Annals of Botany **55**, 433–444, 1985

Effect of Post-anthesis Drought on Cell Division and Starch Accumulation in Developing Wheat Grains

MARC E. NICOLAS, ROSLYN M. GLEADOW
and MICHAEL J. DALLING

Plant Sciences Section, School of Agriculture and Forestry, University of Melbourne, Parkville, Victoria 3052, Australia

Accepted 1 October 1984

ABSTRACT

Wheat plants (*Triticum aestivum* L., cv. Warigal) were subjected to 20 d of water deficit during the period of endosperm cell division. Drought accentuated the differences in final grain weight between spikelets and between grains within spikelets. The distal grains of top spikelets were most affected by drought. The maximum number of endosperm cells was, respectively, 30 and 40 per cent lower in basal grains and distal grains of droughted plants. In basal grains of middle spikelets, the number of large starch granules per cell was unaffected but the number of small starch granules per cell was 45 per cent lower in grains of droughted plants. The initiation of small starch granules was more affected than cell division because severe water deficit occurred earlier during the former process than the latter. Final dry weight appeared to correlate well with the maximum number of endosperm cells, but depended also on the number of starch granules per cell. Consequently, the amount of dry matter per cell was not constant in both treatments.

The concentration of sucrose per endosperm cell was lower only in the droughted distal grains of top spikelets. The supply of sucrose to endosperm cells did not regulate the initiation of small starch granules.

Key words: *Triticum aestivum* L., wheat, drought, grain growth, cell division, starch.

INTRODUCTION

Water deficit during early grain development results in a reduction of grain dry weight at maturity (Asana, Saini and Ray, 1958; Wardlaw, 1971; Brocklehurst, Moss and Williams, 1978; Brooks, Jenner and Aspinall, 1982). During the first 15–20 d after anthesis, cell division occurs in the grain endosperm and large A-type starch granules are initiated (Hoshikawa, 1961; Buttrose, 1963; Evers, 1970; Briarty, Hughes and Evers, 1979). Small B-type granules are initiated from the end of cell division until maturity, and can account for up to 98 per cent of total granule number and up to 40 per cent of the total mass of starch at maturity (Evers and Lindley, 1977; Briarty *et al.*, 1979). Brocklehurst *et al.* (1978) attributed the reduction in final dry weight of droughted grains to the formation of fewer endosperm cells. Indeed, the number of endosperm cells appears to largely determine the capacity of the grain to accumulate dry matter (Jenner, 1979; Gleadow, Dalling and Halloran, 1982; Singh and Jenner, 1982). However, Brooks *et al.* (1982) considered that the reduction in number and size of starch granules affected final grain weight most.

Grains differ in final dry weight depending on their position within the wheat ear (Rawson and Evans, 1970; Bremner and Rawson, 1978; Simmons and Crookston, 1979); this pattern is modified by treatments which reduce assimilate supply (Bremner, 1972). Consequently, differences in grain dry weight are often attributed to differences in assimilate supply to the grains (McPherson and Boyer, 1977; Bremner and Rawson, 1978; Brocklehurst *et al.* 1978), although Singh and Jenner (1982) have cast doubt on this interpretation.

0305-7365/85/030433+12 $03.00/0

The present investigation was undertaken to determine: (i) the effect of drought on the accumulation of dry matter, cell division and starch granule development in grains situated at different positions within the ear; (ii) any relation between assimilate concentration in the grain, number of cells or starch granules, and final grain weight in well-watered and droughted plants.

MATERIALS AND METHODS

Growth conditions

Wheat (*Triticum aestivum* L. cv. Warigal) was grown in 35 cm long × 9 cm diam. pots containing 2 kg of sterilized, air-dried soil. The soil mixture consisted of Mt. Derrimut loam (38 per cent clay, 21 per cent silt and 37 per cent sand), washed quartz sand, vermiculite and perlite in the proportions 2:2:1:1. One plant was grown per pot and tillers were removed as they appeared. Plants received a basal dressing at sowing and a side application of nutrients at the booting stage. The soil was covered with polyethylene beads in order to minimize soil evaporation.

Plants were initially grown in a naturally illuminated glasshouse with the photoperiod extended to 18 h using incandescent lights. The maximum day temperature was 25 °C and the minimum night temperature 10 °C. Two weeks before anthesis plants were transferred to a controlled environment room with 18 h, 24 °C day and a 6 h, 18 °C night. The photosynthetic photon flux density (400–700 nm) at plant height was 450 μmol $m^{-2}s^{-1}$. The relative humidity was approx. 70 per cent. Anthesis was judged as the appearance of exerted anthers in the middle spikelets and occurred over a 5-d period, 50 d after sowing.

