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# Evaluation of spring wheat genotypes for heat tolerance using cell membrane thermostability

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**Key words:** Cell membrane thermostability (CMT), *Triticum aestivum*, Heat tolerant, Heat susceptible, Medium tolerant

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## Abstract

Heat stress causes major loss at reproductive stage in wheat (*Triticum aestivum* L.) productivity. Wheat is a basic staple food in many countries all over the world. The present study was performed to find out the possible effects of heat tolerance on wheat genotypes. During experimentation, 120 spring wheat genotypes were used for evaluating heat tolerance in open field (normal) and in tunnel (heat stress). This resulted in 15 genotypes with diverse genetic makeup as 5 heat tolerant (HT), 5 heat susceptible (HS) and 5 medium tolerant (MT) wheat genotypes based on their relative values of cell membrane thermostability (CMT). ANOVA for CMT depicted highly significant differences among genotypes under both environments. It was concluded that V-13248, MISR 1, AARI- 11, V-13013 and V-12103 could be regarded as heat tolerant whereas V-12056, Millat-11, Chenab- 2000, ND643 and V-12082 as heat susceptible and Shafaq-06, WBLL1, CHIBIA, PBW65 and V-13016 as medium tolerant. These genotypes can cope up the heat stress efficiently and they can be utilized for developing heat tolerant genotypes. CMT was good marker for development of heat tolerant wheat genotypes with addition to grain yield.

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## Introduction

Among different types of stress, heat stress is one of the major threats to production of wheat, which is the basic source of nutrition across the globe (Safari *et al.,* 2018). Wheat is staple food crop of Pakistan and main cash crop of this country so on this crops the economy is dependent. Breeding for heat tolerance along with high yielding genotypes under both normal and heat stress condition needs efficient selection. It is necessary to identify high yielding genotypes, which performed efficiently under both environmental conditions (Thiry *et al.*, 2016). You *et al.* (2009) reported that in wheat, ascent of each 1°C temperature causes 3–10% yield reduction. Population increase also demands more wheat production.

Therefore, need is to work on breeding aspect to increase wheat production in heat stressed condition. Plants utilize various strategies to cope the heat stress. Cell membrane thermostability (CMT) has been used in some experiments for screening against heat stress (Sairam and Srivastava, 2001; Dhanda and Munjal, 2006). Heat stress is a complex mechanism that disturbs cell membrane integrity by altering plant systems (Al-Khatib and Paulsen, 1989). Cell membranes are adversely affected by heat stress and cause injury to chlorophyll, alteration in lipid assembly and denaturation of protein (Wahid *et al.*, 2007). Blum *et al.* (2001), recorded high yield even in increasing temperature during anthesis in wheat. The climate is changing day by day and scarcity of water along with increasing temperatures have developed a problematic situation for the breeders to work accordingly.

Different scientists; Shanahan *et al.*, (1990), Bukhov *et al.*, (1999) reported the heat stress causes changes in membrane fluidity and results in lipid peroxidation and impaired membrane sensitivity. High temperature stress is one of the major cause of yield loss in wheat. Varying climatic conditions that demand varieties, which can withstand the situation and give higher yield as well. Under stressed condition, different changes in metabolism like lipid/oxidation start increasing and results in more membrane damage (Sairam and Saxena, 2000). Availability of genetic diversity in wheat offers opportunities for the breeders to develop genotypes with wider adaptability having resistance to biotic as well as abiotic stresses.

The purpose of this study was to investigate the role of stress sensitivity and stress tolerance indices simultaneously for identification of tolerant, medium tolerant and susceptible genotypes based on cell membrane thermostability analysis. The outcomes of this study could be exploited for the development of new cultivars with heat tolerant genotypes.

## Materials and methods

*Experimental site*

Germplasm consisted of 120 wheat genotypes was evaluated for high temperature at Wheat Research Institute, Ayub Agriculture Research institute, Faisalabad, Pakistan during the cropping season 2014-15. To accomplish the goal, genotypes of spring wheat genotypes were exposed to high temperature stress (heat stress) in the plastic (polythene) sheet tunnel.

