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Selection for Cell Membrane Thermostability and Stomatal Frequency Under Drought and Heat Stress Conditions in Wheat (*Triticum aestivum* L).*

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Key words: cell membrane thermostability, stomatal frequency, heat and drought stresses

Abstract

Two successive cycles of divergent selection for cell membrane thermostability (CMS) were carried out from within F_3 families of five populations of wheat under heat stress as well as under drought + heat stresses. Another cycle of divergent selection for stomatal frequency was also applied under drought + heat stress to the F_4 families selected for CMS in order to generate F_5 families with different combinations of high CMS-high stomata, high CMS – low stomata, low CMS-high stomata and low CMS-low stomata. The descended F_6 families with such combinations were field evaluated for grain yield per plant and 1000 grain weight under favorable, heat stress and drought + heat stress conditions. Significant positive responses to selection for CMS were obtained in the five populations which averaged 26.29% and 26.53% in the F_4 and F_5 high CMS selections, respectively and 25.21% and 24.30% in the F_4 and F_5 selections for low CMS. Positive responses of 19.57% and 8.71% were obtained in the high and low stomatal frequency F_5 selections, respectively, under heat stress. Selection for higher CMS did not produce any significant correlated responses in grain yield per plant under favorable or drought stress conditions although CMS was strongly correlated with grain yield per plant under drought +heat stresses ($r = 0.80$, $p < 0.01$) as well as under drought stress ($r = 0.64$, $p < 0.01$). However, positive correlated responses were obtained in 1000 grain weight under drought + heat stresses which averaged 6.45% but not under drought stress alone. Meanwhile, positive correlated responses to selection for high stomatal frequency were displayed in grain yield per plant as well as in 1000 grain weight under both favorable and heat stress conditions.

Among the four combinations of CMS and stomatal frequency tested, the F_6 families with the high CMS-high stomata were the top in grain yield per

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plant with increments over unselected bulks averaging 17.48% under favorable conditions, 11.85% under heat stress and 17.83% under drought + heat stresses. Similarly, increments in 1000 grain weight averaged 8.89, 8.53 and 9.45% under favorable, heat stress and drought + heat stresses, respectively. Such increments were attributed to the synergistic effects of elevated stomatal frequency and consequently transpirational cooling of canopy with cellular membrane thermostability.

Introduction

Drought due to insufficient soil water supply frequently occurs concurrently with high temperature at the end of wheat growing season in the regions of the world with Mediterranean climate like Egypt. Drought, depending on its timing and duration, causes 10-61% reduction in grain mass (Cseuz *et al*, 2002) while heat stress causes 10-15% reduction in grain yield due mainly to reduced single grain weight (Wardlaw and Wrigly, 1994).

Drought tolerance mechanisms allow the plant to maintain turgor and volume thus continue metabolism even at a low water potential (Turner and Jones, 1980). Such mechanisms include osmotic adjustment and cellular membrane stability, i. e. the ability of the plant to limit cell membrane damage during water stress and regaining membrane integrity and membrane-bound activities quickly upon rehydration (Bewely, 1979).

High temperature affects plants through acceleration of phenology and the impairment of the physiology of photosynthesis and grain filling resulting in yield losses (Stone, 2001). It also causes leaf tissue injuries and increased cellular membrane permeability leading to electrolyte leakage out of the cell. Cell membrane stability is assayed by the electroconductivity of aqueous solution containing leaf discs that were either water stressed *in vitro* by exposure to a solution of polyethylene glycol (Bulm and Ebercon ,1981) or heat stressed by exposure to high temperature (Shanahan *et al*, 1990).

Variation in cellular membrane stability was reported to be related to variation in grain yield in wheat under drought (Blum and Ebercon, 1981; Tripathy *et al*, 2000 and Blum *et al*, 2001) as well as under heat stress (Shanahan *et al*, 1990; Ibrahim and Quick 2001 a, b and Blum *et al*, 2001).

Selection for cell membrane thermostability (CMS) was successfully carried out in wheat by Omara *et al,* (2006) who obtained a 19.5% average response in the F_3 families selected for greater CMS and correlated responses averaging 15.7% in grain weight per spike and 8.25% in 1000 grain weight but the effect on grain yield per plant was rather limited. Moreover, the association between CMS and grain yield under heat stress was reported by Blum *et al*, (2001) to be reasonably strong but not perfect indicating that heat avoidance besides CMS may also support grain yield under high temperature. Among the heat avoidance mechanisms, canopy temperature depression by transpirational cooling was found to be related to grain yield under heat stress in wheat (Lu *et al*, 1994 and Reynolds *et al*, 1998). Since transpiration normally takes place at leaf surfaces via stomata, increasing stomatal frequency per leaf unit area might lead to increasing transpiration rate since the two characters are positively correlated in wheat (Maghsoudi and Maghsoudi, 2008). However, tolerance to drought requires reducing water loss from the plant via transpiration by selecting for smaller stomatal frequency since a 25% decrease in stomatal frequency was found to cause a 24% reduction in transpiration rate (Koy *et al*, 1972) or stomatal closure control (James, 2007).. Thus, it appears that in addition to cellular membrane stability, the stomatal characteristics related to heat tolerance in wheat might be different from those associated with drought tolerance. Evidently, drought and heat tolerance in wheat were not correlated (Blum and Ebercon, 1981). Moreover, Reynolds *et al*, (2001) reported that a drought resistant wheat cultivar might not be heat tolerant. The present study explores the impacts of selection for cell membrane thermostability and stomatal frequency on tolerance to drought and heat stresses in wheat (*Triticum aestivum* L). Two successive cycles of divergent selection for CMS were carried out under drought and heat stresses starting with F_3 families which resulted from an initial cycle imposed on F_2 segregates of five populations under heat stress. Another cycle of divergent selection for stomatal frequency was also applied to the F_4 families selected for CMS under heat stress in order to generate F_5 families with different combinations of high CMS-high stomata, high CMS – low stomata, low CMS-high stomata and low CMS-low stomata. The descended F_6 families with those combinations were field evaluated for grain yield per plant and 1000 grain weight under favorable, heat stress and drought + heat stress conditions. The objectives of the study were:

1- to measure the response to selection for CMS and stomatal frequency under drought and heat stresses.

2- to assess the correlated responses in grain yield and 1000 grain weight under favorable, heat stress and drought + heat stresses.

3- to determine the relative impacts of CMS and stomatal frequency on the grain yield of the selected families having different combinations of the two characters under favorable and stress environmental conditions.

Materials and methods

Plant material

The plant material used in this study consisted of 50 F_3 families which represented the outcome of an initial cycle of divergent phenotypic selection for high and low CMS applied to five F_2 populations of *Triticum aestivum* L grown under heat stress conditions (Omara *et al*, 2006).

The second cycle of selection for CMS:

In the present study, the second cycle of divergent phenotypic selection was applied to the F_3 selected families of the five populations. From within each of the five F_3 families of each population, the highest and the lowest two plants in CMS score (2/15 = 13.3% selection intensity after pooling plants over blocks) were selected and the seeds of the two plants of each family were pooled to form the selected F_4 families.

In 2006-2007 season, seeds of the selected F_4 families of the five populations along with their relevant F_4 unselected bulks were planted in three different environments, namely, favorable, drought stress and combined drought and heat stresses. In the favorable environment, the 11 entries of each population (five high + five low + bulk) were sown into the clay fertile soil of the Faculty Farm in an optimal sowing date (26th November). For the drought stress environment, the entries were sown in 29nd November in the sandy calcareous soil of El-Ghorieb Experimental Station which is located in the eastern desert 25 km south of Assuit where soil contains 80% sand and 19% calcium carbonates. As for the environment with combined drought and heat stresses, the seeds of the 55 entries were sown into the sandy-calcareous soil of the El-Ghorieb Exp. Station in a late sowing date (28th December) so as to allow the drought stressed plants to be exposed to sporadic heat stress waves when temperature rises in March and April while plants were at anthesis. In each environment, seeds were sown in a complete randomized block design with three replications with each entry represented in each block by a row of ten plants spaced 15 cm apart and rows set 30 cm from each other. Flag leaf samples were collected from five randomly chosen plants for each entry grown in the drought + heat environment for CMS assay. After maturity, grain yield per plant and 1000 grain weight were determined in five guarded plants randomly chosen from each entry.

The third cycle of selection for CMS:

The third cycle of phenotypic divergent selection for CMS was practiced under the combined drought and heat stresses conditions. Family selection was practiced by picking the highest family in CMS among the five F_4 high selections and the lowest CMS family among those in the low CMS direction. Meanwhile within each family, the three plants with the highest CMS score were selected to form 3 F_5 high CMS families in each population (1/5*3/15 = 4% selection intensity) and the three plants with the lowest CMS score were also selected from the F_4 family with the lowest CMS score of each population. In 2007-2008 season, seeds of the three plants selected for CMS in each family of each population were sown in the clay soil of the Faculty Farm in two different sowing dates, namely an optimal date (25th November) and a late date (2nd January). Complete randomized block design with three replications was used with each entry represented in each block by a row of ten plants spaced 15 cm apart within rows set 30 cm from each other. Flag leaves were sampled from five random plants of each entry in the heat stressed environment for CMS assay. Grain yield per plant and 1000 grain weight were determined for each entry in the two environments in five guarded plants randomly chosen.

Selection for stomatal frequency:

Selection for stomatal frequency was imposed on the F_4 families selected for high and low CMS which were raised in 2006-2007 under combined drought and heat stresses in the sandy soil. For each of the five populations, the highest and the lowest family in stomatal frequency were selected in each direction (high and low CMS). Within each family, the highest and the lowest three plants in stomatal frequency were selected to form the selected F_5 families of each population (1/10*3/15= 2 % selection intensity).

In 2007-2008 season, seeds of the three F_5 plants selected for stomatal frequency in each population as well as the unselected F_5 bulks were sown into the field of the Faculty Farm in two sowing dates namely a normal (25th November) and a late sowing date (2nd January) so chosen as to allow the selected plants to be exposed to the heat stress resulting when temperature rises later in the growing season. Complete randomized block design with three replications was used within each environment where each entry was represented in each block by a row of 10 plants spaced 15 cm apart within rows set 30 cm apart. Flag leaves of five random plants were sampled from each entry for stomatal frequency determination. Grain yield per plant and 1000 grain weight were determined on five guarded plants randomly chosen.

