

## Morpho-physiological characterization of two wheat genotypes with contrasting trait of heat tolerance.

Suman Bakshi<sup>1</sup>, Rachna Agarwal<sup>2</sup> and Sanjay Jambhulkar<sup>1</sup>

<sup>1</sup>Nuclear Agriculture and Biotechnology Division, <sup>2</sup>Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400 085, India.

### Article history

Received: 17 Sep., 2020

Revised : 10 Dec., 2020

Accepted: 19 Dec., 2020

### Citation

Bakshi S, R Agarwal and S Jambhulkar. 2020. Morpho-physiological characterization of two wheat genotypes with contrasting trait of heat tolerance. *Journal of Cereal Research* 12(3): 270-280. <http://doi.org/10.25174/2582-2675/2020/104899>

### \*Corresponding author

Email: [sji@barc.gov.in](mailto:sji@barc.gov.in)

### Abstract

Various biotic and abiotic stresses adversely affect crop yield across the globe. Wheat is a cool-season cereal crop and sensitive to rise in ambient growth temperature. Ninety genotypes were screened under heat stress and non-heat stress locations. Low heat susceptibility index of <0.5 for HD2189, NI5643, Kenphad39, HD2501, NI179, N5439, HS240, HD2428 and HD2687 indicated that they are heat tolerant and high susceptibility index of >1.5 for PBN142, NI9947, HD2320, GW190, HD2135, NI917 and Batavia indicated that they are heat susceptible. Based on grain yield and heat susceptibility index, two genotypes namely HD2189 and Batavia with contrasting response to heat stress were selected for the further studies. Their morphological and physiological characterization was carried out at seedling stage using 22°C as control and 33°C as heat stress. Under heat stress conditions, reduction for seedling growth traits, relative water content (RWC) and membrane thermostability (MTS) was less in HD2189 (25.3%, 10.2%, 13%) compared to Batavia (55.3%, 34.4%, 30%). HD2189 maintained lower canopy temperature with depression values in the range of 3.75 to 8°C compared to Batavia (2.82 to 5.10°C). The conversion of source to sink was better for HD2189 as displayed by translocated dry matter (TDM), translocation efficiency (TE), contribution of plant assimilates (CPA), longer duration of grain filling, grain yield and yield components under high temperature. Trend for electron transport rate of PSII (ETR<sub>II</sub>) and effective quantum yield (Y<sub>II</sub>) for both the varieties at 22°C and 33°C was similar. However, Batavia plants were adjusting through non-regulated heat dissipation indicating damage to the photosynthetic apparatus whereas HD2189 showed higher regulated heat dissipation. Thus, genotype HD2189 showed heat tolerance at seedling and adult plant stages compared to Batavia.

**Keywords:** High temperature stress, membrane thermostability, relative water content, SPAD, grain yield, photosystem II, translocation efficiency

## 1. Introduction

Wheat is a winter season crop and its full potential for growth and yield is achieved at 12-22°C (Farooq *et al.*, 2011). Heat stress can impair all stages of plant growth from germination to reproduction limiting the productivity of wheat crop (Narayanan, 2018). High temperature stress is a predominant yield limiting factor over more than 40% of total wheat area in the world (Hede *et al.*, 1999). The

rising global temperature would adversely affect wheat yield raising concern to future food security (Asseng *et al.*, 2014). India is the second largest producer of wheat in the world with an annual production of 107.18 million tonnes (IIWBR Annual Report, 2019-20). However, changing climatic conditions, particularly shortening of winter and terminal heat experienced by most wheat growing

areas in the country is continuously threatening the wheat production (Rane *et al.*, 2000; Sharma *et al.*, 2002; Kumar *et al.* 2014). High temperature stress induces many biochemical, molecular and physiological changes in the plant at every developmental stage which in turn affects crop yield and quality. However, the pre-flowering and anthesis are relatively more sensitive to high temperature stress compared to post-flowering stages (Cossani and Reynolds, 2012; Yang *et al.*, 2017). High temperature stress leads to structural and functional instability of biomembranes. It weakens the chemical bonds and denatures the membrane proteins (Savchenko *et al.*, 2002). These biochemical alterations increase the membrane permeability leading to a loss of electrolytes under high temperature stress. Since vital biological functions such as respiration and photosynthesis are primarily occurring in membranes present in mitochondria and chloroplast, these physiological processes are affected to a great extent under high temperature stress. Plant water status is an important measure for estimating the effect of high temperatures on physiological activities of wheat plant (Dwivedi *et al.*, 2017). A genotype with an ability to maintain turgid leaves in heat stressed environment will have physiological advantages such as better stomatal conductance and protected photosystem complex. The process of photosynthesis is primarily susceptible to high temperature stress. Several studies have reported reduction in photosynthesis due to disruptions in the structure and function of chloroplasts and decrease in chlorophyll content (Al-Khatib and Paulsen, 1984; Xu *et al.*, 1995; Djanaguiraman, 2018). Leaf chlorophyll content during grain filling stage shows a high genetic correlation with grain yield in heat stressed environments (Reynolds *et al.*, 1998; Zhang *et al.*, 2009; Dhyani *et al.*, 2013). Heat stress causes rupturing of thylakoid membranes and photosystem II (PSII) thus hampers the activities of all membrane associated electron carriers and enzymes resulting in reduced rate of photosynthesis (Marutani *et al.*, 2012). The measurement of photochemical efficiency is an indirect way to assess heat stress tolerance of a genotype. The leaf maximal quantum yield of PSII (Fv/Fm) has been shown to be a reliable indicator of stress (Krause and Weise, 1984) and associated with heat stress tolerance in wheat (Sharkova, 2001; Sayed, 2003). Canopy temperature is measure of plant water status as it reveals an overall integrated physiological response to drought and high temperature (Balota *et al.*, 2007)

and exhibited association with grain yield (Amani *et al.*, 1996; Ayeneh *et al.*, 2002). Sowing date manipulations (Reynolds *et al.*, 1994; Dubey *et al.*, 2019), growing crop at different temperature regime (Reynolds *et al.*, 1994; Lopes and Reynolds *et al.*, 2012) and controlled chamber studies (Stone and Nicolas, 1994, 1995a, 1995b, 1998) are strategies for estimating effects of high temperature stress on grain yield and components. In the present investigation, effects of high temperature stress were studied on different morpho-physiological characters of two selected wheat genotypes i.e. HD2189 and Batavia.

