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Effect of Heat Stress on Grain Filling in Wheat

E. Millet, P.S. Baenziger, M.D. Clegg

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Investigators' Names
(Principal listed first)

Eitan Millet

Stephen P. Baenziger
Max D. Clegg

Investigators' Institutions

Department of Plant Genetics
The Weizmann Institute of Science
Rehovot 76 100, Israel

University of Nebraska-Lincoln
Department of Agronomy, Crop
Range, Soil and Weed Sciences
Lincoln, Nebraska 68583
U.S.A.

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Signature
Principal Investigator

Signature
Institution's Authorizing Official

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ABSTRACT

Short-term hot spells of very high temperatures (around 40°C) occur during grain-filling both in the Great Plains in the U.S. and in Israel. The effect of such a short episode of elevated temperature on subsequent wheat grain growth in milder temperatures was investigated. Spikes of various wheat genotypes, including cultivars, wild relatives and aneuploids, two weeks after anthesis, were exposed to 96 h of 38°/25°C day/night temperatures using a transparent box and a heater and then transferred to milder ambient temperatures. The heat-induced injury to grain growth and weight was assessed. In addition, spikes growing in the field that were inserted for four days into inverted glass flasks, thereby exposed to higher temperatures, were used to assess the heat-induced reduction to grain weight.

The number and size distribution of endosperm starch granules was determined in several genotypes showing different heat response (in grain weight). Leaf cell membrane thermostability was determined in various genotypes.

Grain growth remained almost unaffected during the heat treatments despite a loss of water in this time. However, following the treatment, grain growth was inhibited or lasted for a shorter period resulting in much smaller grains. Screening of genotypes indicated genotypic variation in the heat-induced injury. Using wheat-*dicoccoides* substitution lines we were able to detect an allele for heat-resistance on the long arm of chromosome 1B and an allele for heat-sensitivity on the long arm of chromosome 6A of *dicoccoides*.

The number of starch granules, especially the smaller (type B) decreased in the heated grains of mainly the heat-sensitive genotypes indicating that at least partly the heat treatment affected directly starch accumulation in the grain.

A differential genotypic variation in membrane thermostability was found in some instances but not in others and was not associated with the heat-response of the grain, indicating a different heat response mechanism in the leaf and the grain.

OBJECTIVES

The purpose of this research is to develop a useful understanding of how a short severe heat stress during grain-filling affects kernel development. The specific objectives of the research are:

1. To determine the effect of such a heat stress, as well as of repeated heat stress (heat hardiness), exerted at various developmental stages of kernel development and grain yield.
2. To screen various wheat germplasm, including wild accessions, and to identify those which are more tolerant of this short, severe heat stress.
3. To study the mechanism of heat damage and tolerance at the tissue and cell levels using organ (spike and grain) culture. During the work we found organ culture unsuitable to our purposes and developed the heating box system.
4. To understand the genetic basis of the tolerance to such heat stress.

The work hypotheses were that tolerance to a short, severe heat stress differs between wheat cultivars (i.e., is under genetic control), and that the main mechanisms of tolerance are largely determined at the kernel level.

ASSESSMENT OF HEAT INDUCED INJURY TO GRAIN GROWTH

Theory

Grain growth follows a sigmoid curve (Fig. 1) with three major phases (Millet & Pinthus, 1984; *J. Cereal Sci.* 2:31): during the first two weeks (duration depends on growth conditions) grain and endosperm morphogenesis takes place and only a small portion of the dry matter of the grain is accumulated. This phase is followed by a linear accumulation of dry matter at a maximum rate until grain desiccation (phase 3).

Heat damage may reduce the rate or the duration of dry matter accumulation or both as demonstrated in Fig. 1. No matter which component is affected, the difference between the treatments in the gain in grain weight after the treatment (Gain C- Gain H) expresses the post treatment damage caused to grain growth by the treatment. This absolute value is related to the gain achieved in the untreated plants (Gain C) to give a

Fig. 1. Scheme for dry matter accumulation in grains of heated (H - dashed line) or unheated (C - solid line) spikes. Spikes were heated 12-16 d after anthesis according to the protocol below. Legend: gwt = final grain weight; gain = gain in grain weight achieved after the treatment.

standard injury value which is independent on intergenotypic variation in grain growth.

According to the described scheme the value is independent of the time of first sampling as long as it is taken during the linear phase (the last sample is taken at maturity). Yet, sampling immediately after the treatment may increase the accuracy since the value is based on a longer growth period.

Protocol for heat treatment and evaluation of heat tolerance in different genotypes

Following the first year's experiments we have discussed the results and agreed on the following protocol to distinguish heat-sensitive and heat-tolerant genotypes:

1. Spikes at about two weeks after anthesis will be enclosed in the heating box for 96 h, starting and ending at mid-day. The method of spike heating was developed by C. F. Jenner at the Waite Institute, South Australia.

2. The heating conditions are about 38°/25°C day/night temperature at a daylength of 12 h. The control box shows the ambient temperature. This was 21°/16°C in a controlled growth chamber in Israel and about 26°/20°C in a cooled greenhouse in Nebraska.
3. The first sample of 10 basal grains is taken immediately after termination of the treatment from the upper half of the spike. The trimmed spike is allowed to mature and a second sample of 10 basal grains is taken.
4. The effect of the treatment on the grain is expressed in a reduction in the final grain weight and in the gain in grain weight after the treatment. Since the gain depends not only on the treatment but also on the genotype, standardization among genotypes is achieved by dividing the difference in the gain between treatments by the grain of the untreated grain of the same genotype as follows:

$$\% \text{ injury} = \frac{\text{gain C} - \text{gain H}}{\text{gain C}} \times 100$$

where gain C is the gain after treatment of the untreated spike
and gain H is the gain after heating the spike

Since the data of heated and the non-heated grains were obtained from different plants, the estimate of the variance is obtained from a development of Taylor's formula for ratio of independent variables:

$$\text{Var. (injury)} = 10^4 \times \frac{\overline{\text{gain H}}}{\overline{\text{gain C}}} \times \left[\frac{\text{Var. (gain H)}}{(\overline{\text{gain H}})^2} + \frac{\text{Var. (gain C)}}{(\overline{\text{gain C}})^2} \right]$$

Materials and Methods

Growth conditions

Israel

Plants of any genotype were grown singly in 1 l plastic bags filled with a mixture of equal volumes of peat, volcanic gravel and vermiculite and fertilized with 2 g of a slow-release fertilizer (Osmocot, 14% N, P & K). The plants were grown outdoors in a screenhouse and were irrigated adequately. During the winter, tillers were removed to provide a unicum plant.

