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Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm

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Abstract

The effect of high temperature on starch accumulation, starch granule populations, and expression of genes encoding key enzymes for starch biosynthesis was examined during grain development in wheat (*Triticum aestivum* L. cv. Butte 86). High temperature applied from anthesis to maturity reduced the duration of starch accumulation. Starch accumulation ceased approximately 6 days earlier for grain produced under a 37/17 °C (day/night) regimen and 21 days earlier under a 37/28 °C (day/night) regimen than for grain produced under a 24/17 °C (day/night) regimen. Compared to the 24/17 °C regimen, starch content was approximately 19% less for mature grain produced under the 37/17 °C regimen and 58% less under the 37/28 °C regimen. Based on relative volume, the smaller type B starch granules were the predominant class in mature grain produced under the 24/17 and 37/17 °C regimens, whereas the larger type A granules were predominant in grain produced under the 37/28 °C regimen. Under the 24/17 °C regimen, steady state transcript levels for ADP-glucose pyrophosphorylase, starch synthases I, II, and III, granule-bound starch synthase, and starch branching enzymes I and II were highest from 12–16 days post-anthesis (dpa). Under the 37/17 °C regimen, steady state levels of these transcripts followed the same temporal pattern, but were substantially lower. Under the 37/28 °C regimen, transcript levels peaked earlier, at 7 dpa. The high temperature regimens reduced the relative levels of transcripts for starch synthase more than the other starch biosynthetic enzymes.
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Keywords: Endosperm development; High temperature; Starch accumulation; Starch granule; *Triticum aestivum* L.

1. Introduction

Starch, which accounts for 65–75% of wheat grain weight and can exceed 80% of the endosperm weight, is a major determinant of wheat yield. High temperatures during grain-fill decrease starch production, reduce the final weight of the wheat grain, and diminish yield [1–5]. In order to develop wheat cultivars with greater yields at high temperatures, it is essential to understand the effects of high temperature on the process of starch accumulation.

Starch is deposited in discrete granules in amyloplasts during grain development. Large type A granules (diameters greater than 16 μm) are initiated early in development, smaller type B granules (diameters between 5 and 16 μm) are initiated during mid-development, and much smaller type C granules (diameters less

Abbreviations: Agp1, ADP-glucose pyrophosphorylase, large subunit; Agp2, ADP-glucose pyrophosphorylase, small subunit; Gss, granule-bound starch synthase; SbeI, starch branching enzyme I; SbeII, starch branching enzyme II; SsI, starch synthase I; SsII, starch synthase II; SsIII, starch synthase III.

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than 5 μm) are initiated late in development [6,7]. Starch is composed of two types of glucose polymers, amylose and amylopectin. Amylose is an almost linear α-1,4 glucan molecule that comprises 25–30% of wheat grain starch. Amylopectin is a much larger glucan polymer that is highly branched and comprises 70–75% of wheat grain starch. Both amylose and amylopectin are synthesized from ADP-glucose [8–10], which is synthesized from glucose-1-phosphate in a reaction catalyzed by ADP-glucose pyrophosphorylase (Agp). Extension of α-1,4 glucan chains by the introduction of glucose units is catalyzed by starch synthases (Ss). The branches on the polymers are formed by starch branching enzymes (Sbe) that cleave α-1,4 bonds on both amylose and amylopectin molecules and reattach the released glucan segments to the same or another glucan chain through the formation of α-1,6 linkages. Several different Ss and Sbe have been characterized in wheat endosperm, some soluble and others localized to the starch granules [8,9,11–13].

The most detailed studies of the effects of high temperatures (27–33 °C) on starch production have been done using Australian wheat cultivars. The decline in starch content that results from exposure to high temperature was suggested to be due to a decrease in the rate of the conversion of sucrose to starch [14] rather than limitations in the supply of sucrose to the head [4] or the availability of sucrose within the endosperm [2,15]. Biochemical studies of starch biosynthesis in the developing wheat grain indicate that high temperature decreases metabolite levels and enzyme activities associated with this pathway [16–21]. The effects of high temperature on the expression of genes that encode the starch biosynthetic enzymes in wheat endosperm were not examined in these studies. In this paper, we report the effect of three temperature regimens applied throughout grain-fill on total starch accumulation, starch composition, starch granule populations, and the expression of genes encoding enzymes for starch biosynthesis in the developing endosperm of a US wheat cultivar.