## 2. Materials and methods

### *Field Experiment*

Ninety genotypes (Table1) were grown at two locations with different temperature regimes to evaluate heat stress tolerance. The first location was Trombay for heat stress (longitude: 72°. 9', latitude: 19°. 0' situated at sea level, temperature: 19.1 to 32.4°C) and the second was agricultural research station, Niphad as control (longitude: 74°.6', latitude: 20°.6', altitude: 549m above mean sea level, temperature: 14.5°C to 30°C). Standard agronomic practices were followed. The experiment was sown on 25<sup>th</sup> November, 2015 in one metre square plots with row-to-row distance of 22cm in both locations. Data on grain yield and its related traits was recorded on 5 randomly selected plants. Their mean and standard error were calculated.

### *Heat susceptibility index (HSI)*

Heat susceptibility index (HSI) was calculated based on grain yield under high temperature stress (Trombay) and non-stress environment (Niphad) using the formula suggested by Fisher and Maurer (1978).

$$\text{Heat susceptibility index} = [l - Y_a / Y_b] / [l - \bar{Y}_a / \bar{Y}_b]$$

Where  $\bar{Y}_a$  and  $\bar{Y}_b$  are the mean grain yield for each genotype under heat stress and non heat stress conditions.  $Y_a$  and  $Y_b$  are the mean grain yield of all genotypes under heat stress and non heat stress conditions.

### *Estimation of translocation efficiency and grain filling*

Ten main tillers that headed on the same day were tagged in each plot. The main tiller stem from each of 10 plants were removed at weekly interval till maturity and divided into spike, grain number, grain weight, chaff weight, flag leaf blade, lower leaves and stem. These plant parts were dried at 70°C and weight of each plant part was taken for

**Table 1.** Heat susceptibility index of wheat genotypes

S.No	Genotype	Ya	Yb	HSI
1	HD2189	9.80	9.24	0.229
2	NI5643	11.70	10.64	0.362
3	Kenphad39	8.90	8.00	0.404
4	HD2501	11.4	10.20	0.421
5	NI179	8.70	7.78	0.424
6	N5439	8.59	7.66	0.435
7	HS240	11.04	9.74	0.470
8	HD2428	8.70	7.64	0.487
9	HD2687	10.06	8.84	0.487
10	HD2656	13.71	11.93	0.519
11	Ajantha	12.60	10.90	0.540
12	HD2270	9.18	7.94	0.542
13	HD2327	12.10	10.30	0.595
14	Kenphad25	8.00	6.80	0.600
15	HD2172	12.85	10.85	0.622
16	HD1949	7.40	6.22	0.638
17	HD1981	13.26	11.10	0.652
18	PBW226	9.03	7.56	0.654
19	PBW138	12.80	10.70	0.656
20	Takari	7.51	6.28	0.656
21	MACS2496	12.80	10.70	0.656
22	Vidisha	9.75	8.07	0.692
23	LOK1	14.78	12.20	0.699
24	HD2669	7.87	6.48	0.705
25	HD2668	12.46	10.26	0.706
26	Raj3077	14.18	11.57	0.738
27	HD1925	9.62	7.82	0.748
28	PBW154	12.8	10.40	0.750
29	C306	5.21	4.21	0.765
30	HI1531	13.97	11.20	0.793
31	HW2003	9.75	7.81	0.796
32	WG357	9.30	7.42	0.810
33	HD2888	11.82	9.39	0.824
34	HD2009	9.48	7.50	0.835
35	PBN4135-1	16.64	13.16	0.837
36	HD2281	9.39	7.42	0.839
37	PBW299	10.02	7.86	0.861
38	Kundan	14.5	11.30	0.883
39	Excalibur	10.17	7.90	0.892
40	PBW373	14.2	10.98	0.906
41	HD2285	8.48	6.56	0.908
42	MP3054	6.62	5.07	0.936
43	HW1085	11.70	8.91	0.953
44	Sonalika	11.20	8.51	0.960
45	HD2680	6.98	5.26	0.984
46	HD2402	11.30	8.51	0.986
47	HD2177	11.44	8.60	0.993
48	RAJ3765	10.48	7.81	1.017
49	HW2004	10.20	7.60	1.020
50	NIAW301	12.36	9.20	1.023
51	HD1941	11.00	8.17	1.029
52	HD2643	11.97	8.80	1.060
53	HD2385	8.40	6.12	1.086
54	PBW435	12.70	9.22	1.096
55	DBW16	14.20	10.27	1.108
56	Kanchan	9.96	7.20	1.108
57	HD1982	10.80	7.80	1.111
58	WH542	12.00	8.62	1.127
59	PBN51	9.57	6.84	1.140
60	UP2338	11.10	7.92	1.146
61	NIAW34	11.69	8.32	1.153
62	Lerma Rojo64	9.94	7.07	1.155
63	Niphad4	7.60	5.40	1.158
64	C518	6.24	4.43	1.160
65	LOK45	12.19	8.60	1.179
66	Sonora64	8.92	6.23	1.207
67	HD2329	10.40	7.07	1.282
68	Kharachia65	7.72	5.17	1.319
69	HW2001	11.77	7.87	1.326
70	HD2745	12.20	8.12	1.338
71	NW1012	15.62	10.37	1.345
72	CPAN1922	7.22	4.72	1.384
73	NP846	13.76	8.98	1.390
74	HD2272	11.71	7.64	1.390
75	N8223	12.60	8.20	1.397
76	Vaishali	11.82	7.56	1.442
77	HD2264	6.02	3.84	1.449
78	HD2667	12.80	8.16	1.450
79	KSona	9.15	5.82	1.456
80	HD2651	12.94	8.22	1.459
81	HD2735	8.20	5.20	1.463
82	NI-747-19	12.60	7.96	1.473
83	HI385	11.80	7.40	1.492
84	PBN142	13.00	8.12	1.502
85	NI9947	12.00	7.48	1.506
86	HD2320	11.20	6.80	1.571
87	GW190	12.77	7.69	1.592
88	HD2135	11.80	6.82	1.688
89	NI917	15.07	8.38	1.776
90	Batavia	7.74	3.8	2.037

