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The Genetics of Heat Shock Proteins in Wheat in Relation to Heat Tolerance and Yield

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Title:

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C. Abstract (main results)

Fifty six diverse spring wheat cultivars were evaluated for genetic variation and heritability for thermotolerance in terms of cell-membrane stability (CMS) and triphenyl tetrazolium chloride (TTC) reduction. The most divergent cultivars for thermotolerance (Danbata-tolerant and Nacozari-susceptible) were crossed to develop an F_8 random inbred line (RIL) population. This population was evaluated for co-segregation in CMS, yield under heat stress and HSP accumulation. Further studies of thermotolerance in relations to HSP and the expression of heterosis for growth under heat stress were performed with F_1 hybrids of wheat and their parental cultivars.

CMS in 95 RILs ranged from 76.5% to 22.4% with 71.5% and 31.3% in Danbata and Nacozari, respectively. The population segregated with a normal distribution across the full range of the parental values. Yield and biomass under non-stress conditions during the normal winter season at Bet Dagan did not differ between the two parental cultivars, but the range of segregation for these traits in 138 RILs was very high and distinctly transgressive with a CV of 35.3% and 42.4% among lines for biomass and yield, respectively. Mean biomass and yield of the population was reduced about twofold when grown under the hot summer conditions (irrigated) at Bet Dagan. Segregation for biomass and yield was decreased relative to the normal winter conditions with CV of 20.2% and 23.3% among lines for biomass and yield, respectively. However, contrary to non-stress conditions, the parental cultivars differed about twofold in biomass and yield under heat stress and the population segregated with normal distribution across the full range of this difference. CMS was highly and positively correlated across 79 RILs with biomass ($r=0.62^{**}$) and yield ($r=0.58^{**}$) under heat stress. No such correlation was obtained under the normal winter conditions.

All RILs expressed a set of HSPs under heat shock (37°C for 2 h). No variation was detected among RILs in high molecular weight HSP isoforms and they were similar to the patterns of the parental cultivars. There was a surprisingly low variability in low molecular weight HSP isoforms. Only one low molecular weight and Nacozari-specific HSP isoform (belonging to HSP16.9 family) appeared to segregate among all RILs, but it was not quantitatively correlated with any parameter of plant production under heat stress or with CMS in this population.

It is concluded that this Danbata/Nacozari F_8 RIL population co-segregated well for thermotolerance and yield under heat stress and that CMS could predict the relative productivity of lines under chronic heat stress. Regretfully this population did not express meaningful variability for HSP accumulation under heat shock and therefore no role could be seen for HSP in the heat tolerance of this population.

In the study of seven F_1 hybrids and their parent cultivars it was found that heterosis (superiority of the F_1 over the best parent) for CMS was generally lower than that for

growth under heat stress, even though CMS was positively correlated with growth under heat stress. Hybrids varied in the rate of heterosis for growth at normal ($15^{\circ}/25^{\circ}$) and at high ($25^{\circ}/35^{\circ}$) temperatures. In certain hybrids heterosis for growth significantly increased at high temperature as compared with normal temperature, suggesting temperature-dependent heterosis. Generally, under normal temperature, only limited qualitative variation was detected in the patterns of protein synthesis in four wheat hybrids and their parents. However, a singular protein (C47/5.88) was specifically expressed only in the most heterotic hybrid at normal temperature but not in its parent cultivars. Parental cultivars were significantly different in the sets of synthesized HSP at 37° . No qualitative changes in the patterns of protein expression under heat stress were correlated with heterosis. However, a quantitative increase in certain low molecular weight HSP (mainly H14/5.5 and H14/5.6, belonging to the HSP16.9 family) was positively associated with greater heterosis for growth at high temperature. None of these proteins were correlated with CMS across hybrids. These results support the concept of temperature-dependent heterosis for growth and a possible role for HSP16.9 family in this respect.

Finally, when all experiments are viewed together it is encouraging to find that genetic variation in wheat yield under chronic heat stress is associated with and well predicted by CMS as an assay of thermotolerance. On the other hand the results for HSP are elusive. While very low genetic variation was expressed for HSP in the RIL population, a unique low molecular weight HSP (of the HSP16.9 family) could be associated with temperature dependant heterosis for growth.

D. Objectives of the Original Research

Heat stress is a major limitation to wheat (*Triticum aestivum* L.) productivity in arid and semi-arid regions of the world including parts of Israel and the U.S. Great Plains. This problem also seriously hinders wheat production in large areas in the sub-tropical and tropical regions and it constitutes an important breeding target for CIMMYT. Consequently, development of heat tolerant cultivars is of major concern in wheat breeding programs in USA, Israel and elsewhere.

The overall objective of this research is to enhance our understanding of the genetic basis of heat tolerance in spring wheat, with strong emphasis on the genetic, physiological, and agronomical implications of heat shock proteins (HSP) in relation to stress breeding. This will facilitate the development of physiological or molecular markers for selection in wheat improvement programs. It is also expected that specific genes conferring heat tolerance will be identified for future genetic manipulation.

We have special interest in HSP work for two important reasons. First, the specific physiological functions of HSPs in plants and the role of HSP genes in crop plant heat tolerance are still not known. In her review of the subject, Vierling (1990) concluded as follows: "In contrast to the many studies relating HSPs to short-term thermotolerance, there is little evidence to indicate that HSPs are involved in the heritable ability of different plant species or plant cultivars to grow and reproduce at high temperature". We are not aware of any report published between 1990 and the present which provides any new information in this respect. Our proposed study offers an opportunity for making a contribution to the understanding of the role of HSPs in relation to plant productivity under heat stress.

Secondly, if HSPs are found to have a role, then this work may bring us closer to using these proteins as molecular probes for the genetic enhancement of heat tolerance in wheat. The unique aspects of this proposal are the genetic approach and the efforts to link molecular, cellular, and whole plant responses in studying the role of HSPs in a major crop plant. Ultimately, this work will help establish the molecular genetic basis of the inherited heat tolerance traits affecting the plant's ability to adapt to hot environments.

E. Results and Discussion

E.1. Heat Tolerance In Spring Wheat I. Estimating Cellular Thermotolerance and its Heritability

ABSTRACT

High temperature is a major environmental stress factor limiting wheat (*Triticum aestivum* L.) productivity. Improvement of heat tolerance in wheat is an important breeding objective. Genetic variation in cellular thermotolerance among 56 spring wheat cultivars was evaluated at the seedling stage of growth by cellular membrane thermostability (CMS) and triphenyl tetrazolium chloride (TTC) assays. A subset of eight lines was evaluated also at the flowering stage using the same assays. All tests were performed after heat-hardening at 34°C for 24 h in the growth chamber. The inheritance of thermotolerance in terms of TTC was investigated using generation means analysis.

Average thermotolerance with both assays tended to decrease from the seedling to the flowering growth stage. However thermotolerance was well correlated between growth stages among the eight cultivars for both CMS ($r=0.92$; $p=0.004$) and TTC ($r=0.84$; $p=0.050$). The correlation between TTC and CMS among eight cultivars tested at the seedling and the flowering growth stages was significant ($r=0.74$; $p=0.031$ and $r=0.75$; $p=0.029$, respectively). The same correlation across 56 cultivars at the seedling stage was less strong though still significant ($r=0.32$; $p=0.014$).

In a study of the cross V747(tolerant)/Barkaee(susceptible), broad sense heritability was estimated for seedling stage TTC at 89%. Most of the genetic variance was accounted for by additive genetic variance.

CMS in seedlings of 16 cultivars was positively and significantly ($p<0.05$) correlated with yields of these cultivars in each of four hot environments in Mexico, Sudan, India and Brazil. The same correlations for TTC were positive but non-significant.

INTRODUCTION

Heat stress is a major limitation to wheat (*Triticum aestivum* L.) productivity in arid, semiarid, tropical, and subtropical regions of the world (Fischer, 1986). Consequently, development of heat-tolerant cultivars is of major concern in wheat breeding programs. A detailed understanding of the genetics and physiology of heat tolerance as well as the use of the proper germplasm and selection methods will facilitate the development of heat-tolerant cultivars of wheat.

Heat hardening or acquired thermotolerance is a well known adaptive phenomenon, whereby exposure to mild heat stress induces tolerance to high temperatures. Heat hardening occurs under natural conditions and the capacity to heat harden is an inherent component of heat tolerance in the field. Cell membrane thermostability (CMS) and triphenyl tetrazolium chloride (TTC) cell viability have been used in various studies to quantify the level of acquired cellular thermotolerance in plants (Blum, 1988).

Exposure of plant cells to heat causes cellular membrane disruptions that are apparently related to temperature-specific phase changes in the membrane lipid bilayer (Suss and Yordanov, 1986). The basic mechanism of cellular membrane disruption under heat stress can impact photosynthetic or mitochondrial activity or decrease the ability of the plasmalemma to retain solutes (Lin et al., 1985). The cellular membrane thermostability test estimates the amount of electrolyte leakage from heat-stressed tissues *in vitro* using a simple conductometric technique. Since the initial work of Sullivan et al. (1977) in the early 1970's, several reports indicate that genetic variation in heat tolerance as measured by the electrolyte leakage method exists in various crops (Onwueme, 1979; Martineau et al., 1979; Wehner and Watschke, 1981; Chen et al., 1982; Wallner et al., 1982; Kuo et al., 1992; Marsh et al., 1985; Milliken, 1987). Blum and Ebercon (1981) developed the method for wheat grown and heat-hardened in the field. Chen et al. (1982) concluded that standardized heat hardening under controlled conditions prior to measurements is essential for assessing genotypic differences in thermotolerance. Reynolds et al. (1994) used controlled growth chamber conditions to heat harden their wheat materials at the seedling and relied on heat hardening *in situ* for field grown adult plants. Saadalla et al. (1990a) concluded that conducting CMS assay with 4 cm long germinated seedlings was appropriate for assaying heat tolerance of wheat and that assays in germinating seedlings predicted the thermotolerance of the fully developed plant. That result is extremely important for selection work, since seedling tests require less resources than assays with fully grown plants. Saadalla et al. (1990a) applied heat hardening to germinated seedlings in a paper towel by immersing their root in a water bath (at 34°C) and covering the bath with a transparent cover. Blum (unpublished) attempted to repeat that method of heat hardening followed by measurements of CMS, as described by Saadalla et al. (1990a). For 24 wheat cultivars there was no correlation

between CMS rating by this procedure and CMS rating in seedlings or fully developed plants hardened by exposure of the whole plant to 34°C in the growth chamber as recommended by Chen et al. (1982). Therefore, in this study we compared methods for measuring thermotolerance at different stages of development after imposing controlled heat hardening of whole seedlings or plants in the growth chamber rather than by immersing roots in a water bath.

The TTC cell viability assay is based on the principles of tetrazolium salt reduction to formazan by dehydrogenase respiratory enzymes. Therefore, the TTC test evaluates the mitochondrial electron transport chain and thus it represents respiratory activity. This assay has a long history of use as an estimate of plant cell and tissue viability at high temperatures (Towill and Mazur, 1974). Chen et al. (1982) reported that genotypic differences existed in the induction of acquired heat tolerance by this test in four crops and that these differences were related to known differences in heat tolerance as evaluated by yield and fruit set under high temperature conditions. Chen et al. (1982) suggested that either the CMS or TTC assays could be used to evaluate heat tolerance in plants. In their studies, both tests gave similar results with a limited number of genotypes. The TTC assay was evaluated in wheat to detect genotypic differences in heat tolerance (Krishnan et al., 1989). However, the two tests were never compared in wheat.

Genetic studies of heat tolerance in wheat are rare (Blum, 1988). Most genetic studies on the physiology of heat tolerance in crop plants have utilized the CMS assay as a measure of relative heat tolerance. Martineau et al. (1979) evaluated segregating populations of soybean [*Glycine max* (L.) Merr.] for membrane stability and found that expected genetic gains for heat tolerance selection ranged from 16 to 18%. It was concluded that there was substantial genetic variability for membrane stability although no estimate was made of the number of genes controlling this trait. Marsh et al. (1985) utilized the same assay with common bean and reported that a large proportion of the variation was controlled by a small number of genes. Heritability of CMS in maize was estimated to be 73%, and several quantitative trait loci (QTL) accounted for 53% of this variation (Ottaviano et al., 1991). In a diallel genetic analysis, Porter et al. (1995) reported that thermotolerance as measured by TTC cell viability assay in winter wheat was controlled mainly by additive genetic effects, suggesting genetic improvement could be made by selection.

The main objectives of this study were: 1) to compare the TTC and CMS assays for estimating the genetic variability for heat tolerance in diverse spring wheat cultivars; (2) to compare thermotolerance rating in seedlings and in fully developed plants; and 3) to characterize the mode of inheritance and determine heritability of heat tolerance based on a TTC assay.

MATERIALS AND METHODS

TTC and CMS Assays in Seedlings

Fifty-six spring wheat cultivars that originated from various production regions of the world were used in this study. Seedlings were germinated and grown in sterilized vermiculite under artificial light, which provided $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with a 14 h photoperiod. Plants were well watered and ambient temperature was maintained between 20° and 25°C . The experimental design was a randomized complete block with three replications.

