Journal of Cereal Science **37** (2003) 9–20
doi:10.1006/jcrs.2002.0483, available online at http://www.idealibrary.com on IDEAL®

Temperature, Water and Fertilizer Influence the Timing of Key Events During Grain Development in a US Spring Wheat

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Received 20 August 2001

ABSTRACT

Controlled environments were used to define the manner in which temperature, water and fertilizer affect the timing of key transition points during grain development and to investigate the effects of combined environmental factors in a US spring wheat (*Triticum aestivum* (L.)). When plants were subjected to very high temperature regimens (37/17 or 37/28 °C day/night) during grain development, the times to maximum kernel water content, maximum dry weight and harvest maturity were shorter than in plants maintained under a 24/17 °C day/night regimen. Starch accumulated at similar rates, but the onset and cessation of starch accumulation occurred earlier. Apoptosis in endosperm tissue also occurred earlier under high temperatures and coincided with physiological maturity. The addition of drought to the 37/17 °C regimen further shortened the time to maximum water content and dry weight and reduced the duration of starch accumulation, but did not influence the timing of protein accumulation or kernel desiccation. Post-anthesis fertilizer had little effect on time to maximum water content, dry weight, apoptosis, or harvest maturity under any of the temperature regimens and did not influence the timing of starch accumulation. However, both the rate and duration of protein accumulation were reduced when post-anthesis fertilizer was omitted.

Keywords: Triticum aestivum L., grain development, environment, apoptosis.

INTRODUCTION

Wheat is grown under a wide range of environmental conditions where climatic factors such as temperature and moisture combined with agronomic inputs such as fertilizer exert diverse effects on plant growth and metabolism. The manifestation of those effects in the developing kernel impacts the value of the crop by influencing yield, grain characteristics and flour quality. Within the kernel, complex programs of gene expression control physiological and biochemical processes, including cell division, water uptake and kernel expansion, accumulation of starch and protein, maturation and desiccation. A better understanding of the genetic program of grain development and the influence of specific environmental variables on that program is required to minimize the effects of environment on yield and quality.

A considerable volume of literature addresses the effects of environment on the developing wheat grain, both in field experiments[1–6] and controlled growth experiments[4,7–16]. In some studies, the effects of environment have been assessed simply in terms of kernel weight, while the rate and duration of grain fill have been evaluated in others. Despite the many studies, a comprehensive picture of grain development under different environmental conditions has

ABBREVIATIONS USED: DPA = days post-anthesis.

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0733–5210/03/010009 + 12 $35.00/0

yet to emerge, particularly for US wheat varieties. One reason is that wheat varieties adapted to diverse growing regions respond differently to environmental cues[9,11,17–19]. Experimental treatments also differ considerably among published reports and may have been applied continuously or for short intervals during grain development. Only a few studies address the interacting effects of environmental variables on grain development in a single variety[14,19].

In the US, wheat is grown with minimal inputs of fertilizer in dryland areas where temperatures often exceed that at which maximum biological activity occurs. However, few controlled growth studies have addressed the effects of temperatures above 30 °C on grain development[4,8,10,15] and only one of these studies utilized a US wheat cultivar[8]. As a result, we conducted a series of experiments in which a US hard red spring wheat was grown under eight different controlled environmental regimens from anthesis to harvest maturity. Our experiments examined grain development under three different temperature regimens, including two regimens with very high daytime temperatures, as well as the interacting effects of water and fertilizer. The maximum daytime temperature of 37 °C used in these experiments is outside of the temperature range in which the degree-day concept can be used. Our assessment focuses on the timing of transition points in the accumulation of water, starch and protein and on the timing of apoptosis in developing kernels since these represent points in the genetic program when changes in gene expression are likely to occur. These data provide the foundation for high-throughput transcript and protein profiling experiments that will examine molecular mechanisms of grain development and the response of the developing wheat grain to the environment.

EXPERIMENTAL

Growth of plants

Wheat seeds (*Triticum aestivum* (L.) cv. Butte 86) were sown in pots, (25 cm diameter × 25 cm deep) containing Sunshine Mix Number 1 (SunGro Horticulture, Inc., Bellevue, WA, USA), a planting mix that contains 70–80% Sphagnum peat moss plus perlite, dolomitic limestone, gypsum and wetting agent. Each pot contained seven plants. Plants were grown until anthesis in a climate-controlled greenhouse under a moderate temperature regimen with a daytime maximum temperature of 24 °C and a nighttime minimum temperature of 17 °C. The maximum and minimum temperatures were maintained for 5 and 11 h, respectively and were separated by 4 h periods at 21 °C. Pots were rotated weekly to minimize positional effects in the greenhouse. Plants were supplied with a dilute solution of Plantex 20-20-20 fertilizer ($0{\cdot}6\ g\ l^{-1}$) with each watering, using an automatic drip irrigation system equipped with a fertilizer injector. Natural light was supplemented with 100 W high-pressure sodium lights to maintain a day length of 16 h. Environmental data was collected at 5-min intervals throughout the experiment using GrowLink *vs.* 2·1 software (MicroGrow Greenhouse Systems, Inc., Temecula, CA, USA). A second greenhouse equipped with similar irrigation, lighting and monitoring systems was used for growth of plants under high temperature regimens. For high daytime temperature treatments, this greenhouse was maintained at a maximum daytime temperature of 37 °C and a minimum nighttime temperature of 17 °C. The maximum and minimum temperatures were maintained for 5 and 11 h, respectively and were separated by 4 h periods at 26·7 °C. For treatments that combined high daytime and high nighttime temperatures, the maximum, minimum and intermediate temperatures were 37, 28, and 30 °C, respectively, and the maximum temperature was maintained for 4 hours. The maximum, minimum and average temperatures recorded in the greenhouse during grain development were 28·9, 15·6 and 20·2 °C under the 24/17 °C regimen, 38·9, 15·6, and 24·0 °C under the 37/17 °C regimen, and 38·9, 26·1 and 30·3 °C under the 37/28 °C regimen. The average humidity recorded during the period of highest daytime temperature was 53·0, 23·5 and 23·6% for the 24/17, 37/17, and 37/28 °C regimens, respectively. The average humidity recorded during the nighttime was 76·1, 67·5 and 37·8% for the 24/17, 37/17, and 37/28 °C regimens, respectively.

