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Identification of Heat-Tolerant Rice Genotypes Through Phenotypic Selection and Multi-Trait Characterization for Genetic Diversity

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Abstract

Rice (*Oryza sativa* L.), a global staple crop, is sensitive to elevated temperatures, particularly during reproductive and grain-filling stages. With climate change projections forecasting significant rises in mean temperature, sustaining rice productivity under heat stress has become a critical challenge. The present study evaluated forty-nine rice genotypes, comprising released varieties and advanced breeding lines, under control and heat-stress conditions. Phenological, morpho-physiological, and yield-related traits, were assessed to identify tolerant genotypes. Results revealed substantial variability among genotypes for chlorophyll content, membrane stability, spikelet fertility, and grain yield under stress. Heat-tolerant checks N22 and Rasi, along with genotypes L 663, L 672, MTU 1239, and CL 448, maintained higher chlorophyll levels, superior membrane integrity, and stable yields. In contrast, genotypes such as Vandana, MTU 1166, and MTU 1001 were highly susceptible, exhibiting severe reductions in chlorophyll content, spikelet fertility, and grain yield. Overall, traits such as minimal chlorophyll degradation, higher membrane thermostability, sustained spikelet fertility, and stable grain yield were strongly associated with heat tolerance. These findings highlight promising donor genotypes and key physiological traits that can be exploited in breeding programs to develop climate-resilient rice cultivars.

Keywords: *Oryza sativa* L.; heat tolerance; phenotypic selection; multi-trait characterization; genetic diversity

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Introduction

Rice (*Oryza sativa L.*) is the second most significant cereal crop globally (Van Nguyen & Ferrero, 2006). It is a primary staple food for over two-thirds of the world's population, often regarded as synonymous with food. It is cultivated extensively across tropical and subtropical regions and is commonly called the "Global Grain" (Shalini & Tulsi, 2008). Due to its relatively small genome, extensive germplasm diversity, abundance of molecular genetic tools, and an efficient transformation system, rice is considered a model crop in cereal research (Paterson et al., 2005). In India, rice is grown on approximately 45 million hectares, with an annual production of 178.30 million tonnes and an average yield of 122 million metric tonnes of milled rice (Ministry of Agriculture, Government of India, 2020).

Rising global temperatures due to climate change have led to an increase of approximately 0.5 °C during the 20th century, with projections suggesting a further rise of 1.5 to 4.5 °C within the current century (Peraudeau et al., 2015). The Inter-governmental Panel on Climate Change (IPCC, 2013) predicts that by 2081–2100, the global average surface temperature could increase by 0.3–1.7 °C under minimal greenhouse gas emissions and by 2.6–4.8 °C under high-emission scenarios, relative to the

1986–2005 baseline. These climatic shifts pose significant threats to crop productivity worldwide. While plants possess inherent adaptive mechanisms that help them cope with environmental stressors, these responses are often insufficient to sustain optimal yield levels. For example, Matsui et al. (2001) observed that plants can reduce panicle temperature by as much as 10 °C through transpirational cooling, which helps maintain spikelet fertility. Additionally, traits such as elongated and upright flag leaf that shield panicles from intense solar radiation have been associated with improved heat tolerance.

Rice is highly susceptible to high temperatures and rising global air temperatures are expected to significantly compromise its yield, grain quality, and nutritional content (Fahad et al., 2015). Although elevated atmospheric CO_2 levels can enhance rice productivity due to its classification as a C_3 plant (Shimono et al., 2009), heat stress often counteracts this positive response (Kadam et al., 2014). High temperatures, particularly during the reproductive and grain-filling phases, adversely affect yield and grain quality (Bahuguna et al., 2015) in tropical and subtropical regions where rice is predominantly cultivated; elevated daytime temperatures during anthesis and grain filling present a significant challenge. For instance, temperature increases of 3.6 °C to 7.0 °C above the critical threshold between heading and mid-ripening stages resulted in reductions in photosynthetic activity by 11.2% and 35.6%, respectively (Fahad et al., 2016). To combat these climate-induced risks, there is an urgent need to strengthen rice resilience through targeted crop improvements in phenological, physiological, and molecular traits.

Photosynthetic pigments are important plant physiology traits, primarily because they capture light energy and generate reducing agents such as ATP and NADPH. However, these pigments particularly chlorophyll 'a' and chlorophyll 'b' are vulnerable to elevated temperatures. Exposure to heat stress alters the balance between chlorophyll 'a', chlorophyll 'b', and carotenoids, often resulting in a noticeable reduction in total chlorophyll content (Farooq et al., 2009). High temperatures also stimulate the production of reactive oxygen species (ROS), which act as secondary stressors. These ROS interact with membrane lipids, primarily unsaturated fatty acids, leading to lipid peroxidation and subsequent accumulation of malondialdehyde. Since biological membranes are susceptible to heat, their structural proteins may undergo conformational changes at the tertiary and quaternary levels. This compromises membrane integrity and increases permeability, as evidenced by greater electrolyte leakage. The resulting rise in electrical conductivity under heat stress conditions reflects a decline in the membrane's thermal stability index (Hemantaranjan et al., 2014).