*Design of experiment*

The germplasm was sown in two sets (one in open field and other in plastic sheet tunnel) adjacent to field in split plot design with 3 replications. All germplasm was sown in single row having row to row distance of 30cm and plant to plant distance of 7.5 cm. Three seeds per hill were placed at the time of planting and later thinned out to single seedling at two-leaf stage. Recommended dose of NPK (120:100:60 Kg/ha) was applied. The genotypes which were sown in tunnel were exposed to heat stress at grain filling stage for almost a period of one month. The temperature inside the tunnel was 5-7°C higher than the ambient temperature.

*Cell Membrane Thermostability (CMT)*

Cell membrane thermostability was recorded by using the method, which was proposed by Saadalla *et al.* (1990) and improved by Petcu and Ciuca, (2009). From selected plants, fully extended leaf sections were taken before anthesis. Leaf discs having size of 10mm diameter were put in falcon tubes with five discs in each tube. Leaf discs were given 2-3 washings with deionized water.

Each tube was filled with 20ml of deionized water and put at room temperature for two hours. After gentle mixing, initial conductance (C1) was recorded. Then samples were autoclaved at 121°C for 15 minutes and then placed at room temperature overnight to record second conductance (C2) reading. Cell membrane thermo-stability was calculated using the formula:

$CMT = 1 - (C1/C2) \times 100$

Where, C1 and C2 are the first and the second readings of conductance respectively

*Data analysis*

Data was subjected to analysis of variance (Steel *et al.*, 1997) and means were compared by the t test (LSD) at confidence interval of 5%.

**Results and discussion**

Modifications in expression of cell membrane stability under different environmental stresses were designated as heat tolerant (high CMT) and heat susceptible (low CMT). Cell membrane thermostability was estimated by diffusion of electrolyte, which resulted in heat stress causing leakage from cell membranes. This method widely used to evaluate and screen wheat germplasm against heat stress (Blum and Ebercon 1981, Saadalla *et al.*, 1990). Increased solute leakage is an indication of decreased cell membrane thermostability and has long been used as an indirect measure of heat stress tolerance in diverse plant species including wheat (Wahid *et al.,* 2007; Blum *et al.,* 2001).

ANOVA depicted a highly significant difference in all of 120 wheat genotypes in both controlled (normal) and stress (heat stress) condition at $P<0.001$ (Table 1). The results of this study revealed the effect of heat stress on cell membrane thermostability. Means comparison of all the genotypes using LSD at 95% confidence interval (CI), showed significant differences (Table 2). Wide range of variability was found among all genotypes for this traits. Kinetic energy of molecules increased across the membrane under heat stress results in membrane loosing due to the rise in amount of unsaturated fatty acids or denaturation of proteins (Savchenko *et al.,* 2002).

High temperature stress decrease cell membrane thermostability and germplasm having more CMT value were considered as heat tolerant genotypes under high temperature stress. Wahid *et al.* (2007) utilized cell membrane thermostability as indirect measure of heat stress tolerance in different crops like wheat, tomato, cotton, potato, sorghum, soybean, cowpea and barley.

**Table 1.** ANOVA of cell membrane thermostability under normal and heat stressed conditions.

| SOV | df | SS | MS |
|---|---|---|---|
| Replication | 2 | 48.3 | 24.16 |
| Genotype | 119 | 24555.7 | 206.35 ** |
| Error (Rep × Gen) | 238 | 4248.2 | 17.85 |
| Treatment | 1 | 3062.5 | 3062.52 |
| Gen × Treatment | 119 | 4837.2 | 40.65 |
| Error (Rep × Gen × Treatment) | 240 | 5213.0 | 21.72 |

