ORIGINAL RESEARCH ARTICLE

Grain and cooking quality analysis in heat-tolerant QTL introgressed restorer of hybrid rice

Jaldhani V[1], Neeraja CN[1], Sanjeeva Rao D[1], Aravind Kumar J[1], Siromani N[1], Beulah P[1], Nagaraju P[1], Manasa Y[1], Rao PR[1], Subrahmanyam D[1], P Sudhakar[2], A Krishna Satya[2], Senguttuvel P*[1]

[1]ICAR-Indian Institute of Rice Research, Hyderabad, India.
[2]Biotechnology department, Acharya Nagarjuna University, Guntur, India.
Corresponding author email: *senguttuvel@gmail.com

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Abstract

Rice grain yield and quality are affected by the high temperature stress to a large extent. Four promising backcross introgression lines (BC_2F_8) of KMR-3R/N22 carrying qHTSF1.1, qHTSF4.1 and HT Score QTLs were evaluated for high-temperature induced rice grain and cooking quality during *Rabi* 2021. Late-sown staggered planting was adopted to ensure uniform high-temperature stress on the improved restorers along with the parents used, which resulted in significant yield loss and grain quality degradation due to high-temperature stress. For cooking quality metrics, ANOVA showed significant variation among genotypes (G), treatments (T), and G×T. Among the backcross introgression lines (BILs), RP6338-9 possesses qHTSF4.1 noted to be promising for grain quality traits under high temperature stress.

Keywords: Hybrid rice, KMR-3R, high-temperature stress, grain quality and cooking traits.

Introduction

Rice (*Oryza sativa* L) is a staple cereal consumed by more than one half of the world's population and providing 35-80 % of total calorie intake (Wassmann *et al.,* 2009). The episodes of high-temperature and altered precipitation levels affect the global food grain security (IPCC 2014). An increase of 1°C of global mean temperature can reduce the yields of maize (7.4%), wheat (6%), rice (3.2%) and soybean (3.1%) which affords two-thirds of human caloric intake globally (Zhao *et al.,* 2017). By the end of the century, the global mean temperature is anticipated to rise by 2.5°C to 5.8°C, and extreme temperatures may be more common (IPCC 2014). Breeding rice for high-temperature stress is a major priority area of rice research in order to sustain global rice production and meet the expected need of the ever-growing population. In comparison to varieties, hybrid rice technology provides more stability in meeting food grain production goals. Hybrid technology's success is attributed to yield heterosis, and hybrids account for a large portion of global rice yield. Hybrid rice occupies 3-4 million hectares in India and contributes 3–4 million tonnes of rice production (Senguttuvel *et al.,* 2019). Despite succumbing to numerous biotic and abiotic stresses, rice hybrids showed a 10-13 percent yield advantage over popular varieties (Virmani 2003; Serraj *et al.,* 2009; Villa *et al.,* 2012). Madan (2012) observed that the yield advantage of hybrids is being affected by the reduced seed setting rate under high-temperatures (38°C) when compared to normal temperatures (29°C - 35°C). Similarly, Zhou (2009) and Hu (2012) also observed the sensitivity of hybrid rice towards high-temperature stress. Rice hybrid seed production is often followed in India during *Rabi* (Dry) season due to dry weather and absence of precipitation. The high-temperature at the anthesis stage affects the pollen viability (Song *et al.,* 2001; Wassmann *et al.,* 2007) anther dehiscence (Matsui *et al.,* 2000, 2005), pollen tube elongation and stigma receptivity which eventually results in low spikelet fertility rate, reduced grain yield and quality (Satake and Yoshida 1978). To develop heat tolerant quality rice hybrids, both the parental lines should possess high level of temperature tolerance (Guan-fu *et al.*, 2015) and this strategy showed significant association in the three-line hybrid rice system (Kuang *et al.*, 2002; Gong *et al.*, 2008). Apart from yield, grain quality also plays a major role in the acceptance of the rice variety or hybrid. Based on this background, the restorer line KMR-3R was introgressed with qHTSF1.1 and qHTSF4.1 for heat tolerance through Marker-Assisted Backcross Breeding (MABB) approach and promising backcross introgression lines (BILs) with heat-tolerance were developed (Jaldhani *et al.*, 2021). In this study, the promising heat-tolerant BILs and their parental lines were evaluated for grain quality under ambient and high-temperature stress.

