyosawa 1972; Silue *et al.*, 1992).

The genetics of rice blast have been extensively studied and two types of resistance have been described, complete (true) and field resistance (Ezuka, 1972; Parlevliet, 1979). Field resistance, incomplete and usually polygenic, is a susceptible infection type, which allows effective control of a pathogen under natural fields conditions and is considered to be durable when exposed to new races of blast. Complete resistance is a hypersensitive reaction, often a complete form of resistance, and is characterized by a resistance infection type.

The existence of many of different blast races has been it difficult to develop durable and long-lasting resistant varieties. However, the resistance of improved varieties easily breakdown after few years released. The precise delineation of pathogenic variability in the target production area is a prerequisite for identifying rice genotypes with a broader resistance spectrum.

Characterization of Vietnam rice varieties based on the selected standard differential blast isolates.

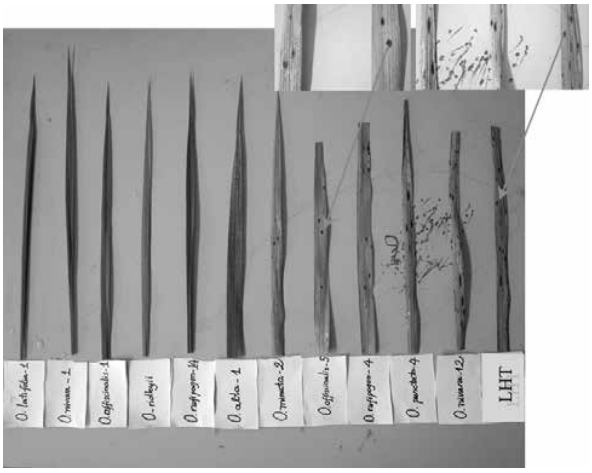

Figure 2. Screening blast for wild rice at CLRRI

A dendrogram based on cluster analysis using hierarchical Ward method was constructed for all rice varieties. The cluster analysis showed significant genetic variation among wild rice ties studied and the genetic distance ranged from 0 to 2.19 (Fig 3). With genetic distance of 1.65, the cluster revealed three major groups, A, B, and C, in characterization of Vietnam wild rice species against the 13 selected standard blast isolates revealed, these accessions classified into 3 groups, group A, B, C (Fig. 3).

Each group showed unique reactions and were differentiated from each other (Table 3). The accessions in group-A was rice accessions showed the most susceptible reaction to blast isolates, and included 3 susceptible control varieties.

Table 3. Classification of rice accessions from Vietnam based on reaction patterns to 13 selected standard differential blast isolates for 101 wild rice accessions

roup	Subgroup	accession	Group	subgroup	accession
A	A1	*O. punctata-1*	B	B1: B1-1	*O. rufipogon-22*
		O. latifolia-1			*O. rufipogon-32*
		O. nivara-1			*O. rufipogon-1*
		Te Tep			*O. rufipogon-8*
	A2	*O. minuta-1*			*O. rufipogon-18*
		O. nivara-2			*O. rufipogon-28*
		O. nivara-3			*O. rufipogon-38*
		O. officinalis-1		B1: B1-2	*O. nivara-10*
		O. ridleyii			*O. rufipogon-51*
		O. nivara-4			*O. rufipogon-52*

Table 3. Classification of rice accessions from Vietnam based on reaction patterns to 13 selected standard differential blast isolates for 101 wild rice accessions *(continued)*