**Table 2.** Cell membrane thermostability mean comparisons for 120 genotypes.

| GEN | Mean | Homogeneous Groups | GEN | Mean | Homogeneous Groups |
|---|---|---|---|---|---|
| HT120 | 68.152 | A | HT73 | 50.232 | LMNOPQRSTUVWXYZabcdefghij |
| HT83 | 67.465 | A | HT24 | 50.172 | LMNOPQRSTUVWXYZabcdefghij |
| HT105 | 66.987 | A | HT117 | 50.163 | LMNOPQRSTUVWXYZabcdefghij |
| HT19 | 66.928 | A | HT86 | 50.018 | MNOPQRSTUVWXYZabcdefghij |
| HT3 | 66.373 | AB | HT8 | 49.952 | MNOPQRSTUVWXYZabcdefghij |
| HT88 | 65.28 | AB | HT72 | 49.654 | NOPQRSTUVWXYZabcdefghijk |
| HT36 | 65.145 | AB | HT50 | 49.572 | NOPQRSTUVWXYZabcdefghijkl |
| HT59 | 64.283 | ABC | HT63 | 49.538 | NOPQRSTUVWXYZabcdefghijkl |
| HT20 | 63.867 | ABC | HT1 | 49.499 | NOPQRSTUVWXYZabcdefghijkl |
| HT92 | 61.787 | BCD | HT29 | 49.383 | OPQRSTUVWXYZabcdefghijkl |
| HT114 | 60.095 | CDE | HT53 | 49.373 | OPQRSTUVWXYZabcdefghijkl |
| HT38 | 58.022 | DEF | HT80 | 49.367 | OPQRSTUVWXYZabcdefghijkl |
| HT60 | 56.294 | EFG | HT95 | 49.316 | OPQRSTUVWXYZabcdefghijkl |
| HT2 | 55.83 | EFGH | HT35 | 49.246 | OPQRSTUVWXYZabcdefghijkl |
| HT27 | 55.824 | EFGH | HT108 | 49.102 | PQRSTUVWXYZabcdefghijkl |
| HT119 | 55.787 | EFGH | HT23 | 49.061 | QRSTUVWXYZabcdefghijkl |

