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## Research Note

# Marker assisted selection and genome recovery for heat tolerance in segregants of rice (*Oryza sativa* L.)

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**Abstract**

In the present global warming scenario, it is urgent to impart heat tolerance into the popular high yielding rice (*Oryza sativa* L.) varieties to reduce yield loss to a great extent. Uma (a prevalent red-grained rice variety in Kerala) was crossed with Nagina 22 (N22), the popular donor for heat tolerance. From the seven promising heat-tolerant $F_3$ plants previously identified through marker assisted selection and field screening, $F_4$ lines (58 nos.) were raised and characterised morphologically. Heat tolerance was scored under natural heat stress during hot summer months in field conditions. Among the evaluated $F_4$ progenies, 26 promising plants registered >75% spikelet fertility. Double panicles were observed in some of the tillers of fourteen $F_4$ plants. During the selection with reported polymorphic markers between parents, all the 26 $F_4$ plants produced the corresponding amplicon for RM5749 (linked marker for heat tolerance in the parental cross Uma x N22) and also for RM9, RM201, RM208, RM225, RM242, RM495, RM3586, RM6100, RM6836, and RM26212, corresponding to the heat tolerant parent N22. Genomic analysis revealed 64% recovery of the Uma genome in seven $F_4$ plants, with maximum genomic regions of Uma on the chromosomes 3 and 5. These promising $F_4$ plants could be forwarded to generate stable and high yielding heat tolerant rice varieties. The identified markers could be used for linkage analysis and QTL mapping in future.

**Keywords:** Heat stress; Rice; spikelet fertility; SSR; QTL

Rice (*Oryza sativa* L.) is the staple food all around the globe, feeding more than one-third of the human population (Sarangi *et al*., 2019). The global demand for rice will be about 584 million tons towards 2050 (Samal *et al*., 2022). Climate change is a threat for food security by intensifying the extreme weather events (Venkateshwarlu *et al*., 2022). Heat stress due to rapid global warming is a serious threat to the world's food security (Xu *et al*., 2021) and a major challenge to the rice breeders. Though rice can grow normally at a temperature range of 27 to 32⁰C, temperature >32⁰C negatively affects all of its growth and development stages (Aghamolki *et al*., 2014). The growth, yield, and quality of rice are influenced by high temperature stress, which disrupts its phenology, physiology, and yield components (Sindhumole *et al*., 2021). In rice, flowering and seed development stages are very sensitive to heat stress, leading to unfilled and chalky grains, which reduce the quantity and quality of seeds.

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Genetically improving the crops for heat tolerance is one of the best approaches to tackle this problem (Chidambaranathan *et al*., 2021). Heat tolerance is a multi-genic trait of which candidate genes are not well known. So, using yield as the selection criterion for developing heat tolerant varieties is usually unreliable during the early stages. Hence, marker assisted selection (MAS) is a better alternative to early generation selection. Fixing the recipient alleles in non-target regions and identifying the genotypes containing cross-overs close to the target genes are the major advantages of using DNA markers (Tanksley *et al*., 1989). As several QTLs associated with heat tolerance traits are with complex inheritance, MAS is more efficient for their screening. SSR markers are used for identifying various abiotic stress tolerance traits in rice (Salam *et al*., 2017; Waghmare *et al*., 2018).

