13

77

BARD

FINAL REPORT
PROJECT NO. IS - 1583 - 88

**PHYSIOLOGICAL AND BIOCHEMICAL MECHANISMS
OF HEAT TOLERANCE IN WHEAT**

A. Blum , H.T. Nguyen

24)

1993

630.72
BAR/BLU

41062

A. BARD Cover Page for Scientific Reports.

Date: 1.6.1993

BARD Project No. IS-1583-88

הספרייה המרכזית
למדעי החקלאות
בית-דגן

Title:

Physiological And Biochemical Mechanisms Of Heat Tolerance In Wheat.

All Investigators' Names
(Principal listed first)

Investigators' Institutions

A. Blum
H.T. Nguyen

The Volcani Center, ARO, Bet Dagan, Israel
Texas Tech University, Lubbock Texas, USA.

Project starting date: September 1989

Type of Report: Final

Signature
A. Blum
Principal Investigator

Signature
Institution Authorizing Official

B. Table of Contents

Chapter	Page
A. Cover page	1
B. Table of contents	2
C. Abstract	3
D. Objectives of the original research	4
E1 Implications of carbon assimilation and cell-membrane thermostability in the heat tolerance and yield of wheat	15
E2. Genetic variability of cellular thermotolerance in cultivated wheat.	30
E3. Stem reserve mobilization support wheat grain filling under heat stress	37
E4. Thermotolerance in wheat seed endosperm utilization and its relations to thermotolerance in the plant	55
E5. Heat shock protein expression in wheat under diurnal heat stress and field	65
F. Description of cooperation	79
G. Evaluation of research achievements	79
H. List of Publications	80

A NOTE OF ADVISE:

This report consists of data which is still subjected to further analysis and interpretation. In that
sense the conclusions expressed here are open to change by the authors.

C. ABSTRACT

Genetic variability in heat tolerance of wheat has been evaluated by plant productivity under chronic heat stress in the field, by cellular membrane thermostability, by cellular viability staining, by growth of juvenile plants under heat stress, by grain growth under heat stress, by seed endosperm utilization and by the production of heat shock protein. Genetic variability in heat tolerance as measured by all of these criteria was very high. Some genotypes, such as V748 or V747 or 'Mustang' sustained heat tolerance according to multiple criteria and may be considered as suitable and agronomic genetic resources for heat tolerance improvement in wheat. Studies with germinating seed and growing photosynthesizing seedlings indicated that tolerance to chronic heat stress in wheat was largely a function of avoiding heat-induced 'carbon starvation'. Heat-tolerant genotypes sustained a larger carbon supply by either better carbon assimilation or by reduced dark respiration. Tolerance during grain filling was largely a function of a high constitutive capability for supporting grain growth from mobilized stem reserves. This capacity was partly dependent on plant performance during the pre-stress plant growth stages.

Heat shock proteins (HSPs) were synthesized in response to heat stress in the field. There were qualitative and quantitative differences in HSPs between heat tolerant (*e.g.* 'Mustang') and heat susceptible (*e.g.* 'Sturdy') wheats. Some unique HSPs were only found in the heat tolerant cultivar. These data provide an evidence for correlation between HSP synthesis and heat tolerance in wheat under a simulated field environment and suggest that further genetic analysis of HSPs in a segregating population is worthy.

D. OBJECTIVE OF THE ORIGINAL RESEARCH PROPOSAL

D.1. The problem

High temperature 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 (Fischer, 1986) and it constitutes an important breeding target for CIMMYT in recent years (Curtis, CIMMYT, 1987 - personal communication). Consequently, development of heat tolerant cultivars is of major concern in more than a few wheat breeding programs.

The main objective of this research was to enhance our understanding of the repercussions of heat stress and heat tolerance in spring and winter wheat, with strong emphasis on plant production and practical problems of breeding for heat tolerance. This main objective was approached by several interfaced areas of investigation which breakdown along several planes - according to plant growth stage, to levels of plant organization and to the expected operative mechanisms of tolerance. Work began by identifying winter and spring wheat genotypes of variable heat tolerance in terms of growth biomass and yield components. Heat tolerance during the pre-flowering stages is important mainly in spring wheat. It was studied under the hypothesis that the plant's carbon balance under heat stress is of major importance in developmental physiology and magnitude of yield components developed at these stages. Heat tolerance during grain filling is important in both spring and winter wheat. It was studied under the hypothesis that the sustained activity of sources for grain filling under heat stress is most important. These sources are flag leaf and spike photosynthesis and stem reserve mobilization.

Known biochemical and physiological responses to heat stress which are involved at the cellular level were studied in the diverse germplasm in order to (a) understand their implications towards wheat productivity under heat stress and (b) open the way for the development of relevant and rapid screening techniques. This involved work with the triphenyl tetrazolium chloride (TTC) cell viability test, membrane thermostability and heat shock proteins (HSP).

Therefore, the specific objectives of this research were:

1. To evaluate genetic variation for heat tolerance at GS1 and GS2 in terms of active growth, phasic development and yield components in relation to plant carbon balance.
2. To evaluate genetic variation for heat tolerance at GS3 and its effect on grain filling as a function of leaf longevity, leaf and spike assimilation and stem reserve mobilization.
3. To characterize genetic variation in HSP, cell viability and membrane leakage, and assess the predictive value of these tests as measures of heat tolerance in relation to the above objectives.

D.2.Literature review

Although high temperature stress is a major cause of yield loss in several crop plants, we have a limited understanding of the genetic and physiological bases of heat tolerance in plants (Marshall, 1982; see also Blum, 1988 for a recent review). The lack of knowledge becomes even more prominent when an attempt is made to understand the effects of heat stress and tolerance on plant production and economic yield.

Wheat, a cool-season C_3 species, is physiologically poorly adapted to high temperatures. Net carbon exchange in wheat leaves has an optimum temperature of 23° to 25°C (Planchon, 1976; Blum, 1986; Kobza and Edwards, 1987). Photosynthetic activities including the rate of CO_2 fixation, RUBP carboxylase activity, and electron transport in flag leaves of winter wheat were significantly reduced as temperatures increased above 25°C (Al-Khatib and Paulsen, 1984). Fisher and Maurer (1976) have shown that a 1° C rise in temperature above the ambient during the period between the end of tillering and the beginning of grain filling reduced yield by 4%. It is very well established that wheat yield reduction at elevated temperatures is strongly associated with the acceleration of plant phasic developmental stages (e.g. Midmore et al, 1984).

Plant growth stage when heat stress occurs is a major determinant of the effect of stress and the impact of tolerance on final plant production. This is because yield components, tissue tolerance, ability to heat harden and ability for recovery vary with plant age and stage of development. In the realm of the weather under which wheat grows, heat stress intensity, duration and total load also vary with plant growth stage. The subject of this research is therefore approached through two main growth stages of wheat: the pre-anthesis stage when tillers develop and spike size is determined and the post-anthesis stage, when grain filling takes place.

The duration of all three major phases of growth (GS1: emergence to double ridge, GS2: double ridge to anthesis, and GS3: anthesis to maturity) is reduced as temperatures increase. A reduction in the size of organs developed at each phase is associated with the reduced duration of that phase, under heat stress. While high temperatures are relatively the least frequent and not critical at GS1, they may cause a reduction in tiller and spike number per plant (Warrington et al, 1977; Shpiler and Blum, 1986). The reduction in the duration of GS2 under the effect of high temperature results in a reduction in the number of spikes per plant and of spikelets and/or grain per spike (Halse and Weir, 1970; Fisher and Maurer, 1976; Warrington et al, 1977; Johnson and Kanemasu, 1983; Shpiler and Blum, 1986). High temperature reduces spikelet number through its effect on both the duration and the rate of spikelet initiation during GS2. Similarly, lower grain weight results from higher temperatures at GS3, with an associated decrease in grain growth duration (Sofield et al, 1977; Pinthus and Sar-Shalom, 1978; Shpiler and Blum, 1986).

The effect of heat on the acceleration of plant phasic developmental stages is very well expressed by thermal units ("heat sum"). Whereas a given sum of thermal units is required for the completion of a

growth phase, high temperatures supply this requirement in a shorter time (Shpiler and Blum, 1986). The acceleration of plant development in hot environments is also well expressed in the association between thermal units and the reduction of leaf phyllochron interval in wheat (Baker et al, 1986) and the general reduction in plant biomass (Rawson, 1986). Growth acceleration under heat stress apparently also involves inherent processes of plant senescence. This is especially noticeable when plant development is near its termination. Thus, for example, higher leaf temperatures after anthesis were associated with reduced yields due to accelerated leaf senescence (Richards et al, 1986). The acceleration of development appears to be associated also with a smaller grain size developed during GS3. Grain development in relation to high temperature is however in a relatively unique situation, as compared with the other phases of growth. This has been recently illuminated by the fact that high temperature inactivates starch synthase in wheat grain (Rijven, 1986), leading to reduced starch accumulation in the endosperm, apparently without any relation to growth acceleration.

The acceleration of plant development and the associated reduction in organ size developed during an accelerated development of a growth phase is therefore an important reason for wheat yield reduction in hot environments. The possibility for improving wheat yield in hot environments by finding and using genetic variation for the response to heat in the nature described above has received only limited attention.

Several investigators found that wheat varieties differed in the effect of heat during GS2 on spikelet number per spike (Halse and Weir, 1974; Halloran, 1977; Rawson, 1986). Bagga and Rawson (1977) found that cv. 'Kalyansona', a popular variety in India, showed excellent stability in grain number per spike at increasing temperatures, as compared with an Australian cultivar ('Condor'). This was confirmed by Midmore (cited in Fischer, 1986) also for cv. 'Siete Cerros' (which may be identical to 'Kalyansona') under hot conditions in Mexico. Further work by Shpiler and Blum (1986) showed that the stability of yield and grain number per spike across increasingly warm environments was well associated with stability in the duration of GS2 of the cultivar. The possibility for genetic modification in yield component stability in hot environments is supported by the isolation of a gamma-ray mutant of wheat that took 50% longer to reach heading and produced 57% more grain, as compared with the source material, when grown under hot conditions (Sheoran et al, 1983).

A further understanding of wheat response to heat in terms of growth acceleration and organ size is essential in order to develop a logical approach to breeding for heat tolerance. The above discussion allows us to hypothesize that variation among wheat genotypes in heat tolerance at GS1 and GS2 may result from either reduced sensitivity to acceleration of phasic development or a reduced sensitivity of organ growth to accelerated development. GS3 and grain development at high temperature are not considered in this context here and will be dealt with as a separate issue described below. This is because active growth and organ differentiation are not corollary at GS3. Sink-source relationship is a

major consideration in GS3. Furthermore, temperatures at GS3 are much higher than that at GS1 and GS2 in the field.

Almost a decade ago, Levitt (1980) discussed "starvation" as a possibly important explanation of reduced plant productivity at supra-optimal ("non-shock") temperatures. He discussed evidence to the effect that the plant's carbon balance becomes shorter as temperatures increase. This was explained by the fact that the temperature optimum for respiration was very often higher than that for photosynthesis. Thus, at higher temperatures when photosynthesis decreases, respiration still increases.

Photosynthesis of the wheat leaf is maximized around 20 - 25°C (Planchon, 1976), depending on temperature pre-conditioning or hardening (Blum, 1986). Photorespiration was found to increase slightly with an increase in temperature from 25 to 35°C (Planchon, 1976). Data on wheat leaf dark respiration at high temperatures is lacking, but as the case is for other cool season plants (Levitt, 1980) it may be expected to peak at temperatures much greater than 25°C. It was estimated that up to 2% of the crop biomass may be lost to respiration daily (Fischer, 1986) and that loss would increase with temperature. Limited evidence shows that wheat genotypes may differ in net leaf carbon exchange rate at high temperatures (Blum, 1986). It follows that the net carbon balance of the wheat plant under high temperature, as affected by genetic variations in net photosynthesis and dark respiration, may possibly be improved by genetic manipulations.

The amount of carbon available at any time during plant development is a critical determinant of growth. Examples are found in studies which demonstrated that a greater sucrose uptake by developing wheat spikes was associated with a greater number of kernels per spike (Radley, 1982; Waters et al, 1984). The quantitative effect of the available carbon pool on growth and plant production is accepted today almost axiomatically, to the extent that it has been included as an important function in plant growth models.

This introduction therefore allows us to propose a working hypothesis that heat tolerant genotypes of wheat are less sensitive to either growth acceleration or organ size reduction under heat stress at GS1 and GS2 because they are less susceptible to carbon starvation. The ability to maintain a larger carbon pool under stress in resistant genotypes is a function of their relatively greater net carbon assimilation under heat stress, as affected mainly by photosynthesis and dark respiration. This hypothesis leads to the work dealing with carbon assimilation in relation to temperature and the association between the carbon pool and plant development in heat tolerant and susceptible wheat genotypes.

The adverse effects of high temperature stress on grain development during GS3 have long been recognized (Evans *et al.*, 1975). In the Southern Great Plains of the United States breeders have emphasized selection for early maturity in winter wheat to escape heat stress at grain filling. But there is a limit to yield stabilization through early maturity because of the danger of spring freeze injury

(Paulsen and Heyne, 1983). Furthermore, earlier genotypes entail some reductions in yield potential. For future improvements of yield potential of winter wheat in the Great Plains of the U.S. we may have to consider later flowering germplasm with a higher level of stress tolerance at grain filling.

While the problem of poor grain filling at high temperature is associated with reduced grain filling duration, an attempt to genetically extend grain filling duration is futile because temperatures increase with time during grain filling. The real issue is therefore the extent to which carbon import into the grain can be sustained at high rates. For wheat grown in the Midwestern U.S. it was suggested (e.g. Wiegand and Cuellar, 1981; Bruckner and Froberg, 1987) that the only avenue open for genetic improvement of grain filling under heat stress is the maintenance of high grain filling rates for the same heat-reduced duration of grain filling. Sustained high filling rates at high temperature depend on sustained sources of carbon for grain filling, which are flag leaf assimilation, spike assimilation and stem reserve mobilization.

Flag leaf activity under heat stress largely depends on the rate at which it senesces (e.g. Al-Khatib and Paulsen, 1984). Loss of photosynthetic capacity in senescing wheat leaves appears to depend largely on loss in chlorophyll and RuBP carboxylase activity under heat stress (Grover *et al.*, 1986). While data on spike senescence in relation to its photosynthetic contribution is totally lacking, it appears that spike assimilation at high temperatures may depend on the amount and activity of awns (Blum, 1986).

Wheat plants use stored stem reserves for grain filling. The contribution of stem reserves to grain filling depends on transient photosynthesis. When the photosynthetic source is limited by stress, both the relative and absolute contribution of stem reserve mobilization to grain filling increases (for review see Blum, 1988). Genetic variation in the ability of wheat to support grain growth in the absence of transient photosynthesis has been revealed by chemical desiccation of plants in the field (Blum *et al.*, 1983a,b). It is now widely recognized that stem reserves offer a very important source for grain filling under post-anthesis drought stress. It has also been found that the ability of disease (septoria leaf blotch) tolerant wheats to support grain filling in spite of heavy disease load may be ascribed to stem reserve mobilization (Zilberstein *et al.*, 1985). However, no information is available on the role of stem reserves in grain filling when the photosynthetic source is limited by heat stress. With the availability of the chemical desiccation method for revealing an ability to support grain growth from stem reserves, it is important to find out whether stem reserve mobilization is an important component of heat tolerance at grain filling.

The above discussion therefore allows us to formulate the working hypothesis for this part of the research which deals with grain filling. It is suggested that heat tolerant wheats during grain filling sustain more viable sources for grain filling which can be seen in reduced flag leaf senescence, sustained flag leaf and/or spike photosynthesis and a greater ability to support grain growth by stem reserve mobilization under heat stress.

The basic cellular-physiological and biochemical consequences of heat injury are being investigated in several laboratories. These aspects are of interest here only to the extent that they can shed more light on heat tolerance of wheat in the field and possibly lead to the development of biochemical probes which would allow progress in selection and genetic manipulation of heat tolerance.

The major effects of heat stress on photosynthesis were identified in photosystem-II activity. This can be probed by chlorophyll fluorescence, provided that fluorescence signals are properly interpreted (Krause and Weis, 1984). Work on chlorophyll fluorescence in relation to heat stress and tolerance led to the conclusion that for certain cases variable chlorophyll fluorescence, as measured under a standard protocol, carries a potential for screening work in plant breeding for heat tolerance (Smillie and Hetherington, 1982). If indeed wheat tolerance to heat will be shown to be associated with an ability to sustain less injury to photosynthesis at high temperatures, then variable chlorophyll fluorescence may be used to develop a rapid screening technique for wheat.

