Received: 01 May 2020 | Revised: 14 Dec 2020 | Accepted: 18 Dec 2020

Electronic Journal of Plant Breeding

Research Article

Identification of QTL linked to heat tolerance in rice (*Oryza sativa* L.) using SSR markers through bulked segregant analysis

Swapnil Gorakh Waghmare[1,5*], P. Sindhumole[2,4], Deepu Mathew[1], M. R. Shylaja[1], Rose Mary Francies[3], P. S. Abida[4] and M. C. Narayanankutty[4]

[1]Centre for Plant Biotechnology and Molecular Biology,
[2]Department of Plant Breeding and Genetics,
[3]Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University, Thrissur - 680 656, India
[4]Regional Agricultural Research Station, Kerala Agricultural University, Pattambi, Kerala - 679 306, India
[5]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, South Bohemian Research Center of Aquaculture and Biodiversity Hydrocenoses, Vodňany 389 25, Czech Republic
***E-Mail:**swapnil.waghmare1856@gmail.com

Abstract
Identification of loci and linked markers enable the marker-assisted selection for transferring the reproductive phase heat tolerance in rice. Microsatellite marker-based bulked segregant analysis (BSA) for heat tolerance was performed in F_3 progenies of the cross between temperature sensitive high yielding variety Uma and tolerant variety N22. High-temperature stress ranged from 23.33 to 38.99 °C at the booting-stage, and spikelet-fertility was considered as a direct measure of tolerance. N22 has exhibited a high spikelet-fertility of 90.92 per cent against 2.62 per cent, 44.78 per cent in Uma and F_3 plants, respectively. Of the 197 microsatellite markers screened, 41 were found to be polymorphic and used for BSA of tolerant and susceptible F_3 bulk along with both the parents. Marker RM5749 on chromosome 4 had co-segregated with tolerance, whereas, previously reported markers for heat tolerance in rice did not have an effect in the study population. The LOD value obtained during single marker analysis for the linkage between marker RM5749 and spikelet fertility was 6.86, indicating a strong linkage with the spikelet fertility under heat stress. The failure of the reported markers in our population suggests that the reproductive phase heat tolerance mechanisms in rice vary across the populations and the markers have to be validated for each population before being employed in MAS.

Key words
BSA, Mapping population, Marker assisted selection, heat tolerance, Simple Sequence Repeats, SSR, Rice breeding

INTRODUCTION

Rice is the staple food for more than half of the world's population and occupies 23.0 per cent of the total area under cereal production in the world (USDA, 2016). Rice has a genome size of 430 Mb (Kurata *et al*., 2002) which is one-tenth the size of the human genome and is a model system for cereal genome analysis. Microsatellite markers extensively used to tag and map the loci affecting quantitative traits. (Mohan *et al*., 1997). SSR markers have been routinely used for the identification of various abiotic stress tolerance and diversity analysis in rice (Rajendra Prasad *et al*., 2016, Salam *et al*., 2017, Waghmare *et al*., 2018, Challa and Kole, 2019, Tripathi *et al*., 2020). Bulked segregant analysis (BSA) is a method to detect the molecular markers linked to the gene of interest by

screening for alterations between two bulked (pooled) DNA samples derived from the segregating population constituted from a single cross.

DNA bulks of ten individuals or lesser are normally bulked, rather than screening dozens of individuals, for linkage analysis with selected polymorphic primers saving time and resources. These pooled DNA samples vary genetically only in a specific region. Segregating population, from which the bulks constituted, is used for validation of linkage between the target loci and the polymorphic marker. Markers for specific loci that are polymorphic and associated with the region can be used to discriminate the individuals constituting the bulks, and this will detect the apparent variances between the bulks (Michelmore *et al*., 1991). Many researchers (Zhang *et al*., 2009; Yang *et al*., 2011; Thakur *et al*., 2014; Dixit *et al*., 2014; Palanog *et al*., 2014; Sandhu *et al*., 2014; Tiwari *et al*., 2016; Pradhan *et al*., 2017) used bulked segregant analysis (BSA) for identification of molecular markers associated with several traits in rice.

Genotypic variations in high-temperature stress tolerance at flowering had been documented in *japonica* and *indica* species (Yoshida *et al*., 1981; Matsui *et al*., 2001; Matsui and Omasa, 2002; Prasad *et al*., 2006; Jagadish *et al*., 2007; Jagadish *et al*., 2008). The molecular markers namely RM7076, RM447, RM160, RM3586, RM430, RM26212, RM3735, RM3692 RM241, RM208, RM5545, RM3340, RM210 RM554, RM5749, RM468 RM7038, RM110, RM229, RM10086, RM147, RM258, RM332, RM3701, RM566, etc. have been reported for heat tolerance in rice (Cao *et al*., 2002; Zhu *et al*., 2005; Zhao *et al*., 2006). Hence, the present work was carried out with the objectives of finding out new markers for reproductive phase heat tolerance in rice and also to validate the reported markers in the present population.

