HEAT SHOCK INDUCED MORPHO-PHYSIOLOGICAL RESPONSE IN INDICA RICE (*ORYZA SATIVA* L.) AT EARLY SEEDLING STAGE

SYED ADEEL ZAFAR[1,3*], AMJAD HAMEED[2*], ABDUS SALAM KHAN[1]AND MUHAMMAD ASHRAF[2]

[1]*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*
[2]*Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Faisalabad, Pakistan*
[3]*Present address: Institute of crop science, Chinese Academy of Agricultural sciences, Beijing 100081, China*
Corresponding author's email: amjad46pk@yahoo.com, adeelzafarpbg@gmail.com

Abstract

Heat stress is a serious threat for rice production in various localities around the world. Present research was conducted to explore the mechanism of heat tolerance in rice at early seedling stage. Forty six rice genotypes including 39 mutants (M5generation) of super basmati and 7 varieties were subjected to heat stress (45±2°C) for 12 h followed by three days recovery under normal temperature (28±2°C). The relative heat tolerance of genotypes was assessed on the basis of various morphological (fresh and dry leaf weight, fresh and dry weight of seedlings) and physiological (relative water contents (RWC), cell membrane thermo-stability (CMTS), photosynthetic pigments and malondialdehyde (MDA)) parameters. Significant variation was observed among the tested rice genotypes for morpho-physiological response. Based on relatively higher CMTS, RWC and photosynthetic pigments along with higher fresh and dry weights of leaves and seedlings, and less MDA content, fifteen genotypes including 11 mutants and four varieties were identified as heat tolerant. HTT-1 was identified as moderately heat sensitive mutant. In conclusion, tested morpho-physiological markers were useful to screen rice germplasm for heat tolerance at early growth stage. In addition, Kashmir basmati and HTT-114 can be used as heat tolerant check and HTT-1 can be used as heat sensitive check in screening experiments at early growth stage.

Key words: Basmati, CMTS, Heat shock, RWC, Mutants.

Introduction

Rice is the second most important crop among cereals after wheat and covers around one tenth of the arable land in the world. It is the primary source of food for 3 billion humans and a major source of calories (35-75%) intake for them (Krishnan *et al.*, 2011). Since rice is the staple food for over half of the world population, an increase of 0.6-0.9% in rice production until 2050 is unavoidable due to increasing population rate (Carriger & Vallee, 2007).

Heat stress has become a serious problem of agriculture in different localities around the world (Ahmad *et al.*, 2016; Zafar *et al.*, 2016). It is an imperative environmental stress that restricts the plant growth, disturb its metabolic activities and production worldwide (Bakhtavar *et al.*, 2015; Hasanuzzaman *et al.*, 2013). Episodes of heat stress which have occurred in the past were predicted to occur more frequently by the end of this century (Semenov & Halford, 2009). The increase in seasonal average temperature by 1°C reduces the yield by 4.1 to 10% in cereals (Wang *et al.*, 2012).High temperature affects various growth related morpho-physiological parameters in plants (Cao *et al.*, 2008; Hameed *et al.*, 2012).

Photosynthesis is an important growth determining phenomenon in crop plants. Several studies showed the inhibitory effects of heat stress on rate of photosynthesis which can be observed by measuring the leaf photosynthetic pigments (Larcher, 2003).Photosynthetic sensitivity to high temperature can be due to destruction to constituents of photosystem II present in the thylakoid membranes of chloroplast (Al-Khatib & Paulsen, 1999).Cell membrane stability and water relations are important physiological processes in plants which are affected by temperature stress (Waqas *et al.*, 2017; Wahid *et al.*, 2007). Heat stress leads to the membrane lipid peroxidation in rice which can be estimated in terms of MDA (Zhou *et al.*, 2011).The plants which show low MDA content can be considered as relatively heat tolerant (Cao *et al.*, 2008). Stress tolerance index (STI) was defined as a very useful tool for determining the yield and stress tolerance potential of genotypes (Fernandez, 1992).A thorough study of physiological responses to high temperature is essential to understand the mechanism of heat tolerance in plants (Wahid *et al.*, 2007). Great variation exists for heat tolerance among the rice genotypes which need to be explored using various strategies (Shah *et al.*, 2011).

So far most of the research on heat tolerance in rice focused on reproductive stage of development. However, the response of rice towards heat stress differ according to the developmental stage. The primary objective of the present research was to study the morpho-physiological responses of Indica rice towards heat stress at early growth stage. The specific objectives were 1) to identify induced rice mutants of Super Basmati with improved heat tolerance based on morpho-physiological response at seedling stage; and 2) to identify efficient and reliable seedling based morpho-physiological markers for quick screening of rice for heat tolerance.

Materials and Methods

Experiment was conducted using 46 rice genotypes including 39 mutants (M5) of super basmati developed by gamma irradiation (using doses of 20-30 Grey) at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan (Table 1).

Raising plant material and growth condition: The seeds were sown in plastic pots filled with equal quantity of autoclaved soil under control conditions in triplicate (15 seedlings per replication) in a growth chamber at normal temperature (28±2°C) in two sets. Both sets were placed in dark till emergence of seedlings (3-4 days). After emergence, 12 h photoperiod (irradiance of 120 μmol m^{-2} s$^-$

¹) was maintained. One set of 10 days old seedlings was subjected to heat stress (45±2°C) for 12 h in another growth chamber running at 45±2°C while other set was kept at normal temperature that served as control. After high temperature exposure, the seedlings were allowed to recover for three days by placing under normal temperature (28±2°C). The relative heat tolerance of genotypes was assessed based on various morphological and physiological parameters.

Table 1. Different rice genotypes used in present study.

