Detection of Blast Resistance genes on Cultivated Rice in Myanmar using SSR and InDel Markers

Thet Yee Khaing[1], Nang Kyu Kyu Win[1], Kyaw Kyaw Win[2], Than Myint Htun[3], Min San Thein[4] and Tin Aye Aye Naing[1]

Abstract

The present study was conducted to detect the presence of blast resistance genes in cultivated rice in Myanmar. Thirteen blast resistance genes — *Pib*, *Pik*, *Pik-h*, *Pik-m*, *Pik-s*, *Pi7*, *Pish*, *Pita*, *Pita-2*, *Piz-t*, *Pi(5)*t and *Piz-5* — were screened on 57 released varieties by using 13 linked markers; 12 Simple Sequence Repeats and one Insertion Deletion, through polymerase chain reaction based methods. The genetic frequencies of these 13 major blast resistance genes ranged from 43.86% (*Pik-m*) to 7.02% (*Piz-t*) whereas *Pita* resistance gene amplicon was not observed on all tested varieties. The *Pik-m* gene was detected as the most prevalent one amongst the genotypes followed by *Pi7* which distributed in frequency of 42.11%, *Pik* , *Pita-2* and *Pi5*(t) (22.81%), *Pik-s* (21.05%) , *Pib and Pik-h* (19.30%), *Pik* (17.54%), *Piz-5* (14.04%) and *Pish* (12.28%). The genotypic variation between the released varieties was detected. Among 57 released varieties, two varieties — Manawthuka and Mote Soe Ma Kyway Pyay line MMK 03-23-3 — possessed seven blast resistance genes the other 12 varieties carried single genes, five varieties five resistance genes, 13 varieties four resistance genes, six varieties three resistance genes and 15 varieties two resistance genes. In the rest four varieties resistance genes could not be amplified by tested markers. This study provided the information of resistant varieties that will be beneficial in pre-breeding program for developing of rice blast resistance varieties.

Keywords: blast, insertion deletion, resistance gene, rice, simple sequence repeats

[1] Department of Plant Pathology, Yezin Agricultural University
[2] Department of Agronomy, Yezin Agricultural University
[3] New Genetics Division, Advanced Centre for Agricultural Research and Education, YAU
[4] Seed Bank, Department of Agricultural Research, Yezin

Introduction

Rice diseases were one of the major yield limiting factors in Myanmar. Among them, blast is one of the most serious when its incidence starts in seedling stage of susceptible variety. The occurrence of blast had been reported in lowland ecosystem in Ayeyawady (CARI 2000). In 2002-2003 cold and dry seasons, leaf blast epidemic followed by neck blast was detected around Yezin area on the variety IR50 (Naing 2004). Aye et al. (2015) reported the occurrence of blast disease in 2013 early summer and 2014 rainy season in Nay Pyi Taw Union Territory. During 2015 to 2018 rice growing season, the leaf blast and neck blast occurrence was observed in Aungban, Pindaya, Taunggyi, Kyaukme, Yezin, Lapputa and Bago (Khaing et al. 2018). Blast disease was a significant problem in temperate regions but at the present time it has been found in different ecosystems such as irrigated lowland, rain fed lowland and upland areas (Zaw et al. 2015; Khaing et al. 2018). In blast disease management; varietal susceptibility, pathogen virulence and climatic conditions play as major roles. Chemical control is practical (Gouramanis 1995), but the use of resistant varieties to disease is the most effective control measure (Hayashi et al. 2006).

The major constraints in using resistant varieties are the rapid adaptability of fungus, durability of genetic resistance and breakdown of resistance due to the newly evolved virulent races (Wang et al. 2014). To develop rice varieties with more durable blast resistance, two or more resistance genes must be incorporated into individual varieties as different resistance genes provide resistance to different isolates. Marker assisted selection (MAS) has been shown to be especially valuable in backcross breeding. In addition, MAS is a powerful tool for pyramiding two or more genes affecting blast resistance. Many type of polymerase chain reaction (PCR) based DNA markers have been developed in which Simple Sequence Repeats (SSRs) markers are cost-effective and useful for applied breeding (Koide et al. 2009). During 2012-2013 growing season, 389 rice test lines were screened against

blast disease at three different locations —Yezin, Aungban and Kyaukme — under natural incidence and it was observed that 48 tested lines showed resistant to disease. Then, those resistance lines had been screened for resistance genes *Pi-9* and *Piz-t* through molecular markers. Moreover, 33 Myanmar landraces have been screened for blast resistance genes; *Pi2-l*, *Pi2-2*, *Pi9-1*, *Pi9-2* and *Piz-t* by using molecular markers (DAR 2018). Currently, a large number of rice varieties resistance to biotic and abiotic stress have been released by Department of Agricultural Research and Department of Agriculture. But the rice varieties resistance to blast is still limited. Therefore, the present study was conducted with the aim to detect the resistant genotypes in cultivated rice in Myanmar to provide the information to develop blast resistant varieties.

Materials and Methods

Plant materials and Genomic DNA extraction

Twenty differentials (IRRI bred blast resistance lines), provided by International Rice Research Institute, were used as checks for their respective genes. Total 77 rice varieties including 57 released varieties provided by Myanmar Seed Bank were pre-germinated in germinator for 3 days at 30°C and sown in plastic bag containing 1.5 kg of soil. Leaves from 4 weeks old seedlings were cut and kept in plastic bag and stored at –20°C refrigerator. Total genomic DNA was extracted from frozen leave tissues using the Cetyl Tri Methyl Ammonium bromide (CTAB) methods with some modification following the protocol described by Ahmadikhah (2008). The quantity and quality of purified genomic DNA was estimated using NanoDrop ND-2000 (Version 1.3.1, Thermofisher scientific, USA).