MATERIALS AND METHODS

Two rice varieties, Uma (popular, high yielding but heat sensitive variety in Kerala, India), and N22 (heat tolerant donor variety from North India) were used in this study. The F_3 progenies derived from the hybrid, Uma × N22, along with parents were used for BSA.

Seedlings of F_3 progenies (>100 numbers) and parents, Uma and N22, were raised during the last week of December 2016 and individually transplanted into pots after three weeks, *i.e.* during the last week of January 2017. Survived F_3 plants (49 numbers) were labelled F_3-1 to F_3-49 and used for the study. Ten seedlings (1 per pot) of each parent were also being transplanted on the same day. At the booting stage, all the F_3 plants and five plants each of the parents transferred to polyhouse and exposed to high-temperature stress from 23.33 to 38.99 °C. Five plants each of both the parental genotypes, retained in ambient conditions, served as control. Thermo-hygrometer was used to record the polyhouse temperature, and the average maximum temperature from January to May 2017 was 35.19 °C. The average temperature recorded during morning time (8.30 to 9.00 am) inside and outside the polyhouse were around 35 °C and 32 °C, respectively. Inside the polyhouse, the temperature ranged from 37.2 to 42 °C during March 2017, and 37.1 °C to 42.7 °C in April (**Table 1**). F_3 plants for bulked segregant analysis were selected based on spikelet fertility percentage. The top seven F_3 plants having the highest and lowest spikelet fertility were selected as tolerant and susceptible plants, respectively.

Table 1. Temperature inside and outside of polyhouse

Month	Temperature (°C)			
	Inside polyhouse		Outside polyhouse	
	Maximum	Minimum	Maximum	Minimum
January 2017	37.67	20.99	34.13	20.93
February 2017	38.08	22.12	35.80	21.58
March 2017	39.44	23.83	35.78	23.52
April 2017	39.91	24.70	35.75	25.46
May 2017	39.89	25.01	34.46	24.70
Mean	**38.99**	**23.33**	**35.19**	**23.24**

Following the CTAB method (Dellaporta *et al*., 1983), genomic DNA was isolated from the young leaves (0.1 g). DNA extraction buffer (4% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2 % PVP and 0.2 % β-mercaptoethanol) preheated at 60 °C was used for the extraction. The quality of extracted DNA was assessed by agarose gel electrophoresis. Quantification and purity check were performed using a NanoDrop spectrophotometer. Initially, 197 RM markers (rice microsatellite markers) were screened to assess their capability to generate polymorphism among the parental line (Waghmare *et al*., 2018). The information on chromosomal location and primer sequences were retrieved from www.gramene.org. These 197 markers were carefully chosen to obtain the uniform coverage of all chromosomes of rice.

The polymerase chain reaction was carried out with the reaction mixture consisting of DNA 50 ng/µl; 10 X Taq

assay buffer; 10 mM dNTPs; 25mM MgCl2, 10 µM each of forward and reverse primers (0.5 µl each). The PCR programme included an initial denaturation at 95 °C for 3 min., followed by 35 cycles of denaturation at 94 °C for 50 seconds, primer annealing ~ 55 to 60 °C (depending upon marker sequence) for 30 seconds, extension at 72 °C for 1 min., and a final extension at 72 °C for 10 min.

Heat tolerant bulk and heat-susceptible bulk were prepared for marker analysis, each bulk containing an equal quantity of DNA (~1500 ng/µl and 2 µl of DNA each sample was used for pooling) from seven extreme individuals of the respective group. Forty-one primers, polymorphic between the parents Uma and N22, were used to detect the linked markers using the bulked DNA of phenotypic extremes for spikelet fertility. Subsequently, the SSR primer pairs, polymorphic between the respective bulks were surveyed on F_3 individuals to analyse the segregation of the markers. Additionally, Single Marker Analysis (SMA) was performed to detect the linkage between marker and trait.

RESULTS AND DISCUSSION

The F_3 progenies and N22 plants reached the booting stage around 70 days of sowing while parent Uma reached the booting stage on the 98th day. F_3 plants were assessed for heat tolerance/susceptibility based on spikelet fertility percentage. Top seven F_3 plants having the highest spikelet fertility were, F_3 - 31, F_3 - 15, F_3 - 41, F_3 - 13, F_3 - 16, F_3 - 12 and F_3 - 45 with spikelet fertility 91.88, 84.98, 82.85, 81.91, 81.18, 80.90, and 78.06 per cent, respectively. The seven F_3 plants having the highest spikelet fertility were used to constitute the tolerant bulk. While the top seven plants having the lowest spikelet fertility were, F_3 - 4, F_3 - 5, F_3 - 11, F_3 - 29, F_3 - 34, F_3- 37, and F_3 - 42 with zero per cent spikelet fertility and used to constitute the susceptible bulk.

