Article AR2028 DOI: HTTPS://DOI.ORG/10.23910/IJBSM/2019.10.5.2028

International Journal of Bio-resource and Stress Management

Crossref

October 2019

Print ISSN 0976-3988
Online ISSN 0976-4038

IJBSM 2019, 10(5):472-480

Research Article *Natural Resource Management*

Screening of Rice Germplasm with Physiological Traits for Identification of Heat Tolerant Genotypes

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Citation: Veronica et al., 2019. Screening of Rice Germplasms with Physiological Traits for Identification of Heat Tolerant Genotypes. International Journal of Bio-resource and Stress Management 2019, 10(5):472-480. HTTPS://DOI.ORG/10.23910/IJBSM/2019.10.5.2028

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

Acknowledgement: DBT-INSPIRE fellowship to the first author was duly acknowledged. The authors are grateful to the Director ICAR-Indian Institute of Rice Research (formely Directorate of Rice Research) for providing research facilities.

Abstract

High temperature as a part of the climate change scenario is imposing a severe threat to rice production and productivity worldwide. Field experiment was conducted in ICAR-IIRR during *rabi* 2016-17 to screen 60 diverse rice genotypes comprising of 2 germplasm lines, 11 green super rice lines, 2 introgression lines, 8 landraces, 2 tropical japonicas and 35 released varieties including 2 checks for identification of high temperature tolerant genotypes. They were tested for their nature of thermotolerance using cell membrane thermostability, PSII activity (F_v/F_m) and paraquat tolerance test as screening criteria. There was a reduction in all the above measured traits in all the genotypes however, among the genotypes the extent of reduction varied. Among the genotypes screened, Rasi, Akshayadhan, HKR47, IR64, Assanchidiya had recorded a higher grain yield under high temperature conditions and hence could be categorized as heat tolerant whereas ADT43, Vandana, NDR359 MTU1001 and IR36 had lower yields and hence were categorized as heat susceptible. The screening traits were correlated with grain yield recorded under high temperature. Positive and significant association was noted between cell membrane thermostability, F_v/F_m measured under high temperature stress with the grain yield. The tolerant genotypes also had a higher cell membrane thermostability and higher F_v/F_m under high temperature conditions. Among the analysed traits, it can be concluded that CMS and F_v/F_m can be used efficiently to screen the germplasm for thermotolerance.

Keywords: Rice, spikelet fertility, high temperature, membrane thermostability, PSII

1. Introduction

Rice (*Oryza sativa* L.) cultivation forms the backbone for more than 50% of the world's population (Sarkar et al., 2009) and is particularly more important in an agricultural country like India. It is the most important cereal crop and fundamental implication for livelihood for majority of the people in the world (Satya et al., 2010). Rice productivity needs to be increased keeping in view the over exploding population (Rosegrant and Cline, 2003). Rice encounters many obstacles in the form of stresses both biotic and abiotic (Sabouri et al., 2011) that restricts its ability to reach its complete yield potential. In this scenario of changing climate, high temperature stress is becoming one of the major threats for food crop production worldwide (Wassmann et al, 2009). Due to climate

Article History

RECEIVED in 25th August 2019 RECEIVED in revised form 10th October 2019 ACCEPTED in final form 17th October 2019

change, by the next century an increase of 1.1 to 6.4 °C in mean global temperature is estimated by the IPCC (IPCC, 2012). Rice is mainly cultivated in tropical and sub-tropical regions of the world and in such climates high temperature is a major constraint for the crops grown in these climates (Wahid et al., 2007).

The optimum temperature for rice cultivation is about 27 to 32 °C (Yin et al., 1996). High temperature has a damaging effect on plant growth and development by affecting majorly the plants physiology, biochemistry as well as at the level of gene regulation that ultimately has an effect on yield of the plant (Bita and Gerats, 2013). Of all the stages, flowering and booting are the most susceptible stages in rice (Farrell et al., 2006). High temperature affects the biological processes as well as hampers the metabolic processes. Major effects of heat stress in rice include reduced germination resulting in also in poor seedling growth, chlorophyll pigment reduction and also its synthesis is affected, imbalance between photosynthesis and respiration, damage to cell membrane, reduction in number of pollen grains and poor anther dehiscence leading to increased sterility and poor grain quality that ultimately leads to reduced yields (Zhou et al., 2012; Matsui and Omasa, 2002).