Test primers

Twelve Simple Sequence Repeat (SSR) markers and one Insertion Deletion (InDel) marker were used to analyze the estimation of resistance genes. The information about the tested markers was shown in Table 1.

Resistance genes detection

PCR amplification was carried out in 10 µl of reaction mixture containing 50 ng template rice genomic DNA, 10X Ex Taq buffer (10X Tris with 20 mM $MgCl_2$), 25 mM dNTPs, 0.5 µl (10 pmol) of each primer, 1 unit of Taq polymerase (Takara) by using Applied Biosystem 2720 thermal cycler. Thermal cycler program for PCR comprised one cycle of 4 minutes at 94°C ; 35 cycles of 95°C for 45 seconds, 55°C to 65°C for 30 seconds, 72°C for 30 seconds and ending up with 7 minutes at 72°C for the final extension. Annealing temperature was adjusted based on the specific requirement of different primer combinations and annealing temperature for each primer pairs were shown in Table 1. The amplified PCR products were loaded by electrophoresis in 3% agarose gel stained with Ethidium Bromide (0.5 µg ml^{-1}) prepared in 0.5X TBE buffer at a constant voltage of 200 V approximately 50 minutes and visualized in UV trans illuminator and SSR bands scores were performed using by a gel documentation system (BIO-RAD, USA).

Results and Discussion

Genotypic screening of 57 released rice varieties scored as (1) for presence and (0) for absence of amplicons generated by 12 SSR and 1 InDel markers was shown in Table 2. The estimation of PCR results for *Pib* blast resistance gene was detected by visualization of amplicons on 173 bp of positive fragments by using SSR primer RM208 on the chromosome number 2. The allele size of individual variety was compared with the allele size of IRBLb-IT 13 check variety by RM208 marker. It was observed that 11 genotypes could be amplified by using RM208 marker while 46 genotypes could not be amplified (Table 2).

For *Pik-s, Pik-h and Pik-m* resistance genes on chromosome 11 were detected by using RM144 and RM206 SSR markers and k2167 InDel marker, respectively. The check varieties were IRBLks-CO for *Pik-s*, IRBLkh-K3 for *Pik-h* and IRBLkm-Ts for *Pik-m* gene. The positive bands were detected at 237 bp on 12 varieties compared with the check IRBLks-

CO (Plate 2), 147 bp on 11 varieties compared with IRBLkh-K3 PCR product (Plate 3) and 310 bp on 25 varieties compared with IRBLkm-Ts's PCR product individually (Plate 4). Out of 57 released varieties, Manawthuka (Code no.1) and MMK 03-23-3 showed the positive product for *Pik-s*, *Pik-h* and *Pik-m* resistance genes (Table 2).

The PCR results of *Pik* genes were estimated by visualization of amplicons produced at 157 bp and 175 bp on chromosome number 11 compared with the product size of check IRBLk-Ku and IRBLk-Ka by amplifying with RM224 and RM1233 respectively. Among 57 varieties, the resistance genes of 10 varieties were amplified as 157 bp of product by RM224 marker (Plate 5) and of 13 varieties were amplified as 175 bp by RM1233 marker (Plate 6 and Table 2).

The genetic diversity of *Pi7* and *Pi5*(t) on chromosome 11 was estimated by positive fragment as 116 bp by amplifying RM229 and 157 bp by amplifying RM21 individually. The corresponding PCR products were linked with the resulted positive bands of check varieties IRBL7-M for *Pi7* and IRBL5-M for *Pi5*(t). The 24 varieties revealed the positive bands for *Pi7* resistance gene (Plate 7) and 13 varieties for *Pi5*(t) genes (Plate 8), whereas presence score (1) of the resistance genes were detected in 5 varieties such as Ayeyarmin, Hnan Kar, Yeanelo 2, Sambha- Mahari Sub1 and Manawthuka (Code no.42) by both SSR markers (Table 2).

The presence or absence of PCR product amplified by RM247 and RM7102 were checked as the size 131 bp and 169 bp of positive band based on their sequence on chromosome 12. None of the genotypes could produce 131 bp amplicon corresponding to the check IRBLta-Ya for *Pita* gene (Plate 9). The presence of 169 bp amplicon was detected on 13 varieties conforming to the check IRBLta2-Pi for *Pita-2* (Table 2 and Plate 10).

The estimation of PCR results for *Piz-5* and *Piz-t* rice blast resistance genes were detected by visualization of amplified product of 233 bp and 140 bp of positive fragment

using SSR primer RM527 and RM225 on chromosome number 6. *Piz-5* gene was scored as presence on 8 released varieties conforming to the resistance check IRBLz5-CA (Plate 11) and for *Piz-t* gene, 4 released varieties were scored as positive corresponding to IRBLzt-IR56 check (Table 2 and Plate 12).

The SSR marker RM5811 is linked to blast resistance gene *Pish* on chromosome 1, and shown the presence of 97 bp fragment specific for *Pish* mediated blast resistance gene in the differential line IRBLsh-B. The presence of positive amplicons was observed in 7 genotypes (Table 2 and Plate 13).