Jagadish *et al*. (2008) have reported that N22 is highly tolerant to temperature stress (64 to 86% fertility at 38 °C) whereas, *Oryza sativa* ssp. japonica cultivars Azucena and Moroberekan are susceptible (<8%) genotypes. Allah *et al*. (2011) also observed 20 genotypes, and they found higher spikelet sterility (14-80%) and pollen sterility (15-54%) under heat stress whereas lesser spikelet sterility (10-18%) and pollen sterility (16-34%) under non-stress conditions. High spikelet fertility was observed for N22 (95.10%) under controlled condition (Yang *et al*., 2011). Vivitha *et al*. (2017) also reported the spikelet fertility in N22 under control and high-temperature treatments, 86.63 and 72.85 per cent, respectively, while in Improved White Ponni as 68.82 per cent and 16.52 per cent, respectively. Some of the individuals of the mapping population showed 60 to 67 per cent spikelet fertility, and they were used for finding the QTLs related to heat tolerance.

N22 is the most heat-tolerant variety of rice found so far and used as a heat tolerance donor in various breeding programmes. During the study of high-temperature stress tolerance at anthesis in rice using physiological and proteomic approaches, some of the heat shock proteins in N22 were considerably upregulated and it may be the reason behind the heat tolerance of N22 (Jagadish *et al*., 2010). Bulked Segregant Analysis and Genomic DNAs of the two parents were initially screened using 197 markers for polymorphism. The primer sets were successful in amplifying one or two alleles of the loci, and the sizes of amplicons ranged from 85bp (RM458) to 478bp (RM166). Forty-one polymorphic SSR markers among the parents (Waghmare *et al*., 2018), were employed in BSA. Among these, RM5749 had shown co-segregation with the trait. This marker was present in the tolerant parent (N22) as well as tolerant bulk at 189 bp (**Fig. 1**) and hence identified as a marker linked with the heat tolerance trait.

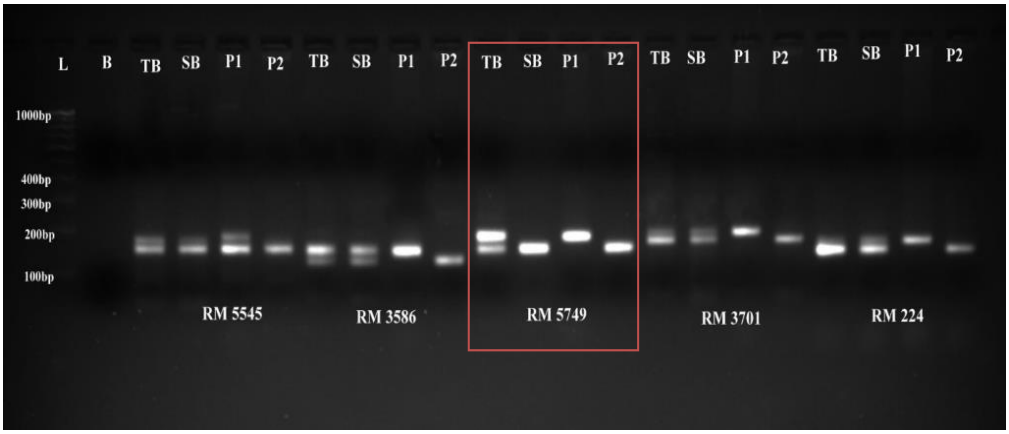

Fig. 1. Marker pattern for RM5749 in BSA using F_3 bulks and parents

L - 100bp ladder, B - blank, P1 - N22 (heat tolerant parent, 189 bp), P2 - Uma (heat susceptible parent, 160 bp), TB - Tolerant Bulk, SB - Susceptible Bulk.

Genotyping of the tolerant parent, susceptible parent, tolerant bulk and susceptible bulk with 41 polymorphic markers has indicated that the RM5749 marker located on chromosome number 4 co-segregates with the trait. This co-segregation pattern of the RM5749 marker indicated that this might be putatively linked to spikelet fertility, which is the vital indicator of heat tolerance. Buu *et al*. (2014) reported markers linked with high-temperature stress tolerance were located mostly on chromosomes 11, 10, 8, 6, 4, and 3. Similar to the current result, Buu *et al*. (2014) also identified RM5749 on chromosome 4, linked to grain yield under heat stress in BC_2F_2 lines obtained from the cross of OM5930 × N22. So, RM5749 can be used efficiently for marker-assisted selection in the subsequent generations of the cross Uma × N22.

