

## Effects of Drought and High Temperature on Grain Growth in Wheat

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### Abstract

The effects of two levels of temperature and of water supply on grain development of wheat (cv. Warigal) were studied by imposing treatments during the early or late period of cell division. High temperature (28°C day/20°C night) accelerated development of the grain. Dry matter accumulation and cell division proceeded at a higher rate but had a shorter duration in the high temperature treatments. Maximum cell number, final cell size and the number of large starch granules per cell were not significantly reduced by high temperature. Drought and drought × high temperature reduced the storage capacity of the grain, with a decrease in number of cells and starch granules in the endosperm. Cell size was also reduced when treatments were imposed late during cell division. Duration of dry matter accumulation and cell division was reduced in the drought and drought × high temperature treatments. The combined effects of drought and high temperature were much more severe than those of each separate treatment. The amount of sucrose per cell was similar in all treatments. It appears unlikely that the supply of sucrose to the endosperm cells is the main limiting factor of dry matter accumulation in both drought and high temperature treatments.

### Introduction

In the semi-arid environment of the Australian wheat-belt, drought and high temperatures occur frequently during grain growth (Nix 1975). Final grain weight of wheat is reduced by drought (Asana *et al.* 1958; Wardlaw 1971; Brocklehurst *et al.* 1978) or high temperature (Sofield *et al.* 1977a; Chowdhury and Wardlaw 1978; Wardlaw *et al.* 1980) that occur during the early period of grain development. The division of endosperm cells and initiation of large (A-type) starch granules take place during the first 15-20 days after anthesis; cell enlargement and initiation of small (B-type) starch granules occur from the end of cell division until maturity (e.g. Evers 1970; Briarty *et al.* 1979).

The few studies concerned with the effects of drought and high temperature on cellular processes in the grain are contradictory. Drought may reduce the number of endosperm cells (Brocklehurst *et al.* 1978) but not always (Wardlaw 1971; Brooks *et al.* 1982) and drought may also reduce the number of small starch granules (Brooks *et al.* 1982). Temperatures above the optimum range of 18/13°C-21/16°C day/night temperatures (Sofield *et al.* 1977a; Chowdhury and Wardlaw 1978; Davidson and Birch 1978) appear not to affect maximum cell number but may reduce cell size and starch granule number (Hoshikawa 1962; Wardlaw 1970; Singh 1982).

High temperatures are unlikely to reduce the accumulation of dry matter by limiting the supply of assimilate to the grain (Spiertz 1974; Ford *et al.* 1976; Wardlaw *et al.* 1980) but drought might (Asana *et al.* 1958; McPherson and Boyer 1977; Brocklehurst *et al.* 1978). In this experiment, the rate and duration of grain growth were related to the division and enlargement of endosperm cells, and the initiation and development of starch granules.

Three main points were investigated in this study:

- (1) The effects of drought and high temperature on the determination of grain size at the cellular level.
- (2) The possible interaction of drought and high temperature. A short period of high temperature may accentuate the effects of drought on grain growth.
- (3) The relations between assimilate concentration in the grains, number of cells or starch granules, and final grain weight under conditions of water deficit or high temperature stress.

## Materials and Methods

### *Growth Conditions*

Wheat plants (*Triticum aestivum* L. cv. Warigal) were grown in pots 60 cm long  $\times$  10 cm diam. containing 6 kg of sterilized, air-dried soil and supplied with a complete nutrient mix at sowing. The soil mixture consisted of Mount Derrimut loam (38% clay, 21% silt and 37% sand), washed quartz sand, vermiculite and perlite in the proportions 2 : 2 : 1 : 1. Two plants were grown per pot and tillers were removed as they appeared. In order to minimize soil evaporation, the soil was covered with polyethylene beads. Plants were initially grown in a naturally lit glasshouse with the photoperiod extended to 16 h by 100-W incandescent lights. Temperature was controlled so that the maximum day temperature was 25°C and the minimum night temperature 10°C. Two weeks before anthesis, the wheat plants were transferred to a controlled-environment growth room. The plants were grown with a 16 h, 23°C day and an 8 h, 15°C night. The average photon irradiance (400–700 nm) at plant height was 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Relative humidity ranged from 50 to 60% during the light period and 75 to 85% during the dark period. Anthesis was judged to have occurred on a particular day when the anthers of the central spikelets had just exerted.

### *Treatments*

The experimental design was  $2 \times 2 \times 2$  factorial, with two levels of water supply and two levels of temperature imposed either during the early period ( $P_1$ ) or late period ( $P_2$ ) of endosperm cell division. Pots were either watered daily to field capacity (control) or allowed to dry for 20 days. Watering was discontinued from day 7 before anthesis until day 13 in the  $P_1$  treatments, and from anthesis until day 20 in the  $P_2$  treatments. The temperature regime of the growth room (23°C day, 15°C night) was taken as control. Plants were exposed to high temperature by transferring them for a 10-day period to a controlled-environment chamber maintained at 28°C day and 20°C night. The 10-day period of high temperature was either from day 3 to day 13 after anthesis during the early period or from day 10 to day 20 during the late period.

### *Sampling Procedures*

The ears of six plants per treatment were harvested every 5 days from anthesis to maturity for measurements of grain dry weight. The ears were sampled at noon to minimize possible diurnal fluctuations in grain sucrose. Three or four of these ears were also used for measurements of endosperm cell number, endosperm cell area and starch granule number.