Sr. no.	Mutant	Sr. no.	Mutant/ variety
1.	HTT-1	24.	HTT-108
2.	HTT-5	25.	HTT-110
3.	HTT-18	26.	HTT-112
4.	HTT-19	27.	HTT-114
5.	HTT-25	28.	HTT-116
6.	HTT-29	29.	HTT-117
7.	HTT-31	30.	HTT-118
8.	HTT-39	31.	HTT-119
9.	HTT-51	32.	HTT-120
10.	HTT-53	33.	HTT-121
11.	HTT-59	34.	HTT-125
12.	HTT-74	35.	HTT-132
13.	HTT-81	36.	HTT-138
14.	HTT-92	37.	HTT-139
15.	HTT-97	38.	HTT-140
16.	HTT-98	39.	HTT-156
17.	HTT-101	40.	Super Basmati
18.	HTT-102	41.	IR-64
19.	HTT-103	42.	Bas-370
20.	HTT-104	43.	Bas-385
21.	HTT-105	44.	Bas-2000
22.	HTT-106	45.	Bas-515
23.	HTT-107	46.	Kashmir Bas

Measurement of different parameters: Morphological parameters (fresh and dry weight of seedlings, fresh, turgid and dry weight of leaves) were measured after harvesting of 10 days old seedlingsusing electrical balance. Seedling and leaf fresh weights were recorded immediately after harvesting to avoid evaporation. For dry weight estimations, pre-weighted plants and leaves were kept at 90°C in brown paper till complete drying. Seedling and leaf dry weights were measured after complete drying when there was no further reduction in weight. Turgid weight of leaves was measuredafter 24 h soaking of leaves in water. The CMTS was calculated using the method of Martineau *et al.* (1979). Details of methodologies for other physiological parameters are given below.

Relative water contents: The relative water contents (RWC) were measured by using formula as described by Yamasaki & Dillenburg (1999).

$$RWC = \frac{LFW - LDW}{LTW - LDW} \times 100$$

where LFW, LDW and LTW represent fresh, dry and turgid weight of leaf, respectively.

Malondialdehyde (MDA) content: The level of lipid peroxidation in the leaf tissue was measured in terms of MDA content using method of Heath & Packer (1968) with minor modifications as described by Dhindsa *et al.* (1981).

Pigments analysis: The concentration of chlorophyll a and b was determined following the method of Arnon (1949) whereas carotenoids were determined following the method of Davies (1976).

Stress tolerance index (STI): The STI for each parameter was calculated using the following formula as described by Fernandez, G.C. (1992).

STI = (Value under stress/value under control) x 100

Heat tolerance index (HTI) percentage: The HTI was calculated using the individual scores of parameters and used for grouping the genotypes according to their relative heat tolerance. HTI (%) was calculated by dividing the sum of individual scores for each parameter by sum of highest score for all parameters and multiplied by 100.

Experimental design and statistical analysis: Forty six rice genotypes were grown in three repeats (15 seeds per replication) using completely randomized design (CRD) under controlled conditions at 28±2°C. Descriptive statistics was applied to analyze and organize the resulting data. Correlation test was performed for all parameters using XL-STAT 2014 software (version 2.06). Significance of data was tested by analysis of variance. Values presented in the figures are mean ± SE.

Results

Response of morphological parameters under heat stress: Significant variation was observed among the rice genotypes in recorded morphological parameters under heat stress (Fig. 1). Leaf fresh weight (LFW) was generally reduced under heat stress in tested genotypes except HTT-101, HTT-110, HTT-112, HTT-117, IR-64 and Kashmir Basmati (Fig. 1a). IR-64 had the highest LFW (24.46 mg), while lowest (2.46 mg) was observed in Basmati-370 under heat stress. The highest percent decrease (86.3%) in LFW was also observed in Basmati-370 under high temperature stress. Highest LFW STI (126%) was recorded in HTT-101, while it was lowest (13.70%) in Basmati-370 (Fig. 1a). Leaf dry weight (LDW) was also reduced under heat stress in most of the tested rice genotypes (Fig. 1b). Highest LDW (4.26 mg) was observed in the genotype HTT-120, while it was lowest (0.43 mg) in the genotype HTT-121 under heat stress. The highest LDW STI (164%) was observed in genotype HTT-98 while it was least (40.21%) in varietyBasmati-370 (Fig. 1b). The highest percent increase (64%) in LDW under heat stress was also observed in the genotype HTT-98. On the other hand Basmati-370 had highest percent decrease (59.8%) in LDW under high temperature which denoted a severe heat stress induced growth inhibition in this genotype.