**Table 1. Details of SSR primers used for polymorphism study**

| S.No. | Primer | Chromo some no. | Sequence |  | Annealing temperature (°C) | Product size (bp) |
|---|---|---|---|---|---|---|
|  |  |  | Forward (5'-3') | Reverse (5'-3') |  |  |
| 1 | RM212 | 1 | CCACTTTCAGCTACTACCAG | CACCCATTTGTCTCTCATTATG | 54. 5 | 136 |
| 2 | RM302 | 1 | TCATGTCATCTACCATCACAC | ATGGAGAAGATGGAATACTTGC | 59 | 156 |
| 3 | RM495 | 1 | AATCCAAGGTGCAGAGATGG | CAACGATGACGAACACAACC | 59 | 159 |
| 4 | RM10346 | 1 | GCTTGATCTGCCCTTGTTTCTTGG | AACTCGAGCGGCCTTCTCAGC | 65 | 292 |
| 5 | RM9 | 1 | GGTGCCATTGTCGTCCTC | ACGGCCCTCATCACCTTC | 59 | 136 |
| 6 | RM3340 | 2 | TCTTGGCAAGCTCTCCTCTC | CCATCATCTCGATCTTGACG | 59 | 117 |
| 7 | RM166 | 2 | GGTCCTGGGTCAATAATTGGGTTACC | TTGCTGCATGATCCTAAACGG | 59 | 321 |
| 8 | RM208 | 2 | TCTGCAAGCCTTGTCTGATG | TAAGTCGATCATTGTGTGGACC | 59 | 173 |
| 9 | RM251 | 3 | GAATGGCAATGGCGCTAG | ATGCGGTTCAAGATTCGATC | 59 | 147 |
| 10 | RM85 | 3 | CCAAAGATGAAACCTGGATTG | GCACAAGGTGAGCAGTCC | 59 | 107 |
| 11 | RM3586 | 3 | GAAGAGAGAGCCAGAGCCAG | ACACGATCGAGCTAGAAGACG | 59 | 118 |
| 12 | RM280 | 4 | ACACGATCCACTTTGCGC | TGTGTCTTGAGCAGCCAGG | 60 | 155 |
| 13 | RM252 | 4 | TTCGCTGACGTGATAGGTTG | ATGACTTGATCCCGAGAACG | 59 | 216 |
| 14 | RM5749 | 4 | GTGACCACATCTATATCGCTCG | ATGGCAAGGTTGGATCAGTC | 58 | 162 |
| 15 | RM518 | 4 | CTCTTCACTCACTCACCATGG | ATCCATCTGGAGCAAGCAAC | 59 | 171 |
| 16 | RM169 | 5 | TGGCTGGCTCCGTGGGTAGCTG | TCCCGTTGCCGTTCATCCCTC | 71 | 167 |
| 17 | RM163 | 5 | ATCCATGTGCGCCTTTATGAGGA | CGCTACCTCCTTCACTTACTAGT | 59 | 124 |
| 18 | RM164 | 5 | TCTTGCCCGTCACTGCAGATATCC | GCAGCCCTAATGCTACAATTCTTC | 60 | 246 |
| 19 | RM13 | 5 | TCCAACATGGCAAGAGAGAG | GGTGGCATTCGATTCCAG | 59 | 141 |
| 20 | RM6836 | 6 | TGTTGCATATGGTGCTATTTGA | GATACGGCTTCTAGGCCAAA | 59.5 | 240 |
| 21 | RM225 | 6 | TGCCCATATGGTCTGGATG | GAAAGTGGATCAGGAAGGC | 58.5 | 140 |
| 22 | RM755 | 6 | AAAGGATAAATGTGGGGATC | ATAACCGTCTGGTTTCACTG | 54.5 | 141 |
| 23 | RM336 | 7 | CTTACAGAGAAACGGC ATCG | GCTGGTTTGTTTCAGGTTCG | 58 | 154 |
| 24 | RM447 | 8 | CCCTTGTGCTGTCTCCTCTC | ACGGGCTTCTTCTCCTTCTC | 58 | 111 |
| 25 | RM256 | 8 | GACAGGGAGTGATTGAAGGC | GTTGATTTCGCCAAGGGC | 58 | 127 |
| 26 | RM242 | 9 | GGCCAACGTGTGTATGTCTC | TATATGCCAAGACGGATGGG | 52.5 | 225 |
| 27 | RM201 | 9 | CTCGTTTATTACCTACAGTACC | CTACCTCCTTTCTAGACCGATA | 54.5 | 158 |
| 28 | RM6100 | 10 | TCCTCTACCAGTACCGCACC | GCTGGATCACAGATCATTGC | 58 | 144 |
| 29 | RM254 | 11 | AGCCCCGAATAAATCCACCT | CTGGAGGAGCATTTGGTAGC | 60 | 165 |
| 30 | RM552 | 11 | CGCAGTTGTGGATTTCAGTG | TGCTCAACGTTTGACTGTCC | 58 | 195 |
| 31 | RM26212 | 11 | GTCGCTCCTCTCCTCCAATCC | GCTCGCTGCTTCTAATCTCTTGC | 62.5 | 180 |
| 32 | RM3701 | 11 | GAGCTAGAGGGAGGAGGTGC | TTGACTGATAGCCGATTGGG | 62.5 | 174 |
| 33 | RM224 | 11 | ATCGATCGATCTTCACGAGG | TGCTATAAAAGGCATTCGGG | 59 | 157 |
| 34 | RM19 | 12 | CAAAAACAGAGCAGATGAC | CTCAAGATGGACGCCAAGA | 58 | 226 |
| 35 | RM17 | 12 | TGCCCTGTTATTTTCTTCTCTC | GGTGATCCTTTCCCATTT CA | 59 | 184 |

Resorting to both genotypic and phenotypic selection gives a better output in breeding programmes. In Kerala, where rice is a major crop, temperature >39ºC causes heat stress-induced spikelet sterility. Among the prominent varieties of the state, Uma is high yielding but very susceptible to heat during reproductive and grain filling phases. To enhance heat tolerance in rice, the rice variety Nagina 22 (N22), which is characterized by better panicle length, panicle weight, and 1000-grain weight under heat stress variety is being used as a donor in several breeding programmes (Vinitha *et al*., 2020).

To incorporate high temperature tolerance, Uma rice variety was crossed with N22 (donor for heat tolerance), and the segregating generations were subjected to early MAS to identify superior progenies with heat tolerance. The present study was undertaken to evaluate the morphological variations in the $F_4$ population of Uma x N22, and to analyse the allelic distribution of various polymorphic marker loci identified between the parents. $F_4$ progenies (58 nos.) raised from the selected seven heat tolerant $F_3$ plants of the cross Uma x N22 were evaluated in pots inside the poly house in the field lab of Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, KAU, Thrissur. This unreplicated experiment was conducted during the summer months of 2018. Hence, the plants were stressed with high temperature (>40ºC), especially during the reproductive phase, and the biometrical observations were recorded.