Exposure of plant cells to heat causes membrane disruptions which are apparently related to temperature-specific phase changes in the membrane lipid bilayer. This is an area of active debate among cell biologists. However, whatever is the basic mechanism of cellular membrane disruption under heat stress, it is expressed in appreciable leakage of various electrolytes from the cell. This leakage may be measured by a very simple (conductometric) method. Since the initial work of Sullivan in the early 1970's (Sullivan *et al.*, 1977), several reports persist in showing that genetic variation in heat tolerance as measured by cell-membrane leakage exists in various crops and that the method is suitable for selection work (Onwueme, 1979; Martineau *et al.*, 1979; Wehner and Watschke, 1981; Chen *et al.*, 1982; Wallner *et al.*, 1982; Marsh *et al.*, 1985; Milliken, 1987). Encouraging results were also obtained for wheat (Blum and Ebercon, 1981). In all plants tested the method involves measurement of leakage after heat shock at near lethal temperature (typically, 1h at 48 C in wheat). A continuous controversy exists on the meaning of the method and the implication of selection by this method towards crop yield under heat stress. The practical implication of cell-membrane stability under a brief heat shock at 48 C is especially unclear for cool season crops such as wheat. While some logical explanations may be derived at the molecular level, breeders still require proof for an association between tolerance by the leakage method and some criteria of plant performance in a hot field environment. This research clearly offers an excellent opportunity to evaluate membrane leakage against various phenomena of heat tolerance, as discussed above and below.

Chen *et al.* (1982) reported that genotypic differences exist in the induction of an acquired heat tolerance system in common bean, potato, soybean, and tomato; and that these differences are related to known differences in heat tolerance as evaluated by their yield and fruit set under high temperature conditions. They suggested that the solute leakage test, or the 2, 3, 5-triphenyl tetrazolium chloride test (TTC cell viability test) can be used to evaluate heat tolerance in plants. In their studies, both tests gave similar results. The addition of the TTC test to this work may therefore provide reasonably good

insight into its value as a logical selection method for wheat, or it may provide support to the use of other methods mentioned here.

The discovery of specific proteins which are expressed under high temperature stress conditions, the heat shock proteins, has caused considerable interest in their role in mechanisms controlling heat tolerance (Kimpel and Key, 1985a; Sachs and Ho, 1986; Lindquist, 1986). The synthesis of HSPs was first discovered in the fruit fly, *Drosophila melanogaster*. When *Drosophila* cells were shifted from their normal growth temperature (25°C) to an increased temperature (37°C), there was a rapid induction of a discrete set of mRNAs coupled with the predominant synthesis of the corresponding proteins. These proteins were subsequently named heat shock proteins (HSPs). Along with the induction of HSP synthesis both the transcription of previously active genes and the translation of pre-existing messages were repressed. When cells were returned to 25°C, transcription and synthesis of normal proteins resumed, and HSP synthesis gradually declined. It is now known that the above heat shock response is highly conserved among both prokaryotic and eukaryotic organisms (Schlesinger *et al.*, 1982; Nover *et al.*, 1984; Atkinson and Walden, 1985; Craig, 1985; and Lindquist, 1986).

In higher plants, most descriptions of HSPs place them in both relatively high molecular weight (70-100 kDa) and low molecular weight (15-27 kDa) groups. The electrophoretic profile and complexity of low molecular weight HSPs varies among plant species, but they are generally more abundant in plant cells than in mammalian, insect or yeast cells (Kimpel and Key, 1985a). The abundant synthesis and accumulation of several low molecular weight HSPs in a variety of crop plants, as reported by Mansfield and Key (1987), suggest that they may have unique physiological functions in plants. In addition, it has been shown that HSP synthesis can be induced either by an abrupt temperature change or by a gradual temperature increase (Altschuler and Mascarenhas, 1985), and that HSPs accumulate in field grown plants (Kimpel and Key, 1985b; Burke *et al.*, 1985). These results suggest that HSP synthesis is a potential biochemical mechanism of heat tolerance and plant adaptation to hot environments.

While substantial progress has been made toward the molecular characterization of HSPs and an understanding of the regulation of heat shock gene expression is rapidly evolving from recent work, the determination of the role of HSP synthesis and the specific physiological function(s) of HSPs has been more difficult to ascertain (Lindquist, 1986; Schlesinger, 1986). This is very well exemplified by a recent report on maize (Bonham-Smith *et al.*, 1987) where heat shock proteins were not required for the establishment of thermal tolerance. Other physiological studies involving higher plant species, *Drosophila*, and yeast have correlated HSP synthesis with the development of thermal tolerance or thermal protection (Lindquist, 1986). It was demonstrated in "Wayne" soybean that the growth of seedlings was protected during a subsequent incubation at 45°C for two hours (normally a lethal treatment) by a heat shock at 40°C for one to two hours (Lin *et al.*, 1984).

Parallel to work by Zivy (1987), research in our laboratory at Texas Tech has provided the first evidence on the genetic diversity of HSP synthesis in the green leaves of wheat plants at the protein level (Krishnan *et al.*, 1987). Subsequent analysis using two-dimensional gel electrophoretic technique revealed substantial qualitative and quantitative genetic differences in HSP synthesis (Nguyen *et al.*, 1988). The amount of low molecular weight HSPs appears to be positively correlated with genotypic differences in cellular thermal tolerance evaluated by the TTC viability test.

D.3. General references for introduction

Al-Khatib, K. and G.M. Paulsen. 1984. Mode of high temperature injury to wheat during grain development. *Physiol. Plant.* 61:363-368.

Altschuler, M. and J.P. Mascarenhas. 1985. Transcription and translation of heat shock and normal proteins in seedlings and developing seeds of soybean exposed to a gradual temperature increase. *Plant Mol. Bio.* 5:291-297.

Atkinson, B.G. and D.B. Walden. 1985. Changes in eukaryotic gene expression in response to environmental stress. Academic Press, New York.

Bagga A.K. and Rawson H.M., 1977. Contrasting responses of morphologically similar wheat cultivars to temperatures appropriate to warm temperate climates with hot summers: a study in controlled environment. *Aust.J.Plant Physiol.* 4:877-887.

Baker J.T. Pinter P.J. Reginato R.J. and Kanemasu E.T. 1986. Effects of temperature on leaf appearance in spring and winter wheat cultivars. *Agron.J.* 78:605-613.

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., Poyarkova, H., Golan, G. and J. Mayer. 1983a. 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., Mayer, J. and G. Golan 1983b. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. II. Relations to drought stress. *Field Crops Res.* 6:149-155.

Blum, A. 1986. The effect of heat stress on wheat leaf and ear photosynthesis. *J. Exp. Bot.* 37:111-118.

Blum, A. 1988. *Plant Breeding for Stress Environments*. CRC Press Inc., Boca Raton, pp.201.

Bonham-Smith, P.C., M. Kapoor and J. D. Bewley. 1987. Establishment of thermotolerance in maize by exposure to stresses other than heat shock does not require heat shock protein synthesis. *Plant Physiol.* 85:575-580.

Bruckner, P.L. and R.C. Frohberg. 1987. Rate and duration of grain fill in spring wheat. *Crop Sci.* 27:451-455.

Burke, J.J., J.L. Hatfield, R.R. Klein, and J.E. Mullet. 1985. Accumulation of heat shock proteins in field-grown cotton. *Plant Physiol.* 78:394-398.

- Chen, H.H., Z.Y. Shen, and P.H. Li. 1982. Adaptability of crop plants to high temperature stress. *Crop Sci.* 22:719-725.
- Craig, E.A. 1985. The heat shock response. *CRC Critical Rev. Biochem.* 18:239-280.
- Evans, L.T., I.F. Wardlaw, and R.A. Fischer. 1975. Wheat. pp. 101-136. In Evans, L.T. (ed.) *Crop Physiology: Some case histories*. Cambridge Univ. Press, London.
- Fischer R.A. and Maurer R.O. 1976. Crop temperature modification and yield potential in a dwarf spring wheat. *Crop Sci.* 16:855-859.
- Fischer R.A. 1986. Physiological limitations to producing wheat in semitropical and tropical environments and possible selection criteria. in *Proc of the Internat. Symp. on Wheat for More Tropical Environments*, CIMMYT/UNDP, Mexico, p.209-230.
- Grover, A., S.C. Sabat and P. Mohanty. 1986. Effect of temperature on photosynthetic activity of senescing detached wheat leaves. *Plant Cell Physiol.* 27:117-126.
- Halse N.J. and Weir R.N. 1970. Effect of vernalization, photoperiod and temperature on phenological development and spikelet number of Australian wheat. *Aust.J.Agric.Res.* 25:687-695.
- Halloran G.M. 1977. Developmental basis of maturity differences in spring wheat. *Agron.J.* 69:899-902.
- Johnson R.C. and Kanemasu E.T. 1983. Yield and development of winter wheat at elevated temperatures. *Agron.J.* 75:561-565.
- Kimpel, J.A. and J.L. Key. 1985a. Heat shock in plants. *Trends Biochem. Sci.* 10:353-357.
- Kimpel, J.A. and J.L. Key. 1985b. Presence of heat shock mRNAs in field grown soybeans. *Plant Physiol.* 79:672-678.
- Kobza, J., and G.E. Edwards. 1987. Influences of leaf temperature on photosynthetic carbon metabolism in wheat. *Plant Physiol.*, 83:69-74.
- Krause, G.H. and E. Weis. 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. *Photosynthesis Res.* 5:139-157.
- Krishnan, M., H.T. Nguyen, and J.J. Burke. 1987. Genetic diversity of heat shock protein synthesis and its relation to thermal tolerance in wheat leaves. In: *Plant Gene Systems and Their Biology*. L. McIntosh and J.L. Key (eds). *UCLA Symposia on Molecular and Cellular Biology*, Vol. 62, A.R. Liss, New York.
- Levitt, E. 1980. *Responses of plants to environmental stresses (Vol. I)*. Academic Press, New York.
- Lin, C.Y., J.K. Roberts, and J.L. Key. 1984. Acquisition of thermotolerance in soybean seedlings. *Plant Physiol.* 74:152-160.
- Lindquist, S. 1986. The heat-shock response. *Ann. Rev. Biochem.* 55:1151-1191.
- 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. *Hort. Sci.* 110:680-683.

- Marshall, H.G. 1982. Breeding for tolerance to heat and cold. In *Breeding plants for less favorable environments*. M.N. Christiansen and C.F. Lewis (eds.). John Wiley and Sons, New York.
- 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.* 19:79-84.
- Midmore D.J. Cartwright P.M. and Fischer R.A. 1984. Wheat in tropical environments. II. Crop growth and grain yield. *Field Crops Res.* 8:207-227.
- Milliken G.A. 1987. Comparison of TTC and electrical conductivity heat tolerance screening techniques in *Phaseolus*. *HortScience* 22: 642-645.
- Nover, L., Hellmund, D. Newmann, K.D. Scharf, and E. Serfling. 1984. The heat shock response of eukaryotic cells. *Biol. Zentralbl.* 103:357-435.
- Nguyen, H.T., M.Krishnan, J.J. Burke, D.R.Porter, and R.A. Vierling. 1988. Genetic diversity of heat shock protein synthesis in cereal plants. In : J.H. Cherry and D.D. Davies (ed) *Biochemical and physiological mechanisms associated with environmental stress tolerance in plants*, Proceed. NATO Workshop. Springer-Verlag (In Press).
- Onwueme I.C. 1979. Rapid plant conserving estimation of heat tolerance in plants. *J.Agric. Sci. Camb.* 92:527-531.
- Paulsen, G.M. and E.G. Heyne. 1983. Grain production of winter wheat after spring freeze injury. *Agron. J.* 75:705-707.
- Pinthus M.J. and Sar-Shalom Y., 1978. Dry matter accumulation in the grains of wheat (*Triticum aestivum* L.) cultivars differing in grain weight. *Ann.Bot.* 42:469-471.
- Planchon, C. 1976. Heterosis, combining ability and photosynthesis in bread wheat. (*Triticum aestivum* L.) In *Heterosis in plant breeding*. A. Janossy and F.G.H. Lupton (eds.). Elsevier, Amsterdam.
- Radley M.E., 1982. Some factors affecting grain set in wheat. In *Opportunities for manipulation of Cereal Productivity*, Monograph 7. Hawkins A.F. & Jeffcoat B. (eds) British Plant Growth Regulator Group, Wantage pp.140-149.
- Rawson H.M. 1986. High-temperature tolerant wheat: a description of variation and a search for some limitations to productivity. *Field Crops Res.* 14:197-212.
- Richards R.A. Rawson H.M. and Johnson D.A. 1986. Glaucousness in wheat: its development and effect on water-use efficiency gas exchange and photosynthetic tissue temperatures. *Aust.J.Plant Physiol.* 13:465-473.
- Rijven A.H.G.C. 1986. Heat inactivation of starch synthase in wheat endosperm. *Plant Physiol.* 81:448-453.
- Sachs, M.M. and T.H. David Ho. 1986. Alteration of gene expression during environmental stress in plants. *Ann. Rev. Plant Physiol.* 37:363-376.
- Schlesinger, M.J., M. Ashburner, and A. Tissieres. 1982. *Heat shock from bacteria to man*. Cold Spring Harbor Lab., New York.
- Schlesinger, M.J. 1986. Heat shock proteins: the search for functions. *J. Cell Biol.* 103:321-325.

- Sheoran I.S. Kuhad M.S. Behl R.K. Nandwal A. and Dalvir Singh. 1983. A high-yielding heat-insensitive mutant of wheat for early sowing. *Indian J.Agric.Sci.*53:1076-1078.
- Shpiler L. and Blum A. 1986. Differential reaction of wheat cultivars to hot environments. *Euphytica* 35:483-492.
- Smillie, R.M. and S.E. Hetherington. 1983. Stress tolerance and induced injury in crop plants measured by chlorophyll fluorescence in vivo: chilling, freezing, ice cover, heat and high light. *Plant Physiol.* 72:1043.
- Sofield I. Evans L.T. Cook M.G. and Wardlaw I.F. 1977. Factors influencing the rate and duration of grain filling in wheat. *Aust.J.Plant Physiol.*4:785-797.
- Sullivan C.Y., N.V. Norcio and J.D. Eastin 1977. Plant responses to high temperatures. in *Genetic Diversity in Plants*. (Eds. Muhammed Askel and Von Borstel) Plenum p.301.
- Wallner S.J., M.R. Bechwar and J.D. Bulter 1982. Measurement of turfgrass heat tolerance in vitro. *J.Am.Soc.Hort.Sci.* 107:608-611.
- Warrington I.J. Dunstone R.L. and Green L.M. 1977. Temperature effects at three development stages on the yield of the wheat ear. *Aust.J.Agric.Res.*28:11-27.
- Waters S.P. Martin P. and Lee B.T. 1984. The influence of sucrose and abscisic acid on the determination of grain number in wheat. *J.Exp.Bot.*35:829-840.
- Wehner D.J. and T.L. Watschke 1981. Heat tolerance of Kentucky bluegrasses perennial ryegrasses and annual bluegrass. *Agron.J.* 73:79-83.
- Wiegand, C.L. and J.A. Cuellar. 1981. Duration of grain filling and kernel weight of wheat as affected by temperature. *Crop Sci.*21:95-101.
- Zilberstein, M., Blum, A. and Eyal, Z. 1985. Chemical desiccation as a simulator of postanthesis speckled leaf blotch stress. *Phytopathology* 75:226-230
- Zivy, M. 1987. Genetic variability for heat shock proteins in common wheat. *TAG* 74: 209-213.

E1. Implications of Carbon Assimilation and Cell-Membrane Thermostability in the Heat Tolerance and Yield of Wheat

A. Blum, L. Shpiler, B. Sinmena, G. Golan, J. Mayer.

ABSTRACT

Photosynthesis and cell-membrane thermostability are generally considered as important components of heat tolerance in crop plants. Their implications in sustaining wheat yield under chronic heat stress are not well documented and were therefore investigated here.

Twenty-four spring wheat (*Triticum aestivum* L.) cultivars were evaluated for leaf carbon exchange rate at 25°C (CER₂₅) and 35°C (CER₃₅), following heat hardening. The ratio of CER₃₅ to CER₂₅ (CER_{35/25}) was taken as a measure of relative heat tolerance in photosynthesis. Cell membrane thermostability was evaluated in heat-hardened leaves by the relative leakage of solutes after heat shock. Thirteen of the cultivars were grown in the field under chronic heat stress during the hot (offseason) summer under full irrigation and data were collected on plant phenology, yield and yield components.

Cultivars differed significantly for CER_{35/25} (range of 38% to 87%) and %leakage (range of 30% to 84%). The correlation across cultivars between %leakage and CER_{35/25} was low and non-significant ($r=-0.41$; $p=0.04$; $n=24$). The variation among cultivars in grain yield under heat stress was affected mainly by variation in kernels ear⁻¹. A general linear model analysis indicated that practically all (97%) of the variation in kernels ear⁻¹ among cultivars could be explained by variation in CER_{35/25} (74%) and by variation in the duration of the period from emergence to 'double ridge' stage of apex differentiation (GS1) (23%). Percent leakage did not have any significant effect on the variation in kernels ear⁻¹ among cultivars, nor was it correlated with any of the other yield components.

It is therefore concluded that genotypic variation in wheat yield under chronic heat stress is largely determined by the state of assimilate supply at the onset of ear differentiation. In this respect, heat tolerant wheats excel in relative carbon assimilation rate and its duration under heat stress. Cell-membrane thermostability was not important in this respect.

