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Evaluation of exotic wheat (*Triticum aestivum* L.) genotypes for heat tolerance on the basis of physiological phenotyping

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Abstract

Plant physiological parameters such as membrane thermostability, canopy temperature depression, leaf chlorophyll content and yield related traits like no. of spikelets per spike, no. of grains per spike, 200-grain weight and grain yield of 18 wheat genotypes were carried out to assess for heat tolerance. Performances of all the genotypes were found to have significant differences for all the traits except canopy temperature depression. But, canopy temperature depression with some other traits like leaf chlorophyll content, no. of grains per spike, 200-grain weight and grain yield per plant demonstrated significant differences when it grown in heat stress condition. In general, genotypes with higher leaf chlorophyll content and enhanced membrane thermostability demonstrated higher 200-grain weight or grain yield. Besides, in spite of having heat tolerant traits, several genotypes performed poor due to their poor genotypic potential. The present investigation has successfully isolated several genotypes *viz.* H024, H023, H022 and H018 with desirable traits related to heat tolerance based on overall performance while grown under heat stress. These genotypes can be used as gene source for future breeding program to improve heat tolerance of the local wheat cultivars.

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop, grown in wide range of environments in the world (FAO, 2013). In Bangladesh, wheat varieties give almost 50% lower yield than the potential yield (Sikder *et al.*, 2010). Among the many other reasons, heat stress is one of the vital constrains for 50% less production (Fischer, 1985). Because, optimum temperature range for wheat cultivation is 18-24^{o}C (Bahar *et al.*, 2011) and for anthesis, grain filling is 12 to 22^{o}C. Above the optimum temperature range is generally known as heat stress (Wahid *et al.*, 2007). Wheat faces heat stress problem due to abrupt increase of daily temperature, late sowing and short winter season. Global climate models predict an increase of mean annual temperatures between 1.5 and 6.0^{o}C by the year of 2100 (IPCC, 2013), whereas in Bangladesh it will be increased by 0.39^{0}C in 2100 (Karmakar *et al.*, 2000). In Bangladesh, wheat plants received the sudden high temperature stress from the very beginning of March or just after end of winter season when plant reaches to grain filling stage. Whereas, with every 1^{o}C increase in temperature from 28^{o}C during grain filling period results 3–4% yield reduction (Reynolds *et al.* 1994, 1998; Wardlaw *et al.*, 1989). Another important problem is getting short winter period due to late planting of wheat, which consequently exposed wheat plants to heat stress and offers short grain filling period. Stone and Nicolas (1994) found that even 5 to 6 days short periods cause 20% or more yield losses in wheat. The consequences for imposing heat stress are shortening grain filling duration (Yang *et al.*, 2002), inducing early flag leaf (Yang *et al.*, 2002), acceleration of grain filling activities (Paulsen, 1994), improper grain filling (Reynolds *et al.*, 2001; Rane *et al.* 2007), constriction of carbon assimilation (Stone, 2001), floret abortion (Wardlaw and Wrigley, 1994), pollen sterility (Saini *et al.*, 1981), tissue dehydration, lower carbon assimilation, depletion of carbohydrate reserve, acceleration of plant growth (Fischer, 1980; Kase *et al.*, 1984) and reduction of plant photosynthetic capacity (Harding *et al.*, 1990) . So, development of heat stress tolerant wheat genotypes based on physiological approaches to minimize the above mention heat-induced effects might be the best way to cope with heat stress problem. There are some physiological traits used as selection criteria for heat tolerance in wheat, and have strong correlation with grain yield under heat stress. *viz.* membrane thermostability (Sharma et al., 2016; Yildirim *et al.*, 2009; Reynolds *et al.*, 1994), canopy temperature depression (Bahar *et al.*, 2008; Reynolds *et al.*, 1994, 2001), chlorophyll content (Yildirim *et al.* 2011), stomatal conductance (Reynolds *et al.* 1994), grain number per spike, grains per spikelet and thousand grain weight and grain yield (Pimentel *et al*., 2015). The main objective of this study was to evaluate some exotic wheat genotypes to identify heat tolerant genotypes for using in future breeding program and to prepare a ranking table on the basis of physiological traits to identify the best genotype.

