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Effects of high temperature after anthesis on starch granules in grains of wheat (*Triticum aestivum* L.)

P. LIU¹, W. GUO^{1*}, Z. JIANG¹, H. PU¹, C. FENG¹, X. ZHU¹, Y. PENG¹, A. KUANG²
AND C. R. LITTLE³

¹ Jiangsu Provincial Key Laboratory of Crop Genetics and Physiology/Wheat Research Institute, Yangzhou University, Yangzhou 225009, Jiangsu, China

² Department of Biology, The University of Texas-Pan American, Edinburg, TX 78539, USA

³ Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

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SUMMARY

The effect of high temperatures (above 25 °C) on starch concentration and the morphology of starch granules in the grains of wheat (*Triticum aestivum* L.) were studied. Wheat plants of cultivars Yangmai 9 (weak-gluten) and Yangmai 12 (medium-gluten) were treated with high temperatures for 3 days at different times after anthesis. The results showed that the starch concentration of grains given a heat-shock treatment above 30 °C were lower than those developing at normal temperature in both cultivars. High temperature lowered starch concentration due to the decrease of amylopectin. Under the same temperature, the effect of heat shock from 6 to 8 days after anthesis (DAA) was the greatest, whereas from 36 to 38 DAA the effect was the least. The effects of high temperatures after anthesis on starch-pasting properties were similar to those on starch concentration, especially after 35–40 °C treatments. The size, shape and structure of starch granules in wheat grains (determined by electron microscopy) after heat shock were visibly different from the control. When given heat shock during development, the starch granules in mature wheat grains were ellipsoid in shape and bound loosely with a protein sheath in Yangmai 9, while they were damaged and compressed with fissures in Yangmai 12, indicating the differences in resistance to high temperature between cultivars. Ratios of large (type-A) and small (type-B) starch granules significantly decreased under heat shock, which limited the potential sink size for dry matter deposition in the grain.

INTRODUCTION

Wheat grain yield and quality are affected by many different environmental factors. Among these, temperature is a major component in environmental variation and has a marked effect on grain filling for wheat. Heat stress reduces the grain weight through a reduction in grain growth duration and grain growth rate (Viswanathan & Khanna-Chopra 2001). High temperatures hasten the decline in photosynthesis, reduce leaf area and decrease shoot and grain mass as

well as the weight and sugar content of kernels (Shah & Paulsen 2003). The rate of cell enlargement and the rate of accumulation of nitrogen (N) in the grains are relatively unresponsive to higher temperatures (Jenner 1994). Within the grains, Feng *et al.* (2000) found that the endosperm cell proliferation rate was increased for a short period after exposure to high temperature, but the duration of cell proliferation was significantly shortened, which resulted in a decreased maximum endosperm cell number and a decline of grain weight. In addition, the grain nitrogen content at maturity increases under heat shock. Viswanathan & Khanna-Chopra (2001) found that gliadin synthesis increased and glutenin synthesis decreased. Protein synthesis

* To whom all correspondence should be addressed.
Email: guows@yzu.edu.cn

Table 1. *Effects of different temperatures on total starch concentration in grains and filling duration in two wheat cultivars during the 2003/04 experiment (D.F. = 3)*

Treated stage (DAA)	Treated temperature (°C)	Yangmai 9		Yangmai 12	
		Starch concentration (mean $\pm$ S.E.M.) (mg/g)	Filling days (day)	Starch concentration (mean $\pm$ S.E.M.) (mg/g)	Filling days (days)
15–17	25	772 $\pm$ 5.5	46	738 $\pm$ 4.8	46
	30	790 $\pm$ 15.8	46	782 $\pm$ 4.4	46
	35	760 $\pm$ 5.2	42	735 $\pm$ 5.3	41
	40	707 $\pm$ 4.2	40	695 $\pm$ 4.7	39
19–21	25	750 $\pm$ 5.0	47	731 $\pm$ 4.6	47
	30	764 $\pm$ 15.3	46	776 $\pm$ 4.5	46
	35	731 $\pm$ 14.6	43	729 $\pm$ 4.6	43
	40	698.0 $\pm$ 4.0	41	685 $\pm$ 3.7	41
25–27	25	751 $\pm$ 5.0	48	744 $\pm$ 4.9	48
	30	779 $\pm$ 15.6	47	772 $\pm$ 5.4	47
	35	711 $\pm$ 4.2	44	713 $\pm$ 4.3	44
	40	670 $\pm$ 3.4	41	666 $\pm$ 3.3	41
33–35	25	794 $\pm$ 5.9	48	755 $\pm$ 4.9	48
	30	803 $\pm$ 6.1	48	797 $\pm$ 5.9	48
	35	777 $\pm$ 15.6	47	748 $\pm$ 5.0	47
	40	728 $\pm$ 4.6	47	709 $\pm$ 4.2	46
Normal temperature		793 $\pm$ 5.8	48	774 $\pm$ 5.5	48