INTRODUCTION

Appreciable progress has been made in understanding the physiological and biochemical nature of heat stress and heat tolerance in plants (*e.g.* Neumann *et al.*, 1989). Much of the recent research has been driven by the impetus for achieving progress in crop plant breeding for improved heat tolerance. However, despite our better understanding of some of the basic

processes involved with heat stress and heat tolerance, their implications in whole plant response to heat stress are not fully resolved. Thus, putative selection criteria for heat tolerance that were suggested on the basis of certain physiological or biochemical responses to heat stress are not being adopted in practice or are being seriously challenged as useful. Evidently, more information is needed on the association between unique physiological components of heat tolerance and crop yield under heat stress.

Depending on the geographical location and the growing season, wheat can be affected by heat stress. Wheat is a cool-season plant, whereas photosynthesis is maximized at 25°C or less (Kobza and Edwards, 1987) and heat shock proteins may accumulate already at 34°C (Krishnan *et al.*, 1989). While the effects of and the recovery from heat shock were studied in various plants rather profusely, chronic heat stress rather than heat shock is a more common environmental situation for certain periods during wheat development. A general and a striking effect of chronic heat stress in wheat is the acceleration of plant development, which is accompanied by early senescence and the reduction in the size of organs developed under heat stress. While the duration of phasic developmental stages decreases, the rate of development increases under heat stress (Ritchie and Nesmith, 1991). However, the increased rate usually does not fully compensate for the decreased duration in achieving a normal organ size, mainly because of the lack of assimilates. 'Carbon starvation' (Levitt, 1980) can therefore be a major determinant of wheat yield under chronic heat stress, and heat tolerant wheats would be expected to excel in assimilate production under heat stress.

Genetic variation for heat tolerance in photosynthesis of wheat is well documented (*e.g.* Rawson *et al.*, 1983; Blum, 1986; Sayed *et al.*, 1989; Alkhatib and Paulsen, 1990). A general association between leaf photosynthesis, plant biomass and single plant yield production under heat stress of 10 diverse winter wheats has been established in the growth chamber (Alkhatib and Paulsen, 1990). However, in that study the effect of photosynthesis on yield was investigated in plants subjected to heat stress only after anthesis, so that kernel weight was the only yield component affected by heat stress. Further information on earlier stages of growth and other yield components is needed.

The cellular membranes are a central site for heat stress perturbations (Raison *et al.*, 1980). For numerous plant species, heat tolerance has been found to be expressed in cell-membrane thermostability (Blum, 1988), which is commonly assessed by the leakage method (Sullivan, 1972). With this method, the relative rate of electrolyte leakage from leaf tissues subjected to heat shock is taken as a measure of tolerance. Despite the extensive evaluation of the method as a putative selection index in various crops (reviewed by Blum, 1988), information on the association between cell membrane thermostability and crop plant production under heat stress is

limited to few reports (Sullivan and Ross, 1979; Martineau *et al.*, 1979; Schaff *et al.*, 1987; Saadalla *et al.*, 1990), which do not seem to agree upon the conclusions.

This research was performed in order to investigate the relationships between carbon exchange rate, cell-membrane thermostability, plant phenology and the yield of diverse spring wheats subjected to chronic heat stress.

MATERIALS AND METHODS

Twenty four spring wheat (*Triticum aestivum* L.) cultivars (Fig.1) were used for this study. Except 'Seri', all cultivars were selected in Israel and those designated by a number were experimental. For several of the cultivars previous information has already been gained on yield response to heat stress (Shpiler and Blum, 1991) and photosynthetic capacity under optimal temperatures (Blum, 1990).

Field Trial

Thirteen of the cultivars (marked by asterisk in Fig.1) were subjected to chronic heat stress under field conditions, as described earlier (Shpiler and Blum, 1986, 1991). Materials were planted at Bet Dagan in the coastal plains of Israel during the hot summer (offseason) on 18 July 1990. Mean weekly temperatures from July to September 1990 ranged from 28.2 to 26.7°C. The experimental design was a randomized complete blocks with four replicates. Each replicate consisted of one row 3 m in length with 90 cm between rows. Fertilizers were applied prior to sowing at the rate of 180 kg ha⁻¹ of N and 150 kg ha⁻¹ of P₂O₅. Irrigation was applied twice a week at the effective rate of 25 to 35 mm per irrigation, which fully accounted for pan-A evaporation. The experiment was kept free of weeds and diseases by the recommended chemical control.

Date of 'double-ridge' formation was determined by dissecting several main stems in each plot. Date of anthesis was determined when 90% of the ears per plot shed pollen. Physiological maturity was determined by the change in ear color (Singh *et al.*, 1984). These data allowed to estimate the duration of GS1 (emergence to "double ridge"), GS2 ("double ridge" to anthesis) and GS3 (anthesis to physiological maturity) phasic developmental stages. Thermal time required for each developmental phase was determined as the sum of mean daily air temperatures above 10°C, based on data from a meteorological station situated 3 km from the site, at the same altitude. Plots were hand-harvested. Ears were counted and threshed. Kernel weight was determined in a sample of grain from each plot.

Physiological measurements

The twenty four cultivars were planted in 15 L pots in a well ventilated greenhouse at Bet Dagan on December 9, 1989 (normal season). Pots contained a well-fertilized local vertisol mixed (1:1) with peat moss. Six plants were established in each pot and were irrigated frequently to avoid water stress. When flag leaves of a given cultivar fully expanded, pots of that cultivar were transferred to the growth chamber for heat hardening at 25/34°C night/day temperatures under 540 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic active radiation (PAR). After 24 h in the growth chamber, main-stem flag leaves were sampled for the measurement of leaf gas exchange and membrane thermostability.

For the measurement of leaf gas exchange, the leaf sheath was cut 10 cm below the flag leaf lamina and placed in a vial with water under a quartz- iodine light source providing $900 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR at the leaf surface. After 15 min of light equilibration, the leaf was used for gas exchange measurements. Preliminary tests had shown that carbon exchange rate (CER) at 25°C or 35°C under $900 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR did not change significantly for at least 1 h, which was more than the time required to perform all measurements with any single leaf.

Leaf gas exchange was measured with an open gas analysis measurement system (Analytical Development Co., UK), as described elsewhere (Blum, 1990). The leaf chamber was clamped onto the leaf. Leaf temperature was regulated by directing a hot/cool air blower to the heat-sink at the base of the leaf chamber. By observing the displayed leaf temperature and by manually adjusting the voltage supply to the heater or its distance from the leaf chamber, leaf temperature was controlled to better than $\pm 0.1^\circ\text{C}$ of the desired nominal temperature. Gas exchange measurements were made at a typical concentration of ambient CO_2 of 335-345 $\mu\text{l L}^{-1}$. After measurements at 25°C the leaf temperature was raised to 35°C and gas exchange was measured following an equilibration period of 15 min. For each leaf two measurements were performed at each temperature and the results averaged. Five leaves (from five different pots) per cultivar were measured.

To verify the effect of high leaf temperature on leaf water status, duplicate measurements of leaf water potential were made with a pressure chamber in leaves which were not subjected to gas exchange measurements and in leaves immediately after gas exchange measurements at 35°C. Leaf water potential was statistically the same before and after gas exchange measurements at 35°C, indicating that gas exchange at 35°C was not influenced by leaf water deficit..

Flag leaves were sampled from heat-hardened plants (see above) for the measurement of cell-membrane thermostability by the electrolyte leakage method according to Blum and Ebercon (1981) as based on Sullivan (1972). Briefly, 10 pieces (measuring about 1 cm^2) taken from one side of the mid-vein of one flag leaf were sampled into a stoppered vial. Five such vials (replications) per cultivar were subjected to heat treatment in a water bath for 1 h at 52°C (treatment). Additional five vials (taken from the other side of the mid-vein) were kept at 10°C as

controls. All vials were then incubated in 20 ml of deionized water for 24 h at 10⁰C, after which the electrolyte leakage from the tissues was measured by the electrical conductivity of the medium. Total background conductivity was measured after autoclaving all vials. Relative (%) leakage caused by heat stress was calculated as:

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

where T and C refer to conductance values for treatment and control, respectively, and the subscripts 1 and 2 refer to initial and background conductance readings, respectively.

All statistical analyses were performed using JMP (SAS Institute, Cary NC, USA) and SuperAnova (Abacus Concepts, Berkeley CA, USA) statistical software for the Macintosh computer (Apple Computer Inc.).

RESULTS AND DISCUSSION

Average yield in the hot summer was 2126 kg ha⁻¹ (Table 1). It compares well with data obtained in previous studies at the same site during the summer (Shpiler and Blum, 1986, 1991), indicating a yield reduction of about 60% as compared with typical yields in the normal (cool) winter season. Similarly, the average duration (in days) of all three phasic developmental stages was short (Table 1), as compared with typical values from the winter growing conditions at the same site (Shpiler and Blum, 1986). The reduction of wheat yield and growth duration under chronic heat stress has already been amply documented and successfully simulated by computers (Ritchie and Nesmith, 1991).

Table 1. Phenology, yield and yield components of 13 wheat cultivars (marked by asterisks in Fig.1) tested under chronic heat stress during the summer (offseason) of 1990 at Bet Dagan.

Trait	Units	Mean	Minimum	Maximum	CV (%) [†]	F ratio
Duration of GS1	Heat units (days)	460(16.9)	328 (12)	695 (26)	25.5	6.71**
Duration of GS2	Heat units (days)	682 (26.8)	626 (25)	787 (29)	4.6	2.66*
Duration of GS3	Heat units (days)	880 (38.2)	685 (35)	993 (43)	7.3	5.54**
Ears m ⁻²	no.	242	177	318	19.2	3.61**
Kernels ear ⁻¹	no.	25.8	13.7	34.5	20.3	4.67**
Kernel weight	mg	33.7	27.5	45.5	14.1	13.80**
Grain yield	kg ha ⁻¹	2126	1064	3001	28.3	5.83**

([†]) Coefficient of variation (CV) among genotypes.

(*, **) Significant at p≤0.05 and p≤0.01, respectively.

Table 2. The general linear model regression of grain yield on its components for 13 wheat cultivars (marked by asterisks in Fig.1) tested under chronic heat stress during the summer (offseason) of 1990 at Bet Dagan. Model $R^2 = 0.96$.

Source of variation in yield	Sum of squares	Sum of squares as % of total	F value
Ears m^{-2}	12,557	20.6	90.6**
Kernels ear^{-1}	39,158	64.2	167.4**
Kernel weight	9,268	15.2	52.5**
Total	60,983	100.0	

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

The thirteen tested cultivars differed very significantly for yield and its three components, as well as for the duration of each of the three phasic developmental stages (Table 1). Of all three phasic developmental stages, the largest variation among cultivars was observed in GS1. A general linear model analysis of the effect of grain yield components on grain yield (Table 2) revealed that the most important grain yield component in affecting yield variations among cultivars under heat stress was kernels ear^{-1} . About 65% of the variation in yield among cultivars under heat stress could be ascribed to variations in kernels ear^{-1} . This value compares extremely well with previous results obtained during the summer at the same site (Shpiler and Blum, 1991) where it ranged between 57.1% and 58.8% in two different years. The importance of kernels ear^{-1} in determining wheat yield under chronic heat stress is also supported by Rawson (1986). On the other hand, under cool conditions during the normal winter season, the most important yield component in affecting yield variations among cultivars was ears m^{-2} (Shpiler and Blum, 1986). Kernels ear^{-1} under heat stress may therefore be taken here as a reliable and sensitive expression of genotypic variation for heat tolerance in terms of yield production under chronic heat stress.

Kernels ear^{-1} was positively correlated with the duration (in thermal time) of GS1 across the 13 cultivars tested ($r=0.75^*$). It was not correlated with the duration of GS2 ($r=0.29$), probably because genotypic variation for GS2 was low (Table 1). These results support previous observations (Rawson 1986; Shpiler and Blum, 1986) on the importance of a longer duration of GS1 in sustaining large ears and grain yield under heat stress.

CER₂₅ (leaf carbon exchange rate at 25°C) under 900 $\mu mol m^{-2} s^{-1}$ of PAR varied significantly ($P \leq 0.01$) among the 24 genotypes studied, ranging from 11.1 to 16.7 $\mu mol m^{-2} s^{-1}$ (Fig.1). For ten of the cultivars, data on CER₂₅ was also available from a previous study.

(Blum, 1990). In that study, the response of CER_{25} to irradiance was measured and expressed as the linear regression of CER_{25} on log irradiance. The slope of that regression reflected the potential for CER_{25} of the different cultivars. It was found now (Fig.2) that CER_{25} values from this study were highly correlated with the slope of the regression of CER_{25} on log irradiance in the previous study, over 10 cultivars. This regression lends credibility to CER measurements as performed here, indicating that the found genotypic differences in CER_{25} are real, repeatable and reflect the photosynthetic potential of the tested cultivars.

Fig.1. Leaf carbon exchange rate (CER) at 25°C and 35°C, CER at 35°C as percent of CER at 25°C and cell membrane thermostability expressed as percent leakage, in 24 diverse wheat cultivars. Vertical bars are S.E.

Fig.2. The association between leaf carbon exchange rate (CER) at 25°C as measured in this study and the slope of the linear regression of CER at 25°C on log irradiance, as obtained in a previous study (Blum, 1990), over 10 wheat cultivars.

On the average, CER₃₅ (leaf carbon exchange rate at 35°C) was reduced by 36% as compared with CER₂₅. CER₃₅ varied significantly among the 24 genotypes tested, ranging from 5.5 to 11.5 μmol m⁻² s⁻¹ (Fig.1). A two-way analysis of variance of CER data (n=24; 5 replicates) indicated a significant ($p \leq 0.05$) cultivar by genotype interaction for CER (not shown). CER₃₅ as percent of CER₂₅ (CER_{35/25}) was therefore taken as the relative heat tolerance of the various cultivars, in terms of CER. CER_{35/25} ranged significantly ($p \leq 0.05$) from 38% to 87%, across the 24 cultivars (Fig.1). For some heat sensitive cultivars (such as 'Dariel') CER₃₅ was relatively high inspite of their low CER_{35/25}, mainly because of their relatively high photosynthetic potential (as reflected in CER₂₅). However, other cultivars (such as 'V652') with a similarly high photosynthetic potential, were not as heat susceptible as 'Dariel', while some cultivars (such as 'Barkae') were most susceptible. Certain tolerant cultivars for CER, such as 'V748-74-1S' and 'V747-16-1', were already noted earlier for their heat tolerance in terms of yield production (Shpiler & Blum, 1986) and flag leaf photosynthesis under high temperature (Blum, 1986).

Percent leakage after heat shock, as an estimate of membrane thermostability, ranged significantly ($p \leq 0.05$) from 30 to 84% across the 24 cultivars tested (Fig.1). The correlation between CER_{35/25} as an expression of the relative tolerance in CER and % leakage as an expression of relative susceptibility in cellular membranes, was negative, low and barely significant ($r = -0.41$; $p = 0.04$; $n = 24$). There is no *a priori* reason to assume that heat perturbation of cell membrane stability is physiologically linked with photosystem response to heat, as already noted earlier (Blum, 1986). Chaisompongpan *et al.* (1990) concluded from their study on heat tolerance in bean that "photosynthetic response to heat stress is independent of plasmalemma damage".

Neither CER_{35/25}, nor CER₃₅, or %leakage were significantly correlated with the duration of GS1 across the 13 cultivars tested in the field ($r = 0.32$, $r = -0.34$ and $r = 0.37$, respectively; $p > 0.05$), indicating relative independence of the duration of GS1 from these physiological attributes of heat tolerance. Therefore, a general linear model analysis of the effect of GS1, CER_{35/25} and %leakage on kernels ear⁻¹ of the 13 cultivars studied in the summer could be considered (Table 3). It was found that about 97% of the variation in kernels ear⁻¹ among cultivars could be accounted for by variation in GS1 and CER_{35/25}. However, CER_{35/25} was three times more effective (74%) than GS1 (23%) in this respect. It therefore seems that a large number of kernels per ear under heat stress can be achieved by sustaining the least reduction in carbon assimilation and by maintaining a sufficiently long duration of assimilation and growth prior to 'double-ridge' formation.

Table 3. The general linear model regression of kernels ear⁻¹ under heat stress on the duration (in thermal time) of GS1, CER at 35°C as % of CER at 25°C (CER_{35/25}) and membrane thermostability (%leakage) across 13 wheat cultivars. Model $R^2 = 0.87$

Source of variation in kernel number	Sum of squares	Sum of squares as % of total	F value
GS1	17.6	23	3.69**
CER _{35/25}	55.6	74	11.67**
%Leakage	2.4	3	0.49

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

Although the ear grows during GS2, the duration of GS2 was not found to be correlated across cultivars with kernels ear⁻¹ ($r = 0.35$; $n = 13$). A longer duration of GS2 is important for sustaining kernels ear⁻¹ under heat stress (Fischer and Maurer, 1976; Shpiler and Blum, 1986). However, in this study the very small variation among cultivars in GS2 (Table 1) may account for its low correlation with kernels ear⁻¹. It therefore appears that a sufficient supply of

assimilates for growth and metabolic activity under chronic heat stress is a key feature of heat tolerance, whereas tolerance in photosystem sustains high rates of assimilation while the maintenance of longer phasic developmental stages provides the necessary time for assimilation. The importance of GS1 in affecting kernels ear⁻¹ under chronic heat stress suggests that the determination of the potential ear size for the given environment may be strongly influenced by the concentration of assimilates in the the plant at the 'double ridge' stage of development. The effect of heat stress on ear differentiation and growth during GS2 could then be realized by the reduction from that potential. This reaffirms the importance assigned to heat tolerance during the first 3 weeks of growth towards yield formation under chronic heat stress (Rawson, 1986; Shpiler and Blum, 1991).