Ten days after emergence, the green seedlings were transferred to an environmental chamber for heat acclimation at 34°C under high relative humidity ($>80\%$) for 24 h. Plants were sampled for assays immediately after acclimation. This protocol was totally different from that of Saadalla et al. (1990a) as they used younger germinated seedlings which were only 4cm long and they applied heat hardening by immersing the seedling roots in hot water.

TTC Cell Viability Assay

The assay was performed according to Porter et al. (1994). A 2.5-cm segment about 0.5 cm from the leaf tip was excised from each seedling. This segment was then quartered, and two quarter segments were placed in 10 mL capped test tubes containing 100 μL distilled water. Treatment tubes were heated in a water bath for 1 h at 50°C , while control tubes remained at 10°C . A 2-mL solution of 0.8% TTC (w/v) dissolved in a 0.05 M phosphate buffer (pH 7.4) was added to all tubes, which were then placed in a vacuum chamber for infiltration of TTC into leaf tissues. Leaf segments were considered to be infiltrated when they settled to the bottom of the tube after release of the vacuum. Tubes were then capped and placed in total darkness for 12 h at 25°C . Subsequently, the TTC solution was drained and leaf segments were rinsed in distilled H_2O . Leaf segments were then returned to the tube, and formazan dye was extracted by addition of 2.5 mL of 95% ethanol. Tubes were capped and allowed to remain in darkness for 24 h at 25°C for extraction. The amount of formazan dye produced by TTC reduction was determined spectrophotometrically at 530 nm. Cell viability as a measurement of thermotolerance was determined as the percent for treated leaves relative to the control absorbency.

Cell Membrane Thermostability (CMS)

Each sample consisted of one leaf segment, 3.5-cm long, washed three times in distilled water and placed in a 10 mL capped vial containing 1 mL of distilled water. The treatment tubes (three per cultivar) were heated in a water bath for 1 h at 52°C , while

control tubes (three per cultivar) remained at 10°C. After treatment, 9 mL of distilled water was added to vials and incubated at 10°C for 24 h. Vials were then brought to room temperature, and solution conductance was measured with a conductivity meter (YSI MODEL 345, Yellow Springs, OH). After the measurements were taken, vials were autoclaved for 15 min and their conductance measured again. The CMS was calculated as the reciprocal of cell membrane injury after Blum and Ebercon (1981):

$$\text{CMS (\%)} = [1 - (T_1/T_2) / 1 - (C_1/C_2)] \times 100$$

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

TTC and CMS Assays at the Flowering Stage

Of the 56 cultivars, eight were selected based on their divergent heat tolerance at the seedling stage. Plants were grown in the greenhouse in a 3.9L pot, one plant per pot. Plants were watered daily and fertilized once a week with a complete solution of Miracle-Gro (15-30-15, Port Washington, NY). Temperatures ranged between 22° and 24°C during plant development. At the boot growth stage, plants were transferred to a growth chamber set at 25/15°C (day/night) with 450 $\mu\text{m}^{-2} \text{s}^{-1}$. At anthesis, plants were transferred to a constant temperature (34°C) and high humidity (>80%) chamber to acclimate for 24 h. Flag leaves were then sampled for TTC and CMS assays as described above. The experiment was a completely randomized design with three replications.

Inheritance of TTC Reduction Thermotolerance Trait

To analyze inheritance of cellular thermotolerance measured by the TTC assay, the heat tolerant variety (high TTC) 'V747' was crossed with the heat susceptible variety 'Barkae' (low TTC). The F₁ hybrid and first generation backcrosses to each parent were made in the greenhouse. Six populations (P₁, P₂, F₁, BC₁, BC₂, and F₂) were developed and used to determine the mode of inheritance and heritability. The TTC measurements were made on an individual plant basis. Fifteen plants for the parents and F₁, 35 plants for each backcross, and 187 plants for the F₂ population were used. All plants were grown under similar conditions and evaluated according to the same protocol at the seedling stage as detailed above. Broad sense heritability was estimated as a ratio of genetic variance to total phenotypic variance (Allard, 1960) as follows: $H^2 = V_g / V_g + V_e$, where: V_g is the genetic variance and V_e is the environmental variance. Genetic variance was estimated by: $V_g = V_{F_2} - V_e$, and environmental variance was estimated by the average phenotypic variance of the two parents and F₁. Additive and Dominant variances were estimated according to Warner (1952).

RESULTS AND DISCUSSION

Comparison of methods and growth stages

Large and significant differences existed among cultivars tested for TTC and CMS at the seedling and the flowering growth stages (Tables 1 and 2). Mean TTC values ranged from 10.7% in Heines Peko to 70.4% in Glennson at the seedling stage (Table 1) and from 18.8% in Barkae to 45.7% in V747 at the flowering stage (Table 2). Mean CMS values ranged from 28.4% in Sonora 64 to 87% in Danbata at the seedling stage and from 75.4% in Danbata to 34.9% in IP 4 at the flowering stage. No consistent differences were seen in TTC or CMS according to origin of the cultivars.

Average TTC over all cultivars did not differ significantly between the seedling and the flowering growth stages (34% and 31.4%, respectively). Average CMS was significantly higher ($P < 0.05$) at the seedling (58.4%) than at the flowering (52.6%) growth stage. The decline in thermotolerance with plant aging has already been documented in wheat (Blum and Ebercon, 1981).

Despite the difference in average thermotolerance by CMS between growth stages, the association between thermotolerance at the seedling growth stages and thermotolerance at the flowering growth stages across cultivars was linear and highly significant for both the TTC assay ($R^2=0.72$; Fig. 1) and the CMS assay ($R^2=0.85$; Fig. 2). Saadalla *et al.* (1990b) found a high correlation between the germinating seedling stage and the flowering growth stage for membrane thermostability across seven cultivars, although cultivar ranking at the two growth stages was not consistent. While our study has been prepared for publication, Reynolds *et al.* (1994) reported a significant correlation for CMS among different wheat cultivars between growth chamber grown seedlings and heat acclimated field plants. In our study, we establish and affirm the correlation between the green seedling (10 d old) growth stage and the flowering growth stage in terms of both CMS and TTC. Basic genotypic variations for cellular thermotolerance in terms of both TTC and CMS assays can therefore be evaluated in growth chamber heat-hardened seedlings, which for this purpose represent the thermotolerance of the fully developed plant. Assays in seedlings are more economical, as they require relatively less time and space.

Results by TTC was well correlated with results by CMS across the eight cultivars tested (Table 2) at both the seedling ($r=0.74$; $p=0.031$) and the flowering ($r=0.75$; $p=0.029$) growth stages (Fig. 3). When all 56 cultivars tested at the seedling stage (Table 1) were examined, the correlation coefficient between TTC and CMS held statistically ($r=0.32$; $p=0.014$), although it was not very high. Chen *et al.* (1982) found that TTC and CMS assays gave similar ranking for cellular thermotolerance when few cultivars and species were compared. There is an association between thermotolerance in

terms of cell membrane stability and thermotolerance in terms of cellular viability, but the strength of the association may depend on the size of the population and its genetic diversity. It cannot be assumed that the cell membrane thermostability and cellular viability under heat stress are necessarily linked genetically or associated physiologically. Some of the cultivars, such as Glennson, Bacanora (from CIMMYT), and V747 (from Israel) were relatively thermotolerant in terms of both TTC and CMS. Danbata (from Nigeria) was thermotolerant in terms of CMS while being intermediate for TTC.

Inheritance of TTC Thermotolerance

The cross V747/Barkaee was studied only for TTC thermotolerance and not for CMS thermotolerance because parents differed more prominently for TTC than for CMS. The mean TTC values of P₁ (V747) and P₂ (Barkaee) as measured in this part of the research were significantly ($p=0.05$) different, 54% and 26.8%, respectively (Table 3). The F₁ mean (46.9%) exceeded the midparent and was not significantly different from P₁ (V747), indicating that gene(s) controlling acquired thermotolerance as measured by TTC assay are dominant. In this population, the mean of BC₁P₁ was intermediate between the means of F₁ and that of P₁ (V747). However, it was not significantly different at the 0.05 level from P₁ (V747). The mean of BC₁P₂ was higher than the mean of P₂ (Barkaee) but not significantly different at the 0.05 level which also indicated the dominant nature of gene action.

Estimates of additive genetic variance, dominance genetic variance, and environmental components showed that most genetic variation could be accounted for by additive genetic variance, suggesting that progress from selection could be made for this trait. Broad-sense heritability was estimated at 89% in this study. These results support the earlier finding by Porter *et al.* (1995). It is important to note that the gene action was dominant, while the genetic variation was largely due to additive variance, as stated by Falconer (1980) "The concept of additive variance does not carry with it the assumption of additive gene action".

CONCLUSIONS

Reynolds et al. (1994) found that the rate of heat injury to cell membranes (the reciprocal of CMS) as measured in heat acclimated seedlings in the growth chamber was negatively and significantly correlated with yield of 16 wheat cultivars grown in several hot environments. All of their 16 tested cultivars were included in our study (Table 4). We have obtained their yield data in the various hot environments from the report by Reynolds et al. (1992) and calculated the correlations between these yields and heat tolerance in terms of CMS and TTC as measured in our study (Table 4). It was extraordinary to find that CMS as measured here was significantly correlated with yields of these cultivars in all of the four stress environments. On the other hand TTC was positively but not significantly correlated with these yields.

We therefore support the results of Reynolds et al. (1994) and conclude that when performed with the proper heat hardening protocol with seedlings, CMS appears to predict reasonably well the relative heat tolerance of wheat genotypes in terms of their yield level in hot environments. While thermotolerance rating by TTC is highly heritable and it is correlated to some extent with that of CMS, TTC is relatively less predictive of plant performance under heat stress.

REFERENCES

- Allard, R.W. 1960. Principles of plant breeding. John Wiley and Sons, New York.
- Blum, A. 1988. Plant breeding for stress environments. CRC Press, Inc., Boca Raton. 209 p.
- Blum, A., and A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21:43-47.
- Chen, H.H., Z.Y. Shen, and P.H. Li. 1982. Adaptability of crop plant to high temperature stress. *Crop Sci.* 22:719-725.
- Falconer D.S. 1981. Introduction to Quantitative Genetics. Longman, London.
- Fischer, R.A. 1986. Physiological limitations to producing wheat in semitropical and tropical environments and possible selection criteria. p. 209-230. In *Proc. Internat. Symp. Wheat for More Tropical Environments*. CIMMYT/UNDP, Mexico.
- Krishnan, M., H.T. Nguyen, and J.J. Burke. 1989. Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol.* 90:140-145.
- Kuo, C.G., H.M. Chen, and H.C. Sun. 1992. Membrane thermostability and heat tolerance of vegetable leaves. p. 160-168. In C.G. Kuo (ed.) *Adaptation of food crops to temperature and water stress*. Proc. Intern. Symp., Asian Vegetable Research and Development Center, Taiwan.
- Levitt, E. 1980. Responses of plants to environmental stresses. Vol. I. Academic Press, New York.
- Lin, C-Y., Y-M. Chen, and J.L. Key. 1985. Solute leakage in soybean seedlings under various heat shock regimes. *Plant Cell Physiol.* 26:1493-1498.

- Marsh, L.E., D.W. Davis, and P.H. Li. 1985. Selection and inheritance of heat tolerance in the common bean by use of conductivity. *HortScience* 110:630-683.
- Martineau, J.R., J.H. Williams, and J.E. Specht. 1979. Temperature tolerance in soybeans. II. Evaluation of segregating populations for membrane thermostability. *Crop Sci.* 19:79-81.
- Milliken, G.A. 1987. Comparison of TTC and electrical conductivity heat tolerance screening techniques in *Phaseolus*. *HortScience*. 22:642-645.
- Onwueme, I.C. 1979. Rapid plant conserving estimation of heat tolerance in plants. *J. Agric. Sci. Camb.* 92:527-531.
- Ottaviano, E., M.S. Gorla, E. Pe, and C. Frova. 1991. Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. *Theor. Appl. Genet.* 81:81-713.
- Porter, D.R., H.T. Nguyen, and J.J. Burke. 1994. Quantifying acquired thermal tolerance in winter wheat. *Crop Sci.* 34:1686-1689.
- Porter, D.R., H. T. Nguyen, and J.J. Burke. 1995. Genetic control of acquired thermal tolerance in wheat. *Euphytica* 83:153-157.
- Reynolds, M.P., E. Acevedo, O.A.A. Ageeb, S. Ahmed, M. Balota, L.J.B. Cervelho, R.A. Fischer, E. Ghanem, R.R. Hanchinal, C.E. Mann, L. Okuyama, L.B. Olugbemi, G. Ortiz-Ferrara, M.A. Razzaque and J.P. Tandon. 1992. Results of the 1st International Heat Stress Genotype Experiments. Wheat Special Report No.14, Mexico, D.F. CIMMYT.
- Reynolds, M.P., M. Balota, M.I.B. Delgado, I. Amani and R.A. Fischer. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Aust. J. Plant Physiol.* 21:7170730.
- Saadalla, M.M., J.F. Shanahan, and J.S. Quick. 1990a. Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability. *Crop Sci.* 30:1243-1247.
- Saadalla, M.M., J.S. Quick, and J.F. Shanahan. 1990b. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. *Crop Sci.* 30:1248-1251.
- Sullivan, C.Y., N.V. Norcio, and J.D. Eastin. 1977. Plant responses to high temperatures. *In* Muhammed Askel and Von Borstel (eds.). *Genetic Diversity in Plants*. Plenum. 301 p.
- Suss, K-H., and I.T. Yordanov. 1986. Biosynthetic causes of in vivo acquired thermotolerance of photosynthetic light reactions and metabolic responses of chloroplast to heat stress. *Plant Physiol.* 81:192-199.
- Towil, L.E., and P. Mazur. 1974. Studies on the reduction of 2,3,5-triphenyl tetrazolium chloride as a viability assay for plant tissue culture. *Can. J. Bot.* 53:1097-1102.
- Wallner, S.J., M.R. Bechwar, and J.D. Butler. 1982. Measurement of turfgrass heat tolerance in vitro. *J. Am. Soc. Hort. Sci.* 107:608-611.
- Warner, J.N. 1952. A method for estimating heritability. *Agron.J.* 44:427-430
- Wehner, D.J., and T.L. Watschke. 1981. Heat tolerance of Kentucky bluegrasses, perennial ryegrasses and annual bluegrass. *Agron. J.* 73:79-83.

