The Pharma Innovation Journal 2023; 12(6): 3256-3259

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 3256-3259

www.thepharmajournal.com
Received: 23-03-2023
Accepted: 28-04-2023

Sairachana R
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Suji KK
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Gokulakrishnan M
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Raveendran M
Directorate of Research, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Gopalakrishnan C
Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Ramalingam J
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
Ramalingam J
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Parental polymorphism survey using rice microsatellite markers between VGD 1 and ADT 55 for marker-assisted backcross breeding program

Sairachana R, Suji KK, Gokulakrishnan M, Raveendran M, Gopalakrishnan C and Ramalingam J

DOI: https://doi.org/10.22271/tpi.2023.v12.i6al.20850

Abstract
Marker-assisted backcross breeding program was anticipated to introgress bacterial blight (BB) resistance genes into an elite fine-grain rice cultivar VGD 1. Pyramided with three bacterial blight (BB) resistance genes (xa5, Xa13, Xa21), ADT 55 was the donor parent. A parental polymorphism survey was executed between VGD1 and ADT 55 using genome-wide simple sequence repeats (SSR) also called microsatellite markers, to identify polymorphic markers. Distributed throughout 12 chromosomes of rice, in the current research 166 SSR markers were employed, out of which 58 are found to be polymorphic between VGD 1 and ADT 55 with 35% parental polymorphism percentage and the polymorphic information content (PIC) is about 0.6. The polymorphic microsatellite markers obtained through this survey could be utilized for background selection of backcross progenies to retrieve the highest recurrent parent genome.

Keywords: Marker assisted backcross breeding, background selection, simple sequence repeats, parental polymorphism, VGD 1, ADT 55, bacterial blight, rice

Introduction
Rice is significant in India since it is valued as both a ceremonial crop and a source of staple food. It is the most consumed cereal grain providing energy for half of the global population. India bangs second position in rice production where China is the leading producer (FAO, 2022) [5]. With a vast 16 million ha area under cultivation, rice is primarily grown in India as an irrigated and rainfed crop. In recent years, because of adverse climatic conditions, production from rainfed rice cultivation is remarkably affected in the world in general and in India in particular (Pradhan *et al*., 2015) [11]. Due to biotic and abiotic stressors, rice yields are declining. The most detrimental of these stressors is bacterial blight (BB), caused by *Xanthomonas oryzae pv oryzae* (Xoo). Under severity, BB can reduce yield by up to 80% (Kumar *et al.*, 2012) [8]. The most efficient and cost-effective way to combat rice with bacterial blight is to employ resistant varieties (Chukwu *et al.*, 2019) [3].

One of the most appealing ideas is marker assisted selection, which makes it easier to incorporate genes into rice with various genetic backgrounds. Rao *et al.*, (2002) [14] reported many successful varieties developed by using MAS in traditional breeding programmes. Marker assisted backcross breeding (MABB) is one such dynamic marker assisted breeding strategy, which works by transferring a few desirable genes/ QTLs from one genetic source, usually denoted as 'donor parent', into an adapted, superior, elite breeding line, which serves as a recurrent parent (Jiang 2013) [7]. A key phase of MABB is background selection, which entails using of genome-wide molecular markers to identify progenies with the greatest amount of recurrent parent genome. This idea of background selection in MABB is alluded to as 'Variety development or enhancement' (Huyen *et al.*, 2013) [6].

DNA segments with a 1-6 nucleotide tandem repeat motif are known as simple sequence repeats (SSRs), also referred to as microsatellites. Genome-wide coverage, co-dominant inheritance, high repeatability, and interspecies transferability are just a few of the enticing quantities that SSR markers offer. Microsatellite loci are valuable as genetic markers because of their high amount of allelic variation, which is one of their key traits. The distinctive sequences around the SSR motifs serve as templates for certain primers that will be used in the polymerase chain reaction (PCR) to amplify the SSR alleles. Numerous applications have made use of these SSR markers, including genetic diversity research, association mapping, linkage mapping, fingerprinting, and background genome recovery studies (Singh *et al.*, 2010; McCouch *et al.*,

1997) [15, 9]. In the current work, polymorphic SSR markers have been discovered to enlist them in strategies of genome restoration.

