



Impact of High Temperature Stress on Morpho-Physiological Components and Yield Traits in Rice

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Abstract

Rice production is being threatened by increase in temperature around the globe. This is affecting the production and productivity of the crop. Changing climate scenario demands the need to identify thermotolerant genotypes to be used as donor in future breeding programmes. The effect of high temperature from panicle initiation to maturity was imposed in twenty-six rice genotypes. The difference in mean monthly maximum and minimum temperature was 3.5 °C and 1.4 °C, respectively between the polyhouse and ambient control. Results revealed a reduction in important physiological traits *viz.*, membrane thermostability as well as chlorophyll content. Of the genotypes tested, MTU 1290, MTU 1153, MTU 1156, JB 683-1, N22 and IL 19211 maintained a higher stability of membranes and lesser reduction in chlorophyll content when exposed to high temperature stress. High temperature resulted in reduction of grain yield as well as yield attributes in rice genotypes. The above genotypes also had a higher spikelet fertility and lesser yield penalty when exposed to high temperature. The results indicated that these genotypes could be selected as thermotolerant and could serve as potential donors for future breeding programmes.

Key words: Chlorophyll content, Grain yield, Membrane thermostability, Rice, Spikelet fertility

Introduction

Rice production is the backbone of Indian agriculture. Majority of the population depends on this cereal crop to meet either their economic needs or for their caloric intake. Rice production is hindered by abiotic as well as biotic stresses. Keeping the climate change scenario in view, abiotic stresses play a major role in decreasing the crop production and productivity resulting in need of a sustainable crop development and resistance to abiotic stress (Randive *et al.*, 2019). Of this, high temperature is one of the major constraints in rice production.

The fifth assessment report of the Inter-governmental Panel on Climate Change (IPCC, 2014) stated that the increase in global average temperature from 1880 to

2012 was 0.85 °C and by 2100 the projected increase of global mean surface temperature is 3-5 °C. This rise in temperature affects the growth and development resulting in morphological, physiological and biochemical changes in plants (Ashraf and Hafeez, 2004).

High temperature affects rice at every growth stage beginning from the inhibition of germination, seedling stage, tillering, reproductive and also at grain filling stage (Dubey *et al.*, 2018). Of all the stages, booting and flowering are the most sensitive stages to high temperature stress which leads to disruption of cell membranes as well as breaking of cell compartmentalization (Larkindale and Huang,

2004). High temperature at flowering stage results in inhibition of anther dehiscence and loss of stigma receptivity resulting in increase of spikelet sterility and lesser number of grains in the panicle. It also reduces the photosynthetic efficiency and ultimately resulting in yield reduction (Narayanan *et al.*, 2016). High temperature results in damage to the thylakoid membrane as well as inhibition of key enzymes in photosynthetic pathway (Bita and Gerats, 2013). In chlorophyll, the thylakoid granum and membranes are degraded to increase in temperature resulting in reduced photosynthesis (Xu *et al.*, 2021).

Imposition of high temperature at reproductive stage leads to reduction in panicle weight, number of spikelets and decreased grain weight (Wang *et al.*, 2019). Lyman *et al.*, (2013) reported that for every 1°C increase in average temperature during rice growing season the paddy yields reduced by 6.2%. It was also reported that in rice spikelet sterility was increased by 80% when high temperatures coincided the reproductive stage of the crop (Xu *et al.*, 2020).

Rice production plays a prominent role in ensuring food security in the country and at this juncture there is a need to identify and breed for thermo tolerant rice varieties that can withstand the future temperature elevations. With this aim, twenty-six genotypes of rice were screened and impact of high temperature was studied in these genotypes to identify thermo tolerant genotypes.

Materials and Methods

Plant material: Twenty-six rice genotypes were screened and tested for high temperature tolerance by conducting a field experiment in *khariif* 2023 at Regional Agricultural Research Station, Maruteru. The experiment was laid out in split plot design with three replications. A spacing of 20 cm between rows and 15cm between hills was maintained. Recommended dose of Nitrogen (N), Phosphorus (P) and Potassium

(K) was applied. Water management as well as crop protection measures were taken up time to time. All the other package of practices recommended for irrigated transplanted rice were followed.

Imposition of treatments: One set of genotype was grown in ambient conditions and another set was subjected to high temperature stress by enclosing a polythene sheet supported by bamboo poles and iron frame at panicle initiation (PI) stage. Temperature inside the polyhouse was monitored continuously using data logger up to physiological maturity.