Seedling fresh weight (SFW) was decreased under heat stress in all the tested genotypes and level of decrease varied among the genotypes (Fig. 1c). Highest heat stress induced decrease (35.29%) in SFW was observed in HTT-39, while the lowest decrease (0.98%) was observed in HTT-118. Highest SFW STI (99%) was observed in genotype HTT-118, while lowest SFW STI (64.70%) was observed in HTT-39. In general, seedling dry weight (SDW) was reduced under heat stress (Fig. 1d). HTT-108 had the highest SDW (14 mg), while lowest was observed in genotype HTT-97 (6.03 mg) under heat stress. Highest SDW STI (156.91%) was observed in genotype Kashmir Basmati while lowest (44.30%) was observed in genotype HTT-125.
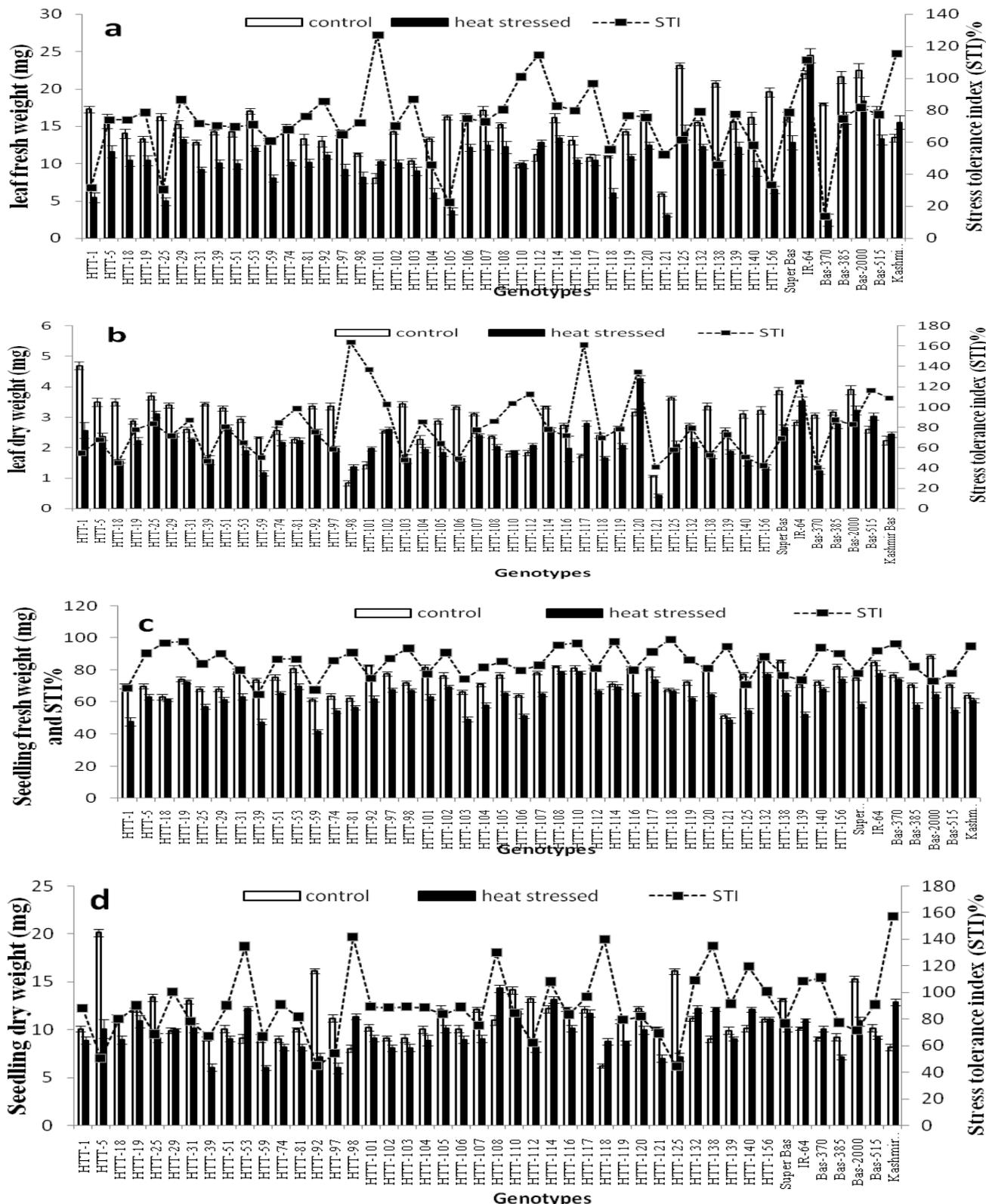

Fig. 1. Effect of heat stress on LFW (a), LDW (b), SFW (c) and SDW (d) in different rice genotypes.

Response of physiological parameters under heat stress: Considerable variation in response of genotypes for the recorded physiological parameters was observed under heat stress (Figs. 2 and 3).Highest CMTS (66.27) was recorded in genotype HTT-19 indicating least heat stress induced injury (33%) in this genotype. While lowest CMTS (14.68) was recorded in HTT-125 indicating highest heat stress induced injury (85%) in this genotype. RWC was found to be the most affected parameter under high temperature. Leaf RWC decreased under heat stress in all genotypes excluding Kashmir Basmati (Fig. 3a). HTT-25 had the lowest RWC (14.32%) under heat stress, while it was highest (77.46%) in HTT-97. Lowest RWC STI (15.85%) was also observed in genotype HTT-25, indicated highest heat stress induced reduction in leaf RWC. Highest RWC STI (100%) was observed in Kashmir Basmati indicating tolerance of this genotype by maintaining water contents under heat stress. MDA content showed considerable variation under heat stress in the tested genotypes (Fig. 3b). The increase in MDA under heat stress as compared to control condition indicated higher membrane lipid peroxidation due to heat stress. The lowest MDA content (31.74 µM/g f. wt.) under heat stress was observed in Basmati-385 which indicated least membrane lipid peroxidation in this genotype. However, HTT-1 displayed highest MDA content under heat stress.

content due to heat stress. Carotenoid content also exhibited a varied response under heat stress among the tested genotypes (Fig. 4d). The highest carotenoid content (57.49 mg/g f. wt.) under heat stress was observed in HTT-139, while lowest carotenoid content (35.57 mg/g f. wt.) under heat stress was observed in HTT-81. The highest carotenoid STI (137.64%) was observed in HTT-19, while lowest carotenoid STI (69.51%) was observed in IR-64.Total chlorophyll content showed a moderate variation under heat stress in tested genotypes (Fig. 4e).It decreased under heat stress in all Basmati varieties except Kashmir Basmati. HTT-102 had the highest total chlorophyll STI (152.38%), while Basmati-385 had the lowest STI (46.89%) for total chlorophyll content.

Correlation test: Correlation analysis depicted significant positive correlation among LFW under heat stress, LTW under heat stress, LDW under heat stress and RWC under heat stress (Table 2). Chlorophyll a content showed significant positive correlation with SFW, RWC and carotenoid under heat stress. MDA under heat stress had significant negative correlation with CMTS, LFW, LDW, chlorophyll a, b and carotenoids under heat stress.

Scale of heat tolerance: On the basis of 19 morpho-physiological parameters (markers) which were significantly correlated, a scale of heat tolerance was developed and the genotypes were grouped in four classes from A to D, being A as heat tolerant, B as moderately heat tolerant, C as moderately heat sensitive and D as sensitive (Table 3). Moreover, a score from 3 to 10 was assigned to each class and value range for each parameter was also set for each class. The tolerant genotypes were given the score 10, moderately tolerant 7, moderately sensitive 5 and sensitive as 3 (Table 3).