2. Materials and methods

2.1. Plant material

Triticum aestivum L. cv. Butte 86, a hard red spring wheat, was grown in a climate-controlled greenhouse [22,23] that had a maximum daytime temperature of 24 °C and a minimum nighttime temperature of 17 °C (24/17 °C regimen); the average daily temperature was 20 °C and maximum head temperature was 22 °C. Water and fertilizer (Plantex 20-20-20, 500 ml of 0.6 g/l per d per pot) were supplied by an automatic drip irrigation system. Natural light was supplemented with 100 W high-pressure sodium lamps to maintain a day length of 16 h; maximum midday light intensity was approximately 1400 μE/m^2. Heads were tagged at anthesis. When the majority of heads had undergone anthesis, some of the plants were transferred to a second climate-controlled greenhouse and subjected to either a high daytime temperature regimen or a high daytime and high nighttime temperature regimen until the grain reached maturity. For the high daytime temperature regimen, daytime temperature was programmed for 5 h at 37 °C and nighttime temperature for 17 °C (37/17 °C regimen); the average daily temperature was 24 °C and maximum head temperature was 33 °C. For the high daytime and nighttime temperature regimen, the nighttime temperature was 28 °C and the high daytime temperature was maintained for 4 h (37/28 °C regimen); the average daily temperature was 30 °C and maximum head temperature was 33 °C. The 24/17 and 37/17 °C comparison was done in May, 1999 and the 24/17 and 37/28 °C comparison was done in June, 2000. Developing heads 3–50 days post-anthesis (dpa) were collected in the morning. Grains were removed from the heads, counted, and weighed. For analysis of gene expression, the endosperm was separated from the embryo and pericarp/testa. Early to mid development, endosperm was squeezed through the cut end of the pericarp/testa of the grain and, late in development, scraped from the pericarp/testa with a spatula after splitting the grain open with a razor blade. Grain and endosperm were frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Starch analysis

Grain was freeze-dried and ground to a powder using a UDY mill (UDY Corporation, Fort Collins, CO). Grain starch content was determined at each time point in triplicate using a kit for assay of total starch (Megazyme International, County Wicklow, Ireland). One hundred milligram samples were pretreated with 80% ethanol to remove sugars and then digested with thermal stable α-amylase followed by digestion with amyloglucosidase. Glucose was determined spectrophotometrically using glucose oxidase, peroxidase, and 4-aminoantipyrine. Weight of free glucose was converted to anhydroglucose using a multiplication factor of 160/180. Amylose/amylopectin ratios of starch were determined using a second Megazyme kit. Flour was placed in a mesh bag, the bag immersed in water, and the bag kneaded to release the starch. The aqueous starch suspension was centrifuged and the pelleted starch freeze-dried. Starch samples were dispersed by heating in dimethyl sulphoxide. Lipids were removed by precipitating the starch in ethanol and lipid-free starch was dispersed in 100 mM sodium acetate, pH 4.5. Amylopectin was precipitated with conconavalin A and amylose was determined before and after removal of

amylopectin. Amylose was enzymatically hydrolyzed to glucose and assayed by colorimetric detection of glucose using a glucose oxidase plus peroxidase detection method. The relative numbers and volumes of type A, B, and C starch granules were determined using dark field microscopy and quantitative image analysis as described by Bechtel et al. [6]. Volume was estimated assuming that the starch granules were oblate spheroids.

2.3. Isolation of clones for the starch biosynthetic enzymes

cDNAs for *SbeI* and *II* (GenBank Accession No. AF286317 and AF286319, respectively) were isolated from a wheat endosperm library using the maize homologues *Sbe1* (pA2-4) and *Sbe2b* (pMAI 1) (kindly provided by Mark Guiltinan, Pennsylvania State University). The library was prepared in Lambda ZAP II by Stratagene from a pool of mRNA isolated from wheat (*T. aestivum* L. cv. Cheyenne) endosperm collected at 5, 10, 15, 20, 25, and 30 dpa. The original library titer was 1×10^7 pfu; all screening was done using an amplified library of 1×10^8 pfu/ml. Plasmid DNA from positively hybridizing clones was sequenced and the identities of the cDNA clones confirmed by BLAST searching of the Genbank database.