Cell membrane thermostability did not contribute to a stable kernel number per ear under heat stress, probably because it was not involved with the plant carbon balance under chronic heat stress (Blum, 1986; Chaisompongpan *et al.*, 1990). The measurement of heat tolerance by tissue leakage after heat shock has been gaining wide attention (Blum, 1988). However, the utility of the leakage test for predicting genetic variations in crop yield under chronic heat stress, is obscure. Schaff *et al.* (1987) obtained a significant negative correlation between %leakage and yield of different bean cultivars when grown under chronic heat stress in Kansas. They did not suggest a reason for this correlation, but it could occur due to the involvement of variable cell membrane thermostability in processes other than the plant carbon balance, such as the heat-induced flower and/or fruit abortion common to the legumes (*e.g.* Warrag and Hall, 1983). On the other hand, correlations between %leakage and yield of segregating soybean populations were nonsignificant, though always negative (Martineau *et al.*, 1979). Sullivan and Ross (1979) indicated a negative association between % leakage and grain yield of several selected sorghum lines under the hot and dry conditions of Nebraska. Saadalla *et al.* (1990) classified winter wheat selections into three classes of heat tolerance in terms of % leakage, and tested them for yield in three locations in Colorado. Field temperatures were relatively high only during grain filling. Grain yield was higher in the tolerant group only in one of the three sites, mainly due to a larger kernel weight. Kernel number per unit area did not differ between groups or was significantly lower in the tolerant group. The authors suggest that drought stress conditions during grain filling may have been involved with their results. In our study (with full irrigation), there was no correlation between %leakage and kernel weight under heat stress ($r=-0.022$; $n=13$). The possibility that % leakage after heat shock may represent genotypic variations in drought tolerance should not be overruled. This has been suggested from work with sorghum (Sullivan and Ross, 1979) and wheat (Blum and Pnuel, 1990).

It is therefore concluded that genotypic variation in wheat yield under chronic heat stress is largely determined by kernel number per ear and that kernel number per ear is strongly affected

by the state of carbon supply at the onset of ear differentiation. In this respect, heat tolerant wheats excel in relative carbon assimilation rate and its duration under heat stress. Tests for heat tolerance which involve tissue reaction to heat shock, such as cell membrane thermostability by the leakage method, should be given further consideration before being used for predicting genotypic variation in plant production under chronic heat stress.

REFERENCES

- Alkhatib, K., and G.M. Paulsen. 1990. Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. *Crop Sci.*, 30:1127-1132
- Blum, A. 1986. The effect of heat stress on leaf and ear photosynthesis. *J. Exp. Bot.*, 37:111-118.
- Blum, A. 1990. Variation among wheat cultivars in the response of leaf gas exchange to light. *J. Agric. Sci. (Cambridge)*, 115:305-311.
- Blum, A., and Y. Pnuel. 1990. Physiological attributes associated with drought resistance of wheat cultivars in a Mediterranean environment. *Aust. J. Agric. Res.*, 41:799-810.
- Blum, A. 1988. *Plant Breeding for Stress Environments*. CRC Press, Boca Raton, 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.
- Chaisompongpan, N., P.H. Li, D.W. Davis, and A.H. Markhart. 1990. Photosynthetic responses to heat stress in common bean genotypes differing in heat acclimation potential. *Crop Sci.*, 30:100-104.
- Fischer, R.A., and R.O. Maurer. 1976. Crop temperature modification and yield potential in a dwarf spring wheat. *Crop Sci.*, 16:855-859.
- Kobza, J., and G.E. Edwards. 1987. Influences of leaf temperature on photosynthetic carbon metabolism in wheat. *Plant Physiol.*, 83:69-74.
- Krishnan, M., H.T. Nguyen, and J.J. Burke. 1989. Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol.*, 90:140-145.
- Levitt, J. 1980. *Response of Plants to Environmental Stresses. I. Chilling, Freezing and High Temperature Stresses*. Academic Press, New York.
- 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.*, 19:79-82.
- Neumann, D., L. Nover, B. Parthier, R. Rieger, K.D. Scharf, R. Wollgiehn, and U.Z. Nieden. 1989. Heat shock and other stress response systems of plants. *Biologisches Zentralblatt*, 108: 1-146.
- Raison, J.K., J.A. Berry, P.A. Armond, and C.S. Pike. 1980. Membrane properties in relation to adaptation of plants to temperature stress. p. 261-278. *In* Turner, N.C., and P.J. Kramer (eds.), *Adaptation of Plant to Water and High Temperature Stress*. New York, Wiley-Interscience.
- Rawson, H.M. 1986. High-temperature tolerant wheat: a description of variation and a search for some limitations to productivity. *Field Crops Res.*, 14:197-212.

- Rawson, H.M., J.H. Hindmarsh, R.A. Fischer, and Y.M. Stockman. 1983. Changes in leaf photosynthesis with plant ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny. *Aust.J. Plant Physiol.*, 10:503-514.
- Ritchie, J.T., and D.S. Nesmith. 1991. Temperature and crop development. p.5-29. *In* Hanks, J., and J.T. Ritchie (eds.), *Modeling Plant and Soil Systems*. Madison Wisconsin, Amer. Soc. Agron.
- Saadalla, M.M., J.S. Quick, and J.F. Shanahan. 1990. Heat tolerance in winter wheat. 2. Membrane thermostability and field performance. *Crop Sci.*, 30:1248-1251.
- Sayed, O.H., M.J. Emes, M.J. Earnshaw, and R.D. Butler. 1989. Photosynthetic responses of different varieties of wheat to high temperature. I. Effect of growth temperature on development and photosynthetic performance. *J. Exp. Bot.*, 215:625-631.
- Schaff, D.A., C.D. Clayberg, and G.A. Milliken. 1987. Comparison of TTC and electrical conductivity heat tolerance screening techniques in *Phaseolus*. *HortScience*, 22:642-645.
- Shpiler, L., and A. Blum. 1986. Differential reaction of wheat cultivars to hot environments. *Euphytica*, 35:483-492.
- Shpiler, L., and A. Blum. 1991. Heat tolerance for yield and its components in different wheat cultivars. *Euphytica*, 51:257-263.
- Singh, V.P., M. Singh, and M.S. Kairon. 1984. Physiological maturity in aestivum wheat: visual determination. *J. Agric. Sci. (Cambridge)*, 102:285-287.
- Sullivan, C.Y. and W.M. Ross 1979. Selecting for drought and heat resistance in grain sorghum. p. 263-281. *In* Mussell, H., and R.C. Staples, R.C. (eds.), *Stress Physiology in Crop Plants*. New York, Wiley-Interscience.
- Sullivan, C.Y., 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. p.247-255. *In* Rao, G.P. and L.R. House (eds.), *Sorghum in the Seventies*. New Delhi, Oxford and IBH Publ. Co.
- Warrag, M.O.A., and A.E. Hall. 1983. Reproductive responses of cowpea to heat stress: genotypic differences in tolerance to heat at flowering. *Crop Sci.*, 23:1088-1092.

E2. Genetic Variability of Cellular Thermotolerance in Cultivated Wheat

Henry T. Nguyen, A. Blum, and Kerry L. Hendershot

ABSTRACT

The main objective of this work was to determine the genetic variability for heat stress response in different wheat cultivars and to establish the suitability of different physiological assays in the measurement of the plant's capacity to acquire thermotolerance. Fifty spring and winter wheat cultivars from diverse geographical locations were screened for the cellular thermotolerance using the TTC cell viability assay and cell membrane thermostability test (CMS). The different wheat genotypes exhibited a broad range in thermotolerance as determined by both assays. In general, a good agreement between previous field performance data during heat stress and TTC data was observed in winter wheat. A good relationship was obtained between TTC and CMS for some wheat lines but the correlation between TTC cell viability and CMS tests was not significant across 12 different wheat genotypes. A strong correlation exists between thermotolerance at the seedling and flowering growth stage as measured by the TTC test.

In conclusion, we have found that extensive natural diversity exists in wheat for physiological thermotolerance in terms of TTC cell viability and CMS. The results suggest that these two traits are genetically independent, and that no singular physiological response may be taken as a descriptor of the total heat tolerance of a wheat genotype.

INTRODUCTION

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 (Fischer, 1986) 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. A detailed understanding of the genetics and physiology of heat tolerance will facilitate the development of specific selection criteria for wheat improvement.

Plant physiologists are aware of the phenomenon of heat acclimation or acquired thermotolerance for a long time, i.e., an exposure to a mild heat stress induces tolerance to higher temperatures. Two physiological tests, namely, membrane thermostability and triphenyl

tetrazolium chloride (TTC) cell viability, have been commonly used to quantitate the level of cellular thermotolerance in plants in term of heat acclimation capacity (Blum, 1988).

Exposure of plant cells to heat causes cellular membrane disruptions which are apparently related to temperature-specific phase changes in the membrane lipid bilayer (*e.g.* Suss and Yordanov, 1986). This is an area of active debate among cell biologists. However, whatever is the basic mechanism of cellular membrane disruption under heat stress, it can impact photosynthetic or mitochondrial activity, or even decrease the ability of the plasmalemma to retain solutes and water (Lin *et al.*, 1985). Cell membrane thermostability (CMS) is well estimated by the rate of electrolyte leakage from heat-stressed tissues *in vitro*, using a simple conductometric technique (see Blum, 1988 for a review). Since the initial work of Sullivan in the early 1970's (*e.g.* Sullivan *et al.*, 1977), an increasing number of reports persist in showing that genetic variation in heat tolerance as measured by the leakage method exists in various crops and that the method is suitable for selection work (Onwueme, 1979; Martineau *et al.*, 1979; Blum and Ebercon, 1981; Wehner and Watschke, 1981; Chen *et al.*, 1982; Wallner *et al.*, 1982; Marsh *et al.*, 1985; Milliken, 1987). Membrane thermostability of wheat has thus been measured at both seedling and flowering stages of growth (Sadalla *et al.*, 1990a). Genotypes were classified as heat-tolerant and heat-sensitive with the heat-tolerant genotypes producing 21% higher grain yield than heat-sensitive groupings. Similarly, heat-tolerant selections of winter wheat, based upon membrane stability, out-yielded heat-sensitive selections by 19% and also had increases in grain volume weight and kernel weight (Sadalla *et al.*, 1990b).

The TTC cell viability assay is based on the principles of tetrazolium salt reduction to formazan by dehydrogenase respiratory enzymes. Therefore, TTC test evaluates the intactness of the mitochondrial electron transport chain and thus correlates with respirational activity. It has a long standing as a valid estimate of plant cell and tissue viability, also at high temperatures (*e.g.* Towill and Mazur, 1974). Chen *et al.* (1982) reported that genotypic differences exist in the induction of an acquired heat tolerance system in common bean, potato, soybean, and tomato; and that these differences are related to known differences in heat tolerance as evaluated by their yield and fruit set under high temperature conditions. They suggested that the solute leakage test, or the 2, 3, 5-triphenyl tetrazolium chloride cell viability test (TTC) can be used to evaluate heat tolerance in plants. In their studies, both tests gave similar results. The TTC assay was used in wheat to detect genotypic difference in heat tolerance (Krishnan *et al.*, 1989).

Genetic studies of heat tolerance in plants are not abundant (Blum, 1988). Predominantly, most genetic studies on heat tolerance of a physiological response have utilized solute leakage as

a measure of membrane stability indicating relative heat acclimation potential. 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 of the number of genes controlling this trait was made. Marsh *et al.* (1985) utilized this same assay with common bean and reported that the major variability was controlled by a small number of genes. A diallel study with six genotypes of winter wheat found that recurrent selection may be an appropriate means of selecting for high temperature tolerance by utilization of chlorophyll fluorescence (Moffatt *et al.*, 1990). Porter (1989) reported from a diallel genetic analysis that the acquired thermotolerance trait as measured by TTC cell viability assay is controlled mainly by additive genetic effects suggesting that genetic progress can be made from selection.

The main objective of this work is to evaluate genetic variability for cellular thermotolerance in a large number of wheat lines using the TTC cell viability and cell membrane thermostability tests. Secondly, we want to determine the relationship between seedling and flowering plant responses using TTC test.

MATERIALS AND METHODS

Plant Materials. Fifty winter and spring wheat cultivars originated from various production regions of the world were used in this study. Seedlings were germinated and grown in sterilized vermiculite for ten days under a light which provided $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with a 14 h photoperiod. Plants were well watered and ambient temperature was maintained between 22° and 25 °C. The experimental design was a randomized complete block with three replications.

Heat Treatments. On the tenth day of growth, seedlings were transferred to an environmental chamber preset at 37 °C with high relative humidity (80-100%) to ensure reduced evapotranspiration for 24h. After this period of heat acclimation, seedlings were removed and 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 test tubes containing 150 μl distilled H_2O . Tubes were subsequently capped and one tube representing each genotype was transferred to a water bath with a preset temperature of 50 °C for 1 h. Previous work in our laboratory indicated that a one-hour treatment at

50 °C was lethal to non-heat acclimated wheat seedlings. Following this 1 h treatment, test tubes were removed and cooled to room temperature (22-25 °C) for utilization in the triphenyl tetrazolium chloride (TTC) cell viability assay.

TTC cell viability assay. This assay was modified from the procedure described by Chen et al. (1982). A brief description follows. Following a specific heat treatment, a 2 ml solution of 0.8% TTC (w/v) dissolved in a 0.05 phosphate buffer, pH 7.4, was added to all tubes. Test tubes 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 H₂O. 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. The amount of formazan dye produced by TTC reduction was determined spectrophotometrically by reading the absorbance at 530 nm. Percentage of cell viability as a measurement of thermotolerance was determined by dividing the A₅₃₀ of the heat acclimated tissues (37 °C for 24 h) which were exposed to 50 °C by the A₅₃₀ of the leaf tissues which were heat acclimated at 37 °C.

Thermotolerance at Seedling and Flowering Stages. Six winter wheat lines were chosen based on their differences in thermotolerance using the above TTC assay to investigate the relationship between cellular thermotolerance at seedling and flowering growth stages. Seedling plants were vernalized in a cold chamber at 4 °C for six weeks then transplanted into pots and grown in the greenhouse until flowering. Temperature in the greenhouse was maintained around 15 to 25 °C. At anthesis, plants were acclimated in a controlled incubator at 37 °C for 24 h and evaluated for TTC reduction as described above.

Cell Membrane Thermostability. A sub-set of 12 wheat lines was used to evaluate the relationship between cell viability measured by TTC reduction and cell membrane thermostability measured by leakage conductivity method. This method was used in wheat by Blum and Ebercon (1981). Plants were grown and exposed to a 37 °C temperature for 24 h and tissues obtained as previously described except that segments of 8 seedlings were placed in a 50 ml tube, two tubes per genotype, containing 1 ml of distilled water. Subsequently tubes were capped and one tube from each genotype was placed in a 50 °C water bath for 1 h. Tubes were removed and 9 ml of distilled H₂O was added to each tube. Tubes were then recapped and placed in a water bath at 10 °C for 12 h after which tubes were removed, warmed to room

temperature, and vortexed. Conductivity of the H₂O bathing the leaf segments was then measured with a conductivity meter. Tubes were then recapped and autoclaved for 12 min. After cooling to room temperature, conductivity of the H₂O containing all released solutes was determined as before. Percentage of cellular membrane injury was determined utilizing the following formula:

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

T_1 = conductivity of acclimated leaf segments exposed to 50 °C after 12 h incubation at 10 °C,

T_2 = conductivity of leaf segments exposed to the same treatment following autoclaving,

C_1 = conductivity of acclimated leaf segments, and

C_2 = conductivity of acclimated leaf segments following autoclaving.

Inheritance of TTC Reduction Thermotolerance Trait. To analyze inheritance of cellular thermotolerance as measured by the TTC assay, a heat tolerant variety, Mustang, was crossed with a heat susceptible variety, Sturdy. The F₁ plant was selfed to obtain F₂ seeds. The parental lines and F₂ population were utilized for studies of inheritance. Essentially 250 F₂ seedlings were analyzed by the TTC assay as well as 25 seedlings of each of the parents. Variances among plants were calculated for each parental and F₂ population to estimate broad-sense heritability on an individual plant basis according to Allard (1960).

RESULTS AND DISCUSSION

The analysis of variance indicated significant genetic variability for the acquired thermotolerance trait (or heat acclimation capacity) in winter and spring wheat germplasm using the TTC cell viability assay (Figure 1). The 50 wheat lines were divided into specific groups of geographical origins of development for least square mean analysis (SAS Institute). It was interesting to note that the hard red winter wheat genotypes developed in the Central and Southern U.S. Great Plains and spring wheat lines developed in Israel had higher levels of acquired thermotolerance when compared to other winter and spring wheat groups.

