Bangladesh J. Bot. **52**(4): 1067-1070, 2023 (December DOI: https://doi.org/10.3329/bjb.v52i4.70610
- Short communication

MOLECULAR CHARACTERIZATION AND DIVERSITY ANALYSIS OF PROMISING TGMS LINES AND HIGH-YIELDING VARIETIES IN RICE BY USING SSR MARKERS

V KARPAGAM AND R KALAIYARASI*[1]

Department of Plant Breeding and Genetics, MIT College of Agriculture and Technology, Vellalapatt, Musiri-621 211 (Tamil Nadu), India

Keywords: Diversity, PIC value, SSR primers, TGMS lines, Two-line breeding

Abstract

The present investigation was intended to assess the extent of genetic diversity present among the selected genotypes of *Oryza sativa* (including TGMS lines) using SSR markers. The SSR markers used for the study gave average Polymorphism Information Content (PIC) value of 0.551. The primer RM 9 followed by RM 536 has given maximum PIC value and the primer RM 29 gave the minimum. It was inferred that the primers RM 9 and RM 536 had been the better choices in this study. The lines, TS09 24 and TS09 26 were in the same cluster as they derived from the same parents. The TGMS lines TS 29 and TNAU 27S are in separate cluster from other parents indicating that these lines are highly diversified and their crosses can give improved cultivars.

Rice is a staple food for about half of the world's population. The rapidly increasing demand for rice and the continuous decrease in rice- growing areas have resulted in searches to improve rice production. The use of hybrid rice has proved to be an effective and economical way to increase rice production output. It is easy to obtain about 10 per cent higher yield just by growing hybrid rice instead of rice varieties. The majority of commercial rice hybrids are producedthrough the Cytoplasmic Genic Male Sterility (CGMS) system. Production of rice hybrids by using this commonly designated "three line system" (A/B/R).

Two-line breeding is one such possibility that emerged following the chance of discovery of a photoperiod sensitive plant called Wongken 58S, in the *japonica* variety Nongken 58S by Shi Min Shong of China, which utilizes Environmentally induced Genic Male Sterility (EGMS) is currently being attempted.

Use of the thermosensitive genie male sterility (TGMS) system in two line breeding is simple, inexpensive, efficient, and eliminates the limitations associated with the cytoplasmic-genetic male sterility (CMS) system in rice. The present study was aimed at isolating and identifyingstable TGMS lines for commercial exploitation of two-line heterosis breeding in rice. Two line hybrid in rice by employing thermo sensitive or photosensitive genic male sterility (TGMS or PGMS) lines is considered as an effective substitute for the three line hybrid using CMS system because of its potential to exploit higher heterosis and simple procedure of hybrid seed production (Yuan 1998, Kalaiyarasi*et al.* 2006)

Among the various types of molecular markers available, microsatellite have recently received greater attention, especially for breeding purposes. Microsatellite markers, also known as simple sequence repeats or SSRs (Littand Luty 1989, Weber and May,1989) are clusters of short (usually 2 to 6) tandem repeated nucleotide bases distributed throughout the genome. Microsatellite markers are in general, co-dominant, multiallelic, and highly polymorphic genetic markers. Microsatellite alleletyping requires small amounts of DNA for straightforward PCR and

*Author for correspondence: <kalaiyarasi_2002@yahoo.com>. [1]Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore - 641 003 (Tamil Nadu), India.

gel electrophoresis analysis (Rafalski *et al.* 1996). Yu *et al.* (2001) established the DNA fingerprints of Ning 2A and Ning 2B with SSR markers and differentiated the two parental lines from other rice varieties by using two SSRs.

The research work was carried out at Paddy Breeding Station, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore with the objective of developing two line hybrids in rice using Thermosensitive genic sterility (TGMS) lines system. The fifteen parents *viz.*, TNAU 27S, TS09 24, TS09 26 and TS 29CO(R) 49, ADT 38, Improved white ponni (IWP), BPT 5204, KDML 105, WGL 14, G 14, T 2006, T 972, T 972, T 360 and CB 87Rraised during *Kharif 2020* in randomized block design with three replications by adopting a spacing of 20 x 15 cm accommodating ten plants per replication and single seedling per hill was planted. The recommended package of practices was followed. The parents were surveyed for polymorphism through SSR markers (Table 1). Total genomic DNA was isolated from the genotypes as described by Gawel and Jarret(1991). Amplification reactions were is in volumes of 15 µl containing nine ng of genomic DNA, 1.5 µl of 10X PCR buffer (including 15 mM $MgCl_2$), 0.6 µl of 10mM each of dATP, dTTP, dGTP and dCTP, 2 µl of forward and reverse SSR primer, 0.3 µl of Taq DNA polymerase (Bangalore Genei Pvt Ltd, Bangalore) and sterile water. Amplifications were performed in Bio-Rad (My Cycler thermal cycler) and Corbett PCR machine. Agarose gel electrophoresis was performed to separate amplification products. Polymorphism information content values were calculated for SSR markers in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. It is the sum of polymorphism information content values of all the markers produced by a particular primer.