was less heat sensitive than starch accumulation (Bhullar & Jenner 1985).

Wheat endosperm contains two types of starch granules: type-A (large granules) and type-B (small granules) (Chiotelli & Le Meste 2002). Environments where temperatures rise above 30 °C during the first 14 days after anthesis (DAA) produce grains with a high proportion of type-A starch granules (Panozzo & Eagles 1998). The proportion of amylose in the total starch content also increases with temperatures above 30 °C during the first 14 days, but is not influenced by temperature to the same extent as granule type (Panozzo & Eagles 1998). However, little is known about the effects of high temperature at different levels during the grain-filling period on starch concentration and traits. The objective of the present study was to assess the effects of different high-temperature treatments at different stages of grain filling on the starch concentration and traits in Yangmai 9 and Yangmai 12, two widely grown wheat cultivars in China with contrasting gluten properties. Such information will aid understanding how heat affects starch concentration and traits, and provide a valuable reference for improving wheat grain yield under high-temperature stress.

MATERIALS AND METHODS

Field traits

The experiment was carried out in the agricultural experimental field at the Jiangsu Provincial Key

Laboratory of Crop Genetics and Physiology (119°26'E, 32°24'N, 7 m asl) of the Yangzhou University from 2003 to 2005. The wheat (*Triticum aestivum* L.) cultivars used in the study were Yangmai 9 (weak-gluten) and Yangmai 12 (medium-gluten). Seeds were obtained from the Wheat Research Institute, Yangzhou University, China. The wheat plants were grown in pots (diameter at the top was 0.3 m and at the bottom was 0.2 m, height 0.27 m) filled with 11 kg loam soil with nitrogenous P₂O₅ and K₂O fertilizer (3.52 g N/pot; 1.32 g P₂O₅/pot; 1.32 g K₂O/pot). Twelve seeds each of Yangmai 9 and Yangmai 12 were sown on 28 October 2003 and 30 October 2004. Plants were thinned to six plants per pot on 30 November 2003 and 3 December 2004. Four pots were used for each treatment and the wheat plants kept in each pot were morphologically uniform. The pots were irrigated when the soil surface appeared dry until mid-April, and then each day thereafter. Both cultivars reached 0.5 anthesis on the same date in both years: 18 April in 2004 and 21 April in 2005.

Treatment levels

Four temperature treatment levels (i.e. daily average temperature) of 25, 30, 35 and 40 °C ($\pm$ 0.5 °C) were set in a phytotron glasshouse. The relative air humidity was set at 0.5 ($\pm$ 0.01), the photosynthetic photon flux density was 800 μ mol/m²/s and the length of photoperiod averaged 12 h. During grain-filling periods, pots with wheat plants were transferred to

Table 2. Effects of varied temperature on total starch concentration in grains and filling duration in two wheat cultivars during the 2004/05 experiment (*D.F.* = 3)