Fig.1. Cell viability (in terms of percent TTC reduction) in different wheat genotypes under the effect of heat stress.

Fig.2. The association between TTC at the seedling stage and TTC at the flowering growth stage across six wheat cultivars.

A strong correlation ($r = 0.90^{**}$) was found between acquired thermotolerance at the seedling and flowering stage of growth using the TTC assay. Previously, Saadalla (1990b) reported in wheat that cell membrane thermostability between seedlings and flowering plants was highly associated. These results suggests that screening for these traits can be done at the seedling stage to predict the cellular heat tolerance potential of mature wheat plants.

The genetic analysis using the parental and F_2 plants resulted in a broad-sense heritability estimate of 72%, suggesting that this trait is highly heritable.

In this study, no simple relationship between the TTC cell viability and cell membrane thermostability was found. The data suggest that these two traits are controlled by different genetic loci. However, some wheat genotypes such as TAM 101, 747, Karl, and Mustang were found to possess both thermotolerance characteristics in term of TTC reduction and cell membrane stability tests.

E3. Stem Reserve Mobilization Support Wheat Grain Filling Under Heat Stress

A. Blum, B. Sinmena, J Mayer, G. Golan, L. Shpiler

ABSTRACT

Wheat (*Triticum aestivum*) grain filling is seriously impaired by heat stress due to reductions in transient leaf and ear photosynthesis at high temperatures. An alternative source of carbon for grain filling is stored stem reserves. This research was done to evaluate the hypothesis that the mobilization of stored stem reserves into the growing kernel is an important source of carbon for supporting grain filling under heat stress. Two spring wheat (*Triticum aestivum*) cultivars (V5 and V2183) of very similar phenology and plant stature were previously found to differ in grain shriveling under drought and heat stress conditions in the field. These cultivars were used to evaluate the hypothesis.

In two experiments (in 1990 and 1991), the rates of stem dry matter and stem total non-structural carbohydrates (TNC), kernel growth and leaf senescence were monitored under low (optimal) and high (stress) temperatures in the greenhouse (1990) and the growth chamber (1991). Cv. V5 as compared with V2183 always sustained a smaller reduction in kernel weight caused by heat stress. V5 as compared with V2183 had a higher stem dry matter weight and TNC content in stems at the onset of grain filling; greater depletion of stem dry matter (or TNC) during grain filling; and longer duration of grain filling, irrespective of temperature. On the other hand, V5 was more heat-susceptible than V2183 in terms of leaf longevity, chlorophyll stability *in vivo* and kernel or floret abortion under heat stress. Therefore, the superior grain filling under heat stress of V5 is a constitutive trait.

In a third experiment (in 1992) five cultivars were subjected to chemical desiccation (0.4% magnesium chlorate spray) of the canopy in the field as means for destroying the photosynthetic source of the plant after anthesis. The same cultivars were subjected to heat stress (25⁰/35⁰) or non-stress conditions (15⁰/25⁰) after anthesis in the growth chamber. It was found that kernel weight reduction by chemical desiccation was predictive of kernel weight reduction by heat stress ($R^2=0.89$).

INTRODUCTION

Wheat grain filling depends on two major sources of carbon: transient photosynthesis in leaves and ears and the mobilization of stored carbohydrates from the stem into the growing kernels.

Photosynthesis in wheat is maximized between 22°C and 25°C (Blum 1986; Kobza and Edwards, 1987) and it decreases drastically above 35°C (Blum 1986; Rawson, 1986; Alkhatib and Paulsen, 1992). Distinct genetic variations exist in wheat leaf and ear carbon exchange rate under heat stress (*e.g.* Blum 1986; Alkhatib and Paulsen, 1992) and it has been generally accepted (*e.g.* Alkhatib and Paulsen, 1992) that heat tolerance in photosynthesis ascribes an advantage in grain filling and yield under heat stress.

It is now fairly established that when the transient photosynthetic source is inhibited by drought stress, grain filling becomes more dependent on mobilized stem reserves (for a review see Blum, 1988). However, the role of stem reserve mobilization in supporting grain filling under heat stress has not been well studied.

Chemical desiccation of plant canopies at the onset of grain filling was developed as a tool for revealing genotypic differences in grain filling from stem reserves in the absence of transient photosynthesis (Blum *et al.*, 1983; 1990). While chemical desiccation has been confirmed to be useful for assessing genotypic diversity in grain filling under drought stress (Hossain *et al.*, 1992; Nicolas and Turner, 1993), it has never been evaluated for heat stress in this respect.

This study was therefore performed to evaluate the role of stem reserve mobilization in supporting grain filling under heat stress in different wheat genotypes. It was also used to evaluate chemical desiccation of the wheat canopy as a predictive method for revealing genetic variations in grain filling from mobilized stem reserves under heat stress.

MATERIALS AND METHODS

1990 experiment

Two spring wheat (*Triticum aestivum*) cultivars (V2183 and V5) were selected for this study. Both were very similar in phenology and plant height. V2183 was a routine pedigree selection out of cross BTL/LKCRCH//BTL/G-40, noted for its tendency to produce shriveled grain under field stress conditions. V5 resulted from a mass selection program under chemical desiccation stress, as described elsewhere (Blum *et al.*, 1990). Unfortunately, the pedigree listing of this selection was lost, but it could only belong to several alternative pedigrees, as listed there. V5 was noted to sustain reasonable grain filling under field stress conditions. Plants were grown in pots (5 per pot) in a well-fertilized commercial potting mixture, placed in an unshaded net house at Bet Dagan Station. Maximum daily temperatures from emergence (December 7, 1989) to anthesis of both cultivars (February 27, 1990) never exceeded 26.6°C (meteorological station situated 4km

from the site, at the same altitude). Plants were well-developed, very green and robust and did not experience any water stress. Upon anthesis, two treatments were imposed each consisting of 15 pots per cultivars. Control treatment consisted of plants remaining to mature under the ambient and relatively cool conditions in the net house. A heat stress treatment consisted of plants placed in an adjacent glasshouse (Fig.1). All pots were irrigated daily with tap water and once a week with half strength Hoagland's nutrient solution.

On five dates during grain filling 10 main stems (taken from five different pots) per cultivar were harvested. Thus, five replicates per date were sampled, each consisting of two stems per treatment x cultivar combination. A midsection of each flag leaf (area of 6 cm²) was dissected and submerged in dimethylformamide for the extraction and the spectrophotometric analysis of total leaf chlorophyll, according to Moran and Porath (1980). From the midsection of each ear 20 kernels were extracted, dried at 80°C and weighed. Stems, leaves and ears were dried separately and weighed. Stems were ground on a Wiley mill and a sample was analyzed for total stem non-structural carbohydrates (TNC) according to Dubois *et al.* (1956). Additional 5 main-stem ears (replicates) per cultivar x treatment combination were threshed at maturity and kernels were counted and weighed after drying.

1991 experiment

Plants of the two cultivars were grown in 15 L pots in the net house at Bet Dagan under the same conditions as described above, only that 6 plants were grown in each pot. Maximum daily temperatures from emergence (December 8, 1990) to anthesis (March 3, 1991) never exceeded 25.9°C. Upon anthesis plants were moved into two growth chambers, maintained at 15°/25°C and 25°/38°C night/day temperatures, with a 12 h day and 460 µmol m⁻² s⁻¹ of photosynthetically active radiation. Relative humidity was maintained above 74% by humidifying the chambers. Six pots (replicates) per cultivar were arranged randomly in each chamber and irrigated as described above. On 6 dates, 6 stems (replicates) were sampled from each cultivar in each growth chamber. The same observations and procedures as in 1990 were performed for each stem, excluding leaf weighing. Each stem was measured for length (to the base of the ear) and specific stem weight was calculated as the ratio of stem weight to stem length.

1992 experiment

Five wheat cultivars (see Fig.9 and its discussion for details) were established in 12 L pots in a net house, as described for the 1990 experiment. Maximum daily temperatures from emergence (December 2, 1991) to anthesis (February 25, 1992) never exceeded 24.9°C. Upon anthesis plants were moved into two growth chambers, maintained at 15°/25°C and 25°/35°C night/day temperatures, with a 12 h day and 460 µmol m⁻² s⁻¹ of photosynthetically active radiation. Relative humidity was maintained above 72% by humidifying the chambers. Four pots (replicates) per cultivar were arranged randomly in each chamber and irrigated as described

above. Upon maturity, all ears were harvested and threshed. Grain were weighed and counted. The rate of reduction in kernel weight due to heat stress at grain filling was calculated for each cultivar by the kernel weight ratio between high to low temperature conditions.

The same cultivars were field-tested at Bet Dagan in a split-plot randomized design with four replicates. Main plots consisted of chemical desiccation and control treatments. Sub-plots consisted of the five cultivars. Plants were grown during the 1992 season under standard management and normal seasonal growing conditions and were fully sprinkler-irrigated to avoid any water stress through maturity. Three seasonal prophylactic standard spray treatments with 'Bayleton' were applied to protect plants against rust and Septoria leaf blotch diseases. At 14 days after anthesis plots of each cultivar under the chemical desiccation treatment were sprayed to full wetting with 0.4% solution of magnesium chlorate (Blum *et al.*, 1983). Within three days the plants were completely (ears, leaves and stem surfaces) bleached. Upon maturity, a sample of plants (yielding about 1 kg of grain) was harvested from each plot and threshed with a stationary ear thresher. Mean kernel weight per plot was determined in a sample of 200 kernels. The rate of reduction in kernel weight due to chemical desiccation was calculated for each cultivar by the kernel weight ratio between desiccated to non-desiccated plants.

RESULTS

1990 experiment

Air Temperature ($^{\circ}\text{C}$)

Fig.1. Maximum daily temperatures ($^{\circ}\text{C}$) in the net house (controls) and the glasshouse (heat stress) during the grain filling period in 1990. Measurements were recorded with shaded thermographs at $\pm 0.3^{\circ}\text{C}$ accuracy.

Maximum daily temperatures under the net house (Fig.1) were considerably moderate for wheat, ranging from about 15°C to 26°C . In contrast, temperatures under the glasshouse (ranging from about 32°C to 42°C) were very high for wheat and indeed constituted severe heat stress conditions. Maximum daily temperatures of 40°C were already noted during the early part of the grain filling period. Under both treatments, minimum night temperatures during grain filling never exceeded 21.2°C .

As already observed in previous trials at Bet Dagan, anthesis occurred on the same date in both cultivars. Final kernel weight under non-stress (controls) conditions was about the same (41 mg) in the two cultivars (Table 1, at end of chapter). Grain weight per ear in the control was higher in V5 (3.04 g) than in V2183 (2.24 g), indicating a relatively higher potential ear productivity in the former cultivar.

Kernel growth differed markedly with cultivars and treatments (Fig.2). While anthesis occurred on the same date in the two cultivars, kernel growth duration in the control was shorter

in V2183 than in V5 by about at least 10 d. Under heat stress, kernel growth rate of V2183 increased slightly and growth duration decreased by about 5 d. While heat stress also reduced kernel growth duration of V5, it still remained longer than that of V2183, by at least 10 d. Evidently, V5 retained a longer kernel growth duration than V2183, irrespective of temperature.

Final kernel weight reduction caused by heat stress was 36.4% in V2183 as compared with only 8.0% in V5 (Table 1). The reduction in grain weight per ear caused by heat stress was 32.6% in V2183 as compared with 24.7% in V5. The heat-affected reduction in grain weight per ear in V5 was proportionally much smaller than the reduction in kernel weight, because of differences between cultivars in kernel number per ear. Kernel number per ear in V5 was reduced by heat stress from 74 to 60 (Table 1), while it was unaffected in this respect in V2183. Therefore, while V5 sustained grain filling under heat stress, it was heat-susceptible in terms of floret or kernel abortion.

Fig.2. Kernel growth (weight) in two wheat cultivars under control and heat stress temperatures (1990).

Fig.3. Stem dry matter weight in two wheat cultivars under control and heat stress temperatures (1990).

Fig.4. The association between stem dry matter weight and total stem non-structural carbohydrates content, across treatments and sampling dates.

Maximum stem weight in V2183 was about 2.4 g and it reduced only slightly during the grain filling period, irrespective of temperature treatment (Fig.3; Table 1). In contrast, maximum stem weight in V5 was significantly higher (about 3 g) and it reduced markedly during the grain filling period. Heat stress apparently shortened the duration of stem weight loss in V5 (Fig.3) and

consequently slightly less stem weight was lost under heat stress as compared with control (not significant; Table 1). Total stem weight loss during grain filling (Table 1) was distinctively smaller in V2183 (0.29 g to 0.31 g) than in V5 (1.55 g to 1.32 g, under controls and heat stress, respectively).

There was a high and positive correlation ($R^2=0.82$) between stem dry matter weight and stem total non-structural carbohydrates (TNC) content across treatments, cultivars and sampling dates (Fig.4). Total TNC loss from stems during the grain filling period (Table 1) did not differ between treatments, but it was about triple for V5 (0.77 to 0.79 g) as compared with V2183 (0.21 to 0.25 g, under controls and heat stress, respectively).

In the controls, total leaf weight per stem on the first sampling date was 0.83 g and 0.90 g in V2183 and V5, respectively. It was reduced on the last sampling date to 0.77 g and 0.89 g, respectively, indicating very small and insignificant leaf weight loss during grain filling. Similarly, small and insignificant leaf weight losses were observed under the heat stress treatment (from 0.82 g to 0.76 g in V2183 and from 0.88 g to 0.91 g in V5). Therefore, no significant amounts of reserves were mobilized from the leaves during grain filling, irrespective of the environment.

Stem dry matter loss as percent of grain weight per ear (Table 1) tended to increase under heat stress as compared with controls, in both cultivars. However, it was more than double for V5 than for V2183. Similar results were obtained for TNC loss as percent of kernel weight per ear (Table 1), only that percentages were generally lower than those for dry matter, in accordance with the ratio between dry matter and TNC (Fig.4). Thus, irrespective of the environment, stem dry matter reserves may have accounted for about half of the grain weight per ear in V5, while it was less than a quarter in V2183. Clearly, the contribution of stem reserves to grain mass was consistently higher in V5 than in V2183 under both stress and non-stress conditions.

Fig.5. Total chlorophyll content in flag leaves during grain filling in two wheat cultivars under control and heat stress temperatures (1990).

Total chlorophyll content in flag leaves reduced with time as kernels developed (Fig.5), indicating the expected progress of leaf senescence. In the control treatment, leaves of V2183 retained higher leaf chlorophyll content than those of V5, at least during the latter half of the grain filling period. Under heat stress, V5 lost more chlorophyll than V2183, already during the first 10 d of stress. Therefore, in terms of leaf chlorophyll content, V5 inherently tended to senesce faster than V2183, and this tendency was amplified by heat stress.

1991 experiment

Fig.6. Kernel growth (weight) in two wheat cultivars under low and high temperatures (1991).

On the average, final kernel weight, kernel weight per ear, kernel number per ear and maximum stem weight in the controls (Table 1) were smaller in this experiment as compared with 1990. This may have been the result of the higher plant density per pot in 1991 or occasionally higher night temperature in control plants in 1991. However, V5 still maintained its superiority over V2183 in potential productivity, as expressed in grain weight per ear.

Kernel growth differed with cultivars and treatments (Fig.6). At low temperatures kernel growth rate was somewhat greater in V2183 than in V5. However, kernel growth duration was longer in V5 than in V2183 by at least a week. When subjected to high temperature stress, kernel weight on the last date was lower than the maximum weight achieved earlier, indicating dry matter loss from the maturing kernel in both cultivars. Kernel growth rate of the two cultivars under high temperatures was very similar. However, V5 maintained longer kernel growth duration than V2183, by about a week. The reduction in final kernel weight due to heat stress was 47.8% in V2183 and 25.7% in V5 (Table 1). These values were very similar to the respective rates of loss in kernel weight per ear. In both cultivars kernel number per ear was unaffected by the high temperature treatment (Table 1).

Fig.7. Stem dry matter weight in two wheat cultivars under low and high temperatures (1991).

Maximum stem weight was higher in V5 than in V2183 (Fig.7, Table 1). The rate of reduction in stem weight with time was greater in V5 than in V2183, at least during the first half of the period of grain filling. The pattern of stem weight loss with time in each cultivar was consistent under the two temperature regimes (Fig.7), with a general tendency for an increase in the rate of weight loss with time under heat stress. Total stem weight loss (Table 1) was greater under high than under low temperatures and it was greater in V5 as compared with V2183. Under low temperatures, stem weight loss could account for 62.3% and 40.2% of grain weight per ear, in V5 and V2183, respectively (Table 1). However, under heat stress these values increased to above 100%. Since kernel number per ear in each cultivar was the same in the two temperature regimes, it may be assumed that an appreciable part of the dry matter lost from stems under heat stress did not reach the growing kernels.

Stem length was statistically the same in both cultivars, 66 ± 2 and 59 ± 2 cm, under low and high temperatures, respectively. Consequently, the greater maximum stem of V5, as compared with V2183, resulted from a corresponding difference in specific stem weight. Depending on sampling date, temperature regime and cultivar, specific stem weights ranged from about 0.015 to 0.043 g cm⁻¹.

