Mapping Temperature Tolerance: Parental polymorphism study in contrasting rice genotypes using micro satellite markers

ABSTRACT

Aims: The current study aims to probe into insights on temperature tolerance in rice by assessing the variation between heat sensitive recipient parent (Manu Ratna) and heat tolerant donor parent (Nagina 22) based on microsatellite (SSR) marker-based polymorphism.
Place and Duration of Study: Department of Plant Physiology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala between January, 2022 and April, 2022.
Methodology: The contrasting rice genotypes namely Manu Ratna (susceptible recipient parent) and Nagina 22 (tolerant donor parent) were used for the study.Leaf samplescollected from 25day old seedlingswere used for DNA extraction. A set of previously identified 44 heat tolerance Rice Microsatellite (RM) markers spanning across the 12 chromosomes of rice were used for the parental polymorphism study. The DNA fragments were amplified by polymerase chain reaction using RM markers, followed by agarose gel electrophoresis. The DNA fragments so amplified were documented using gel documentation system and polymorphism patterns studied.
Results: The study identified significant genetic polymorphism between the rice genotypes Nagina 22 (heat-tolerant) and Manu Ratna (heat-sensitive) using 44 previously identified SSR markers for heat tolerance. Out of these, the two genotypes exhibited polymorphism for 20. The exhibited 45.45% polymorphism highlights the genetic diversity between the parental lines, providing essential markers for QTL mapping and marker-assisted breeding. These results offer a strong foundation for developing heat-tolerant rice cultivars, as a step towards addressing the challenges of climate-induced heat stress.
Conclusion: The study highlights the critical role of micro satellite marker-based analysis in identifying genetic polymorphisms between heat-tolerant and heat-sensitive rice genotypes. The results show the potential of molecular tools in developing heat-resilient rice cultivars, addressing climate-induced challenges to global food security. These findings contribute to enhancing precision in marker-assisted breeding strategies for sustainable agriculture especially helping rice breeders.

Keywords:Global warming, Heat stress, Spikelet fertility, Rice, Parental Polymorphism

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple crops, feeding more than half of the world's population. The productivity and sustainability of rice cultivation are significantly influenced by environmental stressors, among which heat stress has emerged as a critical challenge in the context of global climate change. Heat stress,adversely affe physiological processes in rice, including flowering, photosynthesis, pollination, and grain filling, ultimately lea reduced yield and quality (Jagadish *et al*., 2007., Xu *et al*., 2021). This issue is of particular concern as ~~the~~ climate change is increasing the global mean day and night temperatures, threatening ~~the~~ food security in the heat prone areas.

The heat tolerance ~~mechanism~~ in rice is a complex trait which is governed by multiple physiological and genetic Traditional breeding approaches face limitations due to the polygenic nature of the trait and time-consuming pro breeding. In this context, Marker assisted breeding (MAB) technique has emerged as an effective and promising fa tool to develop heat tolerant rice genotypes. By leveraging molecular markers such as Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs), MAB enables precise selection of desirable traits at the genom thereby reducing the breeding cycle and enhancing the efficiency of cultivar improvement (Collard and Mackill, 200

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Commented [a2]: using microsatellite (SSR) markers. (Avoid repetition of based)

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Commented [a8]: A polymorphism rate of 45.45% highlights (More natural phrasing.)

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Several studies have highlighted the potential of SSR markers in identifying genetic polymorphism associated with heat tolerance in rice. The study by Ravikiran*et al.* (2020) demonstrated the use of SSR markers to detect quantitative trait loci (QTLs) linked to heat stress response during the reproductive stage which is a critical phase for yield determination. Similarly, Waghmare*et al.* (2021) employed SSR markers to explore genetic diversity in rice germplasm under heat-stress conditions, underscoring the utility of molecular markers in understanding the genetic basis of temperature resilience.

Parental polymorphism studies reveal genetic diversity between the contrasting genotypes for specific traits of interest, thereby ~~helping~~ in the selection of ~~diverse~~ contrasting parental lines.They are essential for modern plant breeding, particularly in marker-assisted selection (MAS) and quantitative trait loci (QTL) mapping. Diverse parental lines increase the chances of identifying significant QTLs linked to traits such as stress tolerance, yield, and quality (Collard and Mackill, 2008). Polymorphism studies help in the development of mapping populations like F2, recombinantinbred lines (RILs), F2 derived F3 and backcross populations, which are critical for dissecting the genetic architecture of complex traits (Senguttuvel*et al*., 2020).
Polymorphism studies play a vital role in identifying candidate gene underlying key QTLs. They provide insights into trait-specific genetic mechanisms, facilitating targeted crop improvements (Majhi *et al.,* 2022). These studies are indispensable for developing resilient rice varieties, ensuring global food security through efficient and precise breeding strategies. Despite these advancements, a comprehensive understanding of genetic variability in parental lines remains crucial for the success of marker-assisted breeding programs. Parental polymorphism analysis provides a foundation for the development of mapping populations and the identification of QTLs associated with heat tolerance traits.