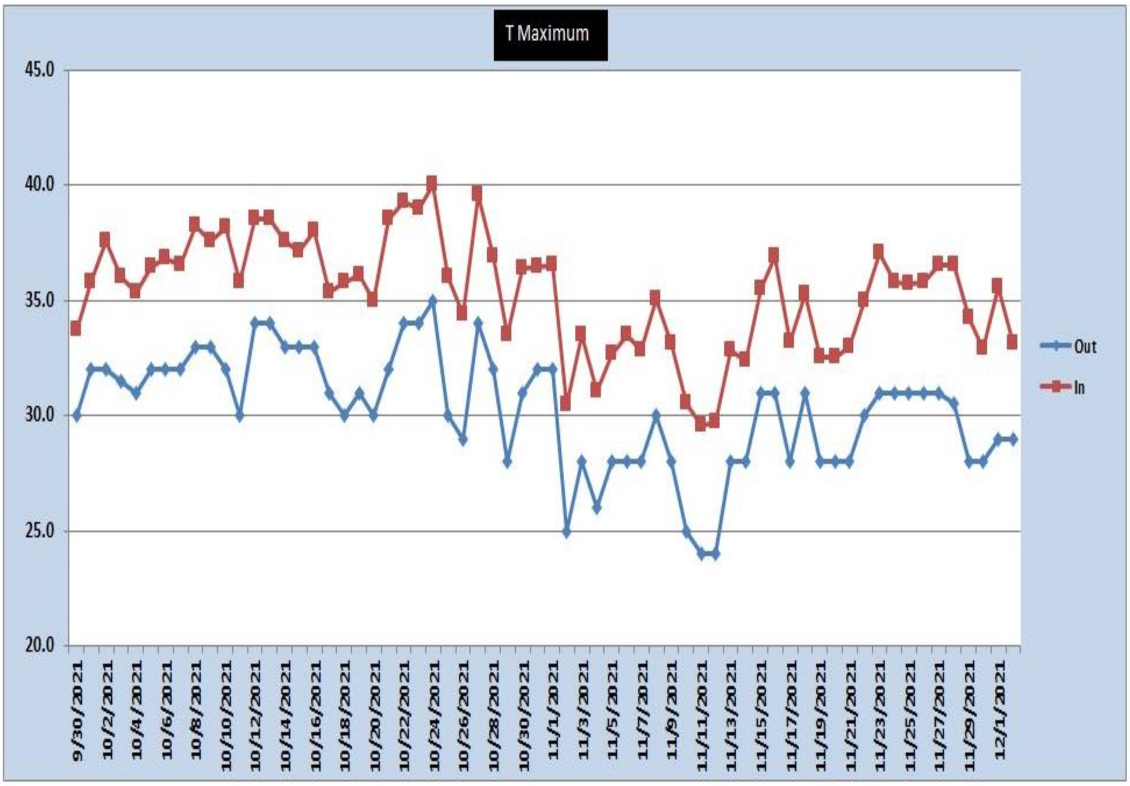
Genetic variability within the rice germplasm is fundamental for the success of crop improvement programs, particularly for enhancing tolerance to high-temperature stress (Mthiyane et al., 2024). This variability is significant during the reproductive stage, where maintaining high spikelet fertility is critical for yield stability and such traits can be effectively targeted through direct phenotypic selection. In addition, correlation analysis between yield and its associated traits under heat stress provides insights into trait interdependence, enabling effective indirect selection strategies. Identifying such genotypes can aid breeders in selecting superior lines with better adaptation to future climate scenarios. Therefore, this study aimed to assess phenotypic response and molecular characterization (Seelam et al., 2024) of rice genotypes under both control and heat stress conditions with stress imposed in the polyhouse during reproductive phase.

Materials and Methods

Plant materials

The study was conducted during kharif-2021 using forty-nine rice genotypes (Supplementary Table S1), comprised released varieties and advanced breeding lines obtained from the Regional Agricultural Research Station (RARS), Maruteru. The experiment was carried out in an artificial polyhouse facility established at RARS. Weather data during the crop growth period were collected from the meteorological observatory at RARS, and polyhouse conditions were continuously monitored with a data logger (RC-4HC) (Figure 1). During the cropping season, the mean monthly maximum and minimum ambient temperatures were 30.60 °C and 25.84 °C, respectively, while during the stress period from panicle initiation to maturity the corresponding values were 30.3 °C and 25.4 °C under ambient conditions and 35.3 °C and 27.9 °C inside the polyhouse, representing an increase of 5.0 °C and 2.5 °C, respectively (Figure 2). The genotypes were evaluated in an augmented design with two replications, and observations were recorded on five randomly selected plants from each entry; mean values were computed and used for analysis. Data were collected on various morpho-physiological and yield-related traits under both conditions. At physiological maturity, plants were harvested manually, threshed by hand, and the grains and straw were cleaned and sun-dried to approximately 14% moisture content for further evaluation.

Figure 1. Inner view of heat stress experimental unit consisting of automatic temperature recorder (Data logger).



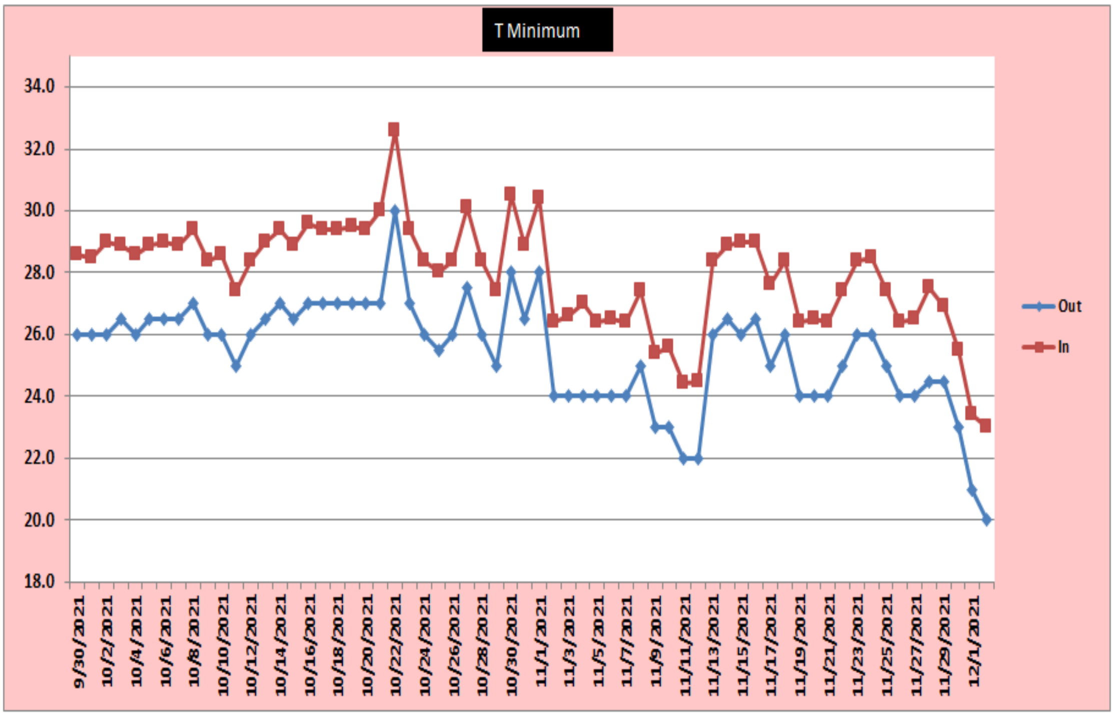


Figure 2. Maximum and minimum temperature (°C) during the crop growing period inside and outside the polyhouse during *kharif* 2021.