It was mentioned that the genes affecting blast resistance are localized on chromosomes 6, 11 and 12 (Kodie et al. 2009). In this study, seven blast resistance genes located on chromosome number 11, two resistance genes on chromosome number 6, two resistance genes on chromosome number 12, one resistance gene on chromosome number 1 and one resistance gene on chromosome number 2 were detected by using molecular markers through PCR based method. The genetic frequencies ranged from 43.86% (*Pik-m*) to 7.02% (*Piz-t*) whereas *Pita* resistance gene amplicon was not observed on all tested varieties. The *Pik-m* gene was resulted as the most prevalent one amongst 57 genotypes followed by *Pi7* which distributed 42.11%. In tested varieties, the resistance genes *Pik* (IRBLk-Ka), *Pita-2* and *Pi5*(t) were detected with the same frequency of 22.81% and the presence of these 3 genes amplicon could be detected in Manawthuka (Code no.42), Yeanelo 2 and Yeanelo 4 (Table 2). The resistance gene *Pik-s* distributes 21.05% followed by *Pib* and *Pik-h* (19.30%), *Pik* (IRBLk-Ku) (17.54%), *Piz-5* (14.04%) and *Pish* (12.28%).

In blast disease management, use of resistant varieties to pathogen is crucial, therefore many researchers conducted molecular screening and found out genetic diversities of major rice blast resistance genes. Singh et al. (2015) screened 10 major blast resistance genes on 192 accessions and reported that the blast resistance gene *Piz-5* was widely distributed

(52.59%) and *Pita* was found to be 19.79% of 192 accessions. Imam et al. (2014) found that the genetic frequency of nine major rice blast resistance genes *Piz, Piz-t, Pik, Pik-p, Pik-h, Pita/Pita2, Pita, Pi9,* and *Pib* ranged from 6 to 97% in the selected set of 84 rice gernplasm.

The results pointed out the genotypic variation between the released varieties. Two varieties namely Manawthuka and Mote Soe Ma Kyway Pyay line MMK 03-23-3 possessed seven blast resistance genes. Among 57 released varieties, five varieties showed positive fragment of five resistance genes, 13 varieties had four resistance genes, six varieties showed the present of three resistance genes and 15 varieties revealed at least two resistance genes and 12 varieties carried single blast resistance genes in their genotypes. The resistance gene in other four released varieties such as Shwe Pyi Hmwe, Shwe Asean, Khun War Taung Pyan and Bay Kyar Taung Pyan could not be detected by the tested markers (Table 2).

As the information of resistant genotype of rice varieties was mainly involved in blast management program, many researchers worked out for blast resistance genes by using microsatellite markers and then more than 100 blast genotypes were identified currently, including 45% from japonica, 51% from indica and 4% from other genotypes (Xioa et al. 2010; Sharma et al. 2012). But the effectiveness of resistance genes varied depending upon their association in rice genotype. Khan et al. (2014) reported that the genes *Pish, Pi9, Pita-2,* and *Pita* were effective in Bangladesh. In addition, Imam et al. (2014) described that the genes *Pi9, Pita-2* and *Piz-t* were more effective than the others in preventing blast infection. Variar et al. (2009) also reported that in India, *Pik-m* multi-gene family and *Pib* genes were moderately distributed among the tested varieties but none of the varieties possessing these genes showed resistance reaction in disease resistance evaluation study.

In Myanmar, more than 100 rice varieties were released for various ecosystems by Department of Agricultural Research. According to surveyed data during 2010-2015, farmers preferred to grow Manawthuka, Sin Thu ka, Sin Thwe Latt, Shwe War Tun, Ayeyarmin,

Thee Htat Yin and Shwe Thwe Yin in pre/post monsoon seasons (Hlaing et al. 2017). Among these varieties, Shwe Thwe Yin was reported to be susceptible to blast disease in Yezin area (Naing 2004; Aye et al. 2014 and Zaw et al. 2015). As benefit cost ratio for Shwe Thwe Yin was calculated as 1.5 (Htwe et al. 2017), this variety should be emphasized in varietal improvement for blast resistance. Zaw et al. (2016) reported that the resistance genes *Piz*, *Pita2*, *Pib* and *Pi-sh* could be used as a source of resistance genes for rice breeding program to manage blast disease in Myanmar. The present study provided the information of resistance varieties that will be beneficial in pre-breeding program for development of rice blast resistance varieties.

Acknowledgements

We would like to acknowledge Myanmar Seed Bank by providing tested rice varieties and Dr. A. Yoshimura, Director, Rice Genome Project, Department of Agricultural Research (DAR), for his kind encouragement for providing tested primers. We also thankful to Daw Tin Tin Myint, Deputy Director General (Retd.), (DAR), and Dr. R. K. Singh for their kind support of providing IRRI bred differential materials.

REFERENCE

Ahmadikhah, A. 2008. A rapid mini-prep DNA extraction method in rice (*Oryza sativa* L.). African Journal of Biotechnology. 8 (2): 234 - 238.

Aye, K. S., N. Win, H. W. Hnin, M. Z. Aung, W. M. Sai, T. T. Maung, S. S. Aye, and T. A. A. Naing. 2015**.** Occurrence of rice diseases in Nay Pyi Taw Council Area. Journal of Agricultural Research, Yezin Agricultural University, Myanmar. 2 (1): 46 - 56.

CARI (Central Agricultural Research Institute). 2000. Research findings of CARI. Myanmar Agricultural Services.