U.S.A.

Plants were grown singly in 2 l pots placed in a cooled greenhouse adjusted at 21°/16° C day/night temperatures and a photoperiod of 15 h light (supplemented lighting was used to extend the day). The actual temperatures that prevailed in the greenhouse are shown in Fig. 2. Tillers other than the main one were removed before the treatment.

Spike treatment procedure

Israel

Only the spike and the uppermost culm were inserted to the heating box for the treatment. Fourteen days after anthesis an equal number of plants were selected for the two treatments. In most genotypes plants flowered uniformly. In a few cases, when small deviations were observed, the average anthesis date was used. When large differences were found the plants were treated individually by their flowering date. Since the heating conditions depended slightly on the number of spikes that were treated, an attempt was made to exert the treatment on related genotypes together.

Selected plants were moved into a walk-in growth cabinet (21°/17°C at 12 h photoperiod) in which the spike heating box was placed. This box maintained a temperature of 38° (36°-40°) C in the light and 25°C (23.5°-26.5°) during the dark hours. The treatment was imposed during 96 h, (from noon) starting and ending with 6 h light, by inserting half of the spikes into the transparent heating box. The other spikes were inserted into a similar but unheated box where the ambient chamber

Fig. 2. Diurnal temperature changes in the two heating boxes and in the ambient atmosphere of the greenhouse in Nebraska from March 5 to March 9, 1992

temperature prevailed (in the second year) or remained exposed to the ambient atmosphere of the chamber (third year).

U.S.A.

The two spike heating boxes were placed in the greenhouse and were adjusted to the same conditions as in Israel. The actual conditions are shown in Fig. 2. Generally, the temperature varied only $\pm 1^{\circ}\text{C}$ in the heating chambers during the experiment. Each heating chamber had its own heater and the two chambers were set at the same temperature (differential $1\text{-}2^{\circ}\text{C}$). As a control, plants were left under normal greenhouse conditions. The heat treatments began 13-14 days after anthesis. The spikes (at least three spikes per treatment within replication) from the main tillers of each plant were randomly inserted into the two heating chambers (replications).

Sampling procedure and calculations

Israel

Immediately after the treatment was ended, the upper half of the spike was cut and dried in an oven at 70°C for 5 d or more. The plant with the trimmed spike was returned to the screen house until maturity. Then the lower half of the spike was removed and dried similarly. Ten basal grains from the five spikelets, adjacent to the cut were removed from each half of the spike, weighed and the gain in grain weight was calculated as the difference between the mean grain weight of the lower half and the upper half. Our experience suggests that the weight of the basal grains is similar in all central spikelets. Thus the difference between halves reflects mainly differences in gain in weight rather than position effect.

U.S.A.

Immediately after the treatment, the spikes were sampled by harvesting the top halves of the spikes and the plants were returned to the ambient greenhouse conditions. Every other grain was taken from the bottom upper half for a total of four grains from every spike. Fresh weight and dry weight (after oven drying) of the immature grain were determined and used to calculate the moisture content in the grain. The bottom half of the spike was allowed to mature. Representative grains were sampled similarly to the top half and were used to determine the final grain weight.

Analysis

Israel

Genotypes that are related (cvs.: wild accessions; addition or substitution lines) and treated at about the same time were grouped for analysis. Although, except during the treatments, the plants were grown in an uncontrolled environment, we believe that for intergenotypic comparisons within groups, the major effect of spike heating is expressed. Even high temperatures during grain filling period were not efficient in masking the treated effect. An analysis of variance procedure and Duncan MRT test (SAS) were used to compare the effect of spike heating in any genotype.

U.S.A.

Two cultivars, Karl and Arapahoe were studied in 1992 and 19 cultivars (Table 3) in 1993. Not all the cultivars flowered on the same day due to multiple planting dates and genetic differences in flowering date. For the analysis of variance, cultivars were considered as fixed effects, days (random) were nested within cultivars and replication (random) were nested within cultivars and days. Heat treatment was considered as fixed effect. Some cultivars were replicated in time, however day to day variation tended to be small as well as the interaction of day with heat treatment and cultivar. Hence, the experiment was analyzed over days and the pooled residual error term was used. Moisture content and relative injury data were transformed using arcsin square root x .

Results

Effect of spike heating on grain growth in two genotypes (Israel)

Grain growth was followed in the two cvs. Miriam and Sun9E in untreated and treated spikes. Five spikes of Sun9E and seven spikes of Miriam were treated in the heating chamber (12-16 days after anthesis) whereas same number of spikes remained untreated. One random spikelet was removed from the central portion of the spike immediately after the treatment and 3, 6, 8, 11, 14 and 17 days after this. The two basal grains were removed, oven-dried and weighed.

The effect of the treatment is shown in Fig. 3. In both cultivars final grain weight decreased due to the treatment. Yet this reduction was caused by a lower rate of dry matter accumulation in Miriam and a shorter dry matter accumulation period in Sun9E. The injury (relative reduction in grain weight after the treatment) was similar in both cultivars (38% in Miriam and 35% in Sun9E). A similarity in the injury values of these cultivars was found also this year in the experiment described below (Table 1) but in other cases (Table 6) Miriam was defined more heat tolerant than Sun9E.

Fig. 3. Accumulation of dry matter in grains of cvs. Sun9E (full symbols) and Miriam (empty symbols) in heated (dashed line) and unheated (solid line) spikes.

Effect of spike heating on six spring wheat cultivars (Israel)

Spikes of six spring cultivars previously (1993 report) defined as heat sensitive or tolerant were exposed to four-day heat treatment 12 d after anthesis. Mean basal grain dry weight was determined in the upper half of the spike immediately after the treatment while the lower half allowed to mature and then sampled. The experiment was repeated twice in two groups of plants sown on two different dates. Table 1 summarizes the measured parameters and the calculated injury values in both experiments. In all the six cultivars and two experiments no significant effect

Table 1: Immediate and after effect of spike heating on grain weight, gain in grain weight after the treatment and the injury caused to grain growth by the treatment in six spring cultivars grown in two experiments.