Materials and Methods

Parent materials

VGD 1 is a superfine grain rice variety with recorded yield superiority, released by the state varietal release committee, Tamil Nadu. It is aroma rice preferred for biryani making with good market value (Mohan *et al.,* 2021) [10]. As this elite cultivar shows high susceptibility to bacterial blight disease, a study was conceptualized to introgress bacterial blight (BB) resistance genes (*xa5*, *Xa13* and *Xa21*) through marker assisted backcross breeding program without compromising on its desirable grain characters. ADT 55 is the first rice variety developed for BB resistance by TNAU using functional marker assisted breeding harboring*xa5, Xa13* and *Xa21* resistance (R) genes and is used as a donor parent. Hence, a survey was designed between these two parents, VGD1 and ADT 55, for polymorphic SSR markers.

Genome wide SSR markers

Genome-wide microsatellite markers were chosen randomly and information on their dispersal throughout 12 rice chromosomes was gathered using the *Gramene* database (https://archive.gramene.org/.). Altogether, 166SSR markers were put forward for the survey (Table 1).

Table 1: List of SSR markers used for polymorphism survey

Linkage group	Simple sequence Repeat (SSR) primer
1	RM 1, RM 5, RM 84, RM 226, RM 323, RM 403, RM 431, RM 443, RM 488, RM 490, RM 493, RM 495, RM 3233, RM 3403, RM 3412, RM 3873, RM 6515, RM 8051, RM 8070, M 8077
2	RM 6, RM 109, RM 207, RM 208, RM 262, RM 279, RM 300, RM 525, RM 1342, RM 2634, RM 3316, RM 3263, RM 6519, RM 6800, RM 13912
3	RM 60, RM 132, RM 218, RM 251, RM 282, RM 468, RM 514, RM 520, RM 545, RM 570, RM 1002, RM 3646, RM 3698, RM 5474, RM 6283, RM 7072, RM 7565, RM 15080
4	RM 127, RM 142, RM 241, RM 307, RM 335, RM 1153, RM 3276, RM 3843, RM 5749, RM 8213, RM 8218, RM 16335, RM 16337, RM 16338
5	RM 13, RM 26, RM 153, RM 164, RM 267, RM 274, RM 289, RM 430, RM 440, RM 507, RM 538, RM 5140, RM 18600,
6	RM 115, RM 204, RM 225, RM 314, RM 400, RM 435, RM 461, RM 469, RM 585, RM 588, RM 3183, RM 4608, RM 8072, RM 19388, RM 19419
7	RM 11, RM 18, RM 134, RM 214, RM 248, RM 234, RM 336, RM 420, RM 432, RM 445, RM 478, RM 505, RM 542, RM 6344, RM 6697, RM 21976
8	RM 44, RM 310, RM 331, RM 404, RM 407, RM 1959, RM 3215, RM 6999, RM 23120
9	RM 107, RM 242, RM 1328, RM 3025, RM 5102, RM 5688, RM 6021, RM 23788, RM 23865, RM 24386
10	RM 147, RM 216, RM 222, RM 228, RM 258, RM 304, RM 333, RM 474, RM 1108, RM 3773, RM 5348, RM 6100, RM 6364, RM 6737, RM 7217, RM 7361
11	RM 144, RM 202, RM 206, RM 286, RM 332, RM 1233, RM 1812
12	RM17, RM 19, RM 20, RM 102, RM 247, RM 463, RM 1302, RM 3813, RM 5196, RM 7003, RM 7102, RM 28048, RM 28102

Molecular analysis

Molecular analysis for identifying polymorphism includes a series of prescribed steps i.e., genomic DNA extraction, quantification and dilution, polymerase chain reaction, gel electrophoresis, and documentation. Leaf samples were collected from the parents grown in pots and DNA extraction was performed following CTAB method (Delloporta *et al.*, 1983) [4]. Quality and the quantity of extracted genomic DNA were taken using a Nanodrop spectrophotometer. DNA concentration as ng/ul and DNA observance at 260 nm was noted down. After measurement, using double distilled water, DNA concentration was finally adjusted to 50 ng/ul to perform a polymerase chain reaction (PCR).