Materials and Methods

Plant material

Four BILs (BC_2F_8) namely RP6338-9 (qHTSF4.1), RP6338-28 (qHTSF1.1), RP6338-48 (qHTSF4.1) and RP6338-66 (HT Score) derived from the KMR-3R/Nagina22 (Jaldhani *et al.*, 2021) were used in this study. These BILs were introgressed with heat-tolerant QTLs and native fertility restoration. KMR-3R is a promising restorer line and Nagina22 (N22) is a potential genetic resource for heat tolerance. N22 is widely employed in heat-tolerance studies (Senguttuvel *et al.*, 2020; Jaldhani *et al.*, 2021).

High-temperature treatment

The study was carried out at the Indian Institute of Rice Research farm, Hyderabad, India (17.53° 19'N latitude and 78.27° 29' E longitude, 542.7 MSL, with a mean temperature of 31.2°C and mean annual precipitation of 988.3 mm) during Dry (*Rabi*) season 2021. The high-temperature stress was imposed by following late sown method in dry season (Senguttuvel *et al.*, 2020). The experiments were replicated thrice in a randomized complete block design (RCBD) with same sets of genotypes in two experiments under normal and late sown method. Standard agronomic practices and integrated pest management was followed throughout the experiment. The weather data recorded throughout the crop duration was indicated in **Figure 1** and **Table 1**.

Grain quality analysis

At physiological maturity, each paddy sample was harvested, thoroughly cleaned from impurities and dried under shade till the moisture content reached to 15%. All the samples were stored under ambient conditions and quality analysis – Milling (M%), Hulling (H%) and Head rice recovery (HRR%), Amylose content (AC), Gel consistency (GC), Kernal length before cooking (KLBC), Kernal breadth before cooking (KBBC), Kernal length after cooking (KLAC), Kernal breadth after cooking (KBAC) were performed at the end of three months aging (Juliano, 1971; Cagampang, 1973).