Morpho-phenological traits: Observations were recorded for various morpho-phenological from the sampled plants tagged in each treatment and genotype. Plant height on tagged plants was recorded at reproductive stage by measuring the height from base of the plant to the tip of the terminal leaf or panicle on main stem and was expressed in centimetres (cm). The number of days taken for 50 per cent of plants to flower in each genotype and each treatment was noted as days to 50% flowering and was expressed in days. The number of days taken from sowing to physiological maturity was recorded and was expressed as days to maturity.

Membrane thermostability: Membrane thermostability (MTS) was measured following the procedure described by Haque *et al.*, (2009). Mature leaves at reproductive stage were collected and first 2 to 4 cm was clipped off. The next 5 cm of leaf was washed three times with deionised water, cut into small pieces and placed in tubes with 10 ml deionized water. Two sets of each sample were prepared, one set designated as control was maintained at 28 °C while, other set was treated in water bath at 52 °C for one hour. Three replications were maintained for both the sets. After the treatment, control and treated tubes were kept at room temperature for 24 h. The initial conductance was measured using conductivity



meter. Thereafter, all the tubes were autoclaved at 121 °C at 15 lb for 20 mins and the next day final conductance was measured. This ensured complete electrolyte leakage from the plant tissue. The MTS was calculated using the following equation (Blum and Ebercon, 1981).

$$\text{MTS (\%)} = (1 - (T1/T2)) / (1 - (C1/C2)) \times 100$$

Where, C1= initial conductance of control sample; C2=final conductance after autoclaving of control sample; T1= initial conductance of high temperature sample (after water bath treatment); T2= final conductance after autoclaving of high temperature sample.

Chlorophyll content: It was estimated in flag leaf at reproductive stage (1 week after anthesis). For this flag leaf was cut into small pieces and 25 mg of leaf sample was weighed. Chlorophyll was extracted by placing the sample in 80% acetone solution as per the methodology described by Porra *et al.*, (1989). The absorbance was measured using a UV-VIS spectrophotometer. Chlorophyll a and chlorophyll b were measured at 663.2 nm and 646.8 nm, respectively and the chlorophyll content was expressed in mg g⁻¹ fresh weight (mg g⁻¹ FW). Chlorophyll a content, chlorophyll b content and the total chlorophyll content was calculated according to Lichtenthaler and Wellburn, (1983).

Yield and yield attributes: Yield and yield attributes such as grain number per panicle, 1000 grain weight, grain yield and total dry matter were recorded. At physiological maturity, panicles were threshed from a demarcated area of one square meter in all the genotypes under control and stress conditions. The number of panicles in one meter square area were threshed, cleaned and the weight of grains was recorded and expressed as grain yield in gm⁻². Five panicles were selected at random in every genotype and all the spikelets were separated from the panicle and filled grains were further separated and expressed

as grain number per panicle. A sample of 1000 seeds at random were taken from every genotype under both the conditions and weighed in gm and expressed as test weight. After harvest the shoot was dried and shoot biomass was recorded. Spikelet fertility was calculated as the number of filled spikelets/ total number of spikelets x 100 and expressed in per cent. Harvest index was calculated as ratio of economic yield to biological yield and expressed in per cent.

Statistical analysis: Two-way analysis of variance (ANOVA) was performed using Statistix 8.1 package. Statistical significance of the parameter means was determined by performing Fisher's LSD test to test the statistical significance.

Results and Discussions

High temperature was imposed at panicle initiation and with the help of data loggers the temperature inside the polyhouse was monitored on daily basis. The mean maximum temperature in the ambient condition was 34.9 °C and the average mean minimum temperature was 25.6 °C. Whereas, under polyhouse the mean monthly maximum temperature was 31.5 °C and the average of monthly minimum temperature was 24.2 °C. The difference in mean monthly maximum and minimum temperature was 3.5 °C and 1.4 °C, respectively (**Figure 1**).