estimating duration of grain filling from anthesis to grain maturity, translocated dry matter, translocation efficiency and contribution of plant assimilates to grain.

Estimation of translocated dry matter (TDM)

Translocated dry matter = Dry matter produced at anthesis [(leaf+culm)+chaff] - Dry matter produced at maturity [(leaf+culm) +chaff].

Translocation efficiency = (Translocated dry matter/dry matter produced at anthesis) × 100.

Contribution of plant assimilates to grain = (Translocation efficiency / grain yield per spike) × 100.

*Soil-plant analysis development (SPAD) and canopy temperature depression (CTD) measurement*

The chlorophyll content of the leaves was measured using a portable chlorophyll meter (Soil-Plant Analysis Development Section, Minolta Camera Co., Osaka, Japan) in terms of SPAD values. It was measured from the midpoint of the flag leaves of five randomly selected plants in each plot at four developmental stages: tillering, boot, spike emergence and grain filling. Canopy temperature was recorded using hand held infrared thermometer-based equipment (Model TI200) by targeting the canopy leaves at 45° angle. Canopy temperature readings were taken on bright sunny and non windy days between 12.00 and 14.00 hrs at tillering, spike emergence, anthesis and grain filling stage, using the following formula:

Canopy temperature depression

$$= \text{Air temperature (Ta)} - \text{Canopy temperature (Tc)}$$

Temperature profile in *rabi* season

The daily temperature data was collected to understand the heat stress experienced by the crop during the season from weather history maintained on wunderground site for Trombay, Mumbai and Niphad location (<https://www.wunderground.com/>).

*Heat treatment in plant growth chamber and seedling trait evaluation*

Based on lowest HSI in HD2189 and highest in Batavia, these genotypes were selected for physiological characterisation. Heat treatments to wheat seedlings were given in an illuminated plant growth chamber at the temperatures of 33°C (high temperature stress) for 16 hours and 22°C of 8 hours with humidity of 70% for

a period of fifteen days. Control plants were grown at optimum temperature of 22°C and 70% humidity in an illuminated growth chamber. The seeds were kept for germination in plastic cups (filled with sand-granules) in a tray. The moisture in the cups was maintained by keeping constant water level in the tray. Water in the trays was replaced with ¼ MS (Murashige and Skoog) medium after 7 days. Seedlings were harvested on 15<sup>th</sup> day and data on shoot length, leaf length, coleoptile length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight was recorded on ten seedlings of each genotype under stress and non-stress conditions.

*Membrane thermostability determination (MTS)*

Leaf sections weighing 0.5g each in three replicates from the temperature treated and untreated seedlings were placed in 10ml deionized water in glass tubes and kept overnight at room temperature to allow exosmosis. Electrolytes released thereafter were measured with conductivity meter (Hanna Instruments, Singapore) that was calibrated with standardized KCl solution. Subsequently, the tubes containing the leaf segments were autoclaved at 0.10MPa for 10min to kill the tissue and release all salts for total conductivity measurement. Membrane thermostability was then estimated as follows:

Membrane thermostability (%) =  $(1 - T1/T2) \times 100$ , where

T1 = conductivity before autoclaving

T2 = conductivity after autoclaving

*Relative Water Content determination (RWC)*

Leaf RWC was determined using the method of Gulen and Eris (2003). Leaf discs from fully expanded uniform leaves of 15-day old seedlings were taken in duplicate from 3 plants for determining fresh weight (FW), turgid weight (TW) and dry weight (DW). Fresh weight was recorded immediately after making the leaf discs and the samples were placed in 10mL distilled water for 6h for cells to gain maximum turgidity and turgid weight was recorded. The leaf samples were then placed in an incubator at 80°C for 24h for complete drying and the dry weight was measured. Leaf RWC (%) was calculated as follows:

Relative water content (%)

$$= [( \text{Fresh weight} - \text{Turgid weight} ) / ( \text{Turgid weight} - \text{Dry weight} )] \times 100$$

### Photochemical efficiency and PAM fluorimetry

The Dual PAM (Pulse amplitude modulated) fluorimeter was used to measure photochemical efficiency (Fv/Fm). The mid-portion of flag leaf was dark adapted for 30 min. before acquiring the data. The measurements were taken from three replicates and mean values were represented in graphs. Light curve of the dark-adapted leaves were also recorded between 50-850  $\mu\text{E}/\text{m}^2/\text{s}$  to understand changes in electron transport rate through PSII (ETR<sub>II</sub>), quantum yield of PSII (Y<sub>II</sub>) and quantum yield of regulated heat dissipation Y(NPQ) and quantum yield of non-regulated heat dissipation (NO).