roup	Subgroup	accession	Group	subgroup	accession
		O. nivara-5			*O. rufipogon-13*
		O. nivara-6			*O. rufipogon-33*
		O. latifolia-2			*O. rufipogon-23*
		O. nivara-7			*O. rufipogon-43*
		O. nivara-8			*O. rufipogon-11*
		O. rufipogon-14			*O. rufipogon-41*
		O. rufipogon-44			*O. rufipogon-21*
		O. rufipogon-24			*O. rufipogon-31*
		O. rufipogon-34			*O. rufipogon-2*
B	B1: B1-1	*O. punctata-2*			*O. rufipogon-7*
		O. latifolia-3			*O. rufipogon-27*
		O. alta-1			*O. rufipogon-17*
		O. rufipogon-10			*O. rufipogon-37*
		O. rufipogon-20			*O. rufipogon-9*
		O. rufipogon-40			*O. rufipogon-19*
		O. rufipogon-54			*O. rufipogon-29*
		O. rufipogon-56			*O. rufipogon-39*
		O. rufipogon-60			*O. rufipogon-50*
		O. rufipogon-64	B	B2	*O. alta-3*
		O. rufipogon-55			*O. rufipogon-57*
		O. rufipogon-59			*O. rufipogon-61*
		O. rufipogon-63			*O. rufipogon-65*
		O. rufipogon-67			*O. nivara-11*
		O. rufipogon-68		B3	*O. punctata-3*
		O. rufipogon-53			*O. officinalis-3*
		O. rufipogon-47		B4	*O. rufipogon-5*
		O. rufipogon-6			*O. rufipogon-48*
		O. rufipogon-58			*O. rufipogon-49*
		O. rufipogon-62	C	C1	*O. officinalis-4*
		O. rufipogon-66			*O. rufipogon-3*
		O. officinalis-2			*O. minuta-2*
		O. nivara-9			*O. rufipogon-15*
		O. alta-2			*O. rufipogon-25*
		O. rufipogon-30			*O. rufipogon-45*
		O. latifolia-4			*O. rufipogon-35*
		O. rufipogon-16		C2	*O. offiscinalis-5*
		O. rufipogon-36			*O. rufipogon-4*
		O. rufipogon-46			*O. punctata-4*
		O. rufipogon-26			*O. nivara-12*
		O. rufipogon-12			*LHT*
		O. rufipogon-42			

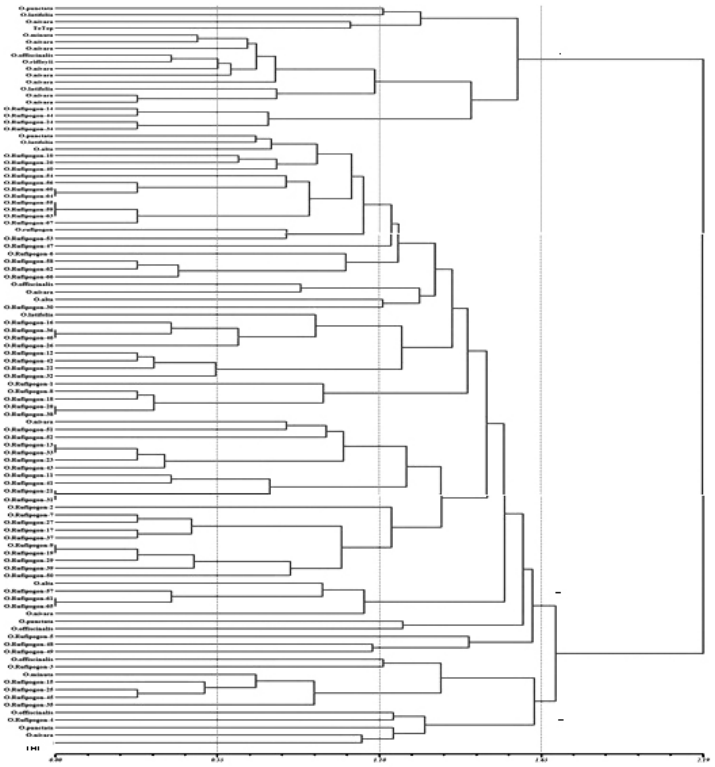

Figure 3. Classification of rice accessions from Vietnam based on reaction patterns to 13 selected standard differential blast isolates

Three groups of Vietnam wild rice species showed resistance and susceptible to 13 selected standard differential blast isolates (Figure 4).