Table 1. Thermotolerance as estimated by the cell viability assay (TTC) and cell stability (CMS) in 56 wheat cultivars at the seedling stage (two pages).

Cultivar	Origin	TTC (%)	Cultivar	CMS (%)
Heines Peko	Germany	10.7 a†	Sonora 64	28.4 a
Nesser	CIMMYT	11.5 a	Nacozari	31.6 ab
IP 4	Myanmar	11.8 ab	Heines Peko	33.4 abc
Aka Komughi	Japan	13.7 abc	Genaro	37.0 abcd
Chinese Spring	USA	16.4 abcd	Kanchan	37.5 abcd
CDN 114/87	Israel	16.8 abcd	IP 4	37.8 abcd
Trigo 3	Phillippines	16.9 abcd	Trigo 3	41.5 bcde
Barkaee	Israel	17.4 abcde	THB/CEP 77	43.6 cdef
Kanchan	China	17.5 abcdef	Barkaee	43.7 cdef
Sampo	Finland	17.6 abcdef	Bansi Palli	44.5 cdef
Sbei	Tunisia	17.7 abcdefg	V2420	45.3 defg
K1056	Israel	18.0 abcdefg	CDN97/87	48.1 defg
Kauz	CIMMYT	18.7 abcdefgh	V2434	49.8 efghi
Bethlehem	Israel	19.0 abcdefghi	Chinese Spring	50.0 efghi
CNO*2/HE1	CIMMYT	19.7 abcdefghi	Debeira	50.0 efghi
Anza	Australia	20.1 abcdefghi	Pavon	51.5 efghij
CDN97/87	Israel	21.7 abcdefghij	K3	53.8 fghijk
K3	Israel	21.8 abcdefghij	Sbei	55.8 ghijkl
Glyndon	USA	22.0 abcedfghij	CIANO 79	56.0 ghijkl
Cook	Australia	22.1 abcdefghij	V748-74-1	56.3 ghijkl
Gutha	Australia	23.0 abcdefghijk	K1056	56.4 ghijkl
V2151-5	Israel	23.4 abcdefghijk	Gutha	56.8 hijkl
Sonalica	Australia	25.0 abcdefghijk	Aka Komughi	57.3 hijkl
Genaro	CIMMYT	25.1 abcdefghijkl	Atir	57.4 hijkl
Niama	Ethiopia	25.1 abcdefghijkl	V5	57.5 hijkl
Nacozari	CIMMYT	25.5 abcdefghijkl	Bethlehem	57.7 hijkl
Lachish	Israel	26.7 bcdefghijklm	Siete Cerros	58.8 hijkl
Sonora 64	CIMMYT	28.0 cdefghijklm	SST 86	59.4 ijklm
Carmo	Brazil	28.5 cdefghijklm	Nesser	60.7 ijklmn
Chuanmail 8/BAU71	CIMMYT	29.4 cdefghijklmn	Glyndon	60.8 ijklmn
CIANO 79	CIMMYT	30.2 defghijklmn	Teboca	60.9 ijklmn
V2420	Israel	32.3 defghijklmn	Sampo	61.3 jklmno
Pavon	CIMMYT	32.4 defghijklmno	Sundor	61.3 jklmno
SST 86	South Africa	23.6 efgghijklmnop	Sinai N1	61.9 jklmno

CONT...

(Continued)

Cultivar	Origin	TTC (%)	Cultivar	CMS (%)
Atir	Israel	33.8 fghijklmnop	Anza	62.6 jklmnop
V748-74-1	Israel	34.0 ghijklmnop	Sunstar	62.6 jklmnop
Danbata	Nigeria	34.8 hijklmnop	Comet	62.8 klmnop
V5	Israel	35.1 ijklmnop	CDN 114/87	64.5 klmnop
V2434	Israel	37.6 jklmnopq	Pea's	65.5 lmnopq
Pea's	CIMMYT	39.3 klmnopqr	V2183	66.0 lmnopq
Siete Cerros	CIMMYT	39.5 klmnopqr	CNO*2/HE1	66.9 lmnopqr
Teboca	CIMMYT	41.4 lmnopqr	V747	67.3 mnoopqr
Sinai N1	Egypt	43.9 mnoopqrs	Prostor	67.4 mnoopqr
Bansi Palli	India	43.9 mnoopqrs	Sonalica	68.9 mnoopqr
Seri 82	CIMMYT	45.1 nopqrs	Fang 60	70.8 nopqr
V2183	Israel	45.3 nopqrs	Chuanmail8/BAU'1	71.0 nopqr
Sunstar	Australia	45.7 nopqrs	Cook	71.5 nopqr
Prostor	South Africa	48.6 opqrs	V2151-5	71.7 nopqr
Fang 60	Thailand	48.8 pqrs	Niama	71.8 nopqr
Debeira	Niger	49.8 pqrs	Kauz	72.5 opqr
Sundor	Australia	52.2 qrs	Carp	73.7 pqr
Comet	Australia	54.4 rst	Bacanora	74.6 qr
THB/CEP 77	CIMMYT	54.7 rst	Lachish	75.0 qr
Bacanora	CIMMYT	57.4 st	Glennson	77.2 rs
V747	Israel	58.8 st	Seri 82	77.9 rs
Glennson	CIMMYT	70.4 t	Danbata	87.0 s
Mean		31.4		58.4
CV (%)		32.5		

(†) Values followed by the same letters are not significantly different according to the new Duncan's multiple range test.

Table 2. Thermotolerance as estimated by the cell viability assay (TTC) and cell membrane stability (CMS) in eight wheat cultivars at the flowering stage.

Cultivar	TTC (%)	Cultivar	CMS (%)
Barkaee	18.8 a†	IP 4	34.9a
Nacozari	21.5 ab	Nacozari	38.0a
Kanchan	24.5 ab	Barkaee	42.9ab
IP 4	25.7 ab	Kanchan	48.1abc
Bacanora	32.7 bc	Bacanora	54.6bcd
Danbata	33.6 bcd	V747	61.7cde
Fang 60	40.1 cd	Fang 60	65.5de
V747	45.7 d	Danbata	75.4e
Mean	30.3		52.6
C.V.(%)	21.1		21.0

(†) Means followed by the same letters are not significantly different according to the new Duncan's multiple range test.

Table 3. Population means and variances for TTC thermotolerance in
'V747'/'Barkaee' wheat population.

Population	No. of Plants	Mean	Variance
P ₁ (V747)	15	54.0 a†	19.4
P ₂ (Barkaee)	15	26.8 d	16.8
Midparent	--	40.4	--
F ₁	15	46.9 ab	20.2
BC ₁ P ₁	35	40.7 bc	88.0
BC ₁ P ₂	35	30.8 d	90.8
F ₂	187	38.3 c	184.2

(†) Means with the same letters are not significantly different at the 0.05 probability level.

Table 4. Correlations (r) across 16 wheat cultivars between the rate of cell membrane injury in seedlings and grain yield in various hot environments as per Reynolds et al. (1994; Table 5) and correlations between the same yields and CMS and TTC values for the same 16 genotypes as measured in this study (Table 1).

Heat stress location	%Injury [†]	CMS [‡]	TTC [‡]
Tlaltizapan (Mexico)	-0.01	0.51*	0.34
Sudan	-0.58*	0.61*	0.40
India	-0.57*	0.57*	0.38
Brazil	-0.57*	0.63*	0.47

[†] as per Table 5 in Reynolds et al. (1994).

[‡] as per Table 1 above.

* significant at P<0.05

Fig. 1. Association in thermotolerance by TTC between the seedling and the flowering stages of growth across eight spring wheat cultivars.

Fig. 2. Association in thermotolerance by CMS between the seedling and the flowering stages of growth across eight spring wheat cultivars.

Fig. 3. The association between TTC and CMS as measured at the seedling and the flowering growth stages across eight wheat cultivars (Table 2). For the seedling growth stage: $y = 27.9 + 0.83X$; $R^2 = 0.56$. For the flowering growth stage: $y = 18.37 + 1.13x$; $R^2 = 0.56$.

E.2. Heat Tolerance In Spring Wheat. II. Grain Filling

ABSTRACT

Heat stress during grain filling is a major constraint of wheat (*Triticum aestivum* L.) yield. Grain filling under heat stress would depend on plant thermotolerance and its capacity to utilize stem reserves for grain filling. This study was performed to evaluate the relationship between stem reserves as a constitutive trait and thermotolerance to sustained wheat grain filling under heat stress.

Seven spring wheat cultivars were tested in two experiments. The percent reduction under heat stress in grain weight per ear and its components was measured as an index of tolerance. The potential capacity for using mobilized stem reserves for grain filling (PSR) was determined by estimating the relative reduction in grain weight per ear in plants devoid of their photosynthetic source. Plant senescence was estimated by flag leaf chlorophyll content reduction during grain growth. Cell membrane thermostability (CMS) was assayed by the electrolyte leakage method in leaves of seedlings and flowering plants after heat-hardening.

Significant variation was seen among cultivars in the reduction in grain weight per ear (RGW), kernel number, and kernel weight under heat stress. Differences in RGW among cultivars were ascribed to variation in the reduction in both kernel number and kernel weight under heat stress. Variation in PSR among cultivars was ascribed to variations in both kernel number and kernel weight under defoliation and ear shading. There was a strong negative correlation across cultivars ($r=-0.96$; $p<0.01$) between RGW and PSR. A significant positive correlation ($r=0.92$; $p<0.01$) was found across cultivars between the rate of chlorophyll loss under heat stress and PSR, indicating that a high potential capacity for utilizing stem reserves for grain filling may be linked with accelerated leaf senescence. There was a strong negative association across cultivars between RGW and CMS at the seedling ($r=-0.98$) and the flowering ($r=-0.92$; $p<0.01$) growth stages. The results indicate that grain filling under heat stress is closely related to the capacity for stem reserve remobilization as a constitutive trait and to plant thermotolerance as expressed by CMS in heat-hardened seedlings or adult plants.

INTRODUCTION

High temperature is one of the major environmental constraints of wheat yield. Heat stress during grain filling is a common occurrence in wheat. High temperatures, typically above 34°C, affect final grain weight by reducing the duration of grain filling, by suppressing current photosynthetic (AL-Khatib and Paulsen, 1984), and by directly inhibiting starch biosynthesis in the endosperm (Jenner 1994; Keeling *et al.*, 1993).

When the photosynthetic source is confined by heat stress, the only major alternative source of carbon for grain filling is remobilized stem reserves. The capacity to support grain filling from remobilized stem reserves is constitutive (Blum *et al.*, 1994), although it may be influenced by stress (Palta *et al.*, 1994). The identification of such a capacity is possible by experimentally inhibiting current photosynthesis during grain filling by leaf removal or chemical desiccation of the canopy (Blum *et al.*, 1983). When such treatments are imposed on non-stressed plants, the ratio of final grain weight under treatment to that of non-treated controls has been accepted as a relative estimate of the potential capacity for supporting grain filling from stem reserves (PSR) (e.g. Hossain *et al.*, 1990; Regan *et al.*, 1993; Nicolas and Turner, 1993).

Cellular membrane stability under heat stress after a pre-hardening treatment (CMS) has been widely accepted as a suitable technique for estimating the cellular thermotolerance of plants (reviewed by Blum, 1988 and Kuo *et al.*, 1992). The method has been applied to wheat (Blum and Ebercon, 1981; Saadalla *et al.*, 1990) and in our previous report in this series (Fokar *et al.*, 1997) we were able to confirm results by Reynolds *et al.* (1994) that CMS predicts reasonably well the relative yield performance of different wheat cultivars in hot environments

This study was performed to re-evaluate the importance of PSR for grain filling under heat stress and to find out if the inherent heat tolerance of the different cultivars in terms of CMS is related to their capacity for sustained grain filling under heat stress.