Genotyping: Genomic DNA of $F_4$ segregants and the parents were extracted as per the CTAB method (Murray and Thompson, 1980). DNA purity was checked by measuring the OD values at 260 and 280 nm using NanoDrop ND100 spectrophotometer (Jenway-Genova Nano). For studying the allelic distribution in $F_4$ generation, 35 SSR markers polymorphic between Uma and N22 (Waghmare *et al*., 2018) were used (**Table 1**). Polymerase Chain Reaction (PCR) was performed with markers in a thermal cycler (Eppendorf Mastercycler® nexus gradient PCR), and the amplified products were subjected to electrophoresis in 2% Agarose gel.

Morphological evaluation: Biometric characters of 58 $F_4$ segregants were recorded and furnished in **Table 2**. Spikelet fertility is the major trait for the identification and selection of plants for heat tolerance. In the current study, heat tolerant parent N22 exhibited the highest spikelet fertility (90.13%), while the heat sensitive parent Uma exhibited a lower spikelet fertility (55.29%) during summer months. Twenty six $F_4$ plants, showing more than 75% spikelet fertility in the field, were selected (**Table 3** and **Fig. 1**). Time taken for the appearance of first panicle in selected $F_4$ plants was equivalent to that of N22 (76 days), whereas no. of filled grains per panicle (79.12) was lesser than both the parents. Mean grain weight per plant of the selected $F_4$ plants was 5.82 g, comparable to Uma.

Usually, only a single panicle is produced in each productive tiller of rice. In contrast, double panicles were observed in some tillers of 14 $F_4$ segregants in this study (**Table 2**). This phenomenon was observed in single tiller of nine plants (Plant Nos. 15.3, 15.4, 16.5, 16.6, 16.9, 31.4, 31.5, 41.1 and 41.2), in two tillers of four plants (Plant Nos. 13.1, 16.3, 16.4 and 31.10) and in three tillers of one plant (Plant No. 31.3). Double panicles per productive tiller was also observed earlier by Sindhumole (unpublished) in the parental $F_2$ (Uma x N22) plant, from which $F_4$ plants of the present study were derived. Similarly, Anup (2022) observed double panicles per tiller in N22 variety while