Similar to the findings of the study, Zhang *et al*. (2009) had also found that the markers RM3735 (chromosome 3) and RM3586 (chromosome 3) are linked with high-temperature stress tolerance at the flowering stage in rice, indicating that the trait was under the control of multiple genes. However, in the present study, though RM3586 was a polymorphic marker between two parents (N22 and Uma), there was no polymorphism among the bulks, suggesting that it cannot be considered as a linked marker to heat tolerance in the present population. On the other hand, RM3735 was monomorphic among the parents themselves .

Among the primers used, some of the markers were recognised by earlier researchers as associated with QTL for heat tolerance. RM 473A was a polymorphic marker between parents for heat tolerance, as identified by Liao *et al*. (2011). Buu *et al*. (2013) reported RM251 as a polymorphic marker between parents for heat tolerance. Poli *et al*. (2013) reported RM225 as a polymorphic marker between parents for heat tolerance. As per the reports of Bharathkumar *et al*. (2014), RM6100 was a polymorphic marker between parents for heat tolerance. Buu *et al*. (2014) had identified RM7076, RM3586, RM26212 and RM5749 as a polymorphic marker for heat tolerance between parents. Wei *et al*. (2013)

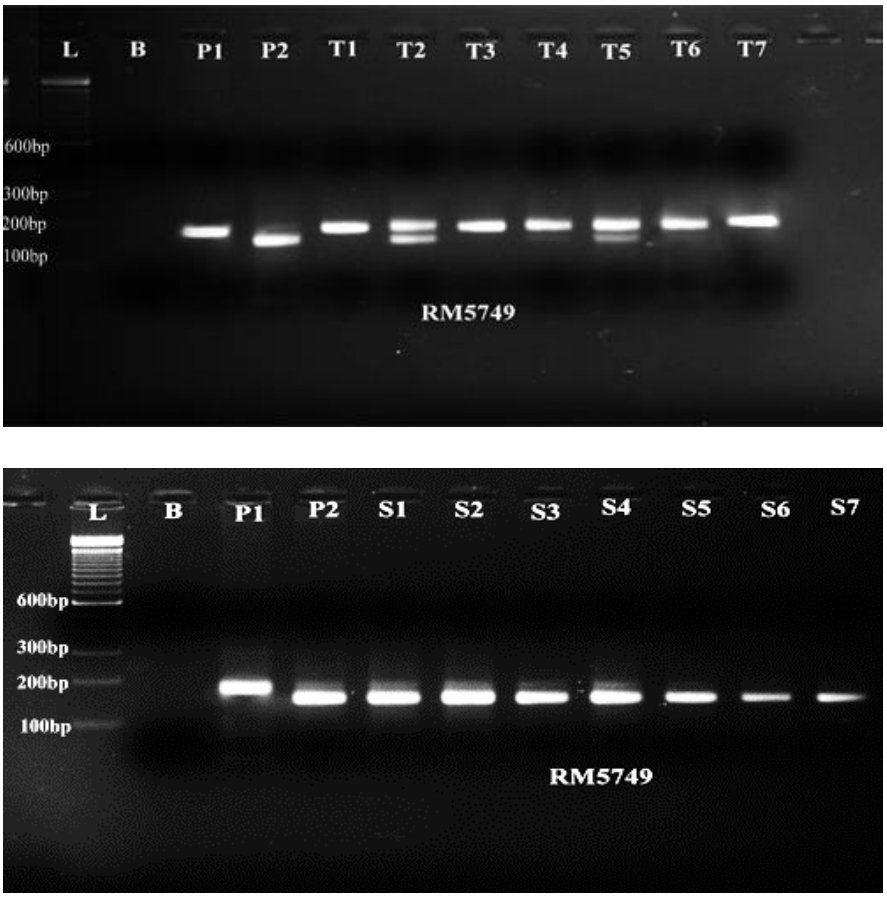

Fig. 2. Marker pattern for RM5749 in BSA using F_3 bulks and parents

L – 100 bp ladder, B - blank, P1 - N22 (heat tolerant parent, 189bp), P2 - Uma (heat susceptible parent, 160 bp), T1 to T7- individuals of heat tolerant bulk, S1 to S7- individuals of heat susceptible bulk

identified RM242 as associated with heat tolerance. Zhao *et al*. (2006) reported RM3340, RM447, RM5545, RM3701 and RM336 as being polymorphic markers for heat tolerance. Various researchers like Zhang *et al*. (2009), Lang *et al*. (2015) and Buu *et al*. (2014) also reported RM3586 as a polymorphic marker linked with heat tolerance.