Fig.8. Total chlorophyll content in flag leaves in two wheat cultivars under low and high temperatures (1991).

Fig.9. The association between kernel weight reduction by chemical desiccation (0.4% magnesium chlorate) of plant canopies in the field and kernel weight reduction

by the exposure of plants to 25/35⁰ (as compared with 15⁰/25⁰) night/day temperatures during grain filling in the growth chamber, across 5 wheat cultivars.

Total chlorophyll content of flag leaves was drastically reduced under high temperature stress (Fig.8). However, irrespective of the temperature regimes, V2183 retained leaf chlorophyll longer, as compared with V5.

1992 experiments.

Results for the effect of chemical desiccation and heat stress on grain weight of five wheat cultivars are presented in Fig.10.

Cultivars differed significantly ($p \leq 0.05$) for percent reduction in kernel weight by both heat stress and chemical desiccation. Cultivars V5 and V114/87 were the least affected while V1861 and V2183 were the most affected by both stresses. Veery-5 was average in response. These results re-confirm the difference in grain filling capacity between V5 and V2183. V114/87 was selected for this experiment, having been observed to sustain low kernel shriveling, while V1861 tended to suffer severe kernel shriveling in previous field tests. No previous information was available for Veery-5.

The association between kernel weight reduction by heat stress and kernel weight reduction by chemical desiccation of the canopy was linear and highly significant. Since chemical desiccation destroyed all green plant surface, it may be assumed that the variable capacity for grain filling under heat stress of these cultivars was highly dependent on the supply of mobilized plant reserves.

DISCUSSION

The two cultivars tested, which differed in their rate of grain shriveling under stress in field trials, also differed markedly in their grain filling under controlled heat stress conditions. Kernel weight reduction caused by heat stress during the grain filling period was distinctively lower in V5 than in V2183. This difference was repeated over three different experiments. In terms of grain filling, V5 appears to be more heat tolerant than V2183.

Compared with V2183, V5 was noted for three important attributes concerning grain filling under heat stress: (a) stem weight and specific stem weight at the onset of grain filling were high (b) stem dry matter and TNC loss during grain filling were large, and (c) kernel growth duration was long.

Since growth conditions up to grain filling were the same, and since the two test cultivars had the same phenology, V5 apparently had an inherently greater capacity for stem reserve storage. Evidently, this capacity was not attained at the expense of grain yield potential, since grain yield

per ear in the controls was consistently higher in V5 than in V2183. Therefore, apart from its effect on transient plant growth and differentiation processes, the maximization of net carbon assimilation prior to anthesis is also important for sustaining grain filling after anthesis. This is in an indirect support of the importance ascribed to 'early plant vigor' for sustaining wheat yields under typical Mediterranean dryland conditions (e.g. Whan *et al.*, 1990).

The higher storage of stem reserves in V5 was also better remobilized from the stem, as compared with V2183, irrespective of temperatures during grain filling. A distinction should be made between maximum stem dry matter weight (or TNC) at the onset of grain filling and the total dry matter depleted from the stem during grain filling. The total amount of dry matter depleted from the stem between its peak weight (normally occurring immediately after anthesis) and maturity may be defined as 'available stem reserves' which are being utilized for metabolism and grain growth.

The available stem reserves were very much influenced by cultivar. As seen in both 1990 and 1991, V5 had more available stem reserves than V2183, irrespective of the environment during grain filling. It is impossible to assess the exact proportion of available stem reserves that contributed to grain filling. Evidently, a proportion was lost to respiration, especially under heat stress conditions. Stem weight loss as percent of grain weight per ear was more than 100% in 1991 but not in 1990. Since the heat treatment night temperatures were higher in 1991 than in 1990, it is suspected that high night temperatures increased the proportion of available stem reserves used in respiration. Undoubtedly, a large amount of available stem reserves at anthesis, as the case was for V5, would also provide for more respiratory carbon losses under heat stress.

Stem dry matter mobilization in support of the growing kernel requires certain synchronization between the rate of stem dry matter depletion on one hand and rate of kernel growth on the other. Irrespective of the environment, V5 tended to have a longer kernel growth duration than V2183. The relatively longer grain filling duration in V5 with about the same final kernel weight as in V2183 would allow more time for importing stem reserves into the grain. Overwhole, V2183 tended to have higher rates of grain filling than V5, at least during part of grain filling period in controls and under heat stress. This was not reflected in stem reserve depletion to the extent that V2183 had higher rate of depletion than V5 (notable are results for 1990). Evidently, the rate of grain filling does not exercise a feedback control on the rate of stem dry matter depletion, and the two processes appear to be independent of each other. In this study, a longer grain filling duration was more important than a high rate of filling when kernel growth depended on mobilized stem reserves. At the same time it must be recognized that a genetically longer duration of grain filling may increase the probability of heat and drought stress during the latter part of the grain filling period where wheat matures as the environment becomes harsh. However, if longer

grain filling duration is indeed complemented by a large amount of available stem reserves, grain filling may be sustained despite the harsh environment.

The capacity of V5 to support grain filling from available stem reserves is a constitutive trait, independent of the environmental conditions during grain filling. It is not an adaptive trait conditioned by exposure to heat stress. In fact, V5 was more heat susceptible than V2183 in terms of flag leaf longevity and chlorophyll heat stability *in vivo*. It was also more susceptible than V2183 in terms of kernel abortion when temperatures reached 40°C soon after anthesis in 1990 (Fig.1). On the other hand, V5 was found to have a higher rate of flag leaf carbon exchange rate (CER) at high temperature (35°C), as compared with V2183 (A. Blum, unpublished data). Heat stability of CER is an important attribute of heat tolerance and it may support kernel growth under heat stress. However, as seen above, the relative advantage of V5 in CER at high temperature was obliterated by its inherently high rate of flag leaf senescence. It may be suspected that a high rate of leaf senescence is probably linked with extensive stem reserve depletion from the stem.

Therefore, V5 is constitutively a more efficient genotype than V2183 in terms of assimilating carbon for the production of larger sinks and the accumulation of available stem carbon storage. In terms of grain filling under heat stress V5 is not a truly heat resistant genotype. At the same time it is expected to excel in grain filling under any stress that inhibits the photosynthetic source during grain filling and compels greater dependence on stem reserve mobilization for grain filling. Genotypes such as V5 may be easily selected for by the chemical desiccation method, as demonstrated in the 1992 experiment, as well as by previous (Blum *et al.*, 1983, 1990) and subsequent (Hossain *et al.*, 1992; Nicolas and Turner, 1993) studies.

REFERENCES

- Alkhatib K. Paulsen G.M. 1992. Photosynthesis and Productivity During High-Temperature Stress of Wheat Genotypes from Major World Regions. *Crop Sci.* 30: 1127-1132.
- Bidinger F.R. Musgrave R.B. and Fischer R.A. 1977 Contribution of stored preanthesis assimilates to grain yield in wheat and barley. *Nature* 270:431-433 1977
- Blum, A. 1986. The effect of heat stress on wheat leaf and ear photosynthesis. *J. Exp. Bot.* 37:111-118.
- Blum, A., Poyarkova, H., Golan, G. and Mayer, J. 1983a. 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., Mayer, J. and Golan, G. 1983b. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. II. Relations to drought stress. *Field Crops Res.* 6:149-155.

- Blum, A. (1988). *Plant Breeding for Stress Environments*. CRC Press, Boca Raton, Florida, 208pp.
- Blum, A., Shpiler, L., Golan, G., Mayer, J. and Sinmena, B.(1990). Mass selection of wheat for grain filling without transient photosynthesis. *Euphytica* 54:111-116.
- Dubois M. Gilles K.A. Hamilton J.K. Rebers P.A. Smith F. 1956 Colorimetric method for determination of sugars and related substances. *Anal.Chem.*28:350-356.
- Hossain A.B.S. Sears R.G. Cox T.S. Paulsen G.M. 1992. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Sci.*30:622-627.
- Kobza, J., and G.E. Edwards.1987. Influences of leaf temperature on photosynthetic carbon metabolism in wheat. *Plant Physiol.*, 83:69-74.
- Moran R. Porath D. 1980 Chlorophyll determination in intact tissues using N,N-Dimethylformamide. *Plant Physiol.*65:478-479.
- Nicolas M.E. 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.
- Rawson H.M. 1986. high-temperature tolerant wheat: a description of variation and a search for some limitations to productivity. *Field Crops Res.*14:197-212.
- Whan B.R. Carlton G.P. Anderson W.K.1990. Potential for Increasing Early Vigour and Total Biomass in Spring Wheat .1. Identification of Genetic Improvements. *Aust.J.Agric.Res.*42:347-361

Table 1. The effect of treatments (1990 and 1991 experiments) and cultivars on final kernel weight, kernel weight reduction by heat stress, grain weight per ear, grain weight per ear reduction by heat stress, kernel number per ear, maximum stem dry matter weight, stem dry matter and TNC loss during grain filling, and loss of stem weight and TNC as percent of grain weight per ear at maturity ($\pm$ Standard Error). A separate analysis of variance was performed for each year.

PART I

Year	Cultivar	Treatment	Final Kernel weight (g)	Reduction in kernel weight by heat (%)	Grain weight per ear	Reduction in grain weight per ear by heat (%)	Kernel number per ear
1990	V2183	Control	40.6 $\pm$ 0.4	-	2.24 $\pm$ 0.042	-	55 $\pm$ 1.37
1990	V2183	Heat	25.8 $\pm$ 1.3	36.4 $\pm$ 1.88	1.51 $\pm$ 0.065	32.6 $\pm$ 1.44	59 $\pm$ 5.62
1990	V5	Control	41.3 $\pm$ 1.5	-	3.04 $\pm$ 0.102	-	74 $\pm$ 2.16
1990	V5	Heat	38.0 $\pm$ 1.8	8.0 $\pm$ 0.94	2.29 $\pm$ 0.048	24.7 $\pm$ 0.69	60 $\pm$ 3.28
Treatment effect, F statistic			75.31*		107.93*		3.88
Cultivar effect, F statistic			10.72*	108.61*	127.77*	154.72*	2.86
Interaction, F statistic			4.95*		0.56		14.98*
1991	V2183	Control	25.1 $\pm$ 0.65	-	0.97 $\pm$ 0.048	-	39 $\pm$ 1.22
1991	V2183	Heat	13.1 $\pm$ 0.56	47.8 $\pm$ 1.88	0.52 $\pm$ 0.064	46.4 $\pm$ 0.085	40 $\pm$ 1.01
1991	V5	Control	27.6 $\pm$ 0.66	-	1.38 $\pm$ 0.045	-	50 $\pm$ 1.11
1991	V5	Heat	20.5 $\pm$ 0.41	25.7 $\pm$ 0.94	1.01 $\pm$ 0.040	26.8 $\pm$ 0.071	49 $\pm$ 2.00
Treatment effect, F statistic			210.07*		80.98*		0.071
Cultivar effect, F statistic			50.76	108.66*	59.14*	15.92*	49.40*
Interaction, F statistic			12.62*		0.048		0.20

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

PART-II

Year	Cultivar	Treatment	Maximum Stem weight (g)	Stem weight loss (g)	TNC Loss (g)	Stem weight loss per grain weight per ear (%)	TNC as %grain weight/ear
1990	V2183	Control	2.40±0.093	0.29±0.023	0.21±0.009	12.9±0.65	9.4±0.60
1990	V2183	Heat	2.45±0.087	0.31±0.006	0.25±0.025	20.7±0.91	16.7±0.44
1990	V5	Control	3.04±0.063	1.55±0.024	0.77±0.030	51.0±2.53	25.3±1.40
1990	V5	Heat	3.00±0.045	1.32±0.041	0.79±0.037	57.6±2.81	34.5±2.01
Treatment effect, F statistic			14.58*	21.51*	1.34	26.89*	14.00*
Cultivar effect, F statistic			80.63*	183.87*	416.44*	244.98*	154.29*
Interaction, F statistic			3.40	25.12*	0.48	9.60*	4.10
1991	V2183	Control	2.22±0.064	0.39±0.047	-	40.2±2.09	-
1991	V2183	Heat	2.06±0.103	0.74±0.039	-	142.3±6.12	-
1991	V5	Control	2.66±0.042	0.86±0.042	-	62.3±2.43	-
1991	V5	Heat	2.34±0.063	1.36±0.096	-	134.6±4.50	-
Treatment effect, F statistic			28.04*	60.36*	-	263.42*	-
Cultivar effect, F statistic			7.68*	114.94*	-	6.75*	-
Interaction, F statistic			2.27	2.00	-	1.87	-

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

E4. Thermotolerance in Wheat Seed Endosperm Utilization and its Relations to Thermotolerance in the plant

A. Blum and B. Sinmena

ABSTRACT

Tolerance of wheat (*Triticum aestivum*) to chronic heat stress and the subsequent effect on growth and yield are largely mediated by the plant carbon balance at high temperature. Seed endosperm utilization for seedling growth in the dark was considered as a simple model for testing tolerance to chronic heat stress in terms of the carbon balance. Seed endosperm utilization efficiency (EUE) was defined as the ratio of gain in seedling (shoot and radicle) weight to loss in seed weight during germination. This study was designed to evaluate heat tolerance in terms of EUE and its relations to heat tolerance in the autotrophic plant.

EUE was measured by periodical sampling of germinating seed in the dark under normal (25°C) and high (35°C) temperatures in 18 wheat cultivars. Heat tolerance of the autotrophic juvenile plant was measured in the same cultivars in terms of plant growth rate (GR) between 17 to 24 days after emergence under normal (15°C/25°C) and high (25°C/35°C) (night/day) temperatures.

Maximum seedling dry matter weight in the dark was greater at low than at high temperature. Total dry matter loss from the seed did not differ much between temperatures, indicating that the lower seedling dry matter weight under high temperature was probably due to a relatively greater loss of carbon to respiration. Averaged over 18 cultivars, EUE was reduced from 0.64 g g⁻¹ at 25°C to 0.53 g g⁻¹ at 35°C. The various cultivars differed significantly in EUE at low and at high temperatures and there was a very significant ($p \leq 0.01$) temperature by cultivar interaction for EUE. EUE at high temperature ranged from 0.30 g g⁻¹ to 0.85 g g⁻¹ among cultivars and it was independent of cultivar kernel weight.

Compared with 15°C/25°C, the percent of GR reduction at 25°C/35°C ranged from null to 53%, depending on cultivar. A negative linear and significant correlation ($r=0.81^{**}$) was found between EUE at 35°C and the rate of GR reduction from low to high temperature, indicating an association across cultivars between heat tolerance in carbon utilization during seed germination and heat tolerance in growth of the autotrophic plant. The common mechanism underlying genotypic variation in heat tolerance at both stages could be in catabolic processes affecting carbon use efficiency.

INTRODUCTION

Wheat is seriously affected by chronic heat stress, which reduces yield in many wheat growing regions in the world. The tolerance of some wheat cultivars to chronic heat stress is well expressed in their yield in warm climates (*e.g.* Midmore *et al.*, 1984; Rawson, 1986; Shpiler and Blum, 1991). Genotypic variation in wheat heat tolerance in terms of yield component development and final biomass and yield was traced to the juvenile plant, 2 to 3 weeks after germination (Rawson, 1986; Shpiler and Blum, 1986). Indeed, there are some strong hints in the literature that heat tolerance in the adult plant may be associated with heat tolerance at the seedling stage. Alkhatib and Paulsen (1990) found a high correlation across 10 wheat genotypes in photosynthetic reductions due to heat stress between the seedling stage (4 weeks) and the flowering stage of growth. Saadalla *et al.* (1990) suggested that the heat tolerance in wheat coleoptiles was predictive of genotypic yield performance under heat and drought stress conditions in the field. Such an association is most intriguing, as it would seem to indicate a genetic entity which ascribes stress tolerance irrespective of plant ontogeny.

A major perturbation to plant productivity under chronic heat stress is ascribed to "carbon starvation" (Levitt, 1982). Chronic heat stress impairs the plant carbon balance by reducing photosynthetic carbon assimilation and accelerating carbon loss by respiration. The result is in reduced growth, reduced sinks and smaller organ and plant size.

The developing wheat seedling during the course of seed germination is totally dependent on the utilization of a given amount of carbon stored in the seed endosperm. Endosperm utilization for seedling growth in the dark proceeds at a given efficiency, which may be calculated as the ratio of the gain in seedling weight to the loss in seed (endosperm) weight in the dark. Seed endosperm utilization for seedling growth in the dark may therefore be considered as a simple model for testing tolerance to chronic heat stress in terms of the carbon balance.

This study was performed to evaluate the hypothesis that the efficiency of wheat endosperm utilization for seedling growth is affected by heat stress; that it depends on the inherent tolerance of the specific genotype; and that heat tolerance in endosperm utilization may be associated with heat tolerance in the autotrophic plant after germination.

MATERIALS AND METHODS

Seed used for this study were produced at Bet Dagan in 1990. They were sorted for homogeneous size within a cultivar and stored at 4°C until used. Seed were surface sterilized by dipping in 1% solution of sodium chlorate for 1 min, followed by 5 min of rinsing in running tap water. Seed dry matter weight was determined for each cultivar used in the experiment by drying a sample of 200 seed at 80°C for 48 h.