Using an Eppendrof thermocycler, polymerase chain reaction was performed. The given reagents were included in ten microliters of PCR reaction mixture: 50 ng of template DNA, 30 ng of forward and reverse primers, 4 ul of Dream Taq green 2 PCR master mix (Thermo Scientific, USA), and 4 µl of nuclease-free water. PCR protocol for amplification of SSR markers starts with one cycle of initial denaturation for 5 mins at 94 °C continued by 35 cycles of primer annealing, extension, and final extension for 1 min @ 55 °C, 1 min @ 72 °C and 7 mins @ 72 °C respectively. Electrophoresis was the technique used to separate amplified PCR products on 2.5% agarose gel stained with ethidium bromide in 1x TBE buffer. The wells of the solidified agarose gel were loaded with 5 microliters of amplified PCR products and a 2 µl of 100 bp DNA ladder. One and a half hours were spent running the gel at 100 volts. Gel documentation unit (Bio- Rad), UV transilluminator was used to visualise bands. The banding pattern was scored visually by comparing it with a 100 bp DNA ladder.

The information about the chromosomal position, primer sequence, repeat motifs, and size of the anticipated product (bp) was gleaned from (https://archive.gramene.org/markers/) and the GGT 2.0 programme was utilised to depict obtained polymorphic markers according to their physical position (Mb) along the length of chromosomes (Figure 2). Finally, Polymorphism Information Content (PIC) and parental polymorphism percentage was calculated.

Results and Discussion

SSR markers are acknowledged as the preferable molecular markers among all the molecular markers that have been studied and used in breeding efforts. Genotyping was done using 166 microsatellite markers spanning over all 12 rice chromosomes. 58 markers showed polymorphism indicating a parental polymorphism percentage of 35% and polymorphism information content is about 0.6. Table 2 shows the frequency distribution and polymorphism percentage of markers on each chromosome. Chromosome 2 indicated the highest genetic variability with 66% of polymorphism with the highest number (10) of polymorphic markers (RM 6, RM 207, RM 208, RM 262, RM 279, RM 300, RM 525, RM 1342, RM 3316, RM

13912). Next to this, chromosome 4 is with 8(RM 127, RM 241, RM 3276, RM 5749, RM 8213, RM 16335, RM 16337, RM 16338) polymorphic markers disclosing 57% polymorphism. Following this, on Linkage group 7 (LG 7) we observed 8 (RM 11, RM 18, RM 234, RM 248, RM 336, RM 432, RM 542, RM 6344)markers to be polymorphic and 8 in LG 1 (RM 5, RM 226, RM 488, RM 490, RM 3233, RM 3403, RM 8051, RM 8070). On the 12th chromosome, there were no polymorphic markers noticed. Thus, polymorphism levels varied from 0% (chromosome 12) to 66% (chromosome 2). There are 2 polymorphic markers found on each of chromosomes 8 (RM 407, RM 3215), 9 (RM 242, RM 5102) and 11(RM 144, RM 206). 3 markers (RM 216, RM 258, RM 1108) are polymorphic on chromosome 10. Finally, to enlist we have 5 polymorphic microsatellite markers each in LG 3 (RM 514, RM 520, RM 570, RM 6283, RM 7072), LG 5 (RM 153, RM 164, RM 267, RM 440, RM 5140) and LG 6 (RM 204, RM 225, RM 585, RM 588, RM 19419).

Results following the previous studies, where, in backcross research by marker assistance to introgress broad-spectrum resistance to bacterial blight into elite variety MR297, 475 SSR markers investigated, showed a total of 17.47% polymorphism dispersed across the 12 chromosomes (Aljumaili *et al*., 2023) [1]. Closer to our study, between the groundnut parental genotypes ICGV 00350 and GPBD 4, 217 markers were examined, and 65 of those markers were polymorphic and no polymorphic primers were found for linkage groups 6 and 20 among the 20 linkage groups for cross-studied (Rajarathinam *et al*., 2023) [12]. A parental polymorphism survey was done using 463 SSR markers for two different parental cross combinations with recurrent parents ADT43 and ASD16 during gene pyramiding for multiple stress tolerance in rice, where 69 and 68 markers were polymorphic respectively (Ramalingam *et al*., 2020) [13]. Further in MABB programme these 59 polymorphic markers are going to be deployed for background selection for opting highest genome from the recurrent parent. Additionally, linkage studies and diversity analysis for a variety of characteristics in rice can also be conducted using the discovered polymorphic microsatellite markers.