Each head was tagged with the date of anthesis. Plants were placed under one of the following growth regimens from the day on which the majority of heads had undergone anthesis until harvest maturity: 24/17 °C day/night with post-anthesis fertilizer, 24/17 °C day/night without post-anthesis fertilizer, 37/17 °C day/night with post-anthesis fertilizer, 37/17 °C day/night without post-anthesis fertilizer, 37/17 °C day/night plus drought with post-anthesis fertilizer, 37/17 °C day/night plus drought without post-anthesis fertilizer, 37/28 °C day/night with post-anthesis fertilizer or 37/28 °C

day/night without post-anthesis fertilizer. For the fertilized plants, each pot received 500 ml Plantex 20-20-20 (0·6 g l^{-1}) every day after anthesis by drip irrigation. All pots were weighed daily and adjusted to 6 kg with water to maintain soil moisture at approximately 80% of capacity except for pots subjected to the drought treatment where the soil was allowed to dry over a 2–3 day period until pot weights reached 2·5 kg. Soil moisture was then maintained at approximately 33% of capacity by adjusting pots to 2·5 kg each day with water.

Determination of fresh and dry weight

At specific time points between anthesis and maturity, kernels from two to seven single heads were collected, counted and weighed. Kernels from single heads were freeze-dried and weighed to determine dry weight. Water contents were calculated by subtracting dry weight from fresh weight. Values from individual heads were averaged. Similar growth curves were obtained in one or two additional experiments (not shown) for each treatment. Kernels from all heads remaining at maturity were bulk harvested and three 1 g samples were counted to determine average kernel weight.

Determination of starch and protein content

Freeze-dried kernels were ground to a powder using an Udy mill (UDY Corporation, Fort Collins, CO, USA). One hundred mg samples were assayed for starch using the Megazyme total starch determination assay kit (Megazyme International, County Wicklow, Ireland). Protein was determined on 30 mg samples by N combustion analysis using a Leco nitrogen analyzer (Leco Corporation, St Joseph, MI, USA) and a protein to N ratio of 5·7. All assays were performed in triplicate and values were averaged for each time point.

Isolation and analysis of DNA

Between 1 and 3·5 g of kernels collected from single heads was ground to a fine powder in liquid N_2 using a mortar and pestle. The resulting powder was mixed with 16 ml of a buffer consisting of 2% cetyltrimethylammonium bromide (CTAB), 1·4 M NaCl, 1% PEG-6000, 2 mM EDTA in 100 mM Tris-Cl, pH 9·5. After extraction with 20 ml phenol:chloroform:isoamyl alcohol (25:24:1), the aqueous phase was recovered by centrifugation. Nucleic acids were precipitated from the aqueous phase with 1 volume of isopropanol at room temperature for 30 min. The resulting pellet was resuspended in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8·0), DNase-free RNase (Boehringer-Mannheim) was added to a concentration of 50 μg ml^{-1}, and the sample was incubated at 40 °C for 30–60 min. The sample was then extracted with phenol:chloroform:isoamyl alcohol. Sodium acetate was added to the resulting aqueous fraction to a concentration of 0·3 M and the DNA was precipitated with ethanol. After washing with 70% ethanol, the DNA was allowed to air dry and resuspended in TE. DNA was analyzed by gel electrophoresis on 1·8% agarose gels in TBE buffer.

RESULTS

Effects of temperature, post-anthesis fertilizer and drought on plant and kernel appearance

Environmental conditions between anthesis and maturity had strikingly different and reproducible effects on the appearance of the plants, because of differences in the timing of leaf senescence and head maturation (Table I). When plants were grown under the 24/17 °C regimen and supplied continuously with fertilizer, leaf tissue appeared dark green until about 40 days post-anthesis (DPA) when pots were allowed to dry. Browning of glumes and awns preceded the senescence of leaves, indicating that maturation of the head occurred independently of leaf senescence. Kernels began to turn from green to brown between 26 and 31 DPA, were entirely brown by 36 DPA and dry by 42–44 DPA. In the absence of post-anthesis fertilizer under the 24/17 °C regimen, both leaves and glumes senesced earlier. There was a pronounced yellowing of lower leaves by 10 DPA that extended to the flag leaves by 20–24 DPA. Senescence of glumes was evident by 24 DPA. By 32 DPA, leaves, glumes and awns all had a bright yellow appearance. Despite these differences, developing kernels from plants that did not receive post-anthesis fertilizer were indistinguishable from kernels from well-fertilized plants.

