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Genetic diversity analysis of rice landraces (*Oryza sativa* L.) for salt tolerance using SSR markers in Bangladesh

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ABSTRACT

Large cultivable area lies in the costal saline zone of Bangladesh where rice cultivation is largely affected by the salinity. This problem can be effectively addressed by identifying salt-tolerant landraces using modern biotechnological method. Assessment of genetic diversity of rice (*Oryza sativa* L.) is an important tool for rice breeding and an essential component in germplasm characterization and conservation. The objective of this study was to assess the genetic diversity among 7 landraces along with 3 released variety of rice using SSR marker. A total of 31 reproducible polymorphic alleles were identified from the loci with an average of 5.167 alleles per locus (ranges from 4–7). The polymorphism information content (*PIC*) value is a reflection of allelic diversity and frequency among the varieties. *PIC* value of each marker was evaluated on the basis of the number of alleles and it varied greatly for all the SSR loci tested. *PIC* values enumerated from the data obtained from allelic variation from 0.595 (RM8094) to 0.797 (AP3206) with an average of 0.697. The average genetic diversity over all SSR loci for the 10 genotypes was 0.740, ranging from 0.660 to 0.820. Positive correlations were found between gene diversity, *PIC* value and number of allele. These findings can have the potential role for further improvement of salinity tolerance rice genotypes through marker-assisted breeding.

Keywords: Rice, genetic diversity, salt tolerance, *PIC*, SSR

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1 Introduction

Rice (*Oryza sativa* L.) hold about one fifth to the total land area covered by cereals and it is the principal staple food for more than 50% of the world's population (Chakravarthi and Naravaneni, 2006). Rice is cultivated under diverse eco-geographical conditions in tropical and subtropical countries like Bangladesh and other Asian countries. Salt stress is a foremost restriction to cereal production worldwide (Tuteja et al., 2012). In Bangladesh, about 2.8 million hectares of coastal soil has become saline due to dense withdrawal of surface and groundwater for irrigation and intrusion of seawater. The total saline area covers a third of the 9 million hectares of total cultivated lands in Bangladesh (ABSPII, 2006). The coastal saline soils are distributed unevenly in 13 districts, covering portions of eight agro-ecological zones of Bangladesh (Seraj and Salam, 2000). Increase in salinity intrusion and expansion in soil salinity will have severe harm-

ful effects on agricultural crops. The food production appears to face a pronounced alarm in near future due to climate change. In Bangladesh, rice production may decrease by 10% and wheat by 30% by 2050 due to salinity (IPCC, 2007).

Assessment of genetic diversity is very important in plant breeding or in biotechnology, if selection is the basis of improvement. For the assessment or analysis of genetic diversity molecular markers have been superior to morphological, pedigree, heterosis and biochemical data (Melchinger et al., 1991). Genetic diversity is generally measured by genetic distance or genetic similarity, which imply that there are either differences or similarities at the genetic level of the plant (Weir, 1990). Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space (Duvick, 1984). As the variation among the genotypes comes from the variations in DNA sequence, therefore, variations in DNA sequence are the basis of genetic diversity analysis (Semagn et al., 2006). Though, rice genome sequence is a valuable (IRGSP, 2005), most researchers are trying to identify particular segment of DNA or gene in a definite chromosome (Semagn et al., 2006). Molecular markers are the molecules that can trace a required gene in observed genotypes.

Molecular markers deliver evidence that can help to define the distinctiveness of germplasms and their ranking according to the number of close relative and their phylogenetic position. DNA-based SSR markers were co-dominant and highly polymorphic; deal an easy, perfect, and measurable degree of the genetic variation within crop plants (Collard and Mackill, 2008). Simple sequence repeat (SSR) markers have been proved to be ideal for making genetic maps (Islam, 2004; Niones, 2004), assisting selection (Bhuiyan, 2005) and studying genetic diversity in germplasms. Besides, SSR markers have been used effectively to map QTLs associated with salt tolerance (Lang et al., 2000, 2001; Singh et al., 2007). A major QTL located on chromosome 1 was identified for salt tolerance using F8 recombinant inbred lines (RILs) of Pokkali/IR29 cross (Gregorio, 1997).This segment of the chromosome 1 was further fine mapped by using near isogenic lines (NILs) of IR29 using Pokkali as the donor with SSR markers (Niones, 2004). For rice, there are nearly about 15,000 SSRs now available (www.gramene.org) and are currently being used to develop high density genetic maps, genotype rice accessions, determine the genetic structure and diversity patterns, optimize the assembly of core collections, and for marker-assisted breeding (McCouch, 2002; Yu et al., 2003; Garris et al., 2005).