Response of photosynthetic pigments under heat stress: The difference in photosynthetic rate can be assessed by measuring the photosynthetic pigments (like lycopene, carotenoids and chlorophyll contents) in plants. Photosynthetic pigments showed variable response under heat stress among the tested rice genotypes (Fig. 4).The highest lycopene value under heat stress was observed in HTT-31 (23.29 mg/g f. wt.) while lowest level of lycopene was observed in Basmati-385 (8.97 mg/g f. wt.). The highest lycopene STI (227.32%) was observed in HTT-102, while lowest lycopene STI (48.49%) was observed in Basmati-385 (Fig. 4a).The level of chlorophyll a generally decreased under heat stress in most of the tested genotypes (Fig. 4b). The lowest chlorophyll a STI (72.37%) was observed in HTT-102 that indicated highest heat stress induced percent decrease in chlorophyll a. HTT-19 showed highest chlorophyll a STI (128.97%) which displayed highest percent increase in chlorophyll a under heat stress. Considerable variation was observed in Chlorophyll b content among the genotypes under control and heat stress (Fig. 4c). The genotype HTT-102 had the highest STI (494%) and percent increase in chlorophyll b content under heat stress as compared to control condition. The lowest chlorophyll b content under heat stress was observed in Bassmati-385 (179.46 µg/g f.wt.). The lowest Chlorophyll b STI (22.73%) was also observed in this genotype that indicated highest percent decrease in chlorophyll b

Heat tolerance index percentage: For classification of genotypes based on relative heat tolerance, a heat tolerance index percentage (HTI%) was calculated by dividing the sum of individual scores for each parameter by total score and multiplied by 100 (Fig. 5). According to HTI, genotypes were classified in three groups on the basis of their response to heat stress. Fifteen genotypes were ranked as heat tolerant with HTI ranging from 73.16% to 83.16%. Kashmir Basmati with highest HTI value (83.16%) was proved to be most heat tolerant genotype. Out of total 190, Kashmir Basmati got the highest score of 158. Kashmir Basmati maintained highest LFW STI, leaf RWC under heat stress, RWC STI, SDW STI, chlorophyll b and total chlorophyll content under heat stress. In case of mutants, HTT-114 was found to be the highest heat tolerant mutant with HTI value 81.58%. Thirty genotypes were classified as moderately heat tolerant with HTI ranging from 70% in HTT-119 to 53.68% in HTT-121 (Fig. 5). HTT-1 was classified as moderately heat sensitive with HTI value 45.26%. HTT-1 has the lowest HTI among all the tested genotypes. This genotype was sensitive for most of the parameters including LFW under heat stress, LFW STI, LDW STI, RWC under heat stress, RWC STI, SFW under heat stress, SFW STI, CMTS, MDA under heat stress and chlorophyll a under control.