At anthesis, plants were distributed between well-watered (control) and droughted treatments. The control plants were watered daily to field capacity. The droughted plants were not rewatered until day 20.

Harvesting procedure

Six plants per treatment were harvested every 3 d from day 6 to day 24, and every 4–5 d until day 50. A final harvest was made on day 70. Two supplementary harvests were made on day 18 and 20 to determine precisely the end of cell division in the endosperm. The flag leaves of three plants per treatment were sampled 1 h after dawn and leaf water potential (ψ) was measured at 24 ± 2 °C using a Wescor HR-33T Dew Point Microvoltmeter with either Wescor C-51 or C-52 sample chambers (Campbell, Campbell and Barlow, 1973). Sample chambers were individually calibrated with NaCl standard solutions. Leaf discs were placed in the sample chambers and allowed to equilibrate for 3 h.

For each treatment, three ears were divided equally into three groups of approx. six spikelets: basal (B), middle (M) and top (T) spikelets. The basal grains (florets a and b) from three middle spikelets on one side of the rachis were weighed immediately, then frozen in liquid nitrogen and freeze-dried. These grains were analyzed later for sucrose content. The difference between fresh weight and dry weight was taken as the water content of the grains. The distal grains (floret c and floret d when it existed) from three top spikelets on one side of the rachis were freeze-dried and analyzed for sucrose. Four basal grains of middle spikelets were sampled on the other side of the rachis and fixed in acetic acid:absolute ethanol (1:3 v/v) for determination of endosperm cell and starch granule number. The same procedure was used for four distal grains of top spikelets but only endosperm cell numbers were determined.

Between day 11 and 19 the pericarp and pigment strand of the grains used for cell counts was peeled off before fixation and freeze dried for determination of sucrose. Three

supplementary harvests were made on day 14, 15 and 17 to measure accurately the sucrose levels in the endosperm. Four basal grains of middle spikelets and four distal grains of top spikelets were sampled, their endosperm was removed and freeze-dried for sucrose estimation. Intact grains at equivalent positions on the other side of the rachis were also analyzed for sucrose content. Spikelets or ears which had not been used in this procedure were oven-dried (80 °C) and kept for measurement of dry weight.

Grain set and grain growth

Grain set was the number of grains expressed as a percentage of the number of florets (Saini and Aspinall, 1982).

Changes in dry weight of the basal grains of middle spikelets were represented by fitting a logistic model (Gleadow *et al.*, 1982).

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). A further 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 per cent 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 0·5 M Na acetate buffer, pH 4·8; A 0·1 ml aliquot 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 Ca acetate containing 4000 units of α-amylase (Calbiochem Behring Corp. La Jolla, CA, USA). After incubation for at least 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 (diam. $< 10\ \mu$m) were distinguished from large starch granules (diam. $> 10\ \mu$m).

Grain sucrose

Freeze-dried grains or pericarp tissue were ground with a mortar and pestle and extracted twice with 80 per cent v/v ethanol at 80 °C. Free glucose and fructose were destroyed by alkali and heat treatment (Jones, Outlaw and Lowry, 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 by a glucose-specific assay (Calbiochem-Behring Glucose S.V.R., NO. 870104).

RESULTS

Water relations of the flag leaf

Under well-watered conditions, ψ declined slowly throughout the experiment period (Fig. 1). In water-stressed plants, ψ declined slowly from day 8 to 16, at a rate of 0·09 MPa d^{-1}, then fell rapidly at a rate of 2·6 MPa d^{-1}. This drought treatment was less severe than those of Munns, Brady and Barlow (1979) and Munns and Weir (1981), but more severe than water deficits recorded in large container or field experiments (Jones and Turner, 1980; Wilson *et al.*, 1980).