Some lines are good for development new varieties for blast resistance such as *O. minuta , O. officinalis, O. nivara, O. ridlegii, O. nivara* selected at Ho Lac (Lam Dong province).

Three accessions *O.nivara* rice species from Holac, Lam Dong and one *O.nivara* from Vung Tau, Con Dao showed durable resistance to 13 selected standard differential blast isolates (Table 4).

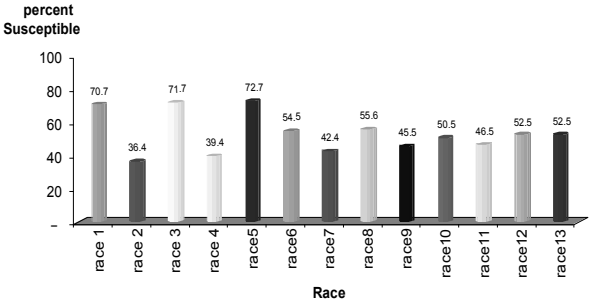

Figure 4. Frequencies of susceptible of 101 wild rice based on the reaction patterns to 13 difference blast

CONCLUSION

PCR based markers have provided valuable information about genetic diversity of rice collection in CLRRI.

In molecular-based analysis, results showed that SSR markers were very useful and effective in characterizing and estimating the extent and distribution of genetic variation in the 101 rice landraces and improvement. Clustering of the accessions based on genetic distance (0.78) allowed grouping of the 101 accessions into two clusters.

From these results, the following recommendations are presented:

(1) Diversity analysis based on agro-morphological traits of rice wild rice need to be continued to further confirm relationships among them.

(20 Extensive molecular marker analysis may be conducted by considering more primers for its relevant application and efficient attainment of breeding objectives in rice improvement.

(3) Continue analysis for the rest of the accessions in the CLRRI genebank. Identification of novel resistance gene in rice germplasm.

(4) Identification of novel resistance gene from wild rice

(5) Development of introgression line harboring resistance gene from wild rice.

Table 4. Selected durable wild rice accessions in Vietnam

		Name of Blast Isolates													
Accession	**Species**	ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10	ID 11	ID 12	ID 13	**Sites selected**
3	*O.minuta*	R	R	R	R	R	R	R	R	R	S	R	R	R	Genbank
9	*O.officinalis*	R	R	R	R	R	R	R	R	R	R	R	R	R	CanTho
10	*O.nivara*	R	R	R	R	R	R	R	R	R	R	R	R	R	Holac, LamDong
12	*O.ridleyii*	R	R	R	R	R	R	R	R	R	R	R	R	R	Gene bank
71	*O.nivara*	R	R	R	R	R	R	R	R	R	R	R	R	R	Vung tau
74	*O.nivara*	R	R	R	R	R	R	S	R	R	S	R	R	R	VungTau
75	*O.nivara*	R	R	R	R	S	R	R	R	R	R	R	R	R	Vung Tau
79	*O.nivara*	R	R	R	R	R	R	R	S	R	R	R	R	R	Vung Tau,
	LTH	S	S	S	S	S	S	S	S	S	S	S	S	S	Susceptible control
Pit	IRBLt-K59	R	R	R	R	R	R	R	S	S	S	R	R	R	Resistance control
Pi20	IRBL20-IR24	R	R	R	S	R	R	R	S	R	R	R	R	R	Resistance control

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GENETIC DIVERSITY AMONG DURIAN (*DURIO ZIBETHINUS* MURR.) CULTIVARS ORIGINATED FROM VIETNAM, THAILAND AND MALAYSIA AS REVEALED BY INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

Van Quoc Giang[1], Mai Van Tri*[2], Huynh Ky[1],
Pham Thi Muoi[2] and Nguyen Loc Hien[1]