Experiment	Cultivar	Number of plants		Grain weight at the end of treatment (mg)		Grain weight at maturity (mg)		Gain in grain weight after treatments (mg)		Injury ¹ % ± SE	Injury ² % ± SE
		Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated		
I	Bethlehem	7	7	15.0	13.1	36.8*	31.8	21.9	18.7	14.5±8.3	22.0±9.3
	Deganit	4	4	11.3	11.9	37.0**	32.0	25.7*	20.0	22.2±6.0	19.9±5.5
	Kalyansona	5	4	16.3	15.8	41.3	36.3	25.0	20.5	18.0±8.6	19.8±9.7
	Miriam	7	7	14.1	12.0	38.7**	30.4	24.6**	18.4	25.3±8.3	32.5±10.4
	Nirit	5	5	12.5	13.0	37.4	34.2	24.9	21.2	14.9±9.2	13.1±7.4
	Sun9E	3	5	7.7	7.5	32.9**	25.3	25.2**	17.8	29.4±5.0	30.1±8.0
II	Bethlehem	6	6	11.9	10.4	43.6**	31.5	31.7**	21.1	33.4±8.3	37.2±10.8
	Deganit	6	6	12.6	12.2	49.6*	41.8	37.0*	29.6	20.0±7.7	21.0±7.5
	Kalyansona	8	7	11.7	11.4	42.1**	36.1	30.4***	24.7	18.8±4.1	19.6±5.9
	Miriam	8	8	12.9	13.5	48.1***	39.4	35.2***	25.9	26.4±3.8	24.9±4.7
	Nirit	8	8	11.2	11.3	41.0**	34.0	29.8**	22.6	24.0±7.3	23.5±7.7
	Sun9E	5	5	7.8	7.8	38.0*	30.5	30.2**	22.8	24.6±7.1	24.8±10.0

¹ Calculated using treatment mean of grain weight after the treatment

² Calculated using the mean values of grain weight after the treatment of both treatments

*, **, *** Significantly higher value than in the heated spike; calculated on varietal MS: ≤0.05, 0.01 or 0.001, respectively

of spike heating on grain weight immediately after the treatment was recorded. However, the weight of the mature grain and gain in grain weight after the treatment were significantly and highly reduced by the spike heating. Since, small differences in grain weight immediately after the treatment significantly affect the calculated injury value, another injury measure was calculated using the mean grain weight immediately after the treatment of the two treatments to calculate the gain in grain weight. The two injury index values indicated intervarietal differences in heat tolerance in any experiment. Yet, the genotype ranking was not completely reproducible in the two experiments: Bethlehem was the most tolerant in the first experiment and the most sensitive in the second.

Viewing Table 1 reveals that the grains on the unheated spikes were always heavier in experiment I than in experiment II, whereas grain weight in the heated spikes was more similar in both experiments. This may indicate an environmental factor that was expressed in the first experiment and differentially prevented the various genotypes to achieve their potential grain weight. This was more pronounced in the unheated spikes since they required a longer grain-filling period. Consequently, we believe that at least in the case of cv. Bethlehem an underestimated injury value was obtained in experiment I.

Effect of spike heating on 17 winter and two spring wheat cultivars (USA)

There was no significant difference between cultivar Arapahoe and Karl for moisture content, fresh grain weight, dry grain weight, mature grain weight and injury index in 1992 (Tables 2 and 3). The exposure to heat decreased moisture content, immature grain fresh weight and mature grain weight, but did not effect immature grain dry weight. The cultivar x treatment interaction was nonsignificant for all traits in the 1992 experiments, hence Karl and Arapahoe responded alike.

In 1993, as in the previous year, the effect of spike heating on immature grain dry weight was small and random. Intervarietal response in fresh weight was however apparent: all but six lines reduced their value. Moisture content invariably decreased due to spike heating.

Although all the lines responded to the treatment by producing smaller grains, the magnitude of the response varied among the genotypes (half of them responded significantly).

Table 2: Analysis of variance of grain characteristics immediately after the treatment, final grain weight and relative injury (RI) in cvs. Arapahoe and Karl in 1992 and in 19 cultivars in 1993 following spike heating.

Source of variation	Grain characteristics at the end of treatment				Grain dry weight at maturity (mg)	RI
	Moisture content (x10 ⁻⁷)	Fresh weight (mg)	Dry weight (mg)	df		
	df					df
1992:						
Cultivar	1	387	152.7	0.25	0.44	1
Day (cultivar)	10	348**	68.2*	44.86**	101.02**	6
Reps (day cultivar)	12	57	20.4	4.32	3.93	8
Treatment	1	1597**	395.0**	0.27	268.31**	1
Cultivar/treatment	1	14	14.1	13.55	6.82	14
Error	22	76	17.6	4.27	6.19	14
Total	47					31
1993:						
Cultivar	18	143	96.5	42.96	128.33**	18
Day (cultivar)	7	45**	56.8*	28.25**	26.67	
Reps (day cultivar)	26	14	20.1	7.26	11.90	
Treatment	1	363**	267.8**	3.76	751.14**	
Cultivar/treatment	18	4	18.0*	4.35*	5.68*	
Error	33	5	8.5	2.29	2.85	27
Total	103					45

*significant at P=0.05; ** highly significant at P=0.01.

greenhouse in 1992 or 1993.

RI values followed by common letter within a year are not significantly different using a Duncan's multiple range test ($p \leq 0.05$)

* Significantly higher than the heated value using Bonferroni test

* Significantly higher than the heated value using Bonferroni test

A conspicuously wide range (70-86%) of relative injury value was found in the studied germplasm. Whereas the Israeli spring cv. Miriam was slightly (n's) more heat tolerant than cv. Bethlehem thus confirming the Israeli results. The two US standard genotypes were not ranked as expected: cv. Arapahoe was more heat tolerant than cv. Karl. No apparent correlation was found between the response in moisture content and the relative injury.