Establishing different combinations of CMS and stomatal frequency:

Within each of the six F_5 families of the five populations

and low stomatal frequency were determined to form two families. The descended F_6 progenies of the 40 entries (four combinations for each of the five populations) along the five F_6 unselected bulks were field evaluated in 2008-2009 season under favorable, heat stress and drought + heat stress conditions. In the favorable environment the 45 entries were sown into the clay fertile soil of the Faculty Farm in an optimal sowing date (28th November). For the heat stress experiment, the 45 entries were sown in a late date (2nd January) in the Faculty Farm. As for the environment with combined drought and heat stresses, the seeds of the 45 entries were sown at the El-Ghorieb Exp. Station in a late sowing date (1rst January) so as to allow the drought stressed plants to be exposed to heat stress when temperature rises in March and April while plants at flowering. Seeds were planted in each environment in a complete randomized block design with three replications where each entry was represented in each block by a row of ten plants spaced 15 cm within rows set 30 cm from each other. Grain yield per plant and 1000 grain weight were determined for each entry in

raised in 2007-2008 season, two plants that combined: high CMS and high stomatal frequency, high CMS and low stomatal frequency, low CMS and high stomatal frequency and low CMS

each block in a random sample of five guarded plants

Cell membrane thermostability assay:

The CMS assay was performed according to the method described by Fokar *et al,.* (1998a). CMS was calculated as the reciprocal of cell membrane injury after Blum and Ebercon (1981), using the formula:

CMS (%) =

$$\frac{\left(1 - {T_1}/{T_2}\right)}{\left(1 - {C_1}/{C_2}\right)} x100$$

Where: T and C refer to heat treatment and control, respectively and 1 and 2 refer to initial and final conductance readings, respectively.

Stomatal frequency measurement

Flag leaf samples were taken from five random plants of each of the selected and bulk F_4 families in the same day, right after flowering was complete. For each sample, a 1 cm segment was taken from the bottom part of the flag leaf, placed in a capped vial containing Carnoy's solution and was transferred to the laboratory for stomatal frequency estimation.

Stomatal frequency was determined on the adaxial surface of the flag leaf along each side of the mid-rib. Before microscopic examination, flag leaf samples were immersed in lactic acid for 15 to 20 minutes. Stomata were counted under the 40 x objective lens of a light microscope in 10 microscopic fields for each sample. The average stomatal frequency per 10 fields was then converted to average stomatal frequency per mm^2 (each field corresponded to 1/6.0172mm^2).

Heritability estimation

Heritability of each character was estimated by two methods:

1- Realized heritability was calculated as:

$$h^2 = \frac{[\overline{H}_S - \overline{L}_S]}{[\overline{H}_B - \overline{L}_B]}$$

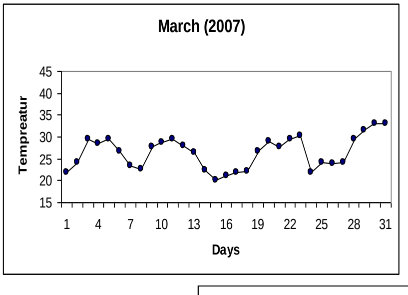
where: $\overline{H}_S$ and $\overline{L}_S$ are the average of the F_n families selected for a trait in the high and low directions, respectively while $\overline{H}_B$ and $\overline{L}_B$ are the average of the F_{n-1} families selected for that trait in the two directions (Ibrahim and Quick, 2001, a).

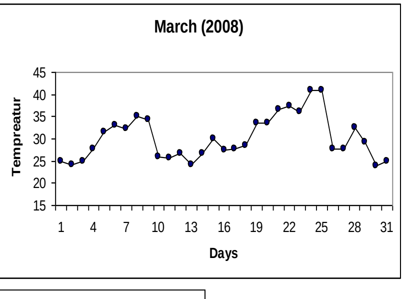
2-Parent-offspring regression (b_{po}) was determined for each character by regressing the means of the F_n selected families on the means of their corresponding F_{n-1} progenitor plants.

The expected response to selection for CMS and stomatal frequency was calculated using the formula: R=h^2S where R is the expected response, h^2 is the heritability and S is the selection differential (**Falconer, 1989**).

Daily temperatures

The recorded temperatures during March 2007, 2008 and 2009 indicated that heat waves have occurred with temperature rising above 34°C for several days which coincided with the post flowering stages of plant development (**Fig. 1**).





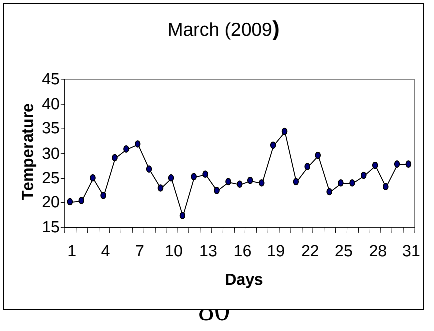


Fig. 1: Maximum daily temperatures during March 2007, 2008 and 2009.

Results

Response to selection in the F_4 and F_5 families for CMS

Significant positive responses to selection for CMS were obtained in the high and low directions in the F_4 families of the five populations raised under combined drought and heat stresses (Table 1).

The observed responses to selection for higher CMS varied considerably among the five populations ranging from 8.27 to 47.58% of population mean with an average of 26.29%. As to selection for lower CMS, the responses were less variable ranging from 18.62 to 40.77% with an average of 25.21%. The observed responses were consistently greater in magnitude than those expected in both the high and low CMS directions (see Table 1). The realized heritability estimates ranged from low in three populations (0.18, 0.35 and 0.32) to moderate in two (0.44 and 0.51). The heritability values as measured by the parent-offspring regression were almost identical to the realized heritabilities.