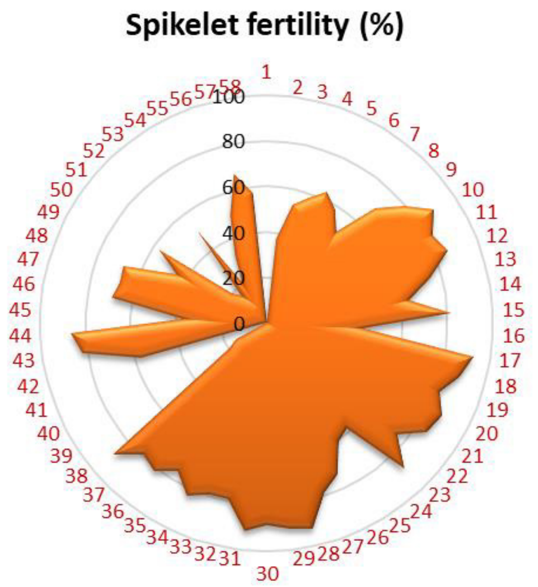


Fig. 1. Variability of spikelet fertility (%) in F4 plants

Table 2. Morphological characters of $F_4$ segregants of Uma x N22 rice hybrid

| S.No. | Plant no. | Tillers/ plant | Panicles/ plant | Panicle length (cm) | Days for flowering | Spikelets/ panicle | Filled grains/ panicle | Partially filled grains / panicle | Sterile grains/ panicle | 100 seed wt (g) | Grain wt/ plant (g) | Spikelet fertility (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 12.1 | 10 | 8 | 19.5 | 70 | 121.1 | 12.2 | 32.6 | 76.3 | 1.50 | 3.10 | 37.00 |
| 2 | 12.2 | 14 | 5 | 16.0 | 69 | 111.8 | 23.8 | 35.4 | 52.6 | 2.50 | 4.30 | 52.96 |
| 3 | 12.3 | 10 | 8 | 18.5 | 70 | 89.2 | 15.2 | 36.4 | 37.6 | 1.90 | 3.80 | 57.85 |
| 4 | 12.4 | 11 | 11 | 19.0 | 75 | 128.2 | 31.0 | 50.2 | 47.0 | 2.00 | 4.60 | 63.34 |
| 5 | 13.1 | 8 | 10$^{\#}$ | 20.5 | 69 | 149.4 | 32.4 | 54.6 | 62.4 | 2.30 | 5.90 | 58.24 |
| 6 | 13.2 | 12 | 7 | 19.2 | 68 | 121.7 | 25.8 | 35.4 | 60.5 | 2.70 | 5.30 | 50.29 |
| 7 | 13.3 | 10 | 8 | 17.0 | 69 | 109.0 | 17.4 | 58.2 | 33.4 | 1.90 | 4.80 | 69.36 |
| 8 | 15.1 | 11 | 10 | 22.5 | 85 | 138.0 | 97.3 | 12.4 | 28.3 | 1.94 | 7.30 | 79.50 |
| 9 | 15.2 | 10 | 8 | 19.6 | 82 | 111.2 | 90.6 | 8.2 | 12.4 | 1.99 | 7.10 | 88.85 |
| 10 | 15.3 | 8 | 9$^{\#}$ | 20.5 | 80 | 164.8 | 108.6 | 27.8 | 28.4 | 1.85 | 7.60 | 82.77 |
| 11 | 15.4 | 9 | 10$^{\#}$ | 21.2 | 81 | 109.0 | 83.2 | 10.4 | 15.4 | 2.01 | 7.08 | 85.88 |
| 12 | 15.5 | 10 | 8 | 21.0 | 81 | 129.3 | 75.4 | 21.2 | 32.7 | 1.58 | 4.01 | 74.71 |
| 13 | 15.6 | 9 | 9 | 19.8 | 80 | 69.2 | 30.0 | 13.8 | 25.4 | 2.12 | 5.80 | 63.30 |
| 14 | 15.7 | 9 | 8 | 22.0 | 80 | 97.8 | 53.6 | 26.0 | 18.2 | 1.73 | 6.60 | 81.40 |
| 15 | 15.8 | 10 | 10 | 21.4 | 79 | 37.8 | 12.0 | 4.0 | 21.8 | 1.91 | 6.90 | 42.33 |
| 16 | 16.1 | 7 | 7 | 20.2 | 69 | 127.0 | 105.4 | 12.0 | 9.6 | 1.98 | 8.21 | 92.45 |
| 17 | 16.2 | 8 | 8 | 19.1 | 73 | 118.7 | 84.0 | 20.5 | 14.2 | 1.94 | 7.30 | 88.04 |
| 18 | 16.3 | 9 | 11$^{\#}$ | 20.5 | 75 | 129.6 | 100 | 6.0 | 23.6 | 2.11 | 7.50 | 81.80 |
| 19 | 16.4 | 8 | 10$^{\#}$ | 20.2 | 70 | 117.6 | 73.2 | 29.4 | 15.0 | 1.68 | 6.51 | 87.25 |
| 20 | 16.5 | 8 | 9$^{\#}$ | 22 | 73 | 117.5 | 62.8 | 38.0 | 17.5 | 1.45 | 4.53 | 85.11 |
| 21 | 16.6 | 7 | 8$^{\#}$ | 18.6 | 75 | 130.6 | 82.0 | 16.4 | 32.2 | 1.90 | 3.16 | 75.35 |
| 22 | 16.7 | 9 | 9 | 19.0 | 76 | 118.4 | 78.0 | 26.4 | 14.0 | 2.70 | 4.64 | 88.18 |
| 23 | 16.8 | 8 | 6 | 22.0 | 76 | 175.2 | 80.0 | 22.0 | 73.2 | 2.22 | 5.93 | 58.22 |
| 24 | 16.9 | 7 | 8$^{\#}$ | 19.5 | 75 | 179.3 | 88.3 | 21.0 | 70.0 | 1.67 | 4.50 | 60.96 |
| 25 | 16.10 | 8 | 8 | 18.8 | 75 | 147.5 | 85.4 | 23.0 | 39.1 | 1.75 | 4.11 | 73.50 |
| 26 | 31.1 | 8 | 7 | 19.5 | 81 | 105.3 | 48.6 | 34.2 | 22.5 | 2.04 | 6.10 | 78.64 |
| 27 | 31.2 | 10 | 8 | 19.6 | 77 | 136.6 | 108.0 | 18.4 | 10.2 | 2.35 | 7.91 | 92.54 |
| 28 | 31.3 | 8 | 11$^{\#}$ | 20.0 | 78 | 149.4 | 104.4 | 31.2 | 13.8 | 1.97 | 5.70 | 90.77 |
| 29 | 31.4 | 9 | 10$^{\#}$ | 23.0 | 77 | 139.0 | 91.0 | 32.6 | 15.4 | 1.90 | 6.81 | 88.93 |
| 30 | 31.5 | 8 | 9$^{\#}$ | 19.5 | 75 | 132.8 | 80.2 | 41.6 | 11.0 | 1.95 | 6.54 | 91.72 |
| 31 | 31.6 | 10 | 9 | 19.2 | 79 | 174.4 | 130.2 | 8.0 | 36.2 | 1.94 | 8.10 | 79.25 |
| 32 | 31.7 | 9 | 8 | 19.7 | 77 | 42.2 | 28.0 | 5.6 | 8.6 | 2.12 | 5.80 | 79.63 |
| 33 | 31.8 | 9 | 7 | 22.0 | 80 | 106.8 | 63.2 | 26.0 | 17.6 | 1.70 | 4.57 | 83.53 |
| 34 | 31.9 | 8 | 7 | 23.5 | 75 | 124.8 | 79.4 | 15.4 | 30.0 | 1.30 | 5.91 | 75.97 |
| 35 | 31.10 | 7 | 9$^{\#}$ | 19.5 | 79 | 114.6 | 59.0 | 34.8 | 20.8 | 2.60 | 4.98 | 81.85 |
| 36 | 31.11 | 8 | 8 | 19.8 | 77 | 93.9 | 61.5 | 13.0 | 19.4 | 2.40 | 6.53 | 79.34 |
| 37 | 31.12 | 12 | 10 | 17.0 | 83 | 128.8 | 90.6 | 23.8 | 14.4 | 1.8 | 7.30 | 88.82 |
| 38 | 41.1 | 7 | 8$^{\#}$ | 20.8 | 81 | 141.0 | 0.0 | 28.0 | 113.0 | 0.00 | 0.00 | 19.86 |
| 39 | 41.2 | 8 | 9$^{\#}$ | 19.2 | 81 | 161.0 | 16.6 | 8.2 | 136.2 | 1.79 | 3.65 | 15.41 |
| 40 | 41.3 | 7 | 6 | 22.0 | 78 | 166.2 | 0.0 | 0.0 | 166.2 | 0.00 | 0.00 | 0.00 |
| 41 | 41.4 | 8 | 8 | 20.9 | 81 | 192.4 | 79.2 | 31.8 | 81.4 | 1.84 | 4.90 | 57.70 |
| 42 | 41.5 | 8 | 7 | 23.7 | 80 | 133.8 | 75.8 | 34.5 | 23.5 | 1.64 | 4.70 | 82.44 |
| 43 | 41.6 | 9 | 8 | 22.4 | 76 | 149.6 | 18.6 | 110.6 | 20.4 | 1.32 | 3.95 | 86.37 |
| 44 | 41.7 | 8 | 7 | 18.6 | 76 | 121.8 | 12.6 | 32.8 | 76.4 | 2.41 | 4.55 | 37.28 |