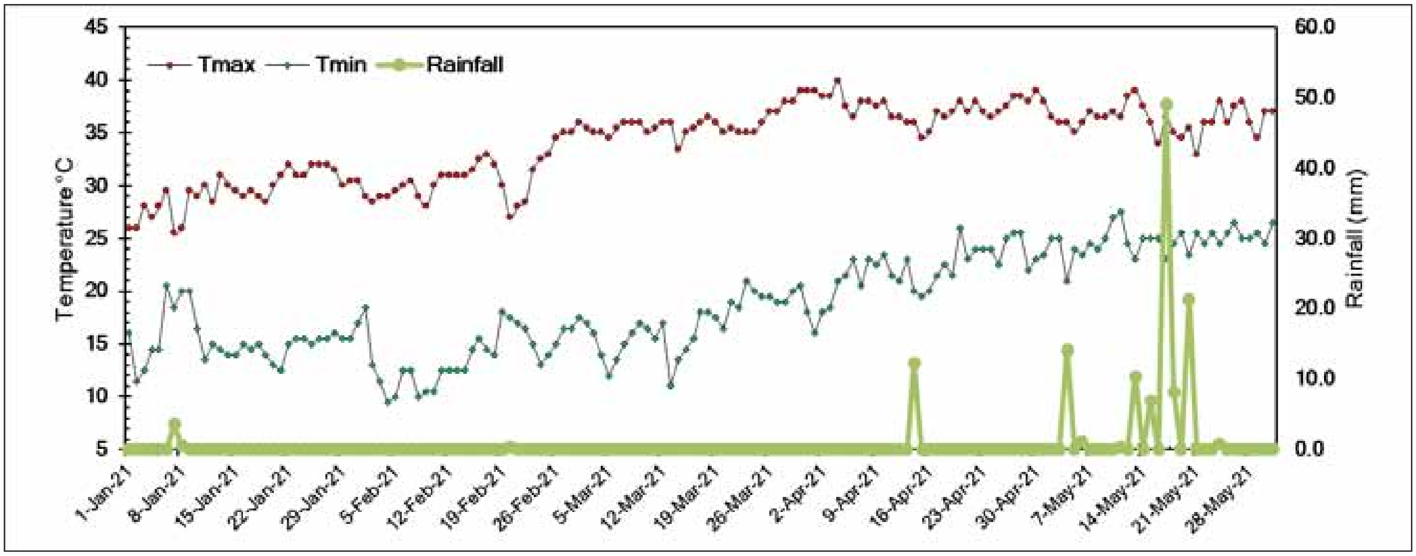

Figure 1: Maximum and minimum temperature (°C), rainfall (mm) during the crop growing period during *Rabi* 2021.

Table 1. Monthly meteorological data recorded during *Rabi* 2021.

Season / Month	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	Rainy Days	Sun Shine (hours)	Wind Speed (Km h^{-1})
	MAX	MIN	I	II				
January	29.5	15.3	95.0	76.0	4.2	2	7.1	3.0
February	30.7	13.8	88.1	41	0.4	1	8.5	3.5
March	35.9	17.1	80.6	30.0	0.0	0	8.0	3.6
April	37.4	22.1	81.1	47.0	12.2	2	7.5	4.4
May	39.6	24.8	89.1	51.1	112.6	6	8.3	6.1
Mean	34.6	18.6	86.78	49.02	-	-	7.88	4.12
Total	-	-	-	-	129.4	11	-	-

Statistical analysis

The data collected on grain quality was analyzed statistically using two-way analysis of variance (ANOVA) (Gomez and Gomez, 2010) in software *Statistix 8.1* (Analytical software, 2003). The derived data from the ANOVA, represented with standard errors of mean (SE) and Tukey's honest significant difference (HSD) (P = 0.05) between treatments and genotypes.

Results and Discussion

Heat tolerant QTL introgressed lines were evaluated in the present study to understand the effect of high temperature on grain quality. As grain quality plays a major role in the acceptance of rice variety, it is imperative to analyze the influence of high temperature on grain quality in comparison with the same variety cultivated under normal conditions (ambient/control). The results of analysis of variance (ANOVA) indicates significant variation among genotypes (G), treatment (T) and G × T for cooking quality parameters (**Table 2** and **Table 3**). Marginal variation (<0.5%) in AC % was observed between RP6338-9 & RP6338-66, whereas AC increased in other samples at high temperature stress except RP6338-28.

Table 2. Mean squares for AC, GC, GLBC, GBBC, GLAC and GBAC in ambient and high temperature methods.

Source	df	AC	GC	GLBC	GBBC	GLAC	GBAC
Rep	2	1.102	39.69	0.004	0.009	0.05	0.003
Genotype (G)	5	46.82***	3591.04***	0.107***	0.027***	0.78***	0.41***
Treatment (T)	1	14.85***	1877.78***	0.001	0.08***	0.20*	0.071***
G × T	5	12.53***	279.04***	0.015*	0.014 **	0.25 ***	0.022 ***
Error	22	0.75	25.39	0.004	0.003	0.03	0.003

df-Degrees of freedom; *P < 0.05; **P < 0.01; ***P<0.001.

Table 3. Effect of high-temperature stress on AC, GC, GLBC, GBBC, GLAC and GBAC.

Trait	Mean		Difference	Grand Mean	CV (%)	Tukey HSD (P<0.05)		
	Control	HT				Genotype (G)	Treatment (T)	G × T
AC	27.79	29.07	1.3	28.43	3.04	1.55	0.60	2.57
GC	57.44	43	-14.4	50.22	10.03	9.06	3.49	14.96
GLBC	4.68	4.67	0.0	4.67	1.39	0.12	0.04	0.19
GBBC	2.14	2.05	-0.1	2.10	2.68	0.10	0.04	0.17
GLAC	7.78	7.63	-0.2	7.70	2.20	0.30	0.12	0.50
GBAC	2.62	2.71	0.1	2.67	1.99	0.10	0.04	0.16