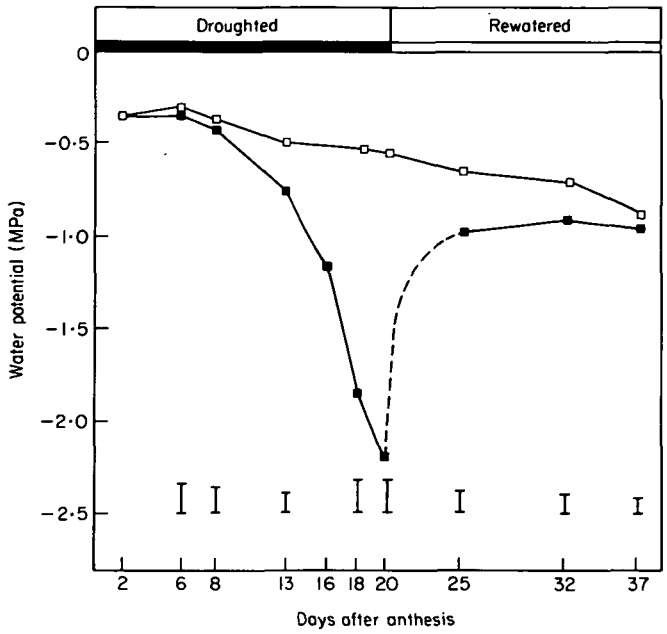

FIG. 1. Total water potential (ψ) of the flag leaf in control (□) and droughted plants (■). The dashed line between day 20 and 25 emphasizes that the shape of the curve is not known with certainty upon rewatering. Error bars represent L. S.D. at $P = 0{\cdot}05$ level.

TABLE 1. *Parameters of dry matter accumulation in basal grains of middle spikelets*

Treatment	Final d. wt K (mg)	Rate of growth at $K/2$ R (mg d^{-1})	Duration of growth (d)	
			D_1	D_2
Control	57·9 ± 1·3	1·48	35·6 ± 1·1	41
Droughted	35·8 ± 1·0	1·04	31·9 ± 1·4	35

Parameters K, R and D_1 calculated using a logistic model of grain growth. K and D_1 are given ± confidence limits at $P = 0{\cdot}05$. Means of K, R, D_1 are significantly different at $P = 0{\cdot}05$ level. D_2 calculated as the time taken to reach the point having for ordinate the lowest confidence limit of K.

Grain set

Drought did not reduce grain set except in the top spikelets of the ear where sterility was 16 per cent higher than in control. The florets of top spikelets flowered 2–3 d after the basal florets of middle spikelets. The grains of top spikelets were in the early phase of cell division when the drought treatment became severe. This may indicate that the early phase of cell division is sensitive to even a mild water deficit.

Grain growth

At maturity the dry weight of the basal grains of middle spikelets was 57·9 mg in the controls and the duration of growth (D_2) was 41 d (Table 1). D_2 values were more realistic than D_1 values as grain growth continued at a slow rate for up to 5 d after the inflexion point was reached on the curve. Grain dry weight at maturity was 33 per cent lower in

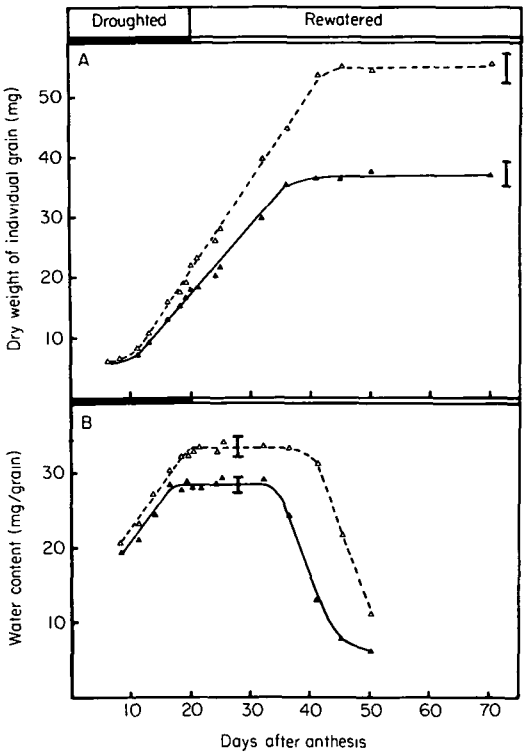

FIG. 2. Dry matter accumulation and water content of the basal grains of middle spikelets in control (△) and under drought (▲). The curves of grain dry weight were fitted using a logistic model. Error bars, representing confidence limits ($P = 0{\cdot}05$) of the asymptotic weight K, were calculated using the model. A, Dry weight; B, water content.