Hence from the available germplasm of rice, donors for thermotolerance are now required. Attempts to identify donors through large scale field screening and studying the genotypic variation among them using a few defined phenotyping traits or methods will enable to ascertain genotypes that can yield substantially under the backdrop of projected climate change scenario. One such is a physiological trait is cell membrane thermostability (CMS) used as a screening tool because of its positive and direct association with thermotolerance in many crops. This is mainly due to the fact that increase in temperature leads to alterations in the permeability of the membrane and leading to electrolytic leakage (Rasheed, 2009) Ibrahim and Quick (2001) used this parameter as an indicator and reported that a heat tolerant genotype is characterized with a higher CMS. Another widely used effective physiological trait to screen crops to various abiotic stresses is the state of PSII photochemistry that indicates the overall photosynthetic efficiency of the plant. The PSII activity is affected by increase in temperature stress by reducing the electron transport rate (Mathur et al., 2011). Exposure to high temperature leads to production of reactive oxygen species that damages the chloroplast membrane that mimicks the damage caused by paraquat treatment in plant. Very few reports are available on the correlation of paraquat tolerance with heat tolerance and its use in phenotyping for heat stress. So, in this study these three traits, CMS, PSII activity and chlorophyll content in the paraquat tested leaves were used as traits to identify the tolerant genotypes from a wide rice germplasm.

2. Materials and Methods

2.1. Experimental design, treatments and growth condition

A field experiment was conducted to screen 60 diverse rice genotypes at ICAR- Indian Institute of Rice Research during *rabi* 2016-17. The farm is geographically situated at an altitude of 542.7 m above mean sea level on 17^0 19' N latitude and 78^0 29' E longitude. The rice genotypes comprises of 2 germplasm lines, 11 green super rice lines, 2 introgression lines, 8 landraces, 2 tropical japonicas and 35 released varieties including 2 checks for identification of high temperature tolerant genotypes. Physiological traits such as CMS, chlorophyll fluorescence and chlorophyll content assay (as an indicator of paraquat tolerance test) were used as screening criteria. The rice genotypes were sown in a split plot design in two sets in *rabi* (November–March) in IIRR field. The genotypes to be screened were sown at 20×10 cm^2 spacing at different sowing dates (normal and late sowing) to expose them to different temperature regimes during reproductive stage (anthesis to physiological maturity). The first sowing was considered as control and second sowing as high temperature stress. Recommended doses of NPK (100:60:60) and Zn in form of $ZnSO_4$ @ 12.5 kg ha were applied. All cultural practices were carried out carefully. A layer of 2-3 cm water was maintained constantly till the establishment of seedlings. Thereafter about 5cm of water was maintained upto dough stage of the crop. For 1st and 2nd sowing, the average mean monthly maximum temperature was 35.2 °C and 38.0 °C respectively and mean monthly minimum temperature was 18.5 °C and 21.3 °C respectively. However, during the critical stages of flowering to grain filling the mean maximum temperature was 36 °C during the 1st sowing and 41.5 °C during the 2nd sowing and the mean minimum was 17.3 °C during the 1st sowing and 21.6 °C during the 2nd sowing. Three replications were maintained for each treatment and each variety.

2.1.1. Cell membrane thermo stability (CMS) analysis

Membrane thermostability 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 de-ionized 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)

$$CMS\ (\%)=(1-(T_1/T_2))\ /\ (1-(C_1/C_2)\times 100$$

Where, C_1= initial conductance of control sample; C_2=final conductance after autoclaving of control sample; T_1= initial conductance of high temperature sample (after water bath treatment); T_2= final conductance after autoclaving of high temperature sample

2.1.2. Chlorophyll fluorescence (F_v/F_m) measurements

Preliminary experiments for optimisation were conducted and a detached leaf protocol for measuring chlorophyll fluorescence was standardised. Fully matured leaf at maximum tillering stage was taken and dark adapted for 30 min at room temperature followed by measurement of F_v/F_m using portable PAM-210. Later all the samples were shifted into a growth chamber maintained at a temperature of 40-42 °C, RH 70% and PPFD of 300µmol m^{-2} s^{-1} for 2 h. After heat stress treatment again F_v/F_m was recorded (Sharma et al., 2012).

2.1.3. Paraquat tolerance test

A modified method of Mohammadi et al. (2007) was followed. Mature leaves were collected and first 2 to 3 cm of leaf was clipped off. The next 5 cm of leaf was used as experimental unit for paraquat treatment. Before treating the leaves with paraquat chlorophyll content was estimated. The treatment strip of leaf was incubated overnight in dark. Next day the leaf was immersed in paraquat solution for 6 h in full sunlight. Later the leaf was washed in distilled water and chlorophyll content was estimated. Paraquat induced reduction in chlorophyll content was calculated.