Photochemical efficiency = (Maximum fluorescence - Minimum fluorescence) / Maximal fluorescence

### 3. Results and discussion

Wheat crop in India is cultivated in five major zones of which peninsular zone comprise states of Maharashtra and Karnataka. The productivity of the peninsular zone is low due to warmer climate and water scarcity experienced during the crop growth. Hence, one of the objectives for wheat improvement for this zone is development of thermo tolerant high yielding varieties. Fortnightly temperature recorded between November 2015 to March 2016 in Trombay, Mumbai and Niphad is presented in Fig 1. In Mumbai, the average maximum temperature observed was more than 30°C except from 3rd February to 16th February 2016. The average minimum temperature was more than 19°C throughout the crop season except the fortnight of 23rd December to 5th January and 20th January to 2nd February, 2016. This indicated that the crop experienced temperature stress which is not suitable for optimum growth. At Niphad, average maximum temperature of less than 30°C from 25th November to 16th February and the maximum temperature showed increase over 30°C from 17th February to 15th March, 2016 and minimum temperature ranged from 10.9°C to 17.8°C during crop season. Ninety genotypes were grown in Trombay fields (High temperature stress) as well as Niphad (No heat stress) during November 2015-March 2016. Heat susceptibility index (HSI) of ninety genotypes in increasing order has been presented in table 1. Under stress condition, per plant grain yield of HD2189 was 9.24g and Batavia was 5.22g whereas it was 9.80g and 8.60g under non-stressed condition for HD2189 and Batavia respectively. It indicated hardly any difference

for grain yield of HD2189 under stress and non stress conditions. Grain yield of rest of the genotypes was in the range of 5.21g to 16.64g under non-stressed condition and 3.80g to 13.1g under stress condition. Heat susceptibility index showed that genotypes namely, HD2189, NI5643, Kenphad39, HD2501, NI179, N5439, HS240, HD2428 and HD2687 were heat tolerant (HSI<0.5) and genotypes PBN142, NI9947, HD2320, GW190, HD2135, NI917 and Batavia were heat susceptible (HSI>1.5). Heat susceptibility index of HD2189 was 0.229 and Batavia was 2.037 indicating heat tolerant and heat susceptible genotypes, respectively. Therefore, HD2189 as heat tolerant and Batavia as heat susceptible were selected for physiological characterization. Two genotypes i.e. HD2189 and Batavia were raised in plant growth chamber under heat stress (33°C) and normal conditions (22°C) and data on fifteen-day old seedlings was recorded. High temperature has substantial effect on fresh and dry weights of shoots and roots (Fig.2). The long-term seedling heat stress for 15 days (33°C) caused growth inhibition as evident from reduction in weight of fresh shoot and roots and dry shoots and roots by 67.4%, 55.3%, 53.1% and 29% in HD2189 and by 63.5%, 70%, 72.2% and 60.0% respectively for Batavia compared to control (22°C). However, reduction in the weight of fresh roots, dry shoots and dry roots was substantially higher for Batavia in comparison to HD2189 (Fig.2). Relative water content (RWC) and membrane thermostability (MTS) of both the genotypes were measured under heat stress and non-stress conditions (Fig. 3). Reduction of RWC of HD2189 leaves grown at 33°C was only ~ 10% compared to control 22°C whereas it was ~ 34% for Batavia indicating the improved ability water retention and osmotic adjustment of HD2189 under heat stress conditions. The reduction of leaf water content in response to heat has been observed previously in wheat plants (Nayyar and Gupta, 2006; Almeselmani *et al.*, 2012; Sattar *et al.*, 2020). Electrolyte leakage was measured in leaves of HD2189 and Batavia under stress and non stress conditions. HD2189 showed a loss of only ~ 13% in MTS values whereas Batavia had a ~ 30% reduction at 33°C compared to 22°C (Fig.3B). The results clearly indicate that seedlings exposed to higher temperature in growth chamber led to a drastic (12 fold) increase in membrane leakiness compared to control. Electrical conductivity has been used as an index of membrane stability to identify heat tolerant genotypes in

wheat. Several studies reported membrane thermostability (MTS) test as a useful screening procedure for selecting heat tolerant genotypes of wheat (Blum *et al.*, 2001; Ibrahim *et al.*, 2001; Shanahan *et al.*, 1990; Tahir *et al.*, 1993). Correlative studies of MTS index with yield have previously indicated that membrane thermostability is a supplemental method to measure tolerance of genotypes to hot climates (Fokar *et al.*, 1998).

Amount of chlorophyll measured through the SPAD values were higher in HD2189 by ~4 %, ~7.5%, ~8.9% and ~ 9.4% than Batavia at tillering, boot, spike emergence and grain filling stage respectively under stress condition indicated thermo tolerance of HD2189 at different crop stages (Table 2). Chlorophyll degradation due to damage of thylakoids under heat stress has been reported in wheat (Ristic *et al.*, 2007). Studies in wheat have established that amount of chlorophyll showed association with grain yield and grain weight and can serve as tool to select heat tolerant genotypes (Shanmugam *et al.*, 2013; Shirdelmoghanloo *et al.*, 2016). On the other hand, canopy temperature depression (CTD) was highest at tillering

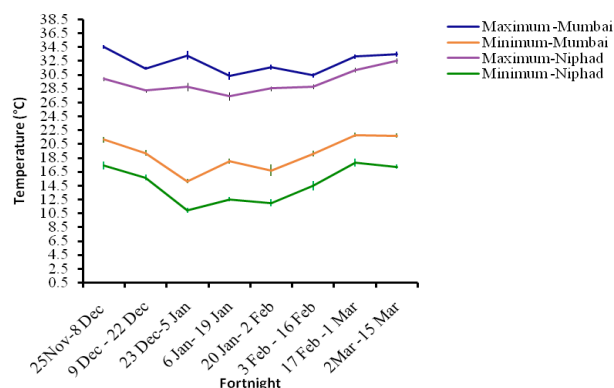


Fig. 1: Average maximum and minimum fortnightly temperature at Trombay, Mumbai and Niphad between 25 Nov. 2015 to 15 Mar. 2016.