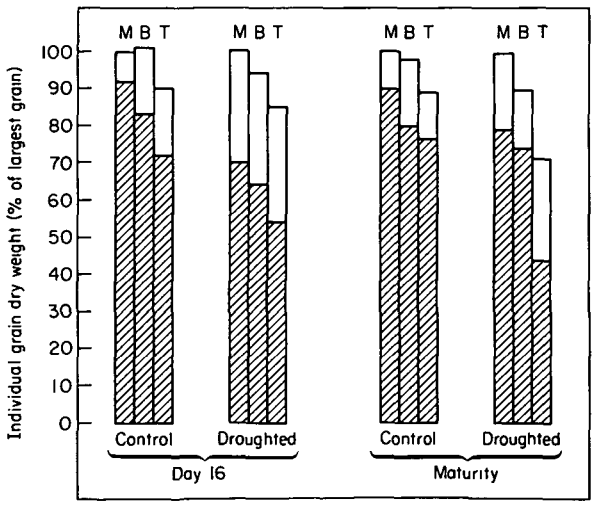

FIG. 3. Individual grain dry weight as a percentage of the largest grain dry weight (basal grains of middle spikelets) for each treatment on day 16 and at maturity. Values on day 19, 21 and 24 were intermediate between values on day 16 and maturity. M, Basal grains of middle spikelets; B, basal grains of lowest spikelets; T, basal grains of top spikelets. Shaded area = distal grains of a given spikelet (M, B or T).

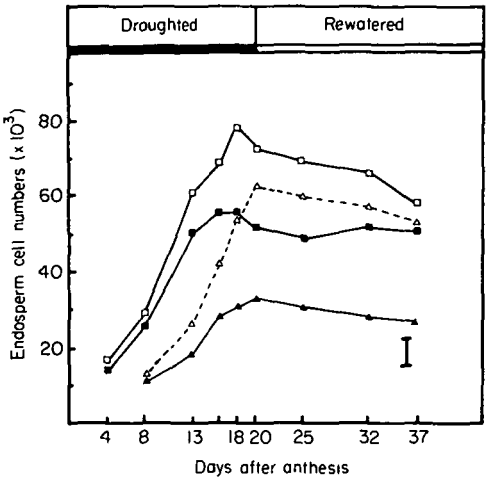

FIG. 4. Number of endosperm cells in basal grains of middle spikelets (□——□ control, ■——■ droughted) and in distal grains of top spikelets (△---△ control, ▲——▲ droughted), Error bar represents L.S.D. ($P = 0{\cdot}05$) between means of treatments at anyone time.

droughted plants. This reduction corresponded to both a shorter duration and to a lower growth rate (Fig. 2; Table 1). There was no increase in growth rate after rewatering. Water content of the grains increased rapidly during the initial 20 d in control and 17 d in the drought treatment and then remained constant until the beginning of grain maturation. The threshold value of water content was significantly higher in control ($33{\cdot}2 \pm 0{\cdot}7$ mg) than under drought ($27{\cdot}2 \pm 1{\cdot}5$ mg). Water loss started 3 d before maximum dry weight was reached in both treatments, and 5 d earlier in the grains of droughted than well-watered plants.

Variation in individual grain dry weight within the ear

Grains from top spikelets were always significantly smaller ($P < 0{\cdot}05$) than those from middle or basal spikelets and within a spikelet the distal grains were always significantly smaller than basal grains (Fig. 3). At maturity, the distal grains of top spikelets showed the largest reduction in dry weight, i.e. 23 per cent relative to the basal grains of middle spikelets. Distal florets reached anthesis 2–3 d later than basal florets. Drought accentuated the differences in dry weight between spikelets and between grains within spikelets. The distal grains of top spikelets were most affected by drought (Fig. 3).

The distribution of individual grain dry weights did not change from day 16 to maturity in the control treatment. During the drought period, basal grains of top spikelets grew faster than distal grains of middle or basal spikelets (Fig. 3, day 16 droughted). After rewatering, this trend was reversed (Fig. 3, maturity droughted) and corresponded to the situation in the control treatments.

Endosperm cell number

The number of nuclei increased for 16–20 d depending on the treatment and the position of the grains in the ear (Fig. 4). Cell division seemed to start later in distal grains than basal grains because there was a 2–3 d delay between anthesis in basal grains (day 0) and anthesis in distal grains. The decrease in nuclei number after the maximum was reached, was highly significant ($P < 0{\cdot}01$) in the control treatment for both grain