2.1.4. Spikelet fertility (%)

Spikelet fertility was worked out by dividing number of filled spikelets by total number of spikelets x 100 and expressed in %.

Grain yield (g m^{-2}): At physiological maturity, panicles from one meter square demarcated area in each plot were harvested, sun dried, threshed, cleaned and weight of was recorded. *Correlation studies:* Relationship between the screening traits and grain yield under high temperature stress was worked out. The data was analyzed using R studio software.

3. Results and Discussion

3.1. Cell membrane thermo stability

High temperature stress led to a significant reduction of CMS in the tested genotypes. Among the genotypes, maximum CMS was noted in N22 (83.0%), Rasi (80.7%), IR64 (74.1%), Khudaridhan (70.3%) and Akshayadhan (66.0%). Compared to normal condition, the membrane damage aggravated in ADT43, Sumati, Sugandhasamba, MTU1001, and Pantdhan12 than other genotypes (Table 1).

An emerging additional challenge to increase rice production is the effect of climate change, especially global warming and associated abiotic stresses. Significant variation exists in the germplasm for high temperature tolerance at different growth stages, which needs to be exploited. Identification of physiological traits that contribute to tolerance to high temperature stress is of paramount importance. In view of climate change scenario, high temperature is having a negative impact on the metabolism of crop plants hence affecting the vital processes such as photosynthesis, respiration, water uptake and other metabolic activities (Sharkey and Schrader, 2006). CMS is used as a screening parameter in many crops as when plants are subjected to high temperature stress it was noted that heat tolerant genotypes possessed better membrane thermostability than heat susceptible ones (Kumar et al, 2012). So this trait has been widely used for phenotyping for high temperature tolerance (Ibrahim and Quick, 2001). Sailaja et al. (2015) also correlated CMS with yield under elevated temperature stress and obtained a high correlation among them and concluded CMS as the most reliable trait to screen for high temperature tolerance in rice. In the present experiment, the genotype possessing a higher CMS were classified as high temperature tolerant and those with low values as susceptible as it was reported that plants with higher electrolyte leakage or lower CMS were susceptible under high temperature stress (Haque et al, 2009).

3.2. Maximum quantum yield of PSII (F_v/F_m)

Compared to normal condition, under heat stress there was a remarkable reduction in F_v/F_m in all the genotypes but it was more in ADT43, ADT49, Mahamaya, MTU1001, RNR6378 and NDR359. The % reduction in F_v/F_m from normal to high temperature ranged from 2.7 to 4.3 in N22, Khudaridhan, Rasi, GSR330, IR64, Assanchidiya, Sonkaichi and Akshayadhan and 33.4 to 56.0 in Vandana, ADT43, ADT49, Mahamaya, MTU1001, RNR6378 and NDR359 (Table 2).

Chlorophyll fluorescence measurement was conducted as it is a powerful and widely used technique for evaluating the photosynthetic performance of plants especially under abiotic stress conditions. Hence it was used as an assay for heat tolerance in this experiment too. PSII indicates the overall photosynthetic efficiency of the plant and of all the components it is affected the most by causing deviations in the oxidation-reduction properties of acceptors located in PSII component resulting in reduction in the efficiency of electron transport chain in both photosystems (Mathur et al, 2011, 2014). F_v/F_m that indicates the PSII photochemistry or PSII photochemical efficiency was used as a selection criterion under heat stress condition (Sharma et al., 2012). They developed a feasible detached leaf protocol and found no significant variation between the F_v/F_m readings obtained on attached or detached leaves. There was a reduction in F_v/F_m under high temperature stress and it was similar in both attached and detached leaf protocol based on which tolerant cultivars were identified. Han et al. (2009) also experimentally proved the reduction of F_v/F_m in rice seedlings when subjected to a high temperature. Similarly reduction in F_v/F_m as well as electron transport rate in rice cultivar (N22) and variety (IR64) was observed under high temperature stress of 44°C at flowering stage, however in the mutant of N22 (NH219), there was no significant reduction and was concluded to be

Table 1: Effect of high temperature on cell membrane thermostability (CMS) (%) in rice genotypes