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Materials and Methods

Experiment was conducted with 18 wheat genotypes among them 14 were exotic (designated as HO18 to HO31; collected from International Maize and Wheat Improvement Center [CIMMYT], Mexico) and 4 were popular genotypes (Shourav, Gourab, Shatabdi, Sonalika, collected from BAU germplasm) in 2014-15 at Field Experimental Laboratory, Dept. of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. Experiment was conducted with three replications and two treatments in a Randomized Completely Block Design (RCBD). Optimum time sowing (15 November, 2014) considered as first treatment and late sowing by one month (15 December, 2014) for imposing heat stress was second treatment. Range of maximum, minimum and mean temperature during the experiment were presented in Fig. 1. Data on physiological traits such as canopy temperature depression, leaf chlorophyll content, membrane thermostability and some other yield contributing characters such as number of spikelets per spike, no. of grains per spike, 200-grain weight and yield per plant (g) were recorded on the basis of following procedures as indicated below. The recorded data on different parameters were analyzed by using MINITAB version 17.0, MSTAT-C and MS Excel® 2007.

According to Pietragalla (2012), infra-red thermometer (Model: CEM DT8818) was used to record the canopy temperature depression (CTD) at 89 DAS in both treatments during which control treatment was in 27^{o}C and 2nd treatment had 29^{0}C day temperature at noon. Leaf chlorophyll content at anthesis (GS65) was measured with a SPAD meter (Minolta-502) from five flag leaves of each genotype in a replication in CCI (chlorophyll concentration index) between 12.00–14.00 h of in fine windless and cloudless days. Membrane thermostability was measured by following the guideline of Yildirim *et al.* (2009) from eight fully expanded leaves of each genotype, collected from randomly selected plants of each replication. The percentage of solute leakage was estimated by using the following equation-

So, Leaf Membrane Thermostability (MTS%) =

$$\left\{1-\frac{T_1}{T_2}\right\}\times 100$$

Where, T refer to electrical conductivity of heat treated samples, and the subscript 1 and 2 refer to electric conductivity readings before and after boiling for 1 hr, respectively.

Number of spikelet's per spike, no. of grains per spike, 200-kernels weight (g), grain yield (g) were measured as per description of Pietragalla and Pask (2012).

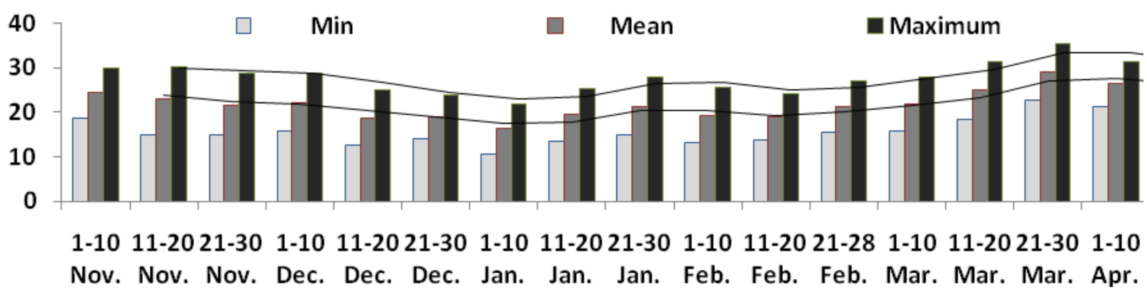

Fig. 1. Bar graph shows the average min., mean and max. temperature ($^{\circ}$C) of every 10 days from 01 November, 2014 to 10 April, 2015 (Experimental period). Data collected from weather division of BAU, Mymensingh. Here, Y= indicates temperature ($^{\circ}$C), X= indicates days

Results and Discussion

Analysis of variance (**Table 1**) showed that, for canopy temperature depression (CTD) there was no significant variation among the genotypes and genotype-treatment interaction does not have any effects on the genotypes. The results obtained from ANOVA table for the trait of leaf chlorophyll content, no. of grains per spike, 200-grain weight, grain yield per plant revealed that highly significant differences existed between genotypes and treatments. These results indicate that genotypes responded differently to the heat stressed conditions. Even, genotype-treatment interaction has significant effect on each genotype except grain yield trait. On the other hand, performance of membrane thermostability and no. of spikelets per spike on genotype was almost similar in different environmental growing condition and the effect of genotype-treatment interaction on genotypes has no differences. But, these two traits performance was different on different genotypes.