Hence, the objective of the study was undertaken to access the genetic diversity of rice landraces along with the check varieties using molecular markers.

2 Materials and Methods

2.1 Plant material

The study was conducted at Plant Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh from June 2015 to December 2015. Ten rice genotypes, among which seven local landraces collected from Satkhira District and three check varieties from BINA and BRRI respectively were used in this study (Table 1).

Table 1. List of plant materials used in the study

Sl.	Genotype	Type	Source
1	Hogla	Landraces	Satkhira
2	DakhShail	Landraces	Satkhira
3	KutePatnai	Landraces	Satkhira
4	Ghunshi	Landraces	Satkhira
5	Mondeshor	Landraces	Satkhira
6	Tal Mugur	Landraces	Satkhira
7	Nona Bokhra	Landraces	Satkhira
8	Binadhan-8	Released variety	BINA†
9	Binadhan-10	Released variety	BINA
10	BRRI dhan47	Released variety	BRRI‡

† Bangladesh Institute of Nuclear Agriculture
‡ Bangladesh Rice Research Institute

2.2 Genomic DNA extraction

DNA was extracted from leaf tissues of 21 days old seedling following Cetyl Trimethyl Ammonium Bromide (CTAB) method (Stewart and Via, 1993).

2.3 Primer selection and DNA amplification

A total of six SSR markers were screened (Table 2) to yield amplification products on the total DNA obtained from the leaf tissues. The following PCR materials were used for PCR: 1.5μl 10$\times$ PCR buffer, 0.5μl Taq DNA polymerase, 1μl forward primer, 1μl reverse primer, 0.75μl dNTPs, 8.25μl sterilized dH_2O and 1μl template DNA for total volume of 14μl. For amplification, the thermal cycler was set at 1 cycle for 5 min at 94 °C as an initial hot start and strand separation step. This was followed by 2nd program having 34 cycles, which comprises denaturation (94 °C) for 1 min, annealing (55 °C) for 1 min and primer elongation (72 °C) for from 2 min. Finally, 1 cycle of 7 min at 72 °C was used for final extension and amplified products were stored at -10 °C until further use. The amplification products were separated on 1.5% agarose gels in 0.5$\times$ TBE buffer. The DNA band patterns were visualized under UV light and photographed using a polaroid camera.

2.4 Analysis of SSR data

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers and 1 kb DNA ladder using AlfaView software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and *PIC* values were determined using POWER MARKER version 3.25 (North Carolina, USA) (Liu and Muse, 2005), genetic analysis software. Molecular weights for microsatellite products, in base pairs, were estimated with Alpha Ease FC 4.0 software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. The *PIC* value described by Botstein et al. (1980) and modified by Anderson et al. (1993) for self-pollinated species was calculated as follows:

$$PIC_i = 1 - \sum \left(P_{ij}\right)^2 \quad (1)$$

where, P_{ij} is the frequency of jth allele for i marker and the summation extends upto the total number of allele for the given marker.

Genetic variation was measured in terms of genetic diversity and was computed by averaging *PIC* estimates over all loci (Weir, 1990). Number of alleles, average *PIC* values, and average *GS* were computed on the basis of different rice gene pools according to the results from cluster analysis and origin of the accessions. Differences in average *PIC* values between the three groups were evaluated by analysis of variance. *PIC* values were calculated for the accessions grouped in each gene pool at each locus. Loci were used as blocks to separate the variation among loci from the error term and increase the sensitivity of the statistical analysis. Heterogeneity (*HG*) by accession and by marker was calculated as percentage of heterogeneous loci per accession across all accessions and loci, respectively.