Sl. no.	Genotype	MTS (%)			S. No.	Genotype	MTS (%)		
		Control	Heat	Mean			Control	Heat	Mean
1,	24K	40.7	31.2	36.0	31.	Lalat	51.4	32.3	41.9
2	ADT43	24.2	19.1	21.7	32.	Mahamaya	50.8	32.6	41.7
3.	ADT49	40.2	31.3	35.8	33.	MTU1001	34.2	20.5	27.4
4.	Akshayadhan	70.8	61.3	66.1	34.	MTU1010	64.3	43.2	53.8
5.	Assanchidiya	58.9	45.3	52.1	35.	N22	85.6	80.5	83.1
6.	Bejaridhan	47.8	35.5	41.7	36	NDR359	39.8	24.4	32.1
7.	BPT5204	65.7	51.1	58.4	37.	Nevaripeeli	60.3	51.3	55.8
8.	Dhaniyadhan	42.3	30.9	36.6	38.	Pantdhan12	34.7	21.1	27.9
9.	E2710	47.6	32.8	40.2	39.	Pantdhan16	45.7	34.5	40.1
10.	E2940	55.7	41.7	48.7	40.	Pantdhan18	48.8	35.2	42.0
11.	GSR304	35.4	28.7	32.1	41.	Pantdhan4	50.3	43.4	46.9
12.	GSR305	42.7	34.5	38.6	42.	Pardeshiya	42.4	32.2	37.3
13.	GSR309	53.0	44.3	48.7	43.	Ranikajal	45.2	33.4	39.3
14.	GSR310	52.6	45.1	48.9	44.	Rasi	82.5	78.9	80.7
15.	GSR312	54.9	42.1	48.5	45.	RNR6378	40.5	30.4	35.5
16.	GSR313	46.6	35.5	41.1	46.	RPHR517	42.4	32.1	37.3
17.	GSR315	55.5	45.4	50.5	47.	S40	55.3	43.2	49.3
18.	GSR319	45.6	33.2	39.4	48.	Sampada	43.9	35.7	39.8
19.	GSR324	62.4	51.2	56.8	49.	Satabdi	41.1	23.5	32.3
20.	GSR328	42.5	32.2	37.4	50.	Sita	59.9	45.7	52.8
21.	GSR330	70.7	59.4	65.1	51.	Sonkaichi	68.9	55.4	62.2
22.	HKR127	55.6	43.7	49.7	52.	Sugandhasamba	32.5	22.1	27.3
23.	HKR47	50.5	40.7	45.6	53.	Sumati	28.4	18.7	23.5
24.	IET21542	40.6	31.3	36.0	54.	Suraj	55.8	42.1	48.9
25.	IR36	34.2	22.4	28.3	55.	Tellahamsa	42.6	28.4	35.5
26.	IR64	78.5	69.8	74.2	56.	TJP139	41.7	31.8	36.7
27.	Jaya	50.9	39.4	45.2	57.	TJP82	60.8	47.7	54.3
28.	Jyoti	45.6	32.5	39.1	58.	Vandana	35.3	23.3	29.3
29.	Khudharidhan	74.2	66.4	70.3	59.	Varadhan	54.2	40.9	47.5
30.	Krishnahamsa	45.3	23.7	34.5	60.	WGL14	43.5	28.5	36.0
Mean							50.2	38.6	
LSD (T)							9.46 (p<0.05)		
LSD (G)							17.8 (p<0.05)		
LSD (TxG)							9.8 (p<0.05)		
CV (%)							8.9		

tolerant to high temperature (Poli et al., 2013). Likewise, in this experiment too genotypes having lesser reduction in F_v/F_m viz., N22, Khudaridhan, Rasi, GSR330, IR64, Assanchidiya, Sonkaichi and Akshayadhan could be identified as high temperature tolerant genotypes. In wheat, mass screening experiment was conducted by Sharma et al. (2012) for high temperature tolerance on 1274 genotypes of diverse origin based on a physiological trait, the maximum quantum efficiency of PSII (F_v/F_m) and identified a set of contrasting cultivars that differ in intrinsic capacity of PSII photochemical efficiency under

Table 2: Effect of high temperature on maximum quantum yield of PSII (F_v/F_m) in rice genotypes