Chlorophyll estimation

Total chlorophyll CHLa and CHLb contents from the leaves were estimated as per porra method (Porra et al., 1989) using spectrophotometer. Chlorophyll content was estimated using the flag leaf collected one week after flowering. One gram of the fresh leaf tissue was cut into small pieces and placed into a volumetric flask containing 25 ml of 80% acetone (Porra et al., 1989) and stored in the dark for 1 to 2 days to ensure complete extraction of leaf pigments The values were expressed in milligram per gram fresh weight. Chlorophyll a content, chlorophyll b content and total chlorophyll content was calculated according to Lichtenthaler and Wellburn (1983)._

$$\text{Chl a } (\mu g/ml) = 12.25\ A_{663.2} - 2.79\ A_{646.8}$$

$$\text{Chl b } (\mu g/ml) = 21.5\ A_{646.8} - 5.1\ A_{663.2}$$

$$\text{Total chlorophyll } (\mu g/ml) = \text{Chl a} + \text{Chl b}$$

Cell membrane thermostability (CMS) analysis

Membrane thermostability (MTS) was assessed following (Haque et al., 2009) with modifications. Leaf samples were washed with deionized water, cut into pieces, and placed in test tubes containing 10 ml deionized water. Two sets of samples were prepared: one maintained at 28 °C (control) and the other incubated at 52 °C for 1 h (heat treatment), with three replications each. After treatment, tubes were kept at room temperature for 24 h, and initial conductance was recorded using a conductivity meter. Samples were then autoclaved at 121 °C (15 lb) for 20 min to ensure complete electrolyte leakage, and final conductance was measured. MTS was calculated using the formula of (Blum & Ebercon, 1981):

$$\mathbf{CMS\ (\%) = [1 - (T1/T2)] / [1 - (C1/C2)] \times 100,}$$

where C1 and C2 represent initial and final conductance of control, and *T1* and *T2* represent initial and final conductance of heat-treated samples.

Days to 50% Flowering: The number of days taken for 50% of plants to flower in each genotype was noted as days to 50% flowering and was expressed in days.

Plant Height (cm): The plant height on the tagged plants was recorded by measuring the height from the base of the plant to the tip of the terminal leaf or panicle on main stem and was expressed in centimeters (cm).

Ear Bearing Tillers Number/ Plant: The ear bearing tillers number per plant was counted and was computed in both heat stress and control conditions and then expressed as ear bearing tillers number/plant.

Spikelet fertility (%)

Spikelet fertility was worked out using the following formula and expressed in per cent.

$$\text{Spikelet fertility} = \frac{\text{Number of filled grains}}{\text{Total number of grains}} \times 100$$

Grain yield (g/plant)

At physiological maturity, panicles from each plant in both stress and non-stress conditions were harvested, sun dried, threshed, cleaned and the weight of grains was recorded and expressed in grams per plant.

Data analysis

Analysis of variance for all the characters was studied as per standard statistical procedure for Augmented Randomized Complete Block Design (Augmented Design II) as given by Federer (1956). The data was analysed using R Studio software.

Results and Discussion

Chlorophyll ‘a’ and Chlorophyll ‘b’

In plants, leaves act as the primary photosynthetic organs, and more than 90% of the dry matter yield in crops is attributed to leaf photosynthesis (Makino, 2011). Chlorophyll is the main pigment in photosynthesis. A higher chlorophyll concentration is necessary for photosynthesis to continue at a regular rate even under heat stress circumstances. In our study, Chlorophyll ‘a’ content varied widely among the 49 genotypes (Supplementary Table S2). Under control conditions, values ranged from 2.41 to 3.97 mg g^{-1} FW (mean 3.20), whereas under heat stress they ranged from 1.88 to 3.68 mg g^{-1} FW (mean 2.75). The highest chlorophyll ‘a’ levels were recorded in CL 447 (3.97 mg g^{-1} FW), MTU 1262 (3.96), and MTU 2077 (3.93) under control, while CL 451 (3.68), MTU 2077 (3.54), and CL 447 (3.52) were superior under stress. MTU 1064 showed the lowest content in both conditions (2.41 and 1.88 mg g^{-1} FW). Consistent with earlier reports (Lohitha et al., 2019), chlorophyll ‘a’ declined significantly under heat stress. Combined analysis revealed that N22 (1.3%), CL 451 (5.4%), and CL 448 (6.0%) had the least decrease, whereas Vandana (33.8%), MTU 1001 (33.5%), and CL 450 (23.1%) showed the maximum reduction.

Mean chlorophyll *b* content was 1.29 mg g^{-1} FW under control and 0.76 mg g^{-1} FW under stress (Table 4.4). MTU 1061 (1.89), L 670 (1.84), and MTU 1190 (1.81) recorded the highest levels under control, while MTU 1140 (1.36), L 677 (1.34), and Rasi (1.32) were superior under stress. MTU 1064 consistently had the lowest values (0.77 control; 0.30 stress). Similar reductions in chlorophyll *b* under heat stress have also been reported, where Zafar et al. (2017) found the lowest levels in the heat-sensitive variety Basmati-385. On the final day of stress application, Thussagunpanit et al. (2015) reported that high temperature decreased chlorophyll *a* by 43.84%, chlorophyll *b* by 49.53%, total chlorophyll ($a + b$) by 45.03%, and carotenoids by 61.84% compared to non-stressed plants, indicating that heat stress impairs the light-harvesting capacity of chlorophyll pigments (Calatayud & Barreno, 2004; Zhu et al., 2011). In the present study, combined analysis showed MTU 1140 (3.6%) had the least decline, lower even than tolerant checks N22 (9.2%) and Rasi (8.3%), while MTU 2077 (72.5%), L 665 (66.8%), and MTU 1224 (65.3%) exhibited the highest reductions.