MATERIALS AND METHODS

Experiment 1

Based on prior screening tests for cell membrane thermostability (Fokar *et al.*, 1997), two heat tolerant ('Danbata', 'Fang-60') and three heat sensitive ('IP4', 'Kanchan', and 'Nacozari') spring wheat cultivars were chosen for this study. Plants were grown in two gallon plastic pots filled with a commercial mix containing sand, soil, peat, and wood bark (Ball Growing-on Mix, Ivy Gardens, Lubbock, TX). Each pot consisted of three plants with sixty-four pots per genotype. Plants were grown in a greenhouse where maximum daily temperatures from emergence to anthesis ranged from 18° to 26°C. Plants were watered and fertilized weekly with a complete solution of Miracle-Gro® (15-30-15 NPK, Port Washington, NY). Orthene was applied three times during pre-anthesis growth stages to control greenbug (*Schizaphis graminum*). At flowering, Prentox® Perm-x 1E (Hummert International, Earth City, MO) was applied to control greenhouse thrips (*Heliothrips haemorrhoidalis*).

Pots were divided randomly at anthesis into two groups. One group (control temperature) remained in the same greenhouse where mean maximum daily temperatures during grain filling ranged from 19° to 28°C with an average of 26°C (Fig.1-A). The second group (heat stress treatment) was moved to an adjacent greenhouse where mean maximum daily temperatures during grain filling ranged from 31° to 42°C with an average of 38°C (Fig.1-B). Plants were irrigated and fertilized as described above. In each greenhouse treatment, pots were arranged in a completely randomized block design with four replicates, where each replicate consisted of eight pots.

Kernel growth was estimated by sampling plants for dry kernel weight on 2, 7, 14, 21, and 49 days after imposing the heat treatment at anthesis. On each date, 10 kernels were extracted from the middle florets of each of four main stem ears (replicates), dried at 80°C for 24 h, and weighed.

Chlorophyll content on a dry weight basis was assayed according to Moran and Porath (1980). Plants were sampled 2, 7, 14, and 21 days after anthesis. On each date, four flag leaves (replicates) were sampled. A midsection of approximately 8 cm² was cut from each leaf and divided into two equal segments. One segment was incubated for 24 h in the dark in 10 mL of N, N dimethyl formamide. Aliquots (1 mL) were used to determine total chlorophyll content spectrophotometrically. The other segment was dried at 80°C for 24 h to determine the dry weight.

Experiment 2

The five cultivars used in Exp. 1 as well as cultivars V5 and V2183 were used in Experiment 2. Cultivar V5 was shown to be superior to cultivar V2183 in supporting grain filling under heat stress from mobilized stem reserves (Blum *et al.*, 1994). Plants

were grown in one gallon, each pot consisted of one plant with 24 pots per genotype. Plants were grown at the same time and under the same conditions as described for the cool greenhouse above. At anthesis, pots were divided randomly into three groups. One group remained in the same greenhouse (control temperature treatment; Fig. 1-A). The second group was moved to an adjacent high temperature greenhouse (heat stress treatment; Fig. 1-B). The third group was manually defoliated at anthesis by excising all leaf laminae and kept in the control greenhouse. Ears of defoliated plants were covered with brown Kraft paper bags to reduce ear photosynthesis. Photosynthetically active radiation inside the bag was 54 to 63 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as compared with 589 to 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$ outside the bag when measured on several dates with a LICOR radiometer. Ears under the bag developed normally and were visibly free of any molds or insects.

In each treatment, pots were arranged in a randomized complete design with four replicates, where each replicate consisted of two pots. At maturity, main stem ears were harvested and seeds were threshed, weighed, and counted. Heat tolerance in terms of grain weight per ear and its components (kernel number per ear and kernel weight) was estimated for each cultivar by the reduction in value by heat stress compared with the controls. The potential capacity for using mobilized stem reserves for grain filling (PSR) was estimated by the percentage ratio of grain weight per ear in defoliated plants with bagged ears to those of the controls.

RESULTS AND DISCUSSION

The two experiments were subjected to the same control and heat stress conditions in the two respective greenhouses (Fig. 1). Maximum daily temperatures under the stress treatment were above 35°C and often reached 40°C or more, while they never reached 30°C in the control. In each greenhouse, all cultivars flowered within two days.

Experiment 1

Heat stress reduced final kernel weight compared with the controls in each cultivar. A typical effect of temperature on kernel dry matter accumulation in two representative cultivars is presented in Figure 2. In each cultivar, kernel weight was reduced under heat stress because of an appreciable reduction in kernel growth duration compared with non-stress controls. There was a slight increase in early kernel growth rate under heat stress, as would be expected, but that increase was not sufficient to compensate for the loss in dry matter accumulation due to reduction in grain filling duration. The reduction in kernel weight on the last sampling date in the heat stressed Nacozari was barely significant, but it could be a real phenomenon as previously observed (Blum *et al.*, 1994). Danbata was more thermotolerant than Nacozari.

Percent reduction in final kernel weight under heat stress compared to the controls is an estimate of the relative sensitivity of the different cultivars in terms of grain filling under heat stress (Table 1). Cultivars differed significantly ($P < 0.05$) in this respect, ranging from 39.3% reduction in Danbata to 58.3% reduction in Nacozari.

Chlorophyll content of flag leaves was a measure of leaf senescence as accelerated by heat stress (Al-Khatib and Paulsen, 1984). Chlorophyll content was reduced with time after anthesis regardless of treatment and cultivar (Fig.3). The rate of reduction was greater under heat stress than in controls, especially in Danbata (Fig.3; Table 1). Percent reduction in chlorophyll content under heat stress compared with the controls differed significantly between genotypes (Table 1). Danbata senesced the fastest under high temperature, compared with Nacozari (Table 1; Fig.3) or IP 4 (Table 1). Therefore, in terms of leaf chlorophyll retention, Danbata appeared to be more heat -sensitive than Nacozari or IP 4 while the opposite trend was seen for the reduction in kernel weight under heat stress in these two cultivars. Such a negative association between chlorophyll retention and grain filling capacity under heat stress has already been observed previously for two extreme cultivars in terms of grain filling under heat stress (Blum *et al.*, 1994). The correlation between percent kernel weight reduction and percent chlorophyll loss under heat stress (Table 1) was negative but not significant ($r = -0.35$) for all five cultivars tested, mainly because data for cultivar IP 4 did not fit the relationship.

Experiment 2

Heat stress and the defoliation and ear shading treatment (henceforth referred to as 'defoliation treatment') reduced grain weight per ear, kernel number per ear, and kernel weight compared with nonstress controls (Table 2). Averaged over all cultivars, defoliation caused a greater reduction in grain weight per ear and kernel weight than with heat stress. Some carbon assimilation may have still taken place in leaves or ears of the heat stressed plants, while practically none occurred under the defoliation treatment. The contribution of the leaf sheaths to carbon assimilation in the defoliated plants was expected to be very small (Araus *et al.*, 1987), especially after flowering.

Kernel number per ear was also reduced more under defoliation (44.3%) than under heat stress (26.9%). Grain filling has been reduced by defoliation and heat stress due to lack of assimilates and a possible direct effect of heat stress on endosperm starch biosynthesis (Jenner 1994; Keeling *et al.*, 1993). Seed set may have been curbed by defoliation and heat through an induced carbon deficiency. The carbon status of plants may affect seed set (Reed and Singletary, 1989), but critical data for wheat in this respect is lacking.

Cultivars differed significantly and appreciably in percent reduction in grain weight per ear and its components under heat stress (Table 2). As in experiment 1, Danbata

demonstrated its relative heat tolerance by sustaining less reduction in kernel number and kernel weight under heat stress compared with most other cultivars. Cultivar V5 again (Blum *et al.*, 1994) expressed its relative heat tolerance in kernel weight under heat stress with somewhat lower tolerance in kernel number per ear compared with Danbata. At the other extreme, Nacozari was again noted for its relatively high sensitivity to heat stress in terms of kernel weight reduction under heat stress.

Cultivars differed significantly and appreciably in rates of reduction in grain weight per ear and its components under the defoliation treatment (Table 2). Both Danbata and V5 sustained the least reduction in kernel weight compared with Nacozari. Danbata also retained its relative superiority in kernel number under this treatment as it did under heat stress. Significant ($p < 0.01$) and often very high correlations existed across cultivars for grain weight per ear, kernel number, and kernel weight reductions between heat stress and defoliation treatments (Table 3). An overall high level of association was seen between reproductive performance under heat stress and that under defoliation treatment across the seven tested cultivars.

Potential capacity for supporting grain filling from stem reserves (PSR) was estimated in this study by the final grain weight per ear under the defoliation treatment as a percent of that of the control. The PSR is the reciprocal of the percent of reduction in kernel weight per ear under the defoliation treatment (Table 2). PSR was highly and negatively correlated across cultivars with rates of reduction in grain weight per ear, kernel number, and kernel weight under heat stress (Fig.4). Danbata had the highest PSR of 45.8% followed by 35.0% in V5. At the other end was Nacozari with a PSR of only 13.4%. The strong negative association ($r = -0.96$; $p < 0.01$) between PSR and the rate of reduction in grain weight per ear under heat stress across the seven cultivars (Fig.4) lends support to the conclusion that the potential (and constitutive) capacity for grain filling from remobilized stem reserves is very important for grain filling under heat stress, as first reported elsewhere for only two cultivars (Blum *et al.*, 1994).

Leaf chlorophyll retention is often regarded as an expression of heat tolerance and delayed leaf senescence. In Experiment 1, leaf chlorophyll content was reduced by heat stress after anthesis and cultivars differed significantly in this respect (Fig.3). However, leaf chlorophyll retention was better in the cultivar Nacozari, which did poorly in terms of sustained grain filling under stress, while it was poor in the cultivar Danbata, which sustained grain filling under heat stress better. A very significant positive correlation ($r = 0.92$; $P < 0.01$) was found for the five cultivars tested for chlorophyll content in Exp. 1 and PSR measured in Exp. 2. This suggested that a high potential capacity to utilize stem reserves for grain filling may be linked with accelerated leaf senescence. Hence, delayed leaf senescence as a genetic trait should not be taken *a priori* as an advantage under heat stress, and it should be reviewed in the context of the whole plant and its grain filling

capacity from stem reserves. While green leaf area retention during grain filling may favor current assimilation, it may be linked with low capacity to support grain filling from stem reserves. This issue warrants further investigation.

While the constitutive capacity of a cultivar in utilizing stem reserves for grain filling is seen here as an important factor for sustaining grain filling under heat stress, it can be assumed that cellular thermotolerance may have also been important in affecting the variation among these cultivars in grain filling under heat stress. However, a quantitative partitioning of the contributions of these two factors is practically impossible here. Some insight may still be derived from present and past data.

Data for thermotolerance in terms of CMS were available from the previous studies with five of the cultivars tested here (Fokar *et al.*, 1997). CMS was measured in seedlings and in flag leaves at flowering. These data were compared with the relative reduction in grain weight per ear under heat stress as measured here (Table 2; Fig.5). It was found that regardless of growth stage, CMS as measured in a previous study was highly and negatively associated with the rate of reduction in grain weight per ear under heat stress in this study. Thermotolerance expressed by CMS was related to the capacity for sustained grain filling under heat stress.

This relationship is supported by two additional sources. First, Shpiler and Blum (1991) tested the relative heat tolerance of various wheat cultivars by comparing their field performance for yield and its components in the normal winter season compared with heat stress conditions in a summer season planting (fully irrigated) at Bet Dagan, Israel. Their data were used to calculate the susceptibility index (S) according to Fischer and Maurer (1978), which estimated cultivar heat susceptibility in terms of kernel weight reductions. For 10 wheat cultivars so tested, Blum (unpublished) measured CMS in leaves of three-week-old seedlings grown and heat-hardened in the growth chamber. A significant, negative (nonlinear) association was apparent (Fig.6) between kernel weight heat susceptibility index and CMS, indicating that thermotolerance was related to grain filling capacity under heat stress.

Finally, work with different winter wheat cultivars tested in Colorado (Saadalla *et al.*, 1990) established a positive association between thermotolerance in terms of CMS in seedlings and grain filling capacity under stress conditions in the field.

We, therefore, suggest that the capacity for grain filling under heat stress is associated with cellular thermotolerance as a heat adaptive trait and with stem reserve utilization potential as a constitutive trait. These associations stand out, especially for cultivars representing the extremes such as the resistant Danbata and the susceptible Nacozari. It is quite possible that while a potential source of carbon must be available as transportable stem reserves, this carbon cannot be synthesized into starch at high temperatures unless thermotolerance is involved. A main endosperm starch synthesizing

enzyme, soluble starch synthase, was found to be inhibited by high temperatures (Jenner, 1994; Keeling *et al.*, 1993) causing the inhibition of starch accumulation at temperatures above 34°C. Stress temperatures during grain filling in the experiment reported here were very often in excess of 34°C (Fig.1). Hence, CMS measured in these cultivars could be either genetically linked or associated in some other fashion with the capacity for carbon utilization towards starch synthesis at temperatures under chronic heat stress.