3 Results and Discussion

SSR markers are widely used for fingerprinting and diversity studies on rice cultivars and wild relatives due to its high polymorphic rates, which can be identified even at individual levels (Ni et al., 2002; Nagaraju et al., 2002). The level of polymorphism among 10 rice genotypes was evaluated by calculating allele number and *PIC* value at each of the 6 different loci. A total of 31 alleles were detected by 6 SSR markers across 10 rice genotypes with an average of 5.167 alleles per locus. The highest number of allele (7) was recorded in locus AP3206, followed by 6 alleles (RM490), 5 alleles (RM10748 & SalT-1) and 4 alleles (RM7075 & RM8094). The lowest number of alleles (4) was detected in loci RM7075 and RM8094 (Table 3). The average number of alleles per locus (5.167) observed, in present study, was very consistent with some earlier reports by Ni et al. (2002) and Kumar et al. (2010), who reported 6.8 and 6.13 alleles per locus, respectively.

The mean alleles (5.167) obtained in our study were higher compared to the result reported by Etemad et al. (2012), who detected 3.57 alleles per SSR locus using 13 Iranian and 13 Malaysian cultivars. Hossain et al. (2012) detected a total of 38 alleles in 12 aromatic rice landraces of Bangladesh using SSR markers. He also found average number of alleles (3.8) per locus, which is lower than our report. Some earlier reports by Pervaiz et al. (2010) and Rahman et al. (2012), who found an average of 4.4 and 4.18 alleles per locus respectively, is also markedly lower than our study. In contrast, the number of alleles detected in the present study was lower than the average number of alleles reported by Jayamani et al. (2007), Zeng et al. (2007) and Prathepha (2012), who reported an average of 7.7 and 11.85, alleles per locus. The variability in the number of alleles detected per locus might be due to the use of diverse genotypes and selection of different SSR primers with scorable alleles. The present study reveals that molecular characterization using SSR markers can be an efficient tools for genotyping the varieties with reasonable accuracy. All the gel pictures can be presented as Fig. 1 by providing (a)∼(f) to the individual gels of markers.

The frequency of most common allele at each locus ranged from 30% (RM490, AP3206 & SalT-1) to 40% (RM7075, RM8094 & RM10748) (Table 4). On an average, 35% of the 10 rice genotypes shared common major allele at any given locus. The genetic diversity of these 6 loci for the 10 rice landraces ranged from 0.660 to 0.820 with an average of 0.740, indicating a moderate level of diversity existing within the genotypes surveyed. The highest genetic diversity (0.820) was recorded in locus AP3206 and the lowest genetic diversity (0.660) was detected in locus RM8094 (Table 4). *PIC* value is a reflection of allelic diversity and frequency among the varieties. *PIC* value of each marker was evaluated on the basis of the number of alleles and it varied greatly for all the SSR loci tested. This the allelic diversity as well as the level of polymorphism among 10 rice genotypes was evaluated using 6 SSR loci and showed variability among markers.

Variation in allele number is also evident from *PIC* values varied widely among loci, which ranged from 0.595 to 0.797 with an average of 0.697. The highest *PIC* value (0.797) was obtained for AP3206, followed by RM8094 (0.595), RM7075 (0.645), RM10748 (0.675), SalT-1 (0.720) and RM490 (0.748) (Table 3). The *PIC* values observed in our study was consistent with previous estimates of SSR marker analysis in rice by Siwach et al. (2004), Jayamani et al. (2007) and Zeng et al. (2007). On the other hand, it was lower than that previously reported by Brondani et al. (2006) and Giarrocco et al. (2007) who observed an average *PIC*

Table 2. Information of SSR markers used for the study

Primer	Expected product size (bp)	Sequence (5′- 3′)	Annealing temp. (°C)
RM490	101	**F:** ATCTGCACACTGCAAACACC **R:** AGCAAGCAGTGCTTTCAGAG	55
RM7075	155	**F:** GCGTTGCAGCGGAATTTGTAGG **R:** CCCTGCTTCTCTCGTGCAGTCG	55
AP3206	167	**F:** GGAGGAGGAGAGGAAGAAG **R:** GCAAGAATTAATCCATGTGAAAGA	55
RM8094	209	**F:** AAGTTTGTACACATCGTATACA **R:** CGCGACCAGTACTACTACTA	55
RM10748	95	**F:** CATCGGTGACCACCTTCTCC **R:** CCTGTCATCTATCTCCCTCAAGC	55
SalT-1	159	**F:** GATGGTATTCATCGGCTACG **R:** AGTCCAAGAATGTCGTTTCG	55