Meanwhile, significant positive responses to selection for both higher and lower CMS were obtained under the heat stress of a late sowing date in clay soil in the F_5 generation of the five populations (Table 1). The response to selection for higher CMS in the five populations ranged from 9.00 to 57.71% of population mean with an average of 26.53%. Meanwhile, for the low CMS direction the responses ranged from 6.73 to 42.98% of population mean with an average of 24.30%.

The observed responses to selection for high CMS in the F_5 generation exceeded those obtained in the F_4 generation by 3.56, 10.13 and 6.61% in pop. No. 1, pop. No. 3 and pop. No. 4, respectively. Similarly, the responses for low CMS in the F_5 generation surpassed those obtained in the F_4 generation by 17.55, 5.48 and 7.87% in pop. No. 1, pop. No. 3 and pop. No. 5, respectively. Evidently, the third cycle of selection which was applied to the F_4 families produced further genetic advance over that resulted from the second cycle of selection in three populations.

Correlated responses to divergent selection for CMS:

1-Grain yield per plant:

Selection for high CMS did not produce any significant correlated responses in grain yield per plant under either favorable or drought stress environmental conditions in any

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of the five in F_4 populations (Table 2).

However, significantly positive correlated responses were observed under combined drought + heat stresses in two of the five populations, namely pop. 2 and pop. 4 which amounted to 23.73 and 17.02% of the population mean, respectively. Meanwhile, selection for lower CMS produced significant

positive correlated responses in grain yield per plant under drought stress in only two of the five populations namely pop. 4 and pop. 5 where the reductions in grain yield amounted to 16.4 and 17.1% of the population mean, respectively and in pop. 1 (12.90%) under drought + heat stresses conditions.

Averaged over all the genotypes tested, grain yield per plant was reduced from 44.82 g in the favorable environment to 19.31 g under drought and further down to 10.96 g under combined drought + heat stresses. Apparently, drought stress of the sandy soil was quite strong resulting in a 56.9% average reduction in grain yield per plant whereas the reduction under combined drought + heat stress in the sandy soil reached 75.5% indicating that the reduction due to heat stress alone reached 18.6%. Cell membrane thermostability was found to be strongly correlated with grain yield per plant under the combined drought + heat stresses ($r=0.80$, $p < 0.01$) as well as under drought stress ($r = 0.64$, $p < 0.01$) or under favorable conditions ($r = 0.77$, $p < 0.01$).

The third cycle of selection for higher CMS F_5 did not produce significant correlated responses in grain yield per plant except in one population under favorable conditions and another population under heat stress (Table 2). Meanwhile, selection for lower CMS produced significant positive correlated response in only one population under heat stress. The mean grain yield per plant as averaged over all F_5 families was reduced from 27.37 g in the favorable environment to 23.15 g under heat stress of the late sowing date in clay soil denoting a 15.4% average reduction. The CMS values of the F_5 high and low selections and the bulks were significantly correlated with grain yield per plant under heat stress ($r = 0.67$, $p < 0.01$) as well as under favorable conditions ($r = 0.57$, $p < 0.05$).

2- 1000- grain weight

The second cycle of selection for higher CMS applied to the F_3 families produced significantly positive correlated responses in 1000 grain weight of the F_4 selection of the five populations under combined drought + heat stresses of the sandy soil (see Table 3) which ranged from 5.02 to 7.52% with an average of 6.45% of the population mean. No significant correlated responses were observed under drought stress alone in any of the five populations. However, under the favorable conditions significant positive correlated responses were manifested in the high CMS F_4 selection of three of the five populations.

Selection for lower CMS resulted in significant correlated responses in 1000 grain weight in the F_4 selection of only two of the five populations, namely pop. 1 and pop. 4 under combined drought + heat stress which reached 4.56% and 6.49%, respectively as well as under drought stress (4.6 and 4.7%, respectively). Meanwhile, a significant positive correlated response was observed under favorable conditions in only one of the five populations (pop. 5) which amounted to 6.17%.

The impact of heat stress on 1000 grain weight was much stronger than that of drought stress. The average of 1000 grain weight over all genotypes was reduced from 46.33 g in the favorable environment to 46.00 g under drought stress and further down to 41.88 under drought + heat stresses indicating a 0.7% reduction due to drought stress against 8.9% reduction due to heat stress.

Significant positive correlated responses in 1000 grain weight were also displayed in the F_5 selections for higher CMS in the five populations under favorable and heat stress conditions except in pop. 4 under favorable conditions (Table 3) which averaged 5.83% of the population mean under favorable condition and 6.40% under heat stress. Meanwhile, selection for lower CMS produced significant positive correlated response in two of the five populations in the favorable environment and in four under heat stress conditions.

The mean 1000 grain weight as averaged over all F_5 families was reduced from 44.27 g in the favorable environment to 42.2 g under heat stress of the late sowing date in clay soil denoting a 4.67% average reduction.