Sl. no.	Genotype	F_v/F_m			S. No.	Genotype	F_v/F_m		
		Control	Heat	Mean			Control	Heat	Mean
1.	24K	0.734	0.592	0.663	31.	Lalat	0.740	0.509	0.624
2	ADT43	0.744	0.466	0.605	32.	Mahamaya	0.747	0.453	0.600
3.	ADT49	0.735	0.457	0.596	33.	MTU1001	0.745	0.417	0.581
4.	Akshayadhan	0.744	0.712	0.728	34.	MTU1010	0.739	0.689	0.714
5.	Assanchidiya	0.746	0.714	0.730	35.	N22	0.767	0.746	0.756
6.	Bejaridhan	0.739	0.570	0.654	36	NDR359	0.761	0.335	0.548
7.	BPT5204	0.731	0.691	0.711	37.	Nevaripeeli	0.715	0.666	0.690
8.	Dhaniyadhan	0.738	0.625	0.681	38.	Pantdhan12	0.754	0.538	0.646
9.	E2710	0.756	0.599	0.677	39.	Pantdhan16	0.732	0.519	0.625
10.	E2940	0.729	0.681	0.705	40.	Pantdhan18	0.749	0.508	0.628
11.	GSR304	0.761	0.636	0.698	41.	Pantdhan4	0.763	0.700	0.731
12.	GSR305	0.736	0.546	0.641	42.	Pardeshiya	0.733	0.514	0.623
13.	GSR309	0.731	0.697	0.714	43.	Ranikajal	0.738	0.670	0.704
14.	GSR310	0.760	0.655	0.707	44.	Rasi	0.763	0.734	0.748
15.	GSR312	0.761	0.689	0.725	45.	RNR6378	0.753	0.390	0.572
16.	GSR313	0.749	0.555	0.652	46.	RPHR517	0.730	0.668	0.699
17.	GSR315	0.747	0.509	0.628	47.	S40	0.742	0.675	0.708
18.	GSR319	0.743	0.639	0.691	48.	Sampada	0.770	0.529	0.649
19.	GSR324	0.759	0.696	0.728	49.	Satabdi	0.756	0.570	0.663
20.	GSR328	0.741	0.672	0.706	50.	Sita	0.758	0.715	0.736
21.	GSR330	0.743	0.711	0.727	51.	Sonkaichi	0.746	0.714	0.730
22.	HKR127	0.770	0.562	0.666	52.	Sugandhasamba	0.733	0.640	0.686
23.	HKR47	0.748	0.691	0.719	53.	Sumati	0.744	0.645	0.694
24.	IET21542	0.737	0.606	0.671	54.	Suraj	0.720	0.662	0.691
25.	IR36	0.737	0.506	0.621	55.	Tellahamsa	0.719	0.600	0.659
26.	IR64	0.741	0.709	0.725	56.	TJP139	0.719	0.607	0.663
27.	Jaya	0.726	0.507	0.616	57.	TJP82	0.739	0.698	0.718
28.	Jyoti	0.738	0.676	0.707	58.	Vandana	0.722	0.482	0.602
29.	Khudharidhan	0.746	0.726	0.736	59.	Varadhan	0.752	0.570	0.661
30.	Krishnahamsa	0.765	0.570	0.668	60.	WGL14	0.732	0.500	0.616
Mean							0.743	0.605	
LSD (T)								0.012 (p<0.05)	
LSD (G)								0.064 (p<0.05)	
LSD (TxG)								0.091 (p<0.05)	
CV (%)								6.79	

high temperature stress treatment.

3.3. Paraquat screening test

The reduction in total chlorophyll content under paraquat treatment was lower in N22 (15.6%), IR64 (16.9%), BPT5204 (17.4%), Rasi (17.4%) and MTU1010 (21.3%). It was higher than 60% in RNR6378, TJP139, Paradeshiya, RPHR517, Sugandhasamba and Vandana (Table 3).

Chlorophyll synthesis as well as the pigment retention is a good indicator of high temperature tolerance in crop plants (Gosavi et al, 2014). In plants exposed to heat stress,

Table 3: Effect of paraquat on total chlorophyll content (mg g^{-1} FW) in rice genotypes