Table 3. Data of summary statistics-I of 10 rice genotypes for 6 SSR markers

Locus	Repeat motif	Character location	Allele size range (bp)	No. of allele†	Rare allele	*PIC*‡
RM490	(CT)13	1	102-115	6	0	0.748
RM7075	(GA)16	7	149-152	4	0	0.645
AP3206	(GT)10	1	162-176	7	0	0.797
RM8094	(AT)31	1	214-224	4	0	0.595
RM10748	(AG)14	1	79-88	5	0	0.675
SalT-1	(GT)10	1	154-162	5	0	0.72
Mean				5.167	0	0.697

† Rare alleles are defined as alleles with a frequency less than 0.05 (5%);
‡ *PIC* = Polymorphism Information Content

Table 4. Data of summary statistics-II of 10 rice genotypes for 6 SSR markers

Locus	Sample size	Major allele†		Availability	Genetic diversity	Heterozygosity
		Size (bp)	Freq. (%)			
RM490	10	101	30	1	0.78	0
RM7075	10	155	40	1	0.7	0
AP3206	10	167	30	1	0.82	0
RM8094	10	209	40	1	0.66	0
RM10748	10	95	40	1	0.72	0
SalT-1	10	159	30	1	0.76	0
Mean	10	147.667	35	1	0.74	0