Response to selection in the F_5 families for stomatal frequency:

Positive responses were obtained in the F_5 families selected for higher and lower stomatal frequency under the favorable as well as under the heat stress conditions in the five populations (Table 4). Under favorable conditions, the response to selection in the high stomatal frequency direction ranged from 5.24 to 18.67% of the population mean with an average of 10.66%. Under heat stress, the % response was greater and ranged from 11.02 to 23.9% with an average of 19.57%. As to the low stomata frequency direction, the % response ranged from 10.73 to 19.38% with an average of 13.67% under the favorable conditions but was much smaller under heat stress where the response ranged from 3.92 to 13.94% with an average of 8.71%. For each of the five populations, the realized heritability of stomata frequency was comparable to the heritability values as estimated by parent-offspring regression in the two environments. Generally, the realized heritability estimates were smaller in the favorable environment (0.27, 0.59, 0.32, 0.34 and 0.41 in the five populations) than under heat stress (0.47, 0.44, 0.42, 0.42 and 0.60).

Correlated response to divergent selection for stomatal frequency:

1-Grain yield per plant:

Selection for higher stomatal frequency produced significant positive correlated responses in grain yield per plant in three of the five populations under favorable conditions, namely pop. 3, pop. 4 and pop. 5 which amounted to 21.69, 34.1 and 19.42% of the population mean, respectively (Table 5). However, under heat stress, selection for higher stomatal frequency produced significant positive correlated responses in grain yield per plant in only two of the five populations, namely pop.2, (19.71%) and pop. 5 (11.29%). Meanwhile, selection for low stomatal frequency did not produce any significant correlated responses in grain yield per plant either under favorable or heat stress conditions in any of the five populations.

Stomatal frequency was significantly correlated with grain yield per plant under heat stress ($r=0.64$, $p < 0.01$) as well as under favorable conditions ($r = 0.53$, $p < 0.05$).

2- 1000 grain weight

Selection for higher stomatal frequency produced significantly positive correlated responses in 1000 grain weight in the F_5 selections in four of the five populations under heat stress (Table 5) which amounted to 6.93, 13.69, 5.02 and 9.29% in pop. No.1 pop. No.2 pop. No.3 and pop. No.4, respectively. However, under the favorable conditions significant positive correlated responses were manifested in the high stomatal frequency F_5 selections in three of the five populations, namely pop. 1, pop. 2 and pop. 4 which amounted to 8.26, 14.21 and 7.12% of the population mean, respectively. Meanwhile, no significant correlated responses were observed in the low stomatal frequency selections under either favorable conditions or heat stress except in population No. 2 (8.99 and 12.39% in favorable and heat stress environments, respectively).

Impacts of the selection for CMS and stomatal frequency on tolerance to drought and heat stresses

1- Grain yield performance:-

The F_6 families combining high CMS with high stomatal frequency proved to be the top yielding under the three environmental conditions (Table 6)

Table (6): Means of grain yield per plant (upper value) and 1000 grain weight (g) (lower value) of the F_6 unselected bulks and the F_6 families with high CMS-high stomata (HH), high CMS-low stomata (HL), low CMS-high stomata(LH) and low CMS-low stomata (LL) of the five populations under favorable, heat stress and drought + heat stresses conditions.

Populations	(1)	(2)	(3)	(4)	(5)	Mean
Favorable						
HH	48.528 54.18	51.866 47.11	40.881 51.58	47.553 46.14	44.311 54.28	46.62 50.66
HL	46.444 51.15	40.383 44.31	30.223 49.76	49.769 46.72	40.044 53.97	41.37 49.18
Bulk	40.906 50.56	39.241 42.3	35.363 47.29	44.121 42.21	38.630 50.24	39.65 46.52
LH	38.530 49.53	45.294 46.29	31.898 46.67	33.203 41.92	35.356 47.83	36.86 46.45
LL	27.968 45.23	29.182 37.71	29.573 45.35	33.670 41.48	34.479 43.77	30.97 42.71
Heat stress						
HH	25.183 47.88	24.194 47.08	32.053 50.49	33.098 47.4	23.976 51.82	27.70 48.93
HL	29.211 47.55	24.157 40.43	31.319 50.2	27.636 46.01	23.430 52.94	27.15 47.43
Bulk	24.656 46.12	22.030 39.89	24.035 46.4	30.754 43.43	22.353 49.6	24.77 45.09
LH	25.801 44.75	25.69 43.7	26.725 50.57	26.871 43.72	22.967 50.51	25.61 46.65
LL	17.650 39.38	17.479 36.46	24.655 46.08	25.615 40.57	15.199 46.42	20.12 41.87
Drought and Heat stress						
HH	23.095 45.43	18.390 44.12	19.863 43.69	24.123 44.73	28.363 48.63	22.77 45.32
HL	17.024 43.76	14.501 39.74	17.943 40.68	27.492 43.24	25.622 48.69	20.52 43.22
Bulk	21.687 42.54	15.303 38.6	13.399 38.98	23.138 40.1	23.078 46.81	19.32 41.41
LH	13.673 40.14	19.291 41.92	12.439 39.61	21.518 39.44	28.754 43.74	19.14 40.97
LL	12.234 33.41	16.525 34.51	13.632 38.96	19.150 37.51	17.334 44.37	15.78 37.95

* P < 0.05   ** P < 0.01

Positive grain yield increments were uniformly displayed over the unselected bulks in the five populations (Table 7). Under favorable conditions, the increments in the high CMS-high stomata families of the five populations ranged from 3.43 to 12.62 g with an average of 6.93 g representing 17.48% over the mean of the unselected bulks. The increments under heat stress ranged from 0.53 to 8.02 g with an average of 2.93 g indicating an 11.85% average increment. Under the combined drought + heat stresses, grain yield increments ranged from 0.99 to 6.64 g with an average of 3.45 g indicating a 17.83% average increment over the bulk. Families that combined high CMS with low stomatal displayed inconsistent changes in grain yield per plant relative to the unselected bulks. Significantly positive increments were confined to only one of the five populations in the favorable environment, two under heat stress and two under combined drought + heat stress where a significant reduction was also obtained in one population (Table 7).

