



RESEARCH PAPER

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

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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:

$$\text{CMT} = 1 - (C_1/C_2) \times 100$$

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	LMNOPQRSTUVWXYZabcdefghijklmnopq
HT83	67.465	A	HT24	50.172	LMNOPQRSTUVWXYZabcdefghijklmnopq
HT105	66.987	A	HT117	50.163	LMNOPQRSTUVWXYZabcdefghijklmnopq
HT19	66.928	A	HT86	50.018	MNOPQRSTUVWXYZabcdefghijklmnopq
HT3	66.373	AB	HT8	49.952	MNOPQRSTUVWXYZabcdefghijklmnopq
HT88	65.28	AB	HT72	49.654	NOPQRSTUVWXYZabcdefghijklmnopq
HT36	65.145	AB	HT50	49.572	NOPQRSTUVWXYZabcdefghijklmnopq
HT59	64.283	ABC	HT63	49.538	NOPQRSTUVWXYZabcdefghijklmnopq
HT20	63.867	ABC	HT1	49.499	NOPQRSTUVWXYZabcdefghijklmnopq
HT92	61.787	BCD	HT29	49.383	OPQRSTUVWXYZabcdefghijklmnopq
HT114	60.095	CDE	HT53	49.373	OPQRSTUVWXYZabcdefghijklmnopq
HT38	58.022	DEF	HT80	49.367	OPQRSTUVWXYZabcdefghijklmnopq
HT60	56.294	EFG	HT95	49.316	OPQRSTUVWXYZabcdefghijklmnopq
HT2	55.83	EFGH	HT35	49.246	OPQRSTUVWXYZabcdefghijklmnopq
HT27	55.824	EFGH	HT108	49.102	PQRSTUVWXYZabcdefghijklmnopq
HT119	55.787	EFGH	HT23	49.061	QRSTUVWXYZabcdefghijklmnopq

GEN	Mean	Homogeneous Groups	GEN	Mean	Homogeneous Groups
HT71	55.481	EFGHI	HT78	48.973	QRSTUVWXYZabcdefghijklmnop
HT11	55.243	FGHIJ	HT107	48.948	QRSTUVWXYZabcdefghijklmnop
HT42	55.132	FGHIJK	HT99	48.935	QRSTUVWXYZabcdefghijklmnop
HT30	54.927	FGHIJKL	HT84	48.742	RSTUVWXYZabcdefghijklmnop
HT46	54.751	FGHIJKLM	HT115	48.651	STUVWXYZabcdefghijklmnop
HT55	54.232	FGHIJKLMN	HT100	48.468	TUVWXYZabcdefghijklmnop
HT43	54.017	FGHIJKLMNO	HT62	48.306	TUVWXYZabcdefghijklmnop
HT74	53.957	FGHIJKLMNO	HT16	48.256	UVWXYZabcdefghijklmnop
HT91	53.896	FGHIJKLMNOP	HT64	48.242	VWXYZabcdefghijklmnop
HT33	53.617	FGHIJKLMNOPQ	HT58	48.2	WXYZabcdefghijklmnop
HT106	53.603	FGHIJKLMNOPQ	HT112	48.193	WXYZabcdefghijklmnop
HT81	53.574	FGHIJKLMNOPQ	HT34	48.084	WXYZabcdefghijklmnop
HT76	53.501	FGHIJKLMNOPQR	HT97	48.075	WXYZabcdefghijklmnop
HT13	53.46	FGHIJKLMNOPQR	HT111	48.024	XYZabcdefghijklmnop
HT90	53.397	FGHIJKLMNOPQRS	HT28	48.023	XYZabcdefghijklmnop
HT12	53.393	FGHIJKLMNOPQRS	HT67	47.908	YZabcdefghijklmnop
HT87	53.318	FGHIJKLMNOPQRS	HT96	47.855	YZabcdefghijklmnop
HT26	53.074	GHIJKLMNQRST	HT47	47.843	YZabcdefghijklmnop
HT98	53.056	GHIJKLMNQRSTU	HT48	47.759	YZabcdefghijklmnop
HT54	53.022	GHIJKLMNQRSTUV	HT61	47.617	Zabcdefghijklmnop
HT6	52.866	GHIJKLMNQRSTUVW	HT68	47.441	abcdefghijklmnop
HT25	52.803	GHIJKLMNQRSTUVWX	HT44	47.433	abcdefghijklmnop
HT82	52.477	GHIJKLMNQRSTUVWXY	HT41	47.344	bcdefghijklmnop
HT52	52.45	GHIJKLMNQRSTUVWXY	HT70	47.3	cdefghijklmnop
HT57	52.382	GHIJKLMNQRSTUVWXYZ	HT32	47.178	defghijklmnop
HT45	52.214	GHIJKLMNQRSTUVWXYZa	HT17	47.146	efghijklmnop
HT21	52.142	GHIJKLMNQRSTUVWXYZab	HT93	47.085	defghijklmnop
HT22	52.052	GHIJKLMNQRSTUVWXYZabc	HT77	46.623	efghijklmnop
HT79	51.994	GHIJKLMNQRSTUVWXYZabc	HT51	46.533	ghijklmnop
HT85	51.991	GHIJKLMNQRSTUVWXYZabc	HT37	46.254	ghijklmnop
HT89	51.873	GHIJKLMNQRSTUVWXYZabcd	HT4	45.947	hijklmnop
HT10	51.817	GHIJKLMNQRSTUVWXYZabcd	HT94	45.543	ijklmnop
HT65	51.738	GHIJKLMNQRSTUVWXYZabcd	HT69	45.474	jklmnop
HT110	51.567	GHIJKLMNQRSTUVWXYZabcd	HT66	44.986	klmnop
HT15	51.393	HIJKLMNQRSTUVWXYZabcde	HT104	44.821	lmnop
HT18	51.314	HIJKLMNQRSTUVWXYZabcde	HT102	44.212	mno
HT7	51.153	HIJKLMNQRSTUVWXYZabcde	HT116	43.634	nop
HT56	51.108	HIJKLMNQRSTUVWXYZabcde	HT103	43.442	op
HT14	50.886	IJKLMNQRSTUVWXYZabcde	HT118	43.014	pqr
HT39	50.818	IJKLMNQRSTUVWXYZabcde	HT101	39.534	qrs
HT31	50.551	JLMNOPQRSTUVWXYZabcde	HT109	39.533	qrs
HT9	50.501	JLMNOPQRSTUVWXYZabcde	HT5	38.523	rs
HT113	50.361	KLMNOPQRSTUVWXYZabcde	HT49	38.32	rs
HT40	50.318	LMNOPQRSTUVWXYZabcde	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).