Table 1. ANOVA for 7 plant characters of 18 wheat genotypes grown under heat stress condition

SL. No.	Traits	Mean Square			
		Genotype d.f.	Treatment d.f.	Gen.* Treat. d.f.	Error
1.	**Canopy temp. depression ($^{\circ}$C) (CTD)**	2.874 ^{NS}	358.978 **	2.061 ^{NS}	2.852
2.	**Leaf chlorophyll content (LChl)**	241.805 **	1050.941 **	39.525 *	22.436
3.	**Membrane thermostability (%) (MT)**	211.347 **	7.938 ^{NS}	48.378 ^{NS}	53.668
4.	**No. of spikelets per spike (Nspk)**	17.162 **	0.231 ^{NS}	2.173 ^{NS}	1.311
5.	**No. of grains per spike (Grspk)**	193.109 **	472.926 **	120.593 *	62.851
6.	**200-grain weight (200grwt)**	2.136 **	94.678 **	1.552 **	0.562
7.	**Yield per plant (g) [Yd]**	214.401**	1409.561**	98.413 ^{NS}	85.72

Legend, * indicates 5% level of significance; ****** indicates 1% level of significance; ^{NS} indicates non-significance

Different mean performances were shown by different varieties for the traits studied (Table 2). Mean of canopy temperature ranged from 24.62^{0}C (H029) to 21.83^{0}C (H018) with an average of 23.414^{0}C. It has been found, in heat tolerant genotypes, leaf stomatal conductance and leaf transpiration rates increased with the increase of temperature (Anjum *et al*., 2008). These activities help plants to keep cooler canopy to avoid heat stress (Anjum *et al.*, 2008). Cooler CTD helps to improve the assimilation rate and yield in stress condition (Kottmann *et al.*, 2013; Sharma *et al*., 2016). Almost similar relationship was found in the Figure 2, where, it is clear that with the increase of CTD of genotype grain yield was reduced. But, H022 genotype showed considerable increase of yield with the increasing temperature. Besides, genotypes H018, H019, H024, H026 and H026 which were placed in Group-1 can maintain cooler canopy and gives higher yield.