The current study focuses on the polymorphic variation between two rice genotypes, Nagina and Manu Ratna, which exhibit contrasting responses to heat stress. By employing SSR markers, we aim to elucidate the genetic diversity and polymorphic loci that can serve as a basis for developing heat-tolerant rice cultivars. The findings from this research will contribute to ongoing efforts to mitigate the adverse impacts of heat stress on rice production and ensure global food security in the face of climate change.

2. MATERIAL AND METHODS

2.1 DNA extraction from leaf samples

The genomic DNA of the parental lines were extracted from 25 days old seedlings by CTAB method of extraction with modification (Doyle and Doyle,1987). Seedlings were raised and 100 mg of leaf tissue was cryogenically ground using a mortar and pestle with 2% polyvinylpyrrolidone (PVP) and preheated 2X DNA extraction buffer. The buffer composition included 2% cetyltrimethylammonium bromide (CTAB), 100 mM Tris-HCl, 20 mM ethylenediaminetetraacetic acid (EDTA), and 1.4 M sodium chloride (NaCl), and the extraction was performed at 65°C. The purity and concentration of DNA were assessed by measuring optical density (OD) at 260 nm and 280 nm using a Nano spectrophotometer.

2.2Parental polymorphism study

The parental polymorphism between Nagina 22 (heat-tolerant) and Manu Ratna (heat-sensitive) with respect to temperature tolerance was assessed utilizing 44 SSR markers (Table 1) previously identified for heat tolerance (Waghmare *et al*., 2018). The sequences and chromosomal locations of these markers were obtained from the GRAMENE (www.gramene.org) database, ensuring comprehensive genomic representation across all 12 rice chromosomes. Primers were designed based on the retrieved sequence information from National Centre for Biotechnology Information (NCBI) and synthesized by Geno Biosciences Pvt. Ltd., Noida, to ensure precision and reliability for downstream analysis.

PCR amplification of the genomic DNA was performed using a Bio-Rad 1000^{TM}Thermal Cycler. Each reaction was carried out in a total volume of 10 µL, consisting of 4.9 µL of sterile water, 0.5 µL each of forward and reverse primers (10 µM concentration), 2.0 µL of genomic DNA (50 ng concentration), 1 µL of 10X Taq buffer containing 15 mM $MgCl_2$, 0.1 µL (3U) Taq DNA Polymerase, and 1 µL of dNTPs (10 µM). The PCR cycling conditions included an initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 50 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 10 minutes, with a final holding step at 4°C.The amplified PCR products were run through 3% agarose gel electrophoresis and subsequently visualized using a Bio-Rad gel documentation system. The resulting banding patterns were carefully documented for downstream analysis, ensuring the reliability and accuracy of the polymorphism study.

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Consider rewording:
Seedlings were raised, and 100 mg of leaf tissue was cryogenically ground using a mortar and pestle. The ground tissue was mixed with 2% polyvinylpyrrolidone (PVP) and preheated 2X DNA extraction buffer.

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The annealing temperature was set at 55°C, though it varied for different SSR markers based on their melting temperatures.

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3. RESULTS AND DISCUSSION

The present study conducted a comprehensive parental polymorphism analysis by genotyping Nagina 22 (heat-tolerant) and Manu Ratna (heat-sensitive) using 44 RM markers previously identified for heat tolerance in Nagina 22 and Uma by Whaghmare*et al*. (2018). ~~The study recorded a 45.45% polymorphism rate between the two parents, Nagina 22 and Manu Ratna.~~ The size of polymorphic markers identified ranged from 97 bp (RM473a) to 292 bp (RM10346). Among the 20 polymorphic markers detected, their distribution across linkage groups was as follows: three in LG-1, two in LG-2, two in LG-3, one in LG-4, three in LG-5, two in LG-6, two in LG-8, two in LG-9, one in LG-11, and two in LG-12. ~~These findings are consistent with those reported by Ravikiran *et al*. (2020), where 31 out of 116 SSR markers screened were found to be polymorphicbetween heat-tolerant NERICA cultivar NL44 and heat-sensitive Pusa Basmati 1.~~ It also revealed five minor QTLs associated with spikelet fertility and stress tolerance index, both of which are critical for heat resilience.

Udawela*et al*. (2018) studied two crosses between SH 527/Bg 90-2 and SH 527/Mengguandamagu and identified 61 and 59 polymorphic markers respectively out of 600 SSR markers screened, facilitating the development of heat-tolerant rice through molecular breeding approaches. Changrong*et al.* (2015) further demonstrated the significance of polymorphism by identifying 178 polymorphic SNP markers out of 384 screened between the rice varieties Milyang23 and Giza178.In another pair of parents IR8 and Giza 178,133 polymorphic markers were detected out of the same 384.These findings emphasize the importance of genetic variation in breeding programs for heat tolerance.