Table 2: List of polymorphic markers between VGD 1 and ADT 55

Chromosome number	Total No. of markers analysed	Number of polymorphic markers	Number of monopoly orphic markers	Polymorphism percentage (%)
1	20	8	12	40
2	15	10	5	66
3	18	5	13	27
4	14	8	5	57
5	13	5	8	38
6	15	5	10	33
7	16	8	8	50
8	9	2	7	22
9	10	2	8	20
10	16	3	13	19
11	7	2	5	28
12	13	0	13	0
Total	166	58	107	35

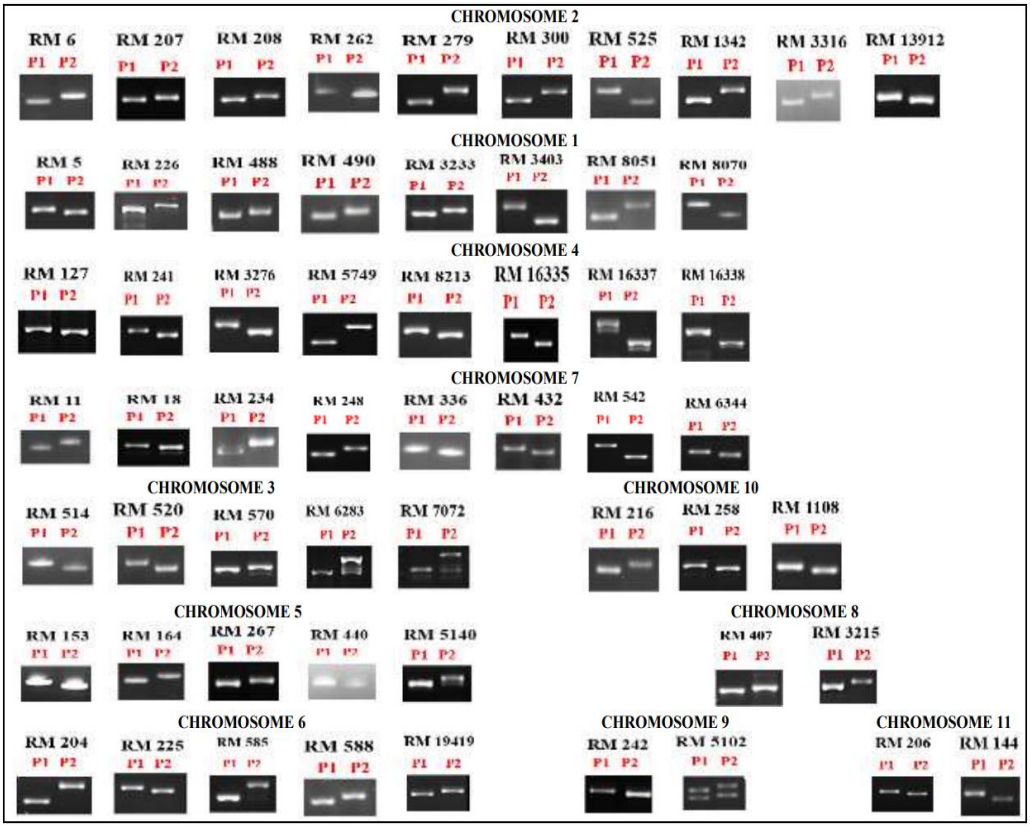

Fig 1: Gel images of amplified PCR products using polymorphic markers between VGD 1 and ADT 55