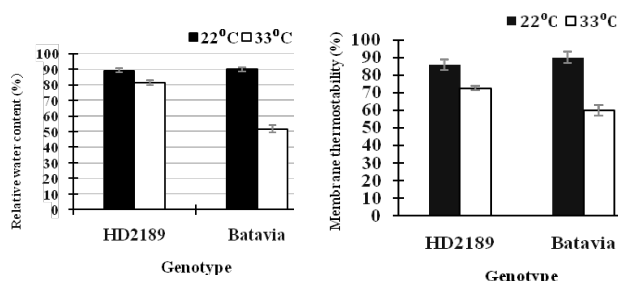


Fig.3: (A) Relative water content (B) Membrane thermostability of HD2189 and Batavia

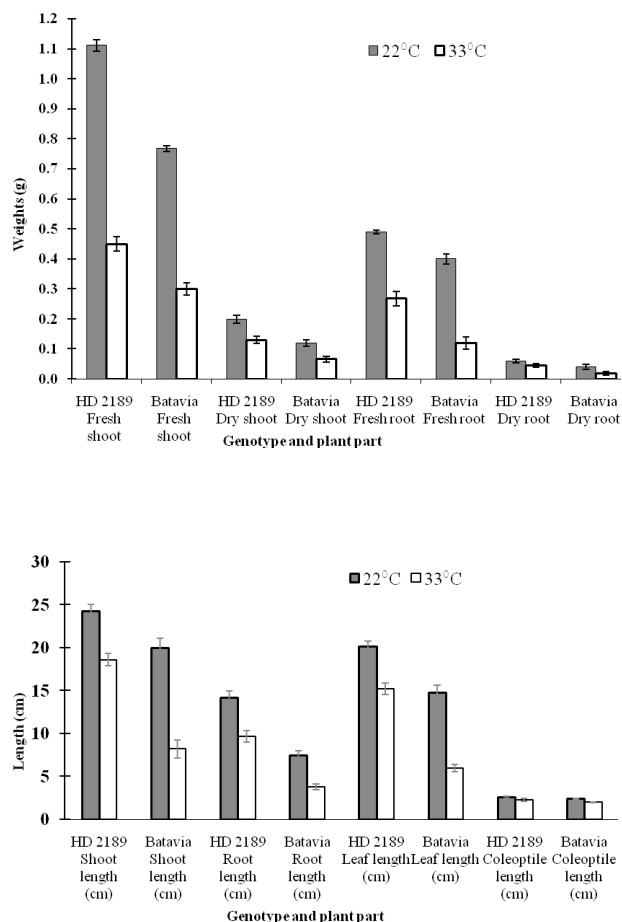


Fig. 2: Morphological trait comparison of HD2189 and Batavia.

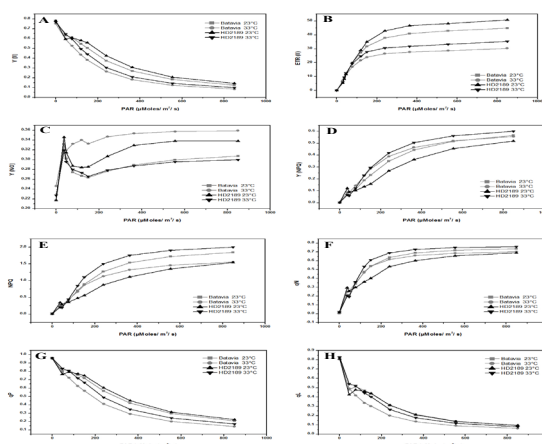


Fig. 4: PSII parameters of HD2189 and Batavia grown under control (22°C) and heat stress (33°C) conditions.

**Table 2.** Chlorophyll content (in terms of SPAD value) and canopy temperature depression (CTD) of genotypes HD2189 and Batavia