The current study identified 20 polymorphic SSR markers (Table 2) distributed across the genome, enabling the identification of genetic loci associated with heat stress tolerance. Ye *et al.* (2012) highlighted the utility of polymorphic markers in mapping QTLs such as ***qHTSF4.1***, a locus associated with spikelet fertility under heat stress, in a population derived from Nagina 22 and IR64. Similarly, the polymorphic information generated from this study can be used for identification of major QTLs associated with heat tolerance in Nagina 22 and Manu Ratna which could help in improving the latter for heat tolerance through molecular breeding techniques. Among the most studied QTLs, ***qHTSF4.1***, located on chromosome 4, has been consistently reported across multiple genetic backgrounds. This QTL is linked to spikelet fertility under heat stress, a critical trait for maintaining yield stability in high-temperature environments (Ye *et al*., 2022; Ye*et al*., 2011; Vijayalakshmi *et al*., 2018). Another significant QTL, ***qHTSF1.1***, located on chromosome 1, has been associated with improved pollen viability, seed set and spikelet fertility during heat stress (Zhao et al., 2006; Ye *et al*., 2012). These traits are crucial for reproductive success and can directly influence grain yield. Chromosomes 6 and 9 also contribute significantly to heat tolerance traits. ***qHT6.1*** is associated with canopy temperature regulation, which minimizes heat damage, while ***qHT9.1*** on chromosome 9 improves root biomass and water uptake efficiency under high temperatures (Collard and Mackill, 2008). Such studies highlight the pivotal role of parental polymorphism analysis in identifying key loci for heat stress resilience.The NERICA cultivar NL44 was noted as a novel source for reproductive stage heat stress tolerance, with several QTLs mapped that contribute to spikelet fertility under heat stress (Ravikiran *et al*., 2020).

The polymorphic SSR markers identified in this study provide a foundation for leveraging these QTLs in breeding programs. Markers associated with QTLs like *qHTSF4.1* and *qHTSF1.1* could be used for marker-assisted backcrossing, enabling the precise introgression of heat-tolerance traits into elite, high-yielding cultivars like Manu Ratna.Therefore this study complements existing research by identifying key polymorphic markers, which align with significant QTLs for heat tolerance. ~~These findings underscore the potential for integrating molecular tools and genomic resources into rice breeding programs, addressing the challenges posed by global climate change and ensuring food security in heat-prone regions.~~

4. CONCLUSION

The present study offers valuable insights into the genetic polymorphism between two contrasting rice genotypes, Nagina 22 (heat-tolerant) and Manu Ratna (heat-sensitive), through the use of SSR markers. ~~By~~ screening 44 microsatellitemarkers across 12 chromosomes~~, the study~~ identified 20 polymorphic markers, contributing to a deeper understanding of the genetic architecture underlying heat tolerance in these varieties. These findings are pivotal in advancing marker-assisted selection (MAS) and quantitative trait loci (QTL) mapping, enabling precise identification of loci associated with temperature resilience.

The significance of this study lies in its applicability to breeding programs aimed at mitigating the adverse impacts of climate change on rice production. The identified polymorphic markers facilitate the development of mapping populations and provide a foundation for discovering candidate genes linked to heat stress tolerance. This not only accelerates the breeding cycle but also enhances the efficiency of developing resilient rice varieties capable of sustaining productivity under elevated temperatures.By employing molecular breeding techniques, it bridges the gap between traditional breeding limitations and modern genetic approaches.

Table1: List of primers screened for polymorphism study

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Commented [a23]: A polymorphism rate of 45.45% was observed between Nagina 22 and Manu Ratna.