Total Chlorophyll Content

Heat stress causes reactive oxygen radicals to be produced in plants due to an imbalance in photosynthesis and respiration, which damages the chloroplast membranes and oxidizes the chlorophyll pigment (Sharkey, 2005; Sun & Guo, 2016; Wang et al., 2018). The ability to synthesize more chlorophyll under high temperature stress is an important criterion for stress tolerance (Zhou et al., 2018). In our study, total chlorophyll ranged from 3.19 to 5.64 mg g^{-1} FW under control, and from 2.18 to 4.54 mg g^{-1} FW under stress (Table 1; Figure 3). MTU 1262 (5.64), MTU 2077 (5.40), and CL 451 (5.27) had the highest content under control, while N22 (4.54) and MTU 1262 (4.49) were superior under stress. MTU 1064 consistently showed the lowest values (3.19 and 2.18). Minimal reductions were observed in N22 (3.5%), Rasi (7.7%), CL 448 (10.2%), while Vandana (42.2%), L 665 (36.8%), MTU 1001 (36.3%), and MTU 1064 (31.5%) showed maximum losses (Table 1). Previous studies also noted similar reductions, with N22 maintaining higher stability compared to sensitive checks (Veronica et al., 2019; Lohitha et al., 2019).

Table 1. Effect of high temperature total chlorophyll content and cell membrane thermostability of rice genotypes.

S. No.	Genotypes	Days to 50% flowering			Plant height (cm)			Ear bearing tillers number/plant		
		Control	Heat stress	Mean	Control	Heat stress	Mean	Control	Heat Stress	Mean
1	MTU 7029	107	107	107	124	121	123	16	12	14
2	MTU 2077	113	113	113	120	118	119	12	10	11
3	MTU 1061	122	121	121	123	128	125	11	10	11
4	MTU 1064	119	119	119	126	124	125	12	11	11
5	PLA 1100	121	120	121	133	122	128	11	9	10
6	MTU 1140	119	118	119	135	119	127	13	11	12
7	MTU 1172	119	119	119	128	130	129	12	10	11
8	MTU 1075	113	110	112	144	137	141	14	12	13
9	MTU 1223	127	120	124	139	132	136	13	10	12
10	MTU 1155	110	109	110	147	137	142	11	10	11
11	MTU1224	109	108	109	125	114	120	11	10	11
12	MTU 1031	127	121	124	123	108	116	12	10	11
13	MTU 1032	112	108	110	142	131	137	11	10	10
14	MTU 1190	108	107	108	145	139	142	13	11	12
15	MTU 1239	115	111	113	136	129	132	12	8	10
16	MTU 1262	129	127	128	132	121	126	13	10	11
17	MTU 1184	128	124	126	163	152	157	12	11	12
18	MTU 1194	122	121	122	137	138	138	11	10	10
19	MTU 1238	112	112	112	146	133	140	12	10	11

S. No.	Genotypes	Days to 50% flowering			Plant height (cm)			Ear bearing tillers number/plant		
		Control	Heat stress	Mean	Control	Heat stress	Mean	Control	Heat Stress	Mean

S. No.	Genotypes	Days to 50% flowering			Plant height (cm)			Ear bearing tillers number/plant		
		Control	Heat stress	Mean	Control	Heat stress	Mean	Control	Heat Stress	Mean
20	MTU 1253	115	113	114	147	138	142	13	11	12
21	MTU 1271	118	114	116	142	133	138	12	10	11
22	MTU 1315	121	118	120	139	132	135	12	9	11
23	MTU 1318	127	127	127	128	122	125	11	9	10
24	MTU 1166	129	127	128	151	144	148	12	11	12
25	MTU 1232	121	115	118	122	118	120	10	9	10
26	L648	129	127	128	142	135	139	10	9	10
27	L 663	112	112	112	149	152	150	12	11	12
28	L 665	118	116	117	140	138	139	13	11	12
29	L 667	124	120	122	152	143	148	14	10	12
30	L 668	124	122	123	151	145	148	12	11	12
31	L 669	122	121	122	151	144	148	13	10	12
32	L 670	120	120	120	145	143	144	12	10	11
33	L 672	121	120	121	146	140	143	10	9	10
34	L 674	117	116	117	149	142	146	10	9	10
35	L 676	112	111	112	143	131	137	10	9	10
36	L 677	112	111	112	140	126	133	12	10	11
37	CL 446	115	109	112	130	126	128	11	8	10
38	CL 447	110	108	109	150	132	141	10	10	10
S. No.	Genotypes	Days to 50% flowering			Plant height (cm)			Ear bearing tillers number/plant		
		Control	Heat stress	Mean	Control	Heat stress	Mean	Control	Heat Stress	Mean
39	CL 448	117	117	117	115	118	117	11	10	10
40	CL 449	114	112	113	154	144	149	11	10	11
41	CL 450	114	112	113	144	139	142	11	10	11
42	CL 451	122	121	122	130	121	126	12	10	11
43	CL 452	122	117	120	107	113	110	12	10	11
44	CL 453	117	115	116	134	121	127	16	11	14
45	CL 454	101	101	101	137	140	138	14	11	12
46	N22	87	87	87	115	114	115	10	7	9
47	Vandana	89	86	89	106	100	103	18	11	14
48	Rasi	92	90	86	100	103	101	21	16	18

S. No.	Genotypes	Days to 50% flowering			Plant height (cm)			Ear bearing tillers number/plant		
		Control	Heat stress	Mean	Control	Heat stress	Mean	Control	Heat Stress	Mean
49	MTU 1001	106	104	105	131	121	126	15	10	12
	Mean	116.0	114.0		135.9	129.7		12.0	10.0	
	Maximum	129.0	127.0		163.0	152.0		21.0	16.0	
	Minimum	87.0	86.0		100.0	100.0		10.0	7.0	
	CD (5%)	2.065	1.381		3.169	1.172		1.143	0.844	
	CV %	9.04	7.40		4.26	4.91		15.88	14.18	