| GEN | Mean | Homogeneous Groups | GEN | Mean | Homogeneous Groups |
|---|---|---|---|---|---|
| HT71 | 55.481 | EFGHI | HT78 | 48.973 | QRSTUVWXYZabcdefghijklm |
| HT11 | 55.243 | FGHIJ | HT107 | 48.948 | QRSTUVWXYZabcdefghijklm |
| HT42 | 55.132 | FGHIJK | HT99 | 48.935 | QRSTUVWXYZabcdefghijklm |
| HT30 | 54.927 | FGHIJKL | HT84 | 48.742 | RSTUVWXYZabcdefghijklm |
| HT46 | 54.751 | FGHIJKLM | HT115 | 48.651 | STUVWXYZabcdefghijklm |
| HT55 | 54.232 | FGHIJKLMN | HT100 | 48.468 | TUVWXYZabcdefghijklm |
| HT43 | 54.017 | FGHIJKLMNO | HT62 | 48.306 | TUVWXYZabcdefghijklmn |
| HT74 | 53.957 | FGHIJKLMNO | HT16 | 48.256 | UVWXYZabcdefghijklmn |
| HT91 | 53.896 | FGHIJKLMNOP | HT64 | 48.242 | VWXYZabcdefghijklmno |
| HT33 | 53.617 | FGHIJKLMNOPQ | HT58 | 48.2 | WXYZabcdefghijklmno |
| HT106 | 53.603 | FGHIJKLMNOPQ | HT112 | 48.193 | WXYZabcdefghijklmno |
| HT81 | 53.574 | FGHIJKLMNOPQ | HT34 | 48.084 | WXYZabcdefghijklmno |
| HT76 | 53.501 | FGHIJKLMNOPQR | HT97 | 48.075 | WXYZabcdefghijklmno |
| HT13 | 53.46 | FGHIJKLMNOPQR | HT111 | 48.024 | XYZabcdefghijklmno |
| HT90 | 53.397 | FGHIJKLMNOPQRS | HT28 | 48.023 | XYZabcdefghijklmno |
| HT12 | 53.393 | FGHIJKLMNOPQRS | HT67 | 47.908 | YZabcdefghijklmno |
| HT87 | 53.318 | FGHIJKLMNOPQRS | HT96 | 47.855 | YZabcdefghijklmno |
| HT26 | 53.074 | GHIJKLMNOPQRST | HT47 | 47.843 | YZabcdefghijklmno |
| HT98 | 53.056 | GHIJKLMNOPQRSTU | HT48 | 47.759 | YZabcdefghijklmnop |
| HT54 | 53.022 | GHIJKLMNOPQRSTUV | HT61 | 47.617 | Zabcdefghijklmnop |
| HT6 | 52.866 | GHIJKLMNOPQRSTUVW | HT68 | 47.441 | abcdefghijklmnop |
| HT25 | 52.803 | GHIJKLMNOPQRSTUVWX | HT44 | 47.433 | abcdefghijklmnop |
| HT82 | 52.477 | GHIJKLMNOPQRSTUVWXY | HT41 | 47.344 | bcdefghijklmnop |
| HT52 | 52.45 | GHIJKLMNOPQRSTUVWXY | HT70 | 47.3 | cdefghijklmnop |
| HT57 | 52.382 | GHIJKLMNOPQRSTUVWXYZ | HT32 | 47.178 | defghijklmnop |
| HT45 | 52.214 | GHIJKLMNOPQRSTUVWXYZa | HT17 | 47.146 | defghijklmnop |
| HT21 | 52.142 | GHIJKLMNOPQRSTUVWXYZab | HT93 | 47.085 | defghijklmnop |
| HT22 | 52.052 | GHIJKLMNOPQRSTUVWXYZabc | HT77 | 46.623 | efghijklmnop |
| HT79 | 51.994 | GHIJKLMNOPQRSTUVWXYZabc | HT51 | 46.533 | fghijklmnop |
| HT85 | 51.991 | GHIJKLMNOPQRSTUVWXYZabc | HT37 | 46.254 | ghijklmnop |
| HT89 | 51.873 | GHIJKLMNOPQRSTUVWXYZabcd | HT4 | 45.947 | hijklmnop |
| HT10 | 51.817 | GHIJKLMNOPQRSTUVWXYZabcd | HT94 | 45.543 | ijklmnop |
| HT65 | 51.738 | GHIJKLMNOPQRSTUVWXYZabcd | HT69 | 45.474 | jklmnop |
| HT110 | 51.567 | GHIJKLMNOPQRSTUVWXYZabcd | HT66 | 44.986 | klmnop |
| HT15 | 51.393 | HIJKLMNOPQRSTUVWXYZabcde | HT104 | 44.821 | lmnop |
| HT18 | 51.314 | HIJKLMNOPQRSTUVWXYZabcdef | HT102 | 44.212 | mnopq |
| HT7 | 51.153 | HIJKLMNOPQRSTUVWXYZabcdef | HT116 | 43.634 | nopq |
| HT56 | 51.108 | HIJKLMNOPQRSTUVWXYZabcdef | HT103 | 43.442 | opq |
| HT14 | 50.886 | IJKLMNOPQRSTUVWXYZabcdefg | HT118 | 43.014 | pqr |
| HT39 | 50.818 | IJKLMNOPQRSTUVWXYZabcdefg | HT101 | 39.534 | qrs |
| HT31 | 50.551 | JKLMNOPQRSTUVWXYZabcdefgh | HT109 | 39.533 | qrs |
| HT9 | 50.501 | JKLMNOPQRSTUVWXYZabcdefgh | HT5 | 38.523 | rs |
| HT113 | 50.361 | KLMNOPQRSTUVWXYZabcdefgh | HT49 | 38.32 | rs |
| HT40 | 50.318 | LMNOPQRSTUVWXYZabcdefghi | HT75 | 37.595 | s |

Means in a column followed by a common letter do not differ ($P \leq 0.05$) by Fisher's protected LSD test.

Under both conditions the genotypes, which performed excellent results for CMT are V-13248, MISR 1, AARI- 11, V-13013 and V-12103. The cell membrane thermostability was recorded for normal (67.79, 67.87, 68.32, 68.97 and 69.32) and heat stressed (64.95, 65.98, 66.61, 65.00 and 66.98) conditions, respectively. These genotypes regarded as heat tolerant genotypes in this experiment (Fig. 1). This indicated presence of genes for heat tolerance as it shows stability under both environments. Our results are in accordance with Blum and Ebercon 1981, Saadalla *et al.*, 1990, Blum *et al.*, (2001), Dhanda and Munjal (2006). Reynolds *et al.* (2001), Blum *et al.* (2001), Dhanda and Munjal (2006) observed positive significant association among cell membrane thermostability and yield in wheat crop at anthesis stage.

It shows that tolerant genotypes of wheat, which have higher CMT values, gave more yield. Cell membrane thermostability (CMT) has also been used in previous studies to evaluate the heat stress (Sairam and Srivastava, 2001; Dhanda and Munjal, 2009).