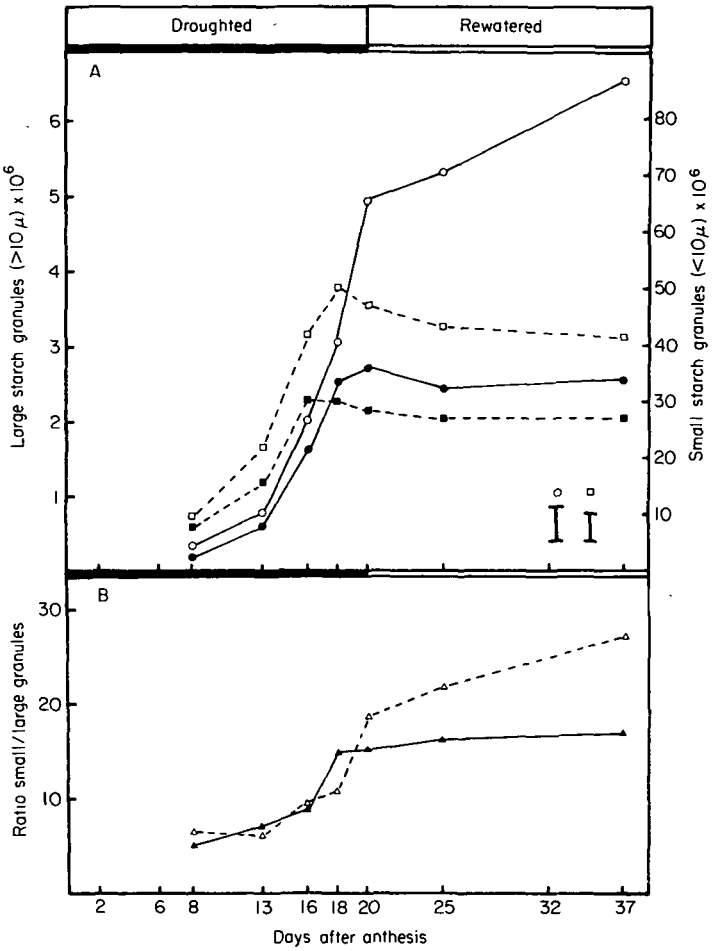

FIG. 5. Number of small (< 10 μm) or large (> 10 μm) starch granules per endosperm, (A), and ratio of small to large starch granules, (B), in basal grains of middle spikelets. (A), Small granules (○——○, ●——●) Large granules (□---□, ■——■) Open symbols, control; closed symbols, drought treatment. (B), Control (△---△); drought (▲——▲). Error bars represent L.S.D. ($P = 0{\cdot}05$) between means of treatments at anyone time for small granules (○) and large granules (□).

positions, but was not significant ($P = 0{\cdot}05$) in the drought treatment. This reduction in number of nuclei was probably due to the rupture of nuclei membranes under the pressure of growing starch granules (Frazier and Appalanaidu, 1965); fewer nuclei would have been disrupted under drought as fewer starch granules were formed (Fig. 5, Table 2).

The best estimate of final cell number appeared to be the maximum number of nuclei (M). The maximum cell number of distal grains represented 80 per cent of that of basal grains in control. M was reduced by 32 per cent in the basal grains and 40 per cent in distal grains under drought. The reduction in M was due to a lower rate of cell division particularly after day 13 (Fig. 4). The average weight per cell (W) ranged from 530 to 742 ng depending on the treatment. As starch accounts for approx. 70 per cent of the total mass of the grain (Peterson, 1965) variations in W reflect to a large extent variation in starch per cell. Distal grains accumulated less dry matter per cell than basal grains, particularly under drought.

TABLE 2. *Number of starch granules per cell in basal grains of middle spikelets*

Treatment	Number large granules per cell	Number small granules per cell
End of cell division*		
Control	46[a]	518[b]
Droughted	42[ab]	381[c]
Grain maturity		
Control	40[ab]	1097[a]
Droughted	37[bc]	623[b]
L.S.D. ($P = 0{\cdot}05$)	6	62

Values within columns with a common superscript are not significantly different at $P = 0{\cdot}05$ using the least significant difference between means.

* 18 and 16 d after anthesis for grains of control and droughted plants, respectively.

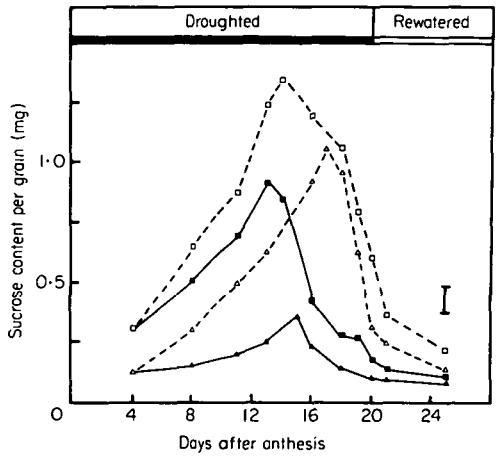

FIG. 6. Sucrose content per basal grain of middle spikelet (□---□ control, ■——■ droughted) and per distal grain of top spikelet (△---△ control, ▲——▲ droughted). Error bar represents L.S.D. ($P = 0{\cdot}05$) between means of treatments at anyone time.