| S.No. | Plant no. | Tillers/ plant | Panicles/ plant | Panicle length (cm) | Days for flowering | Spikelets/ panicle | Filled grains/ panicle | Partially filled grains / panicle | Sterile grains/ panicle | 100 seed wt (g) | Grain wt/ plant (g) | Spikelet fertility (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 45 | 41.8 | 8 | 8 | 22.4 | 83 | 109.1 | 17.5 | 58.0 | 33.6 | 1.84 | 4.29 | 69.21 |
| 46 | 41.9 | 9 | 8 | 17.0 | 85 | 92.9 | 15.0 | 47.2 | 30.7 | 1.36 | 4.07 | 66.96 |
| 47 | 41.10 | 7 | 8 | 20.6 | 80 | 146.1 | 28.0 | 71.6 | 46.5 | 2.75 | 5.01 | 68.18 |
| 48 | 41.11 | 8 | 8 | 22.6 | 79 | 165.6 | 45.0 | 30.2 | 90.4 | 3.71 | 5.40 | 45.42 |
| 49 | 41.12 | 10 | 7 | 18.8 | 79 | 149.3 | 32.4 | 54.0 | 62.9 | 1.96 | 4.28 | 57.81 |
| 50 | 45.1 | 8 | 8 | 19.6 | 77 | 139.4 | 23.2 | 5.4 | 110.8 | 1.80 | 3.00 | 20.52 |
| 51 | 45.2 | 8 | 7 | 19.8 | 79 | 144.3 | 15.6 | 8.5 | 120.2 | 1.10 | 3.10 | 16.71 |
| 52 | 45.3 | 9 | 8 | 20.5 | 77 | 231.0 | 99.2 | 20.4 | 111.4 | 1.70 | 3.80 | 51.78 |
| 53 | 45.4 | 9 | 8 | 17.9 | 76 | 102.0 | 8.0 | 13.1 | 80.9 | 0.80 | 3.50 | 20.69 |
| 54 | 45.5 | 8 | 7 | 22.2 | 76 | 138.0 | 9.0 | 36.4 | 92.6 | 0.50 | 1.20 | 32.90 |
| 55 | 45.6 | 11 | 8 | 18.5 | 79 | 72.0 | 14.8 | 21.0 | 36.2 | 2.20 | 3.90 | 49.73 |
| 56 | 45.7 | 9 | 8 | 16.0 | 80 | 90.6 | 44.2 | 16.4 | 30.0 | 1.10 | 4.20 | 66.89 |
| 57 | 45.8 | 10 | 8 | 20.5 | 80 | 192.4 | 79.2 | 31.8 | 81.4 | 1.90 | 5.80 | 57.70 |
| 58 | 45.9 | 9 | 8 | 18.4 | 79 | 83.6 | 40.2 | 15.0 | 28.4 | 1.40 | 3.60 | 66.03 |

[#] Plant with double panicles in some of its tillers

evaluating N22, Jyothi and their hybrid. Hence, this trait of double panicles per tiller in segregants of Uma x N22 might have transferred from N22. However, exact reason for the appearance of double panicles per tiller in rice plants could not be traced from the available literature.