As to the low CMS-high stomatal frequency families, grain yield per plant was reduced under favorable conditions in four of the five populations where the reduction reached significance in one population. Meanwhile, the changes were rather inconsistent under heat stress where positive increment was displayed in one population while significant reduction was obtained in another. Similarly, positive increments were obtained in two of the five populations under combined drought + heat stresses while a reduction was obtained in one population.

The low CMS-low stomata families of the five populations uniformly displayed considerable reductions in grain yield per plant under the favorable environment which ranged from 4.15 to 12.94 g with an average of 8.68 g marking 21.88% average reduction. Similarly, significant reductions were obtained in four of the five populations under heat stress which averaged 18.76% relative to the unselected bulks. Under drought + heat stresses, significant reductions were manifested in the families of three of the five populations which averaged 18.35% of the bulk mean.

2- 1000 grain weight:-

The high CMS-high stomata families of the five populations uniformly displayed significant increments over the unselected bulks which averaged 8.89% under favorable conditions, 8.53% under heat stress and 9.45% under combined drought + heat stresses (Table 7). Similar but smaller significant positive increments were exhibited by the high CMS-low stomata families averaging 5.72, 5.19 and 4.39% in favorable, heat stress and drought + heat stress environments, respectively.

Evidently, the 38.55% greater stomatal frequency of the high CMS-high stomata families over the high CMS-low stomata families enhanced 1000 grain weight by 3.27, 3.14 and 5.06% in favorable, heat stress and drought + heat environments, respectively. Meanwhile, the low CMS-high stomata families of the five populations displayed inconsistent performance in the three environments. Under the favorable environment, positive increment was displayed in one population while significant reduction was obtained in another. Similarly, positive increments were obtained under heat stress in two of the five populations and a reduction in one population. Under combined drought + heat stress, positive increments were obtained in one of the five populations and a reduction in two. The low CMS-low stomatal frequency uniformly displayed significant reductions in 1000 grain weight relative to the unselected bulks which averaged 8.59, 7.13 and 8.83% under favorable, heat stress and drought + heat stress conditions, respectively.

Discussion

Two successive cycles of divergent selection for CMS were employed in this study on the F_3 families which resulted from an initial cycle carried out by Omara *et al*., (2006) on the F_2 segregates of five populations. The average responses of 26.29 and 25.2% obtained in the high and low CMS F_4 selections, respectively as apposed to the corresponding responses of 19.52 and 11.9% in the F_3 selections reported by Omara *et al*., (2006) indicate the occurrence of farther genetic advances averaging 6.77 and 13.3% in the two directions. Meanwhile, the responses in the F_5 selections which averaged 26.53 and 24.30 in the high and low directions, respectively would indicate a stagnation of selection since the two values are almost equal to those obtained in the F_4 selections.

However, three of the five populations, when individually considered, displayed further genetic advances of 3.56, 10.13 and 6.61% over the corresponding responses in the F_4 selection in the high CMS direction and 17.55, 5.48 and 7.87% in the low CMS direction. Apparently, the additive genetic variation for CMS in those populations was not depleted after the two cycles of selection. The fact that the observed responses were consistently greater than the expected responses would indicate that genes with dominance effects were involved in the control of CMS under combined drought + heat and heat stress conditions. The rather low to moderate realized heritability estimates obtained in the F_4 (ranged from 0.18 to 0.51) and the F_5 (ranged from 0.20 to 0.53) for CMS were similar to those reported by Ibrahim and Qiuck (2001, a) which ranged from 0.27 to 0.47 and by Omara *et al*., (2006) ranging from 0.09 to 0.57.

Selection for greater CMS did not result in any correlated responses in grain yield per plant either under favorable or drought stress conditions while positive responses were obtained in only tow populations under drought + heat stresses. Moreover, selection for smaller CMS did not impact grain yield under favorable conditions while significant reductions were confined to only two of the five populations under drought and one population under heat. Similarly, very limited effects of selection for CMS on grain yield per plant were observed in the F_5 selections of only one population under favorable and another one under heat stress. Evidently, selection for CMS alone cannot improve grain yield in wheat under abiotic stresses in confirmation of the conclusion reached by Blum *et al*., (2001). However, CMS and grain yield per plant were positively correlated in this study under drought + heat stress ($r = 0.80$, $p < 0.01$) as well as under heat stress ($r = 0.64$, $p < 0.01$) or under favorable conditions. Such association is in agreement with that reported by Blum *et al*.,(2001) on CMS being correlated with grain yield under heat stress ($r = 0.53$, $p < 0.01$) but not under favorable conditions. Similar positive association between CMS and grain yield in wheat under drought and heat stresses were also reported by Shanahan *et al*, (1990); Blum and Ebercon, (1981); Tripathy *et al*., (2000) and Ibrahim and Quick (2001 a).

However, CMS was not related in this study to either drought susceptibility index of grain yield ($r = -0.15$) or heat susceptibility index ($r = 0.13$) where both indices which are independent from mean grain yield, were negatively correlated ($r = -0.56$, $p < 0.05$). Evidently, other mechanisms besides CMS are involved in enhancing grain yield under abiotic stresses which are most likely different under heat stress from those operating under drought stress. According to Blum and Ebercon (1981), drought and heat tolerance are not correlated in wheat.