Super Basmati (the parent of mutants used in this study) has the intermediate performance and ranked as moderately heat tolerant with HTI value 66.84%. Twenty mutants were superior than super Basmati under heat stress ranging from HTT-116 (66.84%) to HTT-114 (81.58%) while 19 mutants were inferior ranging from HTT-5 (66.32%) to HTT-1 (45.26%).
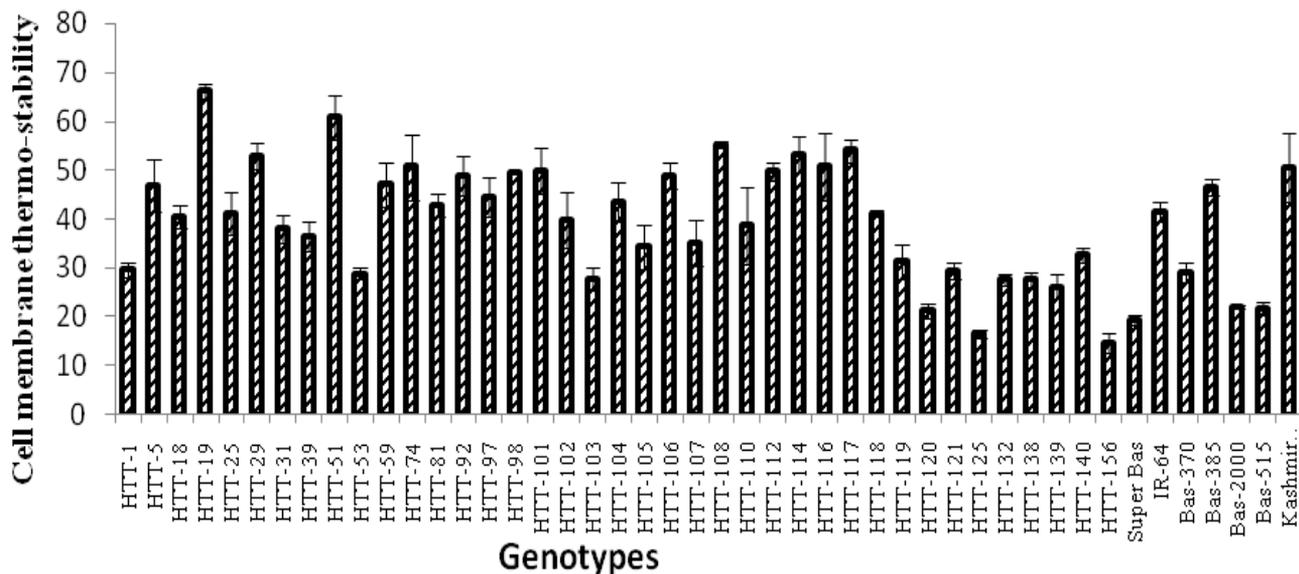
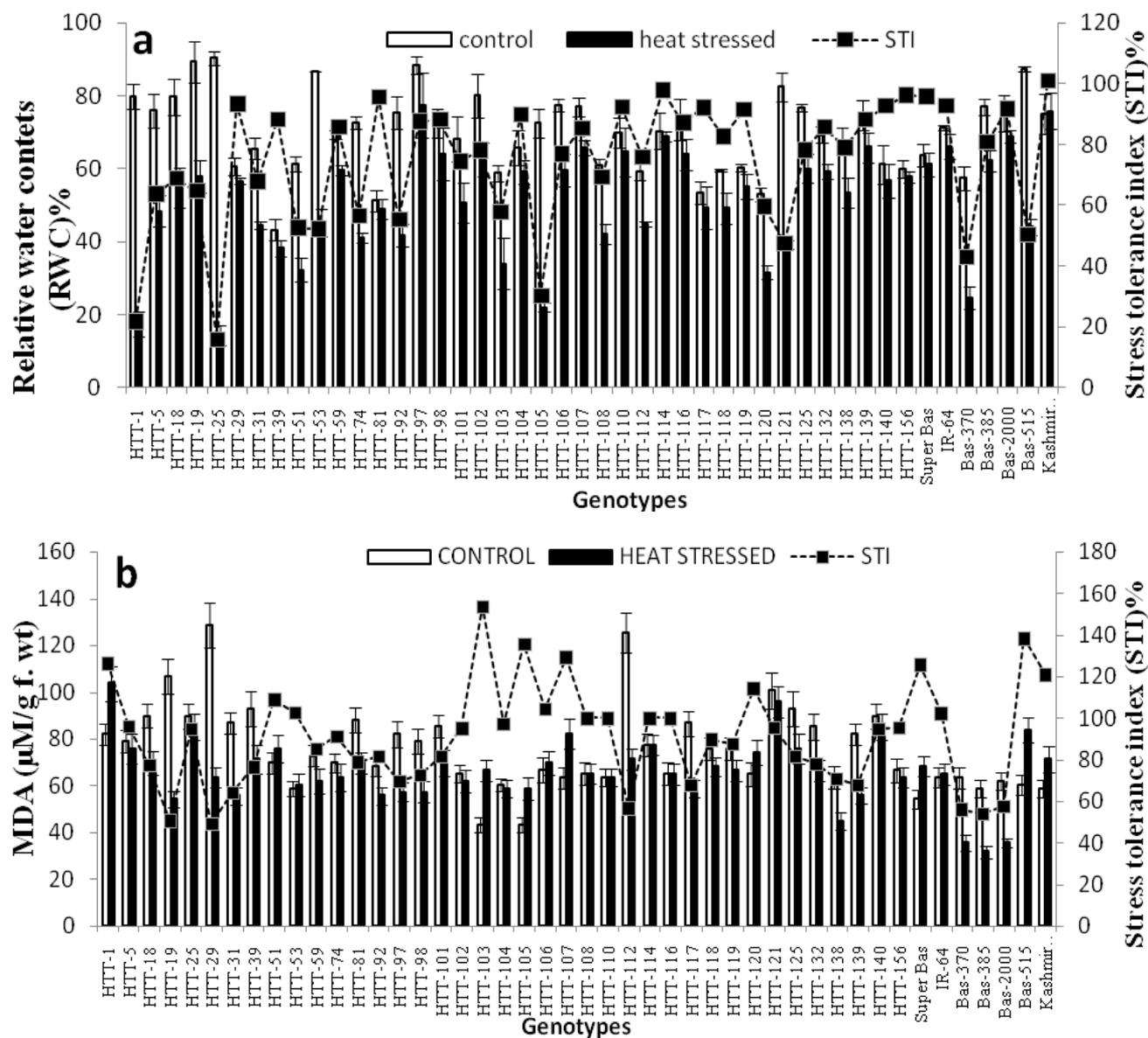Fig. 2. Comparison of cell membrane thermo-stability among rice genotypes.

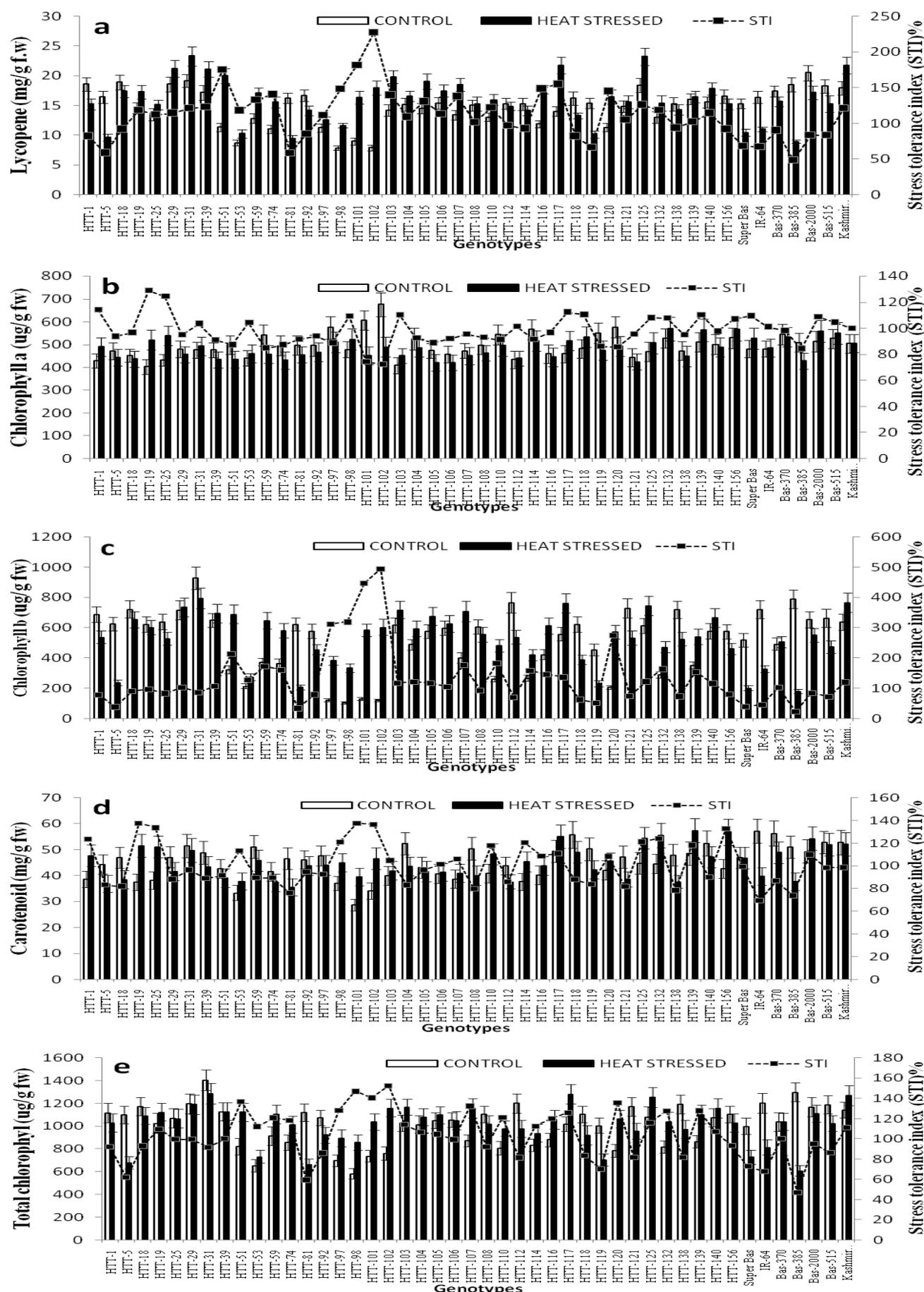Fig. 3. Effect of heat stress on RWC (a) and MDA (b) in different rice genotypes.