The association of marker RM5749 with heat tolerance in rice was validated by screening individuals of tolerant and susceptible bulks and their parents. The five individuals of tolerant bulk F_3 - 12 (T1), F_3 - 15 (T3), F_3 - 16 (T4), F_3 - 31 (T6) and F_3 - 45 (T7) showed monomorphic bands as intolerant parent N22 (**Fig. 2**). While the two individuals namely, F_3 - 13 (T2) and F_3 - 41 (T5) showed heterozygous bands of both parents. The seven individuals of susceptible bulk F_3 - 4 (S1), F_3 - 5 (S2), F_3 - 11 (S3), F_3 - 29 (S4), F_3 - 34 (S5), F_3 – 37 (S6) and F_3 - 42 (S7) showed monomorphic bands like susceptible parent Uma (**Fig. 2**). Again, RM5749 was used for screening all the F_3 individuals for performing single marker analysis (**Fig. 3**). During SMA, RM5749 on chromosome 4, was significantly (*P* <0.001) associated with spikelet fertility under high-temperature stress. Moreover, RM5749 was identified as a tightly linked marker to spikelet fertility, with a LOD value of 6.86 (**Fig. 4**).

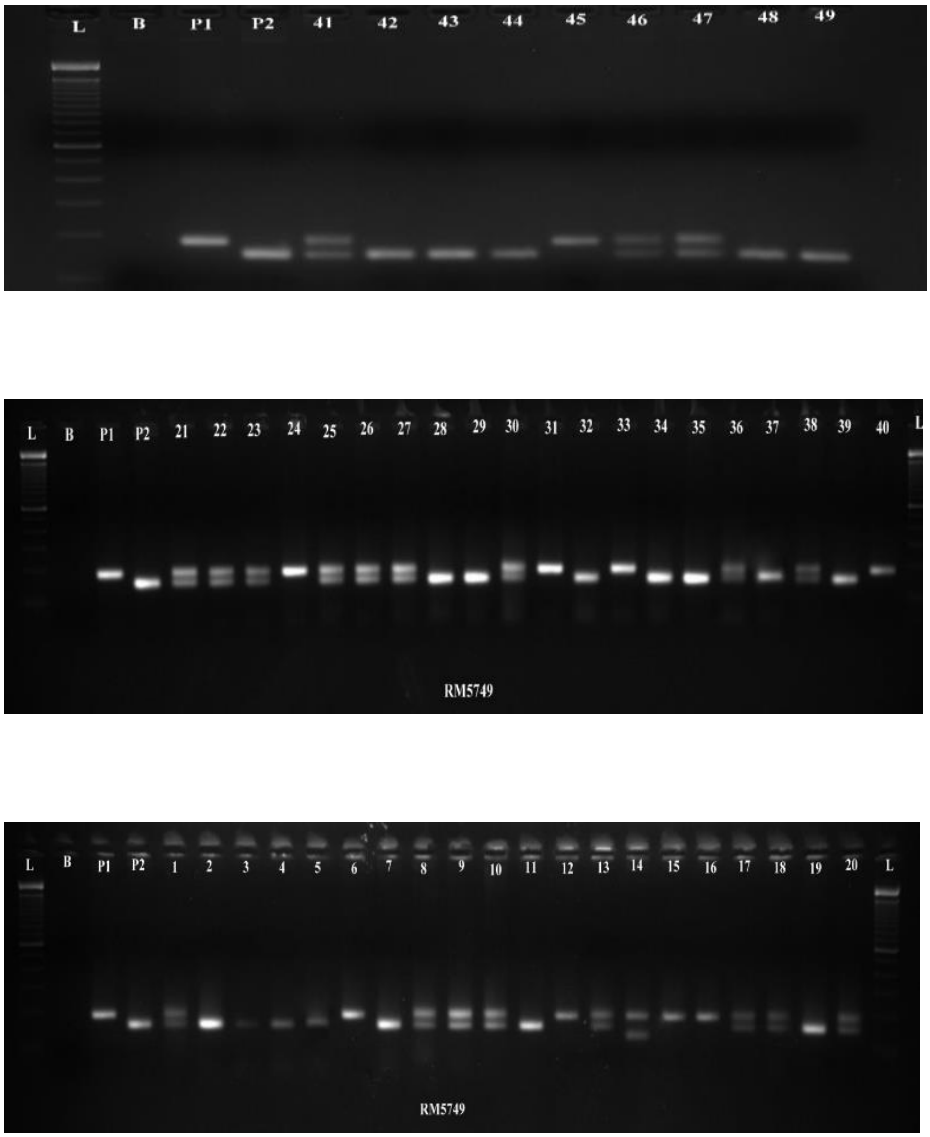

Fig. 3. Marker pattern for RM5749 in F_3 individuals in co-segregation analysis

L- 100 bp ladder (Genei), B- Blank, P1- N22 (Tolerant parent), P2- Uma (Susceptible parent), 1 to 49 - F_3 plants