All measurements were made on basal grains of middle spikelets. The middle spikelets were generally the fourth, fifth and sixth spikelets (numbered from the base) from each side of the rachis. When simultaneous measurements of grain dry weight and cell and starch granule number were made, the ears were treated as follows. The basal grains from the middle spikelets (two per spikelet corresponding to florets *a* and *b*) on one side of the rachis were frozen in liquid nitrogen and freeze-dried. The total dry weight of these grains was measured, and grains were analysed for sucrose. Three of the basal grains from the middle spikelets on the other side of the rachis were fixed in acetic acid–absolute ethanol (1 : 3, v/v) and used to determine endosperm cell and starch granule number. However on day 12, the pericarp plus pigment strand was peeled off the grains before fixation, and freeze-dried for determination of sucrose. The remaining two or three basal grains of middle spikelets from four ears were fixed in Karnovsky's fixative (Karnovsky 1967) and used to determine endosperm cell area. The rest of the ear was oven-dried and kept for measurements of grain dry weight.

### *Grain Growth*

Changes in grain dry weight over time were represented by fitting a logistic model using non-linear regression (Gleadow *et al.* 1982). The model was used to give confidence limits for the asymptotic grain dry weight at maturity ( $K$ ) and the rate of grain growth at half- $K$  ( $R$ ).

### *Transpiration*

Transpiration was determined gravimetrically with a Mettler P10N balance weighing up to 10 kg with an accuracy of 1 g. Pots were weighed daily at dawn until day 24. These measurements were corrected for soil evaporation which was estimated by weighing a parallel set of pots containing soil only.

### *Water Potential*

$\Psi_{\text{soil}}$  was estimated from a previously established relation between soil water content and soil water potential. Pressure plates and pressure membranes were used to establish this relation. Soil water content was determined gravimetrically.  $\Psi_{\text{flag leaf}}$  was measured at dawn by the pressure chamber technique (Turner 1981).

### *Endosperm Cell Number and Starch Granule Number*

The number of nuclei in the endosperm was taken as an indicator of the number of cells in the endosperm. The number of endosperm nuclei was determined using a modification (Gleadow *et al.* 1982; Singh and Jenner 1982) of the method of Rijven and Wardlaw (1966). On day 18 the following modification was introduced to allow the measurement of nuclei number and starch granule number on the same sample. After digestion of the cell walls with 1% cellulysin, the solution was centrifuged (3000 g, 20 min) and the supernatant was discarded. The pellet of nuclei and starch granules was resuspended in 0.6 ml of 0.5 M sodium acetate buffer, pH 4.8. An aliquot (0.1 ml) was taken for starch granule counts and brought to a total volume of 1.0 ml. The remaining 0.5 ml was mixed with 3.0 ml of 12.5 mM calcium acetate containing 4000 units of  $\alpha$ -amylase (Calbiochem-Behring Corp., La Jolla, CA, U.S.A.). After incubation for a minimum of 2 h at 25°C, the nuclei were counted with a haemocytometer. The starch granules were counted as for nuclei after staining with an iodine solution. Small starch granules (diameter < 10  $\mu\text{m}$ ) were distinguished from large starch granules (diameter > 10  $\mu\text{m}$ ).

### *Endosperm Cell Area*

Grains fixed on days 20 and 30 in Karnovsky's fixative were stored at 4°C. The grains were embedded using JB-4 plastic medium (Polysciences Inc., Warrington, PA, U.S.A.). Transverse sections of 4.5  $\mu\text{m}$  thickness were cut with a glass knife, in the mid-region of the grains, on a Cambridge microtome (Cambridge Instruments). The sections were stained using periodic acid-Schiff's reagent and the endosperm cell area measured according to Gleadow *et al.* (1982). Ten grain sections were examined from each of four grains.

### *Deposition of Lipids in the Chalazal Zone of the Grains*

Transverse sections of grains fixed and embedded as above on day 20 were stained with Sudan Black B. The chalazal zone, including the pigment strand and nucellar projection between the vascular bundle in the crease and the endosperm cavity, was examined for the presence of lipids (Barlow *et al.* 1980).

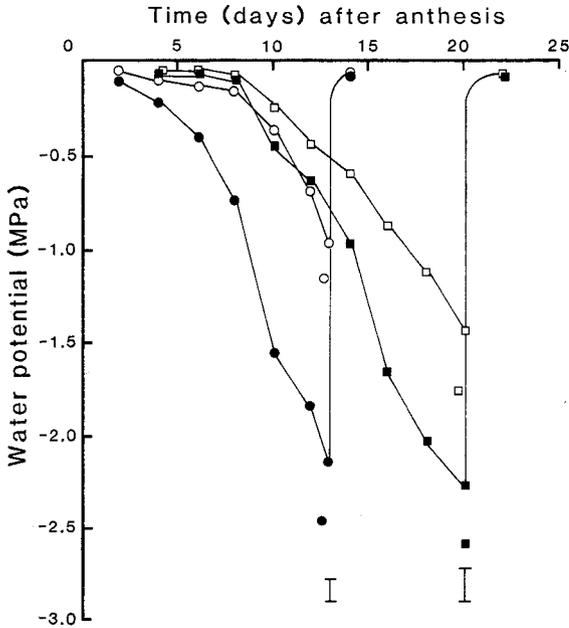
### *Grain Sucrose*

Freeze-dried grains or pericarp tissue were ground and extracted twice with 80% (v/v) ethanol at 80°C. Free glucose and fructose were destroyed by alkali and heat treatment (Jones *et al.* 1977). Sucrose was determined as the glucose released following incubation of the tissue extract with invertase for 1 h at 37°C. Glucose was determined enzymatically by a glucose-specific assay (Calbiochem-Behring Glucose S.V.R., No. 870104).

