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EVALUATION OF SPRING WHEAT GENOTYPES FOR TERMINAL HEAT STRESS

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SUMMARY

Plant breeders exploit available germplasm for the development and improvement of different wheat genotypes. Cell membrane thermostability (CMT) and relative water content (RWC) are good selection criteria for effective selection against heat tolerance. This investigation, 120 spring wheat genotypes were evaluated for the effect of heat stress. Among the 120 spring wheat genotypes, five were identified as heat-tolerant (HT) genotypes, whereas five were found to be heat-susceptible (HS) genotypes on the basis of their relative values of CMT, RWC, and grain yield per plant (GYP). In this experiment, 120 spring wheat genotypes were used to evaluate heat tolerance in an open field (normal) and in a tunnel (heat stress). Analysis of variance depicted highly significant differences among genotypes under both environments. Higher values of CMT and RWC along with GYP were associated with heat tolerance and vice versa. The best-performing HT genotypes under both environmental conditions were V-13248, V-13013, AARI- 11, MIRAJ-08, and V-12103. They could be used as donors for heat stress tolerance in breeding program. Among the 120 wheat genotypes, ND643, V-12082, V-12056, WBLL1, and V-12066 were proven to be HS genotypes in both environments. Given that the identified superior HT genotypes can cope with heat stress, they can be utilized for developing HT genotypes. In addition to grain yield, CMT and RWC are good markers for the development of HT wheat genotypes.

Keywords: Genetic analysis, heat tolerance, heat susceptibility, cell membrane thermostability, relative water content, spring wheat

Key findings: The wheat genotypes V-13248, V-13013, AARI- 11, MIRAJ-08, and V-12103 performed best under normal and heat stress conditions and were identified as HT genotypes. Low values of CMT, RWC, and grain yield revealed susceptible wheat genotypes. Genotypes ND643, V-12082, V-12056, WBLL1, and V-12066 were concluded to be HS genotypes on the basis of their relative performance.

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INTRODUCTION

Wheat has a leading and important position among all cereals. People use wheat to make different kinds of food products due to its nutritional value. In the Indian subcontinent, especially in Pakistan, wheat is one of the most commonly consumed grain cereals (Khan *et al*., 2018). In many parts of the world, heat stress damages wheat at the vegetative and grain-filling stages and causes reductions in yield (Shaukat *et al*., 2018). High-temperature stress is one of major environmental constraints to wheat yield. Heat stress is a major problem for farmers in field cropping systems worldwide because unexpected environmental variations cause reductions in wheat growth and productivity (Parent *et al*., 2010). Each 1 °C increment in ambient temperature results in a 3%–10% reduction in wheat grain yield (You *et al*., 2009). Reynolds *et al.* (2011) reported that approximately 40% of wheat (36 million hectares) grown in temperate environments is facing terminal heat stress. At least a 4% increase is needed to fulfill the demand of our country and its currently growing population (Khan *et al*., 2015). In Pakistan, high temperatures at the grain-filling stage of plant growth severely damage wheat yield. Heat and drought stresses at the grain-filling stage disturb the grain maturity cycle, reduces the number of grains, and decreases grain weight; these effects ultimately cause reductions in grain yield and quality deterioration (Khan *et al*., 2007; Wahid *et al*., 2007; Ozturk and Aydin, 2017).

Cell membrane thermostability (CMT) quantifies the change in electrolyte leakage resulting from the induction of heat stress that changes cell membrane permeability. In wheat, electrical conductivity has been used as an index of CMT to identify heat-tolerant (HT) genotypes (Blum and Ebercon, 1981). High temperatures can cause damage to cell membrane integrity and primary photosynthetic process, changes in lipid composition, and protein denaturation (Wahid *et al*., 2007). Blum *et al.* (2001) reported that genotypes with high CMT values at the anthesis stage in spring wheat are HT genotypes. Ibrahim and Quick (2001) studied membrane thermostability and concluded that it is an indicator of heat stress. HT wheat genotypes with increased grain yield can be developed by using CMT because it is a good marker for selection (Shaukat *et al*., 2018).