Sl. no.	Genotype	Total chl content			S. No.	Genotype	Total chl content		
		Control	Heat	Mean			Control	Heat	Mean
1.	24K	3.31	1.35	59.2	31.	Lalat	2.17	1.25	42.6
2	ADT43	2.65	1.32	50.0	32.	Mahamaya	2.20	1.34	39.1
3.	ADT49	1.80	1.25	30.4	33.	MTU1001	2.00	0.99	50.6
4.	Akshayadhan	2.73	2.01	26.5	34.	MTU1010	1.61	1.27	21.3
5.	Assanchidiya	2.36	1.60	32.2	35.	N22	2.84	2.40	15.6
6.	Bejaridhan	2.53	1.76	30.6	36	NDR359	1.76	1.02	42.0
7.	BPT5204	1.97	1.63	17.4	37.	Nevaripeeli	2.51	1.43	42.9
8.	Dhaniyadhan	2.74	1.56	43.2	38.	Pantdhan12	2.06	1.17	43.0
9.	E2710	2.39	1.26	47.2	39.	Pantdhan16	1.68	1.09	35.3
10.	E2940	2.80	1.93	31.1	40.	Pantdhan18	2.66	1.82	31.7
11.	GSR304	2.88	1.39	51.9	41.	Pantdhan4	2.16	1.63	24.6
12.	GSR305	2.61	1.77	32.2	42.	Pardeshiya	2.96	1.12	62.3
13.	GSR309	2.80	1.75	37.4	43.	Ranikajal	2.74	1.45	47.0
14.	GSR310	2.68	1.73	35.6	44.	Rasi	1.94	1.60	17.4
15.	GSR312	2.64	1.38	47.8	45.	RNR6378	2.75	1.06	61.6
16.	GSR313	2.83	1.73	39.1	46.	RPHR517	2.40	0.85	64.6
17.	GSR315	2.61	1.67	36.0	47.	S40	2.79	2.18	21.8
18.	GSR319	2.71	1.75	35.4	48.	Sampada	2.28	1.54	32.5
19.	GSR324	2.86	2.08	27.4	49.	Satabdi	2.76	1.50	45.7
20.	GSR328	2.65	1.34	49.5	50.	Sita	1.86	1.14	38.7
21.	GSR330	2.66	1.88	29.5	51.	Sonkaichi	2.78	2.00	27.9
22.	HKR127	2.51	1.74	30.7	52.	Sugandhasamba	2.44	0.65	73.4
23.	HKR47	2.76	2.01	27.3	53.	Sumati	2.48	1.23	50.5
24.	IET21542	2.31	1.36	41.1	54.	Suraj	2.63	1.50	43.1
25.	IR36	2.20	0.96	56.4	55.	Tellahamsa	2.79	1.26	54.8
26.	IR64	1.70	1.42	16.9	56.	TJP139	2.62	1.00	62.1
27.	Jaya	1.96	1.17	40.5	57.	TJP82	2.20	1.52	30.9
28.	Jyoti	2.95	1.24	57.9	58.	Vandana	2.83	0.73	74.4
29.	Khudharidhan	2.27	1.76	22.5	59.	Varadhan	2.66	1.52	43.0
30.	Krishnahamsa	2.00	1.12	43.8	60.	WGL14	2.38	1.49	37.3
Mean							2.46	1.46	
LSD (T)							0.050 (*p*<0.05)		
LSD (G)							0.276 (*p*<0.05)		
LSD (TxG)							0.390 (*p*<0.05)		
CV (%)							7.03		

#C- Control, P- Paraquat treated

reactive oxygen radicals are produced due to the imbalance of photosynthesis and respiration, which will damage the chloroplast membranes and oxidizes the chlorophyll pigment. Similarly, paraquat treatment causes oxidative damage by producing highly toxic free radicals which in turn oxidizes the chlorophyll pigments and drastically reduces the chlorophyll content. In the present work paraquat induced changes in chlorophyll content was tested for identification of heat tolerant rice genotypes. The % reduction in chlorophyll content was correlated with grain yield under heat and a significant negative correlation was obtained. However, many reports suggest that paraquat tolerance does not seem to be a powerful technique for heat tolerance screening. Many report suggest a strong correlation between tolerance to paraquat and water stress tolerance Altikut et al. (2001) however under high temperature the repeatability of the data has to be checked. However, it can be used as a preliminary screening parameter.

3.4. Spikelet fertility (%) and Grain yield (g m^{-2})

High temperature had a negative impact on the spikelet fertility of rice genotypes. Rasi (86%) and N22 (86%) followed by Akshayadhan (84.3%) and IR64 (83.0%) had higher spikelet fertility under high temperature was noted whereas lesser in Vandana (50.7%), IR36 (50.7%) and ADT43 (56.7%) (Figure 1a). Substantial mean reduction of grain yield under high temperature was evident (26.7% mean reduction over control). Rasi (640.0 g m^{-2}) followed by Akshayadhan (626.6 g m^{-2}), HKR47 (623.3 g m^{-2}), IR64 (613.3 g m^{-2}) and Assanchidiya (613.3 g m^{-2}) had higher yields and lower were in ADT43 (251.6 g m^{-2}), Vandana (286.6 g m^{-2}), NDR359 (395.0 g m^{-2}), MTU1001 (406.6 g m^{-2}) and IR36 (410.0 g m^{-2}) (Figure 1b).