† Major allele is defined as the allele with the highest frequency

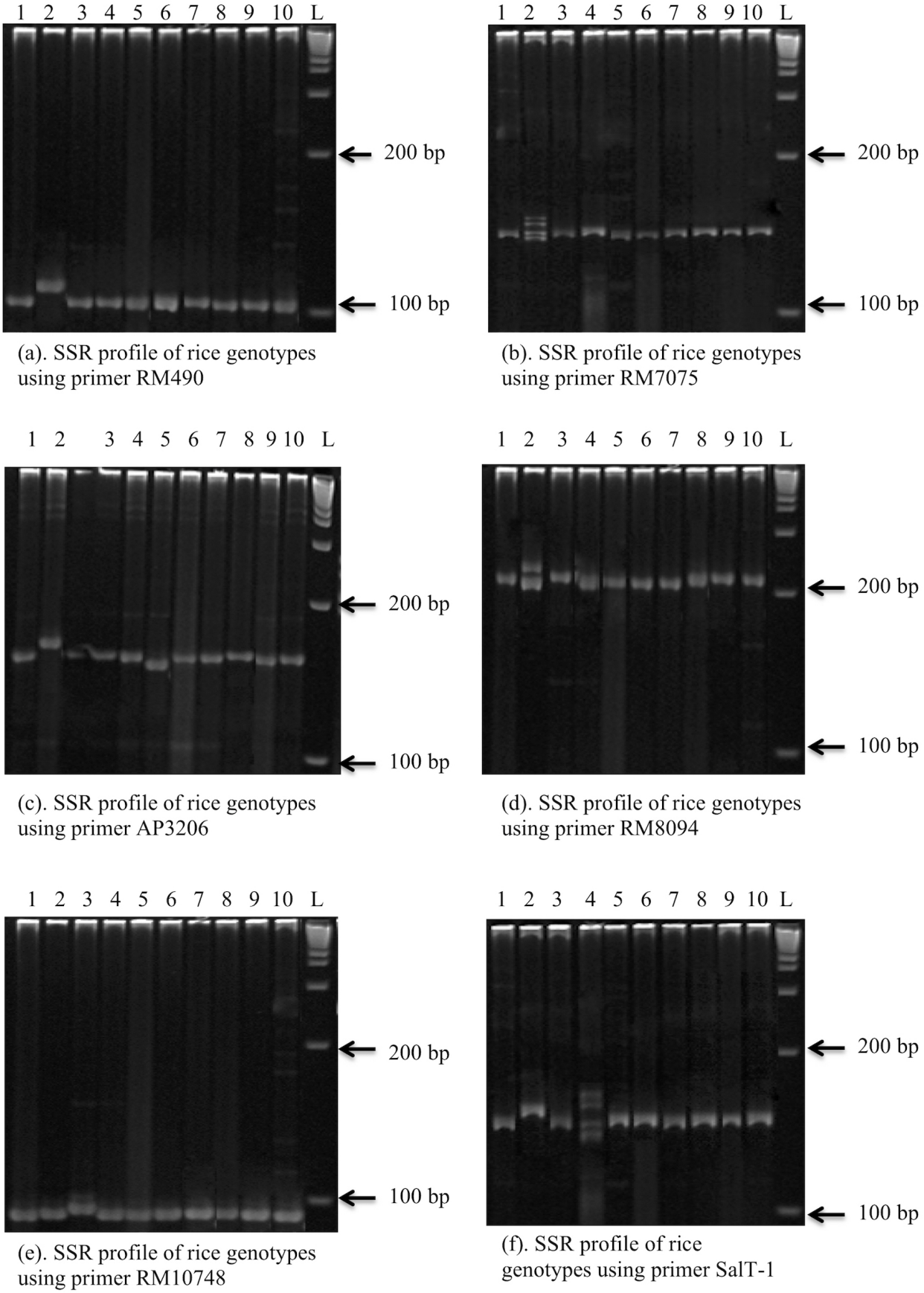

Figure 1. SSR profile of rice genotypes using different primers

value of 0.74 and 0 70, respectively. From the above result and discussion AP3206 was considered the best marker for the identification of 10 rice genotypes, followed by RM490, RM7075, RM8094, RM10748 and SalT-1.

4 Conclusion

An SSR based screening of 10 rice genotypes using 6 SSR markers demonstrated a total of 31 alleles with an average of 5.167 alleles per locus. The highest *PIC* value was recorded for primer AP3206 and that was the lowest for the primer RM8094.Therefore, it can be concluded that AP3206 was the best marker for the identification of rice genotypes, which are salinity tolerance, followed by RM490, RM7075, RM8094, RM10748 and SalT-1. SSR markers used in this study were convenient, polymorphic and associated with salinity tolerance.

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References

ABSPII. 2006. Drought tolerant rice and salinity tolerance rice. Agricultural Biotechnology Support Project II- South Asia, pp. 26.

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. 1993. Optimizing parental selection for genetic linkage maps. Genome 36:181–186. doi: 10.1139/g93-024.

Bhuiyan MAR. 2005. Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. M. Sc. Dissertation, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Human Genet 32:314–331.

Brondani C, Borba TCO, Rangel PHN, Brondani RPV. 2006. Determination of genetic variability of traditional varieties of Brazilian rice using microsatellite markers. Genet Mol Biol 29:676–684. doi: 10.1590/s1415-47572006000400017.

Chakravarthi BK, Naravaneni R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr J of Biotechnol 5:684–688.

Collard BCY, Mackill DJ. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 363:557–572. doi: 10.1098/rstb.2007.2170.

Duvick DN. 1984. Genetic diversity in major farm crops on the farm and in reserve. Econ Bot 38:161–178. doi: 10.1007/bf02858829.

Etemad A, Maziah M, SK D. 2012. Determination of genetic relatedness among selected rice (*Oryza sativa* L.) cultivars using microsatellite markers. Afr J Biotechnol 11:7158–7165.

Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. 2005. Genetic structure and diversity in *Oryza sativa* L. Genetics 169:1631–1638. doi: 10.1534/genetics.104.035642.

Giarrocco LE, Marassi MA, Salerno GL. 2007. Assessment of the genetic diversity in Argentine rice cultivars with SSR markers. Crop Sci 47:853–858. doi: 10.2135/cropsci2005.07.0198.

Gregorio GB. 1997. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). Ph.D. Thesis, University of the Philippines Los Banos. Laguna, Philippines.

Hossain MM, Islam MM, Hossain H, Ali MS, Teixeira da Silva J, Komamine A, Prodhan SH. 2012. Genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.) by microsatellite markers. Genes Genomes Genomics 6:42–47.

IPCC. 2007. Climate change in Asia 'too alarming to contemplate'. Intergovernmental Panel on Climatic Change, pp. 85.

IRGSP. 2005. The map-based sequence of the rice genome. Nature 436:793–800. doi: 10.1038/nature03895.

Islam MM. 2004. Mapping salinity tolerance genes in rice (*Oryza sativa* L.) at reproductive stage. Ph.D. Dissertation, University of the Philippines Los Banos College, Laguna, Philippines.

Jayamani P, Negrão S, Martins M, Maçãs B, Oliveira MM. 2007. Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci 47:879. doi: 10.2135/cropsci2006.04.0236.