Change in amylose content from intermediate under controlled conditions to high under elevated temperature was observed in the parents (N22 & KMR 3) and vice-versa in the case of RP6338-28 (**Figure 2**). High-temperature affected amylose content, amylopectin chain length, immature kernels, chalkiness, kernel dimensions, and fissuring in rice (Wassmann *et al.*, 2009). However, amylose and amylopectin contents were similar in high-temperature than in the ambient condition, which indicates that variation in these components at high-temperature varies among the genotypes/varieties. This underlines the need to verify the fitness of every released variety or developed lines at high-temperature (Jaldhani *et al.*, 2022). The GC % was same in both control and treatment for N22 and RP6338-48, however the values decreased significantly for other samples in control and treatment. Before cooking, there were no significant differences in grain length or grain breadth between the samples under high temperature stress and control conditions. The ratio of grain length to grain breadth indicates that the ILs are of medium bold grain type. Similarly, negligible variations were observed under treatment and control conditions for grain breadth after cooking and grain length after cooking except for RP6338-9 which noted around 0.8 mm more under control conditions.

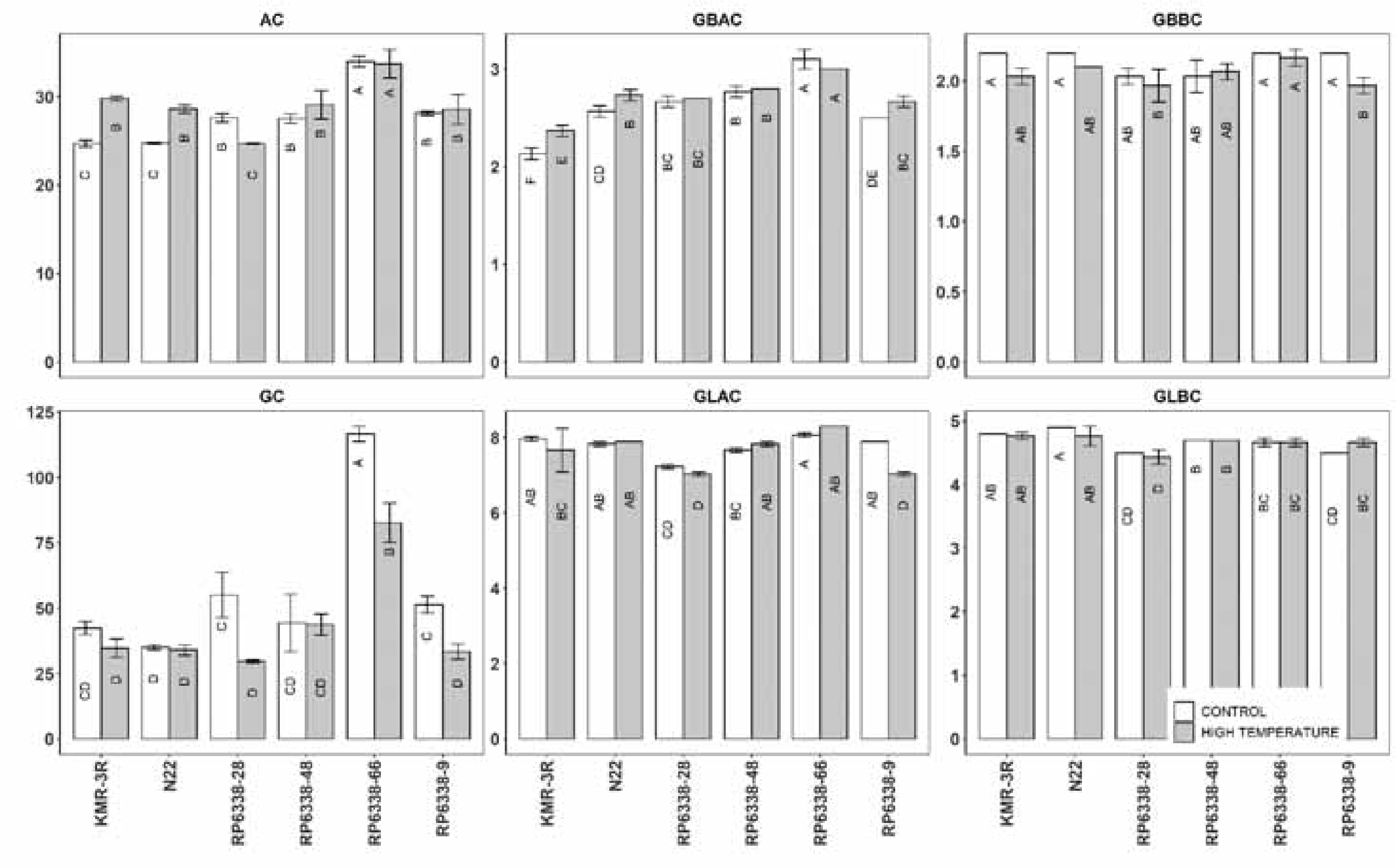

Figure 2: Effect of high-temperature stress on grain quality traits in ILs and parents.

Compared with controlled conditions, marginal variations were observed for Hulling percent and Milling percentage at high-temperature and significant for head rice recovery (**Table 4).** Among the four BILs, only two (RP6338-9 and RP6338-28) noted desired HRR% of ≥60% under controlled conditions. Only one BIL noted (RP6338-9) desired HRR% under high temperature conditions. The reduction in HRR% is largely due to decrease in the density of chalky rice grain that are formed due to high night air temperature. Chalkiness is the opaque region of the brown or polished rice grain. The proportion of chalkiness was high in rice varieties cultivated at high-temperature than the same varieties cultivated at ambient temperature. Variation in grain appearance itself was noticed among the varieties/genotypes. Variation in individual grain weight was also observed under high-temperature (Jaldhani *et al.*, 2022).