Relative water content (RWC) has been used to identify the effect of abiotic stresses, such as drought and heat stress, on wheat. Tolerant cultivars maintain dry shoot weight and RWC of tolerant cultivars under stress conditions (Balouchi, 2010). Arjenaki *et al.* (2012) concluded that reduction in plant vigor is associated with the reduction in RWC contents in crops under heat stress conditions. Ram *et al*. (2017) worked on osmotic potential and concluded that water potential and grain yield are the most important indicators of heat tolerance in wheat. Rad *et al.* (2013) studied the use of RWC as an efficient selection measure for various stress resistance mechanisms. Khakwani *et al*. (2011) suggested that grain yield and RWC are good criteria for heat and drought stress tolerance. In this era of changing climatic conditions, the massive gap between the productivity potential and actual production of various crops should receive focus and treated accordingly (Cattivelli *et al*., 2008). Wheat varieties that can withstand different stresses, such as abiotic stresses, particularly heat tolerance, must be developed to fulfill the food demand in coming years (Iqbal *et al*., 2017). Identifying genotypes that not only have the capability to avoid, escape, and show tolerance against different stresses but also have the potential to provide increased grain yields under heat stress conditions is needed.

This investigation was thus designed to evaluate the genetic behavior of CMT along with RWC and to select HT genotypes for future breeding for the development of new cultivars with desirable attributes.

MATERIALS AND METHODS

Germplasm consisting of 120 spring wheat genotypes was evaluated for heat tolerance at the Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan, during the 2014–2015 cropping season. The germplasm was sown in two sets—one in an open field and another in plastic sheet tunnel adjacent to a field—in randomized complete block design with three replications. Normal agronomic and cultural practices were applied throughout the crop growing season. The genotypes sown in the tunnel were exposed to heat stress at the grain-filling stage. The temperature at the inner and outer sides of the tunnel was recorded on a daily basis. A 5 °C increment in temperature was noted inside the tunnel.

CMT

CMT was recorded by using the method proposed by Saadalla *et al.* (1990) and modified by Ciuca and Petcu (2009).

RWC

RWC was measured by using the protocol and formula described by Ram *et al.* (2017).

Grain yield per plant

Spikes from selected plants were threshed separately at maturity and weighed with an electric balance (Compax- Cx-600) to obtain grain yield.

Statistical analysis

The collected data were subjected to analysis of variance (Steel *et al*., 1997). Relationships between the traits were investigated through correlation among stress selection indexes and grain yield under stress and nonstress conditions (Singh and Chaudhary, 1997). Excel 2016 and Minitab 17.0 software were also used to perform statistical analysis.

RESULTS AND DISCUSSION

Breeding for heat stress tolerance is becoming increasingly important with each passing day owing to global warming. A wide range of variability was found for all traits in all wheat genotypes under study. The mean values for all three traits under normal and heat stress conditions are depicted in Figure 1. This study, membrane stability was measured in the form of CMT to represent high temperature tolerance. The RWCs of 120 wheat genotypes and HT and HS genotypes are shown in Table. 2. Analysis of variance showed highly significant differences among all genotypes under study (Table 1). Screening was performed on different parameters to identify HT and heat-susceptible (HS) genotypes with respect to CMT (Figure 2), RWCs (Figure 3), and grain yield per plant (GYP, Figure 4).

Blum and Ebercon (1981) reported that high CMT values are associated with heat stress when using this method against to screen for heat-stress-tolerant wheat genotypes. Molecules present across the membranes exhibit elevated kinetic energy because increased temperature disturbs the turgidity of cell membranes. This elevation in kinetic energy occurs due to the high incidence of unsaturated fatty acids and the denaturation of protein (Savchenko *et al*., 2002). The genotypes showing high values of CMT were considered to be tolerant to increased temperature. CMT

Table 1. Analysis of variance for CMT, RWC, and GYP under normal and heat stress conditions.

Source of variation	d.f.	CMT	RWC	GYP
Replications	2	41.80	19.46	5.79
Genotypes	119	189.06**	107.87**	14.34**
Error (Rep × Gen)	238	20.38	35.01	3.47
Treatments	1	3168.07	1901.73	96.70
Gen × Treatment	119	43.58	18.20	4.64
Error (Rep × Gen × Treatment)	240	23.10	14.17	3.02

Table 2. Mean values for HT and susceptible genotypes under normal and heat stress conditions.

Genotypes	CMT		RWC		GYP	
	Normal	Heat stress	Normal	Heat stress	Normal	Heat stress
ND643	42.84	37.66	52.22	51.17	19.30	19.34
V-12082	43.15	36.45	52.65	50.99	19.30	19.89
V-12056	43.99	39.87	56.39	51.77	20.61	21.21
WBLL1	46.38	40.50	62.81	65.66	22.08	21.03
V-12066	48.31	37.71	67.83	65.02	24.61	20.78
V-13248	67.79	64.95	72.77	66.19	26.10	26.52
V-13013	68.97	61.26	72.69	72.80	25.53	27.00
AARI-11	68.32	66.61	74.04	71.69	25.77	25.40
MIRAJ-08	67.62	60.95	74.16	71.99	26.43	26.17
V-12103	69.32	62.28	71.77	69.76	27.21	26.40