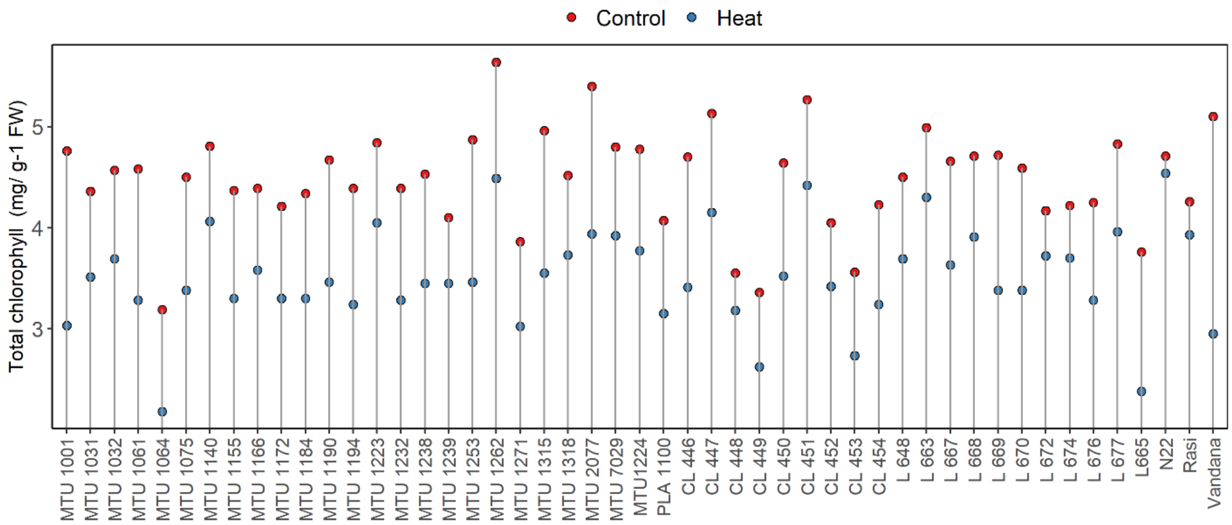


Figure 3. Effect of high temperature on total chlorophyll content of rice genotypes.

Cell Membrane Thermostability (CMS)

Under heat stress, uninterrupted functioning of cellular membranes is essential for photosynthesis and respiration to proceed accurately (Allakhverdiev et al., 2008). Consequently, membrane thermostability has been recognized as a key determinant of heat tolerance. In this study, membrane thermostability was assessed by measuring relative electrolyte leakage caused by stress injury. The genotypes N22, Rasi, and L 672 recorded the highest membrane stability under both control and heat stress conditions. N22 maintained 83.9% under control and 81.4% under stress, while Rasi showed 82.5% and 78.9%, and L 672 displayed 77.7% and 70.5%, respectively. In contrast, Vandana exhibited the lowest membrane thermostability, with only 34.1% under control and 22.1% under stress. When comparing reductions between treatments, N22 had the smallest decline (3.0%), whereas Vandana showed the greatest reduction (35.1%) (Table 1; Figure 4).

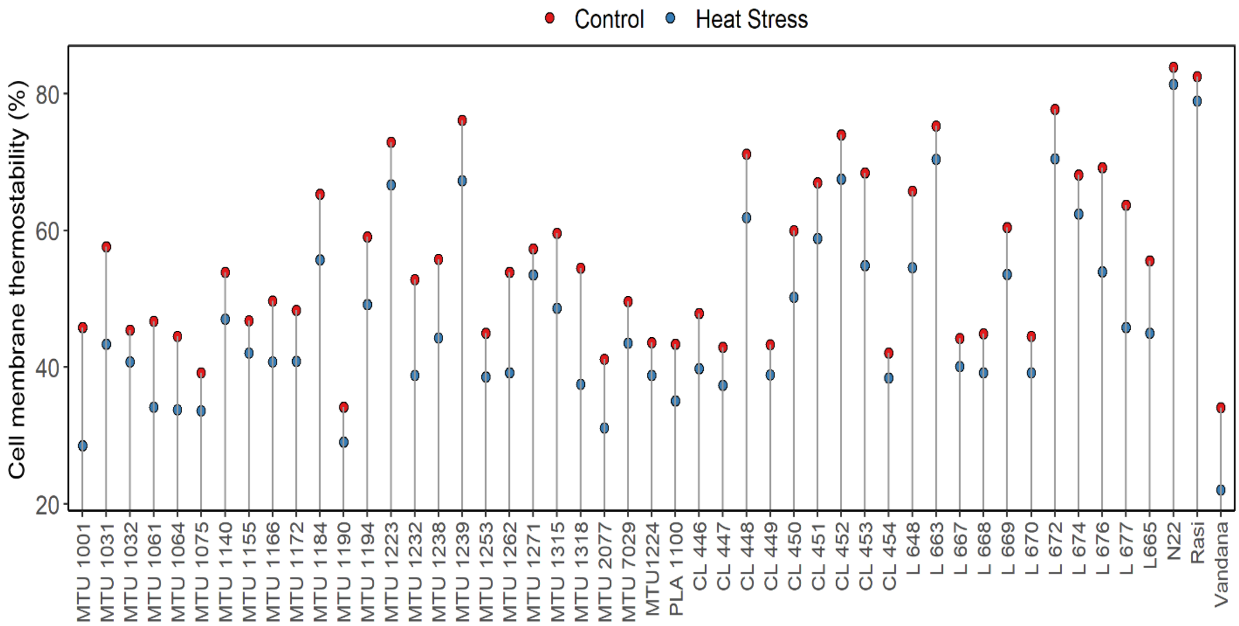


Figure 4. Effect of high temperature on membrane thermostability (%) of rice genotypes.

