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Cell membrane stability- an important criterion for selection of heat tolerant genotypes in wheat (*Triticum aestivum* L.)

Anzer Ul Islam*, Ashok K. Chhabra, Satyaveer S. Dhanda and Renu Munjal

Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar - 125004 (Haryana), INDIA

*Corresponding author. E-mail: anzer.gene@gmail.com

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Abstract: Cell membrane stability, grain filling rate, grain filling duration, canopy temperature and grain yield were used to evaluate performance of 100 diverse bread wheat (*Triticum aestivum* L.) genotypes under timely sown and late sown heat stress conditions for two cropping season. The genotypes differed significantly for all the traits showing considerable variation for improvement of characters. The genotypes WH1165 had significant high grain yield (14.6* g and 11.4g) and (11.3* g and 11.4* g) followed by cell membrane stability under timely sown and heat stress conditions, respectively indicating potential tolerance against heat stress. Correlation coefficients revealed that cell membrane stability (0.451**) and (0.639**) in timely sown and in late sown conditions, respectively were the most important trait followed by grain filling rate (0.882** and 0.744**) under timely sown and late sown conditions respectively. Results revealed that bread wheat genotypes which had high value of cell membrane stability had high grain yield showed potential photorespiration and high grain filling rate under heat stress condition. Twenty two genotypes WH1021, WH1155, VL803, WH787, NW1014, Raj3765, HD1869, 2042, WH1124, HD2285, WH1133, HUW234, 4066, Sonak, UP2425, UP2473, PBW503, PBW373, PBW533, SGP13, HD2643 and WH789 were identified as heat tolerant genotypes based on their relative performance in yield components, grain yield and heat susceptibility indices. These genotypes were found to be ideal candidates to be used in developing heat tolerant wheat varieties. Canopy temperature, membrane thermostability and grain filling rate have also shown strong correlation with grain yield. Because of this association, these traits constitute the best available 'tool' for genetic improvement of wheat suitable for cultivation under heat stressed environments. Thus, these could be used as indirect selection criteria for developing heat tolerant wheat genotypes that would provide sufficient yields to meet the ever increasing wheat demand.

Keywords: Cell membrane stability, Canopy temperature, Grain filling rate, Heat tolerance, Heat susceptibility index

INTRODUCTION

Global warming as a result of climate change negatively affects wheat grain yield, which potentially increases food insecurity and poverty (Ortiz *et al.,* 2008). Wheat is a major dietary component for largest count of population across the world. Grown on more land than any other crop, more than 215 million hectares of wheat are harvested annually to generate a world production of almost 700 million to, making it the third most produced cereal after maize and rice. High temperature stress has a wide range of effects on plants in terms of physiology, biochemistry and gene regulation pathways. However, strategies exist to crop improvement for heat stress tolerance through generation of new varieties with sustainable yield production (Bita and Gerats, 2013; Sareen *et al.,* 2015).

Wheat is a winter season crop and cultivated in the tropical and subtropical zone. By changing climatic scenario wheat in India is undergoing major changes as the cool period for wheat crop is shorten while the threat of terminal heat stress is roll wide (Rane *et al.,* 2000; Sharma *et al.,* 2002; Halford, 2009). Heat stress is a major abiotic stress factor leading yield penalty in wheat production not only in developing countries, but also worldwide. Air temperatures raise significantly due to global warming will even further exasperate the food crises and put pressure on wheat breeders and physiologists to improve yield potential of wheat under heat stress condition. Heat stress is a function of the magnitude and rate of temperature increase, as well as the duration of exposure to the raised temperature (Wahid *et al.,* 2007).

For wheat optimum temperature during anthesis and grain filling ranges from 12 to 22°C. Exposure to temperatures above this can significantly reduce grain yield (Mac˜as *et al.,* 1999, 2000; Mullarkey and Jones, 2000; Tewolde *et al.,* 2006). Increase in this temperature can cause heat stress, particularly when it occurs during reproductive and grain-filling phases. In India, delay sowing shifts the grain filling during high temperature mean time which causes the shortening of grain filling period. Heat stress during the reproductive

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phase can cause pollen sterility, tissue dehydration and injuries of the photosynthetic apparatus diminish source activity and sink capacity, lower CO_2 assimilation and increased photorespiration (Harding *et al.,* 1990, Wardlaw and Moncur, 1995; Zahedi and Jenner, 2003). Therefore, high temperatures spell during anthesis to grain filling and upto grain maturity than optimal temperature in delay planting, deteriorate the grain quality, reduces grain yield which may exceed to 40-50% (Joshi *et al.,* 2007) because of the less time to utilize systematically natural resources.