Effect of spike heating on several whole chromosome- or arm-substitution lines of *T. dicoccoides* in bread wheat (Israel)

Based on last year's results indicating that *T. dicoccoides* (three accessions) and few wheat-*dicoccoides* (acc. TTD09) chromosome substitution lines are more tolerant to spike heating than the bread-wheat cv. Chinese Spring (CS), we have repeated the experiment using only the three most tolerant lines and also the most sensitive line (6A). In addition, we included the available matching chromosome arm substitution lines (these lines are derived from a different *dicoccoides* accession TTD140).

All the lines and the cv. CS were treated in the heating box together, from 15-17 d after anthesis for four days. Cv. CS that was sown and flowered earlier was also treated separately from 15-17 d after anthesis. As shown in Table 4, grain weight immediately after the treatment was higher (ns) in the heated spikes than in the unheated spikes in all the lines (except in 1BL and 2BL). This indicates that under the conditions of this experiment grain growth during the treatment was enhanced in most lines. However, following the treatment, grain growth was highly and significantly reduced in all the lines resulting in significantly lighter grains in the heated spikes. Injury estimates in the two CS groups were similar and much higher than found in all the genotypes tested last year and this year in the other experiments. Yet, despite relatively high error values (Table 4) an intergenotypic variation was evident (e.g. 1BL was less injured; 6AL was most injured). Comparison of the relative injury values in the substitution lines in both years (Table 5) supports that 1B of the *dicoccoides* acc. TTD09 indeed carries a gene for heat tolerance of the grain whereas on 6A of this *dicoccoides* - an allele for heat sensitivity exists. A more closer analysis using chromosome arm substitution lines revealed that the *dicoccoides* 1BL carries the "tolerant" allele and 6AL - the "sensitive" one.

Table 4: Immediate and after effect of spike heating on grain weight, gain in grain weight after the treatment and the injury caused to grain growth by the treatment in cv. Chinese Spring and in eight wheat-*dicoccoides* chromosome or chromosome arm substitution lines.

Line	Substituted chromosome/arm	Number of plants		Grain weight at the end of treatment (mg)		Grain weight at maturity (mg)		Gain in grain weight after treatment (mg)		Injury % $\pm$ SE
		Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated	
CS ¹	-	6	5	16.9	17.9	25.0*	20.4	7.5***	2.5	66.6 $\pm$ 12.1
CS ²	-	3	4	13.8	14.7	26.2*	18.6	12.4*	3.9	68.4 $\pm$ 24.6
TTDDS	1B	5	4	15.5	17.2	27.6***	22.4	12.2***	5.2	57.6 $\pm$ 14.2
	1BL	5	3	17.4	16.6	28.7**	22.2	11.3*	5.6	50.8 $\pm$ 13.4
	1BS	5	4	14.4	15.9	28.3**	21.6	13.9*	5.6	59.4 $\pm$ 15.7
	2B	3	4	18.0	21.1	28.5	24.2	10.5**	3.2	69.7 $\pm$ 25.1
	2BL	4	4	16.3	15.9	25.9***	20.4	9.7*	4.6	52.5 $\pm$ 15.4
	5B	5	4	20.7	21.7	32.6***	24.9	11.9***	3.3	72.6 $\pm$ 15.7
	5BS	5	4	16.7	18.0	32.0***	23.5	15.4***	5.5	64.0 $\pm$ 7.4
	6AL	5	4	18.7	20.5	24.8*	20.8	6.1***	0.3	95.0 $\pm$ 49.5

¹ Flowered on April 12, 1993

² Flowered on April 18, 1993

*, **, *** Significantly higher value than in the heated spike; calculated on varietal MS: ≤ 0.05 , 0.01 or 0.001, respectively

Table 5: Comparison of heat induced injury parameters in two years in wheat-*dicoccoides* (TTD09) chromosome and chromosome arm substitution lines.

Line	Substituted chromosome	Year	Grain weight (mg)		Gain in grain weight (mg)		Injury %
			Unheated	Heated	Unheated	Heated	
CS	—	1992	40.9	37.1	29.0	24.6	15.4
CS	—	1992	40.6	36.0	19.9	14.7	26.1
CS	—	1993	26.2	18.6	12.4	3.9	68.4
TTDDS	1B	1992	46.7	45.2	32.7	29.3	10.4
	1B	1993	27.6	22.4	12.2	5.2	57.6
	1BL	1993	28.7	22.2	11.3	5.6	50.8
	2B	1992	45.4	39.2	28.2	23.5	16.5
	2B	1993	28.5	24.2	10.5	3.2	69.7
	5B	1992	43.3	41.5	30.1	25.4	15.9
	5B	1993	32.6	24.9	11.9	3.3	72.6
	6A	1992	52.0	42.6	38.7	27.7	28.6
	6AL	1993	24.8	20.8	6.1	0.3	95.0

Discussion and Conclusions

This research was aimed at the effect of short period of high temperature exerted on the grains, on grain growth and final weight. There are two phases in which the grains may respond: (1) during the treatment and (2) after the stressful temperature is changed into a milder one (i.e., expression of the after effect of the treatment). It is known that both the rate and the duration of dry matter accumulation during the linear phase of grain growth are temperature sensitive: rate increases and duration decreases with increasing temperature (within a certain limit) usually prevailing in the field. This response is probably the consequence of enhancing of biological processes at higher temperatures. However, beyond a certain critical temperature an irreversible injury to the grain tissues which reduces their synthetic capacity proved to occur. Loss of moisture during the treatment may be one feature of the damaged tissue. During the short hot period, the temperature effect was usually undetectable

(random, small and non significant). This may be accounted for by two opposite effects - the damage to the tissue which is compensated by enhanced biological processes such as starch deposition.

However, it was clearly shown that 4 d episode of elevated temperature that mimics a spring hot spell, has an after effect to decrease grain weight despite that grain development after the treatments occurred at mild ambient temperature.

An injury measure was developed to properly assess the response of the grain. Since intergenotypic differentiation was our major interest, it was important that this injury measure will be independent from the genotypic growth capacity of the grain. Therefore, grain growth in the treated spike was related to that of the untreated one. The rationale behind this measure is described at the beginning of this chapter.