Treated stage (DAA)	Treated temperature (°C)	Yangmai 9		Yangmai 12	
		Starch concentration (mean $\pm$ S.E.M.) (mg/g)	Filling days (day)	Starch concentration (mean $\pm$ S.E.M.) (mg/g)	Filling days (days)
1–3	25	731 $\pm$ 4.5	42	719 $\pm$ 7.0	42
	30	747 $\pm$ 0.1	40	720 $\pm$ 6.5	40
	35	695 $\pm$ 5.3	37	623 $\pm$ 2.8	36
	40	619 $\pm$ 2.0	35	584 $\pm$ 1.3	33
6–8	25	730 $\pm$ 0.8	42	710 $\pm$ 4.9	42
	30	739 $\pm$ 5.4	40	719 $\pm$ 3.6	40
	35	660 $\pm$ 2.2	36	602 $\pm$ 8.0	35
	40	568 $\pm$ 7.9	33	521 $\pm$ 5.7	31
13–15	25	729 $\pm$ 2.5	43	710 $\pm$ 3.1	43
	30	738 $\pm$ 5.5	42	716 $\pm$ 3.6	42
	35	715 $\pm$ 6.0	40	682 $\pm$ 2.3	39
	40	643 $\pm$ 10.2	37	625 $\pm$ 3.5	36
19–21	25	722 $\pm$ 1.3	43	704 $\pm$ 5.2	43
	30	731 $\pm$ 2.5	42	713 $\pm$ 5.8	42
	35	704 $\pm$ 2.9	40	678 $\pm$ 5.4	39
	40	643 $\pm$ 9.5	37	620 $\pm$ 4.3	36
25–27	25	722 $\pm$ 3.9	44	694 $\pm$ 1.8	44
	30	730 $\pm$ 1.2	43	710 $\pm$ 2.2	43
	35	678 $\pm$ 3.2	40	668 $\pm$ 5.5	39
	40	617 $\pm$ 7.2	37	587 $\pm$ 3.6	36
33–35	25	775 $\pm$ 3.6	46	750 $\pm$ 6.1	46
	30	785 $\pm$ 5.7	46	757 $\pm$ 3.1	46
	35	762 $\pm$ 9.6	45	714 $\pm$ 5.6	45
	40	722 $\pm$ 10.2	43	670 $\pm$ 6.4	43
36–38	25	787 $\pm$ 5.6	46	756 $\pm$ 4.5	46
	30	800 $\pm$ 1.4	46	764 $\pm$ 5.4	46
	35	771 $\pm$ 2.8	45	740 $\pm$ 6.2	45
	40	736 $\pm$ 9.4	44	704 $\pm$ 5.2	44
Normal temperature		792 $\pm$ 3.7	46	772 $\pm$ 6.0	46

four temperature-controlled phytotron glasshouses, with the four temperature treatment levels, where they remained for 3 days. Wheat plants were treated with high temperatures on selected DAA (2003/04 experiment: 15–17, 19–21, 25–27 and 33–35 DAA; 2004/05 experiment: 1–3, 6–8, 13–15, 19–21, 25–27, 33–35 and 36–38). After high-temperature treatment, the pots were transferred back to the natural environment (average daily temperature 15–28 °C) outside the phytotron glasshouse. Plants in pots growing under the natural environment, untreated with high temperature, were the controls. The date at which grains attained maximum dry weight was recorded as the date of maturity for the crop. Mature spikes from all plants were hand harvested.

Starch concentration

Wheat flour was produced from 10 g grains from each treatment. Grains were milled on a Brabender

Quadruplex experimental mill, and bran and endosperm were separated by sifting the samples on a strand shaker using a brass standard sieve no. 70. The flour was used for determining starch concentration and pasting characteristics.

The starch concentration of wheat grains was determined based on the double-wavelength method by UV-visible spectrophotometry (Perkin Elmer Lambda 15, Veberlingen, Germany) (He 1985). For each sample, wheat flour (100 mg) was placed in a test tube, to which 10 ml of freshly prepared potassium hydroxide (KOH; 5 M) was added. The tube was heated and mixed for 10 min in a water bath at 100 °C and adjusted to a final volume of 50 ml with sterile distilled water (sdH₂O). A 2.5 ml aliquot of this fraction was mixed with 20 μ l of sdH₂O in two bottles, in which the pH value was adjusted to 3.5 by addition of 1 M hydrochloric acid (HCl). Thereafter, 0.5 ml of a freshly prepared iodine–potassium iodide (I₂–KI) solution (2 g I₂/l + 20 g KI/l) was added to one of the

bottles for colour reaction and the volumes were made up to 50 ml. After 20 min the absorbance was measured (490 and 646 nm for amylose and 574 and 750 nm for amylopectin, respectively) using a UV-visible spectrophotometer.