When plants were exposed to the 37/17 °C regimen, leaf tissue looked similar to that from plants grown at 24/17 °C until at least 32 DPA as long as plants received post-anthesis fertilizer and adequate water. Senescence of glumes and awns occurred several days earlier and kernels matured noticeably faster in plants grown under high

Table I Effect of post-anthesis environment on appearance of plants and kernels during grain development

Environmental regimen			Leaf color[c]			Glume color[c]		DPA when kernels turn from green to brown
Day/night temperature (°C)	Water[a]	Fertilizer[b]	10 DPA	24 DPA	32 DPA	24 DPA	32 DPA	
24/17	+	+	g	g	g	g	g/b	26–31
24/17	+	−	g/y	y/g	y	g/y	y	26–31
37/17	+	+	g	g	g	g/b	b	22–26
37/17	+	−	g/y	b/y	b	b	b	22–26
37/17	−	+	b/g	b	b	b	b	20–24
37/17	−	−	b/g	b	b	b	b	20–24
37/28	+	+	g/b	b	b	g/b	b	16
37/28	+	−	g/b	b	b	g/b	b	16

[a] Plants were well-watered (+) or drought-treated (−).
[b] Plants received post-anthesis fertilizer (+) or did not receive post-anthesis fertilizer (−).
[c] At specific times during grain development; green (g), yellow (y), brown (b), green with some yellow (g/y), yellow with some green (y/g), green with some brown (g/b), brown with some green (b/g), brown with some yellow (b/y).

temperatures. Kernels began to turn from green to brown around 22 DPA, were entirely brown by 30 DPA and dry by 35 DPA. When plants did not receive post-anthesis fertilizer under the 37/17 °C regimen, leaves and glumes senesced earlier than under the 37/17 °C regimen with fertilizer, but the appearance of developing kernels was similar to those from plants that were fertilized. High daytime temperatures advanced the senescence of leaf tissue in the absence of fertilizer resulting in distinct differences in the appearance of plants grown under the 37/17 and 24/17 °C regimens by 24 DPA.

Drought conditions combined with high daytime temperatures exerted the greatest effect on the appearance of the plants, with both leaves and glumes senescing much earlier. Senescence of lower leaves was pronounced by 10 DPA. By 24 DPA, nearly all leaves as well as awns and glumes had turned brown. Developing kernels turned from green to brown by 20 DPA and were brown and dry by 28 DPA. The response was similar whether or not the plants received post-anthesis fertilizer.

Exposure to the more severe 37/28 °C regimen also resulted in the rapid senescence of leaf tissue whether or not the plants were supplied with post-anthesis fertilizer. Kernels began to turn from green to brown by about 16 DPA and were brown and dry by 26–28 DPA.

The timing of grain development under different environmental conditions

Effects of environmental factors on grain development were reflected in changes in the profiles of fresh weight, dry weight, and water accumulation between anthesis and harvest maturity (Fig. 1, Table II). Under the 24/17 °C regimen with post-anthesis fertilizer, kernels accumulated fresh weight in a linear fashion from about 4 to 26 DPA at a rate of 3·3 mg day^{-1}, attaining maximum water content of 36 mg kernel^{-1} by about 23 DPA and maximum fresh weight of 78 mg kernel^{-1} by 30 DPA [Fig. 1(a), Table II]. Kernels continued to accumulate dry weight for several days beyond the time of maximum fresh weight, until about 33 DPA. Over the next 11 days, kernels lost water until they were fully desiccated and had average weights of 48 mg (Table III).

In the absence of post-anthesis fertilizer under the 24/17 °C regimen, kernels also accumulated fresh weight between 4 and 26 DPA at a slightly lower rate of 3·0 mg day^{-1} [Fig. 1(b)]. Omission of post-anthesis fertilizer did not affect the time to maximum water content, fresh weight or dry weight (Fig. 1, Table II) but maximum values were slightly lower in the absence of fertilizer. Mature kernels, obtained after 44 DPA, were of similar average weights for the two treatments (Table III).

Under high daytime temperatures with post-anthesis fertilizer, kernels accumulated fresh weight over a shorter period of time, from 4 to 18 DPA (Fig. 1c) but the initial rate of fresh weight accumulation of 4·0 mg day^{-1} was greater than observed under the 24/17 °C regimen. Maximum water content, fresh weight and dry weight were attained 5–7 days earlier than under the 24/17 °C regimen (Table II) and maximum values were 11–15% less. Mature kernels were obtained after only 35 days and the average kernel weight of 42 mg was 13% less than that of kernels from the 24/17 °C treatment (Tables II, III). In the absence of post-anthesis