Table 4. Effect of high-temperature stress on H%, M% and HRR

	H%		M%		HRR	
	Control	**HT**	**Control**	**HT**	**Control**	**HT**
N22	73.3	75.6	63.5	68.1	59.1	36.3
KMR-3R	75.1	74.5	65.5	66.2	58.6	47.6
RP6338-9	75.1	74.4	66.9	68.1	65.5	59.3
RP6338-28	74.3	74.3	64.3	68.8	62.7	45.4
RP6338-48	72	73.3	63.4	65.5	49.4	48.4
RP6338-66	77.2	76.5	62.4	61.9	39.7	34.5

KMR 3 and N22 had intermediate AC (20-25%) under control conditions, whereas the four ILs exhibited high AC (>25%). Hard GC (35 mm) was observed in N22, soft GC (95 mm) was recorded in RP6338-66, and medium GC was recorded in the other three ILs and KMR 3 (41 to 60 mm). Hybrids with intermediate AC or high AC with soft GC are recommended for release under AICRIP testing, and RP6338-66 with high AC and soft GC is a potential heat tolerant restorer line with desired grain cooking quality. Overall, RP6338-9 possesses qHTSF4.1 and noted to be promising for grain quality under high temperature stress.

References

Cagampang GB, Perez CM, Juliano BO. 1973. A gel consistency test for eating quality of rice. *Journal of the Science of Food and Agriculture*,12:1589-94.

Gomez KA and Gomez AA. 2010. Statistical procedures for agricultural research (2nd ed.). Wiley publications, New York.

Gong HB, Zhou YW, Li C, Hu CM, Sheng SL, Diu LP *et al.*, 2008. Effect of heat stress on the seed-setting rate of indica hybrid rice combinations widely planted in China. *Jiangsu Agricultural Sciences*, 2:23–25. (in Chinese).

Guan-fu F, Cai-xia Z, Yong-jie Y, Jie X, Xue-qin Y, Xiu-fu, Z *et al.*, 2015. Male parent plays more important role in heat tolerance in three-line hybrid rice. *Rice Science*, 22(3):116-122

Hu SB, Zhang YP, Zhu DF, Lin XQ, Xiang J. 2012. Evaluation of heat resistance in hybrid rice. *Chinese Journal of Rice Science*, 26:751–756. (in Chinese with English abstract).