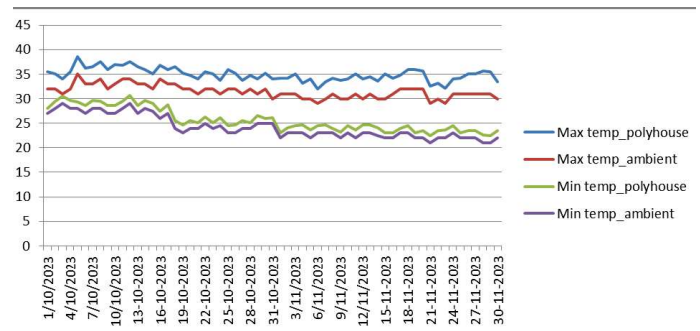


Figure 1: Temperature readings under polyhouse (heat stress) and ambient conditions (control)

Morpho-phenological traits:

The impact of high temperature was not significant on days to 50% flowering but the mean days to maturity

reduced by 3 days. At flowering, plant height reduced under high temperature among the genotypes. When compared to control maximum reduction was of 6 cm was noted in IL 19024 (Table 1). High temperature causes slower growth of the plants resulting in

reduction of plant height and 50% flowering (Pooja and Ajit, 2022). It has been stated that high temperature influenced negatively the growth parameters of rice plants by Korres *et al.*, (2017). In this study reduction of plant height as well as phenological traits was apparent.

Table 1: Impact of high temperature on morpho-phenological characters in rice genotypes

Entry	Days to 50% flowering			Days to maturity			Plant height (cm) at flowering			Chlorophyll content (mg /g FW)		
	C	Ht	M	C	Ht	M	C	Ht	M	C	Ht	M
IET 29859	90	88	89	122	115	119	105	104	104	2.26	1.92	2.09
IL 19019	93	91	92	124	119	122	100	99	99	2.43	2.13	2.28
IL 19020	94	93	93	122	119	121	106	102	104	2.38	1.80	2.09
IL 19021	96	94	95	126	123	124	107	105	106	3.24	2.52	2.88
IL 19022	91	90	91	121	118	120	103	102	103	2.52	2.07	2.30
IL 19024	93	91	92	123	119	121	112	106	109	3.08	2.72	2.90
IL 19185	93	92	93	123	120	122	106	105	106	2.45	2.04	2.25
IL 19198	88	86	87	117	114	116	88	84	86	3.26	2.83	3.05
IL 19202	91	90	91	120	118	119	88	87	87	2.31	1.97	2.14
IL 19211	97	96	97	127	123	125	109	109	109	3.34	3.13	3.24
IL 19247	92	91	92	121	119	120	107	102	105	2.45	2.08	2.26
IL 19396	91	90	91	122	118	120	117	114	116	3.04	2.60	2.82
JB 680-2	90	89	90	121	119	120	112	107	109	2.41	2.00	2.21
JB 683-1	91	90	91	121	118	120	107	105	106	2.65	2.42	2.53
JB 687-3	91	90	90	121	119	120	115	113	114	3.07	2.63	2.85
JB 689-1	96	94	95	124	121	123	112	111	111	2.39	2.00	2.20
JBC 159-11	91	90	90	121	119	120	110	108	109	2.35	1.97	2.16
Krishnahamsa	91	90	90	121	118	120	104	101	102	3.26	2.85	3.06
MTU 1153	94	93	94	124	122	123	110	106	108	3.00	2.70	2.85
MTU 1156	92	91	91	121	119	120	104	102	103	3.20	2.73	2.97
MTU 1273	94	91	93	123	121	122	113	109	111	3.26	2.50	2.88
MTU 1290	92	91	92	122	119	121	112	110	111	2.79	2.61	2.70
MTU 1293	92	91	92	122	119	121	106	103	105	3.01	2.46	2.74
N-22	97	96	96	126	123	125	115	111	113	2.81	2.37	2.59
NLR 3776	97	95	96	127	123	125	110	105	108	2.62	2.13	2.38
NLR 3778	95	94	94	126	123	124	122	118	120	2.59	2.16	2.37
Mean	93	92		123	120		108	105		2.78	2.36	
LSD (T)		NS			0.53			1.04			0.026	
LSD (V)		1.62			1.93			3.76			0.095	
LSD (TxV)		2.29			2.73			5.32			0.135	
CV (%)		1.54			1.39			3.1			3.26	

Physiological traits:

Membrane thermostability: Imposition of high temperature led to reduction in the cell membrane thermostability in all the genotypes. Among the genotypes, MTU 1290 (77.9%), JB 683-1 (75.4%), MTU 1156 (73.9%), Krishnahamsa (73.3%), MTU 1153 (72.9%) and N22 (72.4%) had higher thermostability under heat stress treatment. JBC 159-11 (25.3%), IL 19247 (27.8%) and JB 680-2 (29.4%) had lowest thermostability. Maximum reduction in thermostability when compared to control (more than 50%) was in JB 687-3, JB 680-2, IL 19247 and JBC 159-11. The reduction in thermostability was minimum (less than 12%) in MTU 1290, MTU 1153, MTU 1156, JB 683-1 and N22 (**Figure 2**). The cell membrane is one of the most sensitive structures which is affected by variations in temperature (Hu *et al.*, 2018). In this study it was noted that when subjected to high temperature stress the cell membranes were disrupted leading to a higher electrolyte leakage. Prasertthai *et al.*, (2022) treated 28 days old seedlings of rice at 42 °C for 7 days and noted an increase in electrolytic leakage however N22 showed the highest heat tolerance displaying the lowest increase in electrolyte leakage. In this study also MTU 1153, MTU 1156, JB 683-1, N22 and MTU 1290 recorded lesser reduction in electrolytic leakage that could confer tolerance to increase in temperature.

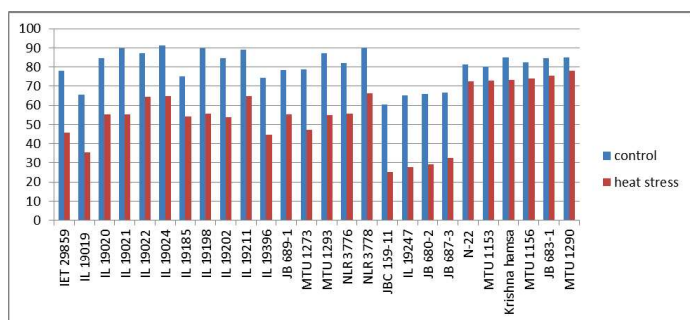


Figure 2: Impact of high temperature on Membrane thermostability (%) in rice genotypes

Chlorophyll content: The mean chlorophyll content reduced from 2.78 in control to 2.36 under heat stress.

Highest chlorophyll content under high temperature stress was noted in IL 19211 (3.13 mg g⁻¹ FW) followed by Krishnahamsa (2.85 mg g⁻¹ FW), IL 19198 (2.83 mg g⁻¹ FW) and MTU 1156 (2.73 mg g⁻¹ FW). Lowest was in IL 19020 (1.8 mg g⁻¹ FW) followed by IET 29859 (1.92 mg g⁻¹ FW) and JBC 159-11 and IL 19202 (1.97 mg g⁻¹ FW). The per cent reduction of chlorophyll content compared to control was less than 10% in MTU 1153, JB 683-1, MTU 1290 and IL 19211. Whereas more than 20% reduction was observed in IL 19021, MTU 1273 and IL 19020 (**Table 1**). Chloroplast is a vital organelle for photosynthesis and contains photosynthetic pigments. The integrity of the organelle as well as biosynthetic pathways for chlorophyll formation is impacted by temperature. In a study conducted by Sánchez-Reinoso *et al.*, (2014), high temperature at 35 and 40 °C resulted in reduction of chlorophyll a, chlorophyll b as well as total chlorophyll content in rice seedlings. Similar chlorophyll degradation was noted in KDML 105 rice seedlings when imposed to heat stress (Taratima *et al.*, 2022). In the present study also there was a reduction in chlorophyll content in the tested entries. However, the reduction of chlorophyll content compared to control was less in MTU 1153, JB 683-1, MTU 1290 and IL 19211 indicating their tolerant nature when compared to other genotypes.

Grain yield and yield attributes:

Spikelet fertility (%): The mean spikelet fertility dropped from 92.0% in control to 75.7% under high temperature stress. Among the genotypes tested, higher spikelet fertility under stress conditions was observed in IL 19211 (87.2%) followed by MTU 1290 (85.4%), JB 683-1 (82.0%). Less than 65% spikelet fertility was noted in JB 680-2 (63.4%) and IL 19247 (64.8%) (**Table 2**).