Pasting characteristics

Pasting characteristics of the flour were measured with a rapid visco analyser (RVA) (Newport Scientific Pty. Ltd., Sydney, Australia). Flour (3.5 g, 0.14 moisture) was placed in an aluminium can containing 25 ml distilled water. The flour suspension was stirred constantly while it was heated at 50 °C for 1.5 min, then the temperature was increased to 95 °C at a constant rate, held at 95 °C for 3 min and cooled at a constant rate to 50 °C for 5.5 min. The primary flour-pasting characteristics derived from the RVA curve included peak viscosity, minimum viscosity, final viscosity (maximum viscosity during cooling to 50 °C), breakdown (peak minus minimum viscosity) and setback (final minus minimum viscosity).

The starch samples were used to determine the size distribution of starch granules and to evaluate the effect of high temperature after anthesis on the volume proportion of different size fractions of starch granules. The low-angle laser light scattering (LALLS) method was used to determine the distribution of starch granule sizes (Analysette 22, Fritsch, Germany). The ratio of starch A-(large) to B (small)-type granules was determined.

Starch granules

Wheat starch granules were examined by environmental scanning electron microscopy (ESEM). Fifteen fresh wheat grains were randomly collected from each treatment and soaked in a fixative containing 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) overnight at 4–10 °C and then dehydrated in a series of acetone. The grains were cut into 2 mm sections. The middle sections were mounted on aluminium stubs and examined in a Philips XL30-ESEM at an accelerating potential of 20 kV.

Temperature treatments for plants were arranged in four replications. Analysis of variance was applied to each data set to assess the effects of the treatments by using SAS (PROC GLM; SAS Institute 1997).

RESULTS

As shown in Tables 1 and 2, plants were premature and the filling duration shortened under heat shock after anthesis in Yangmai 9 (weak-gluten) and Yangmai 12 (medium-gluten). The weight of grains experiencing >25 °C was lower than those grown at normal temperature after anthesis (DAA). Grain weight reduced with increasing temperatures. Under

Fig. 1. Average total starch concentration (amylopectin + amylose) (mg/g) of (a) Yangmai 9 and (b) Yangmai 12 wheat exposed to a range of temperatures (25–40 °C) at 15–17 to 33–35 DAA and average total starch concentration of wheat exposed to normal outdoor temperatures (dashed line) during the 2003/04 experiment. The total starch and amylopectin concentration in temperature above 30 °C are significantly different from that in normal temperature ($P < 0.05$, $n = 4$). Bars represent S.E.M.

the same temperature, the response of grain weight to heat shock from 6 to 8 DAA was the greatest; from 1 to 3 DAA was the second, whereas that from 36 to 38 DAA was the least.

As shown in Figs 1 and 2, high-temperature treatment after anthesis curtailed starch concentration in grains of both Yangmai 9 and Yangmai 12. Amylopectin and total starch concentrations of grains experiencing temperatures >30 °C were significantly lower than those at normal temperature from 1 to 33 DAA. There was little difference in amylopectin and total starch concentrations between grains exposed to 25, 30 and 35 °C and that of the control after 33 DAA,

Fig. 2. Average total starch concentration (amylopectin+amylose) (mg/g) of (a) Yangmai 9 and (b) Yangmai 12 wheat exposed to a range of temperatures (25–40 °C) at 1–3 to 36–38 DAA and the average total starch concentration of wheat exposed to normal outdoor temperatures (dashed line) during the 2004–2005 experiment. The total starch and amylopectin concentration in temperature above 30 °C are significantly different from that in normal temperature ($P < 0.05$, $n = 4$). Bars represent S.E.M.

but they decreased significantly when the temperature was higher than 35 °C. The decline of starch concentration at high temperatures was mainly due to the reduction of amylopectin. Grain starch concentration was highest in grains experiencing 30 °C and lowest in those at 40 °C in both Yangmai 9 and Yangmai 12.

There were differences in starch concentration responses to heat stress at the different filling stages. Under the same temperature at the early filling stage, the effect of heat shock from 6 to 8 DAA was the greatest, and from 1 to 3 DAA was the second highest. At the middle and late filling stages, the effect of heat

shock on starch concentration from 25 to 27 DAA was the greatest, while there was least effect from 36 to 38 DAA. Both cultivars showed the same trends in both years (Figs 1 and 2).