These findings are consistent with previous reports demonstrating that heat stress alters lipid composition of membranes, thereby influencing their stability. For example, creeping bentgrass exposed to 35 °C exhibited higher lipid saturation (Liu & Huang, 2004), while tall fescue showed increased unsaturation under 40/35 °C conditions (Hu et al., 2018), and wheat displayed the opposite trend with reduced unsaturation (Narayanan et al., 2016). Such changes often involve an increase in saturated fatty acids or a decrease in unsaturated fatty acids (Narayanan et al., 2016; Pearcy, 1978). Importantly, Hu et al. (2018) suggested that greater unsaturation enhances photosynthetic membrane stability and may contribute to improved heat tolerance. The superior performance of genotypes such as N22, Rasi, and L 672 in maintaining higher membrane thermostability in this study could therefore be attributed to similar lipid adjustments, enabling them to preserve cellular integrity and function under heat stress.

Days to 50% Flowering

Days to 50% flowering varied widely among genotypes, ranging from 87 to 129 days under control conditions and 87 to 127 days under heat stress (Supplementary Table S3). Under control conditions, N22 (87 days), Vandana (89), Rasi (92), CL 454 (101), and MTU 1001 (106) recorded the earliest flowering. Under stress, Vandana (86 days) and N22 (87 days) maintained early flowering, followed by Rasi (90), CL 454 (101), and MTU 1001 (104). The late-maturing genotypes MTU 1262 and MTU 1166 required 129 days to flower under control, while MTU 1262 and MTU 1318 showed delayed flowering (127 days each) under stress.

Notably, ten genotypes showed no difference in flowering duration, while MTU 1223 recorded the greatest reduction (seven days). These findings align with Sailaja et al. (2015), who reported reduced flowering duration across genotypes under heat stress, and Masuduzzaman et al. (2016), who observed significant variation for this trait among 1217 screened lines. Early flowering under stress, as seen in Vandana and N22, may represent an adaptive escape mechanism, enabling plants to complete reproductive stages before severe stress occurs.

Plant Height

Plant height showed clear reductions under heat stress (Supplementary Table S3). Under control, height ranged from 100 cm (Rasi) to 163 cm (MTU 1184), with a mean of 135.9 cm, while under stress the range was 100 cm (Vandana) to 152 cm (MTU 1184), with a reduced mean of 129.7

cm. Shorter genotypes across conditions included Rasi (101 cm), Vandana (103 cm), CL 452 (110 cm), and N22 (115 cm), while MTU 1184 consistently recorded the greatest height (157 cm mean). The reduction in plant height under elevated temperatures is consistent with earlier reports (Prasad et al., 2006; Sailaja et al., 2015), which attributed shorter stature to suppressed stem elongation and ROS-induced cellular damage. Shorter plants under stress may represent impaired vegetative growth, which can negatively influence biomass partitioning and yield potential (Fageria, 2007; Fu et al., 2016; Wu et al., 2022).

Ear-Bearing Tillers per Plant

Ear-bearing tiller number declined significantly under heat stress, highlighting its sensitivity during panicle initiation. The average number of productive tillers dropped from 12 under control to 10 under stress (Supplementary Table S3). Rasi produced the maximum tillers (21 control, 16 stress), while N22 consistently showed the fewest (10 control, 7 stress). MTU 7029, a popular mega-variety, also showed reductions (16 control, 12 stress). Similar declines in tiller number under high temperature have been reported by Samol et al. (2015) and Oh-e et al. (2007), who observed reduced tillering and fewer productive culms under elevated temperature regimes. Given its role in determining sink capacity, tiller number under stress serves as a key trait for selecting heat-tolerant genotypes (Vishnu Prasanth et al., 2017). Genotypes like Rasi, which sustain relatively higher tiller numbers under stress, may hold promise for breeding programs targeting resilience.

Spikelet Fertility (%)

Reduced spikelet fertility in the panicles is a major consequence of heat stress during reproductive stage. Spikelet fertility was highly sensitive to elevated temperature stress (Table 2). Under control conditions, fertility ranged from 81.3% to 96.0% with a mean of 89.7%. In contrast, under heat stress, values declined drastically, ranging from 22.5% to 84.0% with a mean of 50.4%. Under non-stress conditions, MTU 1166 recorded the highest spikelet fertility (96.0%), comparable to the tolerant check N22 (91.7%). However, under stress, N22 (84.0%), MTU 1239 (77.2%), and Rasi (74.4%) maintained the highest fertility levels. By contrast, MTU 1166 (22.5%) and MTU 1253 (24.3%) showed the lowest spikelet fertility under stress, confirming their susceptibility. Combined analysis revealed that N22 (8.3%), MTU 1239 (12.3%), L 663 (18.0%), Rasi (20.2%), and CL 448 (20.4%) sustained minimal fertility loss and can be considered heat tolerant. In contrast, MTU 1166 (76.5%) and MTU 1253 (74.2%) exhibited the largest declines, reflecting high vulnerability to stress (Figure 5). For phenotyping this trait, spikelets from plots under heat stress and control were harvested separately and compared (Figure 6).

Table 2. Effect of high temperature on spikelet fertility and grain yield per plant of rice genotypes.

S. No.	Score	Genotypes
1	Tolerant (>65 % spikelet fertility, > 19 grams of grain yield, > 65 % MTS)	MTU 1223, MTU 1239, L 663, L 672, L 674, CL 448, CL 452, N22, Rasi.
2	Moderately tolerant (30-65 %spikelet fertility, 12-19 grams of grain yield, 30- 65 % MTS)	MTU 7029, MTU 2077, MTU 1061, MTU 1064, PLA 1100, MTU 1140, MTU 1172, MTU 1075, MTU 1155, MTU 1224, MTU 1031, MTU 1032, MTU 1190, MTU 1262, MTU 1184, MTU 1194, MTU 1238, MTU 1271, MTU 1315, MTU 1318,

		MTU 1232, L 648, L 665, L 667, L 668, L 669, L 670, L 676, L 677, CL 446, CL 447, CL 449, CL 450, CL 451, CL 453, CL 454
4	Susceptible (<30 % spikelet fertility, <12 grams of grain yield, <30 % MTS)	MTU 1253, MTU 1166, Vandana, MTU 1001