Among the selected 26 $F_4$ plants (with >75% spikelet fertility), ten (15.3, 15.4, 16.3, 16.4, 16.5, 16.6, 31.3, 31.4, 31.5 and 31.10) had double panicles, but others were with single panicle per tiller. Moreover, though other four plants (13.1, 16.9, 41.1 and 41.2) had double panicles, their spikelet fertility was not up to the desired level (**Table 2**).

Genotyping: In recent years, genotyping using SSR markers has been proven as the valuable tool for uncovering the hidden information in plants, like domestication features and crop improvement aspects *via* marker assisted breeding (Badri *et al*., 2022). Being co-dominant, SSR markers are ideal for segregation analysis in large population sets (Gupta and Varshney, 2000; Guo *et al*., 2015). Molecular markers are highly effective in assessing genetic diversity, structural analysis, and phylogenetic relations among rice accessions (Bollinedi *et al*., 2020; Shivani *et al*., 2021). In marker assisted breeding programmes, a robust marker-trait association should always be preferred, for exploiting a particular marker to improve a specific trait (Breseghello and Sorrells, 2006).

Foreground selection: Among the segregants of Uma x N22 cross, four $F_4$ plants (Plant nos.16.1, 31.2, 31.3, and 31.5) with >90% spikelet fertility and 22 $F_4$ plants with >75-90% spikelet fertility were subjected to foreground selection along with the parents (N22 and Uma) using the linked marker RM5749. In parents, the corresponding amplicons (157 bp and 129 bp, respectively) were observed for RM5749, located on chromosome 4 (**Fig. 2**). Interestingly, all the 26 selected plants registered only one amplicon (157 bp) corresponding to the heat tolerant parent N22, proving that the heat tolerance in these selected lines was inherited from the donor parent N22.

Thirty five SSR markers identified as polymorphic between the two parental lines by Waghmare *et al*. (2018) (**Table 1**) were used for screening the selected $F_4$ plants. Fourteen among these 35 SSR markers were recognised as associated with QTL for heat tolerance by earlier researchers (Zhang *et al*., 2009; Buu *et al*., 2014 *etc*.). In this study, single marker analysis revealed significant ($P<0.05$) association of spikelet fertility with RM5749, RM9, RM208, RM201, RM225, RM242, RM495, RM3586, RM6836, RM26212, and RM6100. Poli *et al*. (2013) and Bharathkumar *et al*. (2014) suggested RM225 and RM6100 respectively as polymorphic markers for heat tolerance. RM242 (Wei *et al*., 2013) and RM3586 (Lang *et al*., 2015) also were reported as polymorphic markers for heat tolerance.

Xiao *et al*. (2011) reported RM6100 as linked with a major QTL on chromosome 10 for heat tolerance during flowering stage. In conformity with the previous findings of QTL for heat tolerance, the above mentioned eleven markers exhibited the same allelic pattern of the male parent N22 in these 26 fertile $F_4$ plants studied, indicating the similarity in alleles with heat tolerant parent N22 at these marker loci. These 26 lines were further screened based on genome recovery and trait similarities with parents.

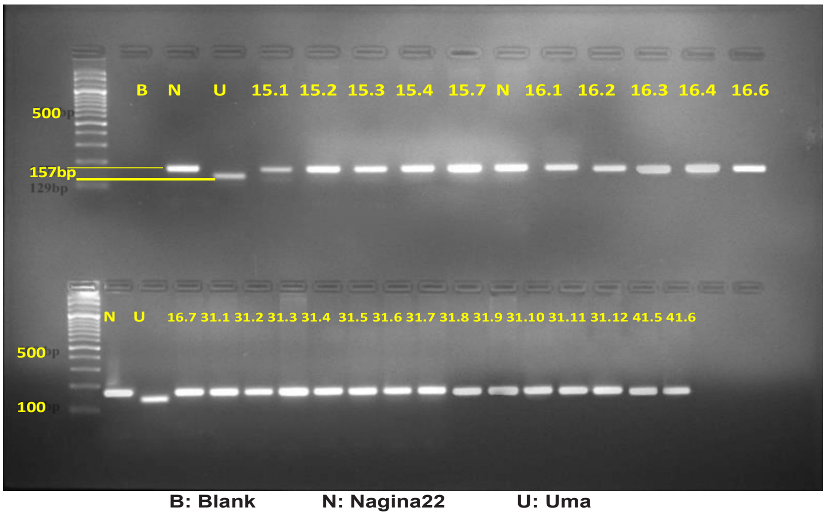


**Fig. 2. Foreground selection of 26 fertile F4 plants using RM5749**

RM5749 (chromosome 4) has been identified by Waghmare *et al.* (2018) as co-segregating with heat tolerance in Uma x N22 and its segregants. This emphasizes its efficiency in MAS programs, especially with the segregating generations of the same cross. The position of RM5749 was reported to be at 22.80 Mb on chromosome 4 (Gramene Database). This study also confirms the findings of Buu *et al*. (2014) that RM5749 was linked to heat tolerance in $BC_1F_1$ lines of the cross OM5930 x N22.