cDNAs for *Agp1* and *2* were amplified using primers based on known nucleotide sequences from wheat (*T. aestivum* L. cv. Chinese Spring). DNA for PCR amplification was obtained by mass excision of the inserts from the cDNA library. For *Agp1* ([24], GenBank Accession No. X66080), the forward primer was GCTTATCCCTCGGCAATG and the reverse GTCTGCTGGAAATCAACTACAAG. For *Agp2* ([25], GenBank Accession No. Z21969), the forward primer was CATTTGATTGATCCGTCGCTTG and the reverse TGTTTGCTCGCTGCCACTTC. The amplified fragments were cloned in pCR2.1 and sequenced in both directions. *Agp1* (GenBank Accession No. AF244997) had minor nucleotide differences compared to X66080 while *Agp2* was identical to Z21969. The cDNA for *GssI* was identified from a collection of wheat endosperm ESTs by BLAST searching of the GenBank database; the full-length clone was sequenced completely in both directions (GenBank Accession No. AF286320).

2.4. mRNA analysis

Total RNA was isolated [26] and the integrity of all RNA samples was confirmed by gel electrophoresis. Total RNA was analyzed on slot blots (500 ng RNA/lane for *Gss* and *Ss*; 600 ng RNA/lane for *Agp1* and *2*, *SbeI* and *II*) using digoxigenin-labeled RNA probes (Genius System, Roche Molecular Biochemicals). Equal loading of total RNA onto the membranes was confirmed by hybridization of the slot blots with an 18S cDNA probe. The 1.7 kb *Agp1* and 2.1 kb *Agp2* cDNAs, both in pCR2.1, were linearized with Mfe I and BsrG I, respectively, and transcribed using T7 polymerase. The 2.3 kb *Gss* cDNA in pBS was linearized with Bbs I and transcribed with T3 polymerase. The 2.8 kb *SbeI* cDNA in pBS was linearized with Bgl II and transcribed with T3 polymerase. The 3.0 kb *SbeII* cDNA in pBS was linearized with BstE II and transcribed with T7 polymerase. The 2.5 kb rice *Ss* cDNA (GenBank Accession No. D16202, kindly provided by Dr Tadashi Baba, University of Tsukuba, Ibaraki, Japan) was subcloned into pSPT 18, linearized with Hind III, and transcribed with T7 polymerase. Initially, Northern blot analysis of wheat endosperm RNA indicated that the probes for *Agp1* and *2*, *Ss*, *Gss*, and *SbeI* and *II* hybridized to mRNAs of 1.7, 2.1, 2.5, 2.3, 2.8, and 3.0 kb, respectively. Because of the specificity of the hybridizations, subsequent analyses were performed using slot blots.

2.5. Reverse transcriptase-polymerase chain reaction

Primer pairs with the following sequences were used to amplify regions of starch synthase genes: *SsI* (Genbank accession No. AJ292521), CCATTCCAGAGCTCATGAGG and ACGTGTAACACGGACAGAGAGG; *SsIIa-3* (Genbank accession No. AJ269504), GGTTGTGGTACCAAGGTATGG and CCGTATGATGTCGTGAAGCC; *SsIII* (Genbank accession No. AF258608), GGCGTTGGATGTGTATATGG and GGTGATGATTCCGACAATAGG. Reverse transcriptase-polymerase chain reaction (RT-PCR) of RNA from 8 dpa developing grains using primer pairs for the *SsI*, *SsII*, and *SsIII* genes yielded single amplification products of 820, 620 and 510 bp, respectively. These amplification products were confirmed to correspond to the desired gene sequences by digestion with Sal I, Acc I, and Sac II (SsI); BstXI, Cla I, and BsaBI (SsII); or BamHI, Nco I, Ava I, and Bst XI (SsIII) (not shown).

RT-PCR was performed according to Altenbach et al. [27] using reagents and enzymes supplied by Applied Biosystems (Foster City, CA). One hundred nanogram of total RNA was reverse transcribed in a reaction containing 50 mM KCl, 10 mM Tris–Cl, pH 8.3, 5 mM $MgCl_2$, 1 mM of each dNTP, 2.5 μM random hexamers, 1 unit/μl RNase inhibitor, and 2.5 units/μl MuLV reverse transcriptase in a final volume of 20 μl. The sample was incubated at room temperature for 10 min, followed by 60 min at 42 °C, 5 min at 99 °C and 5 min at 5 °C in a Perkin Elmer Cetus DNA Thermal Cycler 480.