Abstract

Genetic diversity of 16 durian cultivars originated from Vietnam, Malaysia and Thailand was analyzed by using ISSR markers. Among 181 fragments that were generated by 13 ISSR primers, 165 fragments (91.1%) were shown polymorphism, on average, 12.7 polymorphic fragments per primer. Genetic relationships of 16 durian cultivars were clustered by UPGMA to demonstrate the differentiation of all cultivars and revealed the correlation between molecular grouping and geographical origin. The first cluster grouped the durian cultivars derived from Thailand. The second cluster included three cultivars from Vietnam, Chuong Bo, Sau Huu and Kho Qua, while the third cluster grouped two Malaysian cultivars, D10 and D24. The fourth and fifth clusters only contained D197 cultivar originated from Malaysia and HB11 cultivar supposed from Vietnam, respectively. Furthermore, ISSR primers, such as ISSR B2 and ISSR BB7, can be used to identify HB11 durian cultivar. Thus, DNA profile of 16 durian cultivars based on ISSR markers revealed the potential of digital fingerprinting of all cultivars examined.

Keywords: Durian, genetic diversity, ISSR markers, polymorphism

INTRODUCTION

'The king of fruits', durian (*Durio zibethinus* Murr.) is one of the most popular and economically important fruit crops in the Southeast Asia. Durian is indigenous to the hot equatorial rain forests of Borneo, Malaysia and Indonesia. However, currently durian is mainly grown in Thailand, Malaysia, Indonesia, Philippines and Vietnam (Ahmad and Nanthachai, 1994). Over 200 durian cultivars were named in Thailand (Tinggal *et al*., 1994) but Chanee, Monthong, Kanyao, and Kradumthong are four Thai durian cultivars that are favorite among growers and consumers. In Malaysia, more than 200 durian cultivars were also recorded but only 10 cultivars are recommended for production. In which, D24, D99, D123 (Chanee), D145 (Beserah) and D197 (Raja Kunyit/Musang King) are for general field planting while some cultivars are recommended for specific locations, such as D148 for Perak, D158, D159 and D169 for Kedah, Penang, Kelantan and Terengganu as well as D168 for Johore. In Vietnam, the durian industry is small but rapidly expanded by the effort of Vietnamese government. There are more than 35 durian cultivars were documented in the country but the common cultivars are Chin Hoa (Com Vang Sua Hat Lep), Ri 6, Monthong and Kho Qua Xanh. To date, there is little information on genetic relationship among durian cultivars including common durian cultivars originated from Vietnam, Thailand and Malaysia.

Earlier classification and evaluation of durian relationship were primarily based on the phenotypic expression, such as fruit shape, the size of thorns on durian skins and other morphological characters (Somsri, 2007). However, morphological variation is limited to distinguish genetic relationship among similar individuals. In contrast, molecular markers have become a standard method to study genetic variability among closely related taxa (Weising *et al*., 1995). The relatively similar Inter-simple sequence repeats (ISSR) were introduced as the efficient molecular markers that are based on quick PCR amplification of polymorphic DNA fragments starting from small amounts of templates (Zietkiewicz *et al*., 1994). ISSR technique has been successfully exploited to identify genetic diversity of some crops including mango (Rocha *et al.,* 2011) and durian (Vanijajiva, 2012).

In this study, ISSR markers were to apply for evaluation of genetic diversity and relatedness of 16 durian cultivars originated from Vietnam (10 cultivars), Thailand (3 cultivars), and Malaysia (3 cultivars).

MATERIALS AND METHODS

Plant materials

List of 16 cultivars along with origin and collected locations in this study were presented in Table 1. Fresh leaf samples were used to extract DNA for ISSR analysis.

[1] College of Agriculture and Applied Biology, Can Tho University
[2] Southeast Horticultural Research Center (SOFRI)
* Corresponding author: mv_tri@yahoo.com