Peng et al. (2004) reported that one degree increase in

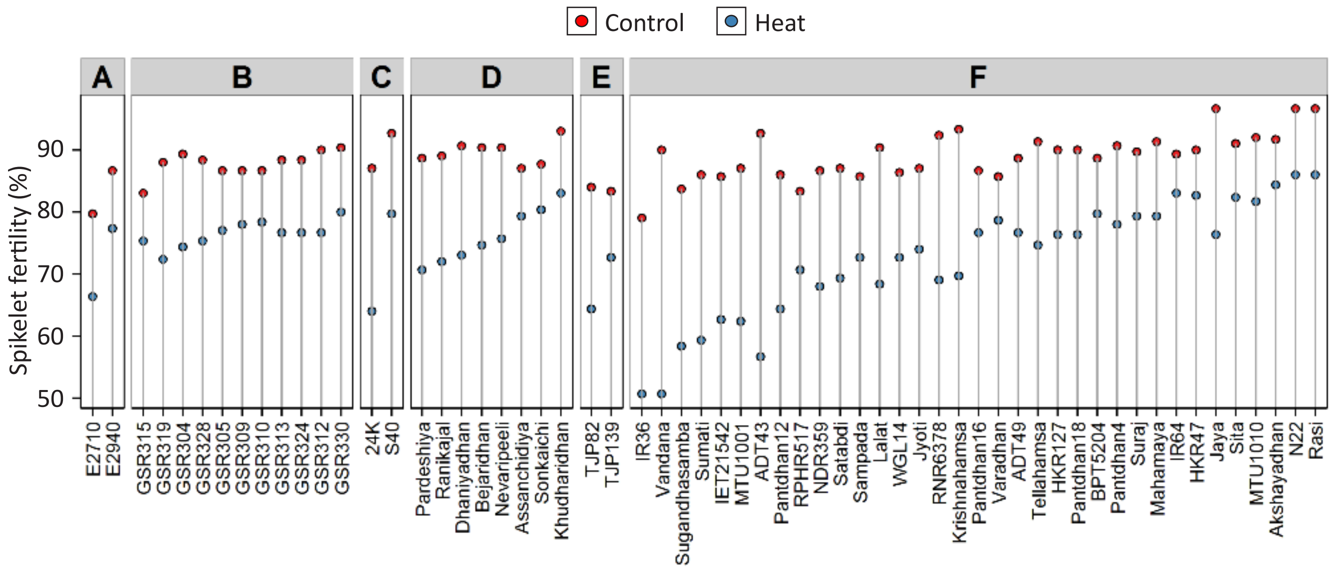

Figure 1a: Effect of high temperature on spikelet fertility (%) of rice genotypes. A: Germplasm lines; B: Green Super Rice; C- Wild introgression; D: Landrace; E: Tropical Japonica; F: Released varieties