Amplifications were performed in 100 μl reaction volumes containing 20 μl of the reverse transcription mix, 2.5 units AmpliTaq DNA polymerase, and 20 pmoles of each oligonucleotide primer. The concentra-

tions of Tris–Cl, pH 8.3 and KCl in the final reaction were adjusted to 10 and 50 mM, respectively. Amplifications were carried out at 95 °C for 90 s, followed by 25 cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 2 min. A final extension was carried out at 72 °C for 7 min and the samples were incubated at 4 °C until analysis. Amplification of each RNA sample without prior reverse transcription confirmed the absence of contaminating DNA. Each RNA sample was also evaluated by RT-PCR using the primers TTCATATCACGTGCTGCATGG and AGACGACTTCGGTGAGACG that amplify a 242 bp region of the 18S rRNA gene. Aliquots of RT-PCR products were analyzed in 1.5% agarose gels in TBE buffer following standard procedures.

3. Results

3.1. Starch accumulation

To assess the effects of temperature on the pattern of starch production, plants were subjected to one of three temperature regimens starting at anthesis and starch content of grain was determined throughout development. In the first set of experiments, starch accumulation was compared in developing grain produced under the 24/17 and 37/17 °C regimens (Fig. 1A). Under the 24/17 °C regimen (Fig. 1A), starch accumulation began at about 10–14 dpa, slowed after 30 dpa, and ceased by approximately 36 dpa. Under the 37/17 °C regimen (Fig. 1A), starch accumulation began earlier, by 9 dpa, slowed after 24 dpa, and ceased by approximately 30 dpa. Grain that was not collected for the time course analyses was harvested at the end of the experiment and average final starch content per grain determined. Starch content was 25.39 ± 0.95 mg for grain produced under the 24/17 °C regimen and 20.39 ± 9.52 mg for grain produced under the 37/17 °C regimen.

A second experiment compared starch accumulation in developing grain produced under the 24/17 and 37/28 °C regimens (Fig. 1B). Under the 24/17 °C regimen, starch accumulation began at 10–12 dpa, slowed after 30 dpa, and ceased by approximately 38 dpa. Under the 37/28 °C regimen, starch began to accumulate rapidly from 7 dpa and starch content per grain was higher than under the 24/17 °C regimen until 14 dpa. However, under the 37/28 °C regimen, starch accumulation slowed after 14 dpa and ceased by approximately 18 dpa. The average starch content per grain at the end of this experiment was 22.06 ± 0.84 mg under the 24/17 °C regimen and 9.18 ± 2.86 mg, or 58.4% less, for grain from the 37/28 °C regimen.

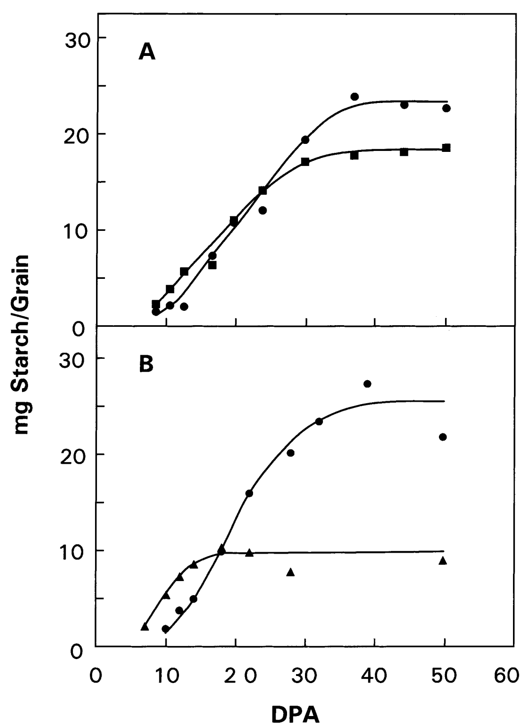

Fig. 1. Accumulation of starch in developing grains from wheat plants grown under different temperature regimens during grain-fill. (A) Comparison of starch accumulation in grains from plants grown under a 24/17 °C (●) or a 37/17 °C (■) regimen. (B) Comparison of starch accumulation in grains from plants grown under a 24 /17 °C (●) or a 37/28 °C (▲) regimen.

3.2. Starch granule distribution

To establish whether or not high temperature had an effect on starch granule populations, the relative number and volume of the three types of starch granules were determined in mature grain produced under the three regimens. The data for the 24/17 °C regimens from the two experiments were very similar and are combined in Fig. 2. Under both the 24/17 and 37/17 °C regimens, the B granules were the predominant type, comprising 63–67% of the relative volume (Fig. 2B). Compared to the 24/17 °C regimen, the relative number of the B granules decreased slightly under the 37/17 °C regimen (Fig. 2A). In addition, the relative volume of the A granules increased slightly and that of the B granules decreased slightly (Fig. 2B). Under the 37/28 °C regimen, the A granules were the predominant type, comprising 78% of the relative volume. Compared to the 24/17 °C regimen, the relative number of A granules increased and B granules decreased under the 37/28 °C regimen (Fig. 2A). In addition, the relative volume of the A granules increased substantially and the relative volume of the B granules decreased substantially (Fig. 2B). Under the 37/17 and 37/28 °C regimens, changes in the relative number and volume of the C granules were small (Fig. 2A and B).