Table 1. List of primers used in the study.

Primers	Sequence	No. of bases
RM10F	TTGTCAAGAGGAGGCATCG	19
RM10R	CAGAATGGGAAATGGGTCC	19
RM29F	CAGGGACCCACCTGTCATAC	20
RM29R	AACGTTGGTCATATCGGTGG	20
RM125F	ATCAGCAGCCATGGCAGCGACC	22
RM125R	AGGGGATCATGTGCCGAAGGCC	22
RM 536F	TCTCTCCTCTTGTTTGGCTC	20
RM 536R	ACACACCAACACGACCACAC	20
RM9F	GGTGCCATTGTCGTCCTC	18
RM9R	ACGGCCCTCATCACCTTC	18

PIC value was calculated using the formula

$$PIC = 1- \sum pi^2$$

where, *pi* is the frequency of the i^{th} allele **(**Smith *et al.* 1997).

Results obtained through SSR analysis showed that the genotype produced two alleles from each primer (Table 2). The polymorphism information content (PIC) value ranged from 0.356 (RM 29) to 0.769 (RM 9). The average PIC value obtained was 0.551 (Table 2). The SSR profiles generated by the primers RM 10 and RM 29 are presented in Fig. 1.

Cluster analysis was performed based on Jaccard´s similarity co-efficient matrices calculated using SSR analysis and it was used to generate a dendrogram of 15 genotypes. The similarity co-efficient ranged from 0 to1.00. The dendrogram separated 15 genotypes into five major clusters (Fig.2). The cluster I consists of five genotypes *viz.*, TS09 26, T360, TS09 24, G14 and KDML105. The cluster II consists of Improved white ponni and T 2006. The cluster III had two

genotypes *viz.*, TS 29 and TNAU 27S. (Sai Rekha *et al.* 2021) The cluster IV consists of CB 87R, T 972, WGL 14 and BPT 5204. The cluster V had CO(R) 49 and ADT 38. CB 87R, T 972 were highly related with each other with a similarity co efficient of 1.00 (Table 2).

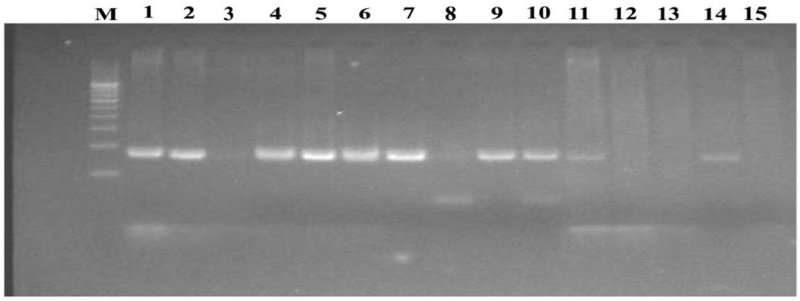

Fig. 1. SSR profiles generated by the Primer RM 9.

Table 2. Polymorphism observed in parents.

Primers	Number of alleles	PIC value
RM10	2	0.497778
RM29	2	0.355556
RM125	2	0.422222
RM 536	2	0.711111
RM9	2	0.768889