Kumar S, Bisht I, Bhatt K. 2010. Assessment of genetic diversity among rice (*Oryza sativa* L.) landrace populations under traditional production using microsatellite (SSR) markers. Electron J Plant Breed 1:474–483.

Lang NT, Li Z, Buu BC. 2001. Microsatellite markers linked to salt tolerance in rice. Omonrice 9:9–21.

Lang NT, Yanagihara S, Buu BC. 2000. Quantitative trait loci for salt tolerance in rice via molecular markers. Omonrice 8:37–48.

Liu K, Muse SV. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129. doi: 10.1093/bioinformatics/bti282.

McCouch SR. 2002. Development and mapping of 2240 new SSR arkers for rice (*Oryza sativa* L.). DNA Res 9:199–207. doi: 10.1093/dnares/9.6.199.

Melchinger AE, Messmer MM, Lee M, Woodman WL, Lamkey KR. 1991. Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. Crop Sci 31:669. doi: 10.2135/cropsci1991.0011183x003100030025x.

Nagaraju J, Kathirvel M, Kumar RR, Siddiq EA, Hasnain SE. 2002. Genetic analysis of traditional and evolved basmati and non-basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc Nat Aca Sci 99:5836–5841. doi: 10.1073/pnas.042099099.

Ni J, Colowit PM, Mackill DJ. 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci 42:601–607. doi: 10.2135/cropsci2002.0601.

Niones JM. 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near-isogenic lines. M. Sc. Dissertation, University of the Philippines Los Banos College, Laguna, Philippines.

Pervaiz ZH, Rabbani MA, Khaliq I, Pearce SR, Malik SA. 2010. Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice lansativa. Electron J Biotechnol 13:1–12. doi: 10.2225/vol13-issue3-fulltext-5.

Prathepha P. 2012. Genetic diversity and population structure of wild rice, *Oryza rufipogon* from Northeastern Thailand and Laos. Aust J Crop Sci 6:717–723.

Rahman MM, Rasaul M, Hossain M, Iftekharuddaula K, Hasegawa H. 2012. Molecular characterization and genetic diversity analysis of rice (*Oryza sativa* L.) using SSR markers. J Crop Improv 26:244–257. doi: 10.1080/15427528.2011.627533.

Semagn K, Bjørnstad Å, Ndjiondjop MN. 2006. An overview of molecular marker methods for plants. Afr J Biotechnol 5:2540–2568.

Seraj ZI, Salam MA. 2000. Growing rice in saline soils. The Biotechnology Directory. Macmillan Reference Ltd., Porters South, Crinan Street, London.

Singh R, Gregorio GB, Jain R. 2007. QTL mapping for salinity tolerance in rice. Phys Mol Biol Plant 13:87–99.

Siwach P, Jain S, Saini N, Chowdhury VK, Jain RK. 2004. Allelic diversity among basmati and non-basmati long-grain Indica rice varieties using microsatellite markers. J Plant Biochem Biotechnol 13:25–32. doi: 10.1007/bf03263186.

Stewart CN, Via LE. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. Biotechniques 14:748–750.

Tuteja N, Singh LP, Gill SS, Gill R, Tuteja R. 2012. Salinity Stress: A Major Constraint in Crop Production. In: Improving Crop Resistance to Abiotic Stress. Wiley-VCH Verlag GmbH & Co. KGaA. p. 71–96. doi: 10.1002/9783527632930.ch4.

Weir BS. 1990. Genetic Data Analysis: Methods for Discrete Population Genetic Data. volume 250. Sinauer Associates, Inc. Publishers, Massachusetts, USA. doi: 10.1126/science.250.4980.575.

Yu SB, Xu WJ, Vijayakumar CHM, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS, Li ZK. 2003. Molecular diversity and multilocus organization of the parental lines used in the international rice molecular breeding program. Theor Appl Genet 108:131–140. doi: 10.1007/s00122-003-1400-3.

Zeng Y, Zhang H, Li Z, Shen S, Sun J, Wang M, Liao D, Liu X, Wang X, Xiao F, Wen G. 2007. Evaluation of Genetic Diversity of Rice Landraces (*Oryza sativa* L.) in Yunnan, China. Breed Sci 57:91–99. doi: 10.1270/jsbbs.57.91.

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