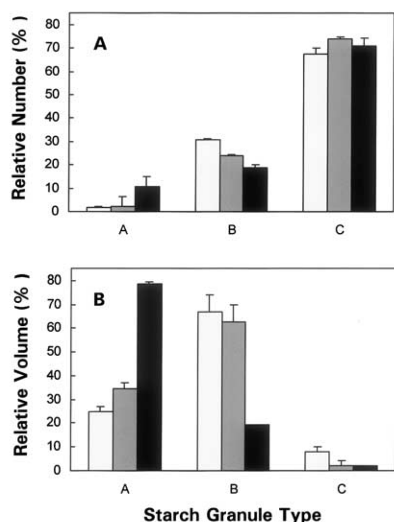

Fig. 2. Effect of high temperatures during grain-fill on the relative number and volume of type A, B, and C starch granules in the mature grain. (□) 24/17 °C regimen; (■) 37/17 °C regimen; (■) 37/28 °C regimen.

3.3. Amylose concentration

The amylose concentration was slightly higher in flour from mature grain produced under the high temperature regimens. In the first experiment, amylose concentration was 27.4 ± 0.7% for flour from grain from the 24/17 °C regimen and 28.5 ± 0.8% for the 37/17 °C regimen. In the second experiment, amylose concentration was 27.8 ± 1.0% for flour from grain from the 24/17 °C regimen and 30.3 + 1.2% for the 37/28 °C regimen.

3.4. Expression of genes for starch biosynthetic enzymes

Transcript levels for the starch biosynthetic enzymes were temporally regulated. Under the 24/17 °C regimen, steady state transcript levels for all of these enzymes peaked early, from 8 to 12 dpa (Fig. 3), preceding the onset of rapid starch accumulation (Fig. 1A). Transcript levels gradually decreased and were detectable at only low levels late in development, at 36–41 dpa (Fig. 3), when starch accumulation ceased (Fig. 1A). *Ss* transcripts decreased more rapidly to low levels than did the other transcripts and the transcript levels for *SbeII* decreased more rapidly than those for *SbeI*. Under the 37/17 °C regimen, steady state transcript levels followed a similar temporal pattern, but transcripts decreased to very low levels earlier in the time course and maximum transcript levels were generally lower. In contrast to the other transcripts, *SbeI* transcript levels were present at high levels later in development, at 19–22 dpa; levels were also reduced by the 37/17 °C regimen. Relative to the other transcripts, *Ss* transcript levels were very low in grain produced under the 37/17 °C regimen.