The results showed that the starch-pasting properties of grains experiencing 30 °C were similar to those grown at 25 °C. The peak viscosity, minimum viscosity, breakdown, final viscosity and setback in the RVA profile of starch were lower with exposure to temperatures over 30 °C in both cultivars. At the same temperature, the effect of heat shock on grain starch-pasting characteristics from 6 to 8 DAA was the greatest, while that from 33 to 35 DAA was the least (Fig. 3). The effect of high temperature after anthesis on the RVA profile was also reflected in the tail of the RVA curve. The bend of the tail for the high-temperature treatment was less than that for the low-temperature treatment in the same cultivar. Resistance to high temperature varied with cultivar; Yangmai 9 was stronger than Yangmai 12.

Starch granules in grains of both heat-shocked cultivars were morphologically different from the granules of the control treatments (Fig. 4) in the 2003/04 experiment. In Yangmai 9, starch granules were only slightly damaged in the 30 °C treatment (Fig. 4), but the granules became small and ellipsoid in shape and were bound loosely with a protein sheath in the 40 °C treatment, in comparison with the grains from a normal environment (Fig. 4). In Yangmai 12, on the first day after the 30 °C heat-shock treatment had ended, the fissures in starch granules were observed, but disappeared at maturity (Fig. 4). However, in the 40 °C treatment, starch granules were compressed with fissures that remained at maturity (Fig. 4).

The results from the 2004/05 experiment were similar to those of the previous year. Heat shock at 30 °C and below had no visible effect on starch grains, while above 30 °C it had a large effect. The starch granules were ellipsoid in shape, and combined loosely with a protein sheath under high temperature in Yangmai 9, in comparison with the grains from the normal environment (Fig. 5). The sizes of starch granules after the 40 °C heat shock were smaller than the control and other treatments. At the early filling stage, the effects of heat shock above 30 °C on starch granules from 6 to 8 DAA were greatest, showing smaller granules loosely combined with a protein sheath (Fig. 5), and those features were also obvious from 1 to 3 DAA (Fig. 5). At the middle and late grain-filling stages, the effect of heat shock was greatest from 25 to 27 DAA (Fig. 5), showing small granules and loosely combined protein sheaths, while there was less effect from 33 to 35 DAA (Fig. 5).

The starch granules in Yangmai 12 had no fissures on the first day after the 30 °C heat-shock treatment had ended, of around 1–3, 6–8, 13–15, 19–21 and

Fig. 3. RVA profile characteristics showing the effect of different temperatures (25–40 °C) after anthesis on the pasting characteristics of grains sampled at different grain-filling periods (1–3, 6–8, 13–15, 19–21, 25–27 and 33–35 DAA for Yangmai 9 (left column) and Yangmai 12 (right column) in the 2004/05 experiment. The y-axis represents viscosity and the x-axis represents pasting time.

33–35 DAA (data not shown) or at maturity, but those after the 40 °C treatment were damaged and compressed with fissures (Fig. 6). However, on the first day during heat-shock treatment with 30 °C and above, from 25 to 27 DAA, fissures on starch granules were observed.

Fig. 4. ESEM micrographs of starch granules showing the effect of temperatures on the morphology of granules from 25 to 27 DAA in Yangmai 9 and Yangmai 12 during the 2003/04 experiment. The column entitled 25–27 represents ESEM micrographs taken one day after treatment. The column entitled Mature represents ESEM micrographs taken at kernel maturity (harvest). Rows represent 30 and 40 °C treatments as well as normal, which represent kernels taken from wheat exposed to normal outdoor conditions. Arrows indicate fissures on starch granules. The columns entitled starch granule indicate images that have been enlarged 3 times of image in the left column in order to accentuate the detailed morphology of starch granule especially fissures. Bars represent 20 µm.

The starch granules had a few fissures in the treatment under 30 °C, but fissures were frequently observed in the treatment at 40 °C. The number of granules with fissures increased with an increase in the duration of high temperatures. On the first day after the 30 °C heat-shock treatment had ended, fissures were observed on granules, but these disappeared on the fifth day after treatment (Fig. 6). However, in the treatment at 40 °C, granules with fissures could still be observed at maturity (Fig. 6).