## Results

### Development of Water Deficit

Soil water potential ( $\Psi_{\text{soil}}$ ) was higher than  $-0.05$  MPa in control and high temperature treatments during the whole experimental period. The  $\Psi_{\text{soil}}$  declined less quickly under drought alone than drought combined with high temperature (Fig. 1). If we assume a  $\Psi_{\text{soil}}$  of  $-0.5$  MPa and lower as indicating a moderate to severe plant water deficit, the duration



**Fig. 1.** Soil water potential in the drought and drought  $\times$  high temperature treatments. Since soil water potential of the control and high temperature treatments was always higher than  $-0.05$  MPa and showed little variation, it was not plotted on the graph. Water potential of the flag leaf just before rewatering is also represented on day 13 (early period) and day 20 (late period) (isolated symbols). Error bars on days 13 and 20 represent the l.s.d. ( $P = 0.05$ ) between means of treatments.

Drought

○ Early period.

□ Late period.

Drought  $\times$  high temperature

● Early period.

■ Late period.

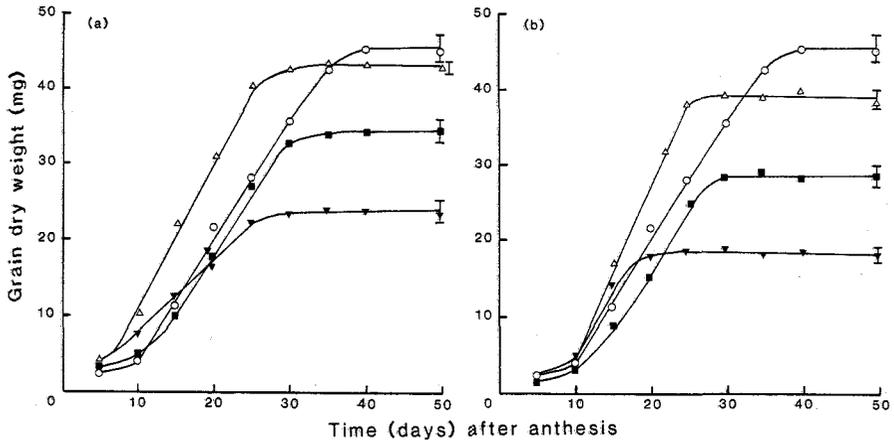
**Table 1.** Components of grain yield per ear

Means within columns with a common superscript are not significantly different at  $P = 0.05$  using the least significant difference between means. The percentage reduction relative to the value in control is given in parentheses

Treatment	Grain yield per ear (mg)	No. of grains per ear	Average grain dry wt (mg)
Control	1882 <sup>a</sup>	43 <sup>a</sup>	43.8 <sup>a</sup>
P <sub>1</sub> HT	1604 <sup>b</sup> (15)	40 <sup>b</sup>	40.1 <sup>b</sup> (8)
Drought	1178 <sup>c</sup> (37)	39 <sup>b</sup>	30.2 <sup>d</sup> (31)
Drought $\times$ HT	788 <sup>e</sup> (58)	35 <sup>c</sup>	22.5 <sup>f</sup> (49)
P <sub>2</sub> HT	1542 <sup>b</sup> (18)	43 <sup>a</sup>	35.9 <sup>c</sup> (18)
Drought	1054 <sup>d</sup> (44)	41 <sup>ab</sup>	25.7 <sup>e</sup> (41)
Drought $\times$ HT	651 <sup>f</sup> (65)	41 <sup>ab</sup>	15.9 <sup>g</sup> (65)
L.s.d. ( $P = 0.05$ )	58	2	2.5

of moderate to severe water deficit was 3 days for P<sub>1</sub> drought, 7 days for P<sub>1</sub> drought  $\times$  HT, 8 days for P<sub>2</sub> drought and 10 days for P<sub>2</sub> drought  $\times$  HT. Drought intensity, as indicated by the value of  $\Psi_{\text{soil}}$  and  $\Psi_{\text{flag leaf}}$  just before rewatering (Fig. 1), was similar in the two drought  $\times$  HT treatments, but was more pronounced in P<sub>2</sub> drought than P<sub>1</sub> drought.

The daily rate of transpiration declined gradually under drought and increased markedly in the high temperature treatments. In the drought  $\times$  HT treatments, transpiration rate increased initially upon imposition of the high temperature treatments, but declined quickly thereafter.



**Fig. 2.** Grain dry weight of plants subjected to treatments during the early period (a) and late period (b) of cell division. Curves were fitted using a logistic model.  $\circ$  Control.  $\triangle$  High temperature.  $\blacksquare$  Drought.  $\blacktriangledown$  Drought  $\times$  high temperature. Error bars, representing confidence limits ( $P = 0.05$ ) of the asymptotic weight,  $K$ , were calculated using the model.