Table 2: Impact of high temperature on yield attributes characters in rice genotypes

Entry	Spikelet fertility (%)			Grain number per panicle			1000 grain weight (g)		
	C	Ht	M	C	Ht	M	C	Ht	M
IET 29859	93.0	76.0	84.5	146	114	130	20.5	18.2	19.3
IL 19019	92.2	73.3	82.8	118	88	103	22.2	20.7	21.5
IL 19020	88.5	68.1	78.3	85	62	73	22.4	21.2	21.8
IL 19021	88.7	71.4	80.0	86	65	75	21.5	20.2	20.9
IL 19022	86.2	65.9	76.1	81	60	71	20.3	18.7	19.5
IL 19024	90.2	75.9	83.1	111	88	100	23.3	22.1	22.7
IL 19185	91.4	73.2	82.3	106	82	94	24.1	23.2	23.6
IL 19198	89.8	72.5	81.2	97	74	86	21.0	19.9	20.5
IL 19202	91.9	81.7	86.8	124	107	115	20.9	19.5	20.2
IL 19211	94.6	87.2	90.9	210	177	193	18.4	17.5	18.0
IL 19247	91.1	64.8	78.0	92	59	76	23.1	21.2	22.2
IL 19396	92.1	74.8	83.4	116	89	103	24.0	22.5	23.3
JB 680-2	92.7	63.4	78.1	139	92	115	22.2	20.6	21.4
JB 683-1	92.6	82.0	87.3	125	109	117	22.1	21.3	21.7
JB 687-3	93.8	78.9	86.4	166	131	149	24.2	22.7	23.5
JB 689-1	91.7	77.3	84.5	132	109	120	21.2	19.3	20.2
JBC 159-11	91.8	78.7	85.3	123	100	112	17.2	15.7	16.5
Krishnahamsa	92.0	78.6	85.3	104	88	96	19.2	17.9	18.6
MTU 1153	94.5	75.6	85.1	155	118	136	22.1	20.6	21.4
MTU 1156	93.3	77.2	85.3	140	112	126	22.2	20.6	21.4
MTU 1273	92.6	75.7	84.1	150	115	132	24.4	22.8	23.6
MTU 1290	94.6	85.4	90.0	140	123	131	24.7	23.3	24.0
MTU 1293	92.5	73.4	82.9	135	102	119	21.2	20.0	20.6
N-22	95.6	78.7	87.2	241	177	209	17.2	15.1	16.1
NLR 3776	93.3	79.0	86.2	166	132	149	22.0	20.1	21.0
NLR 3778	92.6	79.3	86.0	138	111	125	21.3	19.4	20.4
Mean	92.0	75.7		132	103		21.6	20.2	
LSD (T)		5.6			2.86			0.10	
LSD (V)		1.5			10.3			0.37	
LSD (TxV)		2.8			14.5			0.52	
CV (%)		5.1			7.68			1.56	

Grain number per panicle: The average number of grains per panicle dropped from 132 to 103. N22 (177), IL 19211 (177), NLR 3776 (132) and JB 687-3 (131) had a higher grain number per panicle under high temperature stress and IL 19247 (59), IL 19022 (60) and IL 19020 (62) recorded lower number of grains per panicle (Table 2).

1000 grain weight (g): The average test weight reduced from 21.6g in control to 20.2g under high temperature conditions. The test weight was highest

in MTU 1290 (23.3g) followed by IL 19185 (23.2g). Lowest test weight was noted in N22 (15.1 g) and JBC 159-11 (15.7) (Table 2).

Total Dry matter (gm⁻²): The total dry matter reduced from 661 gm⁻² in control to 587 gm⁻² under high temperature conditions. Higher TDM under high temperature conditions were noted in JB 689-1 (680 gm⁻²) followed by Krishnahamsa (667 gm⁻²), IL 19185 (644 gm⁻²). Lowest TDM was in MTU 1293 (490 gm⁻²) (Table 3).



Table 3: Impact of high temperature on grain yield and yield attributes in rice genotypes