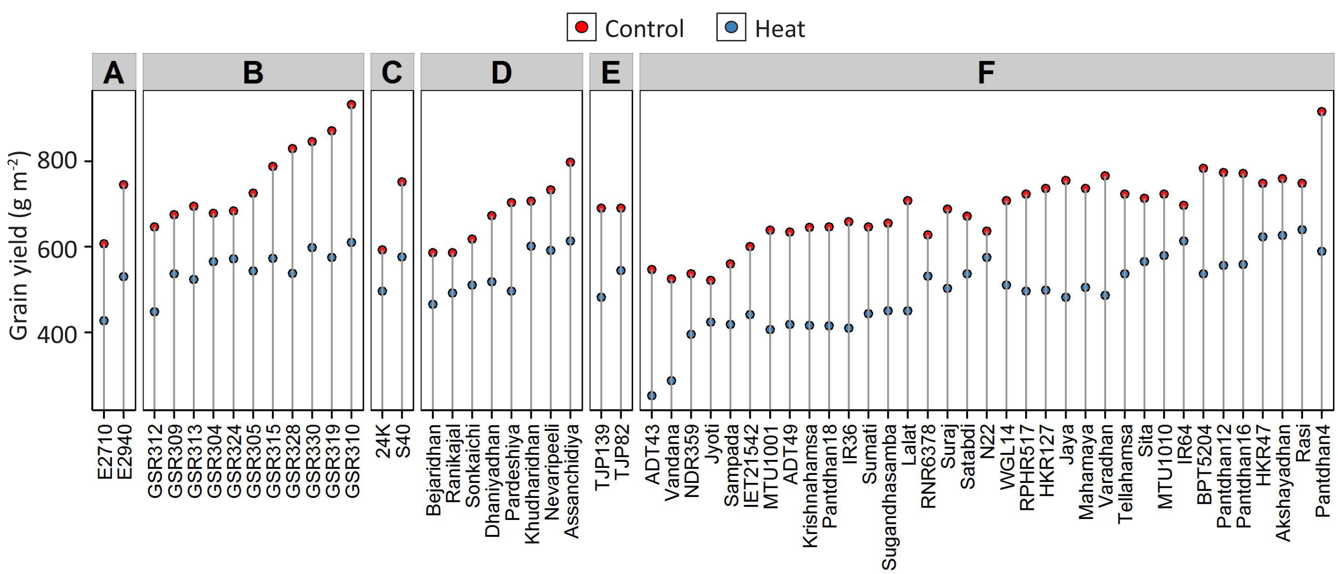

Figure 1b: Effect of high temperature on grain yield (g m^{-2}) of rice genotypes. A: Germplasm lines; B: Green Super Rice; C: Wild introgression; D: Landrace; E: Tropical Japonica; F: Released varieties

the average temperature resulted in 10% reduction in the grain yield. This is mainly due to the negative impact of high temperature on the phenological development processes taking place within. Whereas, Ma et al. (2009) reported that this reduction may be also due to the inhibition in the grain filling process. It was also reported that the susceptible varieties are more severely affected by heat stress when compared to the tolerant varieties. Spikelet sterility under high temperature results due to combined effect of poor dehiscence of anthers as well as less pollen production (Prasad et al., 2006). Reduction in grain yield and spikelet fertility in response to elevated temperature stress in rice was also reported by Sailaja et al. (2015) and Poli et al. (2013). A 3 ^{o}C increase in critical temperature caused a difference of 50% in spikelet sterility between the tolerant genotype Akitakomachi and the susceptible genotype Hinohikari (Matsui et al., 2001). The reduction of spikelet fertility and yield under high temperature conditions are in agreement with the observations in the present study.

3.5. Correlation with grain yield

The relationship between grain yield under heat and other analysed traits under high temperature conditions such as CMS and F_v/F_m along with the % reduction of chlorophyll content in paraquat tested leaves was worked out. The results showed that the relationship between grain yield recorded under high temperature and CMS and F_v/F_m under high temperature was positive and significant (Table 4). Similarly, the relationship between reduction of chlorophyll content after paraquat treatment with grain yield under high temperature stress was also significant but negative. This correlation reveals the reliability on the screening method and they can be used for preliminary screening parameter for high temperature tolerance.

Table 4: Correlation between measured physiological traits and grain yield recorded under high temperature conditions

Param-eters	CMS_ht	Fv/Fm ht	% red	SF_ht	Yld_ht
CMS_ht	1				
Fv/Fm _ht	0.636	1			
% red	-0.717	-0.364	1		
SF_ht	0.746	0.465	-0.716	1	
Yld_ht	0.651***	0.565***	-0.359**	0.719***	1

CMS: Cell membrane thermostability; % red: % reduction in chlorophyll content after paraquat testing; SF: Spikelet fertility (%), Yld_ht: Grain yield under high temperature

Correlation between reproductive stage tolerance measured by spikelet fertility and heat tolerance measured by CMS in 14 rice genotypes was conducted by Prasad et al. (2006). Similarly, in this experiment too the relationship between CMS and grain yield under high temperature was worked out and a positive significant association was noted. However, there may be some other factors besides CMS that may contribute for better yield under high temperature conditions, hence it cannot be exclusively used as screening parameter for high temperature tolerance.

4. Conclusion

Among the genotypes tested, Rasi, Akshayadhan, HKR47, IR64, Assanchidiya had a higher grain yield under high temperature conditions and could be categorized as heat tolerant whereas ADT43, Vandana, NDR359 MTU1001 and IR36 had lower yields and hence categorized as heat susceptible. Among the analysed traits, it can be concluded that CMS and F_v/F_m can be used efficiently to screen the germplasm for thermotolerance.

5. Acknowledgement

DBT-INSPIRE fellowship to the first author was duly acknowledged. The authors are grateful to the Director ICAR-Indian Institute of Rice Research (formely Directorate of Rice Research) for providing research facilities.

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