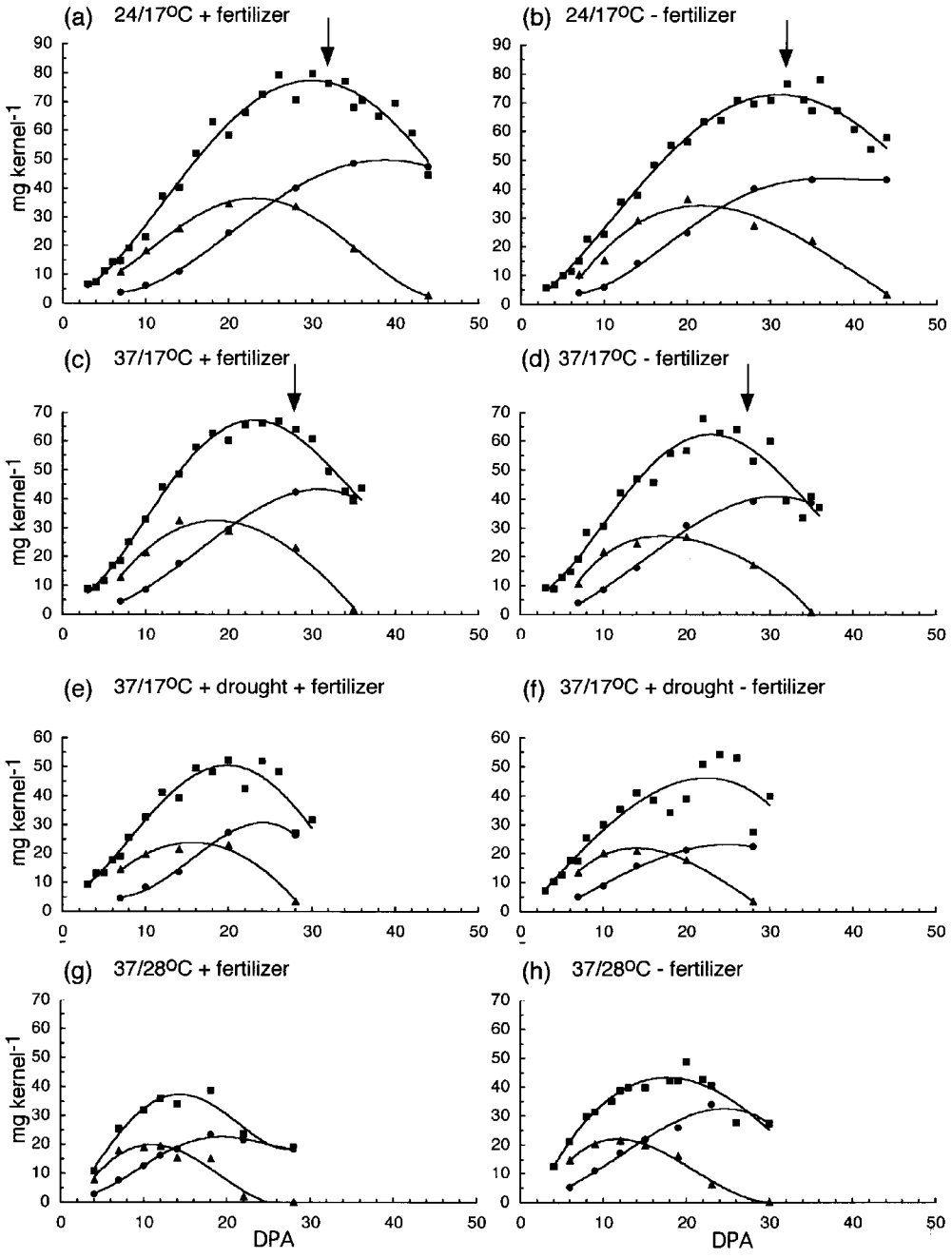

Figure 1 Fresh weight (■), dry weight (●), and water content (▲) of developing kernels from plants grown under the (a, b) 24/17 °C regimen, (c, d) 37/17 °C regimen, (e, f) 37/17 °C regimen plus drought, or (g, h) 37/28 °C regimen. In panels a, c, e, and g, plants were supplied with post-anthesis fertilizer. In panels b, d, f, and h, plants did not receive post-anthesis fertilizer. Each data point represents the average value for a given time in development. Arrows indicate the time that DNA fragmentation characteristic of apoptosis was first observed.

fertilizer under high daytime temperatures, kernels also accumulated fresh weight between 4 and 18 DPA but the rate of fresh weight accumulation was somewhat less, 3·7 mg day^{-1} [Fig. 1(d)]. The times at which maximum water content, fresh and dry weights were attained were similar to those from heat-treated kernels supplied with fertilizer (Table II), but the maximum values were slightly less (Table III). Mature kernels obtained after 35 DPA had average weights of 38 mg (Tables II, III).

Table II Effect of temperature, fertilizer and water on timing of key events during grain development

Environmental regimen				Maximum kernel water content (DPA)	Maximum kernel dry weight (DPA)	Start of starch accumulation (DPA)	End of starch accumulation (DPA)	Start of protein accumulation (DPA)	End of protein accumulation (DPA)	Onset of apoptosis[d] (DPA)	Harvest maturity (DPA)
Day/night temperature (°C)	Water[a]	Fertilizer[b]	Mean daily temperature (°C)								
24/17	+	+	20·2	23	33	13	34	10	36	32	44
37/17	+	+	24·0	18	27	9	28	10	30	28	35
37/28	+	+	30·3	11	18	7	15	7	18	16	26
24/17	+	−	20·2	22	32	13	34	10	24	32	44
37/17	+	−	24·0	17	28	9	28	10	24	28	35
37/28	+	−	30·3	12	20	7	19	7	21	n.d.	28
37/17	−	+	24·0	16	20	9	20	10	30	22	28
37/17	−	−	24·0	14	20	n.d.	n.d.	n.d.	n.d.	22	28

n.d. not determined.
[a] Plants received post-anthesis fertilizer (+) or did not receive post-anthesis fertilizer (−).
[b] Plants were well-watered (+) or drought-treated (−).
[c] Recorded in greenhouse during the period of grain development.
[d] As determined by fragmentation of genomic DNA.