Indeed, using this measure to assess the heat-induced injury revealed intergenotypic variation. In most cases the injury value was reproducible in different growth conditions (different experiment and different years). Yet in a few cases contradicting evaluations were obtained. Therefore, it was sensible first to screen a wide range of genotypes and then validate their relative response in another experiment. Following this habit, it was possible not only to compare genotypes in a specific experiment but also to observe the whole array of selected genotypes and experiments as appears in Table 6. Using these data it was unexpectedly found that cv. Miriam is more tolerant than Bethlehem and that cv. Arapahoe is more tolerant than Karl. The other selected Israeli cvs. Nirit and Deganit exhibited even more heat-tolerance than Miriam. Ten US cultivars were found more heat tolerant than Karl and two more than Arapahoe.

This study concentrated on the heat response of the grain. Hence our techniques required exposure of exclusively the grain to elevated temperatures on one hand and avoiding the effects of other factors, especially humidity, on the other hand. In the first year of this research we have practiced isolated grain culture. This method addresses the two technical problems that were mentioned above. However, using this technique we were unsuccessful to obtain significant information on heat response of the grain. Instead we have adopted the method of heating the whole spike in a chamber. Although it is most likely that the grains were the responsive organ (supported by starch granule counts - below) we cannot exclusively ascribe the response to the grain tissues since the heated spike organs (including the upper part of the peduncle) may indirectly affect also grain growth. The effect of reduced humidity that might prevail in the heating box was also not excluded.

Table 6: Comparison of heat induced injury in different genotypes in several experiments during two years.

Genotype	Experiment*					
	1	2	3	4	5	6
Bethlehem	33.6	30.6			14.5 – 22.0	33.4 – 37.2
Chinese Spring			15.4	26.1	66.6	68.4
Deganit			17.6		22.2 – 19.9	20.0 – 21.0
Karl	24.8	17.6				
Kalyansona					18.0 – 19.8	18.8 – 19.6
Miriam	18.8	23.7			25.3 – 32.5	26.4 – 24.9
Nirit			18.8		14.9 – 13.1	24.0 – 23.5
Sun9E	29.2	29.4			29.4 – 30.1	24.6 – 24.8

* Source of data:

¹ Table 2, 1992

² Table 2, 1992

³ Table 3, 1992

⁴ Table 3, 1992

⁵ Table 1, 1993 (cv. CS, Table 3, 1993)

⁶ Table 1, 1993 (cv. CS, Table 3, 1993)

EFFECT OF HEAT STRESS ON GRAIN WEIGHT IN THE FIELD (U.S.A.)

Introduction

Spike heating boxes served as efficient means to induce a controlled short heat stress on grain growth. However, the number of treated spikes was sometimes a limiting factor to achieve significant results. Parallel to this standard method we have run a set of experiments in which spikes of plants grown in the field were enclosed in inverted flasks, thus experiencing different ambient temperatures. These easy-to-set experiments enabled a cheap evaluation of spike heating effects on grains of various genotypes.

Materials and Methods

1992 experiment

An insulating air bubble was obtained by an inverted 1.0 l glass flask covered with aluminum foil on the lower three-fourths of the flask. Poles were put into the

ground and the flasks tied to the poles at head height. Fifty heads were inserted into each flask and tied together to the pole. Temperatures were measured every two minutes using 10 copper/constantan thermocouples and LICOR data logger model LI-190SA. Thermocouples were placed in the center of flasks, at flag-leaf level to measure the ambient temperature, and also 5 cm below soil surface. Temperature differences between the flask and ambient temperature were about 10°C during the day. Mean ambient night temperature was about 12°C and mean day temperature was about 27°C. The spikes were enclosed in the flasks for 96 hours starting at 10:00 a.m. Cvs. Karl and Arapahoe were used in this experiment in four replications with two flasks per replication. A randomized complete block design with subsampling was used to analyze genotype and treatment effects.

1993 experiments

The flasks were set and the temperature was recorded similarly to the previous year. The thermocouple positions were 15 cm and 8 cm from the top of each flask, at flag-leaf level, 10 cm above the ground and in the ground (5 cm below the surface). Light intensity was recorded. The mean ambient temperatures were about 27°C in the day and 12°C in the night. The temperature in the center of the flask was about 38°C during the day with light of 2000 μ Einstein $m^{-2} s^{-1}$, and about 12°C at night. Soil temperatures were 15°C at night and 20°C during the day time.

Ten spikes were randomly selected from the 50 treated spikes. These spikes and 10 other untreated random spikes (as a control) were harvested at maturity. Two flasks were placed in any plots. Each experiment was replicated four times with two of the replications treated with fungicide.

a. Date of treatment

The purpose of this experiment was to study response to heat stress during different times of the grain-filling period. The four cvs. Karl, Arapahoe, Tam-105 and Redland, were used. Two flasks were placed in each plot 10, 14 and 18 days after anthesis.

b. Flask covering experiment

The purpose of this experiment was to determine what proportion of the flasks should be covered for heat stress experiment. Three covers were used: covering the

bottom three fourths, covering the bottom half, and without covering. The six cvs. Karl, Arapahoe, Arlin, Rawhide, Siouxland and Wichita were used.

c. Genotype screening

The purpose of this experiment was to screen various genotypes for their heat response. The same procedures as described above for flask treatment were used. The bottom three-fourths of the flasks were covered. The sixteen genotypes used are listed in Table 11. A randomized split block design with subsampling was used to analyze the data.

Results and Discussion

1992 experiment

Analysis of variance revealed a significant effect in only the flask treatment and that the two cvs. Karl and Arapahoe responded similarly to the treatment. Since the number of grains per spike was similar in both treatments we assume that a comparable grain population was analysed and that the flask effect was to reduce grain weight (Table 7). The relative reduction in grain weight (RR) was higher in Karl than in Arapahoe.

1993 experiments

a. Date of treatment

A very significant effect of the genotype, date of treatment and a significant interaction between them was found (Table 8). Fungicide control, although interacting with some effects did not interact with genotype x date. Grain weight progressively increased with delaying of the treatment up to 18 days after anthesis (Table 9). Since grain weight was not determined immediately after the treatment it is difficult to claim for a differential developmental response and the decrease may only indicate that more dry matter has already accumulated at later treatment dates.

The two early genotypes Karl and Tam-105 were injured more than the two late ones at any date. This may suggest that the early genotypes were exposed to higher ambient temperatures in the flask under the uncontrolled field conditions.

Table 7. Grain weight of flask-treated and untreated spikes in the field and relative reduction (RR) in grain weight (%) in 1992.