Table 2. Mean value of 18 wheat genotypes for 7 traits studied in the experiment

Sl. No.	Genotype	CTD	LChl	MT	Nspk	Grspk	200grwt	Yd (g)
1	HO18	21.83 b	52.27 a	24.06 a-d	19.17 a	30.0 abc	7.043 d	23.41 abc
2	HO19	23.02 ab	49.07 ab	23.62 a-d	17.67 a-e	28.50abc	7.598 cd	21.64 abc
3	HO20	23.70 ab	41.25 c-f	14.45 d	17.0 cde	38.83 a	8.113bcd	24.37 abc
4	HO21	23.85 ab	48.42 abc	34.17 a	16.8 cde	29.0 abc	8.032bcd	21.79 abc
5	HO22	24.32 ab	44.02 b-e	23.19 a-d	17.67 a-e	38.17 a	8.392 bc	33.37 a
6	HO23	23.45 ab	50.27 ab	18.43 cd	19.00 ab	20.17 c	7.77 bcd	28.84 ab
7	HO24	22.67 ab	50.78 ab	25.47 a-d	18.00 a-d	33.17 ab	7.603 cd	29.05 ab
8	HO25	23.08 ab	53.08 a	26.38 abc	16.17 def	24.50 bc	7.74 bcd	16.64 bc
9	HO26	22.92 ab	43.93 b-e	16.28 cd	19.00 ab	23.67 bc	8.872 ab	21.05 abc
10	HO27	22.57 ab	51.00 ab	21.00 bcd	18.5 abc	24.00 bc	8.425 bc	24.10 abc
11	HO28	23.07 ab	47.43 a-d	17.57 cd	16.83cde	21.00 bc	8.330 bc	15.29 bc
12	HO29	24.62 a	36.97 ef	27.28 abc	16.67cde	25.00 bc	8.383 bc	18.74 abc
13	HO30	23.30 ab	48.68 abc	18.52 cd	17.33 a-e	24.67 bc	8.2 bcd	20.03 abc
14	HO31	23.78 ab	40.62 def	32.22 ab	15.83 efg	26.50abc	7.75 bcd	18.73 abc
15	Shourav	24.30 ab	36.68 ef	33.04 a	14.33 fgh	21.17 bc	8.437 bc	12.12 c
16	Gourab	23.87 ab	34.68 f	27.77 abc	13.33 h	18.67 c	7.678bcd	9.41 c
17	Shatabdi	23.47 ab	36.15 f	27.94 abc	17.17 b-e	28.17abc	8.262bcd	17.98 bc
18	Sonalika	23.65 ab	36.52 ef	18.07 cd	14.00 gh	24.17 bc	9.790 a	16.96 bc
CV (%)		7.21	10.63	30.71	6.77	29.77	9.22	44.62
Maximum		24.62	53.08	34.17	19.17	38.83	9.790	33.37
Minimum		21.83	34.68	14.45	13.33	18.67	7.04	9.41
Mean		23.414	44.545	23.858	16.91	26.63	8.13	20.75
Lsd (0.05)		2.750	7.713	11.93	1.865	12.91	1.221	15.08

Legends,
CTD = Canopy temp. depression, Leaf chlorophyll content (LChl), MT= Membrane thermostability (%) , Nspk = No. of spikelets per spike, Grspk = No. of grains per spike, 200grwt = 200-grain weight, Yd = Yield per plant (g)

Leaf chlorophyll content (Lchl) is another important trait because it helps to improve the grain yield and maturity even when heat stress was imposed (Kumar *et al*., 2010). Wittenbach (1979) reported that, loss of chlorophyll coincides with the development of grain filling. So, improved photosynthesis comes from higher leaf chlorophyll content, which is a major objective for improving the yield potential of wheat (Waddington *et al.*, 1987). Leaf chlorophyll content of the flag leaf varied from 34.68 (Gourab) to 53.08 (HO25) with a mean of 44.545 CCI. Fig. 2 and 3 also depicted the similar findings, where genotypes with lower leaf chlorophyll content give lower grain yield, but higher chlorophyll content brings higher grain yield. Interestingly, Sonalika demonstrated highest 200-grain weight even in lower leaf chlorophyll content and provides higher yield than any other local varieties used in this experiment. In contrast, H022 gave the highest grain yield though has moderate leaf chlorophyll content.

In heat stress condition, membrane thermostability is an important mechanism and measured to determine the adaptation ability of cell. Levitt (1972) reported that, cell membrane is the primary physiological injury site by heat. Because, when heat stress imposed on the cell membrane a series of consequences occurred such as acceleration of the kinetic energy, loosening of chemical bonds within molecules, denaturation of proteins, increasing the unsaturated fatty acids and the amount of fluid lipid bilayer (Savchenko *et al*., 2002). Such types of changes enhance the permeability of membranes, increased the loss of electrolytes and decreased cell membrane thermostability. Membrane thermostability was measured based on the estimation of solute leakage from tissue under heat stress condition. So, the best genotypes leaked out lowest amount of solute from the leaf in stress condition. Sharma *et al*. (2016) reported positive correlation between membrane thermostability and grain yield in heat grown under heat stress. Membrane thermostability had an average of 23.858%, with a range of 14.45% (HO20) to 34.17% (HO21). The lowest relative injury percentage of membrane was showed by HO21 and Shourav *i.e.* 34.17% and 33.04%, respectively. From Fig. 4 it is evident that genotypes with lower membrane membrane thermostability gave lower yield. However, genotypes with lower thermostability also demonstrated higher yield (H022) indicating plants ability to escape the heat stress by early flowering and higher yield potential. In contrast, several genotypes, mostly local genotypes (*viz.* Gourab, Shourav) showed poor yield performance in spite of having higher membrane thermostability, indicating lower yield potential of those genotypes (see Group 4, Fig. 4).