Fig. 4. Effect of heat stress on Lycopene (a), chlorophyll a (b), chlorophyll b (c), carotenoid (d) and total chlorophyll content (e in different rice genotypes.

Table 2. Correlations(pearson) among different morpho-physiological parameters.

	LFW C	LFW H	S.T.I. LFW	LTW C	LTW H	S.T.I. LTW	LDW H	S.T.I. LDW	RWC heat	S.T.I. RWC	SFW H	S.T.I. SFW	CMTS	MDA H	chl a C	chl a H	S.T.I chl b	car C	car H
LFW C	1																		
LFW H	0.445	1																	
S.T.I. LFW	**-0.287**	0.697	1																
LTW C	0.841	0.344	**-0.256**	1															
LTW H	0.350	0.789	0.564	0.339	1														
S.T.I. LTW	-0.352	0.410	0.735	-0.457	0.639	1													
LDW H	0.408	0.586	0.303	0.328	0.677	0.294	1												
S.T.I. LDW	**-0.208**	0.350	0.567	**-0.151**	0.320	0.375	0.517	1											
RWC heat	**0.107**	0.542	0.473	-0.003	-0.031	-0.085	-0.029	0.142	1										
S.T.I. RWC	**0.021**	0.475	0.487	0.193	0.011	-0.179	-0.045	0.190	0.856	1									
SFW H	**0.098**	0.184	**0.163**	0.146	0.018	-0.081	0.186	0.333	0.212	0.215	1								
S.T.I. SFW	**-0.229**	-0.083	**0.040**	-0.198	-0.272	-0.128	-0.118	0.168	0.163	0.132	0.653	1							
CMTS	-0.406	0.062	0.365	-0.407	0.036	0.296	0.011	0.297	0.106	0.074	0.154	0.346	1						
MDA H	**-0.258**	-0.167	**-0.019**	-0.283	0.064	0.244	0.011	-0.065	-0.287	-0.268	-0.306	-0.013	-0.078	1					
chl a C	**0.017**	**0.059**	**0.096**	0.089	-0.132	-0.177	0.159	0.242	0.311	0.311	0.225	0.086	-0.081	-0.191	1				
chl a H	**0.224**	**0.045**	**-0.109**	0.166	-0.152	-0.273	0.219	0.179	0.214	0.176	0.315	0.117	-0.371	-0.119	0.231	1			
S.T.I chl b	**-0.238**	-0.084	**0.141**	-0.275	-0.156	0.072	0.053	0.330	0.177	0.065	0.157	0.052	0.121	-0.022	0.673	0.036	1		
car C	**0.214**	0.146	-0.106	0.363	0.016	-0.332	-0.020	-0.134	0.106	0.249	-0.020	0.065	-0.137	-0.240	-0.025	0.163	-0.510	1	
car H	**0.164**	-0.031	**-0.125**	0.116	-0.194	-0.265	0.123	0.068	0.157	0.132	0.132	-0.068	-0.348	-0.059	0.125	0.862	0.040	0.149	1

Note: values in bold are different from zero with a significant level alpha=0.05.