Allele distribution in selected segregants: The present investigation revealed more allelic diversity in the studied segregants (**Table 4**). Among the 34 polymorphic SSR markers used, nine markers (RM13, RM163, RM164, RM166, RM169, RM212, RM302, RM518, and RM7076) exhibited the same allelic pattern of female parent (Uma) in the selected 26 $F_4$ plants (**Fig. 3**).

Presence of alleles of both the parents was revealed by17 SSR markers, indicating the heterozygous nature of these marker loci in the selected $F_4$ segregants, which were further screened based on genome recovery using the marker data and trait similarities with Uma. Among these, the highest genome recovery of Uma was in plant nos. 16.7(67.14%) and 31.9 (65.71%). Seven plants (Plant no.15.3, 31.3, 31.4, 31.5, 31.7, 31.10, and 41.5) showed 64.29% recovery with Uma, and two plants, 15.4 and 15.7 showed 52.86% and 54.29% recovery of N22 respectively. The GGT(graphical genotyping) software output indicated the maximum genomic regions of Uma on chromosome 3 and chromosome 5 while heterozygous loci were located on chromosomes 3, 5, 11, and 12 (**Fig. 4**).

Thus, during this research programme, the selected $F_4$ segregants inherited the heat tolerance trait from the donor parent N22. Seven $F_4$ segregants were identified with 64.29% recovery of the Uma genome (recurrent parent) and the heat tolerance allele related to the donor parent N22. Hence, these promising $F_4$ lines with better heat tolerance and grain yield can be forwarded to subsequent generations and subjected to phenotyping and genotyping to develop the promising heat tolerant varieties in the background of Uma.

**Table 3. Phenotyping of $F_4$ segregants with more than 75% spikelet fertility**

| Trait | Maximum | Minimum | Mean |
|---|---|---|---|
| Plant height (cm) | 99.0 | 73.6 | 81.11 |
| Tillers/plant | 12.0 | 7.0 | 8.69 |
| Panicles/plant | 11.0 | 7.0 | 8.65 |
| Panicle length (cm) | 23.7 | 17.0 | 20.53 |
| Days for flowering | 85.0 | 69.0 | 77.42 |
| Spikelets /panicle | 174.4 | 42.2 | 123.54 |
| Filled grains/panicle | 130.2 | 18.6 | 79.12 |
| Sterile grains/panicle | 36.2 | 8.6 | 18.94 |
| l00 seed weight (g) | 2.7 | 1.3 | 1.89 |
| Grain weight /plant (g) | 7.6 | 3.1 | 5.82 |

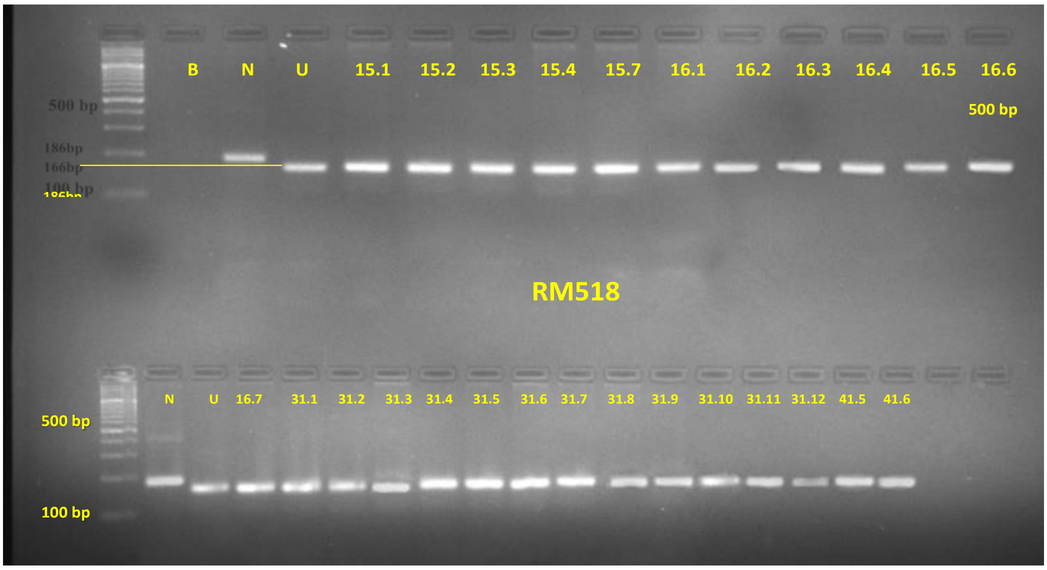
Table 4. Distribution of alleles of marker loci used for background selection in the selected $F_4$ plants