IPCC. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds RK Pachauri and LA Meyer 2014. (Geneva: IPCC).

Jaldhani V, Rao DS, Beulah P, Srikanth B, Rao PR, Subrahmanyam D, Sudhakar P, Satya AK, Neeraja CN, Senguttuvel P. 2021. Assessment of heat-tolerance potential in QTL introgressed lines of hybrid rice restorer, KMR-3R through PS-II Efficiency. *International Journal of Environment and Climate Change*, 11(11), 258-266.

Juliano, B.O. 1971. A simplified assay for milled-rice amylose. *Cereal Science Today*, 16:334-360.

Kuang HC, Wen SS, Liu GM. 2002. Studies on the heat tolerance of Luhui 17 and its cross IIyou 7 at head sprouting. *South west China Journal of Agricultural Science*, 1:106–108. (in Chinese).

Madan P, Jagadish SVK, Craufurd PQ, Fitzgerald M, Lafarge T, Wheeler TR. 2012. Effect of elevated CO2 and high temperature on seed-set and grain quality of rice. *Journal of Experimental Botany*, 63:3843–3852.

Matsui T, Kobayasi K, Kagata H, Horie T. 2005. Correlation between viability of pollination and length of basal dehiscence of the theca in rice under a hot and humid condition. *Plant Production Science*, 8:109–114

Matsui T, OMASA K, Horie T. 2000. High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Production Science* 3:430–434.

Satake T and Yoshida S. 1978. High temperature-induced sterility in indica rices at flowering. *Japanese Journal of Crop Science*, 47:6-17.

Senguttuvel P *et al.*, 2019. Rice Hybrids Released in India. Compendium No. 103/2019. ICAR-IIRR, Rajendranagar, Hyderabad-500 030. India. 127.

Senguttuvel P, Jaldhani V, Raju NS, Balakrishnan D, Beulah P, Bhadana VP *et al.*, 2020. Breeding rice for heat tolerance and climate change scenario; possibilities and way forward. A review. *Archives of Agronomy and Soil Science*. 1-18.

Serraj R, Kumar A, McNally KL, Slamet Loedin I, Bruskiewich R, Mauleon R *et al.*, 2009. Improvement of drought resistance in rice. In *Advances in Agronomy,* 103:41-99. Academic Press.

Song ZP, Lu BR, Chen KJ. 2001. A study of pollen viability and longevity in *Oryza rufipogon*, *O. sativa* and their hybrids. *International Rice Research Notes*, 26:31–32.

Statistix 8.1 User's Manual. Analytical Software, Tallahassee. 2003.

Jaldhani V, D Sanjeeva Rao, P Beulah, P Nagaraju, K Suneetha, N Veronica *et al.*, 2022. Chapter 2 - Drought and heat stress combination in a changing climate, Editor(s): Arun K Shanker, Chitra Shanker, Anjali Anand, M. Maheswari. Climate Change and Crop Stress, Academic Press, Pages 33-70, ISBN 9780128160916.

Villa JE, Henry A, Xie F, Serraj R. 2012. Hybrid rice performance in environments of increasing drought severity. *Field Crops Research*, 125:14-24.

Virmani SS, Mao CX, and Hardy B. (Eds.). 2003. Hybrid rice for food security, poverty alleviation, and environmental protection. IRRI, Philippines.

Wassmann R, Dobermann A. 2007. Climate change adaptation through rice production in regions with high poverty levels. *Journal of Semi-Arid Tropical Agricultural Research*, 4(1):1–24.

Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R *et al.*, 2009. Climate Change Affecting Rice Production: The Physiological and Agronomic Basis for Possible Adaptation Strategies. *Advances in Agronomy*, 101:59-122.

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, *et al.*, 2017 Temperature increase reduces global yields of major crops in four independent estimates. *Pr ceedings of National Academy of Sciences*, USA. 114(35):9326–9331.

Zhou YW, Gong HB, Li C, Hu CM, Lin TZ, Sheng SL. 2009. Influence of thermal damage on seed-setting rate of 67 indica hybrid rice comb nations. *Acta Agriculturae Jiangxi*, 21:23–26. (in Chinese with English abstract).