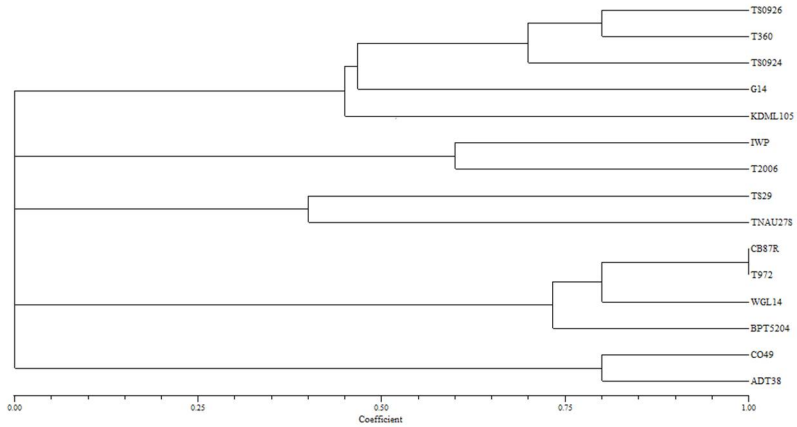

Fig. 2. Association among TGMS lines and varieties by the SSR primers.

For past two decades, the classical strategies of evaluating the genetic variability such as comparative anatomy, morphology, embryology and physiology have been increasingly complemented by molecular markers. The development of molecular markers that are based on polymorphism found in protein, RNA and DNA has greatly facilitated research in all fields of science, from taxonomy to the germplasm evaluation and management. However, the present investigation was intended to assess the extent of genetic diversity present among the selected genotypes of *Oryza sativa* (including TGMS lines) using SSR markers.

The SSR markers used for the study gave average Polymorphism Information Content (PIC) value of 0.551. The primer RM 9 followed by RM 536 has given maximum PIC value and the primer RM 29 gave the minimum. It was inferred that the primers RM 9 and RM 536 had been the better choices in this study (Table 2).

From the dendrogram obtained (Fig.2), it can be inferred that TS09 24 and TS09 26 were in the same cluster as they derived from the same parents. The TGMS lines TS 29 and TNAU 27S are in separate cluster from other parents indicating that these lines are highly diversified and their crosses can give improved cultivars. (Ni *et al*. 2002, Victoria *et al*. 2007, Xu *et al*. 2002, Kumar *et al.* 2016).

References

Kalaiyarasi R and Vaidyanathan P 2006. A novel breeding approach in quest of temperature sensitive genic male lines among rice inter subspecies derivatives. Euphytica **150** (1-2): 173-183.

Kumar P, Nautiyal MK, Kumar P, Kumar K 2016. Morphological and Molecular based Characterization of different Thermo-sensitive Genetic Male Sterile (TGMS) lines in Rice (*Oryza sativa* L.). Vegetos **29**:4

Litt M and Luty JA 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a di-nucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. **44**: 397-401.

Ni J, Colowit MP and Mackill DJ 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci. **42**: 601-607.

Rafalski JA, Hanafey MK, Tingey SV and Wiliams JK 1996. Technology for molecular breeding: RAPD markers, Microsatellites and machines. Plant Genome Anal. 19-27.

Sai Rekha K, SaraswathiR**,** Kumar M**,** Manonmani S, Raveendran M andRobin S2021. Morphological and molecular diversity of thermosensitive genic male sterile lines in rice *(Oryza sativa* L.) Electronic J. Plant Breed. **12**: 177-182.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, MitchellSE, KresovichS andZiegle J 1997. An evaluation of the utility of SSR *loci* as molecular markers in maize. (*Zea mays* L.) Comparisons with data from RFLPs and pedigree. Theor. and Appl. Genet. **95**: 163-173.

Victoria CL, Darshan SB, ToshinoriA, Edilberto DR 2007. Assessment of genetic diversity of Philippines rice cultivars carrying good quality trait using SSR markers. Breed. Sci. **57**: 263-270.

Weber JL and May PE 1989. Abundant class of human DNA polymorhisms which can be typed using the polymerse chain reaction. Am. J. Hum. Genet. **44**: 388-396**.**

Xu W, Virmani SS, Hernandez JE. Sebastian LS, Redona ED and Li ZK 2002. Genetic diversity in parental lines and heterosis of the tropical rice hybrids. Euphytica**127**: 139–148.

Yu YH, Li YH, Ma RR, Wang XY, Hu GC, Si HM, Fu YP and Sun ZX 2001. Establishment of fingerprint map of Ning 2A with simple sequence repeat markers. Chinese J. Rice Sci. 15 **3**: 216-217.

Yuan LP 1990. Progress of two-line system hybrid rice breeding. Sci. Agr. Sin. **3**: 1-6.

(Manuscript received on 10 August, 2023; revised on 12 November, 2023)