Heat stress has negative effect on the plant growth, development and productivity (Hassan and Raffi, 2017; Pimentel *et al*., 2015). Genotypes showed variation for the trait of no. of spikelet per spike from 13.33 (Gourab) to 19.17 (HO18) with a mean of 16.91. HO18 genotype was the best genotype for this trait than followed by HO23 (19.00), HO26 (19.00), HO27 (18.50), HO24 (18.00), HO19 (17.67), HO22 (17.67) and HO30 (17.33) genotypes. The number of grain per spike ranged from 18.67 (Gourab) to 38.83 (HO20) with a mean of 26.63 grains per spike. So, HO20 produced largest number of grains but Gourab had lowest number of grain per spike. Heat stress during grain-filling stage inhibits translocation of photosynthates to the developing grains and starch synthesis within the grains, ultimately which lowers grain weight (Bhullar *et al.,* 1985, Mohammadi *et al.,* 2004). The range of 200-grain weight from 9.790 gm (Sonalika) to 7.043 gm (HO18) and the average weight was 8.135 gm whereas grain yield had a range of 33.37 gm (H022) to 9.41 gm (Gourab), averaging 20.75 gm yield for five plants. The 200-grain weight was highest in Sonalika (9.790 gm) which was followed by HO26 (8.872 gm).

Furthermore, a ranking table (Table 3) for 18 wheat genotypes was made on the basis of mean performances of traits studied. Based on the score, genotypes were arranged from best performer to inferior performer. According to the table best genotype was H024 and inferior was Gourab.

Table 3. Ranking of 18 wheat genotypes for 7 traits under heat stress

Genotype	CTD	LChl	MT	Nspk	Grspk	200grwt	Yield	Total	Ranking
HO18	2.0	6.0	2.5	8.0	2.0	1.0	7.5	29.0	3
HO19	1.5	5.5	2.5	6.0	2.0	1.5	6.5	25.5	6
HO20	1.5	2.5	1.0	5.0	3.0	2.0	9.0	24.0	8
HO21	1.5	5.0	4.0	5.0	2.0	2.0	7.0	26.5	5
HO22	1.5	3.5	2.5	6.0	3.0	2.5	11.0	30.0	2
HO23	1.5	5.5	1.5	7.5	1.0	2.0	10.0	29.0	3
HO24	1.5	5.5	2.5	6.5	2.5	1.5	10.5	30.5	1
HO25	1.5	6.0	3.0	4.0	1.5	2.0	3.0	21.0	10
HO26	1.5	3.5	1.5	7.5	1.5	3.5	6.0	25.0	7
HO27	1.5	5.5	2.0	7.0	1.5	2.5	8.5	28.5	4
HO28	1.5	4.5	1.5	5.0	1.5	2.5	2.5	19.0	12
HO29	1.0	1.5	3.0	5.0	1.5	2.5	5.0	19.5	11
HO30	1.5	5.0	1.5	6.0	1.5	2.0	5.5	23.0	9
HO31	1.5	2.0	3.5	3.0	2.0	2.0	5.0	19.0	12
Shourav	1.5	1.5	4.0	2.0	1.5	2.5	1.5	14.5	14
Gourab	1.5	1.0	3.0	1.0	1.0	2.0	1.0	10.5	15
Shatabdi	1.5	1.0	3.0	5.5	2.0	2.0	4.5	19.5	11
Sonalika	1.5	1.5	1.5	1.5	1.5	4.0	4.0	15.5	13

Legend, Canopy temperature depression = CTD; Leaf chlorophyll content = LChl; Membrane thermostbility = MTS; No. of spikelets per spike = Nspk; No. of grains per spike = Grspk; 200-grain weight = 200grwt; Yield per plant (g) = Yd

*Rating Pattern, Best genotype scored with highest grade for individual trait performance and ranked based on cumulative score.