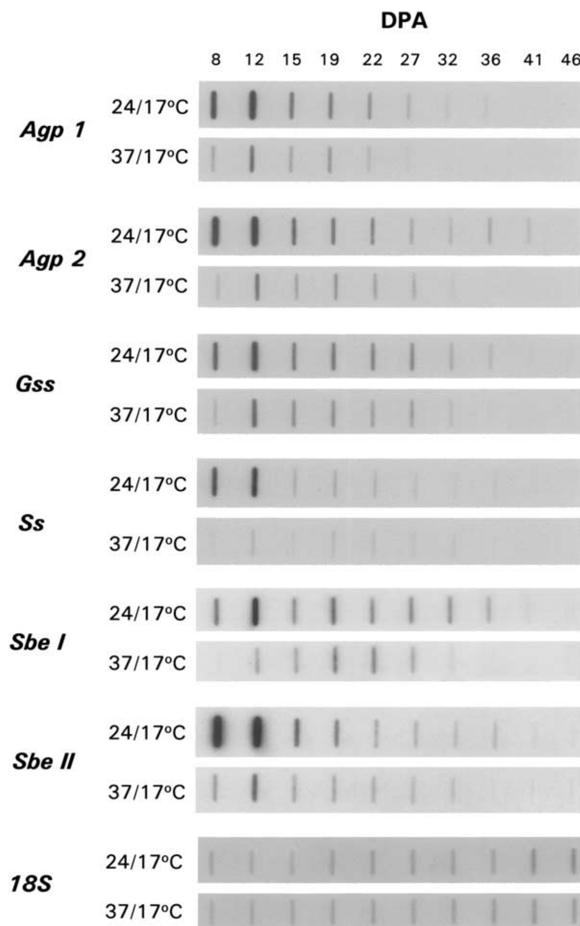

Fig. 3. Comparison of steady state transcript levels for starch biosynthetic enzymes in wheat endosperm collected 8–46 dpa from grain produced under the 24/17 and 37/17 °C regimens. Slot blots were hybridized with probes to *Agp1*, *Agp2*, *Gss*, *Ss*, *SbeI*, *SbeII*, or *18S rRNA*.

Steady state transcript levels for the starch biosynthetic enzymes were also compared in grain produced under the 24/17 and 37/28 °C regimens. The expression pattern for starch biosynthetic enzymes for the 24/17 °C regimen was different from that of the previous experiment. Steady state transcript levels peaked later, at 13–16 dpa for *Agp1* and *SbeII*, 13–23 dpa for *Gss* and *Ss*, and 16–28 dpa for *Agp2* and *SbeI* (Fig. 4). Under the 37/28 °C regimen, steady state transcript levels of the starch biosynthetic enzymes peaked much earlier and decreased to very low levels by 16–20 dpa (Fig. 4), when starch content in the grain was maximal (Fig. 1B). Transcripts for *Agp1* and *2*, *Gss*, *Ss*, and *SbeII* were maximal at 7 dpa, the first time point examined, and transcripts for *SbeI* were again maximal later in development, at 7–13 dpa (Fig. 4). Maximum transcript levels attained for all the starch biosynthetic enzymes, except *Ss*, were similar to or slightly less than those in grain produced under the 24/17 °C regimen. Again, very little *Ss* transcript was detected in endosperm from grain produced under the 37/28 °C regimen.

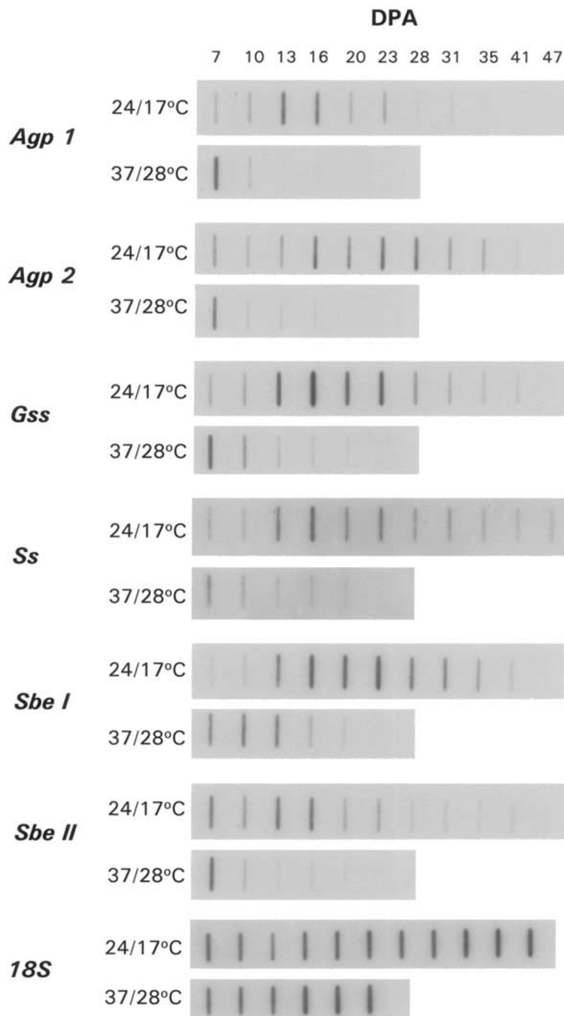

Fig. 4. Comparison of steady state transcript levels for starch biosynthetic enzymes in wheat endosperm collected 7–47 dpa from grain produced under the 24/17 and 37/28 °C regimens. Slot blots were hybridized with probes to *Agp1*, *Agp2*, *Gss*, *Ss*, *SbeI*, *SbeII* or *18S rRNA*.