A number of morpho-physiological traits to be associated with grain yield under heat stress, such as canopy temperature depression (CTD), cell membrane stability (CMS), and leaf chlorophyll content during grain filling, leaf conductance and photosynthesis reported by Reynolds *et al.* (1998). It is difficult to bring progress for increasing grain number and yield components under heat stress condition as these are complex characters and largely influenced by environmental factors. Canopy temperature depression (Reynolds *et al.,* 1998; Bahar *et al.,* 2011; Shefazadeh *et al.,* 2012), cell membrane stability (Dhanda and Munjal, 2006 and Bahar *et al.,* 2011) and chlorophyll fluorescence (Moffatt *et al.,* 1990; Sayed, 1992) have been used as physiological screening techniques for heat tolerance. Membrane leakage is reported as a measure of stress cellular damage (Fokar *et al.,* 1998a). In chloroplasts, heat stress generally reduces the photochemical efficiency of photosystem II (PS II), an important component of photosynthesis (Reynolds *et al.,* 1994; Al-Khatib and Paulsen, 1999). The extent of change in PS II indicated by chlorophyll fluorescence, which refers to the ratio of variable fluorescence to maximum fluorescence (Fv/Fm), and the base fluorescence (F_0) is a physiological parameter that was correlated with heat tolerance (Moffat *et al.,* 1990; Rekika *et al.,* 1997; Mishra *et al.,* 2012).

In view of the changing scenario of the environmental condition, there is a dire need to develop genotypes that are either tolerant to terminal heat stress or that mature early without yield losses and thus escape the stress. To adapt new crop varieties to the future climate, we need to understand how crops respond to elevated temperatures and how plant tolerance to be improved against heat stress (Halford, 2009).

Keeping in view the above facts, the present investigation was undertaken with the following objectives

1. To study cell membrane stability and its role in heat tolerance in bread wheat

1. To determine morpho-physiological indices of heat tolerance

2. Evaluation of promising genotypes for heat stress tolerance under timely and late sown conditions

MATERIALS AND METHODS

Field condition and experiment layout to expose genotypes under heat stress during anthesis and reproductive stage: One hundred diverse bread wheat genotypes were sown in two consecutive cropping season of 2012-13 and 2013-14 in 3 meter single row in three replications under timely sown conditions, E_1 (date of sowing, 15th November, 2012-13) and heat stress conditions, E_2 (date of sowing, 25th December, 2012-13) in randomized block design. Plant to plant distance was kept 10 cm and row to row distance of 22.5 cm was maintained in both season and the environments. The meteorological data on maximum and minimum temperatures and rainfall for the crop period (November, 2012 to April, 2013 and November, 2013 to April 2014) were collected from CCS HAU, Hisar, Agro-Meteorological observatory unit (Table 1).

Calculation of grain filling rate (GFR) and grain filling duration: GFR is source to sink relation into which accumulates are transported from the source to the sink. Grain filling rate was estimated as the ratio between grain yield per plant and grain filling duration. Grain filling duration was calculated as the difference between days to maturity and days to anthesis.

Heat susceptibility index: Heat susceptibility index for grain yield of each genotype were calculated by Fischer and Maurer (1978) formula.

$$\mathbf{HSI} = \frac{1 - \frac{Y}{Yp}}{1 - \frac{X}{Xp}}$$

Where;

Y and Yp is grain yield of individual genotypes under heat stress and normal environment, respectively, X and Xp is mean grain yields of total genotypes under heat stress and normal environment, respectively.

Cell membrane stability: To measure membrane thermo stability, method of Sullivan 1972, (modified by Ibrahim and Quick, 2001 a and b) was followed. Leaf sample were collected from five randomly selected plants, 10 cm long flag leaf sample from each genotype per replication was collected at post anthesis. At field, each sample was collected in sealed plastic bags and immediately kept in ice boxes. In laboratory all the samples were thoroughly dried twice in demonized water. The mid rib of flag leaves were removed gently by hand, 5 cm portion from central flag leaf area was excised and cut in to 5 equal parts. Leaf was kept in glass test tubes. The test tube samples were tightly covered with aluminum foil and submerged in water bath (maintained at 50^{0}C) at depth equal to height of water in test tubes for 30 min time period, after the treatment 10ml de-ionized water was added in each test tube and were held overnight at 4˚C in refrigerator. Conductance (T_1) was measured with an electrical conductivity meter after calibration with a standardized KCL solution. The test tube samples were then autoclaved at pressure of 0.10 Mpa for 10 min to completely kill plant tissues and release all the electrolytes. The conductance (T_2) was measured again after