Cuong, N. M., T. Lang, and B. C. Buu. 2006. Applications of microsatellite and sequence tagged site marker to detect blast resistance in local rice (*Oryzae sativa* L.). Hanoi: Nong Nghiep, pp. 52 - 68.

DAR (Department of Agricultural Research). 2018. Research highlights during 2011-2012 to 2015-2016. Department of Agricultural Research, Yezin, Nay Pyi Taw, Myanmar. Organized by Asian Food and Agricultural Cooperation Initiative (AFACI-ATIN).

Eizenga, G. C., H. A. Agrama, F. N. Lee, W. G. Yan, and Y. Jia. 2006. Identification of novel resistance genes in newly introduced blast resistance rice germplasm. Crop Science. 46: 1870 - 1878.

Fjellstrom, R., A. M. M. Clung, and A. R. Shank. 2006. SSR markers closely linked to the genetic resistance to rice blast disaease. In: Rice - Germplasm, Genetics and Improvement,pp. 195-217.

Fuentes, J. L., F. J. C. Victiria, F. Escobar, G. Prado, G. Aricapa, M. C. Duque, and J. Tohme. 2007. Identification of microsatellite markers linked to the blast resistance gene *Pi-1(t)* in rice. Euphytica. 160: 295 - 304.

Gouramanis, G. D. 1995. Biological and chemical control of rice blast disease (*Pyracularia oryzae*) in Northern Greece. CIHEAM. Options Mediterraneennes. 15p.

Hayashi, K., H. Yoshida, and I. Ashikawa. 2006. Development of PCR-based allele specific and Indel marker sets for nine rice blast resistance genes. Theoretical and Applied Genetics. 113 (2): 251 - 260.

Hlaing, S. M., M. Y. Thwe, P. W. Aung, and T. L. Oo. 2017. History of Department of Agricultural Research and Status of DAR Released Crop Varieties Grown in Myanmar. In Proceeding of the First Agricultural Research Conference. Department of Agricultural Research, Yezin, Nay Pyi Taw. pp. 203 - 210.

Htwe, K. K., K. K. Thet and S. Ni. 2016. Agricultural Situation and Needs Assessment of Farmers in Nay Pyi Taw and Dry Zone Regions in Myanmar. Myanmar Agricultural Research Journal, 2: 233-240.

Imam, J., S. Alam, N. P. Mandal, M. Variar, and P. Shukla. 2014. Molecular screening for identification of blast resistance genes in North East and Eastern Indian rice germplasm (*Oryza sativa* L.) with PCR based makers. Euphytica. 196: 199 -211.

Khaing, T. Y., N. K. K. Win, K. K. Win, and T. A. A. Naing. 2018. Race study of *Pyricularia oryzae* cav. causing rice blast from different rice growing areas of Myanmar. Proceeding of the 11th Agricultural Research Conference 17-18 January, Yezin Agricultural University, Nay Pyi Taw. pp. 57-68

Khan, G. H., A. B. Shikari, R. Vaishnavi, S. Naieeb, B. A. Padder, Z. A. Bhat, G. A. Parray, N. A. Bath, R. Kumar, and N. K. Singh. 2014. Marker-assisted introgression of three dominant blast resistance genes into an aromatic rice cultivar Mushk Budji. Nature. 8 (1): 1 - 3.

Koide. Y., N. X. D. Kobayashi, and Y. Fukuta. 2009. Blast resistance genes and their selection markers in rice (*Oryza sativa* L.). In: JIRCAS working report, 63, pp. 95 - 119.

Liu, S. P., X. Li, C. Y. Wang, X. H. Li, and Y. Q. He. 2002. Improvement of resistance to rice blast in Zhenshan 97 by molecular marker- aided selection. Acta Botanica Sinica, 45: 1346 - 1350.

Naing, T. A. A. 2004. Rice production in Myanmar and effects of intensification on crop health. Cuvillier, Doctoral Dissertation, University of Kassel, pp. 81 - 83.

Sharma, T. R., A. K. Rai, S. K. Gupta, J. Vijayan, B. N. Devanna, and S. Ray. 2012. Rice blast management through host-plant resistance: retrospect and prospects. Agricultural Research, 1: 37 - 52.

Sharma, T. R., M. S. Madhav, B. K. Singh, P. Shanker, T. K. Jana, V. Dalal, A. Pandit, A. Singh, K. Gaikwad, H. C. Upreti, and N. K. Singh. 2005. High-resolution mapping, cloning and molecular characterization of the *Pi-kh* gene of rice, which confers resistance to *Magnaporthe grisea*. Molecular Genetics And Genomics. 274: 569 - 578.

Singh, A. K., P. K. Singh, M. Arya, N. K. Singh, and U. S. Singh. 2015. Molecular screening of blast resistance genes in rice using SSR markers. Journal of Plant Pathology. 31 (1): 12 - 24.

Variar, M. 2007. Pathogenic variation in *Magnaporthae grisea* and breeding for blast resistance in India. In: JARCAS (Japan International Research Center for Agricultural Sciences, Tsukuba, Japan) Working Report, 53, pp. 87 - 95.

Wang, X., S. Lee, J. Wang, J. Ma, T. Bianco, and YulinJia. 2014. Current advances on genetic resistance to rice blast disaease. In: Rice - Germplasm, Genetics and Improvement, pp. 195-217.