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Primer name	Forward primer	Reverse primer	Chromosome number
RM468	CCCTTCCTTGTTGTGGCTAC	TGATTTCTGAGAGCCAACCC	3
RM293	TCGTTGGGAGGTATGGTACC	CTTTATCTGATCCTTGGGAAGG	3
RM5	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAG	1
RM13	AAGGCGAACTGTCCTAGTGAAGC	CAGGATGTTCTTGCCAAGTTGC	5
RM85	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC	3
RM5749	GAAGAGAGAGCCAGAGCCAG	ACACGATCGAGCTAGAAGACG	4
RM9	GGCCCTCATCACCTTCGTAGC	CGTCCTCCCTCTCCCTATCTCC	1
RM3586	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	3
RM473A	TGATTTCTTGGAAGCGAAGAGTGAGG	CCTCCTTGTGCTCAGCCATGC	7
RM164	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG	5
RM3340	CGTCTTCATCATCGTCGCCCCG	GGCCCATCCCGTCGTGGATCTC	2
RM336	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC	7
RM6100	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	10
RM5545	ATCAGCAGCATTCAGCATTTGG	CCGGACGATGTGTATATCTCTTGG	8
RM251	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	3
RM224	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC	11
RM254	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC	11
RM518	CGCACTTGCTTAGAAGTCAATCATCC	ATGCTCTCTCCTTCAGGCCATCC	4
RM447	CACAACCTTTGAGCACCGGGTC	ACGCCTGCAGCTTGATCACCGG	8
RM212	AAGGTCAAGGAAACAGGGACTGG	AGCCACGAATTCCACTTTCAGC	1
RM225	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC	6
RM316	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG	9
RM201	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	9
RM17	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	12
RM19	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	12
RM26212	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC	11
RM7076	GTTCTTCAACTCCCAGTGCG	TGACGATGTGGAAGAGCAAG	3
RM302	TGCAGGTAGAAACTTGAAGC	AGTGGATGTTAGGTGTAACAGG	1
RM10346	GCTTGATCTGCCCTTGTTTCTTGG	AACTCGAGCGGCCTTCTCAGC	1
RM208	ACGGCCCATATAAAAGCCTC	AAGATGGCGGAGTAGCTCAG	2
RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	1
RM166	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	2
RM208	ACGGCCCATATAAAAGCCTC	AAGATGGCGGAGTAGCTCAG	2
RM252	TTCGGAAGTTGGTTACTGATCA	TTGGAGCGGATTCGGAGG	4
RM280	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	4
RM163	GTGACCACATCTATATCGCTCG	ATGGCAAGGTTGGATCAGTC	5
RM169	CCTCACTGACACCAGTATCCTCTCC	GCTCTTGGCAGATGGTGTAGGG	5
RM334	GCGACCGATCAGCTAGCTAG	ATAACTCCTCCCTTGCTGCC	5
RM6836	CCTCGAGCATCATCATCAGTAGG	TCCTCTTCTTGCTTGCTTCTTCC	6
RM242	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC	9
RM 552	AGGTGGTGAGGGTGAACTTG	TGGGATTAGAGCTTTGGTGG	11
RM3701	GGAAGCCTTTCCTCGTAACACG	GAACCTAGGCCGTGTTCTTTGC	11
RM256	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	8
RM3340	CGTCTTCATCATCGTCGCCCCG	GGCCCATCCCGTCGTGGATCTC	3

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Table 2: List of polymorphic markers between Nagina 22 and Manu Ratna

Sl no	Marker Name	Chromosome number
1	RM5	1
2	RM13	5
3	RM3586	3
4	RM164	5
5	RM251	3
6	RM212	1
7	RM225	6
8	RM316	9
9	RM17	12
10	RM19	12
11	RM26212	11
12	RM208	2
13	RM495	1
14	RM166	2
15	RM256	8
16	RM280	4
17	RM163	5
18	RM6836	6
19	RM242	9
20	RM556	8

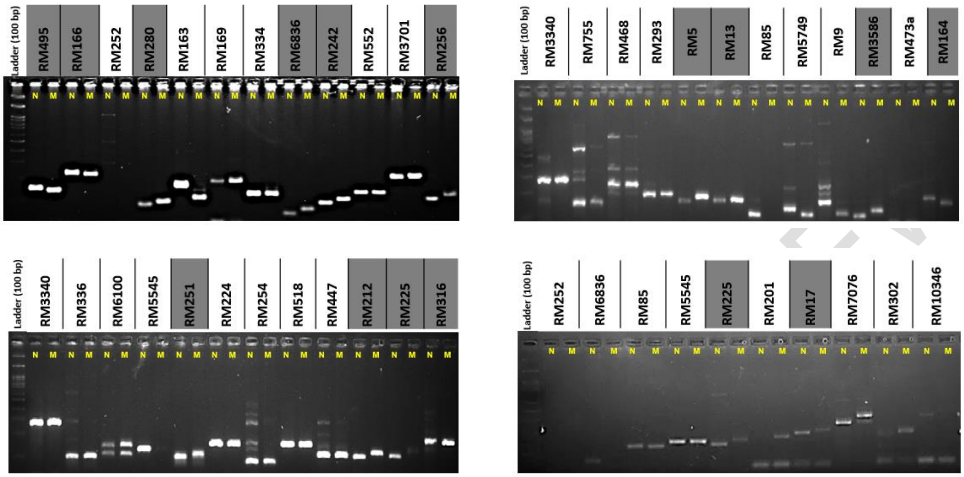

Figure 1: Amplification profile of polymorphic markers. The labels in grey denote polymorphic markers and white denotes monomorphic markers.

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