Where; C: control; H: Heat stressed; LFW: leaf fresh weight; LTW: leaf turgid weight; LDW: leaf dry weight; S.T.I.: Stress tolerance index; SFW: seedling fresh weight; SDW: seedling dry weight; chl a: Chlorophyll a; chl b: Chlorophyll b; car: carotenoid; MDA: malondialdehyde; RWC: Relative water content; CMTS: Cell membrane thermo-stability
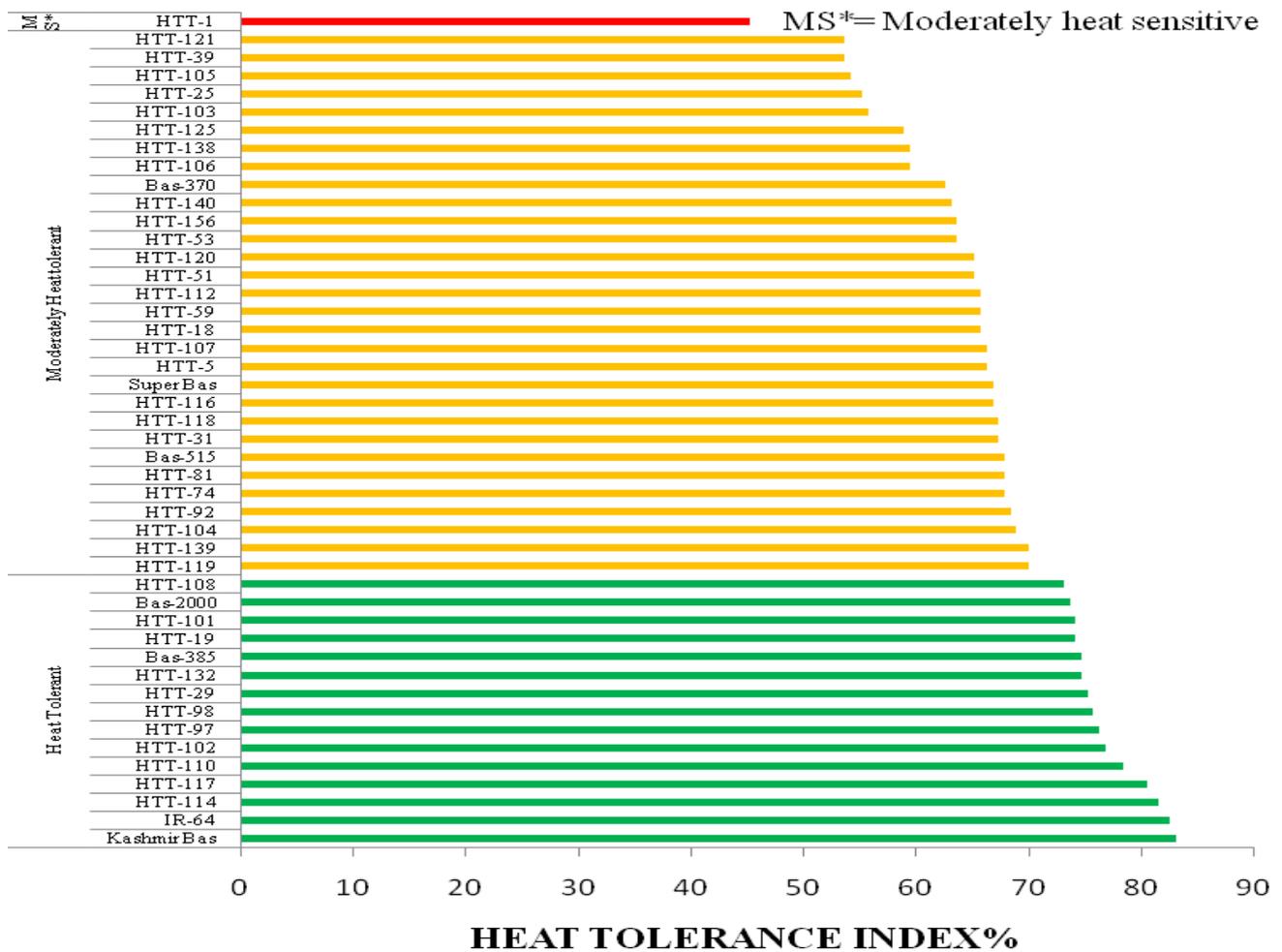
Fig. 5. Ranking of rice genotypes based on HTI.

Table 3. Scale of heat tolerance.

Sr. no.	Traits	Tolerant	Moderately tolerant	Moderately sensitive	Sensitive
	Class	A	B	C	D
	Score	10	7	5	3
1.	LFW C	≥18	14-17.9	10-13.9	<10
2.	LFW H	≥15	11-14.9	8-10.9	<8
3.	S.T.I. LFW	≥80	70-79.9	50-69.9	<50
4.	LTW C	<16	16-19.9	20-24.9	>25
5.	LTW H	<14	14-18.9	19-24.9	>25
6.	S.T.I. LTW	≥90	81-89.9	65-80.9	<65
7.	LDW H	≥2.6	2-2.59	1.6-1.99	<1.6
8.	S.T.I. LDW	≥90	75-89.9	65-74.9	<65
9.	RWC. H	≥50	41-49.9	35-40.9	<35
10.	S.T.I. RWC	≥80	61-79.9	50-60.9	<50
11.	SFW H	≥68	60-67.9	52-59.9	<52
12.	S.T.I. SFW	≥90	84-89.9	70-83.9	<70
13.	CMTS	≥50	40-49.9	30-39.9	<30
14.	MDA H	≤60.9	61-70.9	71-80.9	≥81
15.	chl a C	≥501	500.9-479	478.9-459	≤458
16.	chl a H	≥501	500-475	474-446	≤445
17.	S.T.I chl b	≥150	149-100	99-60	≤59
18.	car C	≥50	49.9-43	42.9-38	≤37.9
19.	car H	≤40	40.9-46.9	47-53	≥54
	Heat tolerance index (%)	**100-71**	**70-51**	**50-31**	**≤30**

where C: Control; H: Heat stressed; LFW: leaf fresh weight; LTW: leaf turgid weight; LDW: leaf dry weight; S.T.I.: Stress tolerance index; SFW: seedling fresh weight; SDW: seedling dry weight; chl a: Chlorophyll a; chl b: Chlorophyll b; car: carotenoid; MDA: Malondialdehyde; RWC: Relative water content; CMTS: Cell membrane thermo-stability

Discussion

Assessment of crop germplasm under natural field conditions is difficult due to sudden fluctuations in temperature and humidity which may influence the reliability of findings. Alternatively, screening for heat tolerance can be performed under controlled environmental conditions on the basis of various morpho-physiological parameters i.e. fresh and dry weight of leaves/seedlings, RWC (Hameed *et al.*, 2012), CMTS (Malik & Saleem, 2004; Ren & Tao, 2016), MDA (Lee *et al.*, 2007; Hameed *et al.*, 2012) and photosynthetic pigments (Cao *et al.*, 2003; Marchand *et al.*, 2005; Scafaro *et al.*, 2010). Heat stress may significantly affect rate of photosynthesis, membrane stability and water relations (Wahid *et al.*, 2007). Reduced leaf fresh and dry weights under heat and cold stress have been reported in rye grass and were proposed as indicators of heat and cold tolerance (Inoue *et al.*, 2004). In our study, LFW was generally reduced under heat stress in tested genotypes. The reason for this reduced LFW seems to be the fact that the leaf was directly exposed and most affected plant part under high temperature stress and high temperature may have increased the transpiration rate from leaves (Ahmad *et al.*, 2016). LFW had significant positive correlation with RWC under heat stress (Table 2).