The results of evaluation of the size distribution of starch granules are shown in Table 3. The volume fraction (proportion of the volume) as A-type starch granules in grains from the 40 °C treatment was lower than that in grains experiencing 30 °C, whereas for B-type starch granules the reverse was true. The volume fraction of B-type starch granules was enhanced significantly ($P < 0.05$), but that of type A starch granules reduced greatly under high temperature. High temperature reduced the volume of type A starch granules, mainly due to the reduction of type A ($> 20 \mu\text{m}$) starch granules. The volume of type A ($10\text{--}20 \mu\text{m}$) starch granules was actually

enhanced. The ratio of types A and B starch granules decreased significantly under heat shock ($P < 0.05$).

DISCUSSION

The yield and quality of wheat grains were dependent on genotype and environmental conditions. High temperature ($> 30 \text{ °C}$) at the time of grain filling is one of the major constraints for increasing productivity of wheat in many countries (Rane & Nagarajan 2004). Increased temperature results in poorly filled, shrivelled grains. Kernels such as these, yield less white flour than filled mature grains (Bayles 1977). Guo *et al.* (1998) showed that at high temperatures, the assimilating efficiency of the flag leaf is reduced and transportation and accumulation of ^{14}C -assimilate were inhibited, which resulted in a significant decrease in grain weight.

The effects of high temperature on the grain growth depended on the timing of the heat shock and the degree of heat stress. Under the high temperature, the grain mass was decreased by reducing the duration of

Fig. 5. ESEM micrographs of starch granules of Yangmai 9 wheat showing the effect of high temperatures on the morphology of granules from different grain-filling periods and harvested at maturity for the 2004/05 experiment. Columns represent 30 and 40 °C treatments. Rows represent different grain-filling periods (1–3, 6–8, 25–27 and 33–35 DAA). The panel labelled normal, mature represents kernels taken from Yangmai 9 wheat exposed to normal outdoor conditions. Bars represent 20 μ m.

grain filling and grain weight decreased significantly, consistent with the findings of Bayles (1977).

Starch (amylose and amylopectin) is the major storage component in wheat grain comprising 0.6–0.7 of dry weight (Morell *et al.* 1995). Changes in starch concentration are indicators of a variety of plant developmental processes, both positive and negative (Rodrigo *et al.* 1997). The composition of starch in wheat is an important determinant of grain quality. The timing and extent of high-temperature stress are crucial in determining its effects on starch concentration in grains. Zhao *et al.* (2006) showed that the concentrations of total starch and amylopectin were markedly reduced, but amylose concentration was affected slightly in higher-temperature treatments. Yan *et al.* (2008) showed that the total starch and amylopectin concentrations decreased significantly,

but the amylose concentration increased in heat treatments in comparison with control. The present study found that high-temperature curtailment of the total starch concentration was due to the decreased amylopectin concentration, whereas the amylose concentration of grains remained relatively constant in both experiments (Figs 1 and 2). A decrease in grain amylopectin concentrations was shown at temperatures above 30 °C, in comparison with those at the normal (outdoor) temperature in all treated days from anthesis to the 33rd DAA and with increased temperature (Figs 1 and 2).

The effects of high temperature during different grain-filling periods on the starch concentration have rarely been reported. The present study found that there were differences in starch concentration in responses to heat stress at the different filling

Table 3. Effects of different temperatures after anthesis on starch granule size distribution in two wheat cultivars (25–27 DAA) (D.F. = 3)

Year	Variety	Temperature (°C)	Volume proportion of different size fractions of starch granules (mean $\pm$ S.E.M.)				Ratio of starch A and B
			< 10 (starch B)	10–20	> 20	> 10 (starch A)	
2003/04	Yangmai 9	30	0.40 $\pm$ 0.005	0.48 $\pm$ 0.007	0.13 $\pm$ 0.012	0.60 $\pm$ 0.005	1.52 $\pm$ 0.03
		40	0.44 $\pm$ 0.003	0.53 $\pm$ 0.008	0.03 $\pm$ 0.011	0.56 $\pm$ 0.003	1.26 $\pm$ 0.02
	Yangmai 12	30	0.35 $\pm$ 0.007	0.50 $\pm$ 0.009	0.15 $\pm$ 0.002	0.65 $\pm$ 0.007	1.83 $\pm$ 0.06
		40	0.40 $\pm$ 0.005	0.55 $\pm$ 0.009	0.05 $\pm$ 0.004	0.60 $\pm$ 0.005	1.52 $\pm$ 0.03
2004/05	Yangmai 9	30	0.39 $\pm$ 0.006	0.48 $\pm$ 0.010	0.13 $\pm$ 0.004	0.61 $\pm$ 0.006	1.58 $\pm$ 0.04
		40	0.44 $\pm$ 0.008	0.55 $\pm$ 0.005	0.02 $\pm$ 0.004	0.56 $\pm$ 0.008	1.27 $\pm$ 0.04
	Yangmai 12	30	0.35 $\pm$ 0.009	0.49 $\pm$ 0.007	0.16 $\pm$ 0.016	0.65 $\pm$ 0.009	1.85 $\pm$ 0.07
		40	0.39 $\pm$ 0.006	0.56 $\pm$ 0.005	0.05 $\pm$ 0.011	0.61 $\pm$ 0.006	1.57 $\pm$ 0.04