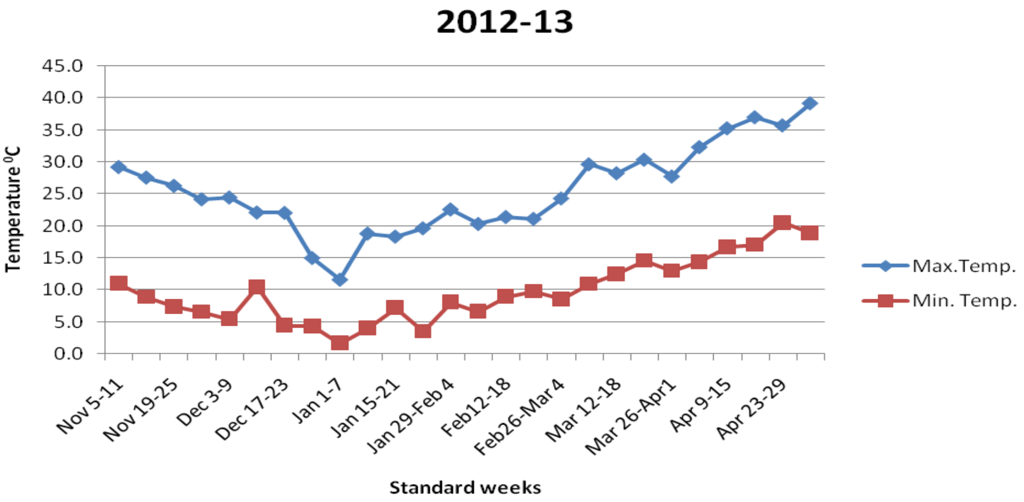

Fig. 1. *Weekly maximum and minimum temperature during the wheat crop seasons of 2012-13 (Source: Agro-Meteorological Observatory Unit CCS HAU, Hisar).*

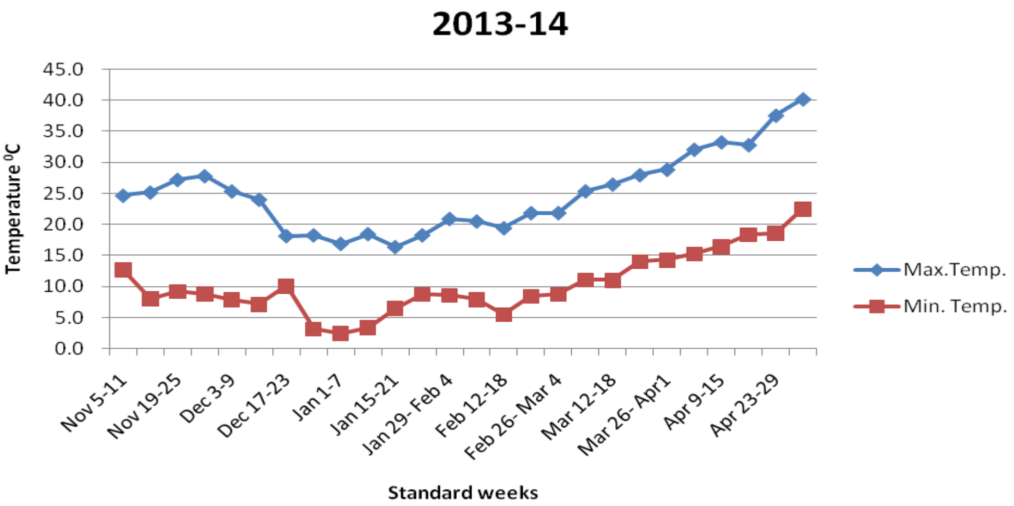

Fig. 2. *Weekly maximum and minimum temperature during the wheat crop seasons of 2013-14(Source:Agro-Meteorological Observatory Unit CCS HAU, Hisar).*

Table 1. Temperature and rainfall during wheat crop season of 2012-13 and 2013-14.