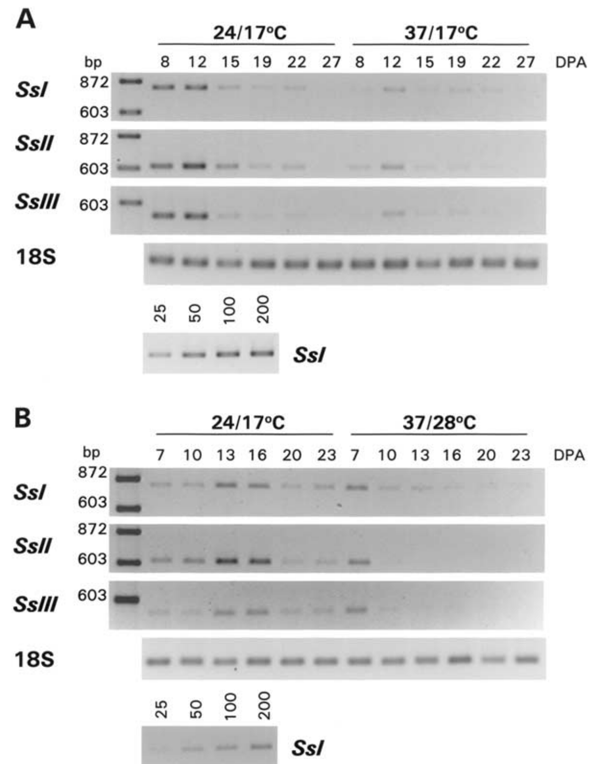

Fig. 5. Comparison of accumulation profiles for starch synthase I, II, and III transcripts in wheat endosperm collected from grain produced under (A) the 24/17 and 37/17 °C regimens or (B) the 24/17 and 37/28 °C regimens. RT-PCR products were produced using primer pairs specific for *SsI*, *SsII*, *SsIII* or *18S rRNA*. Images of agarose gels stained with EtBr were reversed for clarity. RT-PCR products generated with *SsI* primers from 25, 50, 100 or 200 ng of endosperm RNA from 12 dpa (A) or 16 dpa (B) developing grains are shown on the bottom of each panel.

3.5. Expression of genes for soluble starch synthase

The rice probe used to evaluate *Ss* transcripts has approximately 80% similarity to the coding region of wheat *SsI* genes. However, two additional starch synthases that have been characterized in wheat endosperm, *SsII* and *SsIII*, would not be detected by this probe. Consequently, RT-PCR using primers specific for the wheat *SsI*, *SsII*, and *SsIII* genes was used to examine the accumulation profiles of transcripts in grain developing under the different environmental regimens (Fig. 5). In the comparison of grain produced under the 24/17 and 37/17 °C regimens, the accumulation profiles of *SsI* transcripts observed by RT-PCR (Fig. 5A) were similar to those obtained by hybridization analysis (Fig. 3). The expected 820 bp amplification product was easily detected in 8 and 12 dpa grain produced under the 24/17 °C regimen while lesser amounts of amplification product were detected later in development or in samples from grain produced under the 37/17 °C regimen. RT-PCR using the *SsII* and *SsIII* primers generated 620 and 510 bp amplification products, respectively, and the patterns during grain development were strikingly similar to those obtained for *SsI*. In the comparison of grain produced under the 24/17 and 37/28 °C regimens (Fig. 5B), the accumulation profiles of *SsI* transcripts observed by RT-PCR again resembled those obtained by hybridization analysis with the rice probe (Fig. 4) and profiles generated with the *SsII* and *SsIII* primers were similar to those obtained with *SsI* primers. The data suggest that the *SsI*, *SsII*, and *SsIII* genes follow similar patterns of expression during grain development and respond to high temperatures in the same manner.

4. Discussion

High temperatures during wheat grain-fill reduced starch content and altered the size distribution of starch

granules in the mature grain. The decrease in starch content was primarily due to a reduction in the duration of starch accumulation. In addition, we observed a decrease in the relative number of B granules under both high temperature regimens as reported previously by Bhullar and Jenner [28] and Blumenthal et al. [29]. However, under the 37/28 °C regimen, there was also a dramatic effect on the relative volumes of the A and B granules. Under the 37/28 °C regimen, the A granules increased from approximately 25% to nearly 80% of the relative volume under the 24/17 °C regimen and the B granules decreased from 70 to 20% of the relative volume. The A granules appear to have developed and increased in size, but fewer B granules were initiated, suggesting that the program for starch granule formation was interrupted rather than compressed. If the program were compressed, the ratios of A to B granules would be similar to those in grain produced under the 24/17 °C regimen.