REFERENCES

- Al-Khatib, K., and G.M. Paulsen. 1984. Mode of high temperature injury to wheat during grain development. *Physiol. Plant* 61:363-368.
- Araus, J. L., and L. Tapia. 1987. Photosynthetic gas exchange characteristics of wheat flag leaf blades and sheaths during grain filling. *Plant Physiol.* 85:667-673.
- Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton, FL.
- Blum, A., and A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21:43-47.
- Blum, A., H. Poyarkova, G. Golan, and J. Mayer. 1983. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. I. Effects on translocation and kernel growth. *Field Crops Res.* 6:51-58.
- Blum, A., B. Sinmena, J. Mayer, G. Golan, and L. Shpiller. 1994. Stem reserve mobilization supports wheat grain filling under heat stress. *Aust. J. Plant Physiol.* 21:771-781.
- Fischer, R.A., and R.O. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agr. Res.* 29:875.
- Fokar, M., Nguyen, H.T. and A. Blum. 1997. Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. *Euphytica*
- Hossain, A.B.S., R.G. Sears, T.S. Cox and G.M. Paulsen. 1990. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Sci.* 30:622-627.
- Jenner, C.F. 1994. Starch synthesis in the kernel of wheat under high temperature conditions. *Aust. J. Plant Physiol.* 21:791-806.
- Keeling, P.L., P.J. Bacon, and D.C. Holt. 1993. Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. *Planta* 191: 342-348.
- Kuo, C.G., H.M. Chen, and H.C. Sun. 1992. Membrane thermostability and heat tolerance of vegetable leaves. p. 160-168. *In* C.G. Kuo (ed.) *Adaptation of food crops to temperature and water stress.* Proc. Intern. Symp., Asian Vegetable Research and Development Center, Taiwan.
- Moran, R., and D. Porath. 1980. Chlorophyll determination in intact tissues using N,N-Dimethylformamide. *Plant Physiol.* 65:478-479.
- Nicolas, M.E. and N.C. Turner, N.C. 1993. Use of chemical desiccants and senescing agents to select wheat lines maintaining stable grain size during Post-Anthesis drought. *Field Crop Res.* 31:155-171.

- Palta, J.A., T. Kobata, N.C. Turner, and I.R. Fillery. 1994. Remobilization of carbon and nitrogen in wheat as influenced by postanthesis water deficits. *Crop Sci.* 34:118-124.
- Reed, A.J., and G.W. Singletary. 1989. Roles of carbohydrate supply and phytohormones in maize kernel abortion. *Plant Physiol.* 91:986-992.
- Regan, K.L., B.R. Whan, and N.C. Turner. 1993. Evaluation of chemical desiccation as a selection technique for drought resistance in a dryland wheat breeding program. *Aust. J. Agr. Res.* 44:1683-1691.
- Reynolds, M.P., M. Balota, M.I.B. Delgado, I. Amani and R.A. Fischer. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Aust. J. Plant Physiol.* 21:7170730.
- Saadalla, M.M., J.S. Quick, and J.F. Shanahan. 1990. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. *Crop Sci.* 30:1248-1251.
- Shpiler, L., and A. Blum. 1991. Heat tolerance for yield and its components in different wheat cultivars. *Euphytica* 51:257-263.

Table 1. Percent reduction in final kernel dry weight (at maturity) and percent reduction in flag leaf chlorophyll content (at 21 days after anthesis) by heat stress in five wheat cultivars (Experiment 1).

Cultivar	Kernel Weight (%)	Chlorophyll (%)
	Reduction	Reduction
Danbata	39.3	68.8
IP 4	39.5	20.5
Fang-60	42.4	47.8
Kanchan	54.0	41.1
Nacozari	58.3	28.3
LSD (p <0.05)	16.8	23.8

Table 2. Reduction (percent) in grain weight per ear, number of kernels per ear, and kernel weight under heat stress and under the defoliation treatment compared with controls (Experiment 2).

Cultivar	Origin	Heat Stress			Defoliation and Shading		
		Grain weight per ear	Kernels per ear	Kernel weight	Grain weight per ear	Kernels per ear	Kernel Weight
Danbata	Nigeria	32.3	5.1	27.4	53.9	27.2	35.7
Fang-60	Thailand	43.4	16.6	31.7	68.5	36.0	50.2
IP4	Myanmar(Burma)	63.9	40.5	38.6	83.6	51.3	64.4
Kanchan	Bangladesh	68.9	44.7	44.1	83.3	53.9	65.1
Nacozari	Mexico	64.3	29.0	47.7	86.6	55.3	68.9
V2183	Israel	56.3	23.3	42.5	79.1	36.5	65.7
V5	Israel	35.7	29.1	8.9	65.0	50.4	29.5
Mean		52.1	26.9	34.4	74.3	44.3	54.2
LSD (p < 0.05)		7.4	13.2	9.0	6.1	10.8	11.8

Table 3. Phenotypic correlations across seven cultivars among the percent reduction in grain weight per ear, kernel number per ear, and kernel weight under heat (H) and defoliation (D) stresses as compared with controls.

Variable	Grain weight/ear-H	Kernel number-H	Kernel weight-H	Grain weight/ear-D	Kernel number-D
Kernel number-H	0.786*				
Kernel weight-H	0.833*	0.325			
Grain weight/ear-D	0.959**	0.775*	0.746		
Kernel number-D	0.662	0.877**	0.208	0.734	
Kernel weight-D	0.934**	0.534	0.943**	0.908**	0.411

*, ** Correlation coefficients are significant at $P < 0.05$ and $P < 0.01$, respectively.

Fig.1. Maximum and minimum daily air temperatures (°C) during grain development; A- the control (cool) and B- the hot greenhouse.

Fig.2. Kernel growth (accumulated dry matter) in two representative cultivars (Nacozari and Danbata) under heat stress compared with the control treatment. Vertical bars are standard errors (Experiment 1). Vertical bars are SE of the mean.

Fig.3. Flag leaf chlorophyll content with time after anthesis in two representative cultivars subjected to heat stress and control treatment (Experiment 1). Vertical bars are SE of the mean.

Fig.4. The regression of percent reduction under heat stress in grain weight per ear ($y = 82.3 - 1.16x$), kernel number per ear ($y = 48.6 - 0.80x$), and kernel weight ($y = 55.5 - 0.80x$) on the potential capacity for supporting grain filling from stem reserves (PSR) across seven wheat cultivars (Table 4). PSR was calculated as the percent ratio of grain weight in defoliated control plants to grain weight in non-defoliated control plants.

Fig.5. The association across five wheat cultivars between thermotolerance in terms of cell membrane stability (CMS) as estimated by the leakage method (Fokar *et al.* 1996) and the rate of reduction in grain weight per ear under heat stress (Table 2). Regressions were for seedling data $y = -0.62 + 88.0x$; $R^2 = 0.96$, and for flag leaf data $y = -0.81 + 97.5x$; $R^2 = 0.84$.

Fig.6. The association across 10 wheat cultivars between cellular membrane thermostability (% CMS) (Blum, unpublished data), and the heat susceptibility index for kernel weight estimated from field performance under normal and hot (irrigated) conditions at Bet Dagan, Israel (Shpiler and Blum, 1991).

$$y = 0.404x^{0.25}; r^2 = 0.69.$$

E.3. Wheat Thermotolerance in Relations to Yield and Heat Shock Proteins

ABSTRACT

This research was designed first to select parental cultivars for developing a random inbred line (RIL) population and then to evaluate the co-segregation and the relationships among yield, thermotolerance and HSP. Eight wheat cultivars differing in their thermotolerance in terms of cell-membrane stability (CMS) were re-evaluated for their thermostability (CMS) and HSP accumulation. Danbata (heat tolerant) and Nacozari (heat susceptible) were found to be the most different cultivars in thermotolerance and low molecular weight HSP expression after heat shock. They were crossed to develop an F₈ RIL population which was then evaluated for thermotolerance (CMS), HSP accumulation and yield under normal and heat stress conditions in the field at Bet Dagan.

CMS in 95 RILs ranged from 76.5% to 22.4% with 71.5% and 31.3% in Danbata and Nacozari, respectively. The population segregated with normal distribution across the full range of the parental values. Yield and biomass under non-stress conditions during the winter season at Bet Dagan did not differ between the two parental cultivar, but the range of segregation for these traits in 138 RILs was very high and distinctly transgressive with a CV of 35.3% and 42.4% among lines for biomass and yield, respectively. Mean biomass and yield of the population was reduced about twofold when grown under the hot summer conditions (irrigated) at Bet Dagan. Segregation for biomass and yield was decreased relative to the normal winter conditions with CV of 20.2% and of 23.3% among lines for biomass and yield, respectively. However, contrary to non-stress conditions, the parental cultivars differed about twofold for biomass and yield under heat stress and the population segregated with normal distribution across the full range of this difference. CMS was highly and positively correlated across 79 RILs with Biomass ($r=0.62^{**}$) and yield ($r=0.58^{**}$) under heat stress. No such correlation was obtained under the normal winter conditions.

All RILs expressed a set of HSPs under heat shock (37°C for 2h). No variation was detected among RILs in high molecular weight HSP isoforms and they were similar to the patterns of the parental cultivars. There was a surprisingly low variability in low molecular weight HSP isoforms. Only one low molecular weight and a Nacozari-specific HSP isoform (belonging to HSP16.9 family) appeared to segregate among all RILs, but it was not quantitatively correlated with any parameter of plant production or CMS in this population.

It is concluded that this Danbata/Nacozari F₈ RIL population co-segregated well for thermotolerance and yield under heat stress and that CMS was associated with and predicted well the relative productivity of lines under chronic heat stress. For reasons

unknown, this population did not express meaningful variability for HSP accumulation under heat shock and therefore no role could be seen for HSP in the heat tolerance of this population.

INTRODUCTION

This study was designed to evaluate the associations between cellular thermotolerance in terms of cell membrane stability (CMS), yield under heat stress, and HSP expression under heat shock treatment. This was done by the evaluation of various parental candidates for thermotolerance and HSP and by crossing two divergent parents to produce an F_8 random inbred line (RIL) population. The population was used to study thermotolerance in relation to HSP and yield under heat stress in the field.

MATERIALS AND METHODS

Plant materials

Eight spring wheat genotypes which were previously shown (Chapter E1) to diverge for CMS were used here (Table 1). After further testing (see below) two cultivars were selected and crossed. A single-seed descent selection program was started in the F_2 generation by selecting random progeny in each generation. By using a greenhouse environment in Texas and a field environment in Israel 160 F_8 RILs were selected, most of which were used for all analyses reported herein.

Thermotolerance assay

RIL seedlings were germinated and grown in sterilized vermiculite for 10 days in the controlled conditions at 25°C with the photoperiod of 16 hours under approximately 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR. The experimental design was a randomized complete block with three replications. Ten days old seedlings were transferred to an environmental chamber for heat hardening at 34°C under high relative humidity (>80%) for 24 h. Immediately following hardening, three 2.5 cm segments from the primary leaves of three seedlings were sampled, washed in distilled and deionized water, and placed in a capped glass vial containing 1 ml of distilled deionized water. The treatment vials (three per each RIL) were heated in a water bath for 1 h at 52°C while control vials (three per each RIL) remained at room temperature. After treatment, 9 ml of distilled deionized water was added to all vials and they were incubated at room temperature in the darkness for 24 h. Solution conductance was measured with a conductivity meter (YSI model 345, Yellow Springs, OH). After the measurements were taken, all vials were autoclaved for 15 min and their conductance measured again. CMS was calculated as the reciprocal of cell membrane injury after Blum and Ebercon (1981):

$$\text{CMS (\%)} = [1 - (T1/T2) / 1 - (C1/C2)] \times 100$$

where T and C refer to treatment and control conductance, respectively, and 1 and 2 refer to initial and final conductance, respectively.

HSP analysis

All experiments were done with 10-day-old seedlings grown as described above. *In vivo* protein labeling by ^{35}S -methionine incorporation, total protein extraction, two-dimensional polyacrylamide gel electrophoresis and fluorography were performed as described by Vierling and Nguyen (1990) and Jorgensen et al. (1992). Dry gels were exposed with the X-ray film (RM-1, Kodak) for 3-6 days to achieve a total of 3 million dpm x days. Molecular weights and pI values of HSP isoforms were calculated basing on the calibration curves derived from the separation patterns of standard proteins.

Field tests of RILs

138 RILs and the two parent cultivars were planted in the normal winter season of 1996/97 and the hot summer off-season of 1997, at Bet Dagan. The experimental design and plot management were the same in both seasons. The experimental design was randomized blocks with 2 replications. Each plot consisted of one row 2m in length with 1 m between rows. The experiments were fully fertilized and irrigated to avoid any nutritional or water deficit. Plots were chemically protected from diseases by regular prophylactic sprays of Bayleton at 0.5% a.i. At maturity whole plots were harvested and a sample of plants from each was dried at 80°C for 48 h to determine dry matter weight for calculating total biomass. All ears were threshed to determine grain yield per plot.