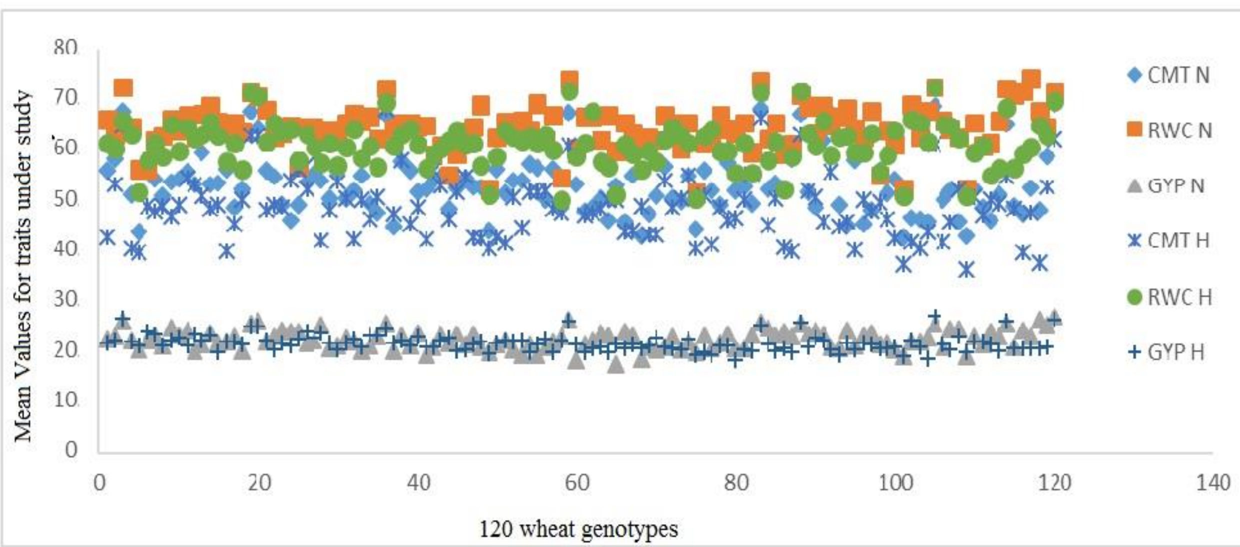

Figure 1. Scatter plot of the mean values of CMT, RWC, and GYP for 120 wheat genotypes under normal (N) and heat stress (H) conditions.

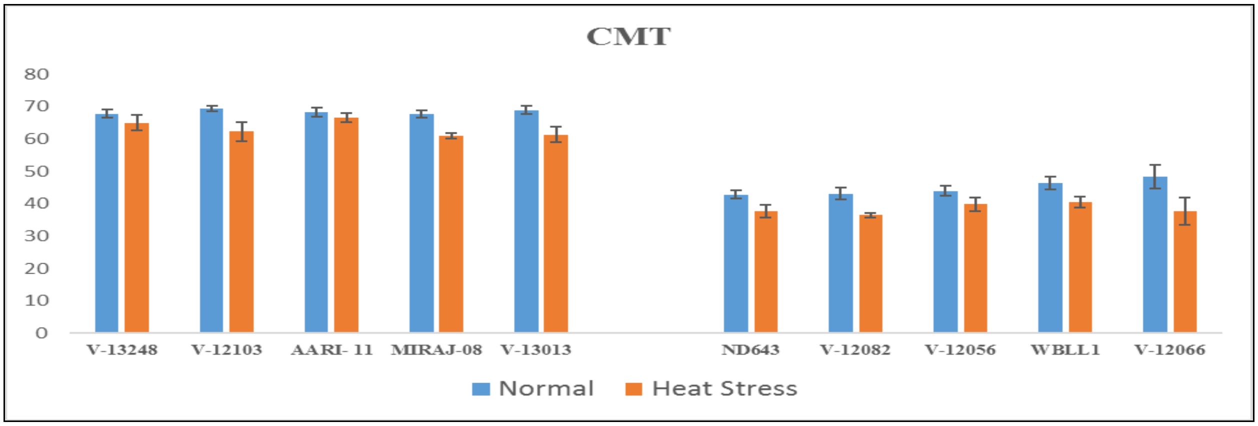

Figure 2. CMT of HT and HS genotypes.