Table III Effect of temperature, fertilizer and water on composition of mature grain

Day/night temperature (°C)	Water[a]	Fertilizer[b]	Single kernel weight (mg)	Starch (mg)	Protein (mg)	Protein (%)
24/17	+	+	48·0	23·3	8·1	16·9
37/17	+	+	41·6	19·7	7·8	18·8
37/28	+	+	20·8	9·4	3·6	17·3
24/17	+	−	47·0	25·6	4·6	9·8
37/17	+	−	37·6	19·5	5·1	13·5
37/28	+	−	25·7	12·0	4·5	17·6
37/17	−	+	29·5	13·5	5·9	20·0
37/17	−	−	28·2	12·6	4·9	17·4

[a] Plants were well-watered (+) or drought-treated (−).
[b] Plants received post-anthesis fertilizer (+) or did not receive post-anthesis fertilizer (−).

The time from anthesis to harvest maturity was only 28 days in plants subjected to drought conditions in addition to high daytime temperatures [Figs 1(e), (f), Table II]. When supplied with post-anthesis fertilizer, kernels from these plants accumulated fresh weight at a rate similar to that of kernels from well-watered, heat-treated plants, but reached maximum water content, fresh weight and dry weight 2, 3 and 7 days earlier, respectively and these values were 23–33% less. Mature kernel weights were about 29% less than those from well-watered plants grown under the high daytime temperature regimen and 39% less than those from well-watered plants grown under the moderate temperature regimen. Post-anthesis fertilizer did not affect the timing of kernel development in plants subjected to the 37/17 °C regimen plus drought [Fig. 1(f), Table II] and mature kernel weights were similar (Table III).

The addition of high nighttime temperatures to the high daytime temperature regimen also shortened the time of grain development (Figs 1(g), (h), Table II). Maximum water content, fresh weight and dry weight were attained by 11, 15, and 18 DPA when plants were supplied with post-anthesis fertilizer and by 12, 17, and 20 DPA when plants did not receive additional fertilizer. Maximum values were significantly less than in kernels from plants grown under either moderate or high daytime temperatures with cool nights. Mature kernels had average weights that were at least 45% less than kernels produced under moderate temperatures.

Starch and protein accumulation

The environmental treatments also influenced the profiles of starch and protein accumulation in the developing kernels (Fig. 2, Tables II, III). In kernels from plants grown under the 24/17 °C regimen with post-anthesis fertilizer, there was an initial lag in starch production until about 13 DPA. Starch then accumulated at a rate of about 1·1 mg/day, reaching maximum levels at about 34 DPA [Fig. 2(a), Table II]. The omission of post-anthesis fertilizer had little effect on the profile of starch accumulation in kernels from plants grown under the 24/17 °C regimen [Fig. 2(a)]. In kernels from plants subjected to the 37/17 °C regimen, starch began to accumulate several days earlier, by 9 DPA, the first time point assayed, at a rate similar to that observed under the 24/17 °C regimen. However, kernels reached their maximum starch contents at 28 DPA [Fig. 2(b)]. Under the 37/17 °C regimen, the profile of starch accumulation also was similar under both fertilizer treatments [Fig. 2(b)]. A comparison of starch accumulation in kernels under the three temperature regimens is shown in Figure 2(c). In plants grown under the 37/28 °C regimen, starch began to accumulate even earlier, by about 7 DPA, at a rate similar to that observed under the 24/17 and 37/17 °C regimens, but reached maximum levels by about 15 DPA [Fig. 2(c), Table II]. Drought also shortened the duration of starch accumulation without influencing the rate [Fig. 2(d)]. Starch reached maximum levels by about 20 DPA in kernels from plants subjected to both high daytime temperatures and drought (Table II).

On a per kernel basis, kernels from heat-treated plants contained less starch at maturity than kernels produced under the moderate temperature regimen. Kernels produced under the 24/17 °C regimen with post-anthesis fertilizer contained an average of 23·3 mg kernel^{-1} while those produced with post-anthesis fertilizer under the 37/17 and 37/28 °C regimens contained 19·7 and 9·4 mg kernel^{-1},