RESULTS AND DISCUSSION

Selection of the parents to produce RIL population

Based on the preliminary analysis of cell membrane thermostability (CMS) under heat stress in a sample of fifty-six spring cultivars originating from the various regions of the world (Chapter E1), 8 spring wheat genotypes contrasting in CMS values were selected for HSP synthesis evaluation by two-dimensional electrophoresis (Table 1). At ambient temperature of 25°C the patterns of protein synthesis was indistinguishable between all cultivars. When subjected to heat stress for 2 h at 37°C, all cultivars expressed a characteristic set of HSPs consisting of high molecular weight HSPs (molecular weights of 100, 90, 85, 80, 70-72 kDa) and low molecular weight HSPs (molecular weight range 16-30 kDa). We did not observe any quantitative or qualitative differences among various cultivars in the high molecular range HSP isoforms (data not shown). While major isoforms of low molecular weight HSPs were invariably expressed in all tested cultivars, there was a significant variation between certain cultivars in specific low molecular weight HSP isoforms (Table 2). In all cases with one exception (see below), the variation

in HSP isoforms was qualitative, *i.e.* a given HSP isoform was observed in one cultivar and completely absent from another. In each pair of cultivars, between 5 and 14 low molecular weight cultivar-specific qualitative HSP isoforms could be detected. Cultivars Danbata and Nacozari were the most divergent in their low molecular weight HSP patterns with 9 Danbata-specific HSP isoforms and 5 Nacozari-specific HSP isoforms. In addition, there was a single Nacozari-specific quantitative HSP variant. This low molecular weight isoform, designated HSP16.9-I, was reproducibly expressed to approximately 10 times higher level in Nacozari, than in Danbata. Based on their contrasting CMS values and HSP patterns, the spring wheat cultivars Danbata and Nacozari were selected as parents for developing the RIL population.

Thermotolerance and yield in RILs

Significant genetic variation ($F=3.49$; $p<0.0001$) was found for CMS among 95 RILs, which were normally distributed for the trait (Fig.1). CMS ranged from 76.5% to 22.4%, while parental cultivars Danbata and Nacozari exhibited 71.5% and 31.3% CMS, respectively.

Mean biomass production of 138 RILs at Bet Dagan was reduced from 676 g m⁻¹ of row in the normal and non-stress (winter) environment to 385 g m⁻¹ of row in the heat-stress (summer) environment (Fig.2). Winter biomass was practically the same in the two parent cultivars, while the RILs biomass ranged nearly six-fold with a CV among lines of 35.3%. In the hot summer (Fig.2) cv. Danbata had about double the biomass than cv. Nacozari. While the variation in biomass among RILs was greatly reduced as compared with the winter season, summer RIL biomass was normally distributed across the full range between the two parental cultivars.

Very similar results were obtained also for yield (Fig.3). Mean population yield was reduced from 314 g m⁻¹ row in the winter to 167 g m⁻¹ row in the summer. The two parental cultivars differed about two-fold in yield under the summer heat stress conditions but not at all under normal winter conditions. The variation in yield among RILs was large under normal winter conditions with a CV of 42.4% among lines. This variation was appreciably reduced under the hot summer conditions (CV of 23.3%). As seen for biomass, summer RIL yield was normally distributed across the full range between the two parental cultivars.

The potential genetic variation for yield in this RIL population was very large when tested under non-stress conditions in the winter, despite the fact that the two parental cultivars yielded the same. Thus, most of the variation in productivity in this RIL population under non-stress (potential) conditions was transgressive, either positive or negative. Under the hot summer conditions the two parental cultivars differed significantly in their yield, in proportion to their relative thermotolerance. The variation in

yield among RILs in the summer was depressed very pronouncedly. Still, yield of the RIL population under heat stress spanned the full range between the two parental values, with little transgressive segregation.

Simple correlation among traits across RILs (Table 3) indicated that there were significant association between plant production in the winter and the summer for both biomass and yield, indicating the contribution of potential productivity towards plant production under heat stress. However, when winter and summer conditions were analyzed as two main treatments, a significant season by RIL interaction was revealed for both biomass ($F=9.74$; $p<0.0001$) and yield ($F=10.08$; $p<0.0001$), indicating specific adaptation of lines to either environment.

There was a fairly high and significant correlation across RILs between biomass or yield in the summer (stress conditions) and CMS (Fig.4). No such correlation was seen for winter (non-stress conditions) biomass or yield (Table 3). The significant association between CMS and plant production in the summer and the lack of it in the winter support the conclusion that the segregation of the RILs for plant production in the summer was largely determined by their relative thermotolerance and that thermotolerance as estimated by CMS can predict the relative plant productivity of wheat genotypes under heat stress. This conclusion is in support of previous observations by (Reynolds et al., 1994) and our results as obtained here under chapter E1 (Table 4 therein)

We have no solid explanation for the different segregation in productivity of this RIL population between the two environments, unless the transgressive segregation under potential conditions was largely affected by non-additive factors which were not fully expressed under heat stress, while additive factors for thermostability (see E1 above) were relatively important in affecting yield under heat stress. The large difference in productivity between the two parental cultivars under heat stress, the segregation of the RIL population over the full span between parental values and the association between CMS and productivity under heat stress all support the conclusion that this RIL population co-segregated very distinctively for cellular thermotolerance and for plant productivity under heat stress.

There are repeated indications in the literature for the general reduction in heritability, or in the apparent genetic variance for yield, under environmental stress (Blum, 1988). Superficially it seems that Fig.2 and Fig.3 support such observations. However, our results show that the genetic variation in thermotolerance and yield under heat stress in this RIL population was fully and well expressed to the full extent of the range span that existed between the parental values. The large apparent variation in yield and biomass of this population when grown under nonstress conditions is the expression of other genetic factors, which may be non-additive and expressed mainly when stress is not present. Thus, the apparent decrease in population variability in yield under heat stress reflects the

inadequate expression of some potential yield factors under heat stress, but at the same time, genetic variation for thermostability became the dominant factor in controlling segregation for yield under heat stress. Therefore, genetic variance for yield under stress is retained if effective factors of resistance to that stress segregate in the population. On the other hand, variance for yield under stress may indeed be reduced (Blum 1988) if no stress resistance factors support yield in the population.

HSP expression in F₈ RILs

All 95 tested RILs expressed a set of HSP after being subjected to a temperature of 37°C for 2 h. No variation was detected among the lines in high molecular weight HSP isoforms and they were similar to the patterns of the parental cultivars. In the low molecular weight region, there was a surprisingly low variability in HSP isoforms. Out of nine Danbata-specific HSP isoforms, none was expressed in the RILs, while all five Nacozari-specific HSP isoforms were invariably detected in the RIL progeny. There was a general quantitative variability among the RILs in the expression of the Nacozari-specific HSP isoform HSP16.9-I. In the study of HSP expression in parental cultivars (above), HSP16.9-I was expressed in Nacozari to approximately 10 times higher than in Danbata. To quantify the expression of HSP16.9-I in the RILs, we visually scored the intensity of the spot corresponding to this protein on the 2-D fluorograms. In order to standardize for the protein loading variability in the individual two-dimensional gels, we used the intensity of the spot corresponding to the small subunit of ribulose biphosphate carboxylase (approximate size 14 kDa). According to our earlier observations, this protein subunit retains a high level of expression under the heat stress and hence is a suitable standard for quantitative comparisons of protein isoforms separated on two-dimensional gels.

Also in this run, Danbata and Nacozari differed in the level of expression of the HSP16.9 isoform with a quantitative rating of 5% in Danbata and 50% in Nacozari. In the RILs, quantities of this subunit varied within the range of values of the parents, from 5% to 50% with a distribution close to normal.

The absence of expression of the Danbata-specific low molecular weight HSP isoforms and the absence of segregation of the Nacozari-specific HSP isoforms (with the exception of HSP16.9-I) in the RIL population derived from the Danbata/Nacozari cross is puzzling. Furthermore, the relatively greater accumulation of HSP16.9-I in the susceptible parent implies that it may have no role in controlling thermotolerance in this materials. Indeed no significant associations could be seen between HSP 16.9-I accumulation and CMS or plant production under stress or nonstress conditions (Table 3).

Thus, while this RIL population expressed very clear co-segregation for thermotolerance and yield under heat stress in relations to thermotolerance, the results for

HSP are disappointing. For some reason unknown the population did not express any effective or important variation in low molecular HSP for which the parental cultivars indeed differed. It remains to conclude that in this population thermotolerance in terms of membrane function under heat stress was a major factor associated with yield under chronic heat stress in the field and that the role of HSP in this respect could not be resolved.

REFERENCES

- Blum A. (1988) Plant breeding for stress environments. CRC Press. Florida.
- Blum, A., and A. Ebercon 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21:43-47.
- Ho T-H.D., and Sachs M.M. 1989. Stress induced Proteins: characterization and regulation of their synthesis. *In* The Biochemistry of Plants: a comprehensive treatise. Marcus A. (ed.) pp. 347-378.
- Jorgensen J.A., Weng J., Ho T-H.D., and Nguyen H.T. 1992. Genotype specific heat shock proteins in two maize inbreds. *Plant Cell Rep.* 11: 576-580.
- Nagao R.T., Kimple J.A., and Key J.L. 1990. Molecular and cellular biology of the heat shock response. *Adv. Genet.* 28: 235-275.
- Reynolds M.P. Balota M. Delgado M.I.B. Amani I. Fischer R.A. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Aust.J.Plant Physiol.* 21:717-730.
- Vierling E. 1991. The roles of heat shock proteins in plants. *Ann. Rev. Plant Physiol. and Plant Mol. Biol.* 42: 579-620.
- Vierling, R.A. and Nguyen H.T. 1990. Heat-shock protein synthesis and accumulation in diploid wheat. *Crop Sci.* 30: 1337-1342.

Table 1. Spring wheat cultivars used for HSP isoform analysis and their rating for thermotolerance as based on CMS.

Cultivar	Origin	Thermotolerance (CMS) rating †
Nacozari	CIMMYT	Sensitive
Heines Peko	Germany	Sensitive
Kanchan	China	Sensitive
IP4	Myanmar(Burma)	Sensitive
Prostor	South Africa	Tolerant
Fang60	Thailand	Tolerant
Bacanora	CIMMYT	Tolerant
Danbata	Nigeria	Tolerant

(†) Based on data presented in chapters D1-D2.

Table 2. Pairwise variability in low molecular weight HSP isoforms of the most divergent spring wheat cultivars.

Tolerant cultivar (T)	Susceptible cultivar (S)	Variable low molecular weight HSP isoforms		
		T-specific*	S-specific*	Total
Danbata	Kanchan	3	7	10
Danbata	Nacozari	9	5	14
Prostor	Kanchan	5	8	13
Prostor	Nacozari	5	8	13
Bacanora	Nacozari	3	2	5

*T-specific and S-specific HSP isoforms are thermotolerant cultivar-specific and thermosusceptible cultivar-specific low molecular weight HSP isoforms, correspondingly.

Table 3. Simple correlations among different productivity and thermostability traits across 98 RILs of Danbata/Nacozari.

Traits	Winter Biomass	Winter Yield	Winter HI	Summer Biomass	Summer Yield	Summer HI	Biomass% Winter	Yield% Winter	CMS%
Winter Yield	0.96**								
Winter HI	0.46**	0.67**							
Summer Biomass	0.39**	0.39**	0.25*						
Summer Yield	0.44**	0.47**	0.40**	0.92**					
Summer HI	0.25*	0.32**	0.43**	0.18	0.54**				
Summer Biomass% Winter	-0.82**	-0.77**	-0.33**	0.13	0.03**	-0.19*			
Summer Yield% Winter	-0.80**	-0.82**	-0.56**	0.07	0.00	-0.13	0.94**		
CMS%	0.15	0.11	-0.07	0.60**	0.53**	0.05	0.21*	0.24*	
HSP16.9-I Intensity	-0.03	-0.01	0.04	0.03	0.05	0.06	0.05	0.03	0.04

(**) Significant at $p \leq 0.05$.

(**) Significant at $p \leq 0.01$.

Fig.1. Frequency distribution of 95 F₈ RILs of the cross Danbata/Nacozari for cell-membrane stability (CMS). Arrows represent parental values.

Fig.2. Frequency distribution of 138 F₈ RILs of Danbata/Nacozari cross for Biomass production under normal (winter) and hot (summer) irrigated growing conditions at Bet Dagan. CV is the coefficient of variation among lines. Arrows represent parental values.

Fig.3. Frequency distribution of 138 F₈ RILs of Danbata/Nacozari cross for grain yield under normal (winter) and hot (summer) irrigated growing conditions at Bet Dagan. CV is the coefficient of variation among lines. Arrows represent parental values.

Fig.4. The association between Biomass or grain yield with cell-membrane stability (CMS) across 79 F₈ DHLs grown under heat stress (summer season) and non-stress (winter season) conditions at Bet Dagan.

E.4. Heterosis for Thermotolerance and its Association with HSP Expression in Wheat

ABSTRACT

The mechanism of heterosis in plants at the molecular level is not well understood. One hypothesis is that heterosis is involved in thermal homeostasis and the rate of its expression may depend on temperature. Homeostasis at high temperatures should involve thermotolerance which in itself can depend on heat shock proteins (HSP). This study was therefore designed to evaluate the expression of heterosis in wheat (*Triticum aestivum* L.) at high stress temperatures and its possible association with HSP.