Xiao, W. M., Q. Y. Yang, H. Wang, T. Guo, Y. Z. Liu, X. Y. Zhu, and Z. Q. Chen. 2010. Identification and fine mapping of a resistance gene to *Magnaporthe oryzae* in a space-induced rice mutant. Molecular Breeding, 28: 303 - 312.

Zaw, M., N. L. Oo, Z. M. Tun, and T. A. A. Naing. 2015. Evaluation of different rice genotypes against rice blast disease in Yezin area. Journal of Agricultural Research, Yezin Agricultural University, Myanmar, 2 (1): 41 - 45.

Zaw, M., S. S. Aye, and M. Matsumoto. 2016. Genetic characteristics of *Pyricularia grisea* and their pathotypes in Myanmar. Bulletin of the Institute of Tropical Agriculture, 39: 103 -111

Table 1 The information of the test primers for analyzing resistance genes on 57 released rice varieties

Linked marker	R gene	Type	Anne-aling tempe rapture (°C)	Forward and Reverse primer sequence	Chromosome no.	Expected PCR product size (bp)	Reference
RM208	***Pib***	SSR	55	**5′-3′**TCTGCAAGCCTTGTCTGATG **3′-5′**TAAGTCGATCATTGTGTGGACC	2	173	Hayashi et al. 2006
RM144	***Piks***	SSR	55	**5′-3′**TGCCCTGGCGCAAATTTGATCC **3′-5′**GCTAGAGGAGATCAGATGGTA GTG CATG	11	237	Hayashi et al. 2006
RM206	***Pik-h***	SSR	55	**5′-3′**CCCATGCGTTTAACTATTCT **3′-5′**CGTTCCATCGATCCGTATGG	11	147	Sharma et al. 2005
k 2167	***Pik-m***	InDel	65	**5′-3′**CGTGCTGTCGCCTGAATCTG **3′-5′**CACGAACAAGAGTGTGTCGG	11	310	Fjellstrom et al. 2006
RM224	***Pik***	SSR	53	**5′-3′**ATCGATCGATCTTCACGAGG **3′-5′**TGCTATAAAAGGCATTCGGG	11	157	Fuentes et al. 2007
RM1233	***Pik***	SSR	51	**5′-3′**TTCGTTTTCCTTGGTTAGTG **3′-5′**ATTGGCTCCTGAAGAAGG	11	175	Fjellstrom et al. 2006
RM229	***Pi7***	SSR	55	**5′-3′**CACTCACACGAACGACTGAC **3′-5′**CGCAGGTTCTTGTGAAATGT	11	116	Hayashi et al. 2006
RM21	***Pi5(t)***	SSR	57	**5′-3′**ACAGTATTCCGTAGGCACGG **3′-5′**GCTCCATGAGGGTGGTAGAG	11	157	Cuong et al. 2006
RM247	***Pita***	SSR	55	**5′-3′**TAGTGCCGATCGATGTAACG **3′-5′**CATATGGTTTTGACAAAGCG	12	131	Eizenga et al. 2006
RM7102	***Pita-2***	SSR	55	**5′-3′**TTGAGAGCGTTTTTAGGATG **3′-5′**TCGGTTTACTTGGTTACTCG	12	169	Liu et al. 2002
RM527	***Piz-5***	SSR	55	**5′-3′**GGCTCGATCTAGAAAATCCG **3′-5′**TTGCACAGGTTGCGATAGAG	6	233	Fjellstrom et al. 2006
RM225	***Piz-t***	SSR	53	**5′-3′**TGCCCATATGGTCTGGATG **3′-5′**GAAAGTGGATCAGGAAGGC	6	140	Hayashi et al. 2006
RM5811	***Pish***	SSR	65	**5′-3′**GGATTTGGTCGAACAGGTTG **3′-5′**TTCGCGCTCTCCAAGCTC	1	97	Fjellstrom et al. 2006

Table 2 The genotypic analysis of 57 released varieties for blast resistance genes