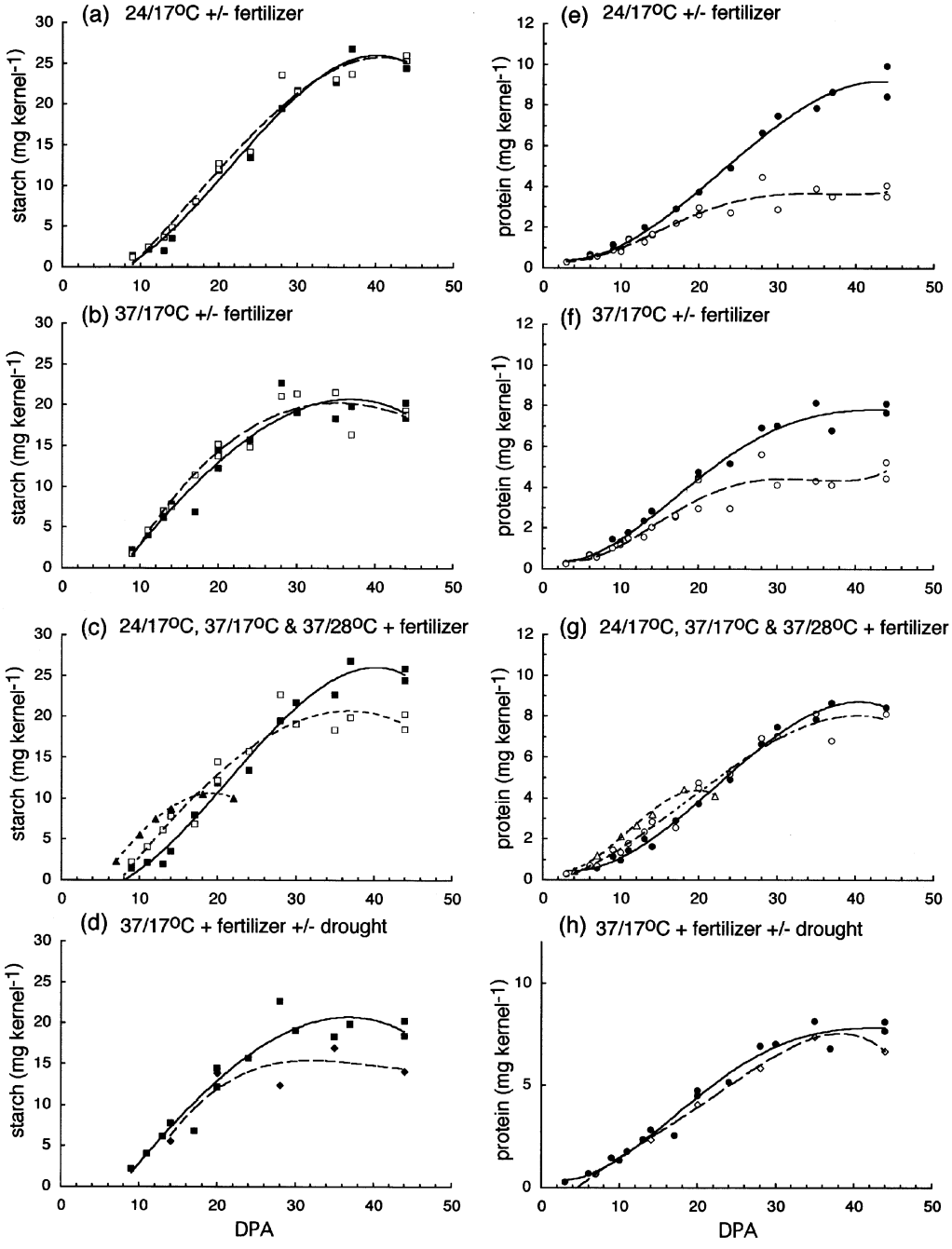

Figure 2 Accumulation of (a–d) starch (■, □, ▲, ◆), or (e–h) protein (●, ○, △, ◇) in developing kernels from plants grown under different environmental regimens. Well-watered plants were grown at (a, e) 24/17 °C or (b, f) 37/17 °C with (–■–, –●–) or without (–□–, --○--) post-anthesis fertilizer. In (c, g), plants were grown with post-anthesis fertilizer at 24/17 °C (–■–, –●–), 37/17 °C (--□--, --○--), or 37/28 °C (--▲--, --△--). In (d, h), plants were grown with post-anthesis fertilizer at 37/17 °C (--■--, --●--) or 37/17 °C plus drought (--◆--, --◇--).

respectively, or 16 and 60% less (Table III). Drought also led to a substantial decrease in the amount of starch in the mature grain. Kernels from fertilized plants subjected to drought as well as high daytime temperatures contained 13·5 mg kernel^{-1}, about 32% less than kernels from well-watered heat-treated plants. The amount of starch in mature kernels did not vary dramatically when plants were grown under the different fertilizer levels under either the 24/17 or the 37/17 °C regimens (Table III).

Protein began to accumulate slightly earlier than starch in kernels from plants grown under the 24/17 °C regimen with post-anthesis fertilizer, from about 10 DPA, and continued until 36 DPA at a maximum rate of 0·28 mg day^{-1} [Fig. 2(e)]. In the absence of post-anthesis fertilizer under the 24/17 °C regimen, the rate of protein accumulation was significantly less, 0·17 mg day^{-1}, and protein levels did not increase after about 24 DPA [Fig. 2(e)]. In kernels from plants grown under the 37/17 °C regimen with fertilizer, the rate of protein accumulation was similar to that observed under the 24/17 °C regimen with fertilizer, but protein was not accumulated beyond about 30 DPA [Figs 2(f), (g)]. In the absence of post-anthesis fertilizer under the 37/17 °C regimen, kernels accumulated protein at a reduced rate, 0·17 mg day^{-1}, and levels did not increase after about 24–26 DPA. Protein accumulated slightly earlier in development in kernels from plants grown under the 37/28 °C regimen, but protein accumulation ceased much earlier, by 18 DPA [Fig. 2(g), Table II]. The addition of drought to the 37/17 °C regimen did not affect the profile of protein accumulation in plants supplied with post-anthesis fertilizer [Fig. 2(h)].