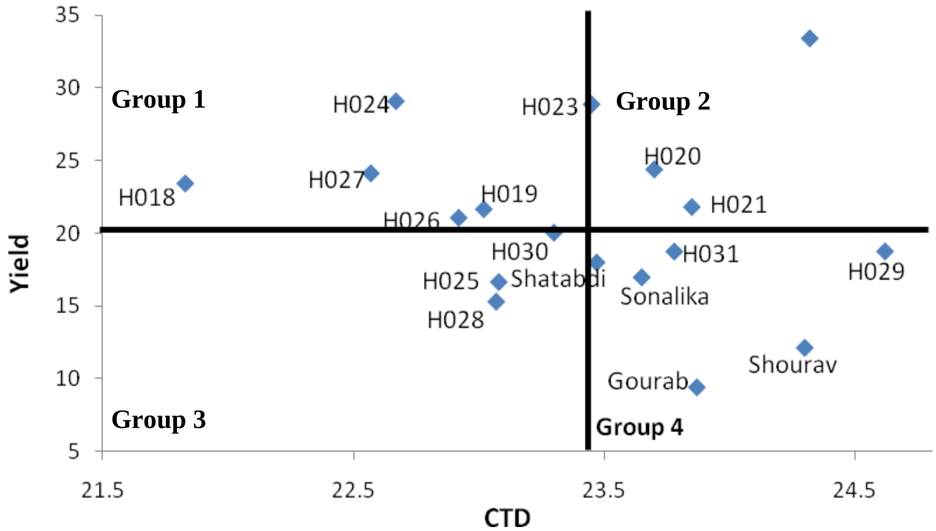

Fig. 2. Performances of 18 wheat genotypes in scatter plot based on of canopy temperature depression and yield

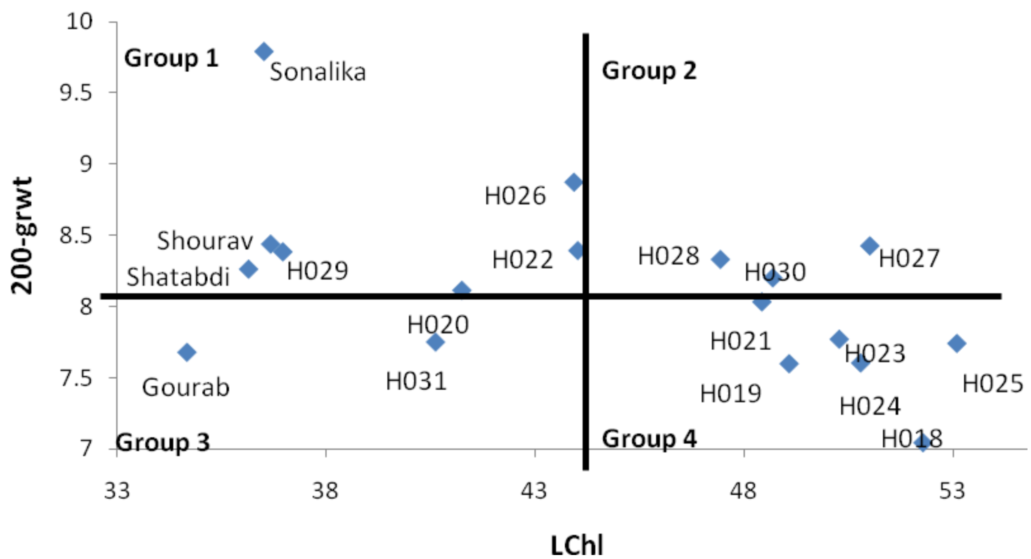

Fig. 3. Performances of 18 wheat genotypes in scatter plot based on of leaf chlorophyll content and 200-grain weight