Sr. No.	Code No.	Variety name	Blast Resistance gene													Total no. of gene
			Pib	*Pik-s*	*Pik-h*	*Pik-m*	*Pik*	*Pik*	*Pi7*	*Pi(5)t*	*Pita*	*Pita-2*	*Piz-5*	*Piz-t*	*Pish*	
1	17	Ayekarihmwe	0	0	1	0	0	0	0	0	0	0	0	0	0	1
2	5	Ayeyarmin	0	0	0	1	0	0	1	1	0	0	1	0	0	4
3	51	Bay Kyar Taung Pyan	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	30	BR 11 Sub 1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
5	57	Bu Kyauk	0	1	0	1	0	0	0	0	0	0	1	0	0	3
6	10	Hmawbi 2	1	1	0	0	0	1	0	0	0	1	0	0	0	4
7	7	Hnan Kar	0	1	0	0	0	1	1	1	0	0	1	0	0	5
8	24	Hsarngankhansinthwelatt	0	0	0	0	1	0	0	0	0	1	0	0	0	2
9	52	Innmayebaw	0	0	1	0	0	1	0	0	0	0	0	0	0	2
10	38	IR 747	0	0	0	1	0	0	0	0	0	0	0	0	0	1
11	28	IRRI 119	0	0	0	1	0	0	0	1	0	1	0	0	0	3
12	48	Khun War Taung Pyan	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	56	Kone Myint 2	0	1	0	1	0	0	0	0	0	0	0	0	0	2
14	35	KTD 6	0	0	0	1	0	0	1	0	0	0	0	0	0	2
15	4	Kyaw Ze Ya	1	1	1	0	0	0	1	0	0	0	0	0	0	4
16	1	Manawthuka	0	1	1	1	0	1	0	0	0	0	1	0	0	5
17	42	Manawthuka	0	1	0	1	1	1	1	1	0	1	0	0	0	7
18	54	MMK 03-23-3	1	1	1	1	0	0	0	1	0	0	1	0	1	7
19	55	MMK 03-25-2	1	1	0	1	0	0	0	0	0	0	1	0	1	5
20	53	MMK 03-8-1	1	1	0	1	0	0	0	0	0	0	1	0	1	5
21	36	MR 9	0	0	0	0	0	0	1	0	0	0	0	0	0	1
22	20	Myaungmyamay	0	0	0	0	1	0	1	0	0	0	0	0	0	2
23	49	Nga Kywe	0	0	0	0	0	1	0	0	0	0	0	0	0	1
24	50	Nga Kywe Taung Pyan	0	0	0	0	0	0	0	0	0	0	0	0	1	1
25	46	Paw San	1	0	0	0	0	0	0	0	0	0	0	1	0	2
26	47	Paw San Latt	0	0	0	1	0	1	0	1	0	0	0	0	0	3
27	8	Paw San Yin	0	0	0	0	0	0	1	0	0	0	0	0	1	2
28	25	Pyi Myanmar Sein	0	0	0	1	1	0	0	1	0	1	0	0	0	4
29	18	Pyi Taw Yin	0	0	0	0	1	0	1	0	0	0	0	0	0	2
30	27	Sambha- Mahari Sub 1	0	0	0	1	0	0	1	1	0	1	0	0	0	4
31	26	Shwe Asean	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	34	Shwe Manaw	0	0	0	1	0	0	1	0	0	0	0	0	0	2
33	41	Shwe Myanmar	0	0	0	0	0	0	0	1	0	0	0	0	0	1
34	16	Shwe Pyi Hmwe	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	32	Shwe Pyi Htay	0	0	1	0	0	0	0	0	0	0	0	0	1	2
36	43	Shwe Pyi Tan	0	1	0	1	0	0	0	0	0	1	0	0	0	3
37	11	Shwe Thwe Yin	0	0	0	0	0	0	1	0	0	0	0	0	0	1
38	6	Shwe War Tun	1	0	0	1	1	0	1	0	0	0	0	0	0	4
39	40	Shwe Yin Aye	0	0	0	1	0	0	0	0	0	0	0	0	0	1
40	14	Sin Aye Kari	0	0	0	0	0	1	1	0	0	1	0	0	0	3
41	2	Sin Thu Ka	0	0	0	0	1	0	1	0	0	0	0	0	0	2
42	3	Sin Thwe Latt	0	0	1	1	0	0	0	0	0	0	0	0	0	2
43	29	Swarna Sub 1	0	0	0	0	0	1	1	0	0	0	0	1	0	3
44	13	TDK 1 Sub 1	0	0	0	0	1	1	1	0	0	1	0	0	0	4
45	9	Thee Htat Yin	0	1	0	0	0	0	1	0	0	0	0	0	0	2
46	19	Thiri Thuka	0	0	0	0	0	0	1	0	0	0	0	0	0	1
47	44	Thuka Hmwe	0	0	0	1	0	0	0	0	0	0	0	0	0	1
48	39	Thuka Yin	0	0	0	1	0	0	0	0	0	0	0	1	0	2
49	33	Yar 2 Tun	0	0	0	1	0	0	0	1	0	0	0	0	0	2
50	15	Yar 8	0	0	1	1	0	0	1	0	0	1	0	0	0	4
51	12	Yatana Toe	0	0	0	0	0	0	1	0	0	1	1	1	0	4
52	21	Yeanelo 1	1	0	1	0	0	1	0	1	0	0	0	0	0	4
53	22	Yeanelo 2	0	0	0	0	1	1	1	1	0	1	0	0	0	5
54	23	Yeanelo 3	1	0	0	1	1	0	1	0	0	0	0	0	0	4
55	45	Yeanelo 4	1	0	0	0	0	1	0	1	0	1	0	0	0	4
56	31	Yet 100	0	0	1	0	0	0	0	0	0	0	0	0	0	1
57	37	Yezin Lone Thwe	1	0	1	1	0	0	0	0	0	0	0	0	1	4
		Total	**11**	**12**	**11**	**25**	**10**	**13**	**24**	**13**	**0**	**13**	**8**	**4**	**7**	

Resistance genes were scored as (1) for presence and (0) for absence of positive fragment of respective marker

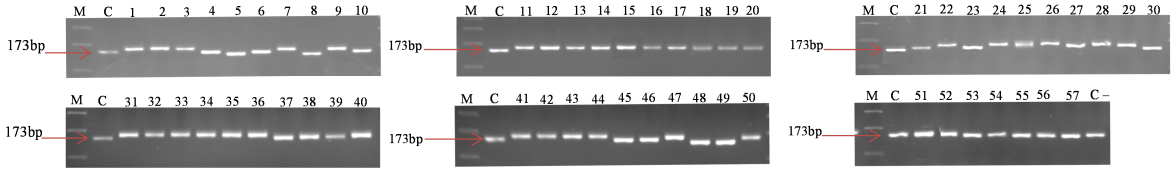

Plate 1 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM208 SSR marker where lane; M= 100bp DNA marker, C= IRBLb-IT 13, C– = CO39 and number1-57 represent code no. of released rice varieties as described in Table 2