Since A granules contain 30–36% amylose while B granules contain 24–27% amylose [30], a possible consequence of the changes in granule composition under the high temperature regimens is an alteration in starch composition in the mature grain. In this study, we found that amylose content increased in grain produced under the high temperature regimens. Although the increases were small, Zeng et al. [31] reported that as little as a 1% change in total amylose content significantly affects starch gelatinization, pasting, and gelation properties. Morris et al. [32] and Zeng et al. [31] have also shown that grain amylose content is influenced by environment as well as genotype.

Because of the large effect of high temperature on starch accumulation observed in our experiments and the decrease in the activities of the enzymes involved in starch biosynthesis reported in previous studies [16,17,19–21], we analyzed transcript levels for the starch biosynthetic enzymes in the endosperm of grain produced under the three temperature regimens. Expression of genes encoding *Agp1* and *2*, *Gss*, *SsI*, *II*, and *III*, and *SbeI* and *II* was temporally regulated. Under the 24/17 °C regimen, maximal transcript levels corresponded to the onset of rapid starch accumulation. Previous studies reported different times for peak expression of transcripts for *Agp* [24,25], *Gss* [33], *SsI* and *IIa* [34,35], *SsIII* [13], *SbeI* [36], and *SbeII* [37]. However, none of these studies examined all of these transcripts in a single experiment using the same cultivar and growing conditions. Differences in the timing of peak expression in different cultivars were reported for *SsIIa* [34] and our results clearly demonstrate that the timing of expression of all of these transcripts is dependent on the temperature regimen during grain-fill. However, there were differences in the expression profiles for grain produced under the two 24/17 °C regimens. In the first experiment (Fig. 3), all transcripts, except SbeI, peaked at 8–12 dpa. In the second experiment (Fig. 4), steady state transcript levels peaked later overall, at 13–16 dpa for *Agp1* and *SbeII*, 13–23 dpa for *Gss* and *Ss*, and 16–28 dpa for *Agp2* and *SbeI*. These variations in expression profiles may be due to the innate variation of living systems and because experiments were carried out in two different years at slightly different times of the growing season. This variability, even under controlled greenhouse conditions, underscores the importance of making comparisons between different regimens within the same experimental set. Under the three temperature regimens used in this study, transcript levels declined well before starch accumulation ceased. It is likely that the mRNAs for these enzymes are translated during the period of rapid starch accumulation and that these enzymes are stable and retain activity until the program for starch accumulation ends. In support of this, Western blots revealed that *SbeI* and *II* increased early and were present at constant levels later in developing wheat grain [38].

Detailed studies demonstrate that high temperatures decrease the activities of enzymes in the starch biosynthetic pathway [16,17,19–21]. Based on the striking correlation between rates of Ss activity and starch synthesis, Jenner et al. [19] and Keeling et al. [20] concluded that Ss is the major site of regulation of starch synthesis in wheat endosperm. In *in vitro* experiments, temperatures above 25 °C decreased the activity of Ss significantly [17,19,20,39]. In addition to decreasing Ss activity, our findings demonstrate that high temperature may regulate *Ss* gene expression at the transcriptional level. Under the 37/17 °C regimen, steady state transcript levels for *Ss* followed the same developmental pattern as for the 24/17 °C regimen, but levels were substantially lower at peak expression. In contrast, under the 37/28 °C regimen, steady state transcript levels peaked earlier and transcripts were present for a much shorter period of time in the developing grain, reflecting the shorter duration of starch accumulation. The three classes of *Ss* that have been identified in wheat endosperm all responded to high temperature in a similar manner. It is intriguing that, despite the effect of high temperature on the accumulation of *Ss* as well as the other transcripts, the rate of starch accumulation was not dramatically different. In addition, starch accumulation began earlier under the high temperature regimens, but the rate did not compensate for the shortened duration of grain-fill. Identification of the specific mechanisms for starch accumulation under high temperature regimens must await quantification of the amounts and activities of the starch biosynthetic enzymes during grain-fill. High temperatures during grain-fill not only affect starch accumulation and transcript profiles for the starch biosynthetic enzymes, but shorten the time to maximum kernel water content, maximum kernel dry weight and

harvest maturity, and alter gluten storage protein transcript accumulation profiles [27], demonstrating that high temperature significantly affects the overall program of grain development. High throughput transcript and protein profiling techniques will be useful in identifying the effect of high temperature on the many genes and gene products that characterize the molecular events central to grain development, including the onset of grain maturation and the endpoint of starch and protein accumulation.

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