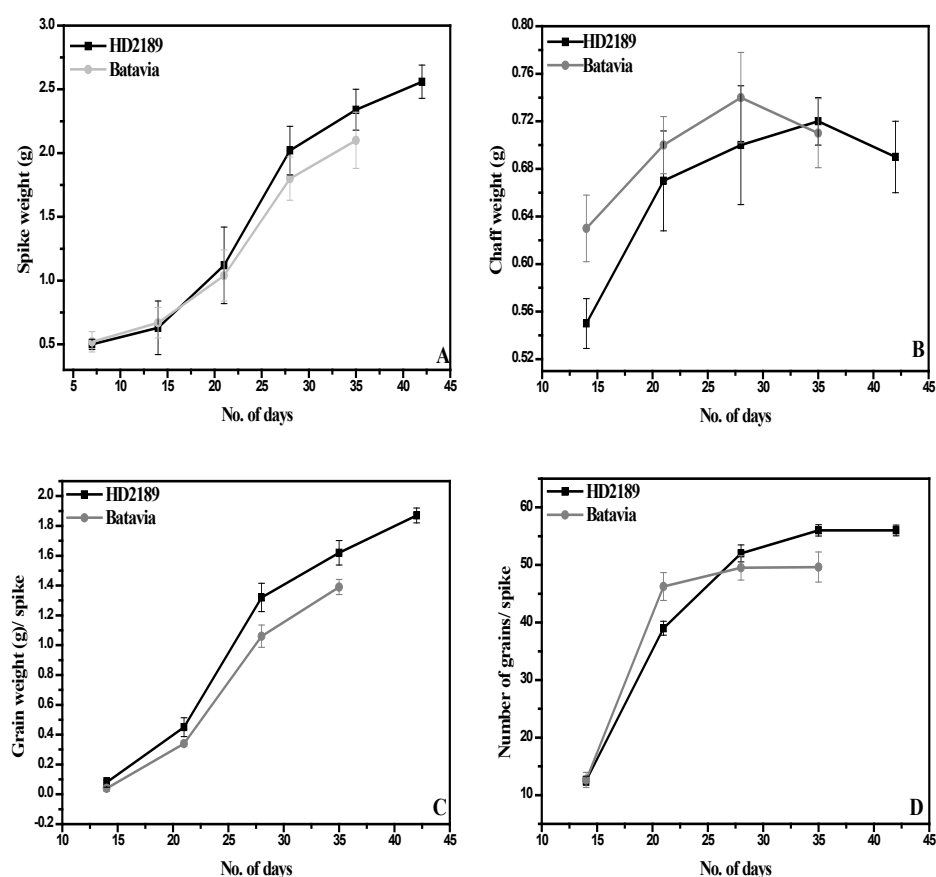
Crop Stage	SPAD values (Mean±S.E.)		CTD values (Mean±S.E.)	
	HD2189	Batavia	HD2189	Batavia
Tillering	43.2±3.2	41.5±2.4	8.00±0.32	5.10±0.24
Boot	43.2±3.2	40.2±3.1	6.20±0.27	4.21±0.18
Spike emergence	46.4±3.4	42.6±3.8	5.80±0.12	3.60±0.09
Grain filling	42.9±2.1	39.2±2.6	3.75±0.08	2.82±0.14

**Table 3.** Photosystem II efficiency (Fv/Fm) of HD2189 and Batavia

	Fv/Fm	
	22°C	33°C
HD2189	0.816	0.813
Batavia	0.803	0.789

**Table 4.** Grain yield and yield contributing traits of HD2189 and Batavia

Plant trait	HD2189 (Mean±SE)	Batavia (Mean±SE)
Plant height (cm)	68.9±1.2	60.2±1.4
Spikelets/spike	19.0±0.6	20.0±0.42
Spike length (cm)	9.5±0.42	8.6±0.27
Grain number/spike	48.0±0.32	41.0±0.43
Grain yield /plant(g)	8.60±0.22	5.22±0.34
100grain weight(g)	4.20±0.24	3.44±0.36
Grain yield /spike (mg)	1890±122	1575±137
Grain yield per plot (g)	112.6±3.0	94.9±5.2
Dry matter/tiller at anthesis (mg)	2800 (NO SE)	2650 (NO SE)
Dry matter /tiller at maturity (mg)	2125 (NO SE)	2330 (NO SE)
TDM (mg)	675 (NO SE)	320 (NO SE)
TE (%)	24.1	12.0
CPA (%)	35.7	20.3



**Fig.5** Grain filling rate and duration were recorded at weekly intervals of HD2189 and Batavia for six weeks. (A) Spike weight (B) Chaff weight (C) Grain weight (D) Number of grains/ spike.

stage and lowest for grain filling stage for both the cultivars, however, HD2189 was maintaining lower temperatures at all growth stages in comparison to Batavia suggesting better physiological efficiency in warmer environment (Table 2). Heat stress causes impairment of photosynthetic machinery through increased thylakoid membrane fluidity and inhibition of electron transport of photosystem II. The potential maximum quantum yield of PSII (Fv/Fm) ratio was recorded on the mid-portion of the flag leaf of both the genotypes. The values were not much different for HD 2189 (0.816 at 33 °C and 0.813 at 22 °C, ~ 0.03% difference) whereas for Batavia ~1.78% reduction in Fv/Fm was observed (Table 3). When the plants are grown in the field, the photon flux density (PFD) varies throughout the day and can be as high as 2000  $\mu\text{moles}/\text{m}^2/\text{s}$ . In order to understand the photosynthetic response of the two wheat genotypes to varying light intensities, light curves were recorded to assess different PSII parameters between 0~ 850  $\mu\text{moles}/\text{m}^2/\text{s}$  (Saturated response) (Fig. 4). The quantum yield Y(II) of PSII was in general higher for HD2189 in comparison to Batavia at both the temperatures and declined similarly with increasing PFD (similar slopes) (Fig. 4). The electron transport rate was higher for HD2189 in comparison to Batavia and reached saturation at ~200  $\mu\text{moles}/\text{m}^2/\text{s}$ . The results indicated that HD2189 had in general better photosystem II performance than Batavia that can account for its better grain yield. However, when Y(NO) and Y(NPQ) were examined, HD2189 showed significantly higher Y (NPQ) at 33°C in comparison to 22°C indicating its potential to adjust to the higher temperature stress via regulated processes of releasing absorbed energy and hence causing less damage to the photosynthetic apparatus (Fig. 4D). On the other hand, Batavia showed a similar increase in Y(NPQ) at both the temperatures, indicating its inadaptability for combating the heat stress in a non-damaging manner. This was established by examining Y(NO), the quantum yield of