Under the 24/17 °C regimen, mature kernels from plants that received post-anthesis fertilizer contained 8·1 mg protein kernel^{-1} while those from plants that did not receive fertilizer contained only 4·6 mg kernel^{-1}, a 43% decline (Table III). Similarly, under the 37/17 °C regimen, mature kernels from plants that received post-anthesis fertilizer contained 7·8 mg protein kernel^{-1} while those from plants that did not receive fertilizer contained only 5·1 mg kernel^{-1}, a 35% decline. Protein amounts in mature kernels were also less in plants that were subjected to drought treatment as well as high daytime temperatures. Kernels from fertilized plants subjected to both high daytime and nighttime temperatures contained the least amount of protein on a per kernel basis, only 3·6 mg.

Kernel protein percentage varied considerably as a result of environmental effects on the accumulation of starch, protein or both during grain development (Table III). With the application of post-anthesis fertilizer, protein percentage increased from 9·8 to 16·9% under moderate temperatures, from 13·5 to 18·8% under high daytime temperatures, and from 17·4 to 20% under high daytime temperatures plus drought. Protein percentage also increased with high daytime temperatures from 16·9 to 18·8% and with high daytime temperatures combined with drought to 20·0% when plants received post-anthesis fertilizer. The increase in protein percentage with high temperatures and drought was greater when plants did not receive post-anthesis fertilizer. Protein percentage increased from 9·8 to 13·5% with high daytime temperatures and to 17·4% with high daytime temperatures and drought. Under the 37/28 °C regimen, protein percentages were between 17 and 18% under both fertilizer levels.

Onset of apoptosis of endosperm tissue in plants grown under different environmental regimens

Apoptosis marks the end of endosperm viability and is a stage in grain development when efforts are directed towards an orderly shut-down of cellular processes[20]. In other plant and animal systems, apoptosis is a well-regulated process that is associated with a cascade of molecular events, including the activation of nucleases and the fragmentation of genomic DNA into discrete ladders. To determine the time at which apoptosis occurred, total genomic DNA was prepared from kernels late in development and examined by gel electrophoresis for signs of internucleosomal fragmentation of the genome. For plants grown under the 24/17 °C regimen either with or without post-anthesis fertilizer, high molecular weight DNA remained intact until 30 DPA. From 32 DPA and beyond, discrete ladders of DNA of 180 bp were apparent [Fig. 3(a)]. In plants grown under the 37/17 °C regimen, these DNA ladders appeared several days earlier, by about 28 DPA [Fig. 3(b)]. Again, there was little difference between plants that received post-anthesis fertilizer and those that did not. The time that DNA fragments first became evident coincided with the time of maximum dry weight or physiological maturity [Figs 1(a)–(d)] and kernels were mostly brown with some green on the undersides at this time. In kernels from plants grown under the 37/28 °C regimen with post-anthesis fertilizer, DNA fragmentation was evident at 16 DPA, the earliest time point that was analyzed (data not shown).

DISCUSSION

Environmental regimens in which temperature, water and fertilizer levels were varied had complex effects on both the plant and the developing kernel. High temperatures and drought changed the period between anthesis and harvest maturity from 44 to

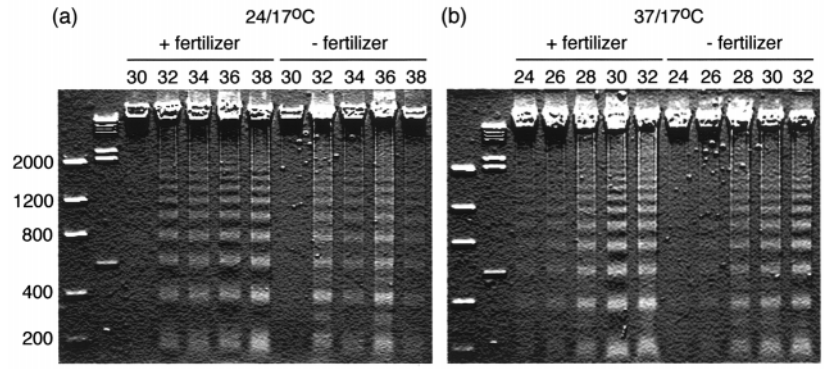

Figure 3 Analysis of total genomic DNA from kernels obtained from plants grown under the (a) 24/17 °C or (b) 37/17 °C regimen, with or without post-anthesis fertilizer. Ages of kernels in DPA are indicated above each lane. Low molecular DNA mass ladder (GibcoBRL) and lambda DNA cut with HindIII were used as molecular weight standards and are shown in the first two lanes in each panel. Sizes in bp of low molecular DNA mass ladder are indicated to the left of the figure.

26 days and had substantial effects on kernel size. In combination with fertilizer, high temperatures and drought also had large effects on kernel composition.