### Grain Growth

The reduction in grain yield per ear ranged from 15 to 65% depending on the treatment (Table 1). Although grain set was reduced when the high temperature and drought treatments were imposed during the early period of cell division ( $P_1$ ), the reduction in

**Table 2.** Parameters of dry matter accumulation in basal grains of middle spikelets  
Confidence limits ( $P = 0.05$ ) for  $K$ , calculated using a logistic model of grain growth are given. Confidence limits for  $R$  calculated from two parameters in the model, were asymmetrical about the rate. Values within columns with a common superscript are not significantly different at  $P = 0.05$  using the confidence limits predicted by the model. Validity of the logistic model was assessed by comparing  $K$  and the predicted final weight, calculated as (rate  $\times$  duration) + (weight at the end of the lag phase)

Treatment	Rate of growth, $R$ (mg day <sup>-1</sup> )	Duration of linear phase (days)	Weight at end of lag phase (mg)	Predicted final weight (mg)	Grain dry weight, $K$ (mg)
Control	1.48 <sup>c</sup>	27	4.0 $\pm$ 0.2	44	46.1 $\pm$ 1.7 <sup>a</sup>
$P_1$ HT	1.72 <sup>b</sup>	21	4.9 $\pm$ 0.2	41	43.3 $\pm$ 1.0 <sup>b</sup>
Drought	1.36 <sup>c</sup>	18	6.5 $\pm$ 0.4	31	34.9 $\pm$ 0.8 <sup>d</sup>
Drought $\times$ HT	0.89 <sup>d</sup>	20	4.2 $\pm$ 0.3	22	24.6 $\pm$ 0.8 <sup>f</sup>
$P_2$ HT	2.14 <sup>a</sup>	16	4.8 $\pm$ 0.3	39	39.2 $\pm$ 0.8 <sup>c</sup>
Drought	1.38 <sup>c</sup>	18	3.2 $\pm$ 0.4	28	29.0 $\pm$ 0.8 <sup>e</sup>
Drought $\times$ HT	1.88 <sup>b</sup>	8	4.0 $\pm$ 0.4	19	18.6 $\pm$ 0.6 <sup>e</sup>

grain set accounted for less than 10% of the reduction in grain yield per ear. Therefore the average grain dry weight appeared to be the main component of grain yield. The high temperature and drought treatments affected both the duration and rate of growth in basal grains of middle spikelets (Fig. 2 and Table 2).

Drought alone significantly reduced final grain weight; the reduction was greater when drought was applied late rather than early during the period of cell division (reduction of 37% and 24%, respectively, relative to control treatment). The reduction in final grain weight was due mainly to a shorter duration of grain growth (Table 2). High temperature alone also significantly reduced the final grain weight but the reduction was smaller (5% in  $P_1$ , 13% in  $P_2$ ) than that caused by the drought treatments. The rates of grain growth were 16% and 44% higher than in the control treatment for  $P_1$  and  $P_2$  respectively, but the increased rates did not entirely compensate for the shortened durations (Table 2).

The combined effect of drought and high temperature was much more pronounced than that of each treatment alone, as final grain weight was reduced by 49% and 60% when the combined treatment was applied during the early and later periods of cell division, respectively. The main effect of the combined treatments was a reduction of 13 and 17 days in duration of grain growth for  $P_1$  and  $P_2$  treatments, respectively.

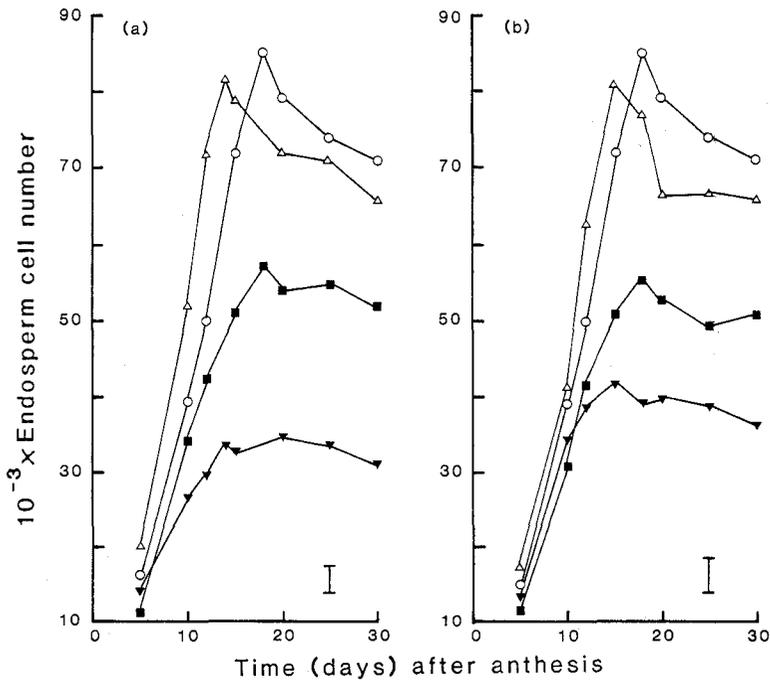


Fig. 3. Number of endosperm cells for plants subjected to treatments during the early period (a) and late period (b) of cell division. Error bars represent the 1 s.d. ( $P = 0.05$ ) between means of treatments at any one time. Symbols as for Fig. 2.

#### *Endosperm Cell Number and Cell Size*

The number of nuclei increased for 14 to 18 days depending on the treatment (Fig. 3 and Table 3), followed by a gradual decline until maturity. While the decrease in nuclei number after the maximum was reached was significant at 1% probability level in the control and high temperature treatments, it was not significant in the drought and drought  $\times$  HT treatments. This reduction in number of nuclei may be due to the rupture of nuclei membranes under the pressure of growing starch granules (Frazier and

Appalanaidu 1965); fewer nuclei would have been disrupted in the drought treatments as much less starch granules were formed (Table 4). Alternatively, the developing embryo may digest neighbouring endosperm cells (Smart and O'Brien 1983); the different rates of decline in nuclei number between treatments would then reflect differences in catalytic activity of the embryo.