Starch granule number in basal grains of middle spikelets

Starch granules were differentiated by light microscopy only on the basis of their size. Small starch granules could have been either small A-type granules or B-type granules towards the end of cell division (Evers, 1971; Brocklehurst and Evers, 1977) and in order to avoid confusion, a division into small or large granules was preferred to a division into A or B-type.

The maximum number of large granules per endosperm was reduced by 37 per cent under drought (Fig. 5) and this was related to a shorter period of large granule initiation. However the number of large granules per cell did not differ significantly between treatments (Table 2). The number of small granules per endosperm differed significantly between treatments at the end of cell division and by day 37 there was a 40 per cent reduction in small granules under drought relative to control (Fig. 5). The initiation of small granules stopped between day 16 and 18 under drought and there were significantly fewer small granules per cell at the end of cell division relative to the control (Table 2).

TABLE 3. *Maximum amount of sucrose per grain and per endosperm cell*

Treatment	Day of maximum sucrose	Amount of sucrose (μg)		
		(per grain)	(per mg d. wt)	(per 1000 cells)
Basal grains of middle spikelets				
Control	14	1336	108	7·4
Droughted	13	915	95	7·5
Distal grains of top spikelets				
Control	17	1060	90	7·2
Droughted	15	360	58	5·2
L.S.D ($P = 0·05$)		144	13	—

The amount of sucrose per endosperm was calculated as the difference between amount of sucrose per grain and amount of sucrose per pericarp+pigment strand on the day of maximum amount of sucrose per grain. The amount of sucrose per 1000 cells was estimated using the cell numbers on day of maximum sucrose (Fig. 4).

As there was no further initiation of small granules after rewatering, the difference in small granules per cell was even more pronounced at maturity between droughted and control grains. The ratio of small:large granules was higher in control after day 20, reflecting the dominant effect of drought on the initiation of small starch granules (Fig. 5).

Grain sucrose

The amount of sucrose per grain increased to a maximum on day 13–17 (Fig. 6) and then declined to 200 μg or less by day 25. The maximum amount of sucrose per grain was reached 2–3 d later in the distal grains than in the basal grains (Table 3) because of the later date of anthesis in these grains. The maximum was reached earlier in grains of droughted plants. The maximum amount of sucrose per grain differed significantly between grain positions in the ear and between treatments (Table 3). It was the highest under control conditions and was particularly low for the distal grains on top spikelets under drought conditions. These differences were not entirely due to differences in grain weight and the distal grains of top spikelets had significantly less sucrose per mg dry weight under drought relative to control or relative to the basal grains of middle spikelets (Table 3).

Sucrose levels were expressed on an endosperm basis rather than a whole grain basis in order to relate the sucrose levels to endosperm cell number and starch accumulation in the endosperm. As grain position and drought affected the number of endosperm cells (Fig. 4), results were expressed per endosperm cell (Table 3). The amounts of sucrose per 1000 endosperm cells were between 7·2 and 7·5 μg, except in the distal grains of top spikelets where the amount of sucrose was appreciably lower.

DISCUSSION

Determination of grain size in wheat

Assuming no limitation to sucrose supply within the endosperm, a high rate of dry matter accumulation would require the creation of enough sites for starch and protein deposition. It should follow that a lower rate of growth under drought or in distal grains, largely reflects a lower number of endosperm cells and starch granules per cell. Indeed, the variation in grain weight between spikelets of control plants was related to the number

of endosperm cells formed. Final grain dry weight and maximum cell number was reduced to the same extent (20 per cent) in distal grains relative to basal grains. A positive correlation between grain weight at maturity and maximum cell number was also found by Brocklehurst (1977), Gleadow *et al.* (1982) and Singh and Jenner (1982).

Under drought, final grain dry weight and maximum cell number were reduced to the same extent for the basal grain (30 per cent), but grain weight was reduced more than cell number for the distal grains: 61 and 48 per cent, respectively. Brocklehurst *et al.* (1978) also found that grain weight at maturity in the wheat variety Val was reduced proportionately more than endosperm cell number under drought. These results can be explained by taking into account the timing of severe water deficit relative to the active phase of cell division. In the experiment of Brooks *et al.* (1982), water deficit became severe only towards the end of the cell division period and reduced markedly the number of small granules, but not the number of cells or large granules. When water deficit was severe just after anthesis, the reduction in grain set was sufficiently large to allow for a compensatory increase in cell number in the remaining grains (Wardlaw, 1971). However, when water deficit became severe after most of grain set had taken place, endosperm cell number and also dry weight per cell were reduced (Brocklehurst *et al.*, 1978).