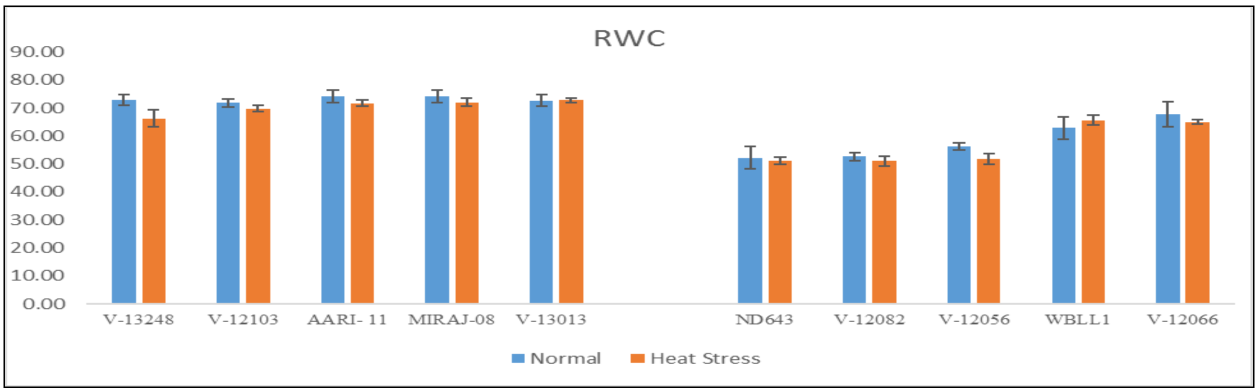

Figure 3. RWC of HT and HS genotypes.

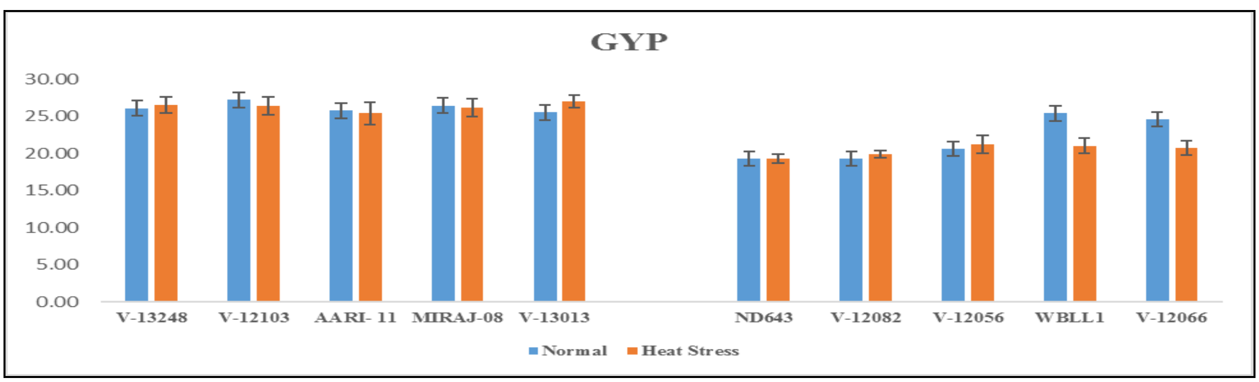

Figure 4. GYP of HT and HS genotypes.

has been used for the indirect selection of HT germplasm of various field crops, such as cotton, wheat, tomato, soybean, potato, and sorghum (Wahid *et al*., 2007). Cultivars V-12103, V-13013, AARI-11, V-13248 and MIRAJ-08 exhibited the highest CMT values, viz., 69.32, 68.97, 68.32, 67.79, and 67.62, respectively, under normal conditions, and CMT values of 62.28, 61.26, 66.61, 64.95, and 60.95, respectively, under heat stress conditions. Genotypes V-12066, WBLL1, V-12056, V-12082, and ND643 presented the lowest CMT values of 48.31, 46.38, 43.99, 43.15, and 42.84, respectively, under normal conditions and CMT values of 37.71, 40.50, 39.87, 36.45, and 37.66 under heat stress conditions, thus showing susceptibility to heat stress (Table 1).

High values of CMT reflect tolerance against heat stress as reported by Bala and Sikder (2017). On the basis of their CMT results, five genotypes were identified as HT genotypes that performed relatively well under normal and heat stress conditions. Five genotypes were designated as HS on the basis of CMT. The range of CMT under heat stress tended to decrease slightly compared with that under normal conditions. Genotypes that could withstand high temperatures were designated as HT and were selected in further breeding programs, whereas those with low values were HS. The results of this study are in line with the results reported by Renu *et al.* (2004), Kaur *et al.* (2008), and Khan *et al.* (2013). CMT is used to evaluate heat stress tolerance in different crops (Blum, 1988). The results of the present work were in agreement with the findings of Behl *et al.* (1993) and showed that heat stress damage to the plasma membrane destroys membrane integrity, thus causing solute leakage from cells.