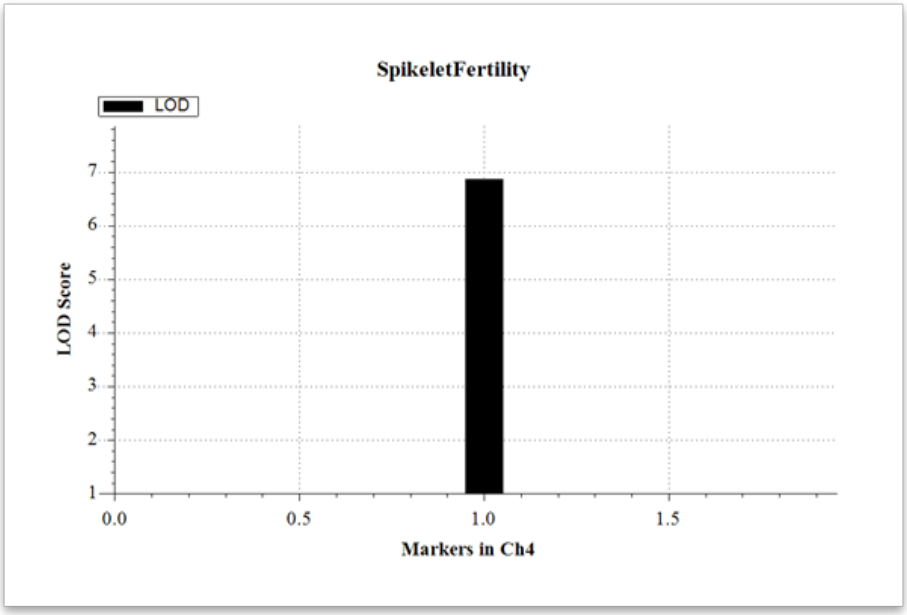

Fig. 4. Association of RM5749 with spikelet fertility

A significant quantitative trait loci (QTL) was identified based on the probability of association of the putative marker with spikelet fertility percentage under high-temperature stress through single marker analysis. LOD value of 6.86 had indicated the higher chances of the presence of QTL on chromosome 4. Hence, it can be inferred that a strong linkage exists for this QTL with spikelet fertility percentage under high-temperature stress. Parallel to the findings of the present study, a QTL on chromosome number 4, at the locus RM5749 was identified as linked to grain yield under high-temperature stress by Buu *et al*. (2014). The same researchers have also identified two QTL for filled grains per panicle on chromosome number 4 (RM241 - RM26212 and RM468 - RM7076), two QTLs regulating sterile grain percentage on chromosome number 3 (RM3686 and RM554) and one QTL for 1000-grain weight on chromosome 6 (RM103). Similar to the findings of the present study, Zhu *et al*. (2005) identified three QTLs for heat tolerance on chromosome number 7, 4 and 1 with LOD scores of 12.86, 11.08 and 8.16, respectively. In addition to this, Zhang *et al*. (2009) found two microsatellites (SSR) markers, RM3586 (on chromosome number 3) and RM3735 (on chromosome number 4) linked to high-temperature stress tolerance in rice through single marker analysis. In the present study, RM241, RM468, RM554, RM3735, and RM103 were found as monomorphic markers and RM26212, RM7076, and RM3586 were found as polymorphic markers between two parents (Uma and N22). As the combination of varieties used in this study was different from the previously reported studies, we got negative results even though using the heat specific SSR markers. The identified 41 polymorphic markers could be further used for the identification of QTLs and linkage analysis in the hybrid progenies of Uma × N22. RM5749 on chromosome number 4 could be used for the marker-assisted selection for heat tolerance in rice. Bulked Segregant Analysis in this population using SSR markers had shown that the marker RM5749 on chromosome number 4 is linked to heat tolerance in rice and (F: GTGACCACATCTATATCGCTCG ; R: ATGGCAAGGTTGGATCAGTC) hence could be used for the marker-assisted selection in rice. SSR markers near RM5749 on chromosome number 4 could be screened for heat tolerance. High-level heat tolerance in N22 could be transferred to high yielding heat susceptible variety Uma, through marker-assisted breeding using RM5749. This finding confirms the previous findings on the linkage of this marker with heat tolerance in rice. The failure of many previously reported markers in our population suggests that the tolerance mechanisms in rice are not universal, but each population vary in their contributing genes. Thus, for marker-assisted selection for heat tolerance in rice, optimization of markers is mandatory for each population.

ACKNOWLEDGEMENT

The senior author gratefully acknowledges the fellowship given by the Department of Biotechnology, Ministry of Science and Technology, Government of India, for this research programme and postgraduate studies.