By day 20, only 35–45 per cent of final dry weight had been accumulated in grains, but the sink size of the grains was determined to a large extent as the number of endosperm cells and number of large granules were fixed. Even the number of small granules was fixed in droughted grains. The relationship between cell number and final grain weight which applies to control plants in this experiment, and to plants subjected to various treatments (see review by Singh and Jenner, 1982) illustrates that sink size determines the subsequent accumulation of dry matter.

Sink size of the grain can be identified with maximum cell number when the number of starch granules per cell is fairly constant. However, when the number of small starch granules was significantly reduced by drought (43 per cent in basal grains), the storage capacity of the grains was smaller and consequently, the dry weight per cell was reduced (10 per cent in basal grains). The weight per cell was less reduced than the number of small granules per cell because of the low weight of small granules (Evers and Lindley, 1977). Although the number of starch granules was not measured in distal grains, the importance of the reduction in weight per cell (24 per cent) indicates that the number of small and probably also large granules was markedly reduced. It can be concluded that the weight per cell is not as constant as indicated by Wardlaw (1970), Brocklehurst (1977), Brocklehurst *et al.* (1978) and Singh and Jenner (1982). It varies under severe environmental stress and as the weight per cell is largely the weight of starch per cell, it depends on the number of starch granules initiated per endosperm cell.

Relationship between sink size and sucrose levels in the grain

In order to test the hypothesis that the rate of cell division or initiation of starch granules is regulated by the supply of assimilates to the grains, we looked at the correlation between number of cells or starch granules and concentration of assimilates in the grain. This approach has been followed by several authors (see Brooks *et al.*, 1982; Singh and Jenner, 1982), but it suffers serious limitations which will be discussed in the light of our results. We measured the concentration of sucrose in the grain or endosperm, as sucrose is the main form of sugar transported to the grain or endosperm and is the main substrate for starch synthesis. Our results (Table 3) show that drought reduced significantly the amount of sucrose per grain, but not the amount of sucrose per unit dry matter or per endosperm cell except in the case of distal grains. However, the existence of a correlation between cell number and sucrose concentration does not allow

us to conclude that the supply of sucrose regulated cell division by changing sucrose levels in the endosperm for at least two reasons.

Firstly, the concentration of assimilates does not necessarily reflect the supply of assimilates to the grains. Sucrose concentration in the endosperm is the net result of the supply to the endosperm and its utilization in the synthesis of starch and cell walls. Thus, a low intracellular concentration of sucrose could be due to a rapid conversion of sucrose into starch or cell walls as well as to a low supply of sucrose. As the supply of sucrose to the grains is not easy to measure directly, information is needed on the utilization of sucrose together with sucrose concentration, before assumptions may be made about the level of supply. In our experiment, the number of endosperm cells and starch granules and the dry weight per cell give an idea of sucrose utilization. The low number of starch granules and the low weight per cell under drought in association with a sucrose concentration per cell equal or lower than in control indicates a lower supply to the grain.

Secondly, a lower supply to the grain does not indicate that assimilate supply *regulates* cell division or starch accumulation. The absence of a correlation between sucrose concentration and for example, cell division, suggests that assimilate supply is not the main limiting factor (Singh and Jenner, 1982). We can conclude in this experiment that assimilate supply did not regulate the initiation of starch granules as the number of small starch granules per cell decreased (Table 2), despite a normal (cf. control) concentration of sucrose per cell (Table 3). However, the existence of a correlation between sucrose level and cell division (Table 3) means either that the sucrose level regulates cell division or that sink size of the grain, i.e. number of endosperm cells, determines assimilate supply and is affected by a regulatory factor other than sucrose. It is possible that the factor inhibiting the initiation of starch granules also inhibits cell division under drought.

LITERATURE CITED

ASANA, R. D., SAINI, A. D. and RAY, D., 1958. Studies in physiological analysis of yield. III. The rate of grain development in wheat in relation to photosynthetic surface and soil moisture. *Physiologia Plantarum* **11**, 655–65.

BREMNER, P. M., 1972. The accumulation of dry matter and nitrogen by grains in different positions of the wheat ear as influenced by shading and defoliation. *Australian Journal of Biological Science* **25**, 657–81.

—— and RAWSON, H. M., 1978. The weights of individual grains of the wheat ear in relation to their growth potential, the supply of assimilate and interactions between grains. *Australian Journal of Plant Physiology* **5**, 61–72.

BRIARTY, L. G., HUGHES, C. E. and EVERS, A. D., 1979. The developing endosperm of wheat – a stereological analysis. *Annals of Botany* **44**, 641–58.

BROCKLEHURST, P. A., 1977. Factors controlling grain weight in wheat. *Nature* (*London*) **266**, 348–9.