RWC has been proposed to be a more important indicator of water status than other water potential parameters under stress (drought and heat) conditions. High RWC is directly associated with the resistance mechanism to drought and heat stress and is the result of increased osmotic regulation in plants (Ritchie *et al*., 1990). V-13248, V-13013, AARI- 11, MIRAJ-08, and V-12103 showed the peak RWC values of 72.77, 72.69, 74.04, 74.16 and 71.77) under normal conditions and 66.19, 72.80, 71.69, 71.99, and 69.76 under heat stress. Thus, these genotypes were considered as HT genotypes. Among the studied germplasm, the poorly performing HS genotypes under normal and heat stress conditions were ND643, V-12082, V-12056, WBLL1, and V-12066 (52.22, 52.65, 56.39, 62.81, and 67.83) and (51.17, 50.99, 51.77, 65.66, and 65.02). CMT and RWC with respect to yield per plant have been used in previous studies to evaluate heat stress (Sairam and Srivastava, 2001; Dhanda and Munjal, 2009).

As concluded from the current investigation, high RWC is associated with plant resistance to heat stress. Almeselmani *et al.* (2012), Saxena *et al.* (2014), Ram *et al.* (2017) and Ramani *et al.* (2017) reported that the RWC of plants decreased under heat stress. The results of our study were in accordance with the results of these scientists.

GYP decreased under heat stress at vegetative and reproductive growth stages. Grain yield reduction can be attributed due to the effects of heat stress during the grain-filling period on growth and developmental processes in wheat, resulting in reduced GYP (Wahid *et al*., 2007). Shpiler and Blum (1991) stated that a reduction in the number of spikelets/spike along with a reduction in the number of kernels/spike in wheat crop under heat stress ultimately causes reductions in GYP. The HT genotypes V-13248, V-13013, AARI-11, MIRAJ-08, and V-12103 showed GYP values of 26.10, 25.53, 25.77, 26.43, and 27.21 under normal conditions and GYP values of 26.52, 27.00, 25.40, 26.17, and 26.40 under heat stress and were thus considered as tolerant genotypes.

The poorly performing HS genotypes under normal and heat stress conditions were ND643, V-12082, V-12056, WBLL1, and V-12066 (19.30, 19.30, 20.61, 22.08, and 24.61) and

Table 3. Correlation coefficients among CMT, RWC, and GYP under normal and heat stress conditions

Stress conditions		RWC		CMT	
		Normal	Heat Stress	Normal	Heat Stress
CMT	Normal	0.5069**	-	-	-
	Heat Stress	-	0.4918**	-	-
GYP	Normal	0.4610**	-	0.4249**	-
	Heat Stress	-	0.5528**	-	0.5710**

* = Significant (<0.05), ** = Highly Significant (<0.01)

(19.34, 19.89, 21.21, 21.03, and 20.78). The results of this study revealed a reduction in grain yield under heat stress. Long-term exposure to high temperature results in drastic wheat yield reduction under changing climatic conditions. The present results were in accordance with the past findings of Bluementhal *et al.* (1995), Gibson and Paulsen, (1999) and Wardlaw *et al.* (2002) in wheat.

CORRELATION

Correlation measures the intensity of the relationship among two or more random variables. Under normal and heat stress conditions, CMT and RWC showed positive and highly significant correlation with GYP as shown in Table 3. CMT and RWC showed a strong positive correlation with GYP in wheat. This result provided positive evidence for the improvement of high-yielding wheat varieties with heat tolerance. Similar to the trends found by Fokar *et al.* (1998) and Yildirim *et al.* (2009) for CMT and RWC, the results of the present work showed significant positive correlations between yield and related traits. In contrast to the present study, previous studies showed that under heat stress, RWC tends to decline and is negatively correlated with grain yield (Sairam *et al.*, 2000; Savicka and Skute, 2012; Ram *et al*., 2017)

CONCLUSIONS

Improving wheat yield under different environmental stresses, among which heat stress is a major stress, is necessary to overcome the need and supply gap of food production. Heat stress during the reproductive and grain-filling stages represent several threats to wheat productivity. Plant breeders exploit available genetic resources for the development and improvement of different wheat genotypes. As concluded from the results for CMT, RWC, and GYP, the following genotypes performed best under normal and heat stressed conditions and were identified as HT genotypes: V-13248, V-13013, AARI- 11, MIRAJ-08, and V-12103. Low values of CMT, RWC, and grain yield reflected the susceptibility of wheat genotypes. On the basis of relative performance, genotypes ND643, V-12082, V-12056, WBLL1, and V-12066 were concluded to be HS.

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