Month	Temperature (°C)				Rainfall (mm)	
	2012-13		2013-14			
	Min	Max	Min	Max	2012-13	2013-14
December	6.0	20.8	7.0	21.8	5.5	0.0
January	4.8	18.4	5.6	18.0	14.4	2.0
February	8.3	21.0	7.6	20.8	0.0	12.5
March	11.9	28.0	12.2	26.3	0.0	47.0
April	17.1	35.0	17.1	34.1	33.3	17.1

autoclaving. Cell membrane stability was expressed in % and measured by the formula given below:

$$CMS = 1 - \frac{T1}{T2} \times 100$$

Where;

T_1=conductivity reading after heat treatment

T_2=conductivity reading after autoclaving

Canopy temperature: Canopy temperature was recorded between 12:00 noon to 14:00 PM in a cloudless, windless and full sunshine daytime. A hand operated infrared thermometer (IRT), model AG-42, Tele temp crop, Fullerton (CA), for rapid indirect determination, and was used for instantaneous measurement of canopy temperature. Measurements were taken when IRT covered 100 per cent canopy and kept at an angle of 45^0 approximately 50 cm above the canopy from hori-

Table 2. Mean sum of squares for various traits under timely sown and late sown conditions.

Source of variation	1	2	3	4	5	6	7	8	9	10
Replication	2	TS	3.94	5.47	2.31	23.34	0.001	665	0.073	0.181
		LS	0.173	7.64	1.29	10.30	0.006	28.79	0.002	
Genotypes	99	TS	722**	6,401**	324**	10,932**	0.508**	4,599**	0.847**	29.92**
		LS	579**	3,066**	356*	10,302**	0.68**	2,225**	0.741**	
Error	198	TS	193	223	127	1,046	0.087	1,383	0.314	11.17
		LS	119	194	172	1,280	0.255	519	0.233	
C.D. (5%)		TS	1.59	1.71	1.29	3.70	0.034	4.26	0.064	0.383
		LS	1.25	1.59	1.50	4.09	0.058	2.61	0.055	
Mean		TS	11.79	99.69	27.36	57.77	0.646	36.79	0.323	0.986
		LS	8.11	87.50	29.99	46.29	0.599	30.12	0.272	

Where;

1	2	3	4	5	6	7	8	9	10
Degree of freedom	Environment	Yield/plant	Days to heading	Canopy temperature	Cell membrane stability	Chlorophyll fluorescence	Grain filling duration	Grain filling rate	Heat susceptibility index

*, **; Significant at 5% and 1% level of probability, respectively; TS; Timely sown, LS; Late sown

Table 3. Genotypic and phenotypic (bold) correlations for various traits under timely sown and late sown conditions.

	Environment	Yield/ plant	Days to heading	Canopy temperature	Cell membrane stability	Chlorophyll fluorescence	Grain filling duration	Grain filling rate	Heat susceptibility index
Yield/plant	TS	1	-0.204**	-0.298**	0.451**	-0.066	0.068	0.744**	0.355**
	LS		-0.029	-0.001	0.639**	0.048	0.031	0.882**	-0.610**
Days to heading	TS	-0.344**	1	0.042	-0.019	0.043	-0.616**	0.261**	-0.001
	LS	-0.108		0.048	-0.075	-0.034	-0.550**	0.222**	-0.043
Canopy temperature	TS	-0.177**	0.136*	1	-0.057	0.026	-0.025	-0.221**	-0.163**
	LS	-0.363**	-0.057		0.007	-0.035	-0.071	0.036	-0.010
Cell membrane stability	TS	0.407**	-0.068	0.074	1	0.003	-0.056	0.399**	-0.095
	LS	0.628**	-0.156**	-0.252**		0.024	0.068	0.545**	-0.318**
Chlorophyll fluorescence	TS	-0.098	0.044	0.039	0.008	1	0.125*	-0.129*	0.058
	LS	0.061	-0.047	-0.113	0.000		0.028	0.023	0.054
Grain filling duration	TS	0.207**	-0.712**	-0.104	0.005	0.194**	1	-0.592**	-0.026
	LS	0.149**	-0.567**	0.087	0.173**	0.033		-0.429**	0.049
Grain filling rate	TS	0.713**	0.230**	-0.094	0.345**	-0.205**	-0.525**	1	0.284**
	LS	0.883**	0.161**	-0.392**	0.522**	0.047	-0.327**		-0.571**
Heat susceptibility index	TS	0.235**	-0.068	-0.042	-0.259**	0.067	0.082	0.142*	1
	LS	-0.602**	0.015	0.331**	-0.281**	0.064	-0.070	-0.543**	

Table 4. List of heat tolerant genotypes based upon heat susceptibility index.