non regulated heat dissipation. The variation for Y(NO) was significantly larger for Batavia than HD2189. These results suggest robustness of photosynthetic apparatus of HD2189 to the high temperature stress primarily due to higher amount of regulated heat dissipation. Data on plant yield and yield contributing traits compared under Trombay conditions are presented in table 4. HD2189 performed better for all the traits except for spikelets per spike in comparison to Batavia such as plant height, spike length, grain number per spike, grain weight per spike, 100 grain weight and grain yield per plot and showed 17% more grains per spikelet, ~ 65% higher grain yield per plant and 22% more hundred kernel weight resulting into ~ 18 % increased grain yield per plot. Translocation of dry matter (TDM) from vegetative plant parts to developing grains and translocation efficiency (TE) was double for HD2189 compared to Batavia in under warm conditions (Table 4). Contribution of pre anthesis assimilates (CPA) to grain was higher for HD2189 (35.7%) compared to Batavia (20.3%). The differences in translocation ability of two genotypes further investigated from rate of grain filling after anthesis for both the genotypes at weekly interval. The weekly monitoring of the plots after ear emergence indicated that senescence occurred in Batavia plants one week earlier than HD2189 and hence no data could be recorded for Batavia for sixth week (Fig. 5A-D). The spike weight remained similar for both the varieties until second week (Fig.5A). However, it increased at a higher rate for HD2189 in the following weeks (third to sixth week) and was ~ 22% more than Batavia at maturity (Fig. 5A). Chaff weight was consistently more in Batavia where as grain number and grain weight was more in HD2189 (Fig. 5C-D). The results indicated better source sink relationship in HD2189 compared to Batavia under heat stress conditions. Thus, morpho-physiological analysis indicated that genotype HD2189 is heat tolerant at seedling and adult plant stages compared to Batavia.

## References

1. Al-Khatib K, GM Paulsen. 1984. Mode of high-temperature injury to wheat during grain development. *Physiology Plant* **61**: 363-368.
2. Almeselmani M, PS Deshmukh, V Chinnusamy. 2012. Effect of prolong high temperature stress on respiration, photosynthesis and gene expression in wheat (*Triticum aestivum* L.) varieties differing in their thermotolerance. *Plant Stress* **6**: 25-32.
3. Amani IR, RA Fischer and MP Reynolds. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. *Journal of Agronomy and Crop Science* **176**: 119-129.



4. Asseng S, F Ewert, P Martre, RP Rötter, DB Lobell, D Cammarano, BA Kimball, MJ Ottman, GW Wall, JW White, MP Reynolds, PD Alderman, PVV Prasad, PK Aggarwal, J Anothai, B Basso, C Biernath, AJ Challinor, G De Sanctis, J Doltra, E Fereres, M Garcia-Vila, S Gayler, G Hoogenboom, LA Hunt, RC Izaurralde, M Jabloun, CD Jones, KC Kersebaum, AK Koehler, S Müller, Naresh Kumar, C Nendel, GO Leary, JE Olesen, T Palosuo, E Priesack, E Eysshi Rezaei, AC Ruane, MA Semenov, I Shcherbak, C Stöckle, P Stratonovitch, T Streck, I Supit, F Tao, PJ Thorburn, K Waha, E Wang, D Wallach, J Wolf, Z Zhao and Y Zhu. 2014. Rising temperatures reduce global wheat production. *Nature Climate Change* **5**: 143-147.
5. Ayeneh A, M van Ginkel, MP Reynolds and K Ammar. 2002. Comparison of leaf, spike, peduncle and canopy temperature depression in wheat under heat stress. *Field Crops Research* **79**: 173-184.
6. Balota M, WA Payne, SR Evett and MD Lazar. 2007. Canopy temperature depression sampling to assess grain yield and genotypic differentiation in winter wheat. *Crop Science* **47**: 1518-1529.
7. Blum A, N Klueva, HT Nguyen. 2001. Wheat cellular thermotolerance is related to yield under heat stress. *Euphytica* **117**: 117-123.
8. Cossani CM and MP Reynolds. 2012. Physiological traits for improving heat tolerance in wheat, *Plant Physiology* **160**: 1710-1718.
9. Dhyani K, MW Ansari, YR Rao, RS Verma, A Shukla and N Tuteja. 2013. Comparative physiological response of wheat genotypes under terminal heat stress. *Plant Signal Behaviour* **8**: 1-6.
10. Djanaguiraman M, DL Boyle, R Welti, SVK Jagadish, and PVV Prasad. 2018. Decreased photosynthetic rate under high temperature in wheat is due to lipid desaturation, oxidation, acylation, and damage of organelles. *BMC Plant Biology* **18** (1). doi:10.1186/s12870-018-
11. Dubey R, H Pathak, S Singh, B Chakravarti, AK Thakur and RK Fagodia. 2019. Impact of sowing dates on terminal heat tolerance of different wheat (*Triticum aestivum* L.) Cultivars. *National Academy Science Letters* doi:10.1007/s40009-019-0786-7
12. Dwivedi SK, S Basu, S Kumar, G Kumar, V Prakash, S Kumar, JS Mishra, BP Bhatt, N Malviya, GP Singh and A Arora. 2017. Heat stress induced impairment of starch mobilisation regulates pollen viability and grain yield in wheat: Study in Eastern IndoGangetic Plains. *Field Crop Research* **206**: 106-114.
13. Farooq M, H Bramley, JA Palta and KHM Siddique. 2011. Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences* **30**: 1-17.
14. Fischer RA and R Maurer. 1978. Drought resistance in spring wheat cultivars-I: grain yield responses. *Australian Journal of Agricultural Research* **29**: 897-912.
15. Fokar M, A Blum and HT Nguyen. 1998. Heat tolerance in spring wheat. II. Grain filling. *Euphytica* **104**: 9-15.
16. Gulen H and A Eris. 2003. Some physiological changes in strawberry (*Fragaria x ananassa* cv. Camarosa) plants under heat stress. *Journal of Horticultural Science and Biotechnology* **78**: 894-898.
17. Hede A, RB Skovmand, MP Reynolds, J Crossa, AL Vilhelmsen, O Stolen. 1999. Evaluating genetic diversity for heat tolerance traits in Mexican wheat landraces. *Genetic Resources and Crop Evolution* **46**: 37-45. doi: 10.1023/A:1008684615643.
18. Ibrahim AMH and JS Quick. 2001. Heritability of heat tolerance in winter and spring wheat. *Crop Science* **41**:1401-1405.
19. Krause GH and E Weiss. 1984. Chlorophyll fluorescence as a tool in Plant Physiol. II. Interpretation of fluorescence signals. *Photosynthesis Research* **5**: 139-157.
20. Kumar SN, PK Aggarwal, DN Swarooparani, R Saxena, N Chauhan and S Jain. 2014. Vulnerability of wheat production to climate change in India. *Climate Research* **59**: 173-187
21. Lopes MS and MP Reynolds. 2012. Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. *Journal of Experimental Botany* **63**: 3789-3798
22. Marutani Y, Y Yamauchi, Y Kimura, M Mizutani and Y Sugimoto. 2012. Damage to photosystem II