—— and EVERS, A. D., 1977. The size distribution of starch granules in endosperm of different sized kernels of the wheat cultivar Maris Huntsman. *Journal of the Science of Food and Agriculture* **28**, 1084–9.

——MOSS, J. P. and WILLIAMS, W., 1978. Effects of irradiance and water supply on grain development in wheat. *Annals of Applied Biology* **90**, 265–76.

BROOKS, A., JENNER, C. F. and ASPINALL, D., 1982. Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. *Australian Journal of Plant Physiology* **9**, 423–36.

BUTTROSE, M. S., 1963. Ultrastructure of the developing wheat endosperm. *Australian Journal of Biological Science* **16**, 305–17.

CAMPBELL, E. C., CAMPBELL, G.S. and BARLOW, W. K., 1973. A dewpoint hygrometer for water potential measurement. *Agricultural Meteorology* **12**, 113–21.

EVERS, A. D., 1970. Development of the endosperm of wheat. *Annals of Botany* **34**, 547–55.

—— 1971. Scanning electron microscopy of wheat starch. III. Granule development in the endosperm. *Die Stärke* **23**, 157–62.

—— and LINDLEY, J., 1977. The particle-size distribution in wheat endosperm starch. *Journal of the Science of Food and Agriculture* **28**, 98–102.

FRAZIER, J. C. and APPALANAIDU, B., 1965. The wheat grain during development with reference to nature, location and role of its translocatory tissues. *American Journal of Botany* **52**, 193–8.

GLEADOW, R. M., DALLING, M. J. and HALLORAN, G. M., 1982. Variation in endosperm characteristics and nitrogen content in six wheat lines. *Australian Journal of Plant Physiology* **9**, 539–51.

HOSHIKAWA, K., 1961. Studies on the ripening of wheat grains. 2. Development of the endosperm tissue. 3. Development of the starch grain and reserve protein particle in the endosperm. *Proceedings of Crop Science Society of Japan* **29**, 415–20.

JENNER, C. F., 1979. Grain filling in wheat plants shaded for brief periods after anthesis. *Australian Journal of Plant Physiology* **6**, 629–41.

JONES, M. G. K., OUTLAW, W. H. and LOWRY, O. H., 1977. Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissue. *Plant Physiology* **60**, 379–83.

JONES, M. M. and TURNER, N. C., 1980. Osmotic adjustment in expanding and fully expanded leaves of sunflower in response to water deficits. *Australian Journal of Plant Physiology* **7**, 181–92.

MCPHERSON, H. G. and BOYER, J. S., 1977. Regulation of grain yield by photosynthesis in maize subjected to a water deficiency. *Agronomy Journal* **69**, 714–18.

MUNNS, R. and WEIR, R., 1981. Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits at two light levels. *Australian Journal of Plant Physiology* **8**, 93–105.

——BRADY, C. J. and BARLOW, E. W. R., 1979. Solute accumulation in the apex and leaves of wheat during water stress. Ibid. **6**, 379–89.

PETERSON, R. F., 1965. *Wheat. Botany, Cultivation and Utilization.* Interscience Publishers, New York.

RAWSON, H. M. and EVANS, L. T., 1970. The pattern of grain growth within an ear of wheat. *Australian Journal of Biological Science* **23**, 753–64.

RIJVEN, A. H. G. C. and WARDLAW, I. F., 1966. A method for the determinations of cell number in plant tissue. *Experimental Cell Research* **41**, 324–8.

SAINI, H. S. and ASPINALL, D., 1982. Sterility in wheat (*Triticum aestivum* L.) induced by water deficit or high temperature: Possible mediation by abscisic acid. *Australian Journal of Plant Physiology* **9**, 529–37.

SIMMONS, S. R. and CROOKSTON, R. K., 1979. Rate and duration of growth of kernels formed at specific positions in spikelets of spring wheat. *Crop Science* **19**, 690–93.

SINGH, B. K. and JENNER, C. F., 1982. Association between concentrations of organic nutrients in the grain, endosperm cell number and grain dry weight within the ear of wheat. *Australian Journal of Plant Physiology* **9**, 83–95.

WARDLAW, I. F., 1970. The early stages of grain development in wheat: response to light and temperature in a single variety. *Australian Journal of Biological Science* **23**, 765–74.

——1971. The early stages of grain development in wheat: response to water stress in a single variety. Ibid. **24**, 1047–55.

WILSON, J. R., LUDLOW, M. M., FISHER, M. J. and SCHULZE, E. D., 1980. Adaptation to water stress of the leaf water relations of four tropical forage species. *Australian Journal of Plant Physiology* **7**, 207–20.