Abernethy et al. (1989) found that heat tolerance in germinating wheat seed was dependent on ontogeny and that the early stage of imbibition (up to 16 h) was highly heat tolerant. The present study was designed to evaluate endosperm utilization by growing seedlings independently of any effects of imbibition on germination under heat stress. A preliminary study was therefore performed to ascertain the effect of heat stress during imbibition and to determine the duration of imbibition in wheat, in order to affect the experimental treatments after full imbibition. Seed of cv. Veery were germinated in petri dishes on filter paper at 20°C, 32°C, 35°C and 38°C in the dark. Seed were sampled periodically up to 48h after the onset of germination and their water content was determined by drying at 80°C for 48 h. Six lots of 20 seeds each (replicates) were sampled for each temperature by sampling time combination. In a second phase of the preliminary study, seed of five cultivars were germinated in petri dishes under three sets of conditions: (1) germination under normal temperature (20°C); (2) germination under high temperature (35°C); and (3) imbibition under high temperature (48 h at 35°C) after which germination continued at normal (20°C) temperature. Final rates of germination were determined in four replicates per treatment, each replicate consisting of 50 seeds.

Three main experiments were performed. In the first two experiments seed were placed for germination on cheesecloth stretched over a cup of de-ionized water, such that the cloth was wet at all times. Upon germination the radicle grew into the water and the germinating seedling could be freely lifted from the medium, intact.

Experiment 1. Seed of cv. V652 were germinated at 25°C and 35°C after having been imbibed for 48 h at 20°C. The individual experimental unit was 10 germinating seeds that were sampled periodically from 3 to 10 days after the onset of germination. Preliminary tests indicated that for various seed lots and temperatures, maximum seedling weight was never attained later than 10 d of dark germination. The shoot and radicle were excised from the seed. The combined seeds and the combined shoots and radicles (defined hereon as seedling) per experimental unit were weighed after drying for 48h at 80°C. Six such units (each from a different cup) per date x treatment combination were weighed. Endosperm utilization efficiency (EUE) was calculated as the ratio of seedling (shoot and radicle) dry matter weight to dry matter weight loss from the seed. Seed dry matter weight loss was calculated as the difference between the germinated seed weight and its initial (non-germinated) weight.

Experiment 2. Different wheat cultivars were tested under the above protocol. Cultivars were of Israeli, South African (Dias, Palmiet, Gamtoos and BSP) or CIMMYT (Veery) origins (Fig.3). Seed were sampled and processed as detailed for Experiment 1, at 3 d and 10 d after germination.

Experiment 3. Plants of the same cultivars as in Experiment 2 were grown in the growth chamber under 12 h day, 15°/25°C night /day temperatures and 520 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of

photosynthetically active radiation. Seed were germinated and grown in 10 cm deep trays filled with vermiculite. Trays were irrigated daily with half-strength Hoagland's nutrient solution. At 14 days after emergence, half of the plants were transferred to a second growth chamber under the same conditions, only at 25⁰/35⁰C night /day temperatures. Shoots were harvested at 17 and 24 d after emergence. For each temperature x cultivar x date combination 6 plants (replicates) were harvested and weighed after drying for 48 h at 80⁰C. Shoot growth rate was calculated from the dry matter weight gained over the 7 d interval. Heat tolerance in terms of growth was estimated by the rate of reduction in growth rate from the low to the high temperature conditions.

All statistical analyses were performed by JMP (V.2) software (SAS Institute, CARY NC, USA).

RESULTS AND DISCUSSION

Preliminary study

Fig.1. The effect of imbibition temperature on water uptake with time by seed of wheat cv. Veery.

The rate of seed imbibition tended to accelerate at temperatures above 20°C (Fig.1). However, irrespective of temperature, water uptake reached the same average maximum rate of 81% of seed dry matter weight. This maximum was reached at 48 h under 20°C and several hours earlier at the higher temperatures tested.

Table 1. Germination of wheat seeds when subjected to 35°C throughout germination or when imbibed for 48 h at 20°C after which they were subjected to 35°C. Data presented is germination rate as percent of germination at 20°C.

Cultivar	Germination at	Germination at 35°C
	continuous 35°C	after imbibition at 20°C
	----- % -----	
V2183-43	17.4	74.4*
C287	24.2	71.2*
Barkaee	48.6	65.2*
C97	53.1	100.3*
V2015-1-4	71.8	88.7*
Mean	43.0	80.0

(*) Significant difference ($p \leq 0.05$) between treatments.

For all five cultivars tested, germination at high temperature (35°C) was significantly lower when imbibition was exposed to high temperature than when imbibition was not exposed to high temperature. Therefore, the stage of imbibition (up to 48 h) was more heat susceptible than subsequent stages of germination. Some variation in susceptibility among cultivars could be seen, as reflected in their germination rates at continuous 35°C. Abernethy *et al.* (1989) found that the early stage of imbibition (up to 16 h) was more heat tolerant than subsequent stages of germination. In the present study imbibition took 48 h and it was more heat susceptible than subsequent stages of germination. Consequently, the following experiments were designed so as to bypass the problem of heat susceptibility or tolerance at imbibition, by performing imbibition at non-stress temperature (20°C) before subjecting the germinating seed to the experimental temperature treatments.

Endosperm utilization (Experiments 1 and 2)

Seedling dry matter increased at a higher rate in 25°C than in 35°C (Fig.2), while the duration of increase was about the same at both temperatures. Consequently final seedling weight was higher at the low than at the high temperature. Seed dry matter loss with time was essentially

the same at both temperatures. Only on the last two days, the seed perhaps lost slightly more dry matter at low than at high temperature. Final endosperm utilization efficiency (EUE) at 10 d was significantly ($p \leq 0.05$) higher at 25°C (0.57 g g^{-1}) than at 35°C (0.48 g g^{-1}). Since the difference between temperature treatments in seed weight loss was much smaller than the difference in seedling dry matter gain it may be assumed that the lower efficiency of endosperm utilization at high temperature resulted mainly from perturbation in reserve assimilation into seedling dry matter rather than from reserve availability from endosperm tissues. The most probable cause for the reduced efficiency at high temperature could have been the relatively greater loss of seed reserve to respiration.

Fig.2. (Experiment 1). The effect of temperature on dark-germinated wheat (cv. V652) seed and seedling (radicle and shoot) dry matter weights in time. Vertical lines are Standard errors (SE) of selected data points. For seed weights, SE were smaller than the data point graphics.

Cultivars differed significantly in initial kernel dry weight (before germination) and in EUE at both high and low temperatures (Table 2; Fig.3). Averaged over all cultivars, EUE was reduced by high temperature from 0.64 to 0.53 g g^{-1} . The variation among cultivars in EUE was roughly the same at both temperatures, about two-fold. There was a very significant temperature by

cultivar interaction for EUE (Table 3), indicating significant variations in cultivar thermotolerance for EUE. The frequency distribution of cultivar EUE at high temperature (Fig.3, inset) was not significantly normal, according to Shapiro-Wilk W test. It displayed one small (high EUE) and one large (medium and low EUE) groups. This would indicate that EUE is not under very complex inheritance.

While the variation among cultivars in kernel weight was very significant, there was no significant correlation across cultivars between kernel weight and EUE at 15⁰/25⁰C ($r=0.12$) or 25⁰/35⁰C ($r=-19$). Therefore, the variation in EUE among cultivars at either temperature could not have been accounted for simply by variations in kernel weight.

Table 2. Initial kernel dry weight and endosperm utilization efficiency (EUE) at two temperatures (experiment #2), plant growth rate (GR) and percent plant growth rate reduction at 35⁰C as compared with 25⁰C (experiment #3). Data presented are cultivar means, minimum, maximum and genotypic coefficient of variation (CV) over 18 cultivars.

Variable	Mean	Minimum	Maximum	CV (%)
Initial dry kernel weight (mg)	37.2**	27.7	45.3	14.2
EUE at 15 ⁰ /25 ⁰ C (g g ⁻¹)	0.64**	0.48	1.00	22.1
EUE at 25 ⁰ /35 ⁰ C (g g ⁻¹)	0.53**	0.30	0.85	28.1
GR at 15 ⁰ /25 ⁰ C (mg plant ⁻¹ d ⁻¹)	4.88**	2.57	7.14	22.1
GR at 25 ⁰ /35 ⁰ C (mg plant ⁻¹ d ⁻¹)	3.83**	1.67	5.67	27.8
Plant growth reduction at 35 ⁰ C (%)	21.4**	0	53.4	76.8

(**) Differences among cultivars are very significant ($p \leq 0.01$).

Table 3. Summary of the two-way analysis of variance of endosperm utilization efficiency (EUE) under a fully randomized factorial experimental design.

Source	Sum of squares	Mean squares	F-Value
Temperatures	0.458	0.458	40.55**
Cultivars	1.317	0.077	6.86**
Interaction	1.519	0.089	7.92**
Residual	1.218	0.011	

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

Seedling Growth (Experiment 3)

Cultivars differed very significantly in plant growth rate (GR) between 17 and 24 d after emergence, under the two temperature regimes (Table 2). Cultivars differed very significantly in the rate of reduction in plant GR under the effect of high temperature, from 0% in K3 to 53% in Barkaee. A strong linear negative correlation ($r=0.81$) was found between EUE at 35°C and the rate of reduction in plant GR at 35°C, across cultivars (Fig.3). Cultivars with low EUE tended to show greater reduction in plant RGR at high temperature. This association held also between EUE at 35°C and plant GR at 35°C ($r=0.57$). On the other hand there was no association between EUE at 25°C and the reduction in plant GR at 35°C ($r=0.08$). Similarly, there was no association between EUE at 25°C and plant GR at 25°C ($r=0.22$). Therefore, the potential (non-stress) EUE bears no relationship to potential plant growth rate. The relationship stands only under heat stress.

Reduction in GR at 25 °/35 °C (%)

Fig.3. The association between endosperm utilization efficiency (EUE) at 35°C and the rate of reduction in plant growth rate (GR) at 25°/35°C as compared with 15°/25°C, over 18 wheat cultivars. Inset: Cultivar frequency for EUE at 35°C. (Experiments #2 and #3)

It is concluded that heat stress reduces EUE, probably because of increased loss of endosperm carbon to respiration. EUE under high temperature may vary with genotypes, and heat tolerance in terms of EUE is probably a result of a moderated loss of endosperm carbon to respiration. Heat tolerance in juvenile plant growth (between 17 and 24 d after emergence) is a reputed measure of heat tolerance in growing wheat plants (*e.g.* Alkhatib and Paulsen, 1990). It was found here to be well associated with heat tolerance in EUE. This association is encouraging. Although heat tolerance can be affected by developmental processes linked with ontogeny (*e.g.* Shpiler and Blum, 1991; Abernethy et al., 1989), certain genes may evidently exercise control over heat tolerance irrespective of ontogeny.

Both EUE and plant growth involve the carbon balance of the system. However, the carbon balance in growing plants under heat stress is affected by photosynthesis and its tolerance to heat stress (*e.g.* Blum, 1986; Rawson, 1986; Alkhatib and Paulsen, 1990), while there is offcourse no carbon assimilation into the endosperm during germination. Therefore, the association seen here between plant growth and EUE under heat stress should draw more attention to the temperature response of catabolic processes affecting carbon use efficiency as an explanation of wheat tolerance to chronic heat stress.

REFERENCES

- Abernethy, R.H., Thiel, D.S., Petersen, N.S., and Helm, K. 1989. Thermotolerance is developmentally dependent in germinating wheat seed. *Plant Physiol.* 89:569-576.
- Alkhatib, K. and Paulsen, G.M. 1990. Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. *Crop Sci.* 30: 1127-1132.
- Blum, A. 1986. The effect of heat stress on wheat leaf and ear photosynthesis. *J. Exp. Bot.* 37:111-118.
- Levitt, J. 1982. Response of plants to environmental stresses. Vol.II. Academic Press NY pp.497
- Midmore, D.J., Cartwright, P.M., and Fischer, R.A. 1984. Wheat in tropical environments. II. Crop Growth and grain yield. *Field Crops Res.* 8:207-227
- Rawson, H.M. 1986. High-temperature tolerant wheat: a description of variation and a search for some limitations to productivity. *Field Crops Res.* 14: 197-212.

Saadalla, M.M., Quick, J.S., and Shanahan, J.F. 1990. Heat tolerance in winter wheat .2. Membrane thermostability and field performance. *Crop Sci.*30:1248-1251.

Shpiler, L. and Blum, A. 1986. Differential reaction of wheat cultivars to hot environments. *Euphytica* 35:483-492.

----- 1991. Heat tolerance in yield and its components in different wheat cultivars. *Euphytica* 51: 257-263.

E5. Heat Shock Protein Expression in Wheat under Diurnal Heat Stress and Field Conditions

Henry T. Nguyen, A. Blum, Jian Weng, and Kerry L. Hendershot

ABSTRACT

The occurrence of heat shock proteins (HSPs) in response to high temperature stress is virtually a universal phenomenon in higher plants and animals and has been well documented. However, with respect to agriculturally important species, less is known about their expression under natural environments. The purpose of this study was to determine if high temperature stress caused the induction of HSPs in flag leaves of a broad range of winter wheats (*Triticum aestivum* L.) collected from diverse geographic backgrounds during field production. Secondly, we want to investigate genotypic differences in HSP expression under a diurnal, chronic heat stress conditions. In the field experiment, flag leaf blade temperatures were obtained and leaf blades collected for northern blot analysis utilizing a ^{32}P -labeled HSP 16.9 as a probe. Temperatures of leaf blades ranged from 32 to 35.5°C under the tested field conditions at New Deal near Lubbock, Texas. Northern blot analysis revealed that HSP mRNAs encoding for a major class of low molecular weight HSPs, HSP 16.9, were detected in all twenty wheat genotypes examined. The results suggested that HSPs are synthesized in response to heat stress under agricultural production, and furthermore, that HSPs are produced in wheats differing in geographic background. In the controlled growth chamber experiment, HSP expression in two wheat lines cv 'Mustang' (heat-tolerant) and cv 'Sturdy' (heat-susceptible) were analyzed to determine if wheat genotypes differing in heat tolerance differ in *in vitro* HSP synthesis (translatable HSP mRNAs) under a chronic, diurnal heat stress regime. Leaf tissues were collected from seedlings over a time-course and poly (A)⁺RNAs were isolated for *in vitro* translation and 2-D gel analysis. The protein profiles shown by 2-D gel analysis revealed that there were not only quantitative differences of individual HSPs between these two wheat lines, but also some unique HSPs which were only found in the heat tolerance line. These data provide an evidence for correlation between HSP synthesis and heat tolerance in wheat under a simulated field environment and suggest that further genetic analysis of HSPs in a segregating population is worthy. In conclusion, the results of this study provides an impetus for the investigation of the roles of heat shock protein genes in heat tolerance in wheat.

INTRODUCTION

The production of heat shock proteins (HSPs) by plants exposed to high temperature stress has been extensively reported for numerous species (see Vierling, 1991 for review). Induction of this response has been accomplished by rapid exposure to a high temperature as well as by exposing plants to a gradual temperature increase over time. The latter approach has been utilized to approximate the manner by which a plant in the natural field environment would be exposed to high temperature stress; however, since a majority of our agricultural crops are field-grown, it becomes prudent to ascertain if HSPs are induced under similar conditions in the field.

Heat shock protein accumulation has previously been reported in field-grown cotton (*Gossypium hirsutum* L.) (Burke et al., 1985) and the induction of HSP mRNA was reported in field-grown soybean [*Glycine max* (L.) Merr] (Kimpel and Key, 1985). In these studies, the presence of HSPs and HSP mRNAs, respectively, indicates that HSPs are the consequence of plant exposure to high temperature stress. Furthermore, profiles of HSP mRNA accumulation in soybean were similar between field-grown and laboratory-grown plants which had been exposed to high temperature (Kimpel and Key, 1985).

Previous experimentation with a winter wheat (*Triticum aestivum* L.) cv 'Mustang' indicated that HSP was induced after 1 hour between the temperatures of 28 and 31°C in seven-day-old seedlings (Hendershot et al., 1992). Similarly, HSP mRNAs were detected in flag leaves of flowering plants after 1 hour at 32°C. The first objective of this study was to document that HSP expression occurs during growth of wheat in the field environment and to demonstrate that HSP induction occurs in a broad range of cultivars collected from major wheat growing regions of the world.

Genetic variability in the synthesis of HSPs in plants has been reported in wheat (Zivy 1987; Nguyen et al. 1989; Vierling and Nguyen 1990), sorghum (Ougham and Stoddart 1986), and maize (Frova et al. 1988). These results provide an avenue for the genetic analysis of HSP genes in plants. Our previous work on *in vivo* labeling wheat HSPs demonstrated a positive correlation between genetic differences in cellular thermotolerance and the levels of LMW HSPs (Krishnan et al. 1989). Our recent results provide a clear molecular evidence for genetic variation in HSP synthesis in hexaploid wheat (*Triticum aestivum* L.) (Weng and Nguyen, 1992). Therefore, the second objective of this study was to investigate genotypic variation in HSP

expression during a cyclic diurnal heat stress treatment in two wheat lines previously characterized as heat tolerant and heat susceptible.