Unlike, the stronger impact of drought on grain yield per plant (56.9% average reduction) as compared with that of heat stress (18.6% average reduction), drought stress have had much weaker effect on 1000 grain weight (only 0.7% average reduction) than that of heat stress (8.9% average reduction). Drought was reported to have little effect on the rate of grain filling since a greater proportion of the grain weight comes from earlier mobilization of non-structural reserve carbohydrates from the stem (Blum *et al*., 1991 and Wardlaw and Willenbrink, 1994) whereas high temperature acts directly on the developing grains by reducing the duration of grain filling and impairing photosynthesis and starch accumulation (Fokar *et al*., 1998 a). The fact that the correlated responses in the F_4 families selected for high CMS in 1000 grain weight were manifested under combined drought + heat stresses (average 6.45% increase) but not under drought stress alone lends further support to the crucial role of CMS in sustaining grain filling under high temperature (Saadalla *et al*., 1990; Shanahan *et al*., 1990; Foker et al., 1998 a,b; Blum *et al*., 2001 and Omara *et al*., 2006).

The rather limited correlated responses to divergent selection for CMS in grain yield per plant as opposed to the positive concurrent responses obtained in 1000 grain weight which averaged 6.4% over the five populations demonstrate further the impact of CMS on grain filling in wheat. An important feature of the results of this study was the significant increase in average stomatal frequency uniformly displayed under heat stress (averaged 9.59%) above that observed under favorable conditions in the F_5 bulks and selections. Such increase in stomatal frequency under heat stress could be adaptive to high temperature via enhancing transpiration and consequently reducing canopy temperature.

Selection for higher stomatal frequency on the adaxial surface of the flag leaf under heat stress produced greater responses under heat stress (19.57% on average) than under favorable conditions (10.66% on average). Evidently, greater responses are expected to occur in the environment in which selection is practiced (Falconer, 1990). However, the responses to selection for lower stomatal frequency was smaller under heat stress (8.75% on average) than under favorable conditions (13.67%). Apparently, this might be due to the antagonistic effect of the environment (heat stress) which increases stomatal frequency with that of the selection which was directed to reducing it. Similar responses to divergent selection for stomatal frequency were reported by Bhagwat and Bhatia (1993). The realized heritability estimates of stomatal frequency obtained in the study were smaller under favorable conditions (0.27 to 0.59) than those under heat stress (0.42 to 0.60). Similarly, low to moderate heritabilities of stomatal frequency in wheat were reported by Bhagwat and Bhatia (1993) and Arminian *et al*, (2008). The positive correlated responses to selection for higher stomatal frequency in grain yield per plant obtained in three of the five populations under heat stress, coupled with the positive correlation between stomatal frequency and grain yield ($r = 0.53$ and $r = 0.64$ $p < 0.05$ in the two environments) indicate a possible role of higher stomatal frequency in grain yield enhancement in wheat. Positive associations between stomatal frequency and grain yield in wheat were reported by Khan *et al.*, (2003); Munir (2006) and Meharun-Nisa *et al*, (2009). However, the lack of correlated responses to selection for lower stomatal frequency in grain yield per plant under favorable or heat stress conditions could be attributed to a possible compensation of increased stomatal length since the two stomatal characters were negatively and strongly correlated under favorable ($r = -0.85$, $P < 0.01$) as well as under heat stress ($r = -0.82$, $p < 0.01$). Similar negative associations between stomatal frequency and size were reported in wheat by Singh and Setwi (1995); Mishra (1997); Arminian *et al.,* (2008) and Maghsoudi and Maghsoudi (2008).

The positive correlated responses to selection for high stomatal frequency in 1000 grain weight which were manifested in four of the five populations under heat stress (ranged from 5.02 to 13.69%) indicate that grain filling might have been enhanced through greater transpirational cooling of the canopy due to elevated stomatal frequency. Meanwhile, increased 1000 grain weight under favorable conditions in the high stomatal frequency selections in three of the five populations (ranged from 7.12 to 14.21%) could be explained as due to greater photosynthetic capacity by gas diffusion due to low stomatal resistance (Yoshida, 1978).

The greatest and uniformly positive increments in grain yield per plant were displayed by the high CMS-high stomatal frequency families which averaged 17.84, 11.85 and 17.83% over the unselected bulks under favorable, heat stress and drought + heat stress, respectively. Since the F_4 families selected for high CMS alone in this study did not produce any significant concurrent responses in grain yield per plant under either favorable or drought stress conditions with limited responses under drought + heat stress, the increments in grain yield per plant in the high CMS-high stomata families are attributed to a possible synergistic effect of elevated stomatal frequency. Evidently, the significant correlated responses to selection for high stomatal frequency in grain yield per plant in this study under favorable and heat stress conditions substantiate that conclusion.

The high CMS-high stomata families also displayed consistently positive increments in 1000 grain weight which were less than those obtained in grain yield per plant being 8.89, 8.53 and 9.45% on average under favorable, heat stress and drought + heat stresses. Evidently, grain yield enhancement is attributable therefore to increases in other yield components besides

1000grain weight, possibly in grain number.