High temperature alone did not decrease significantly the maximum number of endosperm cells: the effect of a shortened duration was compensated by a higher rate of cell division (Table 3; Fig. 3). Drought decreased the maximum number of endosperm cells by about one-third of both P<sub>1</sub> and P<sub>2</sub> treatments and this reduction was probably due to the reduced rate of cell division after day 12 (Fig. 3). Drought had begun to affect cell division before day 10 in P<sub>2</sub> (Fig. 3*b*) and this may account for the severity of the late treatment on cell division. Duration of cell division was the same as in control (Table 3). The combined drought and high temperature treatments led to the largest reductions in maximum cell number, i.e. 60% and 52% reduction for P<sub>1</sub> and P<sub>2</sub> treatments, respectively. The reduction in maximum cell number was due to both a reduced duration and a reduced rate of cell division after day 10 (Fig. 3).

Cell cross-sectional areas ranged from 634 to 919 × 10<sup>-3</sup> μm<sup>2</sup> on day 20 and from 823 to 1033 × 10<sup>-3</sup> μm<sup>2</sup> on day 30, depending on the treatment (Table 3). Cell size on day 30 represented the final cell size for all the stress treatments as the duration of grain growth (Table 2) was shorter than 30 days in these treatments. Cell size was significantly reduced only in the drought and drought × HT treatments during the late period of cell division (reduction of 20% and 8%, respectively, on day 30).

**Table 3. Maximum number of endosperm nuclei, duration of cell division and area of endosperm cells from basal grains of middle spikelets**

Duration of cell division is derived from the data in Fig. 4. Means within columns with a common superscript are not significantly different at  $P = 0.05$  using the least significant difference between means

Treatment	Max. No. of nuclei	Duration of cell division (days)	10 <sup>-3</sup> × Area of cells (μm <sup>2</sup> )	
			Day 20	Day 30
Control	86123 <sup>a</sup>	18	700 <sup>d</sup>	1023 <sup>a</sup>
P <sub>1</sub> HT	81206 <sup>a</sup>	14	793 <sup>b</sup>	1033 <sup>a</sup>
Drought	58552 <sup>b</sup>	18	722 <sup>c</sup>	994 <sup>a</sup>
Drought × HT	34562 <sup>d</sup>	14	753 <sup>bc</sup>	1028 <sup>a</sup>
P <sub>2</sub> HT	82150 <sup>a</sup>	15	884 <sup>a</sup>	995 <sup>a</sup>
Drought	56304 <sup>b</sup>	18	634 <sup>d</sup>	823 <sup>c</sup>
Drought × HT	41679 <sup>c</sup>	15	919 <sup>a</sup>	943 <sup>b</sup>
L.s.d. ( $P = 0.05$ )	6190		50	40

### Starch Granule Number

Both high temperature treatments had no significant effect on the number of large starch granules (*L*), but increased markedly the number of small starch granules (*S*) on day 18, and this resulted in a high *S/L* ratio (Table 4). Both drought treatments decreased significantly the number of large and small starch granules. The decrease in number of large granules was more pronounced when drought occurred during the early period of cell division, whereas the decrease in number of small granules was greatest when drought occurred during the late period of cell division. The number of large granules per cell was similar for P<sub>1</sub> drought and higher for P<sub>2</sub> drought relative to the control treatment (Table 4).

The number of small granules per cell was higher for P<sub>1</sub> drought and lower for P<sub>2</sub> drought. Clearly we need to consider the timing and intensity of water deficit during the period of starch granule initiation to interpret these results.

The combined drought × HT treatments led to the most significant reductions in number of small and large starch granules per endosperm. However, the reduction in number of large granules per endosperm was due not to a reduction in number of large granules per cell but to a reduction in cell numbers. There was in fact a significant compensatory increase in number of large granules per cell (Table 4). Similarly, the decrease in small granules per endosperm for the P<sub>1</sub> treatment was due to the reduction in cell numbers and there was a significant compensatory increase in number of small granules per cell. However in the P<sub>2</sub> treatment, the initiation of small starch granules was affected as well as cell division.

**Table 4.** Number of starch granules per endosperm and per cell on day 18 in basal grains of middle spikelets

Number of granules per cell were estimated using the maximum cell numbers in Table 3. Values within columns with a common superscript are not significantly different at  $P = 0.05$  using the least significant difference between means

Treatment	10 <sup>-6</sup> × No. of starch granules per endosperm		Ratio of small to large granules	No. of starch granules per cell	
	Large	Small		Large	Small
Control	4.3 <sup>a</sup>	45.6 <sup>c</sup>	10.6 <sup>c</sup>	50 <sup>cd</sup>	529 <sup>d</sup>
P <sub>1</sub> HT	4.0 <sup>a</sup>	71.6 <sup>b</sup>	17.9 <sup>a</sup>	49 <sup>d</sup>	882 <sup>b</sup>
Drought	2.8 <sup>bc</sup>	36.2 <sup>d</sup>	12.9 <sup>b</sup>	48 <sup>d</sup>	618 <sup>c</sup>
Drought × HT	2.3 <sup>c</sup>	32.1 <sup>d</sup>	14.0 <sup>b</sup>	66 <sup>a</sup>	929 <sup>b</sup>
P <sub>2</sub> HT	4.3 <sup>a</sup>	84.1 <sup>a</sup>	19.5 <sup>a</sup>	52 <sup>cd</sup>	1024 <sup>a</sup>
Drought	3.5 <sup>b</sup>	25.3 <sup>e</sup>	7.2 <sup>c</sup>	62 <sup>ab</sup>	449 <sup>e</sup>
Drought × HT	2.4 <sup>c</sup>	15.8 <sup>f</sup>	6.6 <sup>c</sup>	57 <sup>bc</sup>	379 <sup>f</sup>
L.s.d. ( $P = 0.05$ )	0.8	6.7	2.5	7	67