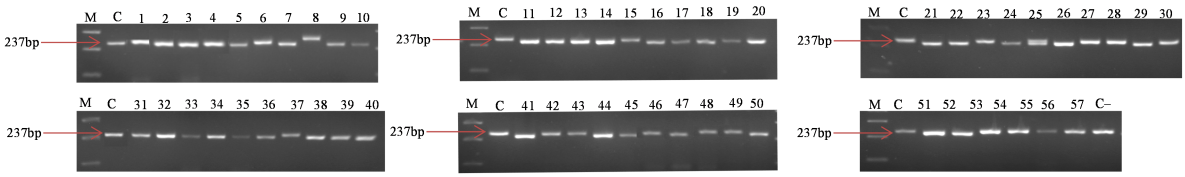

Plate 2 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM144 SSR marker where lane; M= 100bp DNA marker, C= IRBLks-CO, C– = CO39 and number 1-57 represent code no. of released rice varieties as described in Table 2

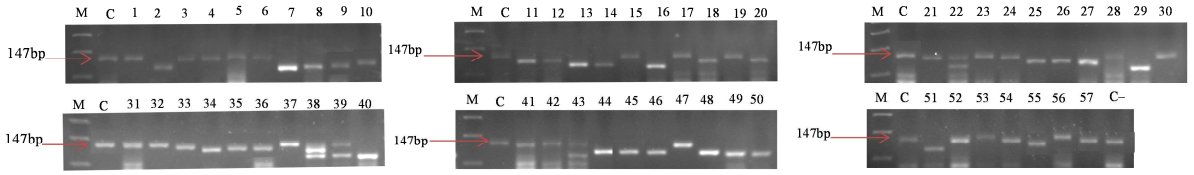

Plate 3 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM206 SSR marker, where lane; M= 100bp DNA marker, C= IRBLkh-K3, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

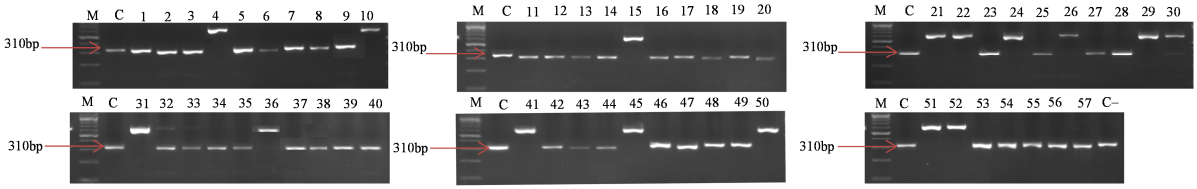

Plate 4 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using k2167 InDel marker, where lane; M= 100bp DNA marker, C= IRBLkm-Ts , C– = CO39, and 1-57 represent code no. of released rice varieties as described in Table 2

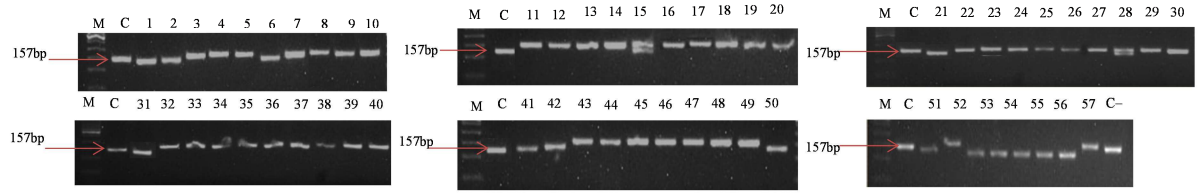

Plate 5 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM224 SSR marker where lane; M= 100bp DNA marker, C= IRBLk-Ku, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

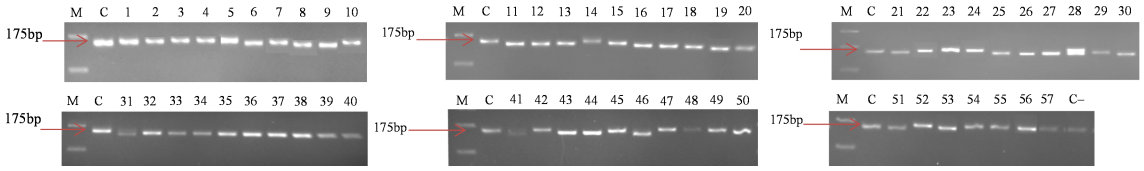

Plate 6 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM1233 SSR marker where lane; M= 100bp DNA marker, C= IRBLk- Ka, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

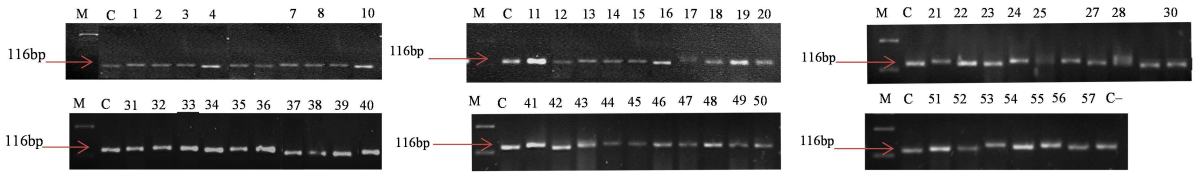

Plate 7 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM229 SSR marker, where lane; M= 100bp DNA marker, C= IRBL7-M, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