LDW was positively correlated with LFW and CMTS (Table 2). The highest percent decrease in LDW was observed in Basmati-370 which was proved to be moderately heat tolerant genotype (Fig. 1b).

In present study, SFW was reduced under heat stress in all genotypes with highest decrease observed in HTT-39. Heat stress also reduces SFW in wheat and considerable variation was observed among the genotypes for percent decrease in SFW (Hameed *et al.*, 2012). Recently, reduction in SFW and SDW were also reported in rice under salt stress and genotypes which maintain high SFW and SDW were considered as salt tolerant (Zafar *et al.*, 2015). Since SFW has significant positive correlation with chlorophyll a under heat stress, the reason for this decrease in SFW may be the reduced photosynthesis under heat stress due to the destruction of photosynthetic pigments (Al-Khatib & Paulsen, 1999). Thus SFW may be utilized as an important selection parameter in the screening of rice germplasm against heat tolerance. RWC were also decreased under high temperature in all genotypes except Kashmir basmati. Reduction in RWC heat stress was also reported in other cereal crops like wheat (Hameed *et al.*, 2012) and barley (Machado & Paulsen, 2001). RWC has positive association with photosynthetic rate (Siddique *et al.*, 2000). CMTS can be used as a good indicator of heat tolerance in plants (Ismail & Hall, 1999; Malik & Saleem, 2004). In the present study, genotypes vary in their relative CMTS. Genotypes with high CMTS can be categorized as better heat tolerant than those with less CMTS (Malik & Saleem, 2004). The genotypes with high CMTS in present study were also proved as heat

tolerant on the basis of heat tolerance index. Therefore, CMTS can be utilized as a reliable physiological marker for heat tolerance in rice at seedling stage. In the present study we also estimated the stress tolerance index (STI) of each trait by using the values of heat stressed and control plants. The STI showed a relative tolerance of a genotype for that trait under heat stress. High STI shows better tolerance of a trait under heat stress and has positive correlation with heat tolerance.

Heat stress causes the lipid peroxidation of cell membrane which is estimated in terms of MDA. The increased MDA content showed high membrane deterioration due to heat stress (Hameed *et al.*, 2012). Increased lipid peroxidation showed that oxidative stress was frequently produced in rice leaves after high temperature exposure (Lee *et al.*, 2007). In our study, there was a considerable variation in MDA among the genotypes. The MDA increased under heat stress in several genotypes, however it remain stable in some genotypes. The highest MDA content was observed in HTT-1 (Fig. 3b) which was moderately heat sensitive genotype and was observed to be the least heat tolerant among tested mutants (Fig. 5). The MDA has significant negative correlation with SFW, CMTS and RWC which were considered important indicators of heat tolerance.Photosynthesis is a very heat sensitive process and significantly contributes in plant growth and yield.Heat shock reduced the photosynthetic pigments and rate of photosynthesis in plants which held to the reduction in vegetative growth (Marchand *et al.*, 2005). In the present study, photosynthetic pigments (chlorophyll a, b and carotenoids) decreased under heat stress in most of the rice genotypes. Lowest chlorophyll a was found in HTT-105 which was least heat tolerant mutant among moderately heat tolerant genotypes. On the other side, highest chlorophyll a content was observed in HTT-132 which was a heat tolerant genotype. Heat stress also led to the reduction in Chlorophyll b in leaves of several rice genotypes. However, there was a great variation in Chlorophyll b content among the genotypes and few genotypes showed a considerable increase in Chlorophyll b (Fig. 4c). The highest percent increase was found in the heat tolerant genotypes. So it can be inferred that high chlorophyll b content is linked with heat tolerance and can be used as an important physiological marker for heat tolerance in rice. Carotenoids are an integral component of photosynthetic membranes and harvest visible light for photosynthesis (Cunningham Jr & Gantt, 1998; Ke, 2003). Carotenoid also act as antioxidant by quenching singlet oxygen and protect plants from oxidative damage by absorbing excited energy from chlorophyll (Davison *et al.*, 2002; Mittler, 2002). In the present study carotenoid content decreased under heat stress in few genotypes but increased in others (Fig. 4d). Heat stress induced reduction in chlorophyll a, b and carotenoids was also observed in other crops like wheat (Xu *et al.*, 2000; Efeoglu & Terzioglu, 2009), fescue (Cui *et al.*, 2006) and bent grass (Liu & Huang, 2000).

Photosynthetic rate at heading stage has positive correlation with heat tolerance in rice (Cao *et al.*, 2003). *Oryzameridionalis* Ng., a wild relative of cultivated rice, *Oryza sativa* L., was reported to be a heat tolerant rice variety because it showed high photosynthetic rate and leaf elongation (Scafaro *et al.*, 2010). Similarly, Karacadag was also reported as a heat tolerant wheat variety on the basis of less reduction in photosynthetic pigments (Efeoglu & Terzioglu, 2009). Thus it can be suggested that the genotypes with high photosynthetic pigments including chlorophyll a, b and carotenoids under heat stress have better heat tolerance in rice and other crops.

Conclusion

Since IR-64, Kashmir Basmati, HTT-114 and HTT-117 were observed to be highly heat tolerant genotypes and LFW, SFW, RWC, and photosynthetic pigments were also least affected in these genotypes under heat stress, it is logical to infer that these attributes were playing a significant role in heat tolerance mechanism in rice. As limited information is available on the assessment of rice germplasm against heat stress at seedling stage, so this new information may be very useful to devise a breeding strategy for rapid screening of rice germplasm against this menace.

Acknowledgments

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