Seven F₁ hybrids and their parent cultivars were measured for growth rate at control (15⁰/25⁰C) and high (25⁰/35⁰C) night/day temperatures. They were also tested for membrane-thermostability by the leakage method following hardening at 34⁰. In four hybrids (reciprocals of two crosses: V5/V2420 and V5/Barkae) the patterns of protein expression was assayed by two-dimensional polyacrylamide gel electrophoresis and *in vivo* labeling in 10 d old seedlings subjected to normal (25⁰) and stress (37⁰) temperatures.

Both hybrids and cultivars varied significantly in their thermotolerance in terms of growth rate and CMS. Heterosis (over best parent) for CMS was generally lower than that for growth under heat stress. Hybrids varied in the rate of heterosis for growth at both temperatures. In some of the hybrids heterosis for growth significantly increased at high temperature as compared with control temperature, suggesting temperature-dependent heterosis. Generally, only limited qualitative variation was detected in the patterns of protein synthesis in four wheat hybrids and their parents under normal temperatures. However, a singular protein (C47/5.88) was specifically expressed only in the most heterotic hybrid at normal temperature but not in its parent cultivars. Parental cultivars were significantly different in the sets of synthesized HSP at 37⁰. No qualitative changes in the patterns of protein expression under heat stress were correlated with heterosis. However, a quantitative increase in certain low molecular weight HSP (mainly H14/5.5 and H14/5.6, belonging to the HSP16.9 family) was positively associated with greater heterosis for growth at high temperature. None of these proteins were correlated with CMS. These results support the concept of temperature-dependant heterosis for growth and a possible role for HSP16.9 family in this respect. Further investigation into the relationship between these proteins, heat tolerance and heterosis under stress is warranted.

INTRODUCTION

Shull (1914) was the first to introduce the term heterosis to describe the special stimulus of heterozygosity upon plant growth, size, productivity and general vigor. By that original definition heterosis is expressed as the superiority of the first generation hybrid over its best parent for the trait in question.

While the phenomenon of heterosis was quickly adopted by breeders to produce commercial F_1 hybrids of many crops (Frankel, 1983), the understanding of the physiological and biochemical basis of heterosis is amazingly limited. Even the genetic explanation of heterosis still remains within the original main hypothesis of Crow (1952) on “dominance” and “overdominance”.

Various biochemical-physiological hypotheses were extended to explain the expression of heterosis for growth, vigor and yield. These include the concept of “physiological bottlenecks” (Mangelsdorf, 1952), the “metabolic balance” concept of Hageman *et al.* (1967) and the more recent “metabolic flux” theory of Rhodes *et al.* (1992). Strong evidence to support either is not available.

Quinby (1974) advocated the idea that the main effect of heterosis in sorghum was controlled by plant growth hormones, probably gibberellins. This was later given partial support by experimental work with maize (Rood *et al.*, 1990) and sorghum (Rood *et al.*, 1992). Srivastava (1981) and McDaniel (1986) both presented a case for cellular organel (mitochondria and chloroplast) complementation as an explanation of heterosis. They drew upon their own extensive work with mitochondrial polymorphism and complementation in relations to heterosis and argued that genomic and intergenomic interactions lead to superior mitochondrial and chloroplast functions in the hybrid. Some of their earlier views and work were challenged by Sinha and Khanna (1975), indicating that the hypothesis could be controversial.

The homeostatic capacity of heterozygotes has long been recognized in evolution and population genetics. Commercial crop hybrids are generally considered to be more “stable” in their yields than or their parental lines or open-pollinated varieties, when stability is defines as consistently good performance irrespective of the environment. However, there is very little information on the interaction between heterosis and environmental stress.

Already in 1963 Griffing and Langridge identified “temperature-dependent heterosis” in *Arabidopsis thaliana*, whereas heterosis for growth increased with temperature between 16°C and 31°C. This was later observed also in maize where heterosis was more pronounced at supra-optimal and sub-optimal than at the optimum temperatures, as well as after heat shock (McWilliams and Griffing, 1965). The positive interaction of heterosis

with non-optimal temperatures was considered by Blum (1989) as an important mechanism of heterosis in sorghum. This reflects upon earlier work with *Drosophila* where the heterozygote sustained greater activity of a heat sensitive enzyme under heat stress as compared with the homozygotes (Singh *et al.*, 1974).

This research was therefore designed as a preliminary study of the possible involvement of heterosis in thermotolerance of wheat, with special reference to the expression of heat shock proteins (HSP) as an important molecular component of thermotolerance (*e.g.* Krishnan *et al.*, 1989; 1992; O'Connel, 1994). While the previous study (E3) employed RIL populations as the genetic materials for exploring the association between HSP and thermotolerance, this study used F₁ hybrids and their parents.

MATERIALS AND METHODS

Growth and thermotolerance

Seed of seven F₁ hybrids of common wheat (*Triticum aestivum* L.) were produced by hand emasculation and crossing among seven parental cultivars (Table 1). Two of the crosses included reciprocals. Seed were planted in trays filled with vermiculite and irrigated every other day with half-strength Hoagland's nutrient solution. Upon emergence trays were placed in two growth chambers both set at 12h day and 480 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR). One chamber was set at 15°/25° (control) and the other at 25°/35° (stress) night/day temperatures. In each chamber seedlings of each hybrid (5 replicates per hybrid by temperature combination) were harvested at 21 and 28 days after emergence and their weight determined after oven drying for 48 h at 80°C. Growth rate was then calculated as dry matter gain per plant per day.

Seedlings were similarly grown under control conditions. When they were at 14 days after emergence growth chamber temperature was raised to 34° for 24h after which leaves were sampled (5 replications per hybrid) for the measurement of cell-membrane thermostability (CMS) by the leakage method (materials and methods in Chapter E1). Heterosis in growth or CMS was calculated as the ratio of the hybrid to its best parent for the trait.

HSP analysis

All experiments were done with the 10-day-old seedlings grown in sterilized vermiculite for 10 days in the controlled conditions at 25°C with the photoperiod of 16 hours under approximately 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR. For *in vivo* labeling, middle parts of the primary leaves were cut into 3 mm segments and placed in the glass tubes with incubation buffer (100 mM chloramphenicol in 20 mM TrisHCl, pH 7.0). Tubes were incubated at 25°C (control) or 37°C (heat) for 15 min in circulating water baths with

lights producing approx. $500 \text{ mmol m}^{-2}\text{s}^{-2}$ of PAR to induce HSP expression. Tubes were then promptly removed from the baths, medium replaced, ^{35}S -methionine (1000 mCi/ml) added and vacuum infiltrated. Tubes were returned to the original water baths and incubation in the same conditions continued. Overall length of temperature incubation was 2 h, label incorporation into proteins was allowed to proceed for the last 1.5 h. After the incubation, leaf segments were extensively washed with 5 mM L-methionine, blotted dry, frozen in liquid nitrogen and stored at -70°C until protein extraction. Total protein extraction, two-dimensional polyacrylamide gel electrophoresis and fluorography were performed as described by Vierling and Nguyen (1990) and Jorgensen et al. (1992). Dry gels were exposed with the X-ray film (RM-1, Kodak) for 3-6 days to achieve a total of 3 million dpm x days. Molecular weights and pI values of HSP isoforms were calculated basing on the calibration curves derived from the separation patterns of standard proteins (2D protein standards, Bio-Rad). Isoelectric point calibration curve was obtained by direct pH measurements in the 0.5-cm segments of Isoelectric focusing gels. Quantification of intensity of the expression of individual protein species was done basing on the visual scoring of the spots corresponding to these proteins in the fluorograms and standardized for protein loading variability in separate two-dimensional gels by using the intensity of the spot corresponding to the small subunit of ribulose biphosphate carboxylase (approximate size 14 kDa). According to our earlier observations, this protein subunit retains a high level of expression under heat stress and hence is a suitable standard for quantitative comparisons of protein isoforms separated on two-dimensional gels.

RESULTS AND DISCUSSION

Heterosis and thermotolerance

Significant variations were found among hybrids in growth rate and heterosis for growth rate under the two thermal regimes (Table 1). Mean growth rate over all hybrids was reduced from $17.4 \text{ mg plant}^{-1} \text{ d}^{-1}$ in the control to $9.3 \text{ mg plant}^{-1} \text{ d}^{-1}$ under heat stress. Hybrid V5/Barkaee expressed heterosis for growth at both temperatures while the reciprocal cross was heterotic only under heat stress. In three of the hybrids the expression of heterosis for growth increased under heat stress as compared with the controls. Temperature by hybrid interaction for growth was significant at $p=0.048$ (Fig.1) indicating that in the two hybrids with V5 as their male parent, heterosis for growth increased with heat stress as compared with the controls.

Cell-membrane thermostability (CMS) varied significantly among hybrids, from 18.3% to 58.2% (Table 1). Heterosis for CMS was generally low (<1), except for hybrid Barkaee/V5 which was marginal in this respect. However, there was a significant linear association between CMS and growth under heat stress as percent of growth in the

controls, across hybrids (Fig.2). This association indicated that CMS was well related to heat tolerance in terms of growth in these hybrids. However, heterosis for CMS was not associated with heterosis for growth.

Protein expression at normal (25⁰) temperature

In the two-dimensional gels, an average of about 700 protein species were expressed at 25⁰. There was a significant level of diversity for protein synthesis between the parental cultivars. A number of protein isoforms specific for cultivar and relatively more abundant in some cultivars are summarized in Table 2. V2420 had the highest number (10) of specific proteins. Even more pronounced differences were observed in the relative abundance of expressed protein isoforms. Cultivar V2420 tended to have relatively higher expression of constitutive forms of HSP70 as compared with V5 and Barkae (Table 3).

Generally, all parental specific proteins recombined in their hybrids. All protein species which were qualitatively different between parental lines were expressed at the intermediate level in reciprocal hybrids with one exception. A protein with Isoelectric point 6.6 and relative molecular weight 30 kDa (designated C30/6.6) was significantly negatively effected in all four hybrids (Table 3). We cannot ascribe any significance to this protein unless it carries a negative effect on growth at 25⁰C.

There was one new protein species (designated C47/5.88; Table 3) observed in hybrids between V5 and Barkae as compared to the parental cultivars. C47/5.88 was absent from all parental cultivars, but it was detected in the two hybrids between V5 and Barkae with much greater expression in V5/Barkae as compared to is reciprocal. It should be noted that V5/Barkae exhibited the highest heterosis for growth under normal temperature among the four hybrids tested for their proteins (Table 1; Fig.1). C47/5.88, being unique for a singular heterotic hybrid, may have a role in heterosis or may serve as marker for seedling growth heterosis at normal temperatures.

Protein expression at stress (37⁰) temperature.

In all genotypes, approximately 400 protein species were found to be synthesized during the incubation at 37⁰C (57 % of that at 25⁰C). A reduction in the total number of proteins expressed under heat stress as compared with normal temperature is a well known phenomenon resulting from the heat shock induced reprogramming of the translation machinery of the cells, repression of the synthesis of the majority of proteins synthesized under normal conditions and a preferential translation of HSP. We observed a typical pattern of HSP synthesis, HSP being the dominating species in the overall set of protein species synthesized. The HSP set included HSP families with relative molecular weights ca.97 kDa, 85 kDa, 80 kDa, 72-74 kDa, 55 kDa, 48 kDa, 40 kDa, 37 kDa, 35 kDa, 20-30 kDa, and 14-17 kDa. Among these, the HSP with molecular weights 20-30 kDa and

14-17 kDa were strictly heat inducible, while some proteins belonging to other HSP families of the higher molecular weight were constitutively expressed at 25°C while their synthesis increased at 37°C.

Generally, variability in protein synthesis was much higher at 37°C than at 25°C. Parental genotypes were significantly different in the sets of specific synthesized HSP (Table 4). Intra-species variability in the HSP expression was previously observed in various species (*e.g.* Jorgenson *et al.*, 1992; O'Connel, 1994), including wheat (Krishnan *et al.*, 1989). A significant number of HSP varied quantitatively among cultivars and hybrids.

No qualitatively new protein expression was observed in any of the hybrids as compared with their parents. All qualitatively different protein variants observed in the parents recombined in the hybrids, with minor exception. A low molecular weight protein H16/5.1 belonging to HSP16.9 wheat HSP family which was expressed in Barkaee but not in V5, was expressed in both reciprocal hybrids at approximately 20% of its intensity in Barkaee.

Under heat stress, the expression of four HSP species was increased to a higher degree in hybrids as compared to parental cultivars (Table 5), which constitutes an expression of heterosis (Table 6). Notably, hybrids Barkaee/V5 and V2420/V5 expressed the highest level of heterosis for the expression of two HSP: H14/5.6 and H14/5.5 (Table 6). These proteins belong to the wheat HSP16.9 gene family. We do not know which HSP family the protein H37/5.85 belongs to.

These two hybrids with V5 as their male parent also expressed the highest increase in heterosis under heat stress as compared with the controls (Table 1; Fig.1). This association is further generalized by the regression of growth under stress as percent of controls on HSP H14/5.5 expression in four wheat hybrids (Fig.3; left). It is also generalized by the regression of the ratio of heterosis for growth under stress to heterosis in the controls (*i.e.* the increase in heterosis for growth under heat stress) on heterosis in HSP H14/5.5 expression (Fig.3; right). Similar correlations were also obtained between these expressions of heterosis for thermotolerance in growth and HSP H14/5.6 (data not shown). Both proteins belong to the HSP16.9 family.