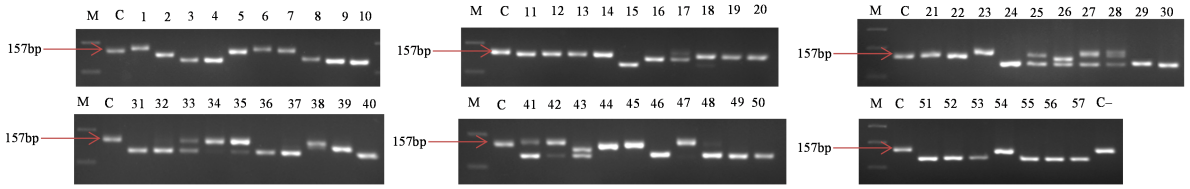

Plate 8 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM21 SSR marker where lane; M= 100bp DNA marker, C= IRBL5-M, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

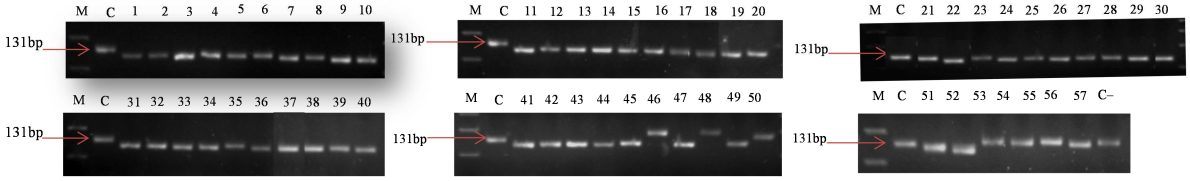

Plate 9 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM247 SSR marker where lane; M= 100bp DNA marker, C= IRBLta-Ya, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

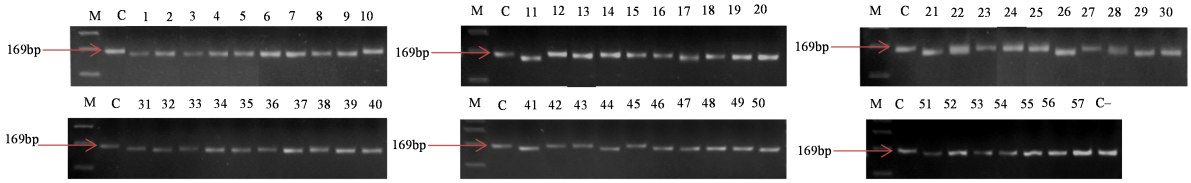

Plate 10 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM7102 SSR marker where lane; M= 100bp DNA marker, C= IRBLta2-Pi, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

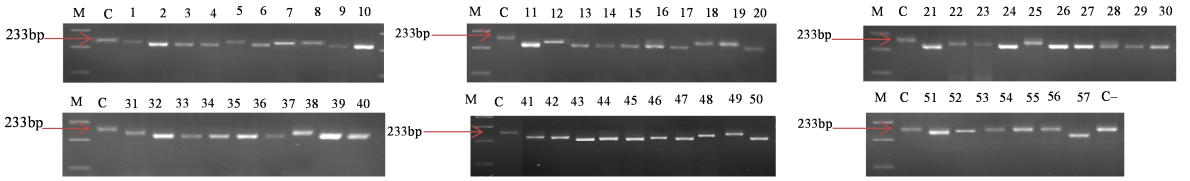

Plate 11 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM527 SSR marker where lane; M= 100bp DNA marker, C= IRBLz5-CA, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

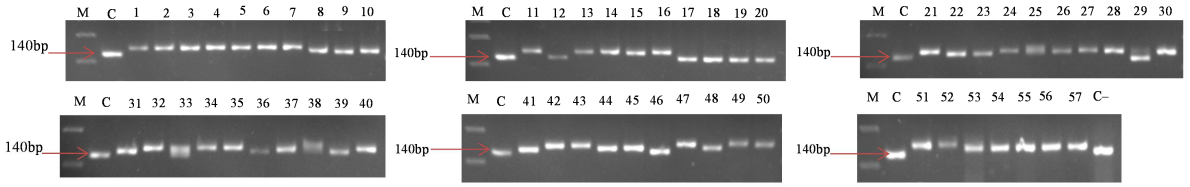

Plate 12 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM225 SSR marker, where lane; M= 100bp DNA marker, C= IRBLzt-IR56, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2

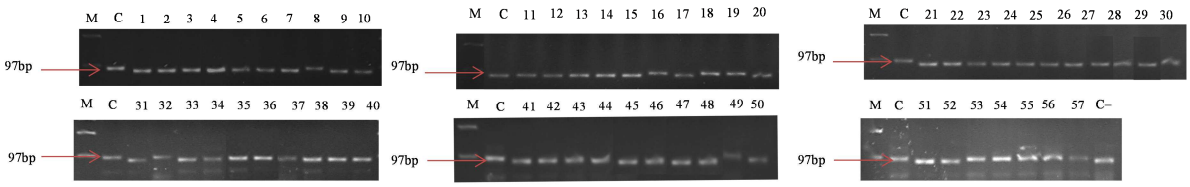

Plate 13 Agarose gel electrophoretic pattern of 57 released rice varieties generated by using RM5811 SSR marker, where lane; M= 100bp DNA marker, C= IRBLsh-B, C– = CO39 and 1-57 represent code no. of released rice varieties as described in Table 2