Genotypes	Seeds per head	Grain weight (mg)		RR (C-T)/C*
		Treated	Untreated	
Karl	20	25.3	30.1	16
Arapahoe	29	26.5	29.1	9

* T - treated; C - Control

Table 8. Analysis of variance of date of treatment experiment.

Source	DF	Mean Square
Fungicide	1	51.51
Block (fung)	2	4.64
Genotype	3	16.77**
Fung x genotype	3	18.10**
Block x var. (fung)	6	0.86
Date	3	586.52**
Genotype x date	9	21.78
Fung x date	3	11.22
Fung x genot x date	9	5.76
Error	24	3.89
Cor. Total	63	

Table 9. Mean grain weight (GW) (mg) in spikes treated 10, 14 or 18 days after anthesis, and relative reduction (RR) in grain weight (%) in 1993.

Genotypes	Anthesis	10		14		18		Control
		GW	RR	GW	RR	GW	RR	GW
Karl	early	5.61	78	12.04	53	12.88	50	25.87
Tam-105	early	3.93	81	11.72	56	14.13	33	21.01
Arapahoe	late	7.45	58	9.15	48	13.77	22	17.75
Redland	late	7.42	57	9.51	46	12.87	27	17.57

b. Flask covering experiment

Very significant effects were attributed only to the genotype and covering. Differences in grain weight were found only between the untreated and any flask treatment but not among the different covering (Table 10).

Table 10. Grain weight (GW) (mg) and relative reduction (RR) in grain weight (%) flask treated spikes with different coverings.

Genotype	Anthesis	Control GW	No covering		1/2 cover		3/4 cover	
			GW	RR	GW	RR	GW	RR
Arapahoe	late	17.75	7.26	59	7.95	55	9.15	48
Karl	early	25.87	13.22	49	9.92	62	12.04	53
Arlin	early	24.93	11.26	55	13.39	46	13.35	46
Rawhide	early	20.49	8.32	59	8.26	59	10.04	51
Redland	late	17.57	7.70	56	8.26	53	9.51	46
Siouxland	late	19.25	8.63	55	10.00	48	9.33	51
Average		20.98a	9.40b	55	9.63b	54	10.57b	50

c. Genotype screening

The 16 tested genotypes flowered at seven different days (Table 11). Since the flask treatment started 14 d after anthesis, different genotypes may have experienced different ambient treatment temperatures, depending on the prevailing environmental conditions. Very significant effects of the flask and the genotype but not of the interaction between them were found (Table 12).

In general, the relative reduction in grain weight was at a similar magnitude in all genotypes despite different anthesis dates (Table 11). However, it is interesting to note that NE88427, an experimental line, known for its good performance in stressful conditions, showed here the lowest reduction in grain weight while cv. Abilene with the same anthesis date exhibited the highest RR.

EFFECT OF HEAT ON STARCH GRANULES (ISRAEL)

The effect of short period of elevated temperatures on the number and size distribution of starch granules in the grain was studied in different genotypes immediately after the treatment and at maturity. Individual grains that reflected the mean of the treatments were selected by their weight from progenies of three heated and three unheated spikes of the four cultivars Dariel, Deganit, Nirit and Sun9E from the cultivar screening experiment in 1992 (Table 3, Second Annual Report). These cvs. were identified as heat sensitive, tolerant and sensitive, respectively. In addition, the number of starch granules were determined in the available wheat-*dicoccoides* (TTD09) chromosome substitution lines from 1992 experiments (Table 5, Second Annual Report).

Procedure for counting starch granules

Enzyme mixture was prepared by mixing, 100 mg. 1% 'Macerozyme' (Yakutt Co., Japan) and 100 mg 1% 'Cellulase' (Yakutt Co., Japan) in 10 ml sodium acetate buffer (pH 4.7). Individual grains were weighed and soaked overnight in 1 ml of the enzyme solution at 37°C. The samples were thoroughly vortexed, grain coat was removed and the samples were centrifuged for 20 min at 3000 g and the supernatant decanted. One ml of sodium acetate buffer was added to the pellet, the sample vortexed and stored up to two days in cold. Before counting, the sample was diluted five to 40 times to enable even spreading of the granules. Each sample was counted three times using

Table 11. Grain weight (mg) and relative reduction (RR) in grain weight (%) of 16 genotypes.

Genotypes	Anthesis*	Control	Treated	RR
Karl	0	25.87	12.04	53
Arlin	1	24.93	13.35	46
Vona	1	23.32	10.53	55
Yuma	1	20.58	10.55	49
Wichita	2	29.17	13.52	54
Tam-105	2	21.01	11.72	44
Rawhide	2	20.49	10.04	51
Scout66	3	24.58	11.34	54
Siouxland	3	19.25	9.33	51
NE88427	4	21.06	12.14	42
Centura	4	22.22	10.84	51
Vista	4	20.03	9.21	54
Abilene	4	16.40	6.59	60
Arapahoe	5	17.75	9.14	48
Redland	5	17.57	9.51	46
Cheyenne	6	14.96	6.35	57
Average		21.2a	10.39b	51

*) Relative anthesis dates 0 to 6 are early to late, respectively.

Table 12. Analysis of variance of grain weight in the genotype screening experiment.

Source	DF	Mean Square
Fungicide	1	20.30
Block (fung)	2	8.88
Genotype	15	63.43**
Fung x genotype	15	11.58
Bloc x geno (fung)	30	10.53
Flask	1	3740.08**
Genotype x flask	15	9.19
Fung x flask	1	1.84
Fun x var x flask	15	10.10
Error	32	7.45
Cor. Total	127	

hemacytometer. The granules were easily divided into two discrete size populations: $> 10\mu$ diameter (type A) and $< 10\mu$ diameter (type B).

Results and Discussion

a. Effect of heat on starch granules in four cultivars

The effect of spike heating on the number of granules and distribution between A and B types at two sampling dates in four genotypes is shown in Fig. 4. As expected, 18 days after anthesis the predominant granule type was A. In most cases (Deganit A-type excluded) the number of both types increased immediately after spike heating. The predominant granule type of the mature grain was, however, B (yet the contribution to grain weight of A granules is apparently greater than of B granules). In most cases (Deganit excluded) starch granule number decreased due to spike heating. This decrease was more exaggerated in the two heat-sensitive cultivars Dariel and Sun9E. In these two genotypes the number of both A and B type granules decreased.