#### *Deposition of Lipids in the Chalazal Zone of the Grain*

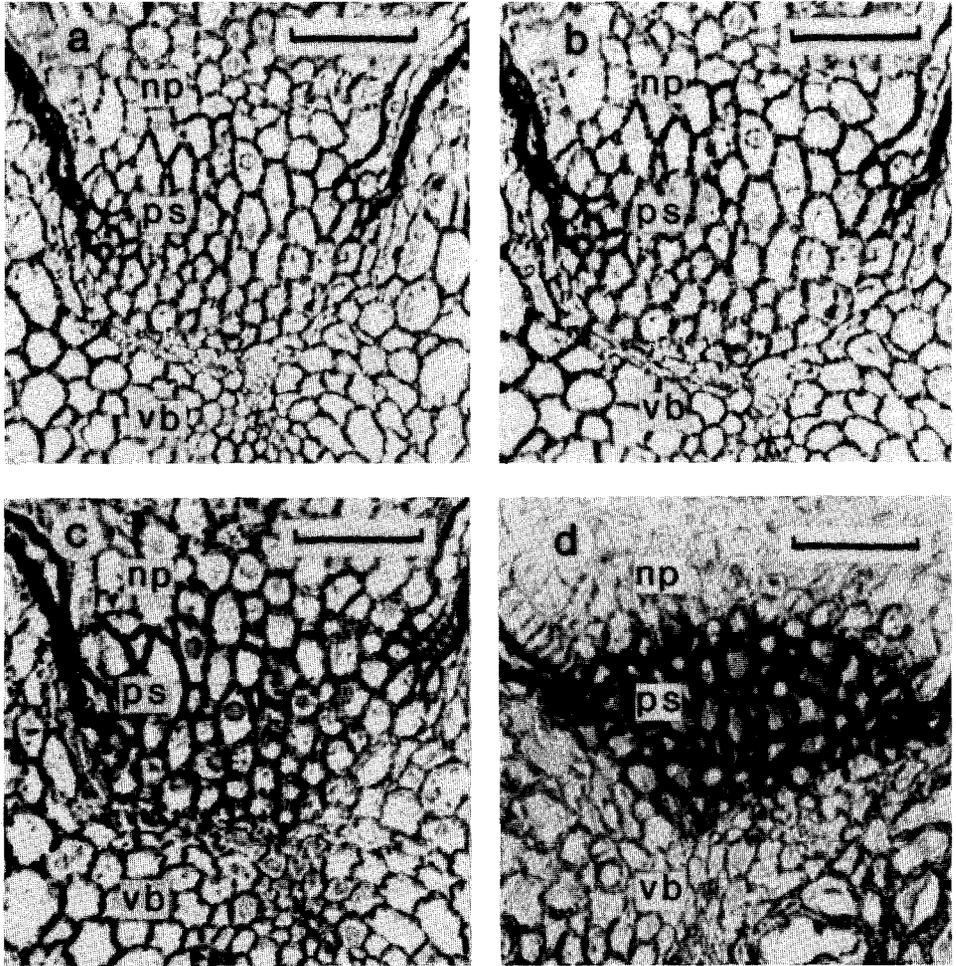
There was no visible deposition of lipids (sudanophilic substances) in the chalazal zone of grains (basal grains of middle spikelets) from control plants on day 20 (Fig. 4). On the other hand, there was considerable lipid deposition in the drought × HT treatments. Results were intermediate for the drought or high temperature treatments alone. Treatments which accelerated markedly grain maturation (Table 2) also accelerated lipid deposition in the chalazal zone of grains.

#### *Grain Sucrose*

The amount of sucrose per grain increased up to a maximum on days 10–14 depending on the treatment (Fig. 5) and then declined to less than 500 µg by day 25. The decrease in amount of sucrose was faster and more pronounced in the high temperature and drought × HT treatments than under control or drought treatments.

The maximum amounts of sucrose per grain differed significantly between treatments (Table 5). Sucrose content was highest in the high temperature and control treatments and lowest in the drought × HT treatments. The differences in sucrose per grain were related mainly to differences in grain dry weight, and the maximum amount of sucrose varied over a much narrower range (Table 5) when expressed on a dry matter basis. Indeed, it differed significantly from the value in control only in the P<sub>2</sub> drought × HT treatment.

Sucrose levels are better expressed on an endosperm basis than a whole grain basis in order to relate the sucrose levels to cell division and starch accumulation in the endosperm. As the high temperature and drought treatments affected the number of endosperm cells (Fig. 4), results were expressed per endosperm cell (Table 5). Values ranged from 7.0 to



**Fig. 4.** Deposition of lipids (sudanophilic substances) in the chalazal zone of the grains. Transverse sections (10  $\mu\text{m}$ ) were cut from grains of control plants (a), or plants subjected to high temperature (b), drought (c) or drought  $\times$  high temperature (d) during the late period of cell division. The endosperm cavity is at the top of the picture. *np*, nucellar projection; *ps*, pigment strand; *vb*, vascular bundle. Horizontal bars represent 100  $\mu\text{m}$ .

7.9  $\mu\text{g}$  sucrose per 1000 cells and tended to be higher in the drought  $\times$  HT treatments. The relationship between endosperm cell number ( $C$ ) and maximum amount of sucrose per endosperm ( $S_E$ ) was linear ( $C = -36420 + 1470S_E$ ,  $r^2 = 0.94$ ).