High temperatures affected all stages of grain development, shortening the duration of water uptake and kernel expansion, dry weight accumulation and kernel desiccation. Apoptosis occurred earlier under high temperature regimens and coincided with physiological maturity. High temperatures also caused a clear shift in the time at which starch began to accumulate. Changes in the onset of protein accumulation were less clear in our experiments, although analyses of gluten transcript accumulation in the same plants revealed that transcripts for the major gluten proteins accumulated slightly earlier under the high temperature regimens[21]. Both starch and protein accumulation also ceased earlier under high temperature conditions. Under both the 37/17 and 37/28 °C regimens, the time to physiological maturity was reduced proportionally to temperature, by between 1·1 and 1·6 days for every 1 °C rise in average daily temperature. The average reduction in grain filling duration in 'Butte 86' was similar to the 1·3 day per °C reported for the US hard red winter wheat 'Karl 92' when grown under 20/20, 25/20, 30/20 and 35/20 °C temperature regimens[8], but shorter than the 3·1 day per °C reported in field studies[5] or the 2·8 day per °C compiled from a variety of field and controlled growth studies[5].

Surprisingly, the high temperature regimens did not have a detrimental effect on the rate of grain growth despite the fact that plants were exposed to maximum temperatures of 37 °C and to either 5 (37/17 °C regimen) or 14 (37/28 °C regimen) hours of temperatures above 30 °C each day. Temperature can affect the overall rate of grain growth by influencing the rates of biochemical reactions within the kernel. At temperatures less than 30 °C, the rate of grain growth has been reported to increase with increasing temperature, although the response is somewhat dependent on cultivar[9,10,19]. When temperatures exceed a maximum threshold, generally around 30 °C, some metabolic processes may be inhibited and there may be a negative effect on the rate of grain growth. A decrease in the rate of grain growth was noted when plants subjected to a 36/31 °C regimen were compared to those grown at either 21/16 or 30/25 °C[10] and when plants subjected to 35/20 °C were compared to those grown at 20/20, 25/20 or 30/20 °C[8]. An immediate decrease in the rate of dry matter accumulation also was reported following only a 3 day treatment of 36/30 °C at 20, 36 or 50 DPA when compared to plants maintained under a 22/16 °C regimen[4]. Several other studies have indicated that reductions in the rate of grain fill at extremely high temperatures are due mainly to inhibition of enzymes involved in starch biosynthesis, in particular soluble starch synthase[22,23]. In our experiments, starch accumulated at about the same rate during early stages of grain development under the 24/17, 37/17 and 37/28 °C regimens. However, starch accumulation ceased much earlier under the high temperature regimens, about 6 days earlier under the 37/17 °C regimen and 16–19 days earlier under the 37/28 °C regimen. Thus, reductions in starch content in 'Butte 86' due to high temperatures resulted from an earlier cessation of starch accumulation rather than from an inhibition of starch biosynthesis.

The effects of drought were additive to the effects of high temperature. When combined with high temperatures, drought reduced the time from anthesis to harvest maturity by an additional 7 days. While high temperatures shortened all stages of grain development, drought primarily condensed the period between maximum water content and physiological maturity. High temperatures altered the profiles of both starch and protein accumulation while drought shortened only the duration of starch accumulation.

Some of the environmental treatments had profound effects on the timing of leaf senescence. However, there was not a clear relationship between leaf senescence and the timing of grain development, supporting the notion that much of the control of grain development resides within the kernel[24]. Leaves senesced much earlier when plants were exposed to the drought treatment or grown without post-anthesis fertilizer. However, drought and fertilizer affected the kernels in very different ways. Drought reduced the duration of starch accumulation while the omission of fertilizer reduced both the rate and duration of protein accumulation with little effect on the timing of grain development or apoptosis.

In controlled environment studies, temperature, water and fertilizer affect processes during grain development in different ways. When multiple environmental factors are combined, some factors may have additive effects while others have mitigating effects. For example, when kernels were grown under the three temperature regimens without post-anthesis fertilizer, the protein percentage of the mature kernels ranged from 9·8 to 17·6%. The application of post-anthesis fertilizer reduced the variation in protein percentage that occurred when plants were grown under the different temperature regimens. In the studies presented here, plants were subjected to specific environmental conditions continuously throughout grain development. The application of environmental factors at different points during grain development would no doubt complicate the response. The complexity increases in a field situation where many more environmental factors come into play.

Undoubtedly, a clear understanding of the molecular mechanisms responsible for limiting kernel expansion, initiating and terminating starch and protein accumulation and inducing desiccation will be required to unravel the complex effects of the environment on grain development. While comparisons of global profiles of gene expression and protein accumulation in kernels developing under different environmental regimens can provide considerable insight into the mechanisms that control these processes, these analyses are complicated because environmental variables alter the program of grain development. Thus, experiments must be designed so that changes in gene expression and protein accumulation that occur as a normal part of grain development can be distinguished from those that represent unique responses to environmental variables. Developmental markers that identify specific stages of grain development will be useful for determining equivalent stages of kernels produced under different environmental conditions. For example, genes activated during apoptosis may be good candidates for developmental markers since apoptosis coincides with physiological maturity in kernels with very different developmental programs. The composite picture of grain development presented in this paper will facilitate the correlation of gene expression data with key developmental events under a number of defined environmental regimens. The data thus provide an essential framework for genomic and proteomic studies that, combined with analyses of flour quality in samples produced under the same conditions, should contribute to a better understanding of the role that the growth environment plays in determining the productivity and quality of one of the world's major crop plants.

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