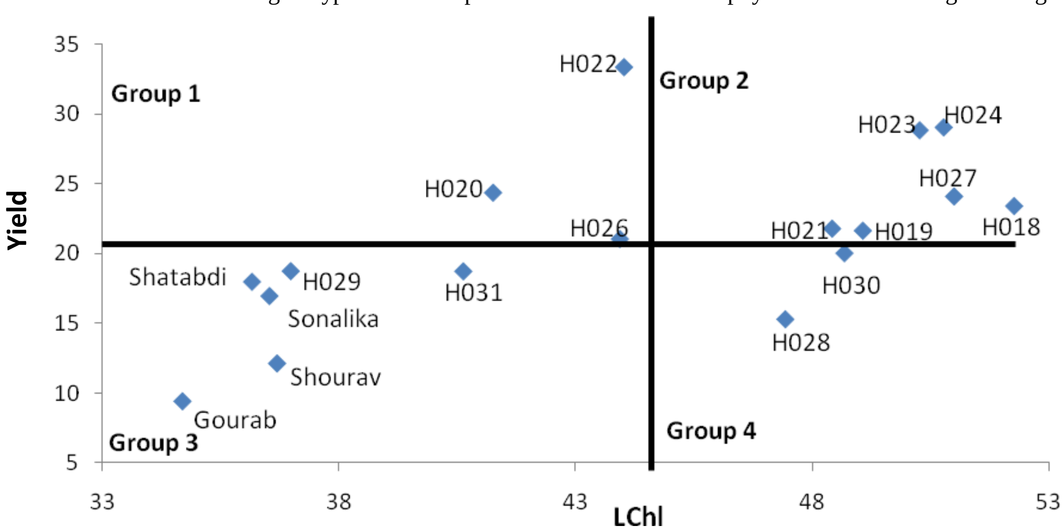

Fig. 4. Performances of 18 wheat genotypes in scatter plot based on of leaf chlorophyll content and yield

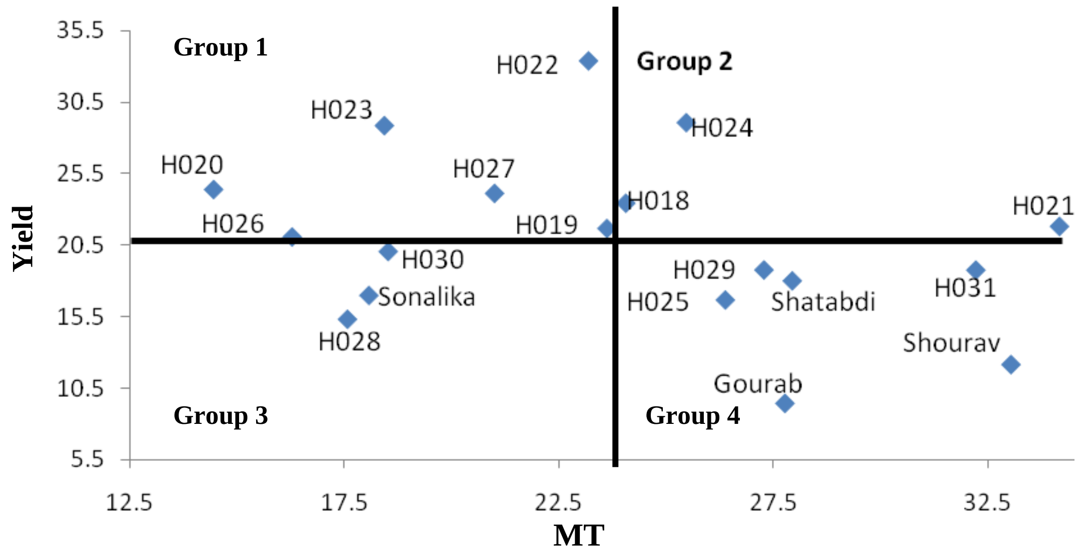

Fig. 5. Performances of 18 wheat genotypes in scatter plot based on of membrane thermostability and yield

Conclusion

Heat tolerance in wheat has been in the prime research priority in wheat growing areas considering global warming and changing cropping pattern. Therefore, wheat genotypes with different heat tolerance traits have great importance in developing heat tolerant wheat varieties. The present study identified several genotypes with different gradients of heat tolerant traits. These genotypes may be used for future breeding program for heat tolerant variety development to mitigate the heat stress problem for increased wheat production.

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