Fig. 4. Number ($\times 10^7$) of A ( ) and B ( ) type starch granules in grains from heated (h) or unheated (c) spikes of four spring wheat genotypes. Samples were taken immediately after the treatment (lower) or at maturity (upper).

The grains selected for starch granule counts reflected the mean grain weight and were smaller in the heated spikes. Since we were most interested to see whether the effect of temperature on grain weight is fully ascribed to the effect on the number of starch granules, the latter was related to grain weight (Table 13). As seen there, the relative number of A-type granules decreased usually (Deganit excluded) in the heated spikes indicating that the effect of the decrease in A granules on grain weight was compensated by another factor. This may be partially ascribed to an increase in B-type granules (characterizing the tolerant cvs. Deganit and Nirit). Even in the sensitive genotypes when the number of B-type granules decreased, their relative number increased indicating another heat induced factor that negatively affect grain weight.

Concluding this study, spike heating reduced the number of starch granules in only the sensitive lines but the decrease could not fully account for the reduced grain weight.

b. Effect of heat on starch granules in *T. dicoccoides* disomic substitution lines

Counting starch granules in mature grains of several selected *T. dicoccoides* disomic substitution lines that were identified last year as heat tolerant or heat sensitive (Table 5, Second Annual Report) revealed intergenotypic variation (Fig. 5). Substitution line 1B and 2B (most tolerant) and 7A (tolerant) were least affected in number of starch granules. Lines 7B and 6A (most sensitive) and 3B (sensitive) were highly affected in number of starch granules. However, not all the substitution lines responded as expected: line 1A (sensitive) showed a similar starch granule response as 1B (most tolerant) whereas 5B (tolerant) severely reduced the number of starch granules. Considering also the heat response of some of the lines in 1993 (Table 5), lines 1B and 2B proved to be tolerant and 5B and 6A are apparently sensitive. These lines reflected their grain weight response to heat also in the number of starch granules. It is obvious that in the tolerant lines 1B and 2B, the number of B-type granules was hardly affected.

Table 13: Mean weight of mature grains selected for starch granule analysis and relative number of starch granules (related to grain weight) in heated and unheated spikes of four genotypes.

Genotype	Grain weight (mg) $\pm$ SE		Heat induced decrease in grain weight %	Relative number of starch granules ($\times 10^6$ mg ⁻¹)					
	Unheated	Heated		Type A		Type B		Total	
				Unheated	Heated	Unheated	Heated	Unheated	Heated
Dariel	57.7 $\pm$ 1.9	42.3 $\pm$ 1.5	27	1.9	1.6	5.5	7.1	7.3	8.7
Deganit	65.3 $\pm$ 1.8	54.3 $\pm$ 1.8	17	1.9	2.6	5.2	7.1	7.1	9.8
Nirit	54.0 $\pm$ 1.0	46.7 $\pm$ 0.7	14	2.5	2.1	4.4	5.6	6.9	7.7
Sun9E	54.0 $\pm$ 1.0	39.7 $\pm$ 1.2	26	2.0	1.6	4.7	5.2	6.7	6.8

Fig. 5. Number ($\times 10^7$) of A ( ) and B ( ) type starch granules in mature grains from heated (h) or unheated (c) spikes of *T. dicoccoides* acc. TTD09 and selected wheat-*dicoccoides* disomic substitution lines (denoted by the alien chromosome).

MEMBRANE THERMOSTABILITY STUDY (U.S.A.)

Materials and Methods

The lab procedure consists of the following steps (Saadalla et al., 1990; *Crop Sci.* 30: 1243).

Plants were grown to heading in the greenhouse or in the field using normal procedures and cultural practices. The greenhouse grown plants (9-10 days after anthesis) were hardened by placing them in a growth chamber for three days with a continuous temperature of 31°C with 12 hrs light/12 hrs dark and then moved into the greenhouse for one day of recovery. This hardening treatment was not applied to plants from the field as they were believed to be naturally hardened. Tillers were harvested and tissue was prepared by first removing the flag leaves or, if the flag leaf was small, the flag and penultimate leaves. The leaves were cut into 1 cm pieces, eight pieces for control and eight pieces for subsequent heat treatment. Leaf sample surface was rinsed with 5 ml of deionized distilled water (ddH₂O) three times, and then placed in a 20 ml vial containing 2 ml of ddH₂O. Heat treated vials were placed in a water bath at a temperature of 48°C, whereas the untreated vials remained at room temperature. After the treatment, 13 ml of ddH₂O was added to the vials and they were incubated for 24 hrs at 10°C. The vials were removed from the incubator and allowed to come to room temperature. The initial conductivity was measured with a digital conductivity meter. The tissue was killed in an autoclave (20 min) (1992 experiment) or in 90°C hot water for one hour (1993 experiment). The final conductivity was read after the vials had cooled to room temperature.

Membrane injury (MI) was calculated as follows:

$$\%MI = [1 - (1 - T_1/T_2) / (1 - C_1/C_2)] \times 100$$

where T₁ is the initial conductivity value of the treated tissue before killing the tissue, T₂ is the conductivity value of the treated tissue after killing the tissue. C₁ is the initial conductivity value of the untreated tissue (control), and C₂ is the conductivity value of the control after killing the tissue.

A wide range of genotypes was used in this membrane study (Table 15). In addition, the effect of leaf age on MI estimates was studied in field-grown plants of

several genotypes (Table 16). Membrane thermostability was determined seven and 14 days after anthesis in 1992 and 0-18 d after anthesis in 1993.

Results and Discussion

A completely randomized design was used to analyze the 1992 and 1993 greenhouse data by SAS. Initially, each sampling date was analyzed individually. However, in 1992, for those lines that were repeated in different dates, the membrane injury values for the genotypes were quite similar. Data for the repeated genotypes were then analyzed using the sampling dates as replications in time. No differences were identified between dates for any of the repeated genotypes. Based upon this result, all the individual sampling date data were combined and analyzed as one large experiment with some lines replicated and others unreplicated. Each year (1992 and 1993) was analyzed separately.