| S. No. | RM Marker | N22 | 15.1 | 15.2 | 15.3 | 15.4 | 15.7 | 16.1 | 16.2 | 16.3 | 16.4 | 16.5 | 16.6 | 16.7 | 31.1 | 31.2 | 31.3 | 31.4 | 31.5 | 31.6 | 31.7 | 31.8 | 31.9 | 31.10 | 31.11 | 31.12 | 41.5 | 41.6 | Uma |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RM302 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 2 | RM212 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 3 | RM495 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 4 | RM10346 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | A | B | A | B | A | A | B | A | B |
| 5 | RM9 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 6 | RM3340 | A | A | B | A | H | A | B | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 7 | RM166 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 8 | RM251 | A | A | A | H | A | H | A | A | H | A | A | A | H | H | B | H | H | B | H | H | B | H | H | B | H | B | B | B |
| 9 | RM208 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 10 | RM85 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 11 | RM7076 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 12 | RM3586 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 13 | RM280 | A | A | H | B | A | H | H | H | H | H | B | H | B | H | A | B | B | H | H | H | A | B | H | B | H | B | B | B |
| 14 | RM252 | A | B | A | B | H | H | H | H | A | H | H | B | H | B | B | B | B | B | B | B | B | B | B | B | B | A | H | B |
| 15 | RM518 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 16 | RM169 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 17 | RM163 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 18 | RM164 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 19 | RM13 | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 20 | RM6836 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 21 | RM225 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 22 | RM7555 | A | B | B | B | B | B | B | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 23 | RM366 | A | A | A | A | B | A | H | B | H | A | B | H | B | B | H | H | H | A | A | H | H | H | B | A | B | B | B | B |
| 24 | RM447 | A | A | A | B | B | B | A | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 25 | RM256 | A | A | B | B | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 26 | RM242 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 27 | RM201 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 28 | RM6100 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 29 | RM254 | A | B | A | B | A | A | H | B | H | H | H | B | B | B | B | H | B | B | B | B | B | B | B | B | B | H | A | B |
| 30 | RM552 | A | B | A | B | A | A | A | A | A | A | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | A | B |
| 31 | RM26212 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | B |
| 32 | RM3701 | A | H | B | A | A | A | H | A | H | H | A | H | H | H | H | H | A | H | H | B | H | B | H | A | A | A | H | B |
| 33 | RM224 | A | B | B | B | B | A | A | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 34 | RM19 | A | B | B | B | H | H | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| 35 | RM17 | A | H | B | B | A | A | B | B | A | A | B | A | B | A | H | H | H | H | H | B | A | B | A | A | B | B | B | B |

A – Alleles similar to male parent N22

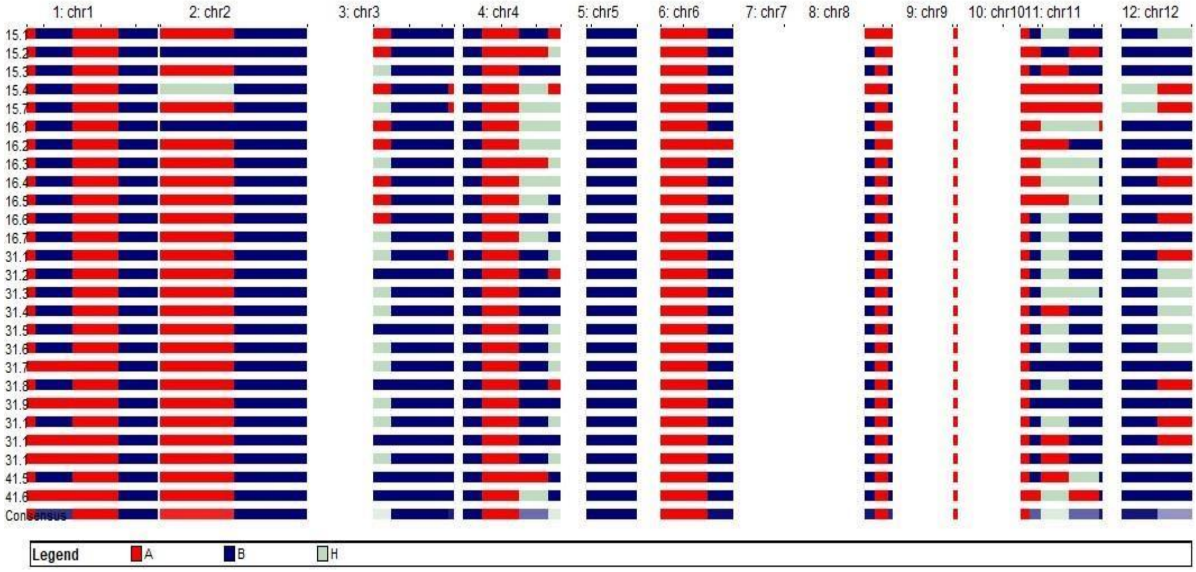
B – Alleles similar to female parent Uma

H – Heterozygous loci



**B: Blank** **N: Nagina22** **U: Uma**

**Fig. 3. Background selection of 26 fertile $F_4$ plants using RM518**



**A - N22 B - Uma H - Heterozygote**

**Fig. 4. GGT software output for the genome of 26 fertile F4 plants of Uma x N22**

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