The rather limited and inconsistent grain yield responses of the high CMS-low stomata families to the three environments as opposed to the uniformly positive increments in 1000 grain weight which averaged 5.72, 5.19 and 4.39% in favorable, heat stress and drought + heat environments, respectively further demonstrate that CMS plays greater role in sustaining grain filling than in supporting grain yield per plant. This conclusion confirms that of Blum *et al,* (2001) and substantiates the evidence that higher stomatal frequency plays greater role in supporting grain yield than enhancing grain filling. The rather contrasting and inconsistent responses in grain yield per plant and 1000 grain weight of the low CMS-high stomatal frequency to heat and drought + heat stresses indicate the vital role of cellular membrane stability when integrated with high stomatal frequency. Apparently, the low CMS-low stomata families displayed consistently negative reductions in both grain yield and 1000 grain weight in the three environments relative to the unselected bulks.

Interestingly, the magnitude of grain yield increments of the high CMS-high stomata families (averaged 17.48, 11.85 and 17.83% in favorable, heat and drought + heat environments, respectively) were almost equivalent to the reductions displayed by the low CMS-low stomata families (21.8, 18.76 and 18.35% in the three environments, respectively). Similarly, the increments in 1000 grain weight of the high CMS-high stomata families (8.89, 8.53 and 9.45%) were almost equivalent to the reductions displayed by the low CMS-low stomata families (8.59, 7.13 and 8.83%). Such parallel impacts demonstrate the integrity of the role of CMS with stomatal frequency on grain yield and grain filling in wheat.

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الانتخاب للثبات الحراري للغشاء الخلوي وتكرار الثغور تحت إجهادات الجفاف والحرارة في قمح الخبز

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مورس الانتخاب ثنائي الاتجاة لدورتين متتاليتين لصفة الثبات الحراري للغشاء الخلوي في عائلات الجيل الثالث في خمس عشائر من قمح الخبز تحت إجهادات الحرارة وكذلك الحرارة مع الجفاف. ومن ناحية اخري مورست دورة انتخاب واحدة لصفة تكرار الثغور تحت اجهادات الحرارة مع الجفاف في عائلات الجيل الرابع المنتخبة للثبات الحراري للغشاء الخلوي وذلك للحصول علي توليفات مختلفة من الصفتين في عائلات الجيل السادس، تشمل عائلات عالية الثبات الحراري للغشاء الخلوي عالية في تكرار الثغور، عالية الثبات الحراري للغشاء منخفضة في تكرار الثغور، منخفضة الثبات الحراري للغشاء عالية في تكرار الثغور واخيرا عائلات منخفضة في كلا الصفتين ثم قيمت هذة العائلات لكل من محصول الحبوب للنبات الواحد ووزن الالف حبة تحت الظروف الطبيعية و إجهادات الحرارة وإجهادات الجفاف مع الحرارة. نتجت استجابات موجبة معنوية عند الانتخاب للثبات الحراري للغشاء الخلوي في عائلات الجيل الرابع والخامس داخل الخمس عشائر وكان متوسط الاستجابة في الاتجاة العالي في الخمس عشائر 26.29% و26.53% وذلك في الجيل الرابع والخامس علي الترتيب و25.21% و24.30% في الجيل الرابع والخامس علي الترتيب في الاتجاة المنخفض. كما نتجت استجابات موجبة ومعنوية للانتخاب لتكرار الثغور العالي والمنخفض في عائلات الجيل الخامس وكان متوسط الاستجابة في الخمس عشائر في الاتجاة العالي 19.57% والاتجاه المنخفض 8.71% تحت الاجهاد الحراري. لم ينتج عن الانتخاب في الاتجاه العالي للثبات الحراري للغشاء الخلوي أي إستجابة متلازمة في محصول النبات للحبوب سواء تحت الظروف المواتية أو الاجهاد الحراري علي الرغم من وجود ارتباط قوي بين محصول النبات للحبوب والثبات الحراري للغشاء الخلوي تحت اجهادات الحرارة وكذلك الجفاف مع الحرارة معا علي مستوي الخمس عشائر مجتمعة حيث كان معامل التلازم 0.64، 0.80 علي الترتيب. بالاضافة إلي ذلك وجدت استجابة متلازمة موجبة في وزن الالف حبة بمتوسط 6.45% في الخمس عشائر تحت بيئة الجفاف مع الحرارة. ومن ناحية اخري وجدت استجابات متلازمة موجبة في المحصول ووزن الالف حبة وذلك عند الانتخاب لتكرار الثغور العالي تحت كل من الظروف المواتية والاجهاد الحراري.

من بين التوليفات المختلفة من الثبات الحراري للغشاء الخلوي وتكرار الثغور اعطت العائلات عالية الثبات الحراري عالية تكرار الثغور أعلي محصول حبوب حيث قدرت الزيادة عن العائلات الغير منتخبة متوسط 17.48% في البيئة المواتية

و 11.85% في بيئة الحرارة و17.83% في بيئة الجفاف و الحرارة معا. وبالمثل كان متوسط الزيادة في متوسط وزن الالف حبة 8.89، 8.53 و9.45% في الظروف المواتية وإجهاد الحرارة وإجهاد الجفاف مع الحرارة علي الترتيب. مثل هذه الزيادات تنسب إلي تآزر آثار تكرار الثغور العالي الذي ارتقي بزيادة عملية التبريد عن طريق النتح مع زيادة الثبات الحراري للغشاء الخلوي.