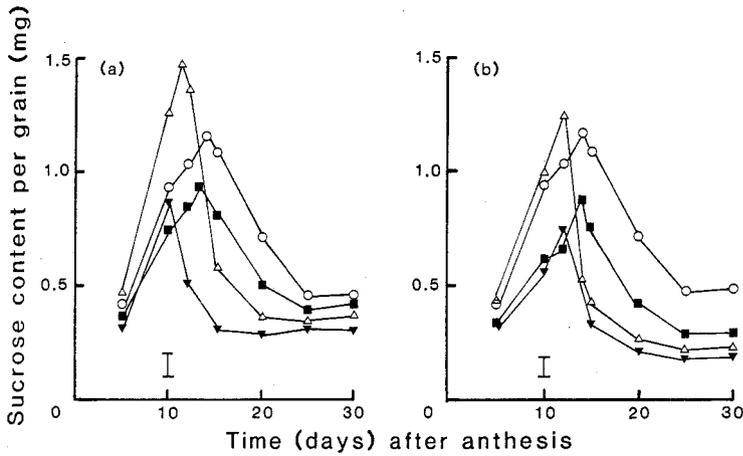


Fig. 5. Sucrose content per grain for plants subjected to treatments during the early period (a) and late period (b) of cell division. Error bars represent the l.s.d. ( $P = 0.05$ ) between means of treatment at any one time. Symbols as for Fig. 2.

Table 5. Maximum amount of sucrose per grain and per endosperm

The amount of sucrose per endosperm was calculated as the difference between the amount of sucrose per grain on day of maximum sucrose and the amount of sucrose per pericarp plus pigment strand on day 12. Amount of sucrose per endosperm cell was estimated using the number of endosperm cells on day of maximum sucrose (Fig. 4). Means within columns with a common superscript are not significantly different at  $P = 0.05$  using the least significant difference between means

Treatment	Day of max. amount sucrose	Grain	Amount of sucrose ( $\mu\text{g}$ ) per: mg dry wt	Pericarp + pigment strand	1000 cells
Control	14	1239 <sup>b</sup>	113 <sup>ab</sup>	777 <sup>b</sup>	7.1
P <sub>1</sub> HT	11	1421 <sup>a</sup>	109 <sup>b</sup>	996 <sup>a</sup>	7.1
Drought	13	949 <sup>c</sup>	118 <sup>a</sup>	627 <sup>c</sup>	7.0
Drought $\times$ HT	11	830 <sup>cd</sup>	104 <sup>bc</sup>	616 <sup>c</sup>	7.7
P <sub>2</sub> HT	12	1230 <sup>b</sup>	123 <sup>a</sup>	774 <sup>b</sup>	7.4
Drought	14	862 <sup>cd</sup>	107 <sup>bc</sup>	517 <sup>d</sup>	7.2
Drought $\times$ HT	12	751 <sup>d</sup>	99 <sup>c</sup>	450 <sup>d</sup>	7.9
L.s.d. ( $P = 0.05$ )		181	10	96	

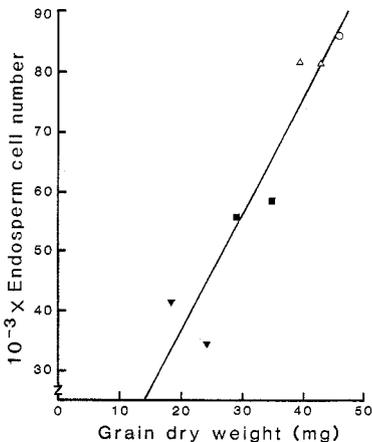


Fig. 6. Relationship between the dry weight of mature grains and the maximum number of endosperm cells.

The regression equation is  $y = -1792 + 1922x$ ,  $r^2 = 0.94$ .

- Control.
- △ High temperature.
- Drought.
- ▼ Drought  $\times$  high temperature.

On the day when sucrose level reached a maximum, the weight per cell in the stress treatments was equal to or higher than that of the control treatment (control, 150  $\mu\text{g}/1000$  cells; P<sub>1</sub> HT, 204; P<sub>1</sub> drought, 174; P<sub>1</sub> drought  $\times$  HT, 309; P<sub>2</sub> HT, 146, P<sub>2</sub> drought, 160, P<sub>2</sub> drought  $\times$  HT, 215). At maturity the weight per cell was very similar for all treatments (Fig. 6).

## Discussion

### *Determination of Grain Size*

Cell division, cell enlargement and starch accumulation determine the storage capacity of the grain which is, at a cellular level, the number of sites available for starch (and protein) accumulation. Assuming no limitation to sucrose supply within the endosperm, the sink size of the grain largely determines the subsequent rate of dry matter accumulation, as clearly shown by the linear relationship between endosperm cell number and dry weight of the mature grain (Fig. 6).

High temperature (28°C day/20°C night) accelerated development of the grain. Higher rate and shorter duration of cell division and dry matter accumulation were measured in the high temperature treatments (Figs 3 and 4). Endosperm cells were nearer to their final size on day 20 in the HT treatments (P<sub>1</sub>, 77%; P<sub>2</sub>, 89% of final size; control, 68% of size on day 30); however, cell size was not significantly different between control and HT treatments on day 30 (Table 3). About 80% of cell enlargement is reported to occur between the end of cell division and end of dry matter accumulation (Hughes 1976); this period of grain growth was noticeably shorter in the HT treatments. Our data suggest that duration may be shorter and rate higher for cell enlargement in the HT treatments, although this point requires further investigation. The faster appearance of new sites for starch accumulation resulted in a higher rate of dry matter accumulation. Bhullar and Jenner (1983) observed a reduced rate of dry matter accumulation following ear warming. This difference in growth response may be related to the higher temperature regime used in their experiments (33°C day/25°C night), or may reflect varietal differences. By contrast, the duration of grain growth was shortened by increase in temperature in the whole range between 15 and 30°C (Chowdhury and Wardlaw 1978).