Differences in membrane injury values were identified among genotypes at $P \leq 0.01$ in both years (Table 14). A great deal of inconsistency features many of the tested genotypes both when compared in the two growth conditions (greenhouse vs. field) and in the two years (Table 15). Best genotype separation was achieved in the greenhouse in 1992. In that study cv. Redland was injured four times more than cv. Arlin and three times more than cv. Siouxland. Yet, in the same year plants from the field showed quite similar MI values (Redland and Siouxland were literally and statistically similar). This year was cool and wet during grain filling period and it is possible that no hardening occurred in the field. The 1993 greenhouse study did not validate 1992 results. Only a few cvs. including Karl and Arapahoe maintained their

Table 14: Analysis of variance of membrane injury in 1992 and 1993.

Source of Variance	1992				1993			
	Greenhouse		Field		Greenhouse		Field	
	df	MS	df	MS	df	MS	df	MS
Genotype	21	0.097**	7	0.014**	18	0.039**	15	0.0075**
Error	184	0.008	40	0.006	85	0.009	89	0.011
Total	205		47		103		104	

Table 15: Membrane injury (%) in plants 14 d after anthesis from the greenhouse and the field in 1992 and 1993.

Genotypes	1992		1993	
	Greenhouse	Field	Greenhouse	Field
Redland	61a ⁺	74bc	59abcd	46def
Rawhide*	49b	72c	60abcd	52cde
Abilene	38c	—	69a	48def
Yuma*	37c	—	—	55bcde
Colt	37cd	85a	—	—
Ram	36cd	—	—	—
Cody	35cde	—	63abc	—
Karl	31cdef	76bc	54bcde	57bcd
NE87612	31cdef	—	—	—
NE86606*	30cdef	—	—	—
NE88584	29cdefg	—	—	—
Tam202	29cdefg	—	—	—
NE86501*	28defg	—	—	—
Arapahoe*	28defg	76bc	42efg	43ef
WI88-181	26efg	—	—	—
Vista	23fg	—	—	52 cde
Tomahawk*	23fgh	—	—	—
Centura	23fgh	80ab	—	30g
NE88427	20gh	—	—	49cdef
XNH1401	20gh	—	—	—
Siouxland	19gh	77bc	55abcde	74a
Arlin	17h	—	57abcde	53bcde
Bethlehem	—	—	65ab	—
Wichita	—	—	55abcde	—
Tam-200	—	—	54bcde	—
Mustang	—	—	50cdef	—
Cheyenne	—	—	48def	39fg
Tam-107	—	80ab	46def	—
Tam-105	—	—	46def	64b
Scout-66	—	—	43efg	57bcd
Miriam	—	—	38fg	—
Sturdy	—	—	32g	—
Vona	—	—	—	59bc
Wichita	—	—	—	53bcde

* These genotypes were repeated in different experiments in 1992. The mean for each genotype from different experiments were not significantly different.

⁺ Genotype means followed by a common letter within a year are not significantly different using a duncan's multiple range test (=0.1)

ranking. Field and greenhouse ranking differed also in 1993 despite high field temperatures that apparently hardened the plants.

Generally speaking MI increased from anthesis onwards in field grown plants in 1992 but decreased in 1993 (Table 16). This observation stresses the importance of the growth conditions on MI estimate. Delaying leaf sampling may have two opposite effects on MI estimate; it may expose the leaf to a natural hardening on one hand but on the other hand leaf senescence increases membrane permeability and mechanical and pathogenic factors may physically damage the tissue.

Table 16: Membrane injury (%) in plants grown in the field 0, 7, 10, 14 or 18 d after anthesis.

Genotype	1992		1993				
	7	14	0	7	10	14	18
Colt	82	85	—	—	—	—	—
Redland	82	74	68	58	52	46	—
Arapahoe	75	76	68	52	40	43	—
Centura	73	80	—	—	—	30	—
Rawhide	72	72	—	—	—	52	—
Siouxland	62	77	—	—	—	74	—
Tam-107	62	80	—	—	—	—	—
Karl	58	76	—	65	54	57	52
Tam-105	—	—	—	63	47	64	54
Abilene	—	—	—	—	—	48	32
Average	71	77*	68	59	48	52	46

* Significantly higher than 7 d after anthesis

DESCRIPTION OF COOPERATION

Cooperation took place in both ways: exchange of knowledge and complementary work. Following a preliminary work we have realized that organ culture is unsuitable for our purposes and adopted the system of the spike heating box. This shift required both conceptual and technical knowhow exchange which consequently produced an efficient system for our purposes. Later on Dr. Millet concentrated on starch granule evaluation whereas Drs. Baenziger and Clegg focussed on membrane thermostability analysis. As to the biological material the U.S. group studied mainly winter wheat while spring wheat was investigated in Israel.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

The main finding of this research is that grain development is inhibited and the final grain weight is reduced following a single episode of elevated temperatures to which only the spike was exposed. Such events are unavoidable, however the presence of genetic variation in the response of the grain itself to the heat stress is exploitable in breeding for such heat tolerance. Wild relatives of wheat showed an enhanced heat tolerance and the allocation of the alleles to chromosomes facilitates the transfer of such genes to cultivated wheats. The basic understanding that the grain itself responds to elevated temperatures and that starch granules are affected will focus further research on the endosperm tissue of this organ.

PUBLICATION

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**Short Term Heat Stress On Wheat Grain Filling. MASRIZAL¹, P.S.
BAENZIGER, M.D. CLEGG, E. MILLET, G. WU, AND M. HORN, Univ.
of Nebraska-Lincoln and Weizmann Inst. of Science, Israel.**

In Nebraska, short periods (2-4 d) of high temperatures ($>35^{\circ}$) often occur during the wheat grainfilling. The temperatures moderate after the heat stress period, but many cultivars never seem to recover and finish kernel development. To study the effect of short term heat stress on kernel development, wheat spikes 14 d after anthesis were placed in heated, plastic boxes in a greenhouse with 12 h at 35°C day, 12 h at 25°C night for 4 d. The control spikes were allowed to grow in ambient conditions (21°C day, 16°C night). The heat treatment tended to increase fresh kernel weight immediately after the treatment, but decrease final kernel weight. No response differences were identified using Karl and Arapahoe; however, additional cultivars and lines are being tested to determine if some cultivars are more tolerant of this stress.

Masrizal, (402) 472-1559.