Entry	TDM (gm ⁻²) at maturity			Grain yield (gm ⁻²)			Harvest index (%)		
	C	Ht	Mean	C	Ht	Mean	C	Ht	Mean
IET 29859	743	583	663	535	345	440	41.9	37.1	39.5
IL 19019	590	522	556	501	351	426	46.0	40.1	43.0
IL 19020	691	597	644	523	387	455	43.1	39.1	41.1
IL 19021	633	596	615	493	408	451	43.7	40.5	42.1
IL 19022	626	588	607	511	396	454	44.8	40.3	42.6
IL 19024	592	537	565	429	348	389	41.9	39.2	40.6
IL 19185	688	644	666	529	388	458	43.4	37.5	40.5
IL 19198	622	577	600	469	386	427	43.0	40.1	41.5
IL 19202	681	614	648	514	403	459	42.9	39.6	41.3
IL 19211	621	582	602	468	416	442	42.9	41.5	42.2
IL 19247	640	566	603	462	364	413	41.9	38.7	40.3
IL 19396	689	585	637	516	407	462	42.8	41.1	41.9
JB 680-2	577	566	572	422	228	325	42.2	28.6	35.4
JB 683-1	679	596	637	515	443	479	43.2	42.8	43.0
JB 687-3	670	544	607	511	366	439	42.9	40.1	41.5
JB 689-1	677	680	679	507	388	448	42.8	36.0	39.4
JBC 159-11	640	574	607	483	364	424	42.9	38.7	40.8
Krishnahamsa	737	667	702	552	434	493	42.9	39.4	41.2
MTU 1153	664	629	647	501	420	460	42.8	40.0	41.4
MTU 1156	622	596	609	486	378	432	43.8	38.7	41.2
MTU 1273	607	630	619	472	345	408	43.3	35.4	39.4
MTU 1290	697	563	630	501	418	460	42.8	40.2	41.5
MTU 1293	711	490	600	551	345	448	43.6	41.4	42.5
N-22	767	598	683	577	392	484	42.9	39.7	41.3
NLR 3776	583	498	540	416	290	353	41.6	36.5	39.1
NLR 3778	751	627	689	571	434	503	43.2	40.9	42.0
Mean	661	587		501	379		43.1	39.0	
LSD (T)		19.6			19.7			0.94	
LSD (V)		70.7			71.3			3.41	
LSD (TxV)		100.0			100.9			4.82	
CV (%)		9.9			14.1			7.27	

Grain yield (gm⁻²): Grain yield reduced from 501 gm⁻² in control to 379 gm⁻² under high temperature stress. Higher grain yield under high temperature was noted in JB 683-1 (443 gm⁻²) followed by NLR 3778 (434 gm⁻²), Krishnahamsa (434 gm⁻²), MTU 1153 (420 gm⁻²) and MTU 1290 (418 gm⁻²). Lower were in JB 680-2 (228 gm⁻²), NLR 3776 (290 gm⁻²) and IET 29859 (345 gm⁻²). More than 35% reduction was in IET 29859, MTU 1293 and JB 680-2 and less than 16% reduction was in MTU 1290, MTU 1153, JB 683-1 and IL 19211 (**Table 3**).

Harvest index (%): Harvest index reduced from 43.1% in control to 39.0% under high temperature conditions. Highest harvest index was noted in JB

683-1 (42.8%) followed by IL 19211 (41.5%). Lowest was in JB 680-2 (28.6%) (**Table 3**).

High temperature during the flowering period reduces the spikelet fertility (Zhang *et al.*, 2018). High temperature leads to poor anther dehiscence and less pollen grains on the stigma resulting in reduction of spikelet fertility (Zhao *et al.*, 2016). The number of spikelets reduced under high temperature majorly due to reduction in pollen production by preventing anther filling during the panicle initiation phase (Wang *et al.*, 2016). Hazra *et al.*, (2016), imposed high temperature at tillering, booting, panicle initiation as well as flowering stage in rice and reported that high temperature stress (36 °C) at PI stage resulted

in higher reduction of grain yield. Similarly high temperature exposure significantly decreased filled grains per panicle, 1000-grain weight and harvest index and increased the unfilled grains per panicle. Mahantashivayogayya *et al.*, (2016), screened forty rice accessions for heat tolerance and results revealed that EC792239, EC792285 and EC792185 which had heat tolerance, recorded more number of panicles and minimum per cent of chaffyness. In this study, reduction in grain yield and yield attributes were evident under high temperature stress and high yielding genotypes had higher spikelet fertility.

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Conclusion

High temperature has a negative impact on rice growth and development. It hampers the cell membranes leading to a higher electrolyte leakage as well as reduction in photosynthetic pigment. Impact of exposure at panicle initiation stage is evident from the reduction of yield and yield attributes of the genotypes tested, MTU 1290, MTU 1153, MTU 1156, JB 683-1, N22 and IL 19211 maintained higher membrane stability, lesser reduction in loss of chlorophyll content, high spikelet fertility and grain yield. These genotypes could thus be identified to be thermotolerant.

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