CONCLUSIONS

In our analysis, we used metabolic *in vivo* labeling techniques to label the proteins which are synthesized in leaf tissue under the given experimental conditions. By doing this, we limit the population of all cellular proteins to those which are being expressed during the incubation period. The first leaves of the ten-day old seedlings which were used here were growing and were metabolically active. Hence, the population of the expressed protein species under normal temperatures should include growth-related protein species.

Only limited qualitative variation was detected in the patterns of protein synthesis in four wheat hybrids and their parents at normal temperature, and such limited variation could not provide a general account for heterosis. However, one protein species (C47/5.88) was unique to the most heterotic hybrids at normal temperature and it varied quantitatively with the reciprocal. This protein should be further investigated in relations to the expression and possibly the tagging of heterosis in wheat, at least in terms of juvenile plant growth at normal temperatures.

Under heat stress, there was a drastic change in the patterns of the expressed proteins with a novel heat inducible set of HSPs as the most prominent feature.

The genetic materials studied here were significantly variable for thermotolerance in terms of CMS and growth. Although CMS and growth under heat stress were related, significant heterosis was expressed for growth, more than for CMS. In two hybrids with V5 as their male parent, heterosis for growth significantly increased under heat stress. This may be defined as temperature (stress)- dependent heterosis.

There was no simple general association between a unique protein and heterosis for growth or CMS across all materials tested. However, heterosis for growth under heat stress or its rate of increase under heat stress (*i.e.* its temperature dependency) was significantly related to the increase in low molecular weight HSP H14/5.5-5.6. This lend support to the concept of temperature-dependant heterosis, which at stress temperatures may be linked to the expression and accumulation of certain HSPs.

REFERENCES

- Blum, A. 1989. The temperature response of gas exchange in sorghum leaves and the effect of heterosis. *J. Exp. Bot.* 40: 453-460.
- Crow, J.F. 1952. Dominance and overdominance. In: J.W. Gowen (ed.) *Heterosis*. Iowa State College Press, Ames, pp.282-297.
- Frankel, R. (Ed.) 1983. *Heterosis: Reappraisal of Theory and Practice*. Springer Verlag, Berlin.
- Griffing, B. and Langridge, J. 1963. Phenotypic stability of growth in self-fertilized species *Arabidopsis Thaliana*, In: Hanson, W.D. and Robinson, H.F., Eds *Statistical Genetics and Plant Breeding*, NAS, NRS, Washington D.C., pp.368-379.
- Hageman, R.H., Leng, E.R. and Dudley, J.W. 1967. A biochemical approach to corn breeding. *Adv. Agron.* 19:45-66.
- Jorgensen J.A., Weng J., Ho T-H.D., and Nguyen H.T. 1992. Genotype specific heat shock proteins in two maize inbreds. *Plant Cell Rep.* 11: 576-580.
- Krishnan M., Nguyen H.T., and Burke J.J. 1989. Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol.* 90: 140-145.

- Mangelsdorf, A.J. 1952. Gene interaction in heterosis. In: J.W. Gowen (ed.) Heterosis. Iowa State College Press, Ames, pp.320-329.
- McDaniel, R.G. 1986. Biochemical and physiological basis of heterosis. *CRC Rev.Plant Sci.* 4:227-246.
- McWilliams, J.R. and Griffing, B. 1965. Temperature-dependant heterosis in maize. *Aust. J. Biol. Sci.* 18:569-583.
- O'Connel M.A. 1994. Heat shock proteins and thermotolerance. In: "Stress-induced gene expression in plants" (Basra A.S., ed.) Harwood Academic Publishers, pp. 163-184.
- Quinby, J.R. 1974. Sorghum improvement and the genetic of growth. Texas A&M Univ.Press College Station pp.108.
- Rhodes, D., Ju, G.C., Yang, W. and Samaras, Y. 1992. Plant metabolism and heterosis. *Plant Breed. Rev.* 10:53-91.
- Rood, S.B., Buzzell, R.I., Major, D.J. and Pharis R.P. 1990. Gibberellins and heterosis in maize: quantitative relationships. *Crop Sci.* 30:281-286.
- Rood, S.B., Witbeck, J.E.T., Major, D.J. and Miller, F.R. 1992. Gibberellins and heterosis in sorghum *Crop Science* 32:713-718.
- Shull, G.H. 1914. Duplicate genes for capsul form in Bursa Bursa Pastoris. *J. Ind. Abst. Vererb.* 12:97-149.
- Singh, R S., Hubby, J L. and Lewontin, R C. 1974. Molecular heterosis for heat-sensitive enzyme alleles. [*Drosophila Pseudoobscura*]. *Proc. Natl. Acad. Sci.* 71:1808-1810.
- Sinha, S.K. and Khanna, R. 1975. Physiological biochemical and genetic basis of heterosis. *Adv. Agron.* 27:123-175.
- Srivastava, H.K. 1981. Intergenomic interaction heterosis and improvement of crop yield. *Adv. Agron.* 34:117-190.
- Vierling, R.A. and Nguyen H.T. 1992. Heat-shock protein gene expression in diploid wheat genotypes differing in thermal tolerance. *Crop Sci.* 32: 370-377.

Table 1. Thermotolerance and heterosis for thermotolerance in terms of growth and cell membrane thermostability (CMS) in seven F₁ wheat hybrids.

Hybrid	Growth rate (mg/d/plant)		Growth under stress as % of control	Heterosis in growth		Heterosis in growth: stress as % of control	CMS (%)	Heterosis in CMS
	Control	Stress		Control	Stress			
F ₁ -Mustang/Sturdy	21.6	10.7	49.6	1.35	0.88	65.4	19.9	0.28
F ₁ -V5/Barkaee	24.8	12.7	51.4	1.35	1.30	96.4	48.4	1.01
F ₁ -Barkaee/V5	15.7	12.5	79.6	0.85	1.28	149.5	58.2	1.21
F ₁ -Barkaee/CDN114	15.4	6.1	39.6	0.84	0.75	88.7	32.0	0.60
F ₁ -V2420/CDN114	24.8	10.1	40.9	1.01	0.88	86.6	18.3	0.33
F ₁ -V2420/V5	9.1	7.1	78.7	0.37	0.62	166.7	45.4	0.82
F ₁ -V5/V2420	10.5	6.0	57.1	0.43	0.52	121.0	39.3	0.69
Significance (p)	0.038	0.049	0.040	0.035	0.029	0.009	0.030	0.027

Table 2. Differences in protein expression at 25°C in spring wheat cultivars.

Cultivar	Number of cultivar specific proteins	Number of quantitatively different proteins	Relative polymorphism (%)*
V5	4		0.57
V2420	10		1.43
V5 vs. Barkaee		13	1.86
V2420 vs. V5		10	1.43

*Relative polymorphism value was obtained as the ratio of the number of polymorphic protein species to 700 which is the estimated number of expressed proteins observed on a single 2D gel.

Table 3. Relative intensities of the synthesis of selected proteins under 25°C.

Accession	C30/6.6†	C47/5.88	HSP70**
V2420	100	nd**	100
V5	50	nd	75
Barkaee	50	nd	50
F ₁ -V5/Barkaee	30	100	60
F ₁ -Barkaee/V5	10	20	60
F ₁ -V5/V2420	30	nd	80
F ₁ -V2420/V5	5	nd	90

(†) Proteins designated by their molecular weight/Isoelectric point.

*constitutive isoforms.

**not detected

Table 4. Variability of HSP patterns at 37°C in wheat hybrids and their parents.

Accession	Number of qualitatively variable HSP species	Number of quantitatively variable HSP species**
Barkaee	15 (3.8%)*	
V5	4 (1%)	
V2420	4 (1%)	
V5 vs. Barkaee	22 (5.5%)	7(1.8%)
V2420 vs. V5	8 (2%)	12 (3%)

*In parenthesis is the level of polymorphism which was calculated as the ratio of the number of polymorphic protein species to 400, the estimated number of expressed proteins observed on a single 2D gel.

**Excluding high molecular weight HSP species. Their expression is described in the text.

Table 5. Expression of four selected HSP species at 37°C in four wheat hybrids and their parents.

Accession	H14/5.6 [†]	H14/5.5	H29/5.75	H37/5.85
Barkaee	20	5	60	nd*
V5	40	50	80	80
V2420	20	40	30	50
F ₁ -V5/Barkaee	50	80	90	50
F ₁ -Barkaee/V5	100	100	100	100
F ₁ -V5/V2420	60	80	100	90
F ₁ -V2420/V5	80	100	100	50

(†) Proteins designated by their molecular weight/Isoelectric point.

*not detected

Table 6. Estimated heterosis for the expression of four selected HSP*

Accession	H14/5.6	H14/5.5	H29/5.75	H37/5.85
F ₁ -V5/Barkaee	1.25	1.60	1.13	0.63
F ₁ -Barkaee/V5	2.50	2.00	1.25	1.25
F ₁ -V5/V2420	1.50	1.60	1.25	1.13
F ₁ -V2420/V5	2.00	2.00	1.25	0.63

* Heterosis with respect to best parent is calculated from values in Table 5.

Fig. 1. Heterosis for growth rate in seven F_1 hybrids of wheat under two temperatures. Hybrid by temperature interaction was significant at $p=0.048$. Vertical bars are S.E. of means.

Fig.2. The association between cell membrane stability (CMS) and growth under heat stress as % of controls, across 7 wheat hybrids (Table 1). $y = 25.2 + 0.84x$; $R^2 = 0.576$

Fig.3. Left: The linear regression of growth under heat stress as percent of controls on the relative quantitative expression of HSP H14/5.5 in four wheat hybrids. Right: The linear regression of the ratio of heterosis for growth under stress to heterosis in the controls (*i.e.* the increase in heterosis for growth under heat stress) on heterosis in the relative quantitative expression of HSP H14/5.5 in four wheat hybrids.

F. Description of cooperation

The cooperation between Texas Tech University (TTU) and The Volcani Center (VC) progressed exceedingly well and according to the original plans of work. The earlier phases of germplasm evaluation of potential parents for developing RIL population were performed in Texas Tech University (TTU) and served as part of the fulfillment of the requirement for thesis work. The development of the RIL population by SSD selection to the F_8 generation testing in a period of 4-5 years was a formidable task. It could not have been carried out if it were not for the close and synchronized cooperation and the detailed planning which allowed the timely shuttling of seed and information between TTU and VC. The agreement of BARD to extend the duration of the project was a significant contribution to its successful completion.

All of the HSP analysis was carried out at TTU. The final field tests of RILs and the study of heterosis in growth and thermotolerance were performed at VC, while seed were sent to TTU for the corresponding tests of HSP. The co-visits by the cooperating investigators at each others labs were very important to the planning and execution of this project.

G. Evaluation of research achievements

Chronic heat stress is an important yield-limiting factor in many wheat growing regions in the world, including USA and Israel. Wheat breeders are seriously concerned with this stress factor. However, progress in breeding heat resistant cultivars has been slow due to the serious lack of resistant germplasm and the lack of proper selection methods and genetic information. Presently, selection is based on yield and growth evaluations in field environments subjected to chronic heat stress. This approach is limited in its progress.

The results obtained in this project are considered as an important contribution to practical breeding for heat tolerance in wheat. We have isolated several important agronomic genetic resources for heat tolerance in wheat, in terms of thermotolerance, grain filling, biomass and yield. Heat tolerance in terms of biomass and yield production under chronic heat stress was found to correlate and co-segregate with cell-membrane thermostability (CMS) as estimated by the simple and rapid leakage method. Despite the constant recent search for physiological selection criteria for environmental stress resistance in selection programs, only a very limited number of selection methods were

developed. This CMS test is the first to be shown as heritable and applicable in the context of wheat yield improvement under chronic heat stress.

The role of heat shock proteins (HSP) in the agronomic domain is not yet resolved. There is general uncertainty if not confusion about the suggested HSP functions at the molecular level (“molecular chaperones”, etc’) and their significance towards plant production under chronic heat stress. No new information has been published in the literature during the duration of this project to shed any light on this issue. This research remains singular in its attempt to examine HSP on the background of crop performance in hot conditions.

In this research we attempted to genetically associate HSP expression and plant production or tissue thermotolerance under heat stress. The results suggest that the role of HSP in plant production under chronic heat stress is elusive. Either because of the specific genetic background of the population used here or because of some incoherent features of how genetic information on HSP is transmitted from one generation to the other, a firm conclusion on the role of HSP cannot be drawn from this research. In this sense this research support the general uncertainty about the role of HSP in whole plants and crops in agriculture. It is however very encouraging that in a limited study of temperature –dependant heterosis we saw that a certain low molecular weight HSP was positively associated with the expression of heterosis for wheat growth, only under heat stress. This is a small window opening for further research to clarify whether HSP has an effective role in plant growth and production under chronic heat stress in the field.

H. List of Publications

1. Fokar, M., Nguyen H.T. and Blum A.. Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. (Euphytica, in revision)
2. Fokar, M., Blum, A. and Nguyen, H.T. Heat Tolerance In Spring Wheat. II. Grain Filling. (Euphytica, in revision).