- due to heat stress without light-driven electron flow: involvement of enhanced introduction of reducing power into thylakoid membranes. *Planta* **236**: 753-761.
23. Narayanan S. 2018. Effects of high temperature stress and traits associated with tolerance in wheat. *Open Access Journal of Science* **8**: 177-186.
  24. Nayyar H and D Gupta. 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. *Environmental and Experimental Botany* **58**: 106-113.
  25. Rane J, J Shoran and S Nagarajan. 2000. Heat stress environments and impact on wheat productivity in India. Guestimate of losses. *Indian Wheat Newsletter* **6**: 5-6.
  26. Reynolds MP, M Balota, MIB Delgado, I Amani and RA Fischer. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Australian Journal of Plant Physiology* **21**: 717-730.
  27. Reynolds MP, RP Singh, A Ibrahim, OAA Ageeb, A Larqué-Saavedra and JS Quick. 1998. Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica* **100**:85-94.
  28. Ristic Z, U Bukovnik, PVVV Prasad. 2007. Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under high temperature stress. *Crop Science* **47**: 2067–2073.
  29. Sattar A, A Sher, M Ijaz, S Ul-Allah, MS Rizwan, M Hussain, K Jabran and MA Cheema. 2020. Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. *PLoS One* **15**(5), e0232974. doi:10.1371/journal.pone.0232974.
  30. Savchenko GE, EA Klyuchareva, LM Abramchik and EV Serdyuchenko. 2002. Effect of periodic heat shock on the inner membrane system of etioplasts. *Russian Journal of Plant Physiology* **49**: 349-359.
  31. Sayed OH. 2003. Chlorophyll fluorescence as a tool in cereal crop research. *Photosynthetica* **41**:321-330.
  32. Shanahan JF, IB Edwards, JS Quick and JR Fenwick JR. 1990. Membrane thermostability and heat tolerance of spring wheat. *Crop Science* **30**: 247-251
  33. Shanmugam S, KH Kjaer, CO Ottosen, E Rosenqvist, DK Sharma and B Wollenweber. 2013. The alleviating effect of elevated CO<sub>2</sub> on heat stress susceptibility of two wheat (*Triticum aestivum* L.) cultivars. *Journal of Agronomy and Crop Science* **199**: 340-350.
  34. Sharkova VE. 2001. The effect of heat shock on the capacity of wheat plants to restore their photosynthetic electron transport after photo inhibition or repeated heating. *Russian Journal of Plant Physiology* **48**: 793-797.
  35. Sharma SN, VK Bhatnagar, MS Mann, US Shekhawat and RS Sain. 2002. Maximization of wheat yields with a unique variety in warmer areas. *Wheat Inform. Ser.* **95**: 11-16.
  36. Shirdelmoghanloo H, I Lohraseb, HS Rabie, C Brien, B Parent and NC Collins. 2016. Heat susceptibility of grain filling in wheat (*Triticum aestivum* L.) linked with rapid chlorophyll loss during a 3-day heat treatment. *Acta Physiologiae Plantarum* **38**(8). doi:10.1007/s11738-016-2208-5
  37. Stone PJ and ME Nicolas. 1994. Wheat cultivars vary widely in their responses of grain yield and quality to short periods of postanthesis heat 1200 stress. *Australian Journal of Plant Physiology* **21**: 887-900.
  38. Stone PJ and ME Nicolas. 1995a. A survey of the effects of high-temperature during grain filling on yield and quality of 75 wheat cultivars. *Australian Journal of Agricultural Research* **46**: 475-492.
  39. Stone PJ and ME Nicolas. 1995b. Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. 1. Grain growth. *Australian Journal of Plant Physiology* **22**: 927-934.
  40. Stone PJ and ME Nicolas. 1998. Comparison of sudden heat stress with gradual exposure to high temperature during grain-filling in two wheat varieties difference in heat tolerance. II. Fractional protein accumulation. *Australian Journal of Plant Physiology* **25**: 1-11.
  41. Tahir M and M Singh. 1993. Assessment of screening techniques for heat tolerance in wheat. *Crop Science* **33**: 740-744.

42. Xu, Q, AQ Paulsen, JA Guikema and GM Paulsen. 1995. Functional and ultrastructural injury to photosynthesis in wheat by high temperature during maturation. *Environmental and Experimental Botany* **35**: 43-54
43. Yang X, Z Tian, L Sun, B Chen, FN Tubiello and Y Xu. 2017. The impacts of increased heat stress events on wheat yield under climate change in China. *Climate Change* **140**: 605-620.
44. Zhang K, Y Zhang, G Chen and J Tian. 2009. Genetic analysis of grain yield and leaf chlorophyll content in common wheat. *Cereal Research Communication* **37**: 499-511