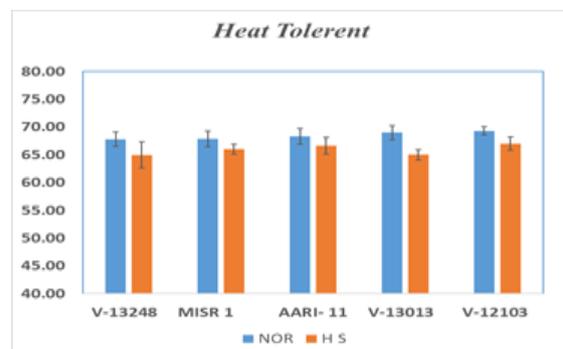


Fig. 1. Graph of five genotypes showing heat tolerance in wheat for cell membrane thermostability.

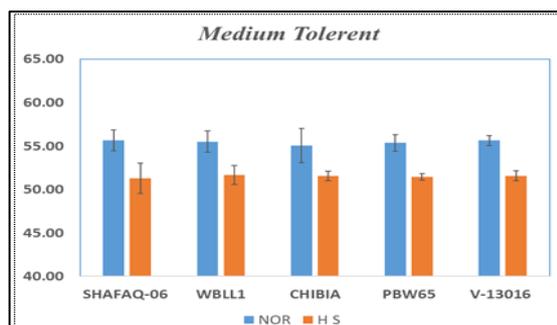


Fig. 2. Graph of five genotypes showing medium tolerance in wheat for cell membrane thermostability.

During heat stress, plants cell membrane ruptures 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.

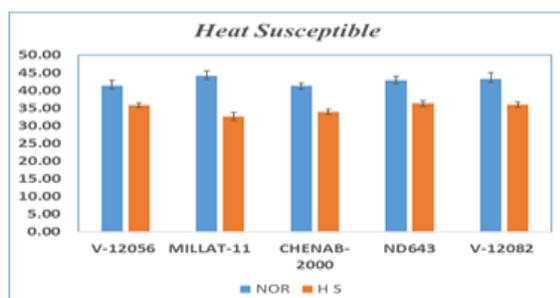


Fig. 3. Graph of five genotypes showing heat susceptibility in wheat for cell membrane thermostability.

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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