REFERENCES

Allah, A.A.A., Badawy, S.A., Elrewainy, I.M.O. and Elkhtyar, A.M. 2011. Identifying heat tolerant rice genotypes under field conditions. *Egyptian J. Agron.,* **33**(2): 167-178. [Cross Ref]

Bharathkumar, S., Pragnya, P.J., Jitendra, K., Archana, B., Singh, O.N., and Reddy, J.N. 2014. Identification of rice germplasms associated with microsatelite (SSR) markers for heat tolerance at reproductive stage and expression of heat stress related gene. *Indian Res. J. Genet. Biotech.* **6**:424-27.

Buu, B.C., Ha, P.T.T., Tam, B.P., Nhien, T.T., Hieu, N.V., Phuoc, N.T., Minh, L.T., Giang, L.H. and Lang, N.T. 2014. Quantitative trait loci associated with heat tolerance in rice (*Oryza sativa* L.*) Plant Breed. Biotech.,* **2**: 14–24. [Cross Ref]

Cao, L.Y., Zhu, J., Zhao, S.T., He, L.B. and Yan, Q.C. 2002. Mapping QTLs for heat tolerance in a DH population from *indica japonica* cross of rice (*Oryza sativa*). *J. Agri. Biotech.,* **10**: 210-14.

Challa, V. and Kole, P. C. 2019. Parental evaluation and polymorphism survey of drought contrasting donor and recurrent parents in rice (*Oryza sativa* L.) using microsatellite markers. *Electronic J. Plant. Breed.,* **10(2)**, 406-412. [Cross Ref]

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA minipreparation: version II. Plant *Mol. Biol. Reporter.,* **1**: 19-21. [Cross Ref]

Dixit, S., Singh, A., Sta Cruz, M.T., Maturan, P.T., Amante, M. and Kumar, A. 2014. Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet* **15:** 16. [Cross Ref]

Jagadish, S.V.K., Craufurd, P.Q. and Wheeler, T.R. 2007. High temperature stress and spikelet fertility in rice. *J Exp Bot,* **58(7)**: 1627-1635. [Cross Ref]

Jagadish, S.V.K., Craufurd, P.Q. and Wheeler, T.R. 2008. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Sci.,* **48**: 1140-1146. [Cross Ref]

Jagadish, S.V.K., Muthurajan, R., Oane, R., Wheeler, T.R., Heuer, S., Bennet, J. and Craufurd, P.Q. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *J. Exp. Bot.,* **61(1):** 143-156. [Cross Ref]

Kurata, N.., Nonomura, K.I. and Ima, Y.I.H. 2002. Rice Genome Organization: the Centromere and Genome Interactions. *Ann. Bot.,* **90**: 427-435. [Cross Ref]

Lang, N.T., Ha, P.T.T., Tru, P.C., Toan, T.B., Buu, B.C., and Cho, Y.C. 2015. Breeding for heat tolerance rice based on marker-assisted backcrossing in Vietnam. *Plant Breed. Biotech.* **3**: 274-281. [Cross Ref]

Liao, J., Zhang, H., Shao, X., Zhong, P. and Huang, Y. 2011. Identification for heat tolerance in backcross recombinant lines and screening of backcross introgression lines with heat tolerance at milky stage. *Rice Sci.* **18(4)**: 279-286. [Cross Ref]

Matsui, T. and Omasa, K. 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: anther characteristics. *Ann. Bot.,* **89**: 683-687. [Cross Ref]

Matsui, T., Omasa, K. and Horie, T. 2001. Comparison of two rice (*Oryza sativa* L.) cultivars with tolerance to high temperatures at flowering or susceptibility. *Plant Prod. Sci.*, **4**: 36-40. [Cross Ref]

Michelmore, R.W., Pran, I. and Kessali, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA*, **88**: 9828-9832. [Cross Ref]

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R. and Sasaki, T. 1997 Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breed.*, **3**: 87-103. [Cross Ref]

Palanog, A.D., Swamy, B.P.M., Shamsudin, N.A.A., Dixit, S., Hernandez, J.E., Boromeo, T.H. and Kumar, A. 2014. Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. *Field Crops Res.*, **161**: 46-54. [Cross Ref]

Poli, Y., Basava, R.K., Panigrahy, M., Vinukonda, V.P., Dokula, N.R., Voleti, S.R., Desiraju, S. and Neelamraju, S. 2013. Characterization of a Nagina 22 rice mutant for heat tolerance and mapping of yield traits. *Rice* **6(36)**: 1-9. [Cross Ref]

Prasad, P.V.V., Boote, K.J., Allen, L.H., Sheehy, J.E. and Thomas, J.M.G. 2006 Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Res.*, **95**: 398-411. [Cross Ref]