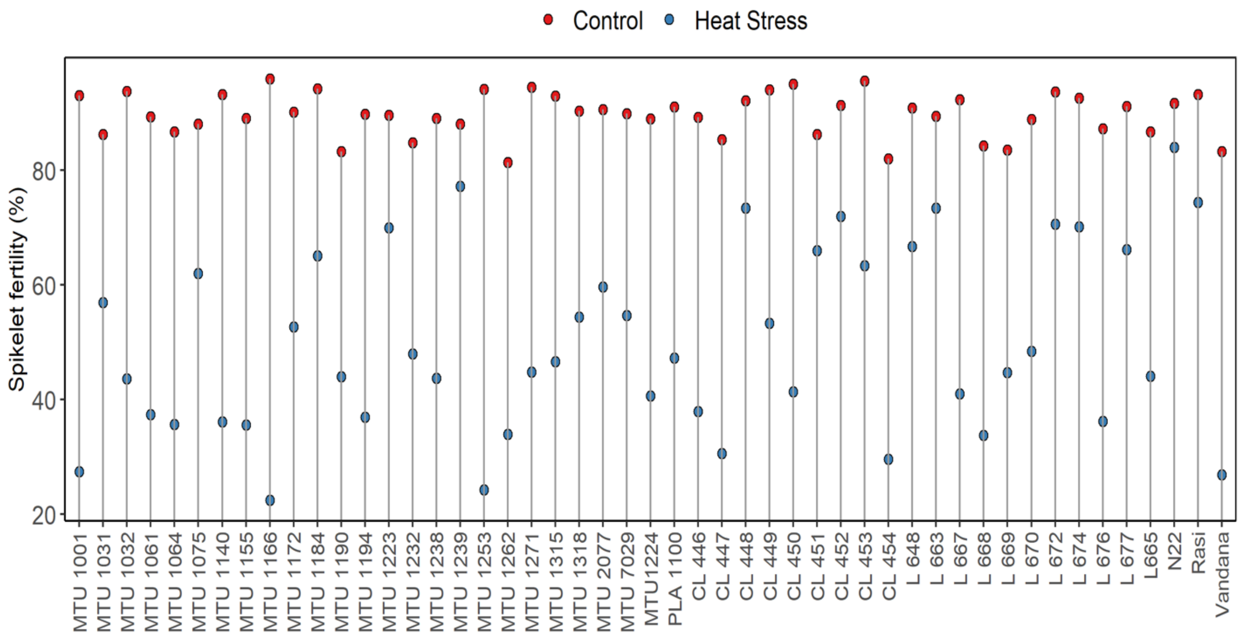
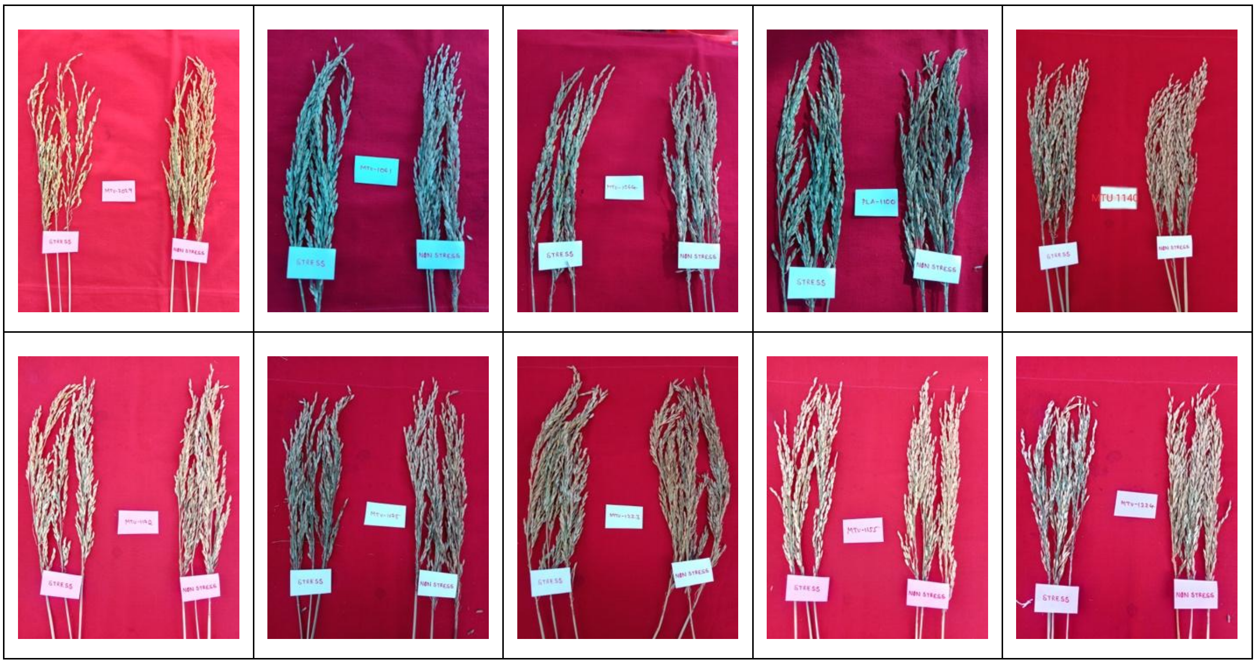


Figure 5. Effect of high temperature on spikelet fertility (%) of rice genotypes.



MTU-1031 STRESS NON STRESS	MTU-1032 STRESS NON STRESS	STRESS NON STRESS	STRESS NON STRESS	STRESS NON STRESS
STRESS NON STRESS	STRESS NON STRESS	MTU-1253 STRESS NON STRESS	MTU-1271 STRESS NON STRESS	MTU-1315 STRESS NON STRESS
MTU-1318 STRESS NON STRESS	MTU-1166 STRESS NON STRESS	MTU-1232 STRESS NON STRESS	MTU-1001 STRESS NON STRESS	L-648 STRESS NON STRESS

Figure 6. Phenotyping of spikelets of various genotypes under Control and Heat Stress Conditions.