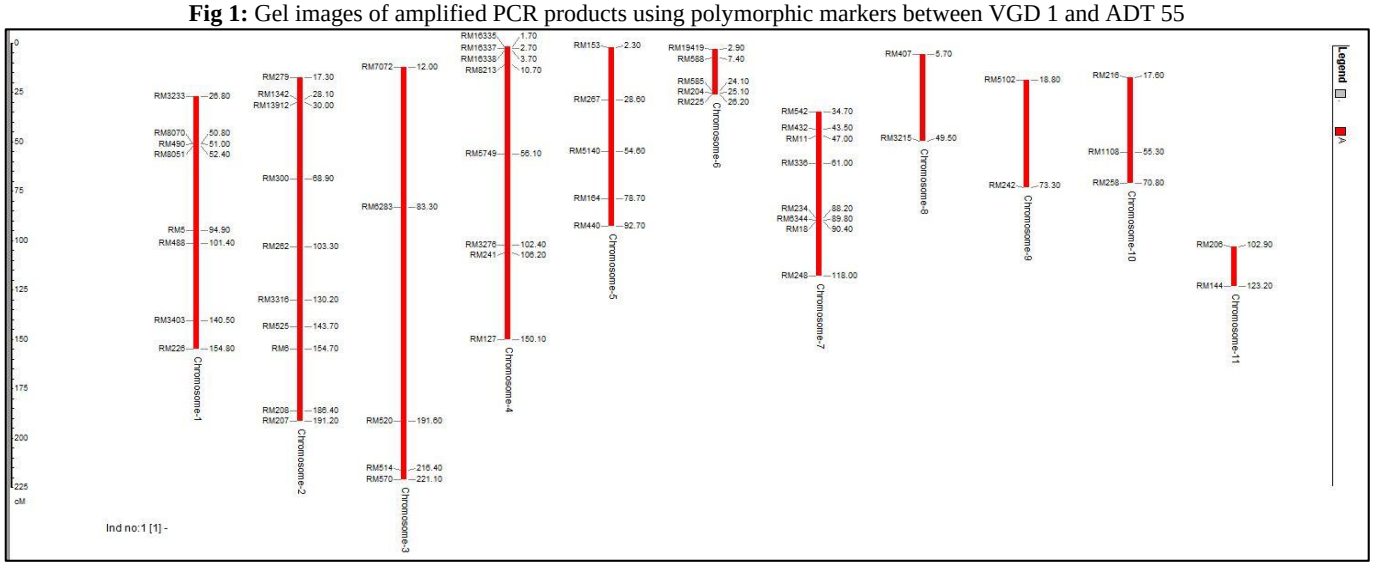

Fig 2: Physical location and distribution of 58 polymorphic markers between VGD 1 and ADT 55

References

1. Aljumaili SJ, Rafii MY, Sakimin SZ, Ahmad K, Al-Jumaily A. Backcrossing with Marker Assistance to Introduce Broad-Spectrum Bacterial Leaf Blight Resistance in the Malaysian Elite Variety MR297. Agriculture. 2023;13(2):372.
2. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American journal of human genetics. 1980;32(3):314.
3. Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Hasan MM, Oladosu YA, *et al*. Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. Molecular biology reports. 2019;46:1519-1532.
4. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: version II. Plant molecular biology reporter. 1983;1:19-21.
5. FAO. FAOSTAT: Production: Crops and livestock products. In: FAO. Rome, Cited October 2022. https://www.fao.org/faostat/en/#data/QCL
6. Huyen LTN, Cuc LM, Ham LH, Khanh TD. Introgression the SALTOL QTL into Q5DB, the elite variety of Vietnam using marker-assisted-selection (MAS). Am J BioSci. 2013;1(4):80À84.
7. Jiang GL. Plant marker-assisted breeding and conventional breeding: challenges and perspectives. Adv. Crop Sci. Technol. 2013;1:e106.
8. Kumar PN, Sujatha K, Laha GS, Rao KS, Mishra B, Viraktamath BC, *et al*. Identification and fine mapping of Xa33, a novel gene for resistance to *Xanthomonas oryzae*

pv. *oryzae*. Phytopathology. 2012;102(2):222-228.
9. McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, *et al*. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant molecular biology. 1997;35:89-99.
10. Mohan MM, Hepziba SJ, Utharasu S, Kumar CR, Ganesamurthy K. A high yielding Seeragasamba variety: VGD 1. Electronic Journal of Plant Breeding. 2021;12(2):263-271.
11. Pradhan SK, Barik SR, Sahoo J, Pandit E, Nayak DK, Pani DR, *et al*. Comparison of Sub1 markers and their combinations for submergence tolerance and analysis of adaptation strategies of rice in rainfed lowland ecology. Comptes Rendus Biologies. 2015;338(10):650-659.
12. Rajarathinam P, Palanisamy G, Narayana M, Alagirisamy M. Marker assisted backcross to introgress late leaf spot and rust resistance in groundnut (*Arachis hypogaea* L.). Molecular Biology Reports. 2023;50(3):2411-2419.
13. Ramalingam J, Raveendra C, Savitha P, Vidya V, Chaithra TL, Velprabakaran S, *et al*. Gene pyramiding for achieving enhanced resistance to bacterial blight, blast, and sheath blight diseases in rice. Frontiers in plant science. 2020;11:591457.
14. Rao KK, Lakshminarasu M, Jena KK. DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. Biotechnology Advances. 2002;20(1):33-47.
15. Singh H, Deshmukh RK, Singh A, Singh AK, Gaikwad K, Sharma TR, *et al*. Highly variable SSR markers suitable for rice genotyping using agarose gels. Molecular breeding. 2010;25(2):359-364.