Pradhan, D., Mathew, D., Mathew, S.K. and Nazeem, P.A. 2017. Identifying the markers and tagging a leucine-rich repeat receptor-like kinase gene for resistance to anthracnose disease in vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. J. *Hortic. Sci. Biotechnol.*, **93(3)**: 225-231. [Cross Ref]

Rajendra Prasad, N. S., Suresh, R., Selvaraj, M. G., Chandra Babu, R. and Shanmugasundaram, P. 2016. Identification of microsatellite markers linked to drought tolerance in rice (*Oryza sativa* L.) through bulked line analysis. *Electronic J. Plant. Breed.,* **7(4)**, 895-903. [Cross Ref]

Salam, S. A., Sindhumole, P., Waghmare, S. G. and Sajini, S. 2017. Molecular characterization of rice (*Oryza sativa* L.) genotypes for drought tolerance using two SSR markers. *Electronic J. Plant. Breed.,* **8(2)**, 474-479. [Cross Ref]

Sandhu, N., Singh, A., Dixit, S., Cruz, M.T.S., Maturan, P.C., Jain, R.K. and Kumar, A. 2014. Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. *BMC Genet.,* **15(1)**: 63. [Cross Ref]

Thakur, P.P., Mathew, D., Nazeem, P.A., Abida, P.S., Indira, P., Girija, D., Shylaja, M.R. and Valsala, P.A. 2014. Identification of allele specific AFLP markers linked with bacterial wilt [*Ralstonia solanacearum* (Smith) Yabuuchi *et al.]* resistance in hot peppers (*Capsicum annuum* L.). *Physiol. Mol. Plant Pathol*., **87**: 19-24. [Cross Ref]

Tiwari, S., Kumar, V., Singh, B., Rao, A. and Mithra, S.V.A. 2016. Mapping QTLs for salt tolerance in rice (*Oryza sativa* L.) by bulked segregant analysis of recombinant inbred lines using 50k SNP chip. *PLoS One*, **11(4)**: e0153610. [Cross Ref]

Tripathi, S., Singh, S. K., Srivashtav, V., Khaire, A. R., Vennela, P. and Singh, D. K. 2020. Molecular diversity analysis in rice (*Oryza sativa* L.) using SSR markers. *Electronic J. Plant. Breed.,* **11(03)**, 776-782.

USDA [United States Department of Agriculture]. 2016. Economic Research Service. Available at https://www.ers.usda.gov/topics/crops/rice.aspx [26 Feb 2017]

Vivitha, P., Raveendran, M. and Vijayalakshmi, D. 2017. Introgression of QTLS controlling spikelet fertility maintains membrane integrity and grain yield in improved White Ponni derived progenies exposed to heat stress. *Rice Sci*., **24(1)**: 32-40. [Cross Ref]

Waghmare, S.G., Sindhumole, P., Shylaja, M.R., Mathew, D., Francies, R.M., Abida, P.S. and Sajini, S. 2018. Analysis of simple sequence repeat (SSR) polymorphism between N22 and Uma rice varieties for marker assisted selection. *Electronic J. Plant. Breed.,* **9(2)**, 511-517. [Cross Ref]

Wei, H., Liu, J., Wang, Y., Huang, N., Zhang, X., Wang, L., Zhang, J., Tu, J. and Zhong, X. 2013. A dominant major locus in chromosome 9 of rice (*Oryza sativa* L.) confers tolerance to 48 °C high temperature at seedling stage. *J.Heredity* **104(2)**: 287-294. [Cross Ref]

Yang, Y.C., Argayoso, M., Redoña, E., Sierra, S., Laza, M., Dilla, C., Mo, Y.J., Thomson, M.J., Chin, J.H., Delaviña, C.B., Diaz, G.Q. and Hernandez, J. 2011. Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breed.,* **131**: 33-41. [Cross Ref]

Yoshida, S., Satake, T. and Mackill, D.S. 1981. High temperature stress in rice. *IRRI Research Paper Series,* **67**: 1-15.

Zhang, G., Chen, L., Xiao, G., Xiao, Y., Chen, X. and Zhang, S. 2009. Bulked segregant analysis to detect QTL related to heat tolerance in rice using SSR markers. *Agric. Sci. China,* **8(4)**: 482-487. [Cross Ref]

Zhao, Z.G., Jiang, L., Xiao, Y.H., Zhang, W.W., Zhai, H.Q. and Wan, J.M. 2006. Identification of QTLs for heat tolerance at the booting stage in rice (*Oryza sativa* L.). *Acta. Agron. Sin*., **32**: 640-644.

Zhu, C.L., Xiao, Y.H., Wang, C.M., Jiang, L., Zhai, H.Q. and Wan, J.M. 2005. Mapping QTLs for heat tolerance during grain filling in rice. *Chinese J Rice Sci* **19**: 117-121.