Fig. 6. ESEM micrographs of starch granules of Yangmai 12 wheat showing the effect of high temperature on the morphology of granules from different grain-filling periods and harvested at maturity for the 2004/05 experiment. Columns represent 30 and 40 °C treatments. Rows represent different grain-filling periods (1–3, 6–8 and 25–27 DAA). The panel labelled normal, Mature represents kernels taken from Yangmai 12 wheat exposed to normal outdoor conditions. Arrows indicate fissures on starch granules. Bars represent 20 μ m.

stages. The results from repeated experiments in the two cultivars (Yangmai 9 and Yangmai 12) showed the same trend, in that high temperatures after anthesis had negative effects on starch concentration,

which varied at different filling stages. The compensating effects of transferring to the normal environment after heat shock could not make up for the loss.

The mature wheat endosperm contains two types of starch granules: large (10–35 µm; A-type) and small (1–10 µm; B-type) (Chiotelli & Le Meste 2002). The work of Zhou *et al.* (2001) showed that amyloplasts are divided into 3 types, I, II and III, according to the axis structure. Type-I amyloplasts are large with an ellipsoid shape; type-II amyloplasts are intermediate amyloplasts with a compressed ellipsoid shape; and type-III are small amyloplasts with a spherical shape. However, little is known concerning the effects of different high-temperature regimes during the grain-filling period on the size and morphology of wheat starch granules. The present study found that two types of starch granules were present: large and small. Additionally, the results show that the size, shape and structure of starch granules in wheat grains are visibly different after heat-shock treatment. Yan *et al.* (2008) found that the volume of type A starch granules were enhanced significantly, but that of type B starch granules were greatly reduced under high temperature. However, the present research results showed that the volume of type A starch granules decreased and that of type B starch granules increased significantly ($P < 0.05$). The volume of type A starch granules in both cultivars was decreased by heat shock, which limited the potential sink size for dry matter deposition in the grain. Based on the present results, the effect of high temperature on the volume of type A and B starch granules appears to be more important than the effect of variety.

Little was known about the effects of high temperature during different grain-filling periods on the morphology of starch granules. The present study observed that the shape and structure of starch granules, regardless of type, in wheat grains after heat shock were visibly different from the control. Heat damage at 30 °C could be considerably compensated for in both cultivars, perhaps implying a protective mechanism existing in the endosperm up to a critical temperature.

Protective mechanisms that operate under adverse starch development conditions have been documented in many plant species. Bowler *et al.* (1992) concluded that the elimination of active oxygen species was an important protective mechanism in plants for numerous stressors including temperature stress. Heat-shock proteins (HSPs) that are induced in many plants were thought to play a function in improving the thermo-tolerance of cells under high-temperature stress (Baler *et al.* 1996; Sung & Guy 2003). It is probable that some protective mechanisms, such as enzymes or HSPs, exist in the endosperm at a critical temperature of 30 °C. The results presented here suggest that a significant change in grain metabolism could take place when temperatures are above 30 °C during grain development, a temperature where HSPs start to be produced in the vegetative tissues of wheat (Hendershot 1992). The resistance to high temperature varied between the wheat cultivars tested in the present study, with Yangmai 9 being more tolerant than Yangmai 12.

It is obvious that improved filling and tighter compaction of starch granules would result in higher starch concentration, whereas changes in morphology and volume of granules caused by high-temperature stress could lead to lower starch concentration and starch viscosity. The present results demonstrated that high-temperature stress altered the morphology and reduced the volume of type A starch granules, and consequently starch deposition was retarded and starch concentration and starch viscosity were decreased.

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