S. N.	Genotypes	Yield/ plant TS	LS	Heat susceptibility index
1	WH1021	13.7	11.4	0.55
2	WH1155	13.7	11.2	0.59
3	VL803	10.7	8.9	0.54
4	WH787	10.8	9.0	0.53
5	NW1014	8.8	7.5	0.47
6	Raj3765	13.9	11.4	0.59
7	HD1869	12.0	9.8	0.60
8	2042	7.4	6.4	0.43
9	WH1124	12.6	10.6	0.50
10	HD 2285	11.9	9.6	0.59
11	WH1133	10.6	8.7	0.57
12	HUW234	10.6	8.8	0.56
13	4066	9.6	7.9	0.58
14	Sonak	11.1	9.0	0.59
15	UP2425	13.3	10.9	0.58
16	UP2473	12.0	10.5	0.39
17	PBW503	12.0	9.8	0.56
18	PBW373	13.8	11.2	0.58
19	PBW533	12.8	10.4	0.58
20	SGP13	7.4	6.0	0.57
21	HD 2643	9.3	8.0	0.48
22	WH789	9.4	8.2	0.42

zontal and at 1m distance from the edge of the plot corner.

RESULTS

Temperature and rainfall during the season: The data of temperature and rainfall during the period of experiments were obtained from the observatory, Department of Meterological Science, CCSHAU, Hisar.

During the months of March maximum temperature (28.0^{0}C) was higher in 2012-13 but minimum temperature (12.2^{0}C) was more in 2013- 14. Experiments faced short episode of higher temperature (> 30^{0}C) during the month of March (at the time of grain filling duration) in both years (Figs. 1 and 2). These periods coincide for taking physiological observation under both timely and late sown environments. Maximum temperature (28.0^{0}C) during March in 2012-13 was comparatively more than 2013-14, but rainfall was more during the year 2013-14 (Table 1).

Analysis of variance (ANOVA): Data for yield, yield components and physiological traits of hundred genotypes were subject to analysis of variance (ANOVA) in timely sown and late sown conditions. In both the conditions ANOVA revealed highly significant genotypic differences among the genotypes for grain yield, yield attributing traits and morpho-physiological characters (Table 2).

Mean performance: Mean value expression of yield per plant in timely sown (11.79 ± 1.59) was higher than heat stress condition (8.11 ± 1.25), Showing a significant effect of heat stress, similarly, days to heading reduce by 12 days under heat stress condition (87.50 ± 1.59) than timely sown condition (99.69 ± 1.71) and mean value of canopy temperature revealed that under timely sown condition plants kept their canopy temperature (27.4^{0}C) low than stress condition (30.0^{0}C) during grain filling duration. Cell membrane conductivity revealed a significant influence of heat stress on yield as the mean value of cell membrane stability reduce in heat stress condition (46.29 ± 4.09) than timely sown (57.77 ± 3.70). Similarly, mean value of chlorophyll fluorescence (0.64 and 0.6), grain filling duration (36.8 and 30.1) and grain filling rate (0.33 and 0.27) were reduced under stress condition, respectively.

The genotypes WH1165 had significant high grain yield (14.6* g) and (11.3* g) under timely sown and heat stress conditions, respectively, while WH 1021 and Raj3765 had high grain yield under late sown conditions (11.4* g) each. In addition, the genotypes WH1021 and Raj3765 were also superior for cell membrane stability (71.2* and 64.6*) and (69.8* and 63.2*) under timely sown and heat stress conditions, respectively and WH1021 had high expression for chlorophyll fluorescence (0.702* and 0.694*) under timely sown and heat stress condition, respectively. WH1021 had significant high grain filling rate (0.389*) in heat stress condition while genotype Raj3765 had significant high grain filling rate under timely sown and heat stress conditions (0.450* and 0.407, respectively) and had considerable low score for heat susceptibility index (0.55* and 0.59*, respectively). The high value of these traits revealed that the genotypes WH1021 and Raj3765 had potent tolerance against heat stress. In bread wheat, genotypes WH1021 had significant high yield, high expression of chlorophyll fluorescence and high cell membrane stability value under heat stress condition was reported by Dhanda and Munjal (2012).