These findings are consistent with earlier studies showing that high temperatures during flowering impair reproductive success. Matsui et al. (2001) and Prasad et al. (2006) reported that reduced pollen germination and poor anther dehiscence under heat stress lead to spikelet sterility. Hurkman et al. (2009) further demonstrated that exposure to >33°C at heading significantly decreased spikelet fertility and increased seed sterility. Similarly, Sailaja et al. (2015) reported declines in fertility and grain yield across multiple rice genotypes under high temperature. Poor development of rice spikelets, especially inferior spikelets caused by heat stress reduced the rice grain yield.

Grain Yield (g/plant)

Heat stress has a significant impact on rice yields because it hinders growth and photosynthesis. Heat stress also had an impact on the reproductive stage of rice, which lowers production. Grain yield per plant showed a marked decline under elevated temperature stress (Table 2). Under control conditions, yield ranged from 19.7 g to 39.3 g with a mean of 29.4 g/plant, while under stress it declined to 10.7–21.5 g with a mean of 15.9 g/plant. Under non-stress conditions, MTU 1075 recorded the highest yield (39.3 g), followed by L 663 (38.7 g), L 669 (37.7 g), and MTU 1001 (37.3 g). However, under heat stress, N22 (21.5 g) sustained the highest yield, closely followed by L 663 (21.0 g), L 677 (20.3 g), and MTU 1223 (20.3 g). Notably, L 663 performed on par with N22, indicating its strong potential as a tolerant genotype. In contrast, Vandana (19.7 g) showed the lowest yield under control conditions, while under heat stress Vandana (10.7 g), MTU 1166 (11.7 g), and MTU 1001 (12.3 g) recorded the poorest performance, confirming their susceptibility. The drastic reduction in yield under stress is primarily due to impaired translocation of soluble sugars to spikelets and reduced starch synthase activity during grain development (Fu et al., 2016). Percent reduction analysis revealed that L 672 (3.2%), N22 (8.5%), and MTU 1239 (23.3%) had the least losses, while MTU 1001 showed the maximum decline (66.9%) (Figure 7). Genotypes with lower yield reduction thus represent stable performers under stress conditions.

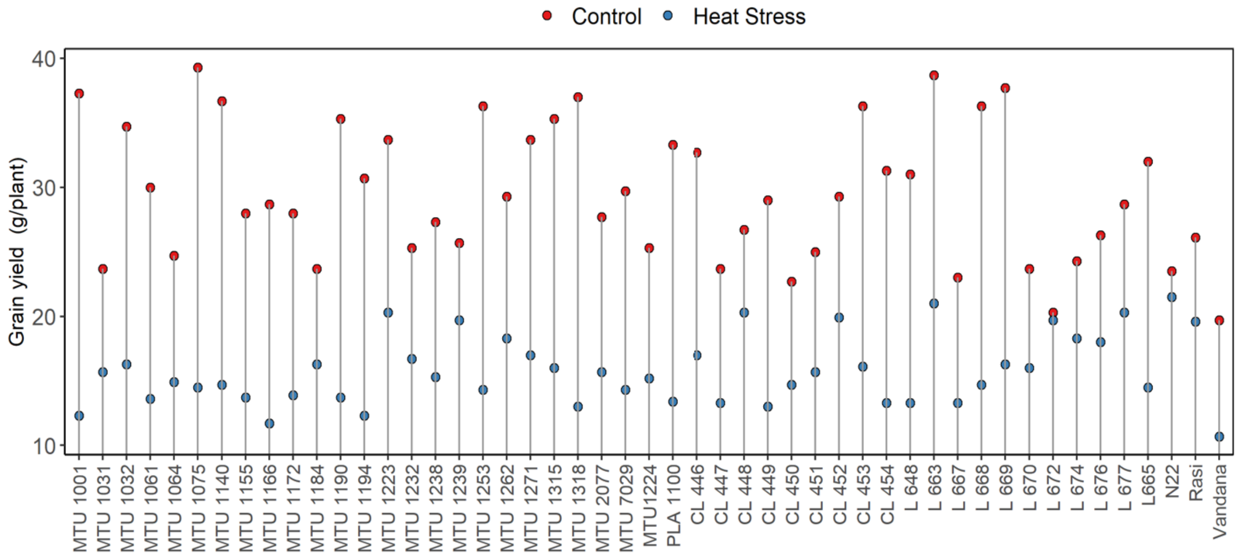


Figure 7. Effect of high temperature on grain yield (g/plant) of rice genotypes.

These findings are consistent with earlier reports. Su et al. (2023) and Peng et al. (2004) demonstrated that yield decreases by ~10% for every 1 °C rise in mean temperature. Ma QiLin et al. (2009) reported such losses due to inhibition of grain filling, while Cao YunYing et al. (2008) and Mohammed and Tarpley (2014) observed severe declines (up to 90%) when rice was continuously exposed to high temperatures during reproductive and grain filling stages. Poli et al. (2013) further reported significant yield reductions in N22 and its mutant under heat stress. Overall, the present study highlights that genotypes MTU 1223, MTU 1239, L 663, L 672, L 674, CL 448, and CL 452 maintained yield stability comparable to tolerant checks N22 and Rasi, making them promising candidates for breeding programs aimed at enhancing heat tolerance (Table 3).

Table 3. Classification of 49 rice genotypes for thermotolerance.

Conclusion

The study demonstrated significant genotypic variation in response to elevated temperatures, underscoring the potential for exploiting existing diversity to enhance heat tolerance in rice. Genotypes such as N22, Rasi, L 663, L 672, MTU 1239, and CL 448 consistently performed well under heat stress, maintaining higher chlorophyll content, better membrane thermostability, superior spikelet fertility, and minimal yield reduction. Conversely, Vandana, MTU 1166, and MTU 1001 were identified as highly heat-sensitive, confirming their unsuitability for cultivation under warming climates. The findings reaffirm that chlorophyll stability, membrane integrity, and spikelet fertility are reliable physiological indicators of thermotolerance and can serve as effective selection criteria in breeding programs. By integrating these traits with molecular tools, as reported in complementary studies, rice improvement efforts can be accelerated to develop cultivars resilient to projected climate scenarios. This research thus provides a valuable foundation for breeding strategies aimed at sustaining rice productivity in the face of global warming.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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