Drought and drought  $\times$  HT reduced the storage capacity of the grain. Maximum cell number and number of starch granules per endosperm were reduced (Tables 3 and 4). Cell size and number of small granules per cell were also reduced when the stress treatments took place during the late period of cell division (P<sub>2</sub> treatments). The degree of reduction in storage capacity and subsequent accumulation of dry matter depended on the intensity and timing of water deficit. Water deficit was more severe when high temperature was combined with drought (Fig. 1), and maximum cell number was reduced by 50–60% under those conditions (Table 3). Timing was also important as severe water deficit coincided only with cell enlargement and initiation of small starch granules in the P<sub>2</sub> treatments. Consequently the latter treatments caused a larger reduction in final cell size and number of small granules per cell.

The reduction in final grain weight was partly due to a shorter duration of grain growth in the drought  $\times$  HT treatments. Duration of grain growth was reduced by as much as 13 and 17 days for the P<sub>1</sub> and P<sub>2</sub> drought  $\times$  HT treatments respectively. The phase of cell division was shorter in the drought  $\times$  HT treatments but not in the drought treatments; the period of grain growth going from end of cell division until maturity was shorter in both drought and drought  $\times$  HT treatments (e.g. 8 and 5 days in P<sub>2</sub> drought and P<sub>2</sub> drought  $\times$  HT compared with 19 days in control). As mentioned earlier, most of cell enlargement occurs during that period. The considerable deposition of lipids in the chalazal zone of the grains observed in the drought  $\times$  HT treatments on day 20 (Fig. 4) could have sealed off the pathway of water and nutrient flow to the endosperm and terminated grain growth (Zee and O'Brien 1971; Sofield *et al.* 1977b; Barlow *et al.* 1980). However, there

was no simple correspondence between lipid deposition and termination of grain growth. The rate of dry matter accumulation was not reduced between days 20 and 24 in the P<sub>1</sub> drought × HT treatment despite a large deposition of lipids, and the reduction in duration of grain growth did not correspond to an important deposition of lipids when drought alone was imposed to the plants. Lipid deposition is unlikely to be the only cause of grain maturation. Although abscisic acid is involved both in the plant response to drought (e.g. Aspinall 1980) and in grain maturation (McWha 1975; King 1979), no clear cause and effect relationship has been demonstrated yet. It is worthwhile to note that, whatever the factors regulating the duration of grain growth, they affected duration both during and after the stress period.

#### *Relationship between Sink Size and Sucrose Levels in the Grain*

The rate of cell division or starch synthesis is frequently assumed to be regulated by assimilate supply but this hypothesis has been tested by only a few authors (Jenner and Rathjen 1978; Bhatia *et al.* 1980; Singh and Jenner 1982). Fewer still have looked at the relationship between the number of cells and starch granules and the sugar concentrations in the grains of droughted plants (e.g. Brooks *et al.* 1982).

In our experiment, sucrose concentrations were measured in the grain and endosperm because sucrose is the main form of sugar translocated to the grains and the main substrate for starch synthesis (e.g. Porter 1962; Duffus 1979). The expression of the results per endosperm cell rather than per grain or unit dry matter was preferred because the stress treatments had a marked effect on dry matter accumulation and maturation of the grains. Differences in sucrose per grain reflected largely differences of grain dry weight, whereas differences in sucrose per unit dry matter reflected differences in rate of starch accumulation. The endosperm cell was a more constant unit as dry matter per cell and number of large starch granules per cell showed little variation (Fig. 6; Table 4).

The maximum amount of sucrose per cell varied within a narrow range and was highest in the most severe stress treatments, i.e. the drought × HT treatments (Table 5). The amount of dry matter per cell on the day of maximum level of sucrose provides an estimate of sucrose utilization per cell. In the stress treatments, dry matter per cell was equal to or higher than in the control treatment and was highest in the drought × HT treatments. When considered together, the maximum amount of sucrose per cell and the amount of sucrose utilized per cell indicate that the supply of sucrose *per cell* was at least equal to the control in the most severe stress treatments. However, the initiation of small starch granules per cell was most reduced in these treatments (Table 4) and this shows that at least one of the cellular processes taking place in the endosperm was not regulated by assimilate supply. This result supports the notion that dry matter accumulation is not limited by assimilate supply (Brooks *et al.* 1982; Singh and Jenner 1982; Bhullar and Jenner 1983).

The linear relationship observed between number of endosperm cells and amount of sucrose per endosperm could mean either that the supply of sucrose regulates cell division or that the number of endosperm cells determines the level of sucrose supply. Although *in vitro* experiments show that a minimum level of sucrose is necessary for cell division to occur (Van't Hof 1966), there is no evidence in the literature that sucrose concentration above a minimum level regulates cell division. The alternative proposition, that the number of cells determines the level of sucrose supply, implies that cell division and initiation of starch granules are regulated by one (or several) factor(s) other than assimilate supply. The possible interaction of abscisic acid coming from droughted leaves and cytokinins in the dividing endosperm cells is being investigated currently.

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