The genotypes WH1155, HD1869, WH1124, HD2285, UP2425, UP2473, PBW503, PBW373 and PBW533 had significant high yield in heat stress conditions (11.17*, 9.80*, 10.58*, 9.64*, 10.93*, 10.53*, 9.81*, 11.23* and 10.43*, respectively) and significant low value for heat susceptibility index (0.59*, 0.50*, 0.59*, 0.58*, 0.39*, 0.56*, 0.58 and 0.58*, respectively). In addition genotypes WH1155 (55.33*), WH1124 (59.07*), UP2473 (52.40*), PBW373 (58.10*) and PBW 533 (60.33*) showed significant high expression for cell membrane stability, while the genotypes WH1124, HD2285, UP2425, UP2473, PBW373, and PBW533 had significantly high value for grain filling rate 0.355*, 0.345*, 0.337*, 0.338*, 0.357* and 0.366*, respectively in heat stress condition. But the canopy temperature and grain filling duration had not much effected during heat stress.

Genotypic and phenotypic correlation coefficients: The results of correlation coefficients revealed the genotypes had high grain yield under timely sown condition due to significant correlation of traits for grain yield. In both environments grain yield were significantly and positively associated with yield attributing traits and physiological traits better performance of these traits associated with tolerance under heat stress *viz.,* cell membrane stability (r = 0.407** and r = 0.628**, respectively), grain filling duration (r = 0.207** and r = 0.149**, respectively) and grain filling rate (r= 0.713** and 0.883**, respectively).

Significant association of grain yield under heat stress condition showed that genotypes having high grain yield under stress condition and had high value of cell membrane stability (r = 0.628**) indicating lowest leakage of solutes from cellular membrane. Canopy temperature were showed negative association with grain yield in both environment timely sown and in late sown (r = -0.177** and r = -0.363**, respectively) indicating the tolerant genotypes were capable to keep their canopy cool during the heat stress condition which is helpful for the plant to perform proper photorespiration. In late sown condition heat susceptibility indices had negative association with less value for grain yield (r = -0.602**) (Table 3).

Out of hundreds (100) diverse bread wheat genotypes 22 genotypes were found heat stress tolerant on the basis of heat susceptibility index. All 22 genotypes had significant low values of heat susceptibility index (Table 4).

DISCUSSION

Heat stress reduces plant photosynthetic ability through metabolic limitations and oxidative damage to chloroplasts and effects the activities of thylakoid membrane leading to diminish number of chloroplast per cell and finally reducing grain yield (farooq *et al.,* 2011). There was also decline in biomass, grain filling duration (GFD) and 1000 grain weight (TGW) under late and very late sowing conditions due to terminal heat stress at anthesis and later stages (Kumari *et al.,* 2013). Grain yield show low heritability (Blum, 1988), and influenced by environmental fluctuations, due to this grain yield cannot be directly used as sole selection criteria (Dhanda and Munjal, 2006).

Cell membrane stability has a reasonable relationship to plant performance under heat stressed environments and has therefore been considered as a possible selection criterion for grain yield under heat stress (Blum *et al.,* 2001). Many researchers have used cell membrane stability test to study the genetics of heat tolerance in wheat (Reynolds *et al.,* 1994; Ibrahim and Quick, 2001; Dhanda and Munjal, 2012). In this study wheat genotypes exhibited significant differences for cell membrane stability under stress condition and genotypes with high cell membrane stability produced high grain yield. The genotypes WH1021 and Raj3765 had the highest value for cell membrane stability (71.2*) and (69.8*) under timely sown condition and WH1021 had high expression for chlorophyll fluorescence (0.702* and 0.694*) under timely sown and heat stress condition, respectively. Stepwise analysis identified that cell membrane stability, grain filling rate, canopy temperature and chlorophyll florescence as traits with significant contribution to total variation in grain yield. Among these traits membrane stability exhibit wide genetic variability and its performance was highly influenced under stress condition. In this backdrop, cell membrane stability is an important physiological trait that could be used as an indirect selection criterion for grain yield under heat stress conditions.

Conclusion

Exposure of wheat to high ambient temperatures adversely affects morpho-physiological traits and grain yield. However, the negative effect of heat stress was statistically different among wheat genotypes and this suggests availability of genetic variability among wheat genotypes which is an essential component in plant breeding and selection for wheat crop improvement. Morpho-physiological traits in this study exhibited a decline under heat stress. Physiological traits such as canopy temperature, cell membrane stability, chlorophyll florescence and grain filling rate have also shown strong correlation with grain yield. Because of this association, these traits constitute the best available 'tool' for genetic improvement of wheat suitable for cultivation under heat stressed environments. Thus, they could be used as indirect selection criteria for developing heat tolerant wheat genotypes that would provide sufficient yields to meet the ever increasing wheat demand. This has therefore generated information on mechanisms of heat stress in wheat that could be used in breeding for developing heat tolerant wheat cultivar/variety.

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