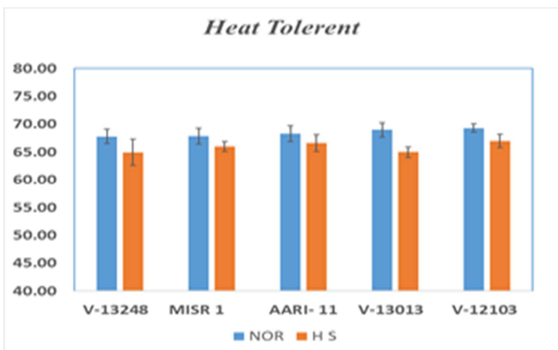


**Fig. 1.** Graph of five genotypes showing heat tolerance in wheat for cell membrane thermostability.

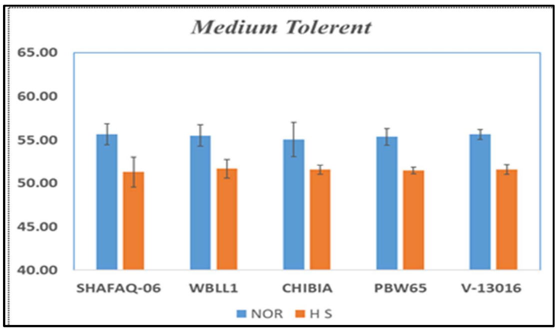


**Fig. 2.** Graph of five genotypes showing medium tolerance in wheat for cell membrane thermostability.

During heat stress, plants cell membrane raptures and leakage of electrolytes from membranes measured by CMT test (Saadalla *et al.*, 1990). Our results also depicted that genotypes; V-12056, Millat-11, Chenab- 2000, ND643 and V-12082 showed their responses toward heat susceptibility and their values of cell membrane thermostability under normal conditions (41.28, 44.06, 41.28, 42.84 and 43.15) and under high temperature; (35.77, 32.58, 33.91, 36.23 and 35.92) these genotypes performance was shown in Fig. 3. These results were in agreement with Behl, *et al.* (1993) also reported heat damage to plasma membrane destroys membrane integrity causing solute leakage from the cells.

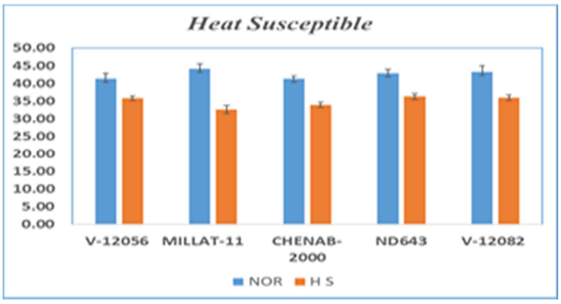


**Fig. 3.** Graph of five genotypes showing heat susceptibility in wheat for cell membrane thermostability.

Wheat genotypes Shafaq-06, WBLL1, CHIBIA, PBW65 and V-13016 have medium performance under normal (55.65, 55.51, 55.06, 55.36 and 55.63) and heat stressed (51.27, 51.64, 51.58, 51.44 and 51.58) conditions for cell membrane thermostability and revealed as medium tolerant genotype. In the present study, membrane stability was measured for high temperature stress through CMT from 120-wheat cultivar.

## Conclusions

This study provides evidence for the use of cell membrane thermostability for identifying different wheat varieties having heat tolerance, susceptibility and medium tolerance. Heat stress caused by rise in temperature, have adverse effect on wheat growth and development. Cell membrane thermostability is one of the major screening parameters for development of heat tolerant wheat genotypes. In this experiment, out of 120 spring wheat genotypes, 15 genotypes were designated as heat tolerant, heat susceptible and medium tolerant on their performance. For heat tolerant genotypes V-13248, MISR 1, AARI-11, V-13013 and V-12103 gave higher CMT values under both normal and heat stress conditions. This stability in two environments represents the presence of heat tolerant genes. Genotypes V-12056, Millat-11, Chenab-2000, ND643 and V-12082 were regarded as heat susceptible whereas Shafaq-06, WBLL1, CHIBIA, PBW65 and V-13016 showed their response towards medium tolerance.

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