MATERIALS AND METHODS

Field Experiment

Twenty wheat cultivars (*Triticum aestivum* L.) were planted in a field in a Pullman clay loam (a fine, mixed, thermic Torric Paleustoll) at the Texas Tech University Teaching and Research Laboratory east of New Deal near Lubbock, Texas in October 1990. Plots were fertilized according to the soil test recommendations for irrigated wheat production in the Texas Southern Plains area. Each plot consisted for four 8-m rows planted at a population of about 60 lbs/A. Plots were irrigated as necessary to prevent water stress. Plants were sprayed with appropriate pesticides to prevent disease and insect problems.

After wheat plants had reached anthesis in May 1991, temperatures of flag leaves were measured in mid-afternoon, using a Licor 1600 porometer (Licor, Lincoln, NE). Each mean leaf temperature measurement represented the average of three individual flag leaves for each cultivar. Simultaneously, flag leaves were collected at the temperature measurement time and frozen in liquid nitrogen until returned to the laboratory. Poly (A)⁺ RNA isolation was conducted as previously described (Weng and Nguyen, 1992) for northern blot analysis using wheat HSP 16.9 cDNA as a probe.

Growth Chamber Experiment

Plant Materials and Diurnal Heat Stress Conditions

Wheat cultivars 'Mustang' (heat tolerant) and 'Sturdy' (heat susceptible) were used as experimental materials because of the previously identified genetic differences in cellular thermal tolerance and HSP synthesis (Krishnan et al., 1989; Weng and Nguyen, 1992).

Wheat seeds were planted in pots containing vermiculite and grown in a controlled environment growth chamber (Conviron Model E-15) which provided about 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD during a 22/18 °C day/night regime with a photoperiod of 16hrs as a normal growth condition. Relative humidity was about 30% of saturation. Seven-day old green seedlings were

used for a cyclic diurnal heat stress treatment and in characterizing HSP profile after seedlings were exposed to a temperature treatment for different time periods. For the chronic, diurnal heat stress treatment, whole pots of seedlings were transferred into a similar controlled growth chamber which was programmed to raise temperature from 20°C to 38 °C at a rate of 3 °C/hr, maintain temperature at 38 °C for 4 hrs, and then return 20 °C at a rate of 3 °C/hr during the 16-hour photoperiod. High humidity (80-100%) was maintained during the photoperiod in order to limit transpirational cooling of the leaves. Samples for RNA analysis and chlorophyll content assay were obtained at the middle time point of the 38 °C period each day for five days. Leaf temperatures reached 37 °C under this stress condition as monitored by an infrared thermometer. For poly (A)⁺ RNA isolation, leaf tissue was excised, immediately frozen in liquid N₂, and held at -80 °C. Leaf tissue of seedlings from the normal growth condition were also collected as control samples.

Poly (A)⁺ RNA Isolation

Poly(A)⁺ RNA isolation was performed as described previously (Weng and Nguyen, 1992). Briefly, total RNA was isolated from each sample of 8 to 10g fresh weight leaf tissue and poly (A)⁺ RNA was further purified using oligo (dT)-cellulose (New England BioLabs, Beverly, MA) chromatography.

In Vitro Translation and 2-D Gel Electrophoresis

In vitro translation of poly (A)⁺ RNA was done using a rabbit reticulocyte lysate translation system (Promega, Madison, WI) in accordance with the manufacturer's instruction. Translated peptides were labelled by supplementing each reaction with 50 µCi of ³⁵S-methionine (ICN, Costa Mesa, CA). The protein products were separated using 2-D gel electrophoresis (Weng and Nguyen, 1992). The IEF dimension effectively separated peptides of pI range about 3.5 to 8.0.

Chlorophyll Content Assay

Chlorophyll was extracted from the leaf tissue of wheat seedlings with 80% acetone and determined spectrophotometrically according to Arnon (1949).

RESULTS AND DISCUSSION

Field Experiment

As seen in Table 1, mean leaf temperatures of wheat cultivars exceeded the established HSP induction temperature (28 to 31 °C) of the cultivar 'Mustang' (Hendershot et al., 1992). Flag leaf temperatures ranged from 32.8 to 35.4 °C during the 45 minute measurement time that started at 1500 h. In general, leaf temperature increased ca. 2 °C from start to finish during the tissue collection.

Since the field environment can produce a variety of stresses, all of which may elicit metabolic responses in the plants, a northern blot analysis with a specific HSP cDNA probe was conducted to determine if HSP mRNAs were induced in the field environment. When mRNAs of wheat flag leaf blades were analyzed for induction of a major low molecular weight HSP 16.9, hybridization with the cDNA probe was observed for all wheat genotypes examined. The results from five wheat cultivars were presented to exemplify the expression of this HSP family (Figure 1).

Our intent in this field analysis was solely to define if HSPs were expressed in hexaploid wheats under natural field conditions. Therefore, no attempt was made to distinguish magnitude of response between cultivars. Furthermore, no other HSPs were analyzed since HSP 16.9 has been previously shown to be very representative of HSP induction in wheat in our lab. However, some quantitative variation in the level of HSP mRNAs was noted, suggesting that the amount of HSP accumulation may vary among wheat cultivars. Future work using carefully controlled temperature environment and specific HSP antibodies will be required to investigate this possibility.

In summary, HSP expression, specifically HSP 16.9 mRNA, was detected in a wide variety of wheat phenotypes with diverse geographical background. As previously surmised, HSP production would seem to be a universal response; however, genetic investigation of this response in genotypes of diverse geographical background could be extremely productive in determining the role of heat shock proteins in thermotolerance of wheat plants during high temperature stress.

Growth Chamber Experiment

Molecular Response of Wheat Seedlings during Diurnal Heat Stress

The molecular response of wheat seedlings to diurnal heat stress was determined by exposing seven-day-old seedling to a cyclic, diurnal temperature stress environment. Two sets of poly (A)⁺RNA were isolated and *in vitro* translations were performed as described in Materials and Methods. Analysis of the *in vitro* translation products separated on 2-D gels revealed that there are qualitative and quantitative HSP differences between 'Sturdy' and 'Mustang' at all time points of the diurnal heat stress (Fig. 2). The data indicate that there is a group of mRNAs ranging for 10 to 14 kDa LMW HSPs (cycle marker) in addition to a group of mRNA ranging for 16 to 18 kDa LMW HSPs accumulated during the stress period, i.e. the middle time point of highest temperature (38°C, 2 hrs) during the cyclic temperature change every day. Some transcripts ranging for 10 to 14 kDa LMW HSPs are also detectable in their controls (Fig. 2, Sc and Mc). In comparison with the cultivar 'Sturdy', 'Mustang' has two unique HSP spots (indicated by arrows) all the time of experiment and another unique spot (star marker) detected only on the last day (Fig. 2, M5) in 10-14 kDa LMW HSP group. There are little qualitative HSP differences between the 'Mustang' and the 'Sturdy' in the range of 16 to 18 kDa, except one spot marked with triangle. In comparison with previous findings in our laboratory, the results indicate that differences in HSP expression may vary between acute and gradual temperature stress conditions.

In the middle range (26 to 28 kDa) of HSPs, only one remarkable spot (indicated by squares) shows the difference between two wheat lines. For the 'Mustang', it appears a strongest spot at the first day of diurnal heat stress, and continuously exists for following several days. For the 'Sturdy', however, it only appears at the second day of the diurnal heat stress treatment.

The significant differences are detected in the high molecular weight range (70 kDa) of HSPs between the 'Mustang' and the 'Sturdy' through the whole diurnal heat stress treatment (indicated by oval cycle). Mustang appears to accumulate a higher level of HSP 70 transcripts.

The Changes of Chlorophyll Contents between 'Mustang' and 'Sturdy' during Diurnal Heat Stress

High temperature stress induces numerous changes in the physiological and cellular processes including photosynthesis. Since chlorophyll content is tightly linked to the efficiency of photosynthesis (Kura-Hotta, et al., 1987), the chlorophyll contents of both wheat lines were examined before and under the cyclic diurnal heat stress treatment (Table 2). After five days of the diurnal heat stress treatment, the chlorophyll content of 'Sturdy' is significantly declined

about 17% when compared with its nonheat-stressed chlorophyll content level; while for 'Mustang', the chlorophyll content almost maintains at the original level. The data indicate that the chlorophyll content is more stable in the heat-tolerant wheat line 'Mustang' than in the heat-susceptible line 'Sturdy' under heat stress. The chlorophyll content data combined with the data from molecular response of HSP gene expression under the cyclic diurnal heat stress described above support the hypothesis that increased HSP levels are accompanied by greater acquired cellular thermal tolerance in wheat (Weng and Nguyen, 1992).

The data presented here, combined with previous data from our laboratory (Krishnan et al. 1989), has demonstrated a positive correlation between increased HSP gene expression and genetic differences in cellular thermal tolerance in winter wheat. Currently, gene-specific DNA probes are being isolated (Joshi et al. 1991; Weng et al. 1991a, 1991b) and recombinant inbred lines of wheat are being developed in our laboratory for further genetic analysis of the role of HSP genes in heritable thermal tolerance in wheat.

REFERENCES

- Burke, J.J., J.L. Hatfield, R.R. Klein, and J.E. Mullet. 1985. Accumulation of heat shock proteins in field grown cotton. *Plant Physiol.* 78:394-398.
- Frova C, Taramino G, Binelli G, Ottaviano E (1988) Heat-shock protein variability in maize. *Maydica* 33: 65-76
- Hendershot, K.L., D.R. Porter, J. Weng, and H.T. Nguyen. 1992. Induction temperature of heat shock protein synthesis in wheat. *Crop Sci.* 32:256-261.
- Kimpel, J.A., and J.L. Key. 1985. Presence of heat shock mRNAs in field grown soybeans. *Plant Physiol.* 79:672-678.
- Kimpel JA, Nagao RT, Goekjian V, Key JL (1990) Regulation of the heat shock response in soybean seedlings. *Plant Physiol* 94: 988-995
- Krishnan M, Nguyen HT, Burke JJ (1989) Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol* 90: 140-145
- Kura-Hotta M, Satoh K, Katoh S (1987) Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. *Plant Cell Physiol* 28: 1321-1329
- Lindquist S, Craig EA (1988) The heat-shock proteins. *Ann Rev Genet* 22: 631-277
- Nagao RT, Kimpel J, Key JL (1990) Molecular and cellular biology of the heat-shock response. In Scandalios JG, ed, *Genomic Responses to Environmental Stress*. Academic Press, San Diego, California, pp 235-274
- Nover L (1991) *Heat Shock Response*. CRC Press, Inc. Boca Raton, Florida.

- Ougham HJ, Stoddart JL (1986) Synthesis of heat-shock protein and acquisition of thermotolerance in high-temperature tolerant and high-temperature susceptible lines of Sorghum. *Plant Science* 44: 163-167
- Rochester DE, Winter JA, Shah DM (1986) The structure and expression of maize genes encoding the major heat shock protein, hsp 70. *EMBO J* 5: 451-458
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning. A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Schoffl F, Baumann G, Raschke E, Bevan M (1986) The expression of heat-shock genes in higher plants. *Phil Trans R Soc Lond B* 314: 453-468
- Vierling E (1991) The roles of heat shock proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* 42: 579-620
- Vierling RA, Nguyen HT (1990) Heat-shock protein synthesis and accumulation in diploid wheat. *Crop Sci* 30: 1337-1342
- Weng, J., H.T. Nguyen. 1992. Differences in the heat shock response between thermotolerant and thermosusceptible cultivars of hexaploid wheat. *Theor. Appl. Genet.* 84: 941-946.
- Weng J, Wang ZF, Nguyen HT (1991a) Nucleotide sequence of a *Triticum aestivum* cDNA clone which is homologous to the 26 kDa chloroplast-localized heat shock protein gene of maize. *Plant Mol Biol* 17: 255-258
- Weng J, Wang ZF, Nguyen HT (1991b) A *Triticum aestivum* cDNA clone encoding a low-molecular-weight heat shock protein. *Plant Mol Biol* 17: 273-275
- Zivy M (1987) Genetic variability for heat shock proteins in common wheat. *Theor Appl Genet* 74: 209-213

Table 1. Mean flag leaf temperatures of 20 hexaploid wheat lines grown in the field at the Texas Tech University Agricultural Research Farm, New Deal, Texas.

Wheat cultivar	Mean leaf temperature (°C)
Mustang	33.4
Arkan	33.7
Siouxland	32.8
Newton	33.0
Bezostaia I	33.7
Sturdy	33.7
Payne	34.5
Pioneer 2157	34.6
Tam 108	35.0
Hawk	34.9
Chisolm	34.3
Vona	34.6
Tam 101	34.2
Tam 105	34.8
Wichita	35.0
Burgas 2	34.9
Sadovo I	35.0
FV 24-10	35.3
Odesskaya S1	35.3
Partizanka	35.4

Table 2. Chlorophyll contents of wheat seedling leaves before and after a cyclic, diurnal heat stress treatment in a controlled growth chamber.

Wheat Lines	Chlorophyll Contents (mg chl/ g dry weight)	
	Control	Day 5th
Mustang	19.9 (± 0.7)	19.8 (± 0.3)
Sturdy	22.0 (± 0.2)	18.3 (± 0.5)*

* Significant at 0.01 probability level by Fisher's LSD and Scheffe F-tests. The mean of chlorophyll content data was obtained from three replications. Standard errors of the means are in the parentheses.

Figure legends

Figure 1. Field blot

Figure 2. Two-dimensional analysis of *in vitro* translation products of mRNA extracted from seven-day old green leaf tissues of 'Mustang' (Mc) and 'Sturdy' (Sc) incubated at 22 °C under the normal growth condition. Comparison of the HS response during diurnal heat stress cycles between the heat-susceptible line 'Sturdy' (S) and heat-tolerant line 'Mustang' (M) by two-dimensional gel analysis. Figures M1/S1, M2/S2, M3/S3, M4/S4, and M5/S5 correspond to day one, day two, day three, day four, and day five in diurnal heat stress, respectively. For each sample, equal cpm of RNA dependent incorporation were electrophoresed and equal exposure time of autoradiograms were performed. Translation products whose qualitative and quantitative level changed consistently across all replicate experiments are indicated by arrows, circles, squares, and oval circles as described in text.

Figure legend:

Northern blot analysis showing accumulation of HSP16.9b transcripts in different wheat genotypes. Leaf tissues were collected from field grown plants and northern blot analysis was performed according to the procedure described earlier (Hendershot et al., 1992). Panels 1 to 5 represent the following genotypes: panel 1, TAM 108; panel 2, Hawk; panel 3, Chisholm; panel 4, Vona ; panel 5, TAM 101.

FIG. 2

77

FIG. 2 (Cont.)

F. DESCRIPTION OF COOPERATION

Work on heat tolerance in terms of plant growth, plant phasic development and its carbon status was performed in Israel. Further testing of the heat tolerance of these materials in terms of cellular viability and membrane thermostability was performed in the USA. A sample of the most heat tolerant and susceptible lines, as based on results from tests in Israel and the USA, were subjected to HSP analysis in USA. The effect of heat stress on grain filling and the role of mobilized stem reserves in this respect was studied in Israel. In summary, the search for heat tolerant genetic stocks and their agronomic and physiological characterization was performed in a coordinated manner in both countries. The final HSP study of the most extreme genotypes was performed in the USA.

The two investigators met for discussing the progress of the work, during the first year in Texas and during the second year in Israel.

G. EVALUATION OF RESEARCH ACHIEVEMENTS

The following were the main objectives of the research:

1. To evaluate genetic variation for heat tolerance at GS1 and GS2 in terms of active growth, phasic development and yield components in relation to plant carbon balance.
2. To evaluate genetic variation for heat tolerance at GS3 and its effect on grain filling as a function of leaf longevity, leaf and spike assimilation and stem reserve mobilization.
3. To characterize genetic variation in HSP, cell viability and membrane leakage, and assess the predictive value of these tests as measures of heat tolerance in relation to the above objectives.

These objectives were not changed during the course of the research. These objectives were fully attained.

Objective #1 is dealt by Chapters E1 and E4.

Objective #2 is dealt by Chapters E1 and E3

Objective #3 is dealt by Chapters E1, E2 and E5.

The significance of the results lies in several domains.

Firstly, Heat tolerant wheat germplasm, in terms of plant performance and yield in the field has been identified. The field tolerance of these materials has been verified by rapid physiological tests of plant tissue heat tolerance. While chronic heat stress has always been assumed to affect the

carbon balance of the plant, we have provided evidence on the nature of this effect and its magnitude on both physiological and agronomic levels.

Secondly, The heat tolerant materials as found here are fully agronomic and are most valuable for further breeding of heat tolerance in wheat.

Thirdly, the association found between heat tolerance in the field and the accumulation of specific HSPs opens the way for better understanding of the possible role of HSP in heat tolerance.

These results also justify further genetic studies of HSP in wheat as a possible molecular genetic marker for improving heat tolerance in wheat.

H. LIST OF PUBLICATIONS.

Chapters E1 to E5 were submitted for publication as separate manuscripts and they are now in review